الشركة القابضة لمياه الشرب والصرف الصحي

برنامج إدارة مياه الشرب و الصرف الصحي

Water and Wastewater Management Program

gtz

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ميكروبيولوجيا المياه – معامل المحطات

Water Microbiology – Plant Laboratories

Jan 2009

ميكربيولوجيا المياه ـ معامل المحطات

Water Microbiology Plant Labratiores

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Jan 2009

Deutsche Gesellschaft für Technische Zusammenarbeit - GTZ
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مقدمه

مقدمـــة

ان القدرة على الحصول على كوب ماء نقي و نظيف هو احد اهم الاحداث التي تواجة البشرية اليوم.

و يتزايد هذا الاحتياج بطريقة حرجة في المستقبل و كذلك بعد الاستخدام المتزايد لمصادر المياه وزيادة الملوثات لمياة الانهار و البحيرات و الينابيع و كذلك مياه الابار .

قلة المياه المنتجة تاثر على الصحة و الشرب و الامن الغذائي و تاثر على ملايين الفراد من العائلات.

كما ذكر السيد كوفي عنان المين العام للامم المتحدة "الماء سيصبح مصدر التوتر و الحروب القادمة بين الدول لو استمرت الاتجاهات الحالية و كذلك قد تصبح نواة التعاون بين الدول".

الفصل الأول

الصفات العامة للخلايا البكتيرية
المظهرية للخلية البكتيرية (المرفوليجية)

الفصل الأول

الصفات العامة للخلايا البكتيرية
المظهرية للخلية البكتيرية (المرفوليجية)

شكل وتجمع الخلايا البكتيرية

1. البكتيريا المستديرة Spheres

Cocci ومفردها Coccus وكثير من البكتيريا لها نظم تجمع لها أهميتها في أغراض التعرف عليها

- أزواج Diplococcus
- سلسة (سبحية) Streptococcus
- مجاميع Tetrads
- أو عناقيد غير منتظمة تشبة عنقود العنب Staphylococcus
- أو في تركيب ذو ثلاثة أبعاد (مكعبات مكونة من 8 خلايا او اكثر) Sarcina

2. البكتيريا العصوية rod like

Bacillus و مفردها Bacilli تشبة العصا يطلق عليها

عادة لا تنتظم الخلايا العصوية في تجمعات مميزة لأجناسها كما يحدث في البكتيريا المستديرة ولاكن أحيانا تشاهد في أزواج ويطلق عليهاDiplobacilli أو في سلاسل Streptobacilli منها شديد الطول ومنها ما طولها أطول قليلا من عرضها

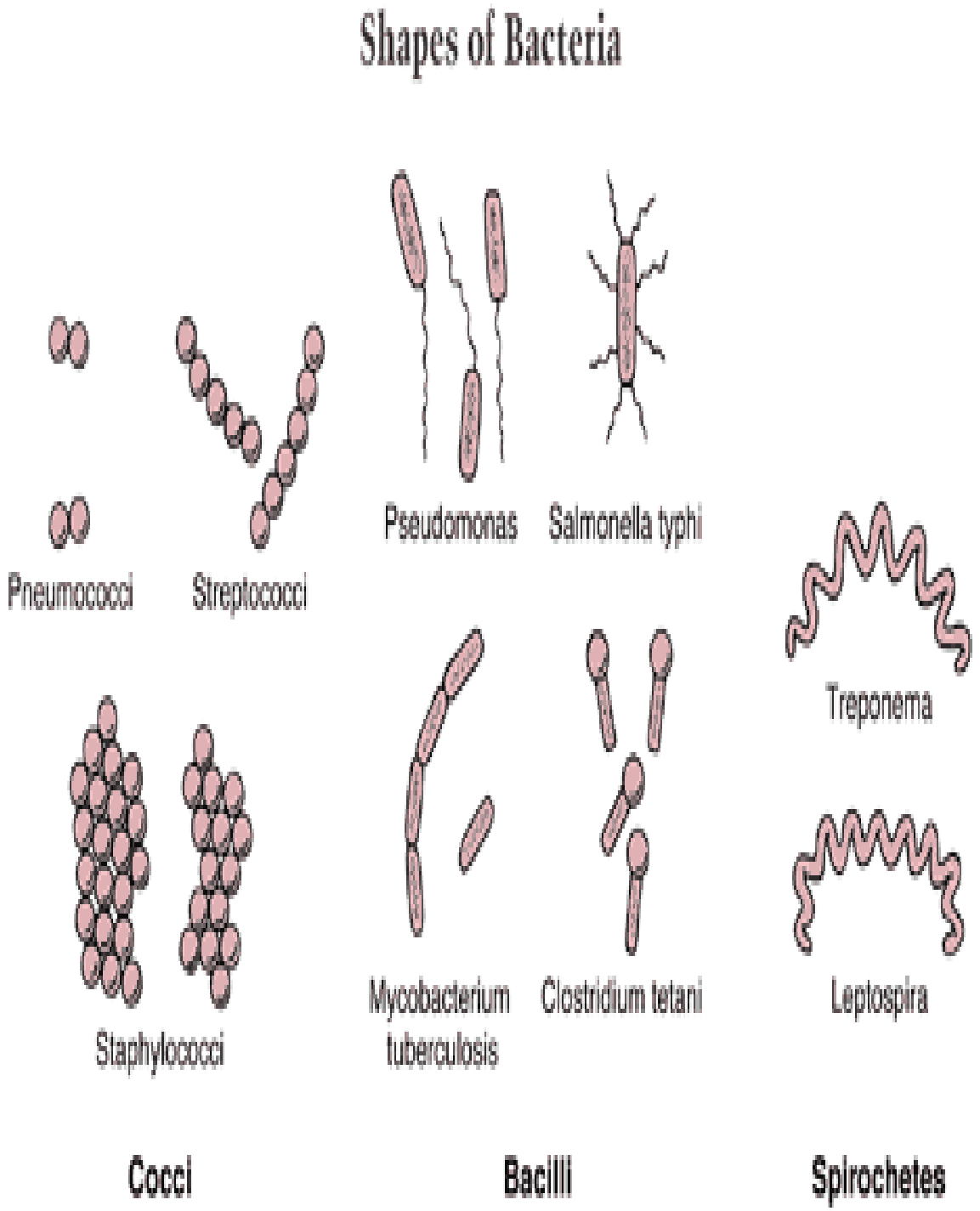
*** بعض البكتيريا العصوية قد تتحول إلى أطوار مقاومة للحرارة تعرف بالجراثيم الداخلية Endospores كما في الجنس Bacillus or Colstridium

3. البكتيريا الحلزونية Spiral

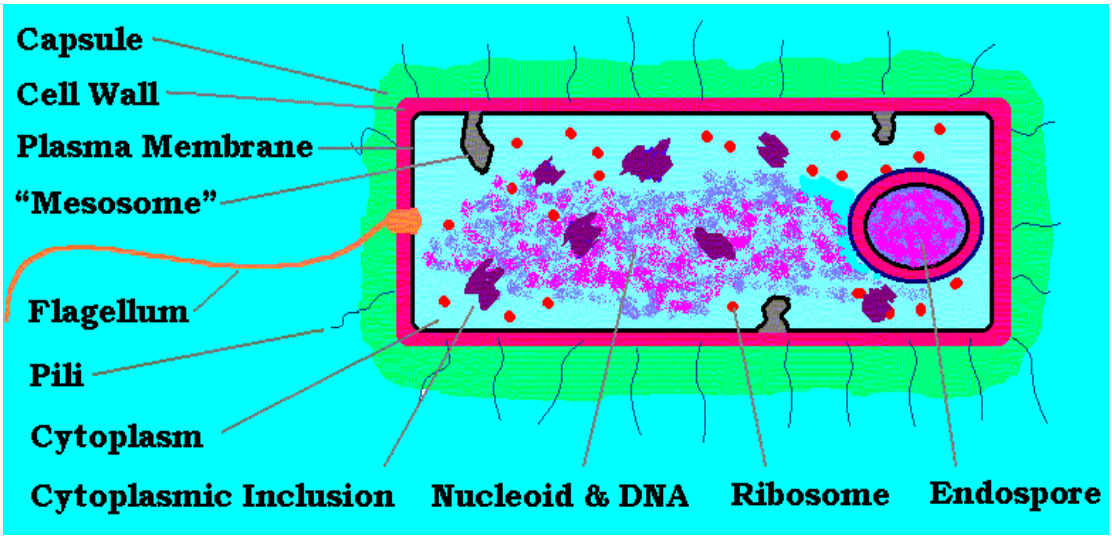
عادتا لا تتجمع خلايا البكتيريا الحلزونية ببعضها بل توجد مفردة دائما ولاكن لها أشكال مختلفة من حيث الطول .

4. أشكال أخرى

- ابلسبيروكيتات Spirochaetes خلايا مرنة طويلة ذات مظهر حلزوني متحركة بدون اسواط.
- الاكتينوميسيتات Actinomycetes شبة الفطريات . تتميز بتكوين خيط أو هيفات.
- الكوريني بكتيرات Coryne bacteria ضمت إلى الاكتينوميسيتات وهي عصويات مستقيمة أو منحنية قليلا.
- الميكوبكتيرات Myco bacteria
- البكتيرات الهلامية


Shapes of Bacteria
Pneumococci
Streptococci
Pseudomonas
Salmonella typhi
Treponema
Staphylococci
Mycobacterium tuberculosis
Clostridium tetani
Leptospira
Cocci
Bacilli
Spirochetes

- **التركيب الداخلى للخلية**



شكل يوضح تركيب الخلايا البكتيلاية

- **النواة:** لا يوجد لها غشاء نووي مميزبل مادة وراثية موجودة في السيتوبلازم(نواة بدائية).
- **الجدار الخلوي:** تحتوي البكتيريا على جدار خلوي يتكون مادة البيبتيدوجليكان.
- **التراكيب الداخلية:** الريبوزومات الجراثيم الداخلية السيتوبلزم النووية و المادة الوراثية.
- **حركة البكتيريا**

الانزلاق gliding movement كما في البكتيريا الهلامية أو حركة دودية flexion movement.

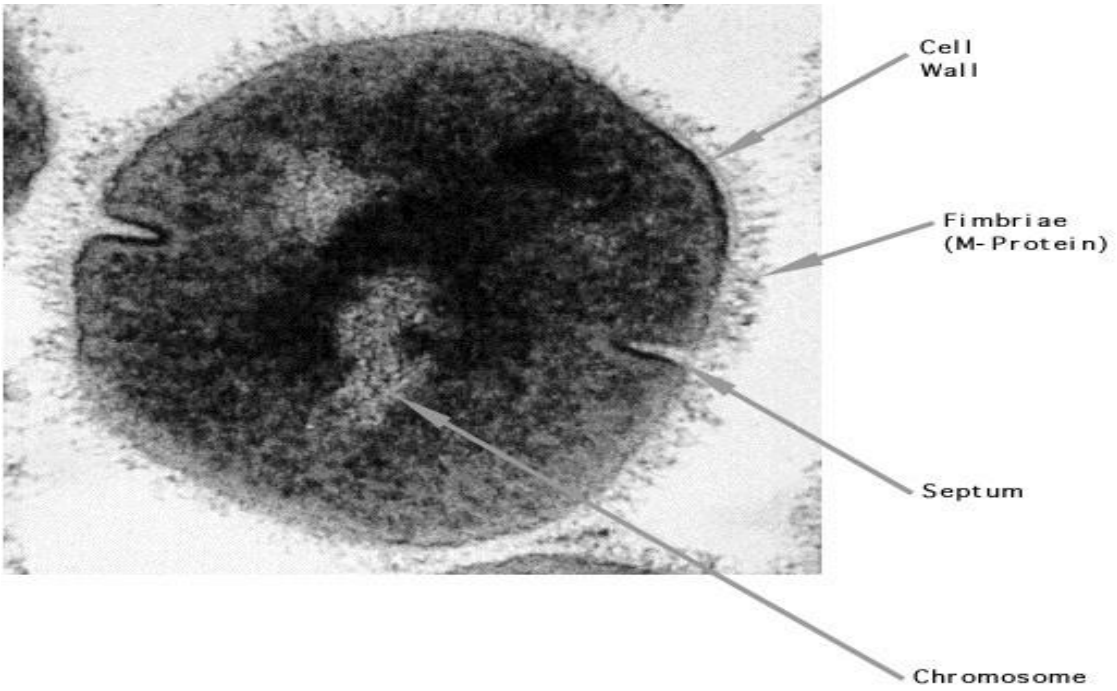
معظم البكتيريا المتحركة تملك أعضاء دقيقة طويلة يطلق عليها الاسواط Flagella و مفردها Flagellum وهي خطوط دقيقة جدا من البروتين.

يوجد توزعين أساسيين للاسواط ويصنف على أساسهما البكتيريا :-

1. عند طرف أو طرفي الخلية.
2. توجد اسواط موزعة عند عدة نقط على امتداد جسم الخلية.

نمو وتكاثر البكتيريا

النمو في البكتيريا :- هو الزيادة في تعداد الخلايا عن القدر الذي بدأت به المزرعة والذي كان موجودا باللقاح الأصلي



صورة لخلية في مرحلة الانقسام الثنائي

- **عملية التكاثر الخلوي**

إن نمو وانقسام الخلايا البكتيرية يمثل عملية دورية cyclical فكل خلية جديدة تتكون تصبح بدورها ذات قدرة على التكاثر . بمعنى أن الخلايا الجديدة الناتجة عن الانقسام تمتلك الخصائص الفسيولوجية التي كانت تميز آباءها القادرة على التكاثر.

أطوار النمو والظروف التي يتاثر :-

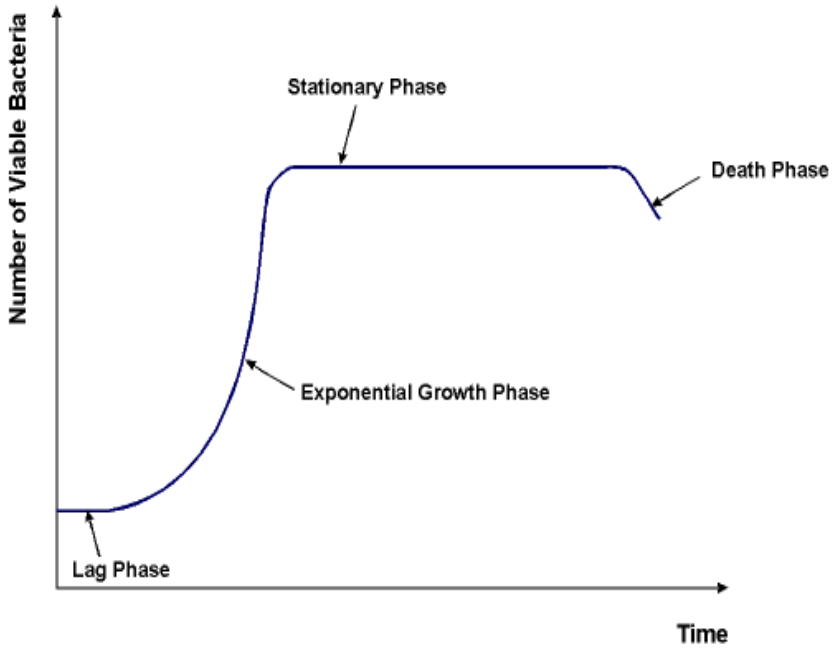
1. **طور الركود Lag phase:** هو مرحلة انتقالية للبكتيريا يحدث بها تباطئ (تلاكئ) في النمو وقد يحدث فقدان لبعض الخلايا البكتيرية وذلك نتيجة عملية النقل للخلايا البكتيرية و فترة التكيف علي الظروف البيئية الجديدة (الحرارة،الرطوبة،الاس الهيدروجيني و العناصر المغذية).

2. طور النمو اللوغارتمي Log phase: هو مرحلة يتم فيها التكاثر البكتيريا باعلى معدلاتة حيث تتزايد اعداد الخلايا البكتيرية تزايدا لوغارتميا وتقل بة اعداد الخلايا البكتيرية الفانية وذلك نتيجة الظروف البيئية الجديدة و وفرة المواد المغذية وعدم التنافس للحصول عليها.

3. الطور الثابت Stationary phase: يحدث ثبات في اعداد الخلايا البكتيرية في هذه المرحلة من النمو وذلك نتيجة تغير الظروف البيئية حيث ينخفض الاس الهيدروجيني (نتيجة تكوين الاحماض العضوية الناتجة عن التمثيل الغذائي لالخلايا البكتيرية) وتنخفض كمية

المغذيات حيث تم استهلاك كمية كبيرة في المرحلة السابقة فيحدث توازن بين الخلايا البكتيرية المتزايدة و الخلايا البكتيرية الفانية.

4. طور تناقص النمو أو طور الموت The phase of decline or death: نتيجة استمرار التغير في الظروف البيئية و انخفاض كمية المواد المغذية يحدث تنافس بين الخلايا البكتيرية لالبقاء فتتزايد اعداد الخلايا البكتيرية الفانية و تقل اعداد الخلايا البكتيرية المزايدة.



شكل بياني يوضح العلاقة بين عدد الخلايا البكتيرية و الزمن في مراحل النمو

- **الاحتياجات الغذائية لالبكتيريا**

1. الهيدروجين

2. الأكسجين

3. الكربون

4. النيتروجين

5. الأملاح

6. بعض العناصر والمعادن

***** **كما هو ملحق بالجدول**

Table Major elements, their sources and functions in bacterial cells

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO2	Main constituent of cellular material
Oxygen	20	H2O, organic compounds, CO2, and O2	Constituent of cell material and cell water; O2 is electron acceptor in aerobic respiration
Nitrogen	14	NH3, NO3, organic compounds, N2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H2O, organic compounds, H2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO4, H2S, So, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Trace Elements

Table ignores the occurrence of trace elements in bacterial nutrition. Trace elements are metal ions required by certain cells in such small amounts that it is difficult to detect (measure) them, and it is not necessary to add them to culture media as nutrients. Trace elements are required in such small amounts that they are present as "contaminants" of the water or other media components. As metal ions, the trace elements usually act as cofactors for essential enzymatic reactions in the cell. One organism's trace element may be another's required element and vice-versa, but the usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, and Mo.

- Oxygen
- Obligate aerobes
- Facultative anaerobes
- Obligate anaerobes

- **الظروف الفيزيائية التى تؤثر على نمو البكتريا**

يتاثر النشاط البكتيري من حيث النمو و التكاثر بالعوامل الفزيائية المحيطة بالخلية حيث ان معدل النمو لالخلايا تتاثر بارتفاع او انخفاض هذه العوامل لذلك يكون هناك مستويات لنمو الخلايا البكتيرية في الظروف المحيطة المختلفة

الحرارة Temperature

أن النطاق الحراري الذي يسمح لنمو معظم البكتيرات هو نفس المعدل الذي يفضلة الانسان

- **بكتريا محبة للبرودة Psychrophile**

تفضل البكتيريا النمو في درجات حرارة **منخفضة.**

- **بكتريا محبة للحراره المتوسطة Mesophiles**

تفضل البكتيريا النمو في درجات حرارة متوسطة.

- **بكتريا محبة للحراره المرتفعة Thermophiles**

تفضل البكتيريا النمو في درجات حرارة **مرتفعة.**

- **درجة الحرارة لاقل نمو Minimum growth temperature**

هي اقل درجة حرارة يمكن حدوث عندها نمو.

- **درجة الحرارة للنمو الامثل Optimum growth temperature**

هي درجة الحرارة التي يحدث عندها افضل نمو لالخلايا البكتيرية.

- **درجة الحرارة لاكثر نمو Maximum growth temperature**

هي اعلى درجة حرارة يمكن حدوث عندها نمو.

- **الاس الهيدروجيني pH**

معظم انواع البكتيريا تعطي افضل نمو لها في حيز ضيق من pH قريب جدا من التعادل حيث انها تعيش مابين 6.5-7.5 القليل من البكتيريا التي يمكن لها ان تعيش في وسط حمضي حتى pH 4.0

- **أنواع الأوساط الغذائية**

يتم تصنيف الأوساط الغذائية علي حسب الأغراض التي يتم الزرع لها

1. الأوساط الغذائية العامة (General Media) :-

وتسمى أيضا بالأوساط الغذائية الأساسية (Minimal Media) حيث يتم عليها تنمية لجميع أنواع البكتيريا وهي عبارة عن خليط من أنواع السكر المختلف (مصدر للكربون والطاقة) والبروتين (مصدر للنيتروجين) والأملاح ومن أمثالهاNutrient Agar

2. الأوساط الغذائية العازلة (Isolation Media)

وهي أوساط غذائية يتم تنمية نوع واحد أو مجموعة واحدة من البكتيريا عليها دون السماح للأنواع الأخرى بالنمو وذلك عن طريق اختيار نوع واحد من السكر و البروتين والأملاح لا يستطيع أي نوع أخر من البكتيري أن يخمرها (Fermentation) ومنها Brilliant Green و E.C. Media و Lauryl Tryptose

3. الأوساط الغذائية الاختيارية (Selective Media)

وهي أوساط غذائية يتم تنمية نوعان أو اكثر من البكتيريا لإجراء بعض التجارب أو المقارنات بينها.

- طرق تحضير وحفظ الأوساط الغذائية

الطرق العامة

أ. تخزين الأوساط الغذائية:-

يجب تخزين الأوساط الغذائية الخالية من الماء "البودرة" في العبوات الخاصة بحيث تكون مغلقة جيدا لمنع تسرب الرطوبة إليها مما ينتج عنه تغيرات كيمائية و فيزيائية غير مرغوبة (تغير اللون والشكل).

1 - تخزين الأوساط الغذائية الخالية من الماء "البودرة" في درجة حرارة اقل من 30 درجة مؤوية ومكان مظلم جاف.

2- لا يمكن استخدام الأوساط الغذائية الخالية من الماء "البودرة" عند تغير لونها أو تصبح كتل " تحجر" أو عندما تفقد خاصيتها .

1. يجب أن تستهلك العبوة في خلال 6اشهر من تاريخ فتح العبوة .
2. تحضر الأوساط الغذائية الكافية لأسبوع "أسبوعيا"
3. إذا حفظت الأوساط المغذية المجهزة في زجاجات مغلقة جيدا بغطاء قلاووظ يمكن أن تصل صلاحية استعمالها حتى 3 شهور من تاريخ التحضير .
4. احفظ الأوساط المغذية المحضرة بعيدا عن ضوء الشمس المباشر و التلوث و التبخير الشديد .
5. في الأوساط الغذائية السائلة تخزن في الثلاجة أو في درجات حرارة منخفضة .
6. يجب حفظ أنابيب الأوساط الغذائية على الأقل ليلة في الثلاجة قبل استخدامها لتجنب تكوين فقاعات هواء داخل الأنابيب .
7. عند بدء استعمال الأنابيب يجب استبعاد الأنابيب الموجود بدخلها فقاعات هواء .
8. عند حدوث تبخير للوسط الغذائي السائل الموجود داخل الأنابيب اكثر من املل تستبعد الأنبوبة .

ب. ضبط تفاعل التحضير :-

1. عن طريق قياس pH للوسط الغذائي .
2. عادتا ما يحدث تغير طفيف في pH الوسط الغذائي بعد التعقيم حسب نوع جهاز التعقيم المستخدم .
3. عند تحضير الأوساط الغذائية يكون التغير في pH عادتا ±0.1 أو± 0.2 لا كن قليلا ما تكون ± 0.3 كما يحدث في الأوساط مزدوجة القوة .
4. يمكن اختبار pH للأوساط الغذائية بواسطة جهاز ال pH .
5. عند خروج معدل التغير في ال pH عن المكتوب على العبوة الخاصة بالوسط الغذائي لا يمكن استخدام هذا الوسط الغذائي .

ج. التعقيم :-

1- لا يتم تخزين الأوساط الغذائية بعد حلها في الماء بدون تعقيم .

2-لا يتم تعقيم الأوساط الغذائية السائلة الموجود بها أحد أنواع السكر مع غيرها

3- تعقم الميديات السائلة في الأوتوكلاف عند درجة حرارة 121درجة مئوية لمدة 15 دقيقة.

4-عند انتهاء التعقيم و انخفاض الضغط (عندما يصل إلى صفر) يتم التبريد. الأوساط الغذائية بسرعة حتى لا يحدث تكسير لمركبات السكر الموجودة به بتعريضها إلى درجة حرارة مرتفعة لفترة طويلة .

5-من اجل الحصول على توزيع جيد لدرجة الحرارة في حالة تعقيم الميديات وأيضا لسهولة تبريدها بعد إتمام عملية التعقيم توزع المديات في زجاجات صغيرة (يفضل 10 مل) و أيضا لا تربط غطاء الزجاجات بشدة .

6-أقصى مده لمكوث الأوساط الغذائية السائلة بتعقيمها في الأوتوكلاف 45 دقيقة (من بدا تشغيل الجهاز حتى إخراج الأوساط منه)

*** يفضل استخدام double- walled autoclave to permit preheating before loading to reduce total needed heating time within the 45-min limit .

- **ملاحظات هامة**

1. عند تحضير الأوساط الغذائية ذات القوة المزدوجة يجب ان يضاعف الوزن مع ثبات كمية المياه
2. تنقع أنابيب الاختبار و أنابيب الدرهم بالماء والصابون لفترة كافية حتى التأكد من نظافتها
3. تغسل بالماء الساخن للتأكد من إزالة الرواسب
4. ضع أنبوبة الدرهم داخل أنبوبة الاختبار بحيث يكون فوهة أنبوبة الدرهم لاسفل
5. أضف الكمية المحددة (10 مل) من الوسط الغذائي داخل الأنبوبة
6. لا تقلب الأنبوبة لاخراج الهواء من أنبوبة الدرهم
7. اغلق أنبوبة الاختبار بحيث يمكن تعقيم الوسط الغذائي في الأوتوكلاف (غير محكم الغلق)

*** يجب اتباع تعليمات الشركة المصنعة في تحضير و تعقيم الميديات .

- **الماء المعامل**

أ. الخصائص:-

1. لتحضير الأوساط الغذائية والكواشف يستخدم ماء مقطر أوDemineralized water التي يتم اختبارها للتأكد من خلوها من آثار المعادن و المواد القاتلة المثبطة للبكتيريا
2. Toxicity في الماء المقطر ربما تحدث من احتواء الماء على فلوريد مع وجود نسبة مرتفعة من السيليكا.
3. المصادر الأخرى للToxicity هي الفضة و الرصاص و المركبات العضوية غير المحددة .
4. الكلور الحر والكلورامينات يمكن ان تكون في الماء المقطر .
5. في حالة وجود مركبات الكلور في الماء المقطر يمكن معادلته بواسطة Sodium thiosulfate OR Sodium sulfate
6. الماء المقطر يجب ان يكون خالي من التلوث بالمواد المغذية للبكتيريا التي يمكن ان تحدث من Flashover of organics أثناء التقطير ويمكن التغلب عليها باستخدام وسادة من الفلتر الكربوني أو إعادة شحن عمود إزالة الأيونات
7. يمكن أن يحدث تلوث عن طريق استخدام زجاجات أو ماطات غير نظيفة أو بواسطة الأبخرة الكيميائية أو الغبار .
8. تخزن المياه المقطرة بعيدا عن ضوء الشمس المباشر لكي يتجنب نمو الطحالب .
9. يجب غلق زجاجات المياه المقطرة جيدا و سريعا

Test	Monitoring Frequency	Limit
Chemical tests: Conductivity	Continuously or with each use	> 0.5 megohms resistance or < 2 µmhos / cm at 25 o C
pH	With each use	5.5-7.5 mg/l
Total organic carbon	Monthly	< 1.0 mg/l
Heavy metals, single (Cd, Cr, Cu, Ni, Pb, and Zn)	Annually*	< 0.05 mg/l
Heavy metals total	Annually*	≤ 0.1 mg/l
Ammonia / organic nitrogen	Monthly	< 0.1 mg/l
Total chlorine residual	Monthly or with each use	< 0.01 mg/l
Bacteriological tests: Heterotrophic Plate Count	Monthly	< 1000 cfu/mL
Water quality test	Annually and for a new source	0.8 -3 ratio
Use test	3 months	Student's t ≤ 2.78

*Or more frequently if there is a problem.

إذا لم تتطابق خواص المياه مع الحدود المذكورة يبحث المسبب ويصحح الوضع.
على الرغم من ذلك فان قياس pH المياه المنقاة وإعطاء قراءات منحرفة جدا فان ذلك يعد دليل على التلوث الكيميائي.

طرق التعقيم

- **فرن الهواء الساخن Hot-air oven**

اختبر الأداء كل 3 شهور باستخدام المتاح سواء شريط الجراثيم أو معلق الجراثيم Spore strips or spore suspensions (Bacillus.subtilis). راقب درجة الحرارة بترمومتر

دقيق عند مجال 160 – 180 مئوية وسجل النتائج. استعمل أشرطة بيان الحرارة Heat-indicating tape لبيان صحة عملية التعقيم لأى مواد تعرض لحرارة التعقيم.

- **الأوتوكلاف Autoclave**

سجل الأشياء المعقمة، الحرارة، الضغط والزمن لكل دورة. استعمل ترمومتر بجهاز تسجيل. أضبط حرارة التشغيل أسبوعيا (أقل/ أقصى) بترمومتر. اختبر الأداء شهريا بشريط أو معلق الجراثيم المشار إليه سابقا. استعمل شريط بيان الحرارة لمعرفة المواد التى تم تعقيمها (عن طريق تغير لون الشريط).

- **لمبات التعقيم بالأشعة الفوق بنفسجية Ultraviolet sterilization lamps**

تفك الوحدة شهريا وتنظف اللمبات باستعمال قطعة قماش مبللة بكحول الايثانول. تختبر اللمبة شهريا بجهاز قياس الأشعة الفوق بنفسجية وتستبدل إذا كانت تشع أقل من 70% من أصل قوتها أو إذا عرضت لها مياه ذات محتوى من الكائنات الدقيقة يتراوح بين 200 – 250 مستعمرة ولم يتم خفضها بواقع 99% خلال دقيقتين من التعرض للأشعة.

* تحذير: على الرغم أن الأشعة فوق البنفسجية ذات الموجات القصيرة (254 نانومتر) معروف أنها أكثر خطورة من تلك ذات الموجات الطويلة (365 نانومتر) ، كلا النوعين يمكن أن يضر العين والجلد وهى مسرطنه قويه (Schmitz et al., 1994). احم عينيك والجلد من التعرض للأشعة الفوق بنفسجية.

- **كابينة الأمان Biohazard hood**

اكشف على الفلاتر شهريا للانسداد ونظفها أو استبدلها حسب الحاجة. عرض شهريا أطباق من الآجار Plate count agar للهواء المنساب من الكابينة لمدة ساعة. حضن الأطباق عند 35 درجة مئوية لمدة 48 ساعة واختبر للتلوث (لا نمو على البيئة إذا كانت الكابينة تعمل جيدا). فك لمبات الأشعة الفوق بنفسجية ونظفها شهريا بقماش مبلل بكحول الايثانول. اكشف عن كفاءة اللمبات بقياس قوة الإشعاع الناتج كما هو مبين سابق. اختبر الكابينة للتسرب ومعدل انسياب الهواء كل 3 شهور. استعمل وسيلة مراقبة الضغط لقياس كفاءة أداء الكابينة. استعمل الكابينة ذات الفلاتر من النوع HEPA. الصيانة كما يرد فى كتالوج المصنع.

- **المواد الكيميائية المستعملة في التعقيم**
- **الكحوليات :-**

يعتبر كحول الايثايل بتركيز 50 – 70 % اكثر الكحولات استعمالا في أغراض التطهير الخارجي لأنه يقوم بتمزيق الغشاء الخلوي للبكتيريا إلا انه لا يمكن الاعتماد علية في عمليات التعقيم حيث ان تركيزاته التي تؤثر على الخلايا الخضرية لا تؤثر على الجراثيم البكتيرية .

الفصل الثانى

الكواشف و الكائنات الكاشفة

الفصل الثاني

الكواشف و الكائنات الكاشفة

Indicator and indicator microorganisms

- من الصعب تحديد البكتريا المسببة للأمراض في الماء لصعوبة التحكم بها داخل المعمل و كذلك لان عدد البكتريا المسببة للأمراض بالنسبة للكائنات الأخرى قليل جدا (لذلك يجب أن يكون حجم العينة كبير)
- لذلك عند اختبار البكتريا المسببة للأمراض يتم استبدالها ببعض البكتريا المثالية التي تستخدم كدليل على جودة المياه (كاشف)
- البكتريا المثالية التي تستخدم ككاشف على جودة المياه يجب أن تتوافر بها عدة صفات : -

1 - يجب أن تتواجد دائما في حالة وجود الكائنات المسببة للأمراض –(كدليل على التلوث)- وان ينعدم وجوده في المياه النظيفة الغير ملوثة .
2 - يجب أن تتواجد بكميات كبيرة في المياه الملوثة بال (fecal materials) .
3 -يجب أن تكون ملائمتها للظروف البيئية و عمليات المعالجة مماثلة للبكتريا المسببة للأمراض.
4 -يجب أن يتم تحديها بطريقة سهلة – بسيطة – غير مكلفة – و تبين نتائج دقيقة في وقت قصير.
5 -يجب أن تكون نسبتها بالمياه عالية بالنسبة للبكتريا الممرضة.
6 -يجب أن تكون ثابتة و غير مسببة للأمراض.
7 -يجب أن تكون مناسبة لكل أنواع مياه الشرب.

من ذلك وجد أن coliform group من البكتريا المثالية للاستخدام ككاشف عن تلوث المياه.

بكتريا القولون TOTAL COLIFORM

- هوائية – لا هوائية اختيارية Facultative anaerobes
- سالبة لصبغة جرام Gram negative
- لا تكون جراثيم Non- spore forming
- بكتريا خلاياها عصوية الشكل Rod – shaped
- تحلل سكر اللاكتوز منتجة غاز و حمض في خلال 48 ساعة عند درجة حرارة 35م.
- تتواجد Coliform group في أمعاء الحيوانات.

- يعتبر تحليل اللاكتوز من الدلائل الأساسية على وجود coliform group لان خاصية تحليل اللاكتوز تعتبر من الصفات الأساسيةلل coliform حيث أن عدد قليل فقط من البكتريا يقوم بتحليل اللاكتوز.
- Coliform group تضم عدة أنواع و أجناس منها E. coli
- تتواجد coliform group في أمعاء الحيوانات و لكن عدد كبير منها يتواجد في البيئة الخارجية (المياه – مخلفات المياه) ، و لكن تعتبر E. coli استثناء أساسي حيث أنها لا تستطيع العيش مدة طويلة خارج الأمعاء إلا في حالة المياه الدافئة

الاستخدام

- تستخدم مجموعة آلColiform لتحديد مدى كفاءة عملية المعالجة وكذلك بالنسبة للشبكات
- وكذلك كدليل على التلوث بالبكتيريا البرازية
- عند خلو المياه المعالجة من Coliform Bacteria يدل ذلك على انخفاض البكتيريا المسببة للأمراض إلى اقل عدد لها

العيوب

- Coliform Bacteria اقل مقاومة لعملية التعقيم من بعض الفيروسات و الحيوانات وحيدة الخلية المسببة للأمراض بأشكالها المختلفة
- ليس بالضرورة إذا وجدت الColiform Bacteria ان يوجد تلوث بال Fecal Bacteria

طريقة أنابيب التخمر المتعددة أو العد الاحتمالى لتقدير مجموعة بكتريا القولون

Multiple Tube Fermentation or Most Probable Number Technique for Determination of Coliform Group

عند استعمال هذه الطريقة فان نتائج اختبار مكررات الأنابيب Replicate tubes والتخفيفات تسجل على صورة العدد الأكثر احتمالا Most Probable Number (MPN) للكائن المتواجد. هذا العدد، يعتمد على نظرية الاحتمالات، وهى تقديرات لمتوسط كثافة البكتريا (القولون أو غيرها) فى العينة. وكثافة بكتريا القولون المتحصل عليها مع بعض المعلومات الأخرى المتحصل عليها من خلال المسح الهندسى أو الصحى ، يمد بافضل طرق تقييم فعالية المعالجة والنوعية الصحية لمصدر المياه.

دقة كل اختبار تعتمد على عدد الأنابيب المستعملة. وأفضل المعلومات يحصل عليها عن استعمال أكبر حجم من العينة فى حقن الأنابيب بحيث تظهر غاز فى بعض منها أو كلها فى حين أن أضغر حجم من

العينه يستعمل فى حقن الأنابيب لا يظهر غاز فى كل الأنابيب أو معظمها. وكثافة البكتريا تقدر من معادلة أو من جدول باستعمال عدد الأنابيب الموجبة فى التخفيفات المتعددة. عدد جزئيات العينة Sample portions المختارة سيحكم بدرجة الدقة فى النتيجة. وجداول العدد الاحتمالى تعتمد على افتراض توزيع بويسون Assumption of a poisson distribution (الانتشار العشوائى). وعلى ذلك ، اذا لم يتم رج العينة بطريقة مناسبة قبل أخذ الجزئيات منها أو اذا وجدت تجمعات من خلايا البكتريا ، فان قيمة العدد الاحتمالى ستكون بعيدة عن الكثافة الحقيقية للبكتريا.

المياه من نوعية مياه الشرب Quality of Drinking Water

عند تحليل مياه الشرب لتقدير ما اذا كانت النوعية تتطابق مع المواصفات التى وضعتها وكالة حماية البيئة الأمريكية ، يستعمل 10 أنابيب مكررة تحتوى على 10 ملل، 5 مكررات من أنابيب تحتوى على 20 ملل أو زجاجة واحدة تحتوى 100 ملل من العينة. وعند اختبار مياه الشرب يجرى الاختبار التأكيدى على كل الأنابيب أو الزجاجات التى تعطى نمو مع وجود أو عدم وجود نتيجة ايجابية لتكون حامض أو غاز . ويجرى الاختبار الكامل Completed test على ما لا يقل عن 10 % من العينات الموجبة كل 3 شهور. وتعتبر نتيجة EC broth أو EC MUG الايجابية كبديل للنتيجة الايجابية للاختبار الكامل.

وللاختبار الروتينى لمصادر مياه الشرب العامة فان الهدف من الاختبار لبكتريا القولون الكلية هو تقدير كفاءة عمليات المعالجة التى تتم فى المحطة وكذلك سلامة نظام التوزيع . وكذلك يستعمل كمانع لوجود التلوث البرازى. تواجد جزء كبير من بكتريا القولون فى نظام التوزيع ربما يكون غير مرتبط بفشل المعالجة أو البئر المصدر، ولكن مرتبط باستعادة النمو للبكتريا . ولصعوبة التفرقة ما بين استعادة بكتريا القولون للنمو والتلوث الجديدن يفترض أم نواجد بكتريا القولون هو تلوث جديد الا اذا اثبت.

- **المياه الأخرى خلاف مياه الشرب**

فى اختبار المياه خلاف مياه الشرب يحقن سلسلة من الأنابيب بتخفيفات متتالية عشرية من المياه (10و 1 و 1، و 01، .....)اعتمادا على كثافة بكتريا القولون المحتملة . يجرى الاختبار الكامل أو النهائى على 10% على الأقل من العينات الموجبة على أساس موسمى . والهدف من اختبار المياه خلاف مياه الشرب هو اختبار كثافة التلوث البكتيرى، تقدير مصدر التلوث، الالزام بنوعية المياه القياسية، أو تقدير حيوية Survival للبكتريا. اختبر عدد كاف من العينات للحصول على نتائج ممثلة . وعموما، فان قيمة المتوسط للنتائج من عدد من العينات يعطى قيمة يكون فيها يتدنى الاختلاف من عينة الى أخرى .

- **العينات الأخرى**

فى حالة اختبار المواد الصلبة أو شبه الصلبة يتم عمل تخفيف وزنم حجم من محلول منظم أو ماء الببتون 1،% ويتم الخلط باستعمال خلاط بسرعة منخفضة (8000 لفة فى الدقيقة) لمدة 1 – 2 دقيقة ومنه يتم عمل التخفيفات المتتالية بسرعة حتى لا يحدث ترسيب.

طريقة التخمر القياسية لتقدير بكتريا القولون الكلية

Standard Total Coliform Fermentation Technique

- **المرحلة الافتراضية Presumptive Phase**

استعمل بيئة Lauryl tryptose broth فى المرحلة الافتراضية لاختبار الأنابيب المتعددة. اذا كانت البيئة مبردة بعد التحضير والتعقيم، تترك على درجة حرارة الغرفة (20 مئوية) خلال الليل وقبل الاستعمال. يتخلص من الانابيب التى يظهر بها نمو أو فقاعات أو كلاهما.

- **تركيب بيئة مرق اللوريل تربتوز :**

Tryptone	..20	g
Lactose	. 5	g
Dipotassxium hydrogen phosphate	2.75	g
Potassium dihydrogen phosphate	2.75	g
Sodium chloride	5	g
Sodium lauryl sulphate	.0.1	g
Reagent-grade water	1	L

أضف المكونات الى الماء واخلط جيدا، سخن للذوبان. pH يجب أن يكون 6,8 +/- 2، بعد التعقيم. قبل التعقيم وزع البيئة بكميات كافية فى أنابيب التخمر ويكون بكل انبوبة انبوبة درهام مقلوبة وتكون حجم البيئة يغطى نصف الانبوبة أو ثلثاها على الأقل بعد التعقيم. وكبديل اهمل وضع انبوبة درهام وأضف 01، جرام / اللتر من برومو كريزول بيربل الى البيئة للكشف عن انتاج حموضة والذى يعتبر دليل على ايجابية هذا الاختبار. وتغطى الانبوبة بغطاء معدنى أو بلاستيكى مقاوم للحرارة. حضر اللوريل بتركيز بحيث لا يؤثر اضافة 100 ملل، 20 ملل ، أو 10 ملل على تركيز مكونات البيئة

9221:I. Preparation of Lauryl Tryptose Broth

Inoculum *mL*	Amount of Medium in Tube *mL*	Volume of Medium + Inoculum *mL*	Dehydrated Lauryl Tryptose Broth Required *g/L*
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

- **الطريقة**

رتب الأنابيب فى صفوف من 5 أو 10 أنابيب فى حامل أنابيب. عدد الصفوف وحجم العينة يعتمد على نوعية وخصائص المياه المختبرة. لمياه الشرب استعمل 5 أجزاء من العينة كل 20 ملل، 10 كل 10 ملل، أو زجاجة واحدة 100 ملل، وللمياه الغير مستعملة فى الشرب استعمل 5 أنابيب لكل تخفيف (10، 1، 1، ملل أو غيرها)

لعمل التخفيفات وقياس حجم العينات المخففة اتبع ما ذكر سابقا. رج العينة والتخفيفات جيدا حوالى 25 مرة. لقح كل انبوبة فى المجموعة بحج متكرر من العينة . اخلط العينة مع البيئة بالهز برفق.

حضن الأنابيب الملقحة أو الزجاجات عند 35 +/- 5، مئوية . وبعد 24 ساعة +/- 2 ساعة اختبر الأنابيب أو الزجاجات لوجود نمو، غاز، تفاعل حامضى (لون أصفر)، اذا لم يكن هناك غاز أو حامض ، أعد التحضين ،اعد الكشف فى نهاية 48 ساعة +/- 3 ساعة. سجل النتيجة.

انتاج تفاعل حامضى أو غاز فى الأنابيب أو الزجاجات خلال 48 ساعة +/- 3 ساعة يدل على التفاعل الافتراضى الموجب. النابيب الموجبة يجرى لها الاختبار التأكيدى.

غياب تكون الغاز أو الحموضة فى نهاية التحضين يدل على النتيجة السلبية للاختبار.
أكد الأنابيب أو الزجاجات التى أظهرت غياب الغاز وغياب الحموضة ولكن أثبتت أن هناك نمو.

- **المرحلة التأكيدية Confirmed phase**

- **بيئة الزراعة :** استعمل بيئة مرق البريلينت جرين لاكتوز بيل للتخمر فى أنابيب للمرحلة التأكيدية وتركيبها كما يلى

Peptone ……..	10	g
Lactose ……	10	g
Oxgall ……..	20	g
Brillient green ……...	0.0133	g
Reagent grade water ……1		L

أضف الكونات الى الماء واخلط جيدا، سخن لاذابة الكونات واضبط pHبحيث يكون 7،2 +/- 2، بعد التعقيم. قبل التعقيم وزع فى أنبيب وبهاانبوبة درهام مقلوبة ، كمية من البيئة كافية لتغطى نصف الى ثلثى انبوبة درهام بعد التعقيم. غطى الأنابيب بأغطية معدنية أو بلاستيك مقاوم لحرارة التعقيم.

- **الطريقة Procedure :**

كل الأنابيب الايجابية أو الزجاجات التى أعطت نتيجة ايجابية فى المرحلة الافتراضية Presumptive phase (أية كمية من الغاز- عكارة- حامض) خلال 24 +/- 2 ساعة (أو قبل ذلك وينصح بالفحص بعد 18 +/- 1 ساعة للايجابية) من التحضين يتم اجراء الاختبار التأكيدى. واذا ظهرت أنابيب ايجابية أخرى عند 48 +/- 3 ساعة من التحضين يجرى لها هى الأخرى الاختبار التأكيدى.
هز برفق الأنابيب الايجابية من المرحلة الافتراضية (حامض وغاز) لتعليق النمو من الكائنات فى الأنابيب. باستعمال لوب معقم (قطر فتحتها 3 – 3,5 مم) انقل لوب واحدة أو أكثر من المزرعة من كل انبوبة ايجابية الى انبوبة بريلينت جرين (يمكن استعمال ناقل خشبى Applicator معقم بغمسه لمسافة 2,5 سم على الأقل فى المزرعة ووضعه فى بيئة البريلينت جرين ثم ازالته). ويكرر ذلك بالنسبة لكل الأنابيب الايجابية من المرحلة الافتراضية.
حضن انابيب البريلينت جرين عند 35 مئوية +/- 5، درجة ، تكون غاز فى انبوبة درهام بعد فترة تحضين (من 6 +/- 1 سلعة الى 24 +/- 2 ساعة) وخلال 48 ساعة +/- 3 ساعة تعتبر تلك الأنابيب ايجابية للاختبار التأكيدى. احسب العدد الاحتمالى من الأنابيب الايجابية من بيئة البريلينت جرين.

- **الطريقة البديلة :**

تستخدم تلك الطريقة بالنسبة للمياه الملوثة ومياه الصرف الصحى . اذا كانت كل الأنابيب فى المرحلة الافتراضية ايجابية لتخفيفين متتالين من العينة المختبرة يتم اجراء الاختبار التأكيدى للانابيب الايجابية من الأنابيب المحقونة بالكمية الأقل من العينة والموجبة جميعها الى جانب الأنابيب الايجابية من الصف الثالث (المحقونة بكمية أقل من العينة) ويتم عليها ما تم سابقا.

- **المرحلة الكاملة أو النهائية Complete phase**

يجرى هذا الاختبار كبيانات للتحكم فى الجودة على 10% على الأقل من الانابيب الايجابية من المرحلة السابقة. يتم حقن انابيب بريلينت جرين لبكتريا القولون الكلية و مرق EC أو بيئة مرق EC with MUG لايشيريشيا كولاى (كلاهما يتم التحضين عند 44,5 درجة مئوية) والنتيجة الايجابية الايجابية لبكتريا القولون الكلية (انظر الشكل التالى) فى الحالات الثلاث تدل على أن الاختبار النهائى ايجابى .

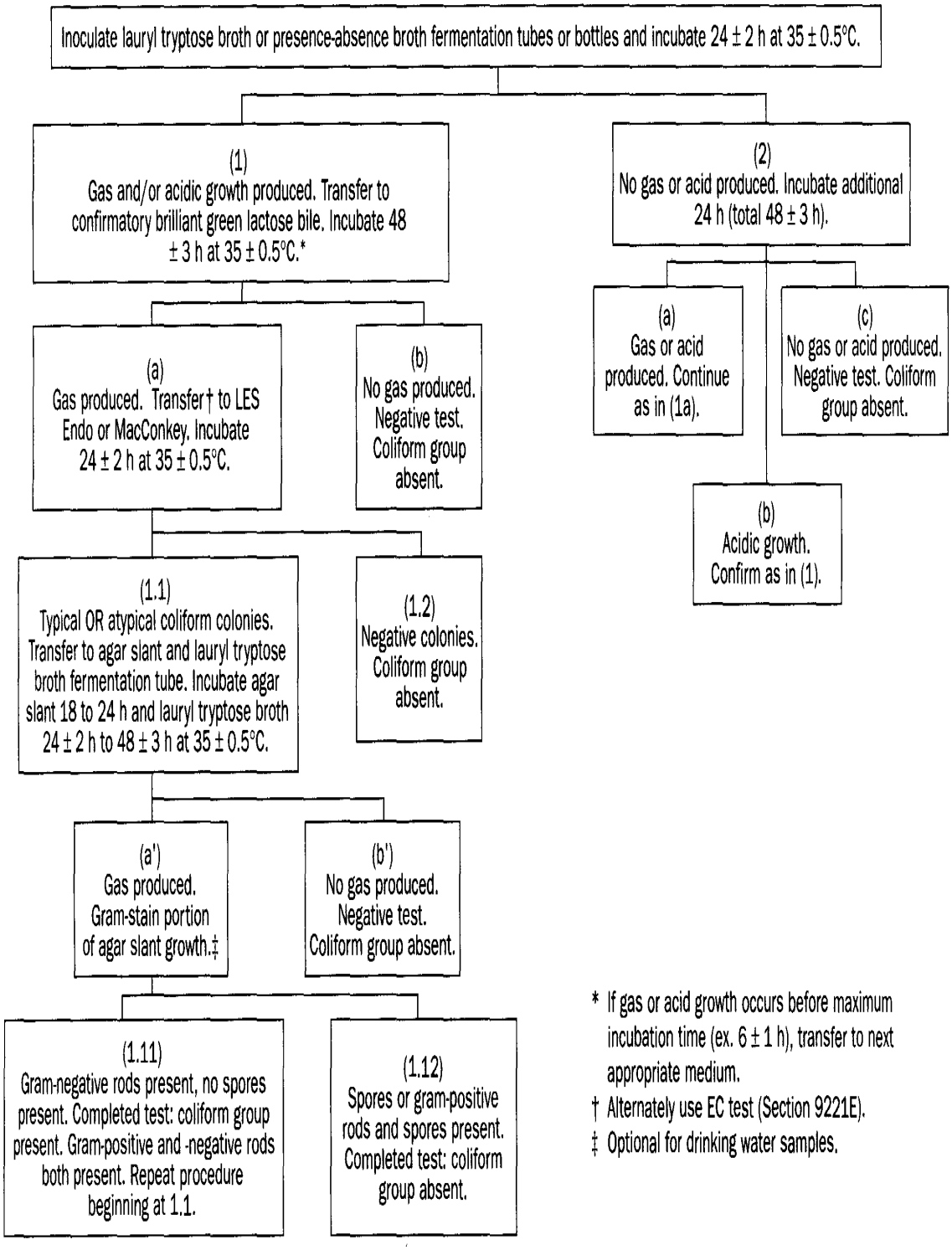


Figure 9221:1. Schematic outline of presumptive, confirmed, and completed phases for total coliform detection.

وايجابية أنابيب البرينت جرين فقط دل على

LES Endo agar:

Yeast extract ……1,2 g
Casitone or trypticase ……3,7 g
Thiopeptone or thiotone …… 3,7 g
Tryptose …… 7,5 g
Lactose …… 9,4 g
Dipotassium hydrogen phosphate …… 3,3 g
Potassium dihydrogen phosphate …….. 1.0 g
Sodium chloride …….. 3,7 g
Sodium desoxycholate ……0,1 g
Sodium lauryl sulphate …… . 0,05 g
Sodium sulfite …… 1,6 g
Basic fuchsin …….. 0,8 g
Agar …… 15.0 g
Reagen grade water …… 1 L

اضف الكونات الى 1 لتر ماء وأضف 20 ملل ايثانول 95 %. سخن الى قرب الغليان لاذابة المكونات ثم اخرجها من حمام الماء وبرد الى 45 – 50 درجة مئوية. لا تعقم فى الأوتوكلاف. pH النهائى 7,2 +/- 2،. وزعها 5 – 7 ملل فى أطباق 60 مم. فى حالة استعمال أطبا ق أخرى مختلفة الحجم ضع كمية من البيئة بيحيث يكون عمق البيئة 4 – 5 مم. احفظ الأطباق بحيث لا تعرض الأطباق الى أشعة الشمس، برد فى الظلام، يفضل حفظها فى أكياس بلاستيك محكمة الغلق لخفض الفقد فى الرطوبة. لا تزيد مدة الحفظ عن اسبوعان أو يتخلص منها بمجرد الاحساس بفقد الرطوبة ، تغير فى اللون أو التلوث.

MacConkey agar

Peptone …….. 17 g
Proteose peptone …….. 3 g
Lactose …… 10 g
Bile salts …….. 5 g
Sodium chloride …… 1,51 g
Agar …… 13,5 g
Neutral red ……0,03 g
Crystal violet ……0,001 g
Reagent grade water …… ..1 L

أضف المكونات الى الماء واخلط جيدا، سخن الى الغليان لاذابة المكونات. عقم فى الاوتوكلاف لمدة 15 دقيقة عند 121 مئوية. لطف الحرارة Temperعقب التعقيم وصب البيئة فى أطباق .
pH البيئة يجب أن يكون 7,1 +/- 2و بعد التعقيم.

Nutrient agar

Pesptone	5	g
Beef extract	3	g
Agar	15	g
Reagent grade water	1	L

أضف المكونات الى الماء ، اخلط جيدا وسخن للذوبان . pH يجب أن يكون 6,8 +/- 2، بعد التعقيم.قبل التعقيم وزع البيئة فى أنابيب بقلاووظ . بعد التعقيم أمل الأنابيب لتعطى سطح منحنى Slant . اغلق الأنابيب باحكام بعد أن تبرد وخزنها فى مكان بارد محمى.

Gram stain reagents (يراجع Module 2)

- **الطريقة**

أ . فى ظروف معقمة ازرع على سطح طبق LES Endo agar أو MacConkey agar كل انبوبة موجبة من البريلينت جرين فور ظهور الغاز بها باستعمال لوب 3 مم أو ابرة منحنية قليلا (لا يؤخذ الريم على سطح الانبوبه، تغمس اللوب الى العمق ويخطط الطبق للحصول على مستعمرات متباعدة). حضن على 35 مئوية +/- 5، درجة لمدة 24 +/- 2 ساعة.

ب . المستعمرات التى تنشأ على LES Endo agar تكون نموذجية Typical(قرمزية الى أحم ر قاتم مع اللمعة المعدنية) أو غير نموذجية Atypical (قرمزية، أحمر، أبيض، أو عديمة اللون بدون اللمعة المعدنية) بعد تحضين 24 ساعة. المستعمرات المخمرة للاكتوز النموذجية والتى تنشأ على بيئة الماكونكى تكون حمراء وربما تكون محاطة بمنطقة معتمة أو ترسيب املاح الص فراء. من كل طبق التقط مستعمرة أو أكثر من بكتريا القولون النموذجية تكون منعزلة ، وفى حالة عدم تواجد تلك المستعمرات التقط مستعمرات أقرب ما يمكن للشكل المشار اليه وكل مستعمرة تلقح فى انبوبة تخمر من مرق لوريل تريبتوز وعلى سطح آجار مغذى مائل Nutrient agar وتحضن 24 ساعة عند 35 مئوية. حضن على 35 +/- 5و درجة مئوية لمدة 24 +/- 2 ساعة وافحصها لتكن غاز وأعد الفحص اذا كانت سالبة عند 48 +/- 3 ساعة. ويجرى الصبغ بجرام من انبوبة الآجار المغذى (يمكن عدم اجراء تلك الخطوة فى حالة مياه الشرب).
تكون غاز فى انبوبة مرق اللوريل تـربتوز خلال 48 +/- 3 ساعة واعطاء خلايا سالبة لجرام، غير متجرثمة ، عصوية تعتبر موجبة للاختبار التكميبى وتؤكد وجود أعضاء من مجموعة القولون.

- **حساب كثافة البكتريا Estimation of Bacterial Density**
- **دقة اختبار أنابيب التخمر Precision of fermentation tube test**

اذا لم يتم اختبار عدد كبير من جزئيات العينة ، فان دقة اختبار أنابيب التخمر تكون منخفضة . مثلا، اذا أختبر 1 ملل من زجاجة تحتوى على خلية واحدة من بكتريا القولون /ملل، فان حوالى 37 % من الأنابيب المحتوية على 1 ملل من العينة من المتوقع أن تنتج نتائج سلبية نتيجة للتوزيع العشوائى للبكتريا فى العينة، كل املل من العينة. وعندما يكون هناك 5 أنابيب كل منها به 1 ملل من العينة فانه النتيجة السلبية تحت تلك الطروف فان التوقع أن تكون النتائج سلبية بالكامل لا يزيد عن 1%.

وبالتالى يكون هناك حرص عند اسنتاج المغزى الصحى لنتائج الاختبار لبكتريا القولون والمتحصل عليها من بضع انابيب قليلة من كل تخفيف من العينة، خاصة عندما يكون عدد العينات من نقطة معينة محدود.

- **حساب وتسجيل العدد الأكثر احتمالا Computing and Recording of MPN**

لحساب كثافة بكتريا القولون يعبر عنها فى صورة عدد أكثر احتمالا MPN . قيم العدد الأكثر احتمالا لسلسلة من نتائج الزرع، تتضح فى الصفحة التالية.

وتلك الجداول تشتمل على 95 % حدود ثقة Cofidence limits لكل قيمة مقدرة من قيمة العدد الأكثر احتمالا المقدرة. اذا كان حجم العينة المستعملة من تلك الموجودة فى الجداول، سجل النتيجة منسوبة الى عدد النتائج الايجابية والسلبية فى السلاسل كعدد أكثر احتمالا / 100 ملل أو سجل النتيجة كوجود أو غياب بكتريا القولون الكلية أو البرازية (P/A) .

حجم العينه التى تظهر فى الجداول فى الصفحه التاليه

TABLE 9221:II. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 20-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 5 of 20 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	<1.1	0	3.0
1	1.1	0.05	6.3
2	2.6	0.3	9.6
3	4.6	0.8	14.7
4	8.0	1.7	26.4
5	>8.0	4.0	Infinite

TABLE 9221.III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN TEN 10-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	<1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

TABLE 9221.IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)

Combination of Positives	MPN Index/ 100 mL	95% Confidence Limits	
		Lower	Upper
0-0-0	<2	—	—
0-0-1	2	1.0	10
0-1-0	2	1.0	10
0-2-0	4	1.0	13
1-0-0	2	1.0	11
1-0-1	4	1.0	15
1-1-0	4	1.0	15
1-1-1	6	2.0	18
1-2-0	6	2.0	18
2-0-0	4	1.0	17
2-0-1	7	2.0	20
2-1-0	7	2.0	21
2-1-1	9	3.0	24
2-2-0	9	3.0	25
2-3-0	12	5.0	29
3-0-0	8	3.0	24
3-0-1	11	4.0	29
3-1-0	11	4.0	29
3-1-1	14	6.0	35
3-2-0	14	6.0	35
3-2-1	17	7.0	40
4-0-0	13	5.0	38
4-0-1	17	7.0	45
4-1-0	17	7.0	46
4-1-1	21	9.0	55
4-1-2	26	12	63
4-2-0	22	9.0	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9.0	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360
5-3-3	170	80	410
5-4-0	130	50	390
5-4-1	170	70	480
5-4-2	220	100	580
5-4-3	280	120	690
5-4-4	350	160	820
5-5-0	240	100	940
5-5-1	300	100	1300
5-5-2	500	200	2000
5-5-3	900	300	2900
5-5-4	1600	600	5300
5-5-5	≥1600	—	—

مرتبط أساسا بالمياه النهائية . ويبين قيم العدد الأكثر احتمالا لنتائج ايجابية وسلبية عند اختبار خمسة من 10 ملل، وخمسة 1 ملل، خمسة 1، ملل. عندما تكون التخفيفات المتتالية مختلفة عن تلك الموجودة فى الجدول، اختار قيم العد الأكثر احتمالا الجدول السابق للعدد من النابيب الموجبة واحسب طبقا للمعادلة التالية:

Example	1 mL	0.1 mL	0.01 mL	0.001 mL	Combination of positives	MPN Index /100 mL
a	5/5	5/5	2/5	0/5	5-2-0	5000
b	5/5	4/5	2/5	0/5	5-4-2	2200
c	0/5	1/5	0/5	0/5	0-1-0	20

In *c*, select the first three dilutions so as to include the positive result in the middle dilution.

MPN value (from table) X $\frac{10}{\text{Largest volume tested in dilution Series used for MPN determination}}$ = MPN / 100ml

عنداستعمال أكثر من 3 تخفيفات فى سلسلة متدرجة من التخفيف، استعمل نتائج 3 فقط منها فى حساب العد الأكثر احتمالا . لاختيار الثلاث تخفيفات لاستعمالها فى تقديلا العد الأكثر احتمالا، اختار أعلى التخفيفات التىتعطى نتائج ايجابية فى كل الخمس اجزاء من العينة المختبرة والتخفيفان التا ليان الناجحان الأعلى فى التخفيف. استعمل نتائج لهذه الحجوم الثلاثة فى حساب العدد الأكثر احتمالا . وفى المثال المبين أسفل (الجدول التالى) ، الثلاث تخفيفات المختارة تظهر بخط سميك. فى حالة C اختار الثلاث تخفيفات الأولى بحيث تكون الأنابيب الايجابية فى التخفيفات الوسطى.

وفى حالة مثل الموجود أسفل أسفل (مثال d) فان الأنابيب الايجابية تتواجد فى تخفيف أعلى من الثلاث المختارة طبقا للقاعدة وفى حالة e استخدم الثلاث قيم الأولى
عندما يراد تلخيص نتائج بالنسبة لمجموعة متسلسلة على صورة قيمة واحدة يحسب المتوسط.

الجدول التالى يظهر الثلاث التخفيفات المختارة . اذا تحصل على قراءات بعيدة الاحتمال بنسبة تزيد على 1% فانها دليل أن هناك خطأ فى الطريقة أو أن الافتراض الاحصائى والذى تحته حسبت قيمة العدد الاحتمالى لم يتحقق. واذا كانت هناك قراءات للأنابيب الموجبة لا تتواجد فى الجدوب فانه يمكن حسابها من المعادلة التالية:

والجداول لقيم العدد الأكثر احتمالا وكذ1لك المعادلة السابقة يمكن استعمالها لتقدير أية مجموعة أخرى خلاف بكتريا القولون.

$$\text{MPN/100 mL} = \frac{\text{no. of positive tubes} \times 100}{\sqrt{\left(\begin{matrix}\text{mL sample in} \\ \text{negative tubes}\end{matrix} \times \begin{matrix}\text{mL sample in} \\ \text{all tubes}\end{matrix}\right)}}$$

اختبار التواجد – عدم التواجد لبكتريا القولون
Presence-Absence (P-A) Coliform Test

اختبار التواجد-عدم التواجد لمجموعة بكتريا القولون هى تطوير لطريقة العدد الأكثر احتمالا . تبسيط، باستعمال حجم كبير واحد (100 ملل) فى زجاجة زرع للحصول على معلومات نوعية عن تواجد أو غياب بكتريا القولون، ضبطت على نظرية أنه لا بد من غياب بكتريا القولون فى 100 ملل من عينة مياه الشلرب . واختبار التواجد- عدم التواجد يوفر ايضا الفرصة لزراعة وعزل اللائل البكتيرية الأخرى (بكتريا القولون البرازية، ايروموناس، ستافيلوكوكى، سيدوموناس، السبحيات البرازية، والكلوستريديم) وعلى نفس الأساس النوعى وليس الكمى . وهناك مميزات أخرى تشمل امكانية اختبار عدد كبير من العينات فى وحدة الوقت . اجريت دراسات مقارنة بطريقة أغشية الترشيح Membrane filtration أظهرت أن اختبار التواجد – الغياب ربما تعطى أقصى فرصة لكشف تواجد بكتريا القولون فى وجود العديد من البكتريا الأخرى والتى ربما تتفوق فى النمو على بكتريا القولونوتسبب مشاكل فى الكشف عنها.

اختبار التواجد-الغياب وضع للاستعمال فى الاختبار الروتينى للعينات المجمعة من نظام التوزيع أو محطات معالجة المياه . عندما تنتج العينات المجمعة من موقع معين نتائج ايجابية لاختبار التواجد-الغياب لبكتريا القولون، فانه ربما ينصح بأن تقدر كثافة بكتريا القولون فى عينات متكررة . والمعلومات الكمية ربما تظهر مدى التلوث.

المرحلة الافتراضية Presumptive phase

- **بيئة الزرع Culture media**

P-A broth

Beef extract	3	g
Peptone	5	g
Lactose	7.46	g
Tryptose	9.83	g
Dipotassium hydrogen phosphate	1.35	g
Potassium dihydrogen phosphate	1.35	g
Sodium chloride	2.46	g
Spdium lauryl sulphate	0.05	g
Bromocresol purple	0.0085	g
Reagent grade water	1	L

تحضر البيئة ثلاثية التركيز (القوة) عند اختبار 100 ملل من العينة. تذاب بيئة مرق التواجد-الغياب فى الماء بدون تسخين، تستعمل وسيلة تقليب. توزع 50 ملل من البيئة المحضرة فى زجاجات بغطاء قلاووظ 250 ملل. وضع داخل كل زجاجة انبوبة تخمر (درهام) غير ضرورى. عقم فى الاوتوكلاف لمدة 12 دقيقة عند 121 مئوية ومدة بقاء البيئة فى الأوتوكلاف لا يجب أن تزيد عن 30 دقيقة. pH يجب أن يكون 6,8 +/- 2و بعد التعقيم. وعند تعقيم البيئة بالترشيح تستعمل بيئة سداسية التركيز. وزع 20 ملل من البيئة السداسية التركيز فى زجاجات معقمة 250 ملل .

Lauryl tryptose broth (see 8. 1 . 1).

- **الطريقة Procedure**

رج العينة بشدة حوالى 25 مرة ولقح 100 ملل فى زجاجة بيئة التواجد- الغياب. اخلط جيدا بقلب الزجاجة مرة أو مرتين للحصول على توزيع تام للبيئة ثلاثية التركيز خلال العينة . حضن عند 35 +/- 5، درجة مئوية وافحص بعد 24 ساعة و 48 ساعة للتفاعل الحامضى.

- **تفسير النتائج Interpretation**

تكون لون أصفر فى البيئة عند تواجد الظروف الحامضية تتواجد بعد تخمر اللاكتوز . اذا تكون غاز أيضا ، هز الزجاجة برفق سوف يظهر رغوة او فوران . أية كمية من الغاز مع /أو حامض يعتبر الاختبار موجب ويحتاج الى التأكيد.

مرحلة التأكيد Confirmed phase

- **بيئة الزراعة** : استعمل بيئة مرق البرلينت جرين لاكتوز أملاح الصفراء فى أنابيب
- **الطريقة Procedure** انقل كل المزارع الموجبة (التى أعطت حامض أو حامض وغاز) الى بيئة البريلينت جرين وتحضن عند 35 +/- 5و درجة مئوية .
- **تفسير النتائج Interpretation** انتاج الغاز فى أنابيب البريلينت جرين بعد 48 +/- 3 ساعات يؤكد وجود بكتريا القولون . يكتب التقرير على صورة اختبار التواجد /الغياب (موجب أو سالب) لبكتريا القولون فى 100 ملل من العينة.

Fecal Coliforms and E.coli

- البكتيريا القولونية البرازيةFecal coliform Bacteria هي جزء من المجموع الكلية Total coliform Bacteria
- E.Coli هي الجزء الأكبر من البكتيريا القولونية البرازيةFecal coliform Bacteria
- يمكن تعين كلا من E.Coli و Fecal coliform في المعمل عن طريق قدرتهم على النمو في درجة حرارة 44.5 مؤوية
- يعتبر كلا من E.Coli و Fecal coliform دلائل على وجود تلوث برازي افضل من الTotal coliform ولاكن لا يدلان إذا كان التلوث ادمي أو حيواني
- لا تستخدم E.Coli و Fecal coliform كدليل على كفاءة عملية المعالجة (على عكس ال Total Coliform) وذلك لان نسبة وجودها اقل من Total Coliform
- وكذلك طبقا لقاعدة Total coliform عندما تعطي العينات نتائج إيجابية لل Total Coliform group يجب ان يتم عمل اختبار لE.Coli و Fecal coliform

الاختبار:-

يتم اختبار وجود Coliform group عن طريق :-

1- Multiple - tube fermentation technique. 2 - Membrane filter technique

- تستخدم طريقة الأنابيب المتعددة لإيجاد العدد الاحتمالي للColiform group (MPN)
- (MPN) هو عدد احتمالي يوضح كثافة Coliform فى العينة و منها يتضح مدى تأثير عملية المعالجة على المياه0.

1 . الاختبار لبكتريا القولون البرازية باستخدام بيئة EC

FecalColiform Test (EC medium)

اختبار بكتريا القولون البرازية يستعمل لتفرقة الجزء من بكتريا القولون الكلية الذى ينتمى لبكتريا القولون البرازية . استعمل كطريقة سريعة لاختبار مياه المحاريات ، المخلفات السائلة المعالجةShellfish بيئة1 A كاختبار مباشر.

1 . 1 بيئة EC وتركيبها:

Tryptose or trypticase	20	g
Lactose	5	g
Bile salt mixture or bile salt No.3.....	1.5	g
Dipotassium hydrogen phosphate	4 .	g
Potassium dihydrogen phosphate.....	1.5	g
Sodium chloride	5	g
Reagent-grade water 1		L

تضاف المكونات الى المياه، تخلط جيدا، تسخن لتذوب . pH يجب أن يكون 6,9 +/- 2، بعد التعقيم. قبل التعقيم وزع فى أنابيب تخمر، كل واحدة بها انبوبة درهام مقلوبة ، كمية من البيئة كافية لتغطى الأنبوبة المقلوبة أو على الأقل جزئيا بعد التعقيم. سد الأنابيب بغطاء معدنى أو بلاستيكى مقاوم لحرارة التعقيم.

2 . الطريقة Procedure

ارسل انابيب الاختبار الفرضى Presumptive testالتى تظهر نتيجة ايجابية بان يكون فى أنابيب درهام أية كمية من الغاز، نمو، أو حموضة خلال 48 ساعة من التحضين لاجراء اختبار بكتريا القولون البرازية.

2 . 1 . هز أنابيب الاختبار الفرضى برفق أو الزجاجات التى أظهرت غاز، نمو، أو حموضة . استخدم لوب قطر فتحتها 3 – 3,5 مم لنقل النمو من كل انبوبة أو زجاجة موجبة

2 **. 2 .** حضن الأنابيب الملقحة (EC) فى حمام مائى عند 44,5 +/- 2و درجة مئوية لمدة 24 +/- 2 ساعة. ضع كل أنابيب مرق EC الملقحة فى الحمام المائى خلال 30 من الانتهاء من حقنها. احرص على أن يكون فى الحمام المائى كمية من المياه تكفى لتغطية مستوى البيئة فى الأنابيب المحضنة.

تفسير النتائج

انتاج الغاز مع النمو فى بيئة مرق EC خلال 24 +/- 2 ساعة أو أقل يعتبر الاختبار موجب لبكتريا القولون البرازية . الفشل فى انتاج غاز (مع وجود نمو قليل أو معدوم) يعتبر التفاعل سالب. اذا استعملت الأنابيب المتعددة ، يحسب MPN من عدد أنابيب EC الموجبة وتقرأ من الجداول السابقة. وعند استعمال انبوبة واحدة من اختبار Presumptive تذكر النتيجة على صورة وجود أو غياب لبكتريا القولون البرازية.

3. اختبار يكتريا القولون البرازية المباشر
Fecal-coliform Direct Test (A-1 medium)

3 . 1 . البيئة

تستخدم بيئة مرق A1، ليس هناك مرحلة افتراضية Presumptive phase. وتركيب بيئة A 1 كما يلى:

Lactose ..... 5 g
Tryptose ..... 20 g
Sodium chloride ..... 5 g
Salicin ..... 0.5 g
Polyethylene glycol p-isooctyl phenyl ether (Tween 100) ..... 1 ml
Reagent-grade water ..... 1 L

سخن لاذابة المكونات الصلبة، اضف توين X 100 ، واضبط pH عند 6,9 +/- 1،. . قبل التعقيم وزع البيئة فى أنابيب تخمير بها انابيب درهام مقلوبة بكمية تكفى لغمر انبوبة درهام على الأقل جزئيا بعد التعقيم. غط الأنابيب بأغطية معدنية أو بلاستيك مقاوم لحرارة التعقيم . عقم لمدة 10 دقائق فى الأوتوكلاف عند 121 درجة مئوية. خزن الأنابيب فى الظلام عند درجة حرارة الغرفة بحيث لا تتعدى فترة التخزين 7 أيام. تجاهل تكون راسب .

حضر البيئة بتركيز مضاعف حتى يمكن استعمال 10 ملل من العينة بحيث لا يتأثر تركيز البيئة باضافة العينة.

3 . 1 . الطريقة

لقح الأنابيب من بيئة A 1 كما ذكر سابقا حضن لمدة 3 ساعات عند 35 +/- 5و درجة مئوية ثم انقل الأنابيب الى حمام مائى عند 44,5 +/- 2و درجة مئوية وحضن لمدة 21 +/- 2 ساعة.

3 . 2 . تفسير النتائج

انتاج الغاز فى الأنابيب خلال 24 ساعة أو أقل يعتبر علامة على أن الأنبوبة ايجابية للاختبار لبكتريا القولون البرازية. احسب العدد الأكثر احتمالا MPN من عدد الأنابيب الايجابية .

طريقة أغشية الترشيح لمجموعة بكتريا القولون

Membrane Filter Technique of the Coliform Group

1 . مقدمة

طريقة أغشية الترشيح يمكن استعمالها مع حجوم كبيرة نوعا ما من العينات، وعادة تنتج نتائج أكثر حقيقة من طربقة الدد الأكثلا احتمالاز طريقة أغشية الترشيح مفيدة جدا فى مراقبة مياه الشرب وعديد من نوعيات المياه الكيعية. ومع ذلك، فان طريقة أغشية الترشيح لها بعض المحدوديات، خاصة عند اختبار المياه ذات العكارة العالية أو أعداد البكتريا التى لا تنتمى الى مجموعة بكتريا القولون Noncoliform (background) . وعند استخدام طريقة أغشية الترشيح سابقا، كان من المرغوب اجراء اختبارات من التى يستخدمها المعمل فى نفس الوقت مع طريقة الأغشية ثبات امكانية تطبيقها وللمقارنة.

2 . تعريف Definition

بالنسبة لطريقة أغشية الترشيح فان مجموعة بكتريا القولون عرفت على أنها تلك الاهوائية اختيارا ، سالبة لجرام، غير مكونة للجراثيم ، عصوية الشكل والتى تنتج مستعمرات حمراء بلمعة ذهبية Mettalic (golden) sheen خلال 24 ساعة عند 35 مئوية على بيئة الاندو Endo medium والتى تحتوى على سكر اللاكتوز . بعض أعضاء مجموعة بكتريا القولون ربما تنتج مستعمرات بلون أحمر قاتم Dark red ، مخاطية Mucoid أو ذات نواه Nucleated دون اللمعة المعدنية. وهذه تصنف على أنها مستعمرات بكتريا القولون ولكنها غير مثالية Atypical. وعنج اختبار مزارع بكتريا القولون المنقاة Purified cultures فانها تكون سالبة لانزيم سيتوكروم أكسيديز Cytochrome oxidase negative وموجبة لانزيم B-galactosidase. وبصفة عامة ، فانه فى تلك الطريقة MF تعتبر المستعمرات ذات اللون الأحمر الوردى Pink الغير مخاطية Nunmucoid، الزرقاء، البيضاء، أو عديمة اللون والتى ينقصها اللمعان فانها تعتبر ليست من بكتريا القولون.

3. التطبيق Application

العكارة المتسببة عن وجود الطحالب ، الجزيئات أو أى مواد متداخلة ربما لا تسمح باختبار حجم من العينة كافى لانتاج نتائج معنوية. والتقديرات المنخفضة لبكتريا القولون ربما تتسبب عن وجود البكتريا الأخرى والتى لا تنتمى لبكتريا القولون، أو عم وجود مواد سامة .

طريقة أغشية الترشيح قابلة للتكبيق لا ختبار المياه المالحة Saline water، **ولكن ليس للمخلفات السائلة التى اجرى عليها معتاجة مبدئية** متبوعة بمعالجة بالكلور بسبب العكارة عند استخدام حجوم كبيرة من العينات أو مخلفات سائلة تحتوى على معادن سامة أو مواد عضوية سامة مثل الفينولات . وللكثف عن تواجد بكتريا القولون الكلية المضارة Stressed total coliforms فى مياه الشرب المعالجة وناتج المعالجة الثانوية من المخلفات السائلة المعالجة بالكلور فان الطريقة صممت للكشف عن تواجد تلك المجموعة (ستناولها فى Module 4).

وحجم العينة اللازم اختباره فى حالة مياه الشربالمرشحة هو 100 ملل. ولاغراض المراقبة الخاصة، مثل حل مشاكل نوعية المياه أو تصنيف بكتريا القولون فى التركيزات المنخفضة من خلال خطوات المعالجة، ربما يكون من المرغوب اختبار 1 لتر من العينة. اذا كانت المواد المعلقة تمنع ترشيح اللتر من العينة خلال مرشح واحد، تقسم العينة الى الى أجزاء صغيرة ، 250 ملل مثلا للتحليل. وتجمع اعداد الترشيح (مقارنة الجداول التالية) والنتائج بالنسبة لتقييم نوعية المياه بتلك الطريقتين متماثلتان ولكن الأعداد الاحصائية بطريقة الأغشية أكثر دقة من طريقة العدد الأكثر احتمالا.بكتريا القولون الناتجة عن المرشحات المستخدمة لترشيح العينة ليكون لديتا العدد/اللتر. وأحجام أصغر من العينات تكون لازمة فى حالة مياه المتنزهات Recreational water أو مصدر المياه Source water أو المخلفات السائلة والتى تحتوى على أعداد عالية من بكتريا القولون. والدراسات المقارنة ما بين طريقة لمرشحات الغشائية وطريقة العدد الأكثر احتمالا أظهرت زيادة دقة طريقة أغشية الترشيح (مقارنة الجداول التالية) والنتائج بالنسبة لتقييم نوعية المياه بتلك الطريقتين متماثلتان ولكن الأعداد الاحصائية بطريقة الأغشية أكثر دقة من طريق ولكن الأعداد الاحصائية بطريقة الأغشية أكثر دقة من طريقة العدد الأكثر احتمالا

TABLE 9222:II. CONFIDENCE LIMITS FOR MEMBRANE FILTER COLIFORM RESULTS USING 100-mL SAMPLE

Number of Coliform Colonies Counted	95% Confidence Limits	
	Lower	Upper
0	0.0	3.7
1	0.1	5.6
2	0.2	7.2
3	0.6	8.8
4	1.0	10.2
5	1.6	11.7
6	2.2	13.1
7	2.8	14.4
8	3.4	15.8
9	4.0	17.1
10	4.7	18.4
11	5.4	19.7
12	6.2	21.0
13	6.9	22.3
14	7.7	23.5
15	8.4	24.8
16	9.2	26.0
17	9.9	27.2
18	10.7	28.4
19	11.5	29.6
20	12.2	30.8

TABLE 9221.III. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN TEN 10-mL PORTIONS ARE USED

No. of Tubes Giving Positive Reaction Out of 10 of 10 mL Each	MPN Index/ 100 mL	95% Confidence Limits (Approximate)	
		Lower	Upper
0	<1.1	0	3.0
1	1.1	0.03	5.9
2	2.2	0.26	8.1
3	3.6	0.69	10.6
4	5.1	1.3	13.4
5	6.9	2.1	16.8
6	9.2	3.1	21.1
7	12.0	4.3	27.1
8	16.1	5.9	36.8
9	23.0	8.1	59.5
10	>23.0	13.5	Infinite

الطريقة القياسية لبكتريا القولون باستخدام أغشية الترشيح
Standard Total Coliform Membrane Filter Procedure

1 . الأدوات المعملية Laboratory apparatus

للتحليل بأغشية الترشيح استعمل أدوات زجاجية وأي أجهزة أخرى تكون مصنوعة من مواد خالية من المواد السامة والتى ربما تؤثر على نمو البكتريا.

- **زجاجات العينات Sampling bottles (**QA/ QC Notesَ)
- **زجاجات التخفيف Dilution bottles (**QA/ QC Notesَ)**.**
- **الماصات والمخابير المدرجة** QA/QC **Pipets and graduated cylinders**
  (Notes) قبل التعقيم ، يغطى السلندرات بورق كرافت أو الومونيوم وتكون التغطية غير محكمة، وبعد التعقيم مباشرة احكم التغطية.
- **أوعية بيئة الزرع Containers of culture medium**
- **أطباق الزرع Culture dishes**
- **وحدات الترشيح Filtration units**
- **أغشية الترشيح Membrane filter**
- **الوسادة الماصة Absorbent bads**
- **الملاقط Forceps**
- **الميكروسكوب ومصدر الاضاءة Microscope and light source**

2. المواد وبيئات الزرع Materials and Culture Media

لا تلجأ الى تحضير البيئة من مكوناتها طالما البيئة الجاهزة متوافرة على نطاق تجارى . خزن البيئة التى تم فتح عبوتها فى مجفف. أنظر QC/QA للتحكم فى نوعية البيئة.
اختبر كل لوط جديد من البيئة باستعمال اللوط القديم المقبول كمرجع (QC/QA) . مع كل لوط جديد من بيئة من نوع الاندو Endo type تحقق Verify على الأقل من 10% من مستعمرات بكتريا القولون المتحصل عليها م ن العينات الطبيعية أو عينات بها اضافات معروفة، لمقارنة اللوطات المختلفة للبيئة وقدرتها على الاستخلاص Recovery .
قبل الاستعمال، اختبر كل عملية تحضير Batch للبيئة التى ستستخدم مع M F باستخدام مزرعة معروف أنها تعطى نتيجة ايجابية وأخرى معروف أنها تعطى نتيجة س لبية. اختبر تلوث بكتريا القولون عند بداية ونهاية كل سلسلة من الترشيح بترشيح 20 – 30 ملل من محلول التخفيف أوالشطف خلال المرشح. اذا ظهر تلوث فى المرجعيات Controls ارفض أية نتائج واطلب عينة أخرى.

2 . 1. بيئة LES Endo agar

2 . 2 . بيئة M-Endo

Tryptone or polypeptone	10 g
Thiopeptone or thiotone	5 g
Casitone or trypticase	5 g
Yeast extract	1,5 g
Lactose	12,5 g
Sodium chloride	5 g
Dipotassium hydrogen phosphatr	4,375 g
Potassium dihydrogen phosphate	1,375 g
Sodium lauryl sulphate	0,05 g
Sodium desoxycholate	0.1 g
Sodium sulfite	2,1 g
Basic fuchsin	1,05 g
Agar	15 g
Reagent-grade water	1 L

تحضير الآجار- تضاف المكونات الى 1ذ لتر ماء يحتوى على 20 ملل 90% كخحول ايثايل. سخن الى قرب الغليان لاذابة الآجار، وترفع وتبرد الى 45 – 50 درجة مئوية. وزع كميات 5 – 7 ملل فى الأطباق الزجاج أو بلاستيك قطر 60 مم. اذا استعملت اطباق بأقطار مختلفة يراعى وضع كمية من

البيئة تعطى نفس السمك . لا تعقم البيئة فى الاوتوكلاف. pH النهائى 7,2 +/- 2،. من العادى وجود راسب .

برد البيئ النهائية فى الظلام ويستبعد الغير مستعمل من البيئة بعد اسبوعين.

تحضير المرق_ حضر كما ذكر سابقا مع عدم اضافة الآجار ، وزع البيئة السائلة (2 ملل لكل طبق) على الوسائد الماصة (ارجع الى خصائصها QA/QC) وباحتراس يزال الزائد من البيئة . المرق قد يكون به رواسب ولكنها لا تتداخل مع كفاءة البيئة اذا كانت الوسائد الماصة يشهد لها بأنها خالية من الكبريتات أو أى مواد سامة بتركيزات يمكن أن تقضى على نمو البكتريا . المرق المبرد ربما يخزن الى 4 أيام.

3 . العينات (راجع QA/QC)

4 . تعريف بكتريا القولون

البكتريا التى تنتج مستعمرات حمراء مع لمعة ذهبية (معدنية) Metallic sheen خلال 24 ساعة من التحضين على 35 درجة مئوية على بيئة من نوع الاندو تعتبر عضومن مجموعة بكتريا القولون. اللمعة قد تغطى المستعمرة كلها أو قد تظهر على مركز المستعمرة فقط أو على الحواف . هذا التعريف لبكتريا ا لقولون يعتمد على انتاج الألدهيدات من تخمر اللاكتوز . وبينما هذه الخاصية البيوكيماوية هى جزء من طريق عملية التمثيل الغذائى لانتاج الغاز فى اختبار العدد الأكثر احتمالا، فان بعض الاختلاف فى اللمعان للمستعمرات يلاحظ بين سلالات بكتريا القولون. وعلى ذلك، فان هذا الاختلاف البسيط فى تحديد الدليل لا يذكر على أنه خطير لتغيير مغزاه من ناحية الصحة العامة، خاصة اذا اجريت دراسات مناسبة لايجاد العلاقة بين النتائج المتحصل عليها بطريقة أغشية الترشيح والأخرى المتحصل عليها بطريقةانابيب التخمر المتعددة.

5 . الطريقة Procedure

1 . 5 . اختيار حجم العينة : يحكم حجم العينة بكثافة البكتريا المتوقعة. فى تحليل مياه الشرب يحكم حجم العينة بالعكارةن أو بنمو البكتريا التى لا تنتمى الى بكتريا القولون على البيئة (الجدول التالى) . وللأغراض التنظيمية، 100 ملل هو الحجم الرسمى.

TABLE 9222:I. SUGGESTED SAMPLE VOLUMES FOR MEMBRANE FILTER TOTAL COLIFORM TEST

	Volume (X) To Be Filtered *mL*							
Water Source	100	50	10	1	0.1	0.01	0.001	0.0001
Drinking water	X							
Swimming pools	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
Bathing beaches			X	X	X			
River water				X	X	X	X	
Chlorinated sewage				X	X	X		
Raw sewage					X	X	X	X

الحجم الأمثل من العينة سينتج 20 – 80 مستعمرة من بكتريا القولون وليس أكثر من 200 مستعمرة من كل الأنواع على سطح المرشح الغشائى. تحليل مياه الشرب بترشيح من 100 الى 1000 ملل، أو بترشيح مكررات من عينات صغيرة الحجم مثل اثنان كلا 50 ملل أو 4 كلا 25 ملل. تحليل المياه الأخرى بترشيح 3 حجوم مختلفة (مخففة أو غير مخففة) ، يعتمد على كثافة البكتريا المتوقعة . فى حالة ترشيح أقل من 10 ملل من العينة (مخففة أو غير مخففة) أضف حوالى 10 ملل محلول تخفيف معقم الى قمع الترشيح قبل الترشيح أو انقل الحجم من العينة الى زجاجة تخفيف ورشح ناتج التخفيف جميعه. هذه الزيادة فى حجم المياه يساعد على انتظام توزيع معلق البكتريا على سطح الفلتر .

5 . 1 . وحدات الترشيح المعقمة Sterile filtration units

استعمل وحدات ترشيح معقمة فى بداية كل سلسلة من الترشيحات المتتالية كحد أدنى من الحيطة لمنع التلوث. سلسلة الترشيح تعتبر معترضة اذا مرت فترة 30 دقيقة أو أكثر بين عمليات الترشيح المتتالية. واذا1 حدث ذلك عامل عملية الترشيح التالية على الرغم من انتمائها الى عملية سابقة على أنها عملية جديدةوبالتالى عقم كل الجهاز (راجع QALQC).

5 . 2 .ترشيح العينة Filtration of sample

باستعمال ملقط معقم ، ضع مرشح غشائى معقم Sterile membrane filter (السطح المقسم الى أعلى) على الجزء المدعم المنفذ من الجهاز . بحرص ضع الجزء المتحرك من القمع على الجزء الحامل للفلتر وثبته جيدا مع مراعاة عدم السماح بوجود أى تسريب . رشح العينة تحت تفريغ جزئى. مع بقاء الفلتر فى مكانه اغسل السطح الداخلى للقمع من خلال ترشيح 3 دفعات كل مل بين 20 – 30 ملل من محلول التخفيف المعقم . وكبديل، اشطف القمع بتيار من محلول التخفيف المعقم من زجاجة غسيل Washing bottle مع الاحتراس من التلوث خلال الاستعمال . الشطف بين العينات يمنع من انتقال التلوث. مع انتهاء الشطف النهائى وعملية الترشيح اوقف التفريغ، افتح وزل القمع، والتقط الفلتر الغشائى بملقط معقم .، وضعه على البيئة المختارة بحركة دائرية لمنع حجز الهواء بين الفلتر والبيئة وهذا يمنع انتقال البيئة الى الخلايا على سطح الفلتر . اغلق الطبق واقلبه وحضن لمدة 22-24 ساعة عند 35 +/- 5، درجة مئوية.

فى حالة استخدام البيئة السائلة، ضع الوسادة الماصة Filter pad فى الطبق وشبعها باستعمال 2 ملل من البيئة (M-Endo) وباحتراس ازل الزائد من البيئة ولم يمتص (بالتجفيف). ضع الفلتر المحضر مباشرة على الوسادة المشبعة بالبيئة، اقلب الطبق، وحضن عند 35 +/- 5، درجة مئوية لمدة 24 ساعة.

تمييز المستعمرات سواء فى حالة استخدام البيئة فى صورة صلبة أو سائلة ربما بفقد أو يتعذر اذا حضنت المزارع أكثر من 24 ساعة.

بعد عشر عينات اختبر تعقيم مياه الشطف بترشيح 100 ملل واختبارها كمصدر للتلوث بنحضين أطباقها تحت نفس ظروف تحليل العينة.

لعينات المياه التى تستعمل فى اغراض أخرى خلاف الشرب وبسبب وجود أعداد كبيرة من بكتريا القولون يجب تعقيم وحدة الترشيح بعد كل عينة. وكبديل، استعمل محلول منظم Buffer rinse لشطف الوحدة بعد ازالة الفلتر ومنع نقل التلوث بين العينات.

5 . 3 .طريقة التخصيب البديلة Alternative enrichment technique

ضع وسادة ماصة معقمة فى غطاء طبق بترى معقم وشبعها بحوالى 2 ملل من بيئة مرق لوريل تربتوز محضر كما ذكر مسبقا ضع المرشح الغشائى الذى رشحت عليه العينة على الوسادة الماصة ولا تقلب الطبق وحضن لمدة 1,5 الى 2 ساعة عند 35 +/- 5، درجة مئوية فى جو رطوبته 60% على الأقل.

اذا استعمل بيئة اندو آجار، ازل الفلتر من على سطح الوسادة الماصة بعد التحضين وضعه بحركة دائرية على سطح بيئة اندو آجار مع الحرص بعدم السماح بوجود هواء بين الفلتر والبيئة.

أما اذا استعملت البيئة السائلة ، فانه يتم وضع وسادة جديدة فى الطبق وتشبع بالبيئة M-Endoثم يزال الفلتر من على الوسادة القديمة ويقلب الطبق ويحضن تحت نفس ظروف استخدام البيئة الصلبة.

سواء استخدمت البيئة الصلبة أو السائلة اقلب الأطباق وحضن لمدة 20 الى 22 ساعة عند35 +/- 5، درجة مئوية.

5 . 4 . العد Counting

لعد المستعمرات على سطح المرشحات الغشائية استعمل قوة تكبير 10 – 15 مرة من خلال
Wide-field dissecting microscope or other optical devices
مع استعمال لمبة فلورسنت بيضاء كمصدر للضوء وتوجه الاضاءة حتى ترى أوضح صورة .
مستعمرات بكتريا القولون المثالية لها لون قرنفلى Pink الى أحمر قاتم مع لمعان معدنى على السطح. ز عد كلا من المستعمرات المثالية وغير المثالية Typical and atypical. المنطقة اللامعة ربما تختلف فى الحجم من نقط فى حجم رأس الدبوس الى كل سطح المستعمرة. مستعمرات بكتريا القولون غير المثالية يمكن أن تكون ذات لون أحمر قاتم، مخاطية Mucoid أو ذات نواة دون لمعان. عامة المستعمرات القرنفلية، الزرقاء، البيضاء أو عديمة اللون والتى يغيب عنها اللمعان تعد على أنها ليست بكتريا القولون. عدد كل المستعمرات (بكتريا القولون وغيرها) على بيئة الاندو ليس له علاقة بعدد البكتريا الكلى فى العينة الأصلية. العدد العالى من البكتريا الغير قولونية ربما يتداخل مع نشوء أقصى أعداد من بكتريا القولون. تبريد المزارع (بعد 22 ساعة تحضين) مع العدد المرتفع من البكتريا الغير قولونية لمدة 5، الى 1 ساعة قبل العد ربما يمنع الاحتشاد ويساعد على تمييز اللمعان
Deter spread of confluence while aiding sheen discernment.
عينات المياه المطهرة أوناتج المخلفات السائلة المعالجة ربما يحتوى على كائنات مضارة Stressed organisms والتى تنمو ببطء وتنتج أقصى لمعان فى 22 – 24 ساعة. الكائنات من المصادر الغير مطهرة ربما تنتج اللمعان بعد 1ذ6 – 18 ساعة، ويبهت اللمعان بعد 24 – 30 ساعة.

5 . 5. التحقق من بكتريا القولون Coliform verification

على فترات، ربما تظهر المستعمرات اللامعة المثالية عن نمو بكتريا لا تتبع بكتريا القولون والمستعمرات غير المثالية (مستعمرات لونها أحمر قاتم أو مستعمرات ذات نواة دون لمعان) ربما تكون بكتريا القولون. ويفضل أن يتحقق من كل نوعيات المستعمرات المثالية وغير المثالية عن طريق مسح الفلتر الغشائى أو التقاط على الأقل 5 مستعمرات مثالية وخمسة مستعمرات غير مثالية من مزرعة فلتر غشائى. وفى حالة المياه الأخرى خلاف مياه الشرب، كحد أدنى، تحقق من 10 مستعمرات لامعة (وممثلات للمستعمرات الغير مثالية والمختلفة فى مظهرها) من عينات المياه الموجبة شهريا (QA/QC notes). اعتمادا على الحاجة ونوعية العينة، ربما تطبق المعامل مقاييس متشددة (مثلا، التحقق من مستعمرة واحدة من كل نوع Typeمثالى أو غير مثالى من مزرعة من الترشيح الغشائى، تحقق من 10% من العينات الموجبة). أضبط الاعداد بناء على نتيجة التحقق.

4 . 6 . 1. اختبارات التحقق Verification tests

5 . 6 . 1 . 1 . تخمر اللاكنوز

انقل نمو من كل مستعمرة أو امسح غشاء الترشيح باستعمال Swab قطن (فى حالة اختبار التواجد والغياب فى عينات مياه الشرب) وضعها فى مرق لوريل سلفات، وحضن عند 35 +/- 5، درجة مئوية لمدة 48 ساعة\. انتاج غاز فى مرق اللوريل سلفات والتأكيد فى مرق بريلينت جرين لاكتوز (راجع بند 8 . 2) وخلال 48 ساعة تحقق من المستعمرة كبكتريا القولون. ومن الممكن زرع البيئتان فى نفس الوقت والتحقق من انتاج الغاز فى كلاهما. واضافة حقن أنابيب EC broth والتحضين عند 44,5 +/- 2، درجة مئوية لمدة 24 ساعة يعطى نتيجة عن وجود بكتريا القولون البرازية، وحقن انابيب من نفس البيئة مضاف اليها MUG والتحضين تحت نفس الظروف السابقة يعطى نتيجة عن وجود ايشيرشيا كولاى.

5. 6 . 1 . 2 . بديل عن التحقق من بكتريا القولون

استعمل تلك الطريقة لعزل مستعمرات على أغشية الترشيح. اذا شك بأن المزرعة مختلطة أو اذا كانت المستعمرات المفصولة أقل من 2 مم، ازرع من النمو على بيئة M-Endo أو ماكونكى آجار لتأكيد نقاء المزرعة أو اجرى اختبار انابيب التخمر.

5. 6 . 1 . 3 . الاختبار السريع

-التحقق من المستعمرات باستعمال اختبار التفاعل للسيتوكروم أكسيديز وبيتا جالاكتوسيديز الموجب خلال 4 ساعات تحضين للانبوبة أو طريقة اختبار البقعة Spot test .

5 . 6 . 1 . 4 . نظم الاختبار المتعدد Commercial multi-test systems

تحقق من المستعمرة بزرعها للتنقية . اختار مستعمرة معزولة جيدا، احقنها فى نظام الاختبار الم تعدد لمجموعة Enterobacteriaceae والتى تشمل اختبارات تخمر اللاكتوز و / أو بيتا جالاكتوسيديز وسيتوكروم أكسيديز.

6 . حساب كثافة بكتريا القولون Calculation of Coliform Density

احسب العدد، باستعمال المرشحات الغشائية التى بها 20 – 80 مستعمرة كوليفورم وليس أكثر من 200 مستعمرة من كل الأنواع لكل مرشح غشائى باستعمال المعادلة:

بكتريا القولون الكلية / 100 ملل = عدد مستعمرات بكتريا القولون 100X

حجم العينة المرشحة ملل

اذا لم تتواجد مستعمرات لبكتريا القولون يكتب التقرير أقل من 1 من بكتريا القولون/ 100 ملل.

ولعد التحقق طبق المعادلة التالية:

نسبة بكتريا القولون المتحقق منها = عدد المستعمرات / العدد الكلى لمستعمرات الكوليفورم المعرضة للتحقق ×100

6. المياه من نوعية مياه الشرب

نظم EPA تحتم خلو المياه من بكتريا القولون / 100 ملل فان ذلك يتطلب تقدير كثافة بكتريا القولون فى عينات متكررة . هذا مهم اذا كانت بكتريا القولون مستوطنة نظام التوزيع . المعلومات الكمية تعطى مؤشر عن مدى حدث التلوث.

المياه الجيدة محتواها من بكتريا القولون يكون أقل ما يمكن. لذلك ، تعد كل مستعمرات بكتريا القولون ولا يؤخذ فى الاعتبار الحد الأدنى المذكور سابقا (20 مستعمرة) وتستعمل المعادلة لمعرفة كثافة بكتريا القولون.

اذا كان النمو مغطى لكل مسطح الفلتر الغشائى أو جزء منه والمستعمرات غير محددة تكتب النتيجة نمو طاغ مع وجود بكتريا القولون أو عدم وجودها

Confluent growth with (or without) coliform

اذا كان العدد الكلى لبكتريا القولون وغيرها يزيد عن 200 على سطح المرشح الغشائى أو اذا كانت المستعمرات محددة لعدها تكتب النتيجة أعداد كبيرة جدا لعدها too numerous to count Confluent or TNTC على الترتيب.

فى مياه الشرب، وجود بكتريا القولون دون لمعان ربما تؤطد عن طريق نقل قليل من المستعمرات الى انبوبة ملاق بريلينت جرين مع الصفراء أوكبديل تؤخذ Swap من سطح المرشح الغشائى وتوضع فى انبوبة مرق بريلينت جرين مع الصفراء وتحضن عند 35 +/- 5، درجة مئوية لمدة 48 ساعة وانتاج غاز معناه وجود بكتريا القولون.

وعدم الحصول على نتيجة يحتم الحصول على عينة أخرى من نفس الموقع خلال 24 ساعة.

للاقلال من الازدحام على سطح المرشح الغشائى نتيجة ترشيح 100 ملل، قسم العينة على 4 أغشية ترشيح مثلا واجمع النتائج على المرشحات الغشائية المستخدمة.

6 . 2 . المياه خلاف مياه الشرب Water of other thsan drinking water

اذا كانت الأعداد الظاهرة يمكن عدها وكان قد استعمل فى تحليل 100 ملل من العينة 2 مرشح غشائى وتم ترشيح 50 ملل من كلا منهما وظهر على الأول 3 مستعمرات والثانى 5 مستعمرات فان العدد الكلى يكون 8 مستعمرة / 100 ملل .

وفى حالة الحصول على نتيجة من ترشيح 10 ملل، 1 ملل، 1، ملل – 40 ، 9، وأقل من واحد من مستعمرات بكتريا القولون فان الحساب يتم على اساس نتيجة 10 ملل وهى 40 فيكون العدد/ 100 ملل 400 . واذا كان العدد الكلى للبكتريا على هذا المرشح أكبر من 200 تكتب النتيجة أكبر من أو تساوى 100/400 ملل.

تأخر التحضين لبكتريا القولون الكلية

Delayed-Incubation Total Coliform Procedure

تطوير طريقة المؤشحات الغشائية القياسية يسمح بنقل العينة بعد ترشيحها الى مكان النعنل للتحليل وبالتالى يتم نقل المرشح الغشائى الى البيئة المتخصصة والتحضين واكمال الاختبار . هذا الاختبار الخاص بتأخير التحضين يمك ن استعماله عندما لا يكون عمليا استخدام الطرق التقليدية ، ويمكن استعماله أيضا

(أ) عندما لا يمكن حفظ العينة على درجة الحرارة المطلوبة أثناء النقل

(ب) عندما يكون الوقت اللازم لعملية النقل أطول من الوقت المسموح به

(ج) عندما يكون مكان أخذ العينة نائى عن منطقة المعمل.

أجريت دراسات باستخدام مياه مالحة ومياه عذبة ووجد أن نتائج طريقة التأخير فى التحضين مماثلة لنتائج الاختبار الفورى للعينة. اكمال الاختبار لبكتريا القولون بنقل غشاء الترشيح من بيئة النقل الى LES Endo والتحضين عند 35 +/- 5، درجة مئوية لمدة 20 – 22 ساعة وعد المستعمرات المثالية Typical والغير مثالية Atypical.

بيئة النقل صممت لتسمح ببقاء بكتريا القولون حية ولكن لا تنتج مستعمرات مرئية خلال النقل . المواد الموجودة فى بيئة النقل تقتل البكتريا الأخرى المصاحبة لبكتريا القولون ولكن لا تقتلها هى ذاتها الى وقت نقلها الى بيئة اجراء الاختبار فى المعمل . ويجدر الاشارة الى أن طرق الاختبار للعينة بعد وصولها المعمل من الممكن أن يتم باتباع الطرق العادية أو باتباع الكرق البديلة التى سيأتى ذكرها.

1 . الأدوات

أطباق بترى بلاستيكية

محكمة للمرشحات الغشائية أو زجاجيةٍ معقمة وملفوفة فى فيلم بلاستيك.

وحدة ترشيح فى الحقل

تعقم بين العينات اذا كانت معدنية (كحول ميثيل ويشعل ثم يطفأ لتكوين فورمالدهيد أو يكتفى بغليانها فى الماء) أو لا يعاد استخدامها اذا كانت من البلاستيك . ويمكن اسنخدام الأشعة الفوق بنفسجية فى التعقيم اذا توافر مصدر للتيار الكهربى. ويمكن ايجاد وسيلة للتفريغ باستخدام طلمبة يدوية.

2 . المواد وبيئة النقل

M-Endo methods . 1 . 2

2 . 1 . 1 . M-Endo preservative medium : حضر بيئة M-Endo كما سبق ، بعد تبريدها الى تحت 45 درجة مئوية أضف 3,48 جرام بنزوات الصوديوم /لتر . اخلط جيدا ووزعها 5 – 7 ملل فى أطباق صغيرة واحفظها فى الثلاجة ويهمل مالم يستعمل منها بعد 96 ساعة.

2 . 1 . 2 . محلول صوديوم بنزوات : أذب 12 جرام بنزوات صوديوم فى كمية من الماء Reagent-grade water واكملها الى 100 ملل. عقم فى الاوتوكلاف أو بالترشيح خلال مرشح غشائى 22، ميكرون ولا يستعمل بعد 6 شهور من تحضيره.

2 . 1 . 3 . سيكلوهكساميد Cyclohexamide اختياريا أضف سيكلوهكساميد الى بيئة M-Endo المضاف اليها البنزوات . ويضاف للتخلص من الفطر والخميرة فى حالة اعتياد تلويثها للعينة. أضف السيكلوهكساميد بواقع 50 مجم/100 ملل من البيئة . خزن محلول السيكلوهكساميد فى الثلاجة ويتخلص من المتبقى بعد 6 شهور.

M-ST method . 2 . 2

M-ST holding medium

Sodium dihydrogen phosphate ……جرام, 1
Dipotassium hydrogen phosphate .....جرام 3
Sulfanilamid ..... 1 جرام
Ethanol 95% ……. 10مل
Tris aminomethane ……..……3جرام
Reagent-grade water……1 لتر
ر

أذب المكونات فى الماء. عقم فى الأوتوكلاف لمدة 15 دقيقة عند 121 مئوية . pH النهائى يجب أن يكون 8,6 +/- 2،. وزع بواقع 2 ملل على الوسادة الماصة Absorbent pad ويمكن حفظها فى الثلاجة مدة 96 ساعة.

3 . الطريقة

ضع الوسادة الماصة المعقمة فى طبق معقم وشبغها ببيئة Selected coliform holding med. يزال غشاء الترشيح بعد انتهاء الترشيح ويوضع على سطح الوسلدو المشبعة السابقة وغلق الطبف باحكام. وفى حالة استخدام الأطباق الزجاجية يحكم غلق الطبق باستخدام شريط لاصق . يرسل الطبق أو اللأطباق بعد وضعها فى صندوق محكم وترسل الى المعمل للتحليل . يمكن أن تتحمل العينة 72 ساعة دون حدوث نمو.

بعد وصول الأطباق الى المعمل يرفع غشاء الترشيح ويوضع على بيئة M-Endo or LES-Endo وتحضن على 35 +/- 5و درجة مئوية لمدة 20 – 22 ساعة.

سجل وقت أخذ العينة، وقت وصولها، وقت اختبارها فى المعمل . احسب النتيجة وسجل الوقت ا لدى مر قبل اختبار العينة فى المعمل.

طريقة أغشية الترشيح لتقدير بكتريا القولون البرازية

Fecal Coliform Membrane Filter Procedure

بكتريا القولون البرازية ربما يتم تقديرها باستخدام الأنابيب المتعددة Multiple-tube procedure أو باستخدام طريقة أغشية الترشيح Membrane filte technique. ويتم التحضين على درجة 44,5 +/- 2، درجة مئوية لمدة 24 ساعة. ولأهمية ضرورة التحضين عند تلك الدرجة فان الأطباق يتم وضعها فى أكياس بلاستيك غير منفذة للماء ويستخدم حمام مائى وتحضن الأطباق تحت سطح الماء. ويمكن استخدام أية حضانة تحقق الاشتراطات السابقة.

1 . المواد وبيئة الزرع

M-FC medium . 1 . 1

يستخدم فى التحضير البيئة الجاهزة ولا يلجأ الى تحضير البيئة من مكوناتها كالما توجد البيئة جاهزة تجاريا. وتركيب البيئة كالتالى:

Tryptose or Biosate .....10 g
Proteose peptone No. 3 or polypeptone ..... 5 g
Yeast extract .....3 g
Sodium chloride ..... 5 g
Lactose .....12,5 g
Bile salt No, 3 or bile salt mixture ..... .1,5 g
Aniline blue ..... 0,1 g
Agar (optional) ....15 g
Reagent-grade water ..... 1 L

تضاف المكونات الى ا لتر ماء مضاف اليه 10 ملل من محلول 1 % Rosalic acid مذاب فى 2، نورمال صودا كاوية . سخن لقرب الغليان وبرد الى أقل من 50 درجة مئوية. لا تعقييم للبيئة فى الأوتوكلاف. اذا استخدم الآجار توزع البيئة فى أطباق بترى 50 12X مم بكمية 5 – 7 ملل وتترك لتتصلب. pH النهائى 7,4 +/- 2، . البيئة النهائية تحفظ فى الثلاجة فى أكياس محكمة للمخحافظة على الرطوبة، ويتخلص من البيئة السائلة الغير مستعملة بعد 96 ساعة فى حالة البيئة السائلة واسبوعان فى حالة اضافة آجار.

بختبر كل لوط من البيئة مقارن باللوط السابق المقبول (QC/QA) ويعمل تخفيف من مزرعة E,coli ويرشح حجم مناسب ليعطى 20 – 60 مستعمرة على الفاتر .ومع كل لوط تحقق من 10 مستعمرات أو أكثر متحصل عليها من عينات طبيعية مختلفة، لتأكيد عدم وجود ن تائج ايجابية غيلا حقيقية False positive. لمعظم العينات تستخدم بيئة M-FC بدون Rosalic acid مع اعتبار أن ليس هناك تداخل من الخلفية البكتيرية. وهذا التداخل يتوقع فى حالة اختبار Storm water.

قبل الاستخدام يختبر كل Batch من البيئة لكفاءته باستخدام مزرعة ايجا بية واخرى سلبية. ويختبر للتلوث فى نهاية وبداية كل سلسلة من الترشبح بامرار 20 – 30 ملل من محلول الشطف المعقم وفى حالة وجود تلوث تهمل النتائج للعينات المتأثرة بالتلوث ويعاد طلب عينات.

1 . 2 . أطباق الزرع Culture dishes

تستخدم الأطباق البلاستيكية الحكمة ال غلق حتى لا يفقد رطوبة من البيئة ويلف كل طبق فى كيس بلاستيك محكم الغلق حتى لا تنفذ منه المياه بتحضينه تحت سطح الماء.

1 . 3 . الحضانه Incubator

التخصص فى اختبار بكتريا القولون البرازية يرجع الى استخدام حرارة التحضين . استخدام حضانة هوائية فى التحضين ربما يترتب عليعا مشاكل نتيجة طبقات الحرارة فى الحضانة، انخفاض معدل انتقال الحرارة من هواء الحضانة الى البيئة، والتغير فى حرارة الحضانة مع كل عملية فتح لها طوال اليوم. ولذلك فان استخدام حضانة مائية (حمام مائى) هو ما ينصح به بالنسبة لهذا الاختبار الشديد الحساسية ويكون مغطى بغطاء جمالون لتقليل الفقد فالحرارة وفى المياه.

2 . الطريقة Procedure

2 . 1 . اختيار حجم العينة اختار حجم العينة بالاسترشاد بالجدول التالى :

والحجم المختار يعطى ما بين 20 – 60 مستعمرة بكتريا قولونية يرازية على الفلتر.

عندما تكون كثافة البكتر يا غير معروفة ، رشح عدة حجوم أو تخفيفات للحصول على كثافة يمكن عدها. احسب التخفيف والحجم المتوقع أن يعطى عد معقول على الفلتر واختار كميتان أخرتان تمثل عشر وعشرة أضعاف الحجم المختار .

2 . 2 . ترشيح العينة Filtration of sample اتبع نفس الطريقة والاحتياطات المشار اليها فى الاختبار لبكتريا القولون الكلية.

2 .3. تحضير طبق الزرع Preparation of culture dish ضع وسادة ماصة فى كل طبق وشبعها بحوالى 2 ملل من لبيئة (M-FC). ضع بطريقة معقمة الفلتر الذى تم ترشيح العينة من خلاله على الوسادة واتخذ الاحتياطات المشار اليها مسبقا. ويمكن استعمال البيئة المضاف اليها الآجار بدلا من الوسادة الماصة.

2 . 4 . التحضين Incubation توضع الاطباق فى كيس بلاستيك غير منفذ للمياه واغلقه باحكام ويحضن تحت سطح الماء فى الحمام المائى عند 44,5 +/- 2، درجة مئوية لمدة 24 +/- 2 ساعة. يراعى وضع كل الأطباق المستعملة خلال 30 دقيقة من الترشيح.

2 . 5 . العد Counting بكتريا القولون البرازيةعلى بيئة M-FC تأخذ درجات مختلفة من اللون الأزرق. بكتريا القولون الغير برازية تأخذ لون رمادى- كريم. قليل من مستعمرات بكتريا القولون الغير برازية سوف ترى على بيئة M-FC بسبب حرارة التحضين المرتفعة ووجود Rosalic acid ز استعمل للعد قوة تكبير 10 -15 باستخدام Binocular wide field.

2 . 6 . التحقق Verification تحقق من المستعمرات الزرقاء المثالية وأية مستعمرات غير مثالية Atypical رمادية الى خضراء .

TABLE 9222:III. SUGGESTED SAMPLES VOLUMES FOR MEMBRANE FILTER FECAL COLIFORM TEST

	Volume (*X*) To Be Filtered *mL*							
Water Source	100	50	10	1	0.1	0.01	0.001	0.0001
Lakes, reservoirs	X	X						
Wells, springs	X	X						
Water supply intake		X	X	X				
Natural bathing waters		X	X	X				
Sewage treatment plant			X	X	X			
Farm ponds, rivers				X	X	X		
Stormwater runoff				X	X	X		
Raw municipal sewage					X	X	X	
Feedlot runoff					X	X	X	
Sewage sludge						X	X	X

2 . 7 . حساب كثافة البكتريا القولونية البرازية احسب عدد المستعمرات فى المجال المحدد 20 – 60 مستعمرة ويلاحظ أن الأعداد المسموح بها فى حالة بكتريا القولون الكلية 20 – 80 ويرجع ذلك لكبر حجم الخلية على بيئة M-FC.

تأخر التحضين للبكتريا القولونية البرازية

Delayed-Incubation Fecal Coliform Procedure

الطريقة مشابهة لطريقة البكتريا القولونية الكلية Total coliformز تستعمل فى حالة عدم امكان عمل الاختبار فى الحقل أو اذا كان هناك حالة خاصة ينصح فيها بأنه سيتم التحقق من المستعمرات . ترشح العينة ويوضع الفلتر على بيئة M-ST وترسل الى المعمل لاجراء الاختبار بنقل الفلتر الى بيئة M-FC والتحضين على 44,5 +/- 2، درجة مئوية لمدة 24 +/- 2 ساعة وتعد مستعمرات البكتريا القولونية البرازية. وبيئة M-ST تحافظ على حيوية البكتريا القولونية البرازية ولا تسمح لها بالنمو وتكوين المستعمرات ويمكن أن تستمر حيويتها على تلك البيئة لمدة 3 أيام والتأثير ضئيل.
جميع الخطوات بعد ذلك هى الخطوات العدية السابق ذكرها بالنسبة لبيئة M-FC.

طرق سحب العينات

أولا الزجاجات:-

1. العينات الكيماوية :- تسحب العينات في زجاجات حسب المكتوب عليها في الجدول الملحق
2. زجاجات البكتريولوجي :- زجاجات لا تقل عن 100 مل وتكون بغطاء قلاووظ محكم الغلق .

طرق غسيل الزجاجات

1. تغسل الزجاجات بالماء الساخن والصابون جيدا حتى تتأكد من النظافة
2. تغسل بعد ذلك بماء ساخن حتى تزيل باقي رواسب الغسيل
3. تغسل بعد ذلك بماء ذو ضغط عالي

تعقيم الزجاجات

تعقم الزجاجات بوضعها في الوعاء الخاص بها عند درجة حرارة 170 مْ لمدة لا تقل عن ساعتين في الفرن

تعقم الزجاجات بوضعها بصورة مباشرة في الفرن عند درجة حرارة 170مْ لمدة لا تقل عن ساعة

تعقم الزجاجات (غير البلاستيك) بوضعها في الأوتوكلاف عند درجة حرارة 121مْ لمدة 15 دقيقة

عند استعمال زجاجات بلاستيك في الأوتوكلاف لا تضع الغطاء حتى لا تتلف

** تستخدم زجاجات ذات غطاء قلاووظ لإحكام غلق الزجاجة .

** تكون الزجاجات المستخدمة لسحب العينات نظيفة ومعقمة .

ثانيا:- إزالة الكلور من العينات

1- يضاف عامل اختزال لإزالة الكلور المتبقي في مياه الشرب أو آي هالوجين آخر
2- Na2 S2 O3 هو عامل جيد لإزالة الكلور المتبقي الذي يعادل (فيستهلك) آي هالوجين متبقي (أو موجود) لعدم تلويث العينة بأي مادة قاتلة للبكتريا أثناء انتقال العينات
3- لعينات مياه الشرب يكون تركيز عامل إزالة الكلور 0.1 مل من 3 % من محلول الصوديوم ثايو سلفات كافي لإزالة الكلور في عينة 120 مل .
4- في حالة التعقيم الطارئ بتركيز اكبر من الكلور. أضف كميات كافية من عامل اختزال الكلور إلى العينة .
4- عندما تكون العينة بها نسبة عالية من النحاس أو الزنك أو معادن ثقيلة يجب إضافة EDTA على العينات لكي تختزل نسبة المعادن (السموم) الموجودة في العينة إذا كانت سوف تحلل بعد أكثر من 4 ساعات
5- يجب ضبط EDTA عند pH = 6.5 قبل الاستخدام
6- يضاف 0.3 من محلول 15% EDTA على زجاجة 120 مل قبل التعقيم وحدة أو مع Na2 S2O3

ثالثا:- طريقة سحب العينات وتعقيم الحنفيات

تقاس درجة الحرارة و الكلور المتبقي و pH عند سحب العينة و تكتب علي الزجاجات سحب العينات البكتريولوجي

1. عند سحب العينات لابد من ترك مسافة لا تقل عن 2.5 سم بها هواء لسهولة رج العينة جيدا قبل الاستخدام مباشرة
2. أضف ثلج مجروش إلى شنطة العينات (لا تقل عن 2سم) حتى تحفظ العينات لكي تمثل العينة عند وقت السحب
3. التأكد من غلق زجاجة العينة جيدا
4. عند سحب العينات لا تفتح الزجاجة قبل تعقيم مصدر الماء وثبوت تدفقه
5. يعقم المصدر سواء بلهب أو بكحول 70%
6. افتح الزجاجة وأحرص على عدم تلوث الغطاء من الداخل أو الزجاجة
7. أملئ الزجاجة بدون غسلها ثم غلق الزجاجة والحرص على عدم تلويث العينة

8. عند سحب العينة من الشبكة أختار مصدر موصل مباشرا إلى الشبكة وتأكد من عدم وجود خزان أو طلمبة حبشية أو تكون مصدر المياه من شركة أخرى بحيث تتأكد من أن العينة تمثل مياه شبكة الشركة مع مراعاة النقط الميتة في الشبكة

9. افتح صنبور المياه تماما وأترك المياه لمدة دقيقتين أو ثلاث دقائق حتى ثبوت درجة الحرارة لمدة 30 ثانيه

10. قلل من شدة تدفق المياه بحيث تصبح ثابتة ومتوسطة التدفق .

11. لا يجوز سحب العينات من حنفيات بها تسرب أو كسور أو ضغط المياه بها شديد جدا(غير منتظم) أو ضغط المياه بها ضعيف

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ARAB REPUBLIC OF EGYPT
German Technical Cooperation

ميكربيولوجيا المياه - معامل المحطات

Water Microbiology Plant Labratiores

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Annexes

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SECTION II

WHAT MICROBIOLOGY IS ALL ABOUT

GENERAL

Microbiology is the science of the invisible world and its effect on other forms of life. In its broadest sense, it is the science that deals with the study of all kinds of microorganisms — *bacteria, viruses, yeasts, molds, fungi, protozoa*, *algae* and *prions.* The term “microorganism” refers to any of the microscopic forms of life found in nature. There are very few places where some form of microscopic life does not exist. Bacteria are found everywhere — in soil, in the air, in every kind of organism, from humans to plants, living or dead. It is sometimes hard to accept their existence, but special methods have been developed to demonstrate their presence. Single cell, free-living bacteria, are one of the simplest life forms, existing long before human life began. Without bacteria, our world, as we know it, could not exist for bacteria perform many varied functions. Decomposition of matter, for example, is a basic bacterial activity, returning to nature materials necessary to the revitalization of the earth. The gardener’s compost heap, through bacterial action, becomes rich mulch to fertilize the soil.

Survivability of Bacteria

The survival and persistence of different types of bacteria over the ages reflect their ability to live and multiply under a great variety of conditions. Some can survive in a range of temperatures from freezing to almost boiling. Under optimum conditions bacteria can double their number in 15 minutes with or without oxygen. Some can cease growth and go into a kind of hibernation — a virtual state of suspended animation known as a spore. During this “hibernation”, bacteria can survive cold intense enough to liquefy air (–320ºF), and tolerate dry heat of over 200ºF. Bacteria serve a useful purpose from a human’s point of view — from sewage disposal to cheese making. Some are even used to make antibiotics that kill pathogens. Only a small percentage of bacteria fall into the disease-producing class. When bacteria are disease producing, they are often called germs or pathogens.

How Bacteria Transmit Disease in Humans

Gain entrance: usually through the nose, eyes, mouth, sweat glands, hair follicles, wounds, and cuts or through sexual contact. Adapt and multiply: adjust to their new environment and multiply to possibly cause infection and illness. Find a satisfactory exit point: such as the respiratory or gastrointestinal tract, exiting through a cough, a sneeze, in feces, other body fluids, and, in some cases, through sexual organs. Have an effective carrying mechanism: water, dust, food, airborne droplets, insects, used hand towels and other animate or inanimate objects can all carry bacteria, viruses or fungi.

CHARACTERISTICS OF BACTERIA

Forms of Bacteria

The description of bacteria in the following sections relates, in a very general way, to all of these microorganisms. There are actually thousands of types of bacteria, but the basic information should be adequate for most purposes.

Bacteria come in three principal shapes:
Cocci (kock'-si): Spherical bacteria are called cocci, which means "berries" in Greek. Under the microscope they look like miniature berries. Among the cocci group of bacteria are the

Streptococci. Some forms can cause abscesses in teeth, sore throats, and upper respiratory infections, while other forms result in turning milk into buttermilk and turning sweet milk into sour milk. *Staphylococcus aureus* is also a coccus and is a common pathogen that causes pneumonia, meningitis and food poisoning, among others.

Bacilli (ba-sil'-i): The rod-shaped cylindrical bacteria are called bacilli, from the Latin word meaning "little rod". Among the diseases caused by this type of bacteria are typhoid fever, some kinds of diarrhea, cholera, dysentery and eye infections. *Pseudomonas aeruginosa* and

Salmonella choleraesuis are examples. They are the leading cause of bacterial diarrhea in the United States.

Spirilla, Spirochetes: Spiral, curved or corkscrew shaped bacteria are called Spirilla or Spirochetes. This type of microorganism causes trench mouth and syphilis, among others.

Size of Bacteria

Bacteria are so small that 100 cocci placed side by side would be no thicker than a page of this manual. An average coccus (singular of cocci) magnified 500 times would be about the size of the period at the end of this sentence. Bacteria are measured in microns: a micron is 1/25,000 of an inch. Even with today's microscopes, many bacteria are so tiny that dyes must be used so they can be seen.

Structure of Bacteria

A bacterium (singular of bacteria) is a one-cell organism made up of:
Cell wall: a kind of skin for the cell. Plasma membrane: this part of the cell, inside the cell wall, regulates food passage and elimination and is best at picking up a dye or stain to make the cell visible under the microscope.
Cytoplasm: the "insides" of each cell.

SPORES: A small number of bacteria of the rodshaped family have an additional structural capability that you should know about because it relates particularly to sterilization. These bacteria produce a special cell structure, usually described as a "resting body" since it is totally

inactive while in this form. This type of cell is called a *spore* or an *endospore. Bacillus* species and *Clostridium* species are the two most common spore-forming bacteria. It is a miniature suit of armor worn around the cytoplasm that makes this cell the most resistant of all living things to heat, chemicals and drying. Some spores can withstand steam at 212ºF for an hour or more. Some have been found to survive for many years — possibly for centuries! The spore will remain a spore — a resting body — until a proper growth environment exists.

Spirals
(Spirilla or Spirochetes)
Spheres (Cocci) Rods (Bacilli)
It will then, once again, come to life and behave like its ancestors (good or bad), multiplying in the environment of the body or other hospitable area. Common means of destroying spores are irradiation, autoclaving, gas and chemical sterilization. Disinfectants typically have little or no effect on bacterial spores.

Reproduction of Bacteria

Bacteria reproduce by simply dividing. The cell reaches its maximum size, then starts to draw together in the middle, and eventually separates into two. A newborn bacterium becomes an adult in 15 to 30 minutes. Overpopulation in the bacteria world is easy to see.

In 15 Minutes 1 Becomes 2
In 30 Minutes 2 Become 4
In 45 Minutes 4 Become 8
In 60 Minutes 8 Become 16
In 75 Minutes 16 Become 32
In 90 Minutes 32 Become 64
In 105 Minutes 64 Become 128
In 120 Minutes 128 Become 256
In 135 Minutes 256 Become 512
In 150 Minutes 512 Become 1,024
In 165 Minutes 1,024 Become 2,048
In 180 Minutes 2,048 Become 4,096
In 195 Minutes 4,096 Become 8,192
In 210 Minutes 8,192 Become 16,384
In 225 Minutes 16,384 Become 32,768
In 240 Minutes 32,768 Become 65,536
In 255 Minutes 65,536 Become 131,072
In 270 Minutes 131,072 Become 262,144
In 285 Minutes 262,144 Become 524,288
In 300 Minutes 524,288 Become 1,048,576
5 hrs

An Example of the Rate of Reproduction*

** Keep in mind the above example represents the theoretical growth under optimum conditions. A situation such as that indicated above would not normally occur in nature, as bacteria would reach a level of equilibrium based on their environment.*

"Food" for Bacteria — In five hours, one bacterium turns into over 1,000,000 bacteria. And, of course, starting with just one would be strange indeed — even slightly dirty hands might have anywhere from 500 to 1,000 bacteria on them. Moisture is necessary for the growth and reproduction of bacteria because food material must be dissolved in a fluid environment before the bacterial cell can absorb it. Drying, therefore, interferes with bacteria growth. Delicate bacteria such as *Pseudomonas* can survive drying for only a few hours at the most. Bacteria also require carbon, nitrogen, hydrogen, phosphorus and some mineral salts. Some bacteria also require vitamins, just like people. Aerobes and Anaerobes — Bacteria that grow in the presence of atmospheric oxygen are called *aerobes. Pseudomonas aeruginosa* is an example of an aerobe. Some bacteria, however, cannot grow and are even killed in the presence of free atmospheric oxygen. This second group is called *anaerobes.* The botulism organism and tetanus organisms are anaerobic, pathogenic bacteria. *Clostridium* bacteria cause botulism and tetanus. A third group is *facultative anaerobes.* They use oxygen when available. When oxygen is not available, they grow as anaerobes.

Staphylococcus aureus and *Salmonella choleraesuis* are examples of facultative anaerobes. Different types of bacteria have preferences in climate, too. Those that attack the human body are, naturally, most comfortable and thrive best at 98.6ºF or 37ºC (body temperature). Some types of bacteria that are responsible for food spoilage may also grow in cool temperatures. Cold slows down bacterial growth; warmth speeds growth. Very high temperatures, however, are more destructive to bacteria than very low temperatures. Disease-producing bacteria that do not produce spores are killed when exposed to watery liquids at 140ºF (60ºC) for 30 minutes. This is the basis of pasteurization.

Bacterial Activity in Disease

When pathogenic microorganisms enter the body, opposing forces are set in motion. The bacteria try to invade the tissues, multiply, and colonize. The body tries to prevent the invasion by destroying the germs and casting them off. If the microbes win, infection sets in and disease results. Microorganisms cause disease in a number of ways, but in most cases, the ability to cause infection and disease is associated with chemical substances released by the bacteria into the body. In a sense, it is a kind of poisoning or toxic effect.

KEY BACTERIA IN ENVIRONMENTAL SANITATION

In order to understand label claims and disinfectant test reports, and to discuss the more common problems of bacterial contamination, the names, categories and diseases produced by certain key organisms should be familiar. Some background on the importance of these organisms may be helpful.

Bacterial Contamination in Hospitals

In hospitals, the combination of harmful microorganisms and people with low resistance is the major problem. When a patient develops an infection during a hospital stay — an infection which was neither present nor in the incubation stage at the time of admission — the infection is described as “nosocomial” (hospital or institutionally acquired). Approximately 5-10% of the people who enter a hospital come down with a nosocomial infection resulting in over 1 million infections and 80,000-100,000 deaths annually.

Nosocomial infections account for billions of dollars in additional health care costs. Various categories of nosocomial infections include:

- Urinary tract infections
- Respiratory infections
- Gastroenteritis
- Skin and subcutaneous infection
- Intra-abdominal infection
- Septicemia

Most surveillance reports identify the urinary tract as the most common site of nosocomial infection. The next most common site of nosocomial infection is the surgical wound. Respiratory infections are the third major type of nosocomial infection.

Antibiotic Resistant Strains

In hospitals, extended care facilities, schools, hotels, restaurants and other public-service establishments, the problem of infection associated with antibiotic-resistant strains also exists. These organisms, although resistant to antibiotics, *pose no resistance to being destroyed by disinfectants.* Although nonhospitalized people are presumably in better health than hospital patients, the danger still exists. The following material will give you the basics on some key bacteria. A more complete listing of other important organisms appears in the Glossary, Section X.

Note: There are two additional classifications of bacteria about which you will often hear: *Gram positive* and *Gram negative.* This is simply a common way to help classify and identify bacteria.

Gram Positive/Gram Negative/Acid Fast

The Gram stain is the dye (named after its developer Dr. Gram) used to color some kinds of bacteria so they can be seen under the microscope. The bacteria are stained with a blue dye called crystal violet. If the bacteria become permanently stained blue they are known as Gram positive. If the stain can be readily removed (by treatment with alcohol) and are counter-stained pink with a red dye called safranin, they are known as Gram negative. Most Gram negative bacteria are found in the intestines. Most Gram positive bacteria are found elsewhere in the body. Fungi and viruses are not included in this type of classification.

Gram Positive Bacteria
Staphylococcus aureus (Staf-ill-o-cock-es oar-ee-us) is considered a major cause of hospital infections and can develop resistance to antibiotics. It causes boils, carbuncles, blood poisoning and most food poisoning. Keep in mind there are many other types of Gram positive bacteria, such as

Streptococcus and *Bacillus.*
Gram Negative Bacteria
Salmonella choleraesuis (Sal-mon-el-la coll-erah-soo-iss) is the standard test bacteria used in germicide evaluation procedures because its resistance to germicides is typical of the Gram negative group. The Salmonella group, as a whole, is associated with a variety of gastrointestinal and other disorders.

Pseudomonas aeruginosa (Soo-do-moan-us airoo- gin-o-sa) is an organism which is very difficult to destroy and causes diseases similar to those caused by Staphylococcus. It is a definite problem for hospitals and other public facilities and a major concern in hospital burn units.

Acid Fast Bacteria
Mycobacterium tuberculosis (My-co-bac-teer-eeum toob-er-ku-lo-sis) is the bacteria that causes tuberculosis (TB) and is spread primarily through the air. Due to its waxy cell wall, TB bacteria do not stain Gram positive or Gram negative. Another type of stain called an acid-fast stain is used which turns the cells green. This organism is extremely difficult to kill with disinfectants. As a result, it is often used as a benchmark for germicidal efficacy.

HOW BACTERIA MOVE

Bacterial Movement

Most bacteria are completely dependent on getting a free ride. Although some kinds of self movement are possible for bacteria, for our purposes we can assume that they are moved only by air currents, water, animals, insects, people, or objects — never under their own power. In the air, there are three ways for bacteria to get around:
• They can be moved around individually which is not considered a hazard since the drying effect of the air tends to destroy them, in a short time.
• They can cling to dust particles moving through the air and be protected by the dust.
• They can attach themselves to droplets of moisture. For example, when a person sneezes or coughs, the shower of droplets contain bacteria. The smaller drops lose most of their moisture when they hit the floor. But even droplets barely larger than the individual bacteria, called droplet nuclei, can provide the moisture and nourishment bacteria require. Bacterium and droplet nuclei are easily blown about in the air. This is the means by which TB bacteria are transmitted.

Movement on Objects

When we speak of objects carrying bacteria we must include practically anything: furniture, dishes, wheelchairs, instruments and shoes are just a few examples. Linens, especially soiled linens, are considered a particular hazard, especially in hospitals. For example, a person with a Staphylococcus infection sheds literally millions of bacteria onto sheets and blankets. If linens

are not properly handled and bagged within the institution or hospital room, these potentially hazardous bacteria can be spread throughout the facility as the linen is removed to the laundry. Moveable objects that hold or carry bacteria are called *fomites.*

Movement on People

People are also dangerous carriers of bacteria. It can be very difficult to detect a human carrier of harmful germs. In the hospital, doctors, nurses and aides are the most frequent offenders, sometimes carrying a *Staphylococcus* colony in their nose and throat. Even without showing any signs of illness, the staff can spread these germs to patients. One of the most famous cases of an unidentified carrier was “Typhoid Mary”. This helpful lady worked in public kitchens from 1900 to 1907, and, without ever suffering from the disease herself, managed to infect a large number of people with typhoid fever over the years.

Animal, Insect and Bird Carriers

Animals, insects and birds can also carry pathogenic bacteria capable of infecting humans or other animals. Veterinarians, kennel workers, poultry farmers, etc., should be aware that the same rules apply — pathogenic bacteria represent a danger to all creatures, and can be just as costly and painful in animals as in people. Animals, insects and birds that carry pathogenic agents are called vectors. It should be noted that viruses and fungi can also be moved in any of the ways described above.

VIRUSES, FUNGI AND PRIONS

In addition to the three basic types of bacteria *(cocci, bacilli and spirilla/spirochetes),* there are three additional kinds of microorganisms that are likely to cause problems in humans — *viruses, fungi* and *prions.*

Viruses

Viruses are the simplest form of life. Their structure, method of replication, and size, among other factors, are different from the three basic bacteria. The most significant difference is that viruses must get inside other living cells in order to multiply, while bacteria can multiply most anywhere. Viruses are responsible for many diseases with colds and flu being two of the more common. Some scientists and textbooks claim that viruses are not “alive” and therefore it is difficult to say that they are “killed” by treatment with disinfectants. Viruses are made up of certain types of organic materials (e.g. lipids and proteins) which help to protect their genetic material (e.g. DNA or RNA), but they lack other functions which would allow them to grow on their own. If a virus is left on a surface by itself, it will not be able to grow. For this reason, many people say that viruses are not alive because they can’t reproduce on their own. However, if a virus is able to get into a person’s body, through the mouth or a cut, it can start an infection and reproduce millions of copies of itself within the body. Because viruses are able to start infections and reproduce themselves with the help of a human host, many scientists do consider them “living” organisms.

Viruses are not equal in their susceptibility to chemical disinfectants. The chemical make-up and structure of the virus account for this. Viruses can be divided into three broad categories:

Lipophilic — those surrounded by a lipid envelope. Examples of lipophilic viruses are: Hepatitis B, Influenza, Vaccinia, Herpes, Respiratory Syncytial Virus (RSV) and Human Immunodeficiency Virus (HIV-1 AIDS Virus).
The latter virus should be referred to as HIV, not HIV Virus.
Hydrophilic — do not have a lipid envelope. These viruses are very difficult to inactivate. Examples include: Poliovirus, Echovirus, Hepatitis A, and Canine Parvovirus. High concentrations of ethanol and strong concentrations of chlorine are among the few germicides that will inactivate these viruses. Intermediate — do not have a lipid envelope but do have some lipophilicity in the virus' outer coat. Phenolic disinfectants generally inactivate these viruses. Examples of intermediate viruses are Adenovirus and Rotavirus.

Fungi

Fungi (plural of fungus) are altogether different from bacteria and viruses. In fact, fungi are more closely related to humans than to bacteria. Fungi form mold and mildew. Yeast and mushrooms are examples of fungi. Fungi also produce diseases of the skin, lungs, and mucous membranes and can in rare cases actually invade the entire body.

Trichophyton mentagrophytes (Try-co-fi-ton or Try-cough-it-on men-ta-grow-feet-es), also known as *Trichophyton interdigitale (Try-co-fiton or Try-cough-it-on inter-dij-i-tallee)* is the fungus that causes athlete's foot fungus. It is used as a test organism to represent the fungus class in germicidal evaluation tests.

Prions

Prions are transmissible pathogenic agents that cause a variety of neurodegenerative diseases including Creutzfeldt-Jakob Disease in humans, bovine spongiform encephalopathy (BSE) in cows (also known as "Mad Cow Disease") and scrapie in sheep. They are unlike the other pathogens discussed above because they are composed entirely of protein. An abnormal configuration of a normal cellular protein acts as a "template" to convert the normal protein into the abnormal form. Accumulation of the abnormal protein in the brain leads to disease. Conventional sterilization and disinfection practices are not effective against prions. Some researchers recommend the use of a 1:2 dilution of bleach (~20,000 ppm) or 1-2 N sodium hydroxide (NaOH) for extended periods of time (>1 hour).

SECTION III

METHODS FOR CONTROLLING BACTERIA IN THE ENVIRONMENT

AGENTS THAT KILL BACTERIA OR SLOW THEIR GROWTH

Light

Sunlight is a great natural disinfecting agent. Direct sunlight destroys many bacteria. Unfortunately, the sun's irregular performance does not make it a dependable method for disinfecting. Ultraviolet lamps are sometimes used to prevent the spread of germs in the air, particularly tuberculosis (TB) bacteria. Neither visible nor ultraviolet light rays have much ability to penetrate surfaces, so their action is limited to organisms on or near the light. Ultraviolet light strong enough to disinfect can also damage skin and eyes.

Cold

Cold is a useful agent in inhibiting growth of bacteria and can prevent the dangers of active bacteria. Cold over a long period of time suspends or significantly reduces the growth of bacteria. As evidence, frozen foods spoil rapidly after thawing when bacteria come back to full activity. Even fragile bacteria have been maintained for years frozen in dry ice (–169ºF) and then brought back to life.

Heat

Heat is widely used as a sanitizer and is a very effective sterilizing agent. At high temperatures, *sterilization* is the process of destroying all microorganisms (bacteria, including spore forming bacteria, viruses and fungi) on a substance or surface. Heat for sterilizing may be moist or dry. Moist Heat may be applied either as hot water or steam. Steam under pressure is the preferred and most often used sterilization method. Autoclaves used in hospitals for instruments work with moist heat in the form of steam under pressure. Materials that are harmed by heat or moisture, of course, cannot be sterilized in this way. Boiling kills *vegetative* forms of bacteria (those bacteria not in spore form and able to multiply), fungi and viruses, in a few minutes. Spores, as mentioned, may take many hours to kill even at boiling temperatures, and still not every one may be dead. Dry Heat (hot air sterilization) is done in a kind of oven. Temperatures ranging from 320ºF (160ºC) to 356ºF (180ºC) must be applied for at least 1.5 hours to kill spores.

Chemical Agents

A number of chemical agents are used to destroy or slow the growth of bacteria. The name of the product — disinfectant, sanitizer, etc. — indicates its degree of action against bacteria.

Disinfectant — A chemical agent that destroys pathogenic microorganisms (not spores). The term is generally used for an agent that destroys organisms on inanimate objects (surfaces) rather than on people or animals. Antiseptic — A chemical agent, such as surgical scrubs, antiseptic hand soaps, antimicrobial soaps and ointments that kills microorganisms when applied to the body. Germicide/Bactericide — Chemical agents that kill bacteria. These terms are often synonymous with the term disinfectant.

Bacteriostat — A chemical agent that prevents microbes from multiplying. The term may be used in referring to the action of certain antibiotics, as well as to antimicrobial action on surfaces. Also used as a preservative in household and cosmetic products. Sanitizer — An agent that reduces (through killing) the number of bacteria to a safe level. This means a 99.9% kill as set by public health requirements. This term is also applied to agents used to control the microbial population in food service, food preparation and food processing areas. These are called food-contact surface sanitizers and require a 99.999% kill. Sterilizer — An agent or device that destroys all living things, including vegetative bacteria, spores, fungi and viruses. It is an absolute term; that is, there is no such thing as “almost sterile”. Preservative — A chemical agent that inhibits microbial growth. It generally refers to agents used to prevent the deterioration of foods, drugs, cosmetics, household products, chemicals and other products.

Radiation and Gas

Cobalt radiation is often used to sterilize materials, as is ethylene oxide (EtO) gas. While EtO is often used within hospitals, radiation is used primarily by manufacturers of disposable medical equipment.

HOW TO REMOVE AND/OR DESTROY BACTERIA

There are three basic ways to control harmful microorganisms:
• Destroying bacteria in the environment with heat or disinfectants.
• Physical removal of bacteria from the environment, for example, by filtering air or cleaning.
• Destroying bacteria after a person has been infected by using antibiotics.

SECTION IV

COUNTING BACTERIA ON SURFACES

WHY A COUNT?

There are times when it's desirable to know just how well a disinfectant, sanitizer or cleaning procedure works. Since bacteria are invisible, only indirect methods can be used to show the level of cleanliness achieved. In these cases, being able to do a bacterial count, or, at least, to understand the procedure, is important. Bacterial counts in one area can be compared with a neighboring area or bacteria can be counted on a surface before and after cleaning. Most people tend to think that bacteria are spread evenly over a surface. This is not so. Unless adequate samples are taken across an entire area, results can be very misleading. For example, in order to get a reasonably accurate estimate of a floor area only 8 to 10 square feet in size, it is necessary to take at least a dozen random samples within that area.

METHODS OF BACTERIAL COUNTING

Individual bacteria, as we know, are too small to be seen. Therefore, it is obviously not practical to try to count them one at a time. Since bacteria multiply so quickly, and form colonies (clumps) when they have moisture and food, it is possible to do a count at the stage when these colonies have become big enough to be seen with the naked eye.

Swab Method

With the swab method, a sterile swab is dipped in sterile solution and then used to "wash" the surface. This lifts the bacteria onto the swab. The swab is rubbed over a selected area, rolling back and forth and criss-cross to thoroughly cover the few square inches involved. The swab is dipped back into the sterile solution several times during the cleaning so that the bacteria are rinsed off into the tube. The final step is to break off the tip of the swab and place it in the solution. The tube is shaken hard to rinse all of the bacteria out of the swab and into the solution. The solution is then poured onto the culture media plate. A culture media plate is simply a small plastic or glass plate with a cover which is filled with melted (warm) culture media, called agar. As the media cools it forms a gel.

The solution is poured over the gel. The lid is put on and the plate is kept in a warm place for about 24 to 48 hours. The bacteria then multiply and become colonies that can be seen and counted.

Rodac Plate

With a *Rodac* plate, the melted agar media is poured almost to overflowing into a plastic disposable dish. When the agar media solidifies, it forms a too-full dish of media. This is then pressed firmly and steadily onto the flat surface so bacteria will stick to it to be counted. The lid is then put on, and the plate is put in an incubator (preferably about 98ºF to 110ºF) for about 24 to 48 hours. The Rodac plate is very simple to use, but it does not fit every situation. It only works on flat or barely rounded surfaces where the gel can be rolled over the test surface. If this cannot be done, the swab method should be used. Generally, the swab method is used for “tight” spots and in food service areas, and Rodac plates for flat, nonfood surfaces.

COMPARING COUNTS

It should be noted that Rodac counts cannot be compared with counts from a swab method. With the swab method, a good deal more bacteria are removed from the surface than is possible with a Rodac plate — so swab counts will be higher than Rodac counts. In addition, swabbing usually breaks up small clumps of bacteria and each part forms another colony, which is counted as a separate colony. With the Rodac plate method the clump is undisturbed, and, as it multiplies it grows together and is counted as only one colony. Always compare Rodac counts with Rodac counts, and swab counts with swab counts in order to obtain a true comparison. When conducting counts in hospitals, keep in mind that there are no fixed numbers or acceptable/ nonacceptable counts, only rough guidelines. Incubating swab or Rodac plates under aerobic conditions will only show aerobic or facultative anaerobic bacteria. In order to obtain counts for anaerobes, the plates must be incubated under anaerobic conditions. There are special chambers that provide the right set of circumstances for their growth. However, in most cases anaerobic plate counts are not very important because their bacteria do not grow or die quickly in aerobic environments. Some anaerobic bacteria such as Clostridium are pathogenic and it may be helpful to conduct such tests. Fungi can also be detected in a swab or Rodac plate, but viruses cannot.

OTHER METHODS FOR DETERMINING NUMBER OF BACTERIA AND VIRUSES

Today, other methods exist to count bacteria and viruses other than the plate count method. Viruses, unlike bacteria on Rodac or agar plates, cannot be counted because of their “parasitic” way of life. The new methods are based on molecular biology and genetics. For example, one method is called PCR or Polymerase Chain Reaction. In this procedure the genetic material — DNA or RNA of the bacteria or virus — are collected and replicated. Specific regions of the genetic code unique to the particular organism are then located. This identifies and, in some cases, counts the number of organisms.

IDENTIFYING SPECIFIC BACTERIA — PRESENCE OF SPORES

Ordinarily there is no need to identify the type of bacteria counted. However, if persistently high counts occur when cleaning procedures seem to be perfectly correct, the laboratory can help by identifying the specific bacteria, or unusual strains of bacteria. This is particularly significant when numerous spores are found on analysis. Spores, those bacteria whose “armor plates” resist all disinfecting attempts was discussed in an earlier section of this manual. Many sporeforming bacteria are harmless, but since they cannot be killed, they can produce high counts that may not be meaningful in terms of sanitation. Some examples of harmful spores are *Clostridium difficile* and *Clostridium tetani.*

OTHER INFLUENCES ON BACTERIAL COUNTS

Bacteria clump together. Many times when a floor is mopped, a clump of bacteria is broken up and each section has the potential to start a new colony. As a result, it is possible to obtain higher counts after mopping than before. The number of samples taken and the method of cleaning/ disinfecting, especially mopping, are important considerations if this type of result is found.

Another important factor is the contamination of mops. Unless mops are thoroughly cleaned and dried, they can become contaminated even if they are always used with a disinfectant. It is not possible to kill all the bacteria trapped in the fibers and pores of a mop without sterilization or hot air drying. Mop contamination and other dirty cleaning tools often account for strange results in bacterial counts. Factors such as utilizing clean mops and wipe rags are important for complete infection control procedures.

SECTION VII

TYPES OF DISINFECTANTS AND SANITIZERS

GENERAL INFORMATION

Before discussing the various types of disinfecting and sanitizing products available, a few general points should be made to put the picture in perspective. First, there is no “ideal”product. If one product could do everything for every use, there would be no need for so many products. There are many variables between products — even products of the same type, between procedures, and from one type of facility to another. There are advantages and disadvantages to all disinfecting/sanitizing products. It’s important to understand these differences in order to select the best product to satisfy your needs and meet your objectives. It’s also important to know what can be expected from the different types of products available.

PHENOLICS

Phenolic disinfectants are the standard for disinfectant performance. They have had the advantage of being among the first products to be called “hospital disinfectants” which has added to their stature in many industries. Current phenolics are primarily synthetic in nature. They are excellent disinfectants that have hospital antimicrobial action. Unlike dilutable quaternaries, phenolics are tuberculocidal. This is a very important factor in their selection, particularly in the health care setting. The OSHA Bloodborne Pathogens Standard requires the use of a hospital disinfectant including tuberculocidal efficacy for clean up of blood or OPIM (Other Potentially Infectious Material) spills. Phenolics couple up well with selected detergents to make effective combination cleaner-disinfectant products. They are, however, inactivated by nonionic synthetic detergents, so although they are not manufactured in such combinations, care must be taken to avoid improper mixing which can result in poor disinfectant performance. This can be avoided by properly cleaning equipment, and not mixing anything but water with a phenolic disinfectant or cleaner-disinfectant (or, for that matter, *any* disinfectant regardless of chemical type).
Straight phenolic disinfectants, such as Professional AMPHYL® Disinfectant Cleaner or Professional AMPHYL® Hospital Bulk Disinfectant Cleaner, manufactured by Reckitt Benckiser Professional, are widely used in many industries for general disinfecting. It should be noted that any disinfectant, sanitizer or simple cleaner can produce problems for certain people with sensitive skin. For this reason, all of today’s disinfectant labels warn against possible skin irritation and recommend that employees wear rubber gloves. All label precautions should always be followed.

QUATERNARIES

Quaternary ammonium compounds are a second major class of germicides. They are not tuberculocidal in dilutable formulations. Quaternaries are effective against *Staphylococcus, Salmonella, Pseudomonas,* some lipophilic viruses, fungi and other common pathogens. Properly formulated, quaternaries are considered to be very good cleaners and deodorizers. As with any disinfectant/sanitizer product, there are differences between brands of quaternaries. Proof of equal germicidal effectiveness cannot be taken for granted; lists of EPA-approved kill claims should be reviewed to make comparisons. LYSOL® BrandI.C.™ Quaternary Disinfectant Cleaner is effective for general health care use. Quaternaries are also used in the food processing area, in supermarkets, and in restaurants. Professional LYSOL® Brand Antibacterial All Purpose Cleaner and Professional LYSOL® Brand No Rinse Sanitizer are authorized by the USDA and registered with NSF for food processing area use.

ACID DISINFECTANT CLEANERS

Acids disinfect by changing the environment for germs, rather than by attacking the cell walls, as do phenolics or quaternaries. Reckitt Benckiser Professional's acid-based cleaners are EPAregistered and provide disinfecting action. Professional LYSOL® Brand Disinfectant Toilet Bowl Cleaner and Professional LYSOL® BrandII Basin Tub & Tile Cleaner contain highly active acids, which destroy germs. In addition, other actives such as quaternaries are added for extra germicidal activity.

IODINES AND IODOPHORS

Most iodine-type disinfectants now in use are called iodophors — compounds of iodine and nonionic synthetic detergents. These compounds, often promoted as "tamed" iodine products, have fewer disagreeable characteristics than simpler iodine products. They are relatively low in toxicity and less corrosive. In order to be active germicidally, iodophors must be kept on the acid side of the pH scale. This is usually done by including phosphoric or hydrochloric acid in the formula resulting in the potential for corrosion problems. Iodophors are only fair detergents compared to quaternary or phenolic detergent-disinfectants. This relates partly to the acid pH. Phenolics and quaternaries are on the alkaline side of the scale, which contributes to their cleaning ability. The reddish brown iodine color of iodophors can be an advantage or disadvantage. The depth of color allows the user to see the degree of concentration since the shade relates directly to the amount of iodine in the solution, and therefore, to the amount of germicidal activity remaining. On the down side, however, these products do stain temporarily, sometimes even permanently. Some plastics will be permanently stained. Some white paints will be temporarily yellowed. Starched fabrics will show dark blue stains. Iodophors, used at proper dilution, can generally be depended upon to kill most microorganisms.
The greatest use of iodophors is in disinfecting and sanitizing food contact surfaces and food preparation equipment. These surfaces, however, must be cleaned first and rinsed with fresh water before iodophor disinfectants are applied.

CHLORINE

Chlorine, also known as bleach or sodium hypochlorite, is a chemical that has received much attention due to the issuance of the OSHA Bloodborne Pathogens Standard-29 CFR 1910.1030. This standard, along with numerous previous recommendations for its use, promotes chlorine as an effective method of decontaminate on of surfaces. During disease outbreaks such as Legionnaires Disease and the initial discovery of HIV-1 (AIDS Virus), chlorine was the initial product recommended by many government agencies since, at the time, no other disinfectants, or chlorine for that matter, had been tested against these organisms. It seems that, when it is unknown what will effectively kill a bacterial or viral organism, chlorine is the standard product recommended. Although chlorine can be an effective disinfectant, its chemistry, and particularly its stability, leave much to be desired.

Chlorine's use in swimming pools and potable water systems is well known. However, it is quite unstable when exposed to light, heat or organic soil. It is for this reason that chlorine must be added to swimming pools regularly on hot, sunny days or when they receive excessive use. This increased use causes significant reduction in the pool's chlorine level. As people enter a pool they carry with them soil, body oils, and dirt. This equates to an increase in organic soil levels, which quickly reduce chlorine's bactericidal efficacy. The Bloodborne Pathogens Standard calls for disinfection of contaminated surfaces with a "freshly prepared" 1:10 solution of sodium hypochlorite. However, no indication is given as to what is considered "freshly prepared". Most sources suggest daily preparation. Chlorine is also corrosive and/or damaging to numerous surfaces including steel, carpet fiber, clothing and human skin. In addition, it is extremely reactive if inadvertently added to other chemicals such as acids. The following chart lists many properties of chlorine as compared to other chemical disinfectants including phenolics and quaternaries. Please keep in mind that this discussion does not cover the subject of chlorine used in food service sanitation.

Characteristics	Phenolics*	Quats*	RTU Quats*	Chlorine
Dilution Stability	Stable, EPA recommends	Stable, EPA recommends	Stable Use solutions should be used promptly.	daily use daily use High temperatures preparation and light accelerate instability
Concentrate Stability	Over 2 years	Over years	Indefinitely	Should be stored in a cool dark location in closed containers, i.e., shielded from ultra violet light
Disinfection Level	Hospital strength including TB	Hospital strength some including TB	Hospital strength recommended, not all are EPA registered	While OSHA Disinfectants
Effective in presence of Organic Soil	Yes stability/efficacy	Yes	Yes	Presence of organic material can effect
Wetting Ability	Good	Good	Good	Poor or none
Odor	Clean/medicinal	Pleasant	Clean	Harsh
Cleaning Ability	Good	Very Good	Exceptional	Some color/stain removal

*Refers to products manufactured by Reckitt Benckiser Professional

OTHER DISINFECTANT AND SANITIZING AGENTS

There are a number of other agents used for limited purposes, a few of which are mentioned below.

• Glutaraldehyde products are used for cold sterilization of heat sensitive instruments and equipment. They are not recommended for environmental surfaces. Glutaraldehydes are a very effective germicide, but have a distinctly unpleasant odor and are very harsh. There are special precautions that must be taken when handling glutaraldehydes. Although glutaraldehydes are sporicidal, the sporicidal activity is available only with a 10-hour soak. Disinfection is achieved, in most cases, after a 20 to 45 minute soak. Most glutaraldehydes must be prepared by adding a separately bottled “activator”.

• Glycols are used in some aerosol air-sanitizing products. The presence of 5% to 10% glycols in such products reduces airborne bacteria. When this percentage is in the formula, the product is considered to be an air sanitizer under EPA regulations.

• Antimicrobial hand soaps use ingredients that reduce bacterial counts on skin but have no application for surface disinfection or sanitizing, such as Professional LYSOL® Brand I.C.™ Antimicrobial Soap.

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http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index_e.html

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Laboratory Procedure MFLP-41 B
July 2006
HEALTH PRODUCTS AND FOOD BRANCH
OTTAWA
ENVIRONMENTAL SAMPLING FOR THE DETECTION OF MICROORGANISMS;
PREPARATION OF SAMPLING MATERIAL

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1. APPLICATION

This method is applicable to the preparation of necessary material in order to take environmental samples in food processing plants in support of compliance activity relative to Section 7 of the Food and Drugs Act. This revised method replaces MFLP-41B dated April 1998 and the Supplement to Method MFLP-41B dated September 1999.

2. DESCRIPTION

This procedure describes methods of preparation for material to be used when environmental sampling is done in a food plant environment for microbiological evaluation. For information on how to take a sample from a food contact surface in a food plant environment please refer to MFLP-41A.

3. PRINCIPLE

It is essential that the methods and materials used for environmental sampling be standardized since the presence of pathogens or high numbers of bacteria in a food plant establishment may be a sign that foods MFLP-41B - 2 - July 2006 have been produced under poor sanitary conditions or that the plant houses microbial niches. Methods used will demonstrate the presence or estimate the number of viable microorganisms sampled on food contact surfaces. Samples obtained are then inoculated into or onto selective media specific to the type of microorganism(s) of interest. It is assumed that each viable microorganism will then multiply under specified conditions of incubation and give rise to visible growth which can be measured, counted and identified. This method is still considered semi-quantitative since varying proportions of the total number of viable cells may be recovered. However, identification of

microorganisms isolated may provide valuable information. It is essential that microbiological analysis be initiated as soon as possible after sampling in order to avoid any loss due to die-off.

4. BEFORE SAMPLING

Before initiating environmental sampling, the person responsible for performing the environmental sampling must notify and consult with laboratory personnel.

5. MATERIALS AND SPECIAL EQUIPMENT

5.1 Sterile material may be prepared by laboratory personnel or purchased

1) Spoons (short- and long-handled), sterile
2) Forceps, sterile
3) Knives (Victorinox or equivalent), sterile
4) Plastic cups (8 oz., Becton Dickinson Labware, #4015, or equivalent), sterile
5) Swabs (cotton, calcium alginate, Dacron or Rayon)
6) Sponges (celluose or polyurethane). Commercially available with or without neutralizing buffer (Qualicum Scientific, Oxoid) or equivalent.
7) Swatches (J-cloths, gauze or cloths)
8) Jars, polypropylene or other unbreakable material (Nalgene or equivalent)
9) Whirlpak™ bags, sterile
10) Screw-cap tubes, polypropylene or other unbreakable material for swabs
11) Disposable overalls, head cover, overshoes, facial hair-cover (if sterile clothing is needed)
12) Prepackaged surgical gloves (wrist), sterile
13) RODAC™ plates (Falcon)
14) Petrifilm™ plates, various types
15) Neutralizing Buffer, commercially available (Difco, Qualicum Scientific, Oxoid) or equivalent
16) D/E Neutralizing Agar, commercially available (Difco, Qualicum Scientific, Oxoid) or equivalent.
17) Microbial Content Test Agar, commercially available (Difco, Qualicum Scientific, Oxoid) or equivalent.
18) Violet Red Bile Agar, commercially available (Difco, Oxoid) or equivalent.

19) Dichloran Rose-Bengal Chloramphenicol (DRBC) Agar Base, commercially available (Difco, Oxoid) or equivalent.
20) Baird Parker Agar Base, commercially available (Difco, Oxoid), or equivalent.
21) Letheen Broth/Agar, commercially available (Difco) or equivalent.
22) Transport medium, commercially available (Difco) or equivalent.

5.2 Surface Treatment for Chemical Germicides

If sampling is to be carried out on surfaces previously subjected to chemical germicide treatment, appropriate neutralizers should be incorporated into the medium. Neutralizing media are usually commercially available. Although efficacy of the neutralizers for agar contact sampling has not been demonstrated definitively, it has been found useful.
See Table 1 for a list of media, neutralizers, the compounds which are neutralized and the reference.

6. PREPARATION OF BACTERIAL CARRIERS

It is recommended that each lot of bacterial carriers (sponges, swatches, swabs) be tested for inhibitory properties against the selected bacteria by using the method of Libras and Rose (8.1, Appendix 1) or another acceptable method. See Appendix 1 of this method.

6.1 Sponges
6.1.1 Put sponge(s) (approx. 4 cm X 8 cm) in a wide mouth Nalgene jar containing 10 to 15 mL of Neutralizing Buffer or any other buffered rinse solution which contains neutralizers to completely moisten each sponge. Sterilise jars at 121°C for 15 minutes. Alternately, place pre-sterilized sponges and Neutralizing Buffer into sterile Whirlpak bags or jars.
Indicate the sterility of the sponges with autoclave tape. Mark each container with the preparation or expiry date.
Alternatively, individual packaged sponges, pre-moistened with neutralizing buffer, are commercially available.

6.2 Swatches (J-cloths, gauze and cloths)
6.2.1 Cut J-cloths 35 X 60 cm in half in order to obtain a cloth of 17.5 X 30 cm. Fold the swatches in such a way that they can be easily removed by inspectors. Five swatches can be put in a sampling jar with the last side folded on the top to facilitate removal while using forceps or gloves.

6.2.2 Add 200 mL of neutralizing buffer per sampling jar. Label each jar with the identity of the media, the date and the number of cloths in the container. Autoclave at 121°C for 15 min.

6.3 Swabs
6.3.1 Swabs of approximately 2 cm with the head firmly attach to an applicator stick 12 to 15 cm long may be used. Swabs made of calcium alginate fibres are soluble in aqueous solutions containing 1% sodium hexametaphosphate (or sodium glycerophosphate, or sodium citrate, or 1% of any mixture of these) allowing the release of the captured organisms. Pre-sterilized swabs in various transport media are commercially available.
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6.3.2 For sterile dry swabs, prepare screw-capped plastic vials containing 20 mL of sterile Neutralizing Buffer or any other buffered rinse solution which contains neutralizers.
Also, prepare screw-capped tubes of sterile transport media to contain the swab used to sample the environmental surface.

6.4 Keep bacterial carriers refrigerated until picked up by the inspector. Ship bacterial carriers to inspectors with ice packs.

7. SAMPLING
There are two types of sampling: qualitative and quantitative. Qualitative sampling includes liquid media which promote the growth of the organism of concern. Quantitative sampling estimates the number of bacteria isolated from a determined sampling area using Petrifilm™ and RODAC™ plates.

7.1 Qualitative Sampling and Procedure
Note: It is imperative that the “Application Section” of each method be reviewed before use to determine the method’s applicability to the food or environmental sample in question. Applicability of methods to new matrices requires validation.

7.1.1 Bacterial carriers and controls must be refrigerated when received by the laboratory and analyzed as soon as possible.

7.1.2 Add media or supplements to media as appropriate, and incubate following the appropriate method for the microorganism(s) of concern.

7.1.3 Detection of Microorganisms; table 2 lists appropriate steps to be taken when samples are received at the laboratory after inspection. Usually qualitative environmental sampling is done when the presence of *Listeria monocytogenes*, *Salmonella*, or *Staphylococcus aureus* is suspected. Therefore, only those are listed in the table. Other determinations can also be performed depending upon recommendations made by laboratory personnel to the inspector, and is usually determined on a case by case basis.

7.2 Quantitative Sampling and Procedure

7.2.1 Perform quantitative sampling using RODAC (Replicate Organism Detection and Counting plates) or Petrifilm plates.

7.2.2 Preparation of RODAC™ plates

Fill the disposable plastic RODAC™ plates aseptically with 15.5 to 16.5 mL of the appropriate sterile media. The meniscus of the agar should rise above the rim of the plate to give a slightly convex surface in order to allow a good contact with the surface to be sampled. Prefilled plates with test medium are also commercially available. Media for some methods are listed in Table 3 and the formulae can be found in the Compendium, Appendix G (8.2). When sanitizer residues are present on the contact surface, choose an appropriate neutralizer to add to the media from Table 1. Media with neutralizers are also commercially available.

7.2.3 Preparation of Petrifilm™

Follow the manufacturer's instructions. Request the technical bulletin on SURFACE SAMPLING PROCEDURES from the manufacturer,and pay particular attention to the recommended diluents when sanitiser residues may be present.

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7.2.4 Detection of Microorganisms

Count all developing colonies. Spreading colonies should be counted as one but care should be taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bicoloured colonies and halo type spreaders. It is generally agreed that 200 colonies is the approximate maximum that can be counted on contact plates. Colony counts may be recorded by:

1) Individual counts,
2) Number of viable particles per selected area,
3) Means and standard deviations.

8. REFERENCES

8.1 Libras, C. M. and Rose, B. E. 1989 . Antibacterial Properties of Retail Sponges. J. of Food Prot. **52**(1):49-50.

8.2 Health Canada. 2006. Appendix G In Volumes 1, 2 and 3. *Compendium of Analytical Methods*. Website: http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index_e.html.

8.3 American Public Health Association. 2001. Compendium of Methods for the Microbiological Examination of Foods; Fourth Edition. F.P. Downes and K. Ito (eds.). American Public Health Association Inc., 1015 Fifteenth Street, Washington, D.C. 20005.

8.4 Difco Laboratories. 1998. Difco Manual, Dehydrated Culture Media and Reagents for Microbiology; Eleventh Edition. Difco Laboratories Inc., Detroit, Michigan 48232.

8.5 Health Canada. 2006. Appendix K In Volumes 1, 2 and 3. *Compendium of Analytical Methods*. Website: http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index_e.html.
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Table 1: Neutralizers of sanitisers for media

Media	Neutralizers	Compounds Neutralized	Ref.
Bacto D/E Neutralizing Agar (Dey and Engley Agar)	Sodium thyoglycollate Sodium thiosulphate Sodium bisulfite Lecithin (soybean) Tween 80	Quaternary ammonium, phenols, iodine, chlorine, mercurials (Merthiolate), formaldehyde, gluteraldehyde	APHA (8.3) Difco (8.4)
0.1% peptone water	For surfaces containing fatty materials		
0.5% Tergitol Anionic 7 0.5-1.0% Tween 80		Phenols	APHA (8.3)
Letheen Broth/Agar	Lecithin Tween 80	Quaternary Ammonium, phenols, formalin, hexachlorophene, ethanol	Difco (8.4)
Bacto Neutralizing Buffer	Monopotassium phosphate, Sodium thiosulfate, Aryl Sulfonate Complex	Chlorine and quaternary ammonium	Difco (8.4)
Bacto Microbial Content Test Agar	Lecithin Tween 80	Quaternary ammonium, phenols, formalin,	

hexachlorophene, ethanol
Difco (8.4)
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Table 2: Methods for Qualitative Analyses
Determination Receiving Analysis
Listeria monocytogenes Add LEB (or the appropriate broth) at 100 mL / unit (sponges) or 10mL/unit (swabs), and incubate at 30°C for 48 hours (or the temperature specified in the selected method). Proceed as per method chosen from the Compendium Appendix K
(8.5) *Listeria* species and *Listeria monocytogenes*.

Staphylococcus aureus Incubate for 3 hours at 35°C in 50 mL of double-strength TSB. Add 100 mL of a solution of TSB single strength containing 20% salt (sponges) or 10mL/unit (swabs). Incubate at 35°C for 24 hours. (8.3) Proceed as per method chosen from the Compendium Appendix K

Staphylococcus aureus
Salmonella Add Nutrient broth (or the appropriate broth) at 100 mL / unit (sponges) or 10mL/unit (swabs), and incubate at 35°C for 18-24 hours. Proceed as per method chosen from the Compendium Appendix K

Salmonella
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Table 3: Methods for Quantitative Analyses
Determination Petrifilm/Rodac Media Method (or equivalent)
Coliforms RODAC™
Petrifilm™
Petrifilm™
Violet Red Bile (VRB)
Coliform Count (CC)
plates
High Sensitivity
Coliform Plates
See manufacturer's
procedure
MFHPB-35
MFLP-85
Escherichia coli Petrifilm™ *E. coli* count (EC)
plates
MFHPB-34
Aerobic Colony Count RODAC™
Petrifilm™
Microbial Content Test

Agar (MCTA)
Aerobic Count (AC)
See manufacturer's procedure
MFHPB-33 Yeasts and Moulds RODAC™
Petrifilm™
DRBC Agar
Yeast and Mould (YM)
Count Plates
See manufacturer's
procedure
MFHPB-32
Staphylococcus
aureus
RODAC™
Petrifilm™
Baird Parker (BP)
Staph Express plates
See manufacturer's
procedure
MFLP-21
Other bacteria, as
needed
See this Compendium for
suitable methods
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APPENDIX 1

VERIFICATION OF INHIBITORY PROPERTIES

Published studies by Libras and Rose (8.1) have indicated that some sponges sold at retail outlets for environmental sampling may contain antibacterial agents. In order to prevent problems caused by inhibition from the use of sponges or similar material, each lot of bacterial carriers (sponge, J-cloth, etc.) should be tested for inhibitory properties using one of the two methods presented below. Before initiating the inhibitory test, bacterial carriers should be moistened with neutralizing buffer, put in a Nalgene jar (or equivalent) and autoclaved for 30 minutes.

PREPARATION OF CONTROL CULTURES

Keep cultures on TSA at 4°C. For each test, each culture must be transferred into test tubes containing 9 mL of TSB and incubated at 35°C overnight. Sample 0.1 mL from the first tube of TSB and transfer it into a second tube containing 4 mL of TSB. Incubate the second tube at 35°C overnight. Adjust the optic density of cultures at 0.5-0.6 at 600 nm wavelength. Adjust cell density by either diluting with sterile TSB or centrifuging and resuspending the cell pellet in a smaller volume of sterile broth.

METHODS

From Libras and Rose (8.1) The following two methods are suggested:

1. Solid Media

1) After the optical density of each bacterial culture is adjusted, a sterile swab is dipped into the culture. Drain excess liquid from swab by pressing the tip against the inside of the tube just above the level of the broth. The plate is swabbed in three directions to obtain a uniform lawn of growth. Three plates should be prepared for each bacterial strain.

2) Aseptically place two to three sponges, *ca*. a 1 cm square piece of the sponge, per plate for each bacterial strain. Incubate plates upright at 35°C for 20-24 h.

3) Roughly measure the inhibition zones, in millimetres, around the edges of the piece of sponge.

2. Liquid Media

1) Sterilize Neutralizing buffer at 121°C for 30 minutes and inoculate (MUST be cooled or you will kill the organisms) with appropriate bacterial strain in order to obtain approximately 20 cells per millilitre. The material analyzed should be completely submerged. The material tested (previously moistened and sterilized) is immersed into that suspension, pressed against the inside of the tube just above the level of the broth, draining the excess liquid in order to give a good impregnation. Follow the same procedure for each trial in peptone water without bacteria as a negative control.

2) After being immersed into the bacterial suspension, the bacterial carrier (sponge, Q-tips, J-cloth, etc.) is put into the appropriate nutrient broth i.e. enrichment broth for *Listeria* (LEB) to isolate *Listeria monocytogenes* or Nutrient Broth (NB) to isolate *S. aureus, E. coli* and *Salmonella*. Repeat this procedure three times in 3 different jars for each bacteria and each type of bacterial carrier tested. For each trial, a sample of 5 mL of inoculated peptone water is put into a nutritive media as a positive control.

3) LEB is incubated at 30°C and NB at 35°C for 24 hours.

4) In order to check for the growth of *L. monocytogenes*, LEB is inoculated onto PALCAM agar and Oxford agar. NB is streaked on Baird-Parker (BP) agar to check for the growth of *S. aureus* or on MacConkey (MC) agar to check for the growth of *E. coli* and *Salmonella*.

LAB 3: OBTAINING PURE CULTURES FROM A MIXED POPULATION

DISCUSSION

As stated in Lab 2, microorganisms exist in nature as mixed populations. However, to study microorganisms in the laboratory we must have them in the form of a pure culture, that is, one in which all organisms are descendants of the same organism.

Two major steps are involved in obtaining pure cultures from a mixed population:

1. First, the mixture must be diluted until the various **individual microorganisms become separated** far enough apart on an agar surface that after incubation they form visible **colonies isolated from the colonies of other microorganisms**. This plate is called an **isolation plate**.

2. Then, an isolated colony can be aseptically **"picked off"** the isolation plate (see Fig. 1) and **transferred** to new sterile medium (see Fig. 3). After incubation, all organisms in the new culture will be descendants of the same organism, that is, a **pure culture**.

Animation showing a portion of a single colony being "picked off."

A. STREAK PLATE METHOD OF ISOLATION

The most common way of separating bacterial cells on the agar surface to obtain isolated colonies is the streak plate method we used in Lab 2 to inoculate a petri plate. It provides a simple and rapid method of diluting the sample by mechanical means. As the loop is streaked across the agar surface, more and more bacteria are

rubbed off until individual separated organisms are deposited on the agar. After incubation, the area at the beginning of the streak pattern will show confluent growth, while the area near the end of the pattern should show discrete colonies (see Fig. 2).

B. THE POUR PLATE AND SPIN PLATE METHODS OF ISOLATION

Another method of separating bacteria is the pour plate method. With the **pour plate method**, the bacteria are mixed with melted agar until evenly distributed and separated throughout the liquid. The melted agar is then poured into an empty plate and allowed to solidify. After incubation, discrete bacterial colonies can then be found growing both on the agar and in the agar.

The **spin plate method** involves diluting the bacterial sample in tubes of sterile water, saline, or broth. Small samples of the diluted bacteria are then pipetted onto the surface of agar plates. A sterile, bent-glass rod is then used to spread the bacteria evenly over the entire agar surface (see Fig. 4) in order to see isolated colonies (see Fig. 5). In Lab 4 we will use this technique as part of the plate count method of enumerating bacteria.

C. USE OF SPECIALIZED MEDIA

To supplement mechanical techniques of isolation such as the streak plate method, many **special-purpose media** are available to the microbiologist to aid in the isolation and identification of specific microorganisms. These special purpose media fall into four groups: selective media, differential media, enrichment media, and combination selective and differential media.

1. Selective media

A selective medium has agents added which will **inhibit the growth of one group of organisms while permitting the growth of another**. For example, **Columbia CNA agar** has the antibiotics colistin and nalidixic acid added which inhibit the growth of gram-negative bacteria but not the growth of gram-positives. It is, therefore, said to be selective for gram-positive organisms, and would be useful in separating a mixture of gram-positive and gram-negative bacteria.

2. Differential media

A differential medium contains additives that **cause an observable color change in the medium when a particular chemical reaction occurs**. They are useful in differentiating bacteria according to some biochemical characteristic. In other words, **they indicate whether or not a certain organism can carry out a specific biochemical reaction** during its normal metabolism. Many such media will be used in future labs to aid in the identification of microorganisms.

3. Enrichment media

An enrichment medium contains additives that **enhance the growth of certain organisms**. This is useful when the organism you wish to culture is present in relatively small numbers compared to the other organisms growing in the mixture.

4. Combination selective and differential media

A combination selective and differential medium **permits the growth of one group of organisms while inhibiting the growth of another**. In addition, it differentiates those organisms that grow based on whether they can **carry out particular chemical reactions**. For example, Eosin Methylene Blue (EMB) agar is selective

for gram-negative bacteria. The dyes eosin Y and methylene blue found in the medium inhibit the growth of gram-positive bacteria but not the growth of gram-negatives. In addition, it is useful in differentiating the various gram-negative enteric bacilli belonging to the bacterial family Enterobacteriaceae (see Labs 12 & 13). The appearance of typical members of this bacterial family on EMB agar is as follows:

- Escherichia coli: large, blue-black colonies with a green metallic sheen
- Enterobacter and Klebsiella: large, mucoid, pink to purple colonies with no metallic sheen
- Salmonella and Shigella and Proteus: large, colorless colonies
- Shigella: colorless to pink colonies

The color changes in the colonies are a result of bacterial fermentation of the sugar lactose while colorless colonies indicate lactose non-fermenters. Fermentation reactions will be discussed in more detail in Lab 8.

There are literally hundreds of special-purpose media available to the microbiologist. Today we will combine both a mechanical isolation technique (the streak plate) with selective and selective-differential media to obtain pure cultures from a mixture of bacteria. In future labs, such as 12 - 16, which deal with the isolation and identification of pathogenic bacteria, we will use many additional special-purpose media.

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MEDIA

One plate of each of the following media: Trypticase Soy agar, Columbia CNA agar, and EMB agar.

ORGANISMS

A broth culture containing a mixture of one of the following gram-positive bacteria and one of the following gram-negative bacteria:

- Possible gram-positive bacteria:
  - Micrococcus luteus. A gram-positive coccus with a tetrad or a sarcina arrangement; produces circular, convex colonies with a yellow, water-insoluble pigment on Trypticase Soy agar.
    - Micrococcus luteus growing on TSA
    - Close up of Micrococcus luteus growing on TSA
  - Staphylococcus epidermidis. A gram-positive coccus with a staphylococcus arrangement; produces circular, convex, non-pigmented colonies on Trypticase Soy agar.
    - Staphylococcus epidermidis growing on TSA
    - Close up of Staphylococcus epidermidis growing on TSA
- Possible gram-negative bacteria:
  - Escherichia coli. A gram-negative bacillus; produces irregular, raised, non-pigmented colonies on Trypticase Soy agar.
    - Escherichia coli growing on TSA
  - Enterobacter aerogenes. A gram-negative bacillus; produces irregular raised, non-pigmented, possibly mucoid colonies on Trypticase Soy agar.

- Enterobacter aerogenes growing on TSA

During the next three labs you will attempt to obtain pure cultures of each organism in your mixture and determine which two bacteria you have. **Today** you will try to separate the bacteria in the mixture in order to obtain isolated colonies; **next lab** you will identify the two bacteria in your mixture and pick off single isolated colonies of each of the two bacteria in order to get a pure culture of each. The **following lab** you will prepare microscopy slides of each of the two pure cultures to determine if they are indeed pure.

PROCEDURE (to be done in pairs)

1. First attempt to obtain isolated colonies of the two organisms in your mixture by using mechanical methods on an all-purpose growth medium, Trypticase Soy agar. Streak the mixture on a plate of Trypticase Soy agar using one of the two streaking patterns illustrated in Lab 2, Fig. 4 and Fig. 5.

Animation showing how to streak an agar plate for isolation.

2. Streak the same mixture for isolation (see Fig. 4 and Fig. 5) on a plate of Columbia CNA agar (selective for gram-positive bacteria).

- Micrococcus luteus growing on Columbia CNA agar.
- Staphylococcus epidermidis growing on Columbia CNA agar.

3. Streak the same mixture for isolation (see Fig. 4 and Fig. 5) on a plate of EMB agar (selective for gram-negative bacteria and differential for certain members of the bacterial family Enterobacteriaceae).

- Escherichia coli growing on EMB agar.
- Enterobacter aerogenes growing on EMB agar.

4. Incubate the three plates **at 37°C** until the next lab period.

Trypticase Soy agar
Observations
Conclusions
Columbia CNA agar
Observations
Conclusions
EMB agar
Observations
Conclusions

Return to Menu for Lab 3

RESULTS

1. Observe isolated colonies on the plates of Trypticase Soy agar, Columbia CNA agar, and EMB agar. Record your observations and conclusions.

2. Using any of the three plates, **pick off a** single isolated colony **of each of the two organisms in your original mixture and aseptically transfer them to separate plates of Trypticase Soy agar** (see Fig. 3). When picking off single colonies, **remove the top portion of the colony without touching the agar surface itself** to avoid picking up any inhibited bacteria from the surface of the agar. Use your regular plate-streaking pattern to inoculate these plates and incubate **at 37°C** until the next lab period. These will be your pure cultures for Lab 5 (Direct and Indirect stains).

Animation showing a portion of a single colony being "picked off."

PERFORMANCE OBJECTIVES FOR LAB 3

After completing this lab, we will be able to complete the following objectives:

DISCUSSION

1. Given a mixture of a gram-positive and a gram-negative bacterium and plates of of Columbia CNA, EMB, and Trypticase Soy agar, describe the steps you would take to eventually obtain pure cultures of each organism.
2. Define: selective medium, differential medium, enrichment medium, and combination selective-differential medium.
3. State the usefulness of Columbia CNA agar and EMB agar.
4. Describe how each of the following would appear when grown on EMB agar:

    a.Escherichia coli

    b. Enterobacter aerogenes

    c. Salmonella

PROCEDURE

1. Using the streak plate method of isolation, obtain isolated colonies from a mixture of microorganisms.

2. Pick off isolated colonies of microorganisms growing on a streak plate and aseptically transfer them to sterile media to obtain pure cultures.

RESULTS

1. When given a plate of Columbia CNA agar or EMB agar showing discrete colonies, correctly interpret the results.

Dilution Theory – Page 2:

More Dilution Plating

John L's Bacteriology Pages >
Selected General Topics > Dilution Theory:

Dilution Theory per se:
• Page 1 – Dilution Plating
• Page 2 – More Dilution Plating
• Page 3 – The MPN Method

Supplementary Pages:
• Five-Tube MPN Table
• Practice Set 1 (Plating)
• Practice Set 2 (Plating&MPN)

A quick review of highlights from the previous page ("Dilution Theory–Page 1"):

- Inoculating plates from increasing dilutions (decreasing concentrations) of a sample is equivalent to plating successively smaller amounts of the sample.
- We treat the units **grams** and **milliliters** as equivalents. This is done **for convenience**. One ml of water does indeed weigh one gram and vice versa. In real life however, the same may not apply to other things – especially solid samples.
- We need to have "countable" plates – having preferably between 30 and 300 colonies.

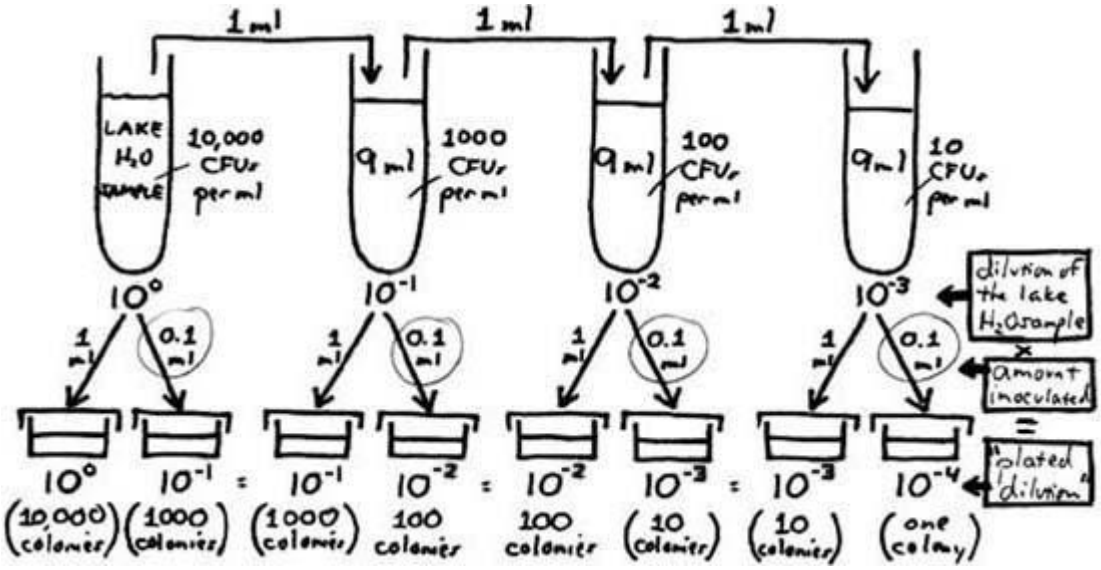
On this page:

- Inoculating other than 1.0 ml into/onto our plates.
- Using handy dilution formulas which introduce the terms "plated dilution" and "dilution factor." We note the fact that the **actual amount of original, undiluted sample plated** is the "**plated dilution**" and vice-versa.
- Checking your answer!

When we inoculate plates already containing medium (the usual case in our lab courses), we find that it would take too long for a one ml inoculum to soak into the medium. So, we generally plate **0.1 ml** from each dilution made. For each plate, you can readily see that we are then inoculating **one-tenth the number of CFUs** there would have been in a one ml inoculum.

In the following diagram, we have built on the last illustrated example given on Page 1 by adding inoculations of 0.1 ml from each of the dilutions into respective plates. (The numbers of

colonies in parentheses are either "too many" or "too few" for counting – remembering our "30-300 rule" above.)



Instead of the letters we labeled our plates with previously, we have labeled each plate with the **dilution** it represents – as if **one ml** had been inoculated from that dilution. **For example**, a plate inoculated with one ml of a 10^{-2} dilution would have the same label (10^{-2}) as a plate inoculated with 0.1 ml of a 10^{-1} dilution, as they are equivalent plates. This value (10^{-2}) has been traditionally called the "**plated dilution**." (A more fitting term we have come up with – and may officially substitute some day – is "**virtual dilution**"!)

Remembering our discussion on Page 1, you can see that the value of the "plated (virtual) dilution" is equivalent to the actual amount (in ml or g) of undiluted sample that is being plated out. For example, a plate labeled "10^{-2}" represents 10^{-2} ml or gram of sample being inoculated onto the plate.

A quick example problem: Suppose you inoculate a plate with 0.1 ml of a 10^{-1} dilution of a sample of milk. After incubation, you find that 80 colonies have arisen on the plate. How may CFUs were there per ml of the milk?

Solution: As plating **0.1 ml of a 10^{-1} dilution** is the equivalent of plating **1 ml of a 10^{-2} dilution** which is in turn equivalent to plating **10^{-2} ml of the original, undiluted sample** of milk, then you could say that there would have been 80 CFUs per 10^{-2} ml of the sample, and – proportionately – there would have been **10^2 times as many** CFUs (i.e., 8000 or 8.0 X 10^3) **per ml** of the undiluted milk sample.

Looking at the problem this way:
IF 80 colonies arise from plating 0.01 ml of the milk,
THEN there were 8.0 X 10^3 CFUs per one ml of the milk.

From the foregoing explanation and examples, one can figure out the concentration of CFUs (i.e., the number of CFUs per ml or gram of the sample) in **any dilution and plating problem** – by knowing **just three things** about our setup and results:

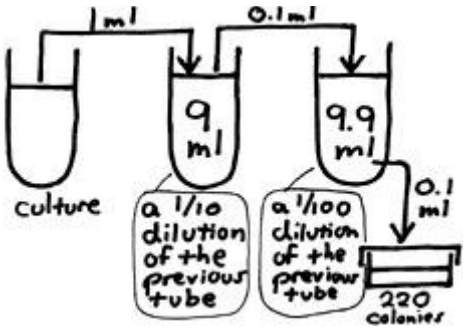
- The COLONY COUNT
- The AMOUNT INOCULATED into the plate that was counted
- The DILUTION OF THE SAMPLE from which the inoculation was made

Often it is handy to utilize **formulas** to work out dilution problems. In Bacteriology 10, we show continuously that the following set of formulas always work (if they are used properly). We could use one "universal" formula, but we have traditionally used these: the first (already used above) to find what portion of our sample is being analyzed (expressed as our so-called plated or virtual dilution) and the second to inflate our colony count **proportionately**, resulting in the number of CFUs that were in one gram or ml of the original, undiluted sample. (Don't just take our word for it. Spend a little time here and see how ultimately we can always come up with the number of CFUs **per one ml or one gram** of the undiluted sample.)

dilutions made	**X**	**amount inoculated**	**=**	**"plated dilution"**
dilution factor (simply the inverse of the plated dilution)	**X**	**# colonies**	**=**	**# CFUs/ml**(or gram) of the original undiluted sample

SOME EXAMPLES:

I. **The following is a sample problem from Bacteriology 102 worked out with the formulas:** One ml of a bacterial culture was pipetted into a 9 ml dilution blank. One-tenth ml of this dilution was pipetted into a 9.9 ml dilution blank. From this dilution, one-tenth ml was plated with 25 ml of culture medium. 220 colonies arose after incubation. How many colony-forming units were present per ml of the original culture? (Does the amount of medium in the plate matter in the calculations?)



dilutions made	**X**	**amount inoculated**	**=**	**"plated dilution"**
1/10 X 1/100	**X**	1/10	=	1/10,000 or 10^{-4}
dilution factor	**X**	**# colonies**	**=**	**# CFUs/ml**
10^4	**X**	220	=	2.2 X 10^6

You can also look at the problem this way: If 220 colonies arose from plating (the equivalent of) 10^{-4} ml of the culture, then (proportionally) there would have been 220 X 10^4 or 2.2 X 10^6 CFUs **per one ml** of the culture. **This is the reasoning behind the second of the two dilution formulas.**

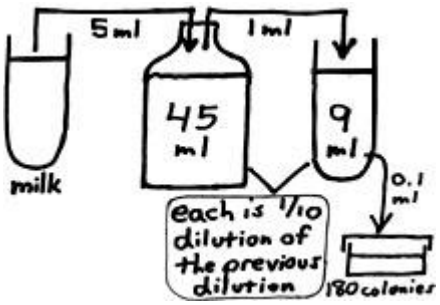
II.

When <u>checking your answer</u>, which you can always do as follows for such problems: Start with the sample which you have determined to contain 2.2 X 10^6 CFUs per ml. Then, see if you wind up with the stated number of colonies on the plate, after making the specified dilutions in which the number of CFUs per ml is sequentially reduced.

- The first, 1/10 dilution contains 2.2 X 10^5 CFUs **per ml**.
- The second, 1/100 dilution contains 2.2 X 10^3 CFUs **per ml**.
- As **0.1 ml** of the second dilution was inoculated into the plate, we would expect the number of CFUs in the inoculum to then be 2.2 X 10^2 which is 220, the number of colonies we counted on the plate.

III. **Here is a problem where we start out with something other than 1 ml or 1 gram of sample being diluted:** Five ml of milk were pipetted into 45 ml of diluent. One ml of this dilution was pipetted into 9 ml of diluent. From this dilution, 0.1 ml was plated. After incubation, 180 colonies were counted. Determine the number of colony-forming units per ml of the original milk sample.

A 1/10 dilution is achieved when 5 ml of sample are added to 45 ml of diluent. Remember that a **1/10 dilution** can be made in a variety of ways – as long as there is **one part of sample added to 9 parts of diluent**. Even if we had a dilution we could not so reduce – e.g., something like 3 grams of hamburger added to 80 ml of diluent which would result in a 3/83 dilution – we can still simply "plug it into" the formula and we could wind up with the answer. And remember that the formulas will always give the answer as no. of CFUs per one ml (or one gram) no matter what amount we start with. Wouldn't this problem have the same answer if we had put 1 ml of milk into 9 ml of diluent?



dilutions made	**X**	**amount inoculated**	=	**"plated dilution"**
1/10 X 1/10	**X**	1/10	=	1/1000 or 10^{-3}

dilution factor	**X**	**# colonies**	=	**# CFUs/ml**
10^{3}	**X**	180	=	1.8 X 10^{5}

As for the above problem, you can look at this one as follows: If 180 colonies arose from plating (the equivalent of) 10^{-3} ml of the milk, then (proportionally) there would have been 180 X 10^{3} or 1.8 X 10^{5} CFUs **per one ml** of the original, undiluted milk sample.

IV. **For a problem presented on the previous page (as no. III) in which <u>no dilutions</u> were made, we can still work it out with the formulas:** Five ml of an undiluted spring water sample were added to a petri dish to which 15 ml of melted Plate Count Agar were then added. Fifty colonies were counted after incubation. How many CFUs were present per ml of the original, undiluted spring water sample?

dilutions made	**X**	**amount inoculated**	=	**"plated dilution"**
1	**X**	5	=	5

dilution factor	**X**	**# colonies**	=	**# CFUs/ml**
1/5	**X**	50	=	10

V. Note that when there are no dilutions, we indicate "1" – not zero! – for the dilutions made. As always, the "dilution factor" is the <u>inverse</u> of the so-called "plated dilution" (according to how we defined our terms), and the "plated dilution" always represents the amount of sample being plated.

Dilution Theory / Supplementary Pages:

A Five-Tube MPN Table

John L's Bacteriology Pages >
Selected General Topics > Dilution Theory:

Dilution Theory per se:
• Page 1 – Dilution Plating
• Page 2 – More Dilution Plating
• Page 3 – The MPN Method

Supplementary Pages:
• Five-Tube MPN Table
• Practice Set 1 (Plating)
• Practice Set 2 (Plating&MPN)

At the right is a 5-tube MPN table, taken from the **Standard Methods for the Examination of Water and Wastewater**, 15th edition (1980) and adapted for use to determine the most probable number of positive organisms per inoculum of the middle set of tubes. The same rules apply as previously stated in that the actual amount of sample inoculum decreases ten-fold with each succeeding set of tubes – which can be accomplished by (for example) making one ml inoculations from decimally-increasing dilutions as in the following example.

As an example problem: Suppose 5 tubes of an all-purpose medium are each inoculated with **1 ml** of a 10^{-2} dilution of a water sample, and 5 more are likewise inoculated from a 10^{-3} dilution as are 5 more from a 10^{-4} dilution. If the results show 5 positives for the first set of tubes, 3 for the second and 1 for the last, the 5-3-1 combination matches with the MPN value of 1.1 which, according to the table, means that there would be (on the average) approximately 1.1 positive organisms per ml of the 10^{-3} dilution. Therefore, the number **per ml** of the original, undiluted water sample would be 1.1 X 10^{3}. Remember that such a value is really a rough estimate.

No. of Tubes Positive in			MPN in the inoculum of the middle set of tubes
first set	middle set	last set	
0	0	0	‹0.01
0	0	1	0.02
0	1	0	0.02
0	2	0	0.04
1	0	0	0.02
1	0	1	0.04
1	1	0	0.04
1	1	1	0.06
1	2	0	0.06
2	0	0	0.05
2	0	1	0.07
2	1	0	0.07
2	1	1	0.09
2	2	0	0.09
2	3	0	0.12
3	0	0	0.08

Lower and upper 95% confidence limits listed for each combination of positive results in Standard Methods can be different by ten-fold or more.

Return to the Main MPN Page.

Page last modified on 5/19/01 at 4:00 PM, CDT.
John Lindquist: new homepage, complete site outline.
Department of Bacteriology, U.W.-Madison

3	0	1	0.11
3	1	0	0.11
3	1	1	0.14
3	2	0	0.14
3	2	1	0.17
4	0	0	0.13
4	0	1	0.17
4	1	0	0.17
4	1	1	0.21
4	1	2	0.26
4	2	0	0.22
4	2	1	0.26
4	3	0	0.27
4	3	1	0.33
4	4	0	0.34
5	0	0	0.23
5	0	1	0.31
5	0	2	0.43
5	1	0	0.33
5	1	1	0.46
5	1	2	0.63
5	2	0	0.49
5	2	1	0.7
5	2	2	0.94
5	3	0	0.79

5	3	1	1.1
5	3	2	1.4
5	3	3	1.8
5	4	0	1.3
5	4	1	1.7
5	4	2	2.2
5	4	3	2.8
5	4	4	3.5
5	5	0	2.4
5	5	1	3.5
5	5	2	5.4
5	5	3	9.2
5	5	4	16
5	5	5	›24

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This document is also available in pdf format:

Ch7_1.3.pdf

7.1.3
IDENTIFICATION AND ENUMERATION METHODS

The membrane filtration (MF) and most probable number (MPN) methods are used for the presumptive identification, confirmation, and enumeration of indicator bacteria. For general use, the MF method is preferable to the MPN method. The MPN method is preferred if toxic substances are present in the sample or if, after filtration, a residue heavy enough to block the micropores of the membrane filter is visible. The MPN method is described in Standard Methods for the Examination of Water and Wastewater, 18th edition (American Public Health Association and others, 1992, p. 9-45 to 9-53) and in Britton and Greeson (1989). Procedures for analyzing water samples by use of MF methods are described below.

Indicator bacteria for presumptive identification and enumeration are cultured on selective media after filtration of several different sample volumes onto gridded membrane filters. Detailed confirmation, identification, and enumeration of these bacteria require additional culturing and biochemical testing, the details of which are beyond the scope of this manual. However, additional confirmation procedures are needed under certain circumstances, such as use of the data in support of environmental regulation and enforcement.

The fecal indicator bacteria are operationally defined by the method employed for identification and enumeration, as follows:

- The total coliform bacteria are defined as the organisms that produce red colonies with a golden-green metallic sheen within 24 ± 2 hours when incubated at 35.0 ± 0.5°C on m-Endo medium.
- The fecal coliform bacteria are defined as the organisms that produce blue colonies in whole or part within 24 ± 2 hours when incubated at 44.5 ± 0.2°C on m-FC medium.
- E. coli are defined as the organisms that produce yellow or yellow-brown colonies that remain so when placed on a filter pad saturated with urea substrate broth for 15 minutes after rescusitation at 35.0 ± 0.5°C for 2 hours and incubation for 22 to 24

hours at 44.5 ± 0.2°C on m-TEC medium.

- E. coli are defined as the organisms that produce a blue fluorescent margin around a darker colony center within 4 hours when incubated at 35 ± 0.5°C on NA-MUG medium after primary culturing as total coliform bacteria on m-Endo medium.
- The fecal streptococci are defined as the organisms that produce red or pink colonies within 48 ± 2 hours when incubated at 35.0 ± 0.5°C on KF medium.
- Enterococci are defined as the organisms that produce pink to red colonies with a black or reddish-brown precipitate after primary culture for 48 to 50 hours at 41.0 ± 0.5°C on m-E medium followed by incubation for 20 minutes at 41.0°C on EIA medium.

7.1.3.A
PREPARATION OF MEDIA AND REAGENTS

MF analysis requires the use of several types of media and reagents, the types being dependent on the indicator. The necessary media and reagents include sterile buffered water, agar- or broth-based selective and differential growth media, and media and reagents for additional biochemical identification.

Sterile buffered water (buffer) is used to dilute samples and to rinse the membrane-filtration apparatus and utensils. **Purchase sterile buffered water from the Quality of Water Service Unit (QWSU).** It is provided in 250-mL bottles and in 99-mL dilution bottles. There are two types: phosphate buffer to be used for total and fecal coliform, and fecal streptococci tests; and saline buffer to be used for E. coli and enterococci tests. Buffer exceeding the expiration date should not be used. When sterile buffered water is not obtained from the QWSU, it can be prepared ahead of time and sterilized by autoclaving. Preparation instructions for sterile buffered water are described in Britton and Greeson (1989, p. 18) and Standard Methods for the Examination of Water and Wastewater (American Public Health Association and others, 1992, p. 9-17).

Culture media for enumeration of fecal indicator bacteria should be purchased in kits from the QWSU. The QWSU provides instructions for media preparation with each kit. Otherwise, dehydrated media can be purchased from scientific suppliers. Guidelines for storage of media and reagents are as follows:

- Store media kit (supplied by QWSU) and dehydrated, commercially prepared media in a desiccator. Store other reagents in a dust-free laboratory cabinet (not in a field vehicle).
- Label all media with the date received, date opened, and preparer's initials. Discard media and reagents with an expired shelf life.
- Refrigerate reagents when necessary. Use buffered dilution water immediately after opening; discard any remainder. Storing an opened bottle is not recommended.
- Mark all plates to identify the media type, the preparation date, and the preparer.
- Store prepared petri dishes upside down in a plastic bag before use and refrigerate.

7.1.3.B
PREPARATION, HOLDING TIMES, AND SPECIFICATIONS FOR CULTURE MEDIA

The preparation of selective and differential culture media for indicator bacteria is an important part of analysis. Adhering to proper preparation, storage, and holding-time requirements will help ensure the quality of the analysis. Instructions for the preparation of 100 mL of primary culture media for five MF tests and additional confirmation media or broth for three MF confirmation tests are described in section 7.1.5, entitled "Instructions for Media Preparation."

Quality control. Supplies of dehydrated media purchased from the QWSU or through catalogs have been quality-control tested. Media prepared fresh by the analyst must also be quality-control tested. If sterile buffered water is prepared in the laboratory, quality-control procedures must be used to ensure it will provide a suitable medium for transfer of bacteria from samples to filters. **Sterile buffered water should be tested for sterility by use of blanks of 100 mL, processed along with each set of samples.** Quality-control procedures applicable to microbiological testing

can be found in the 18th edition of "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association and others, 1992, p. 9-7 to 9-13).

7.1.3.C
MEMBRANE FILTRATION PROCEDURE

After collecting the sample and selecting the appropriate sample volumes, label the petri dishes with the station number (or other identifiers), the volume of sample filtered, date, and time. Select those sample volumes that are anticipated to yield one or two plates in the ideal colony count range. General information on the concentrations of fecal indicator bacteria in surface water and contaminated surface water is given in table 7.1-1.

A suitable work area inside the field vehicle and out of direct sunlight and wind is best.

- Before and after processing the samples, clean countertops in field vehicles with an antibacterial cleaning solution; for example, a 7-percent phenolic solution, 50 to 70 percent isopropyl or ethyl alcohol; 5 percent bleach; or a 7-percent ammonia solution.
- Preheat incubators for at least 2 hours before beginning analysis, according to specifications for each test (table 7.1-5). Portable heater-block incubators must not be left on in closed, unventilated vehicles when the outside temperature is less than 15°C or greater than 37°C.

  Technical Note: Review past analyses for the site to help determine the number of sample volumes to be filtered. Where past analyses of samples from a site have shown a small variation in the number of fecal indicator bacteria, the filtration of as few as three or four different sample volumes may suffice. However, where past analyses have shown the variation to be large or where the variation is not known, the filtration of five or more different sample volumes is recommended.

Table 7.1–5. Incubation times and temperatures for fecal indicator tests

[m-Endo, total coliform media; ±, plus or minus; °C, degrees Celsius; NA-MUG, *E. coli* confirmation media (nutrient agar-4-methylumbelliferyl-β-D-glucuronide; m-FC, fecal coliform media; m-TEC, *E. coli* media; KF, fecal streptococcus media; m-E, enterococcus media; EIA, enterococcus confirmation media]

Test (media)	Incubation time and temperature
Total coliform bacteria (m-Endo)	24 ± 2 hours at 35.0 ± 0.5°C
Escherichia coli (NA-MUG)	4 hours at 35± 0.5°C after primary culture on m-Endo media
Fecal coliform bacteria (m-FC)	24 ± 2 hours at 44.5 ± 0.2°C
Escherichia coli (on urea substrate broth after primary culture on m-TEC media)	First resuscitate for 2 hours at 35.0 ± 0.5°C, and then incubate for 22 to 24 hours at 44.5 ± 0.2°C After 22 to 24 hours, transfer filter to urea substrate broth for 15 to 20 minutes before counting
Fecal streptococci (KF media)	48 ± 2 hours when incubated at 35.0 ± 0.5°C
Enterococci (m-E and EIA)	48 to 50 hours at 41.0 ± 0.5°C on m-E medium. Transfer filter to EIA medium for 20 minutes at 41.0°C before counting

The steps required for membrane filtration are depicted in figure 7.1-2 (pages 28 and 29) and listed below. Quality-control samples must be collected as part of the filtration procedure (see Technical Note, step 16).

Steps to follow when filtering samples and making colony counts are listed below (and summarized in fig. 7.1-2):

1. Select sample volumes (table 7.1-6) to result in at least one filter having colonies in the ideal counting range. The ideal range and number of sample volumes to filter depend on the test and the expected bacterial concentrations. Record on the petri dish and on the record sheet the site name, date, time of sample collection, and sample volume. Record the time of sample processing on the record sheet. Also label equipment and procedure blanks and other quality-control samples.

2. Assemble filtration equipment by inserting the base of the filter-holder assembly into a flask. Vacuum is supplied by use of a hand-held pump, vacuum, or battery-operated peristaltic pump. If flame sterilization was used, rinse the inside of the filtration apparatus with sterile buffered water to remove any residue of formaldehyde.

3. Sterilize stainless steel forceps by immersing tips in a small bottle or flask containing 70 or 90 percent ethanol; then pass forceps through the open flame of an alcohol burner. Allow alcohol to burn out and allow the forceps to cool for several seconds to prevent heat damage to the membrane filter. Resterilize forceps before each use. Return cooled forceps to alcohol container between transfers. **Do not set forceps on the countertop.**

Table 7.1–6. Recommended sample volumes for membrane filtration analyses based on ideal colony count and concentration range

[<, less than; col/100 mL; colonies per 100 milliliters; mL, milliliters]

Ranges of observed fecal indicator concentrations					
<1 to 60,000 col/100 mL		<1 to 80,000 col/100 mL		<1 to 200,000 col/100 mL	
Ideal counting ranges for number of colonies per membrane filter					
20–60 colonies		20–80 colonies		20–100 colonies	
Sample volume (mL)[1]	Added as (mL)[2]	Sample volume (mL)[1]	Added as (mL)[2]	Sample volume (mL)[1]	Added as (mL)[2]
100	100	100	100	100	100
30	30	25	25	20	20
10	10	6.0	6.0	5.0	5.0
3.0	3.0	1.5	1.5	1.0	1.0
1.0	1.0	0.4	4.0 of 1:10 dilution	0.25	2.5 of 1:10 dilution
0.3	3.0 of 1:10 dilution	0.1	10 of 1:100 dilution	0.05	5.0 of 1:100 dilution
0.1	10 of 1:100 dilution				

[1]All sample volumes less than 1.0 mL require dilution in a 99-mL bottle.

[2]Sample volumes smaller than those indicated may be needed when bacterial concentrations are greater than those listed.

4. Remove the sterilized funnel from the filtration apparatus. Always hold the funnel in one hand while placing or removing the membrane filter. (Placing the funnel on anything but the filtration apparatus might result in contamination of the funnel.)
    - Using sterile forceps, place a sterile, gridded membrane filter (47-mm diameter) on top of the filter base, grid-side up. Be sure to use the correct pore-size membrane filter for the test procedure (table 7.1-7).
    - Carefully replace and secure the filter funnel on filter base. Avoid tearing or creasing the membrane filter.

- Rinse funnel with 100 mL of sterile buffered water before filtering sample volumes to obtain a filtration assembly equipment blank (filter blank).
- Filter sample in order of smallest to largest sample volume.

5. If the sample volume is less than 1.0 mL, prepare dilutions with sterile buffered water in a 99-mL dilution bottle and transfer appropriate volume of dilution to the membrane filter (fig. 7.1-3 and table 7.1-8).

   a.
   When preparing dilutions, use a sterile pipet to measure each sample volume.
   b.
   After each sample-volume transfer, close and shake the dilution bottle vigorously at least 25 times.
   c.
   Filter diluted samples within 20 minutes after preparation. Keep dilution bottles out of sunlight and do not transfer dilute sample volumes with pipets used to transfer concentrated volumes.

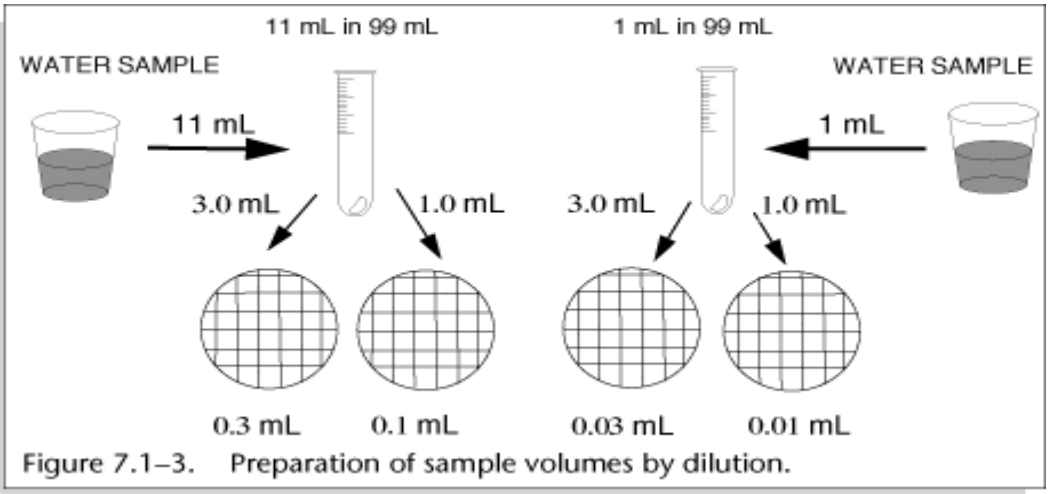


Figure 7.1–3. Preparation of sample volumes by dilution.

6. Shake the sample vigorously at least 25 times before each sample volume is withdrawn in order to break up particles and ensure an even distribution of indicator bacteria in the sample container. Proceeding from smallest to largest sample volume, deliver the sample volume to the membrane filter by use of a pipettor or pipet bulb with a valve for volume control.
    - Allow the pipet to drain, and touch the tip to the inside of the funnel to remove remaining sample. Pipets of the TD (to deliver) type will have a small amount of liquid left in the tip after dispensing the liquid.
    - **If the volume of sample to be filtered is 10 mL or more--**transfer the sample with a sterile pipet or graduated cylinder directly into the funnel.
    - **If the volume of sample to be filtered is between 1.0 and 10.0 mL**--pour about 20 mL of sterile buffered water into the funnel before pipetting the sample to facilitate distribution of bacteria on the membrane filter. Refer to table 7.1-6 for appropriate sample volumes for each test.

Table 7.1–8. Preparation guidelines for dilution of samples to volumes less than 1.0 milliliter for fecal indicator bacteria analysis

[mL, milliliter]

Dilution factor	Volume (mL) of sample added to 99 mL sterile dilution water	To obtain this dilution, filter this volume
1:10	11.0 mL of original sample	1.0 mL of 1:10 = 0.1 mL 3.0 mL of 1:10 = 0.3 mL
1:100	1.0 mL of original sample	1.0 mL of 1:100 = 0.01 mL 3.0 mL of 1:100 = 0.03 mL
1:1,000	1.0 mL of 1:10 dilution	1.0 mL of 1:1,000 = 0.001 mL 3.0 mL of 1:1,000 = 0.003 mL

7. Apply vacuum with a hand, peristaltic, or vacuum pump. To avoid damage to bacteria, do not exceed a pressure of about 5 lb/in^2 (25 cm of mercury).
8. Rinse inside of funnel twice with 20 to 30 mL of sterile buffered water while applying vacuum. If a graduated cylinder was used, rinse the cylinder with sterile buffered water and deliver rinse water to the filtration apparatus.

9. Remove the funnel and hold it in one hand. Do not set the funnel on the countertop. Remove the membrane filter with sterile forceps. Release the vacuum. Releasing the vacuum after removing the filter prevents backflow of sample water onto the filter. Unnecessarily wet filters promote confluent growth of colonies and poor results. Replace funnel on filter base.
10. Open petri dish and place membrane filter grid side up on medium by use of a rolling action, starting at one edge. Avoid trapping air bubbles under the membrane filter. If air is trapped, use sterile forceps to remove the membrane filter and roll it onto the medium again. **Do not expose prepared plates to direct sunlight.**

Do not pipet by mouth.

11. Close petri dish by pressing top firmly onto bottom. Invert the petri dish. To avoid growth of interfering microorganisms, incubate within 20 minutes.
12. Continue to filter the other sample volumes in order, from smallest to largest volume. Record on the field forms the volumes filtered and time of processing.
13. After filtrations are complete, place a sterile, gridded-membrane filter on the funnel base and rinse the funnel with 100 mL of sterile buffered water to obtain a procedure blank.
14. After the sample volumes and blanks have been filtered, place the inverted petri dishes in a preheated aluminum heater-block or water-bath incubator. Incubate at the prescribed times and temperatures (table 7.1-5). Wash, then flame sterilize or autoclave filtration apparatus. Wash countertop between each sample and wash hands with bacteriocidal soap.
15. After incubation, remove the petri dishes from the incubator. Count and record on the field forms, for each sample volume filtered, the number of typical colonies (table 7.1-9). Recount until results agree within 5 percent. Recounting is done by turning the plate 90 degrees to obtain a slightly different angle. Count by use of a preset plan (a side-to-side pattern along grid lines is suggested) (fig. 7.1-4). **Make the counts with the aid of 5 to 15 magnifications and a fluorescent illuminator placed as directly above the filter as possible.**

- For total coliform colonies, enhance sheen production by removing filters from media and placing them on absorbent pads to dry for at least 1 minute before counting.
- If the optional NA-MUG test is done for E. coli, transfer the total coliform filter onto NA-MUG plates and incubate for 4 hours at 35°C. Afterward, count under a long-wave ultraviolet light in a completely darkened room (U.S. Environmental Protection Agency, 1991b).
- For E. coli and enterococci, additional biochemical tests are required by use of confirmation media. For E. coli, transfer the filter to a filter pad saturated with urea-phenol reagent; count only yellow colonies after 15 to 20 minutes at room temperature (U.S. Environmental Protection Agency, 1985).
- For enterococci, transfer the filter to EIA media after incubation for 20 minutes at 41°C; count colonies from the underside of the plate placed over a fluorescent illuminator.

16. Check quality-control blanks for colony growth, and report results on the field forms.
    - The presence of colonies on blanks indicates that results of the bacterial analyses bracketed by positive blanks are suspect and should not be reported.
    - It is not valid to subtract colony counts on blanks from results calculated for samples.

TECHNICAL NOTE: It is necessary to collect equipment, filter, and procedure blanks. The equipment and filter blanks measure the effectiveness of sterilization. One or more colonies on this type of blank indicates inadequate sterilization of either the equipment or the buffered water. The procedure blank measures the effectiveness of the analyst's rinsing technique. One or more colonies on the procedure blank indicates either inadequate rinsing or contamination of equipment or buffered water during sample processing.

Table 7.1–9. Test (media type), ideal colony count, and typical colony color, size, and morphology for indicator bacteria colonies

[m-Endo, total coliform media; mm, millimeters; NA-MUG, nutrient agar-4-methylumbelliferyl-β-D-glucuronide; m-FC, fecal coliform media; m-TEC, *E. coli*. media; KF, fecal streptococcus media; m-E, enterococcus media; EIA; enterococcus confirmation media]

Test (media type)	Ideal count range (colonies per filter)	Typical colony color, size, and morphology
Total coliform bacteria (m-Endo)	20–80	Colonies are round, raised, and smooth; 1 to 4 mm in diameter, and red with a golden-green metallic sheen.
Escherichia coli After primary culture as total coliform colonies on m-Endo (NA-MUG)	None given but much fewer in number than total coliforms on same filter	Colonies are cultured on m-Endo media as total coliform colonies. After incubation on NA-MUG, colonies have blue fluorescent margins with a dark center. Count under a long-wave ultraviolet lamp in a completely darkened room.
Fecal coliform bacteria (m-FC)	20–60	Colonies are round, raised, and smooth with even to lobate margins; 1 to 6 mm in diameter, and light to dark blue in whole or part. Some may have brown or cream colored centers.
Escherichia coli (m-TEC)	20–80	Colonies are round, raised, and smooth; 1 to 4 mm in diameter, yellow to yellow brown; may have darker raised centers.
Fecal streptococci (KF media)	20–100	Colonies are small, raised, and spherical; about 0.5 to 3 mm in diameter; glossy pink or red in color.
Enterococci (m-E and EIA)	20–60	Colonies are round, smooth, and raised; 1 to 6 mm in diameter; pink to red with a black or red-dish-brown precipitate on underside.

17.

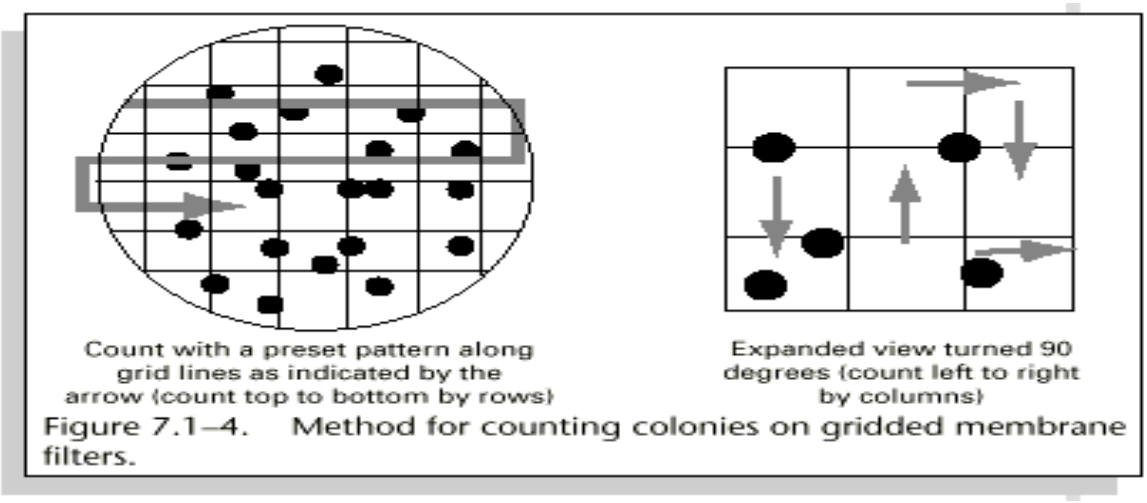


Figure 7.1–4. Method for counting colonies on gridded membrane filters.

18. Calculate the number of colonies per 100 mL of sample as shown in section 7.1.4, "Calculation and Reporting of Fecal Indicator Bacteria."
19. Put all plates to be discarded in an autoclavable bag. Freeze or chill the plates to be discarded until they can be autoclaved in the laboratory. Autoclave all cultures at 121°C for a minimum of 30 minutes before discarding.

Quality control. In addition to blanks, collect and analyze samples in duplicate at a minimum frequency of 5 percent (1 in every 20 samples). Periodically purchase and analyze a pure culture containing Escherichia coli or Enterococcus faecalis (formerly Streptococcus faecalis to ensure that the test procedure is acceptable.

PROCEDURE

1. Preheat incubator, prepare work areas.
2. Select sample volumes. If needed, prepare dilutions for filtration of sample volumes less than 1.0 mL. (Tables 7.1–6 and 7.1–8; and figure 7.1–3.)
3. Label petri dishes.
4. Assemble, and if not sterile, sterilize filtration apparatus.

PROCEDURE

5. Place sterile filter on filtration apparatus using sterile forceps.

TECHNICAL NOTE: a small hand pump is preferred over a syringe as a vacuum source.

PROCEDURE

6. Shake sample 25 times and deliver to filtration apparatus by use of graduated cylinder or pipet. Add 20 mL sterile buffered water to filtration apparatus before filtering sample volumes less than 10 mL.

Figure 7.1–2. Steps in membrane-filtration procedure (taken from Millipore, 1973, and published with permission).

PROCEDURE

7. Apply vacuum, and afterwards, rinse filtration apparatus and cylinder twice with sterile buffered water.

PROCEDURE

8. Sterilize forceps and remove filter. Replace funnel on filtration apparatus.

PROCEDURE

9. Roll filter onto media in petri dish. Place inverted petri dish in incubator.

PROCEDURE

10. Repeat steps 4–9 for each sample volume in order of the smallest to the largest volume. A filter blank is processed before each sample. Filter a procedure blank after every 20 samples or once per day or at each site, according to study objective. Filter a duplicate sample after every 20 samples or at each site, according to study objective. Use a hand pump instead of a syringe.

Figure 7.1–2—Continued. Steps in membrane-filtration procedure (taken from Millipore, 1973, and published with permission).

Streak Method for Agar Plates

The streak plate is used primarily for isolating microorganisms in pure culture from specimens or samples containing mixed flora. Obtaining isolated colonies on plates allows colonial morphology and hemolytic reactions to be examined, and biochemical / serological testing to be performed.

1. With a sterile inoculating loop, streak a loopful of the sample across the surface of an agar plate. The four-quadrant streak is the most common, and accomplished by streaking and rotating the plate in four sections, one quarter at time, slightly overlapping the original streak area. The fourth quadrant contains the greatest dilution of microorganisms, and usually provides isolated colonies for further testing.

2. Incubate plates under favorable growth conditions.

3. Examine plates for isolated colonies.

Spread Plate Technique

The spread plate technique is used for enumerating microorganisms.

1. Drop 0.1 mL aliquots from serial dilutions onto the surface of an agar plate.

2. Aseptically spread inoculum across the surface using a bent glass rod or sterile inoculating loop. By spreading the suspension over the plate, a dilution gradient is established to provide isolated colonies.

3. Incubate plates agar inverted in appropriate conditions.

4. Count colonies and calculate the number of microorganisms in the original suspension.

Pour Plate Technique

The pour plate technique is also used for enumeration of microorganisms in a particular sample. In this technique, test samples or suspensions of microorganisms are mixed with molten agar (45-50°C). The agar is allowed to solidify, trapping the bacteria at separate discrete positions within the matrix of the medium. While the medium holds bacteria in place, it is soft enough to permit growth of bacteria and the formation of discrete isolated colonies.

1. Perform serial dilution of sample.

2. Aseptically pipette microorganism dilutions into labeled petri dishes.

3. Add melted agar that has been cooled to approximately 44–45°C.

4. Mix well by slightly rotating plate with bacteria and agar mixture.

5. Allow the agar to solidify, trapping bacteria at separate discrete positions within the medium.

6. Incubate plates in a favorable environment.

7. Count the number of colonies and calculate the number of microorganisms in the original sample.

Streak / Stab Method for Agar Tubes

Tubed media may be in the form of solid agar slants, semisolids, or broths. Depending on the type of medium used and the purpose of the inoculation, use an inoculating loop or needle.

1. For agar slants, place the loop at the base of the tube surface and draw it up the agar surface while moving it from side to side.

2. For semisolid media, insert the loop into the medium to approximately one-fourth of its depth. If testing motility, use an inoculating needle and stab it in the center of the agar tube to the bottom. Draw the needle out carefully, keeping it straight.

Media Inoculation

Inoculation of Broth Media

Broth media are generally used as enrichments, general cultivation and sterility testing.

1. Aseptically inoculate appropriate broth media with the sample or specimen using sterile pipette, syringes or forceps.

2. Incubate inoculated broth at the appropriate atmospheric conditions, temperature, and time.

3. Examine broth for any signs of growth including, turbidity with or without gas bubbles, “puff-ball” appearance, hemolysis (in blood cultures), pellicle formation and precipitate on the bottom of the tube or bottle.

Membrane Filtration Method

The membrane filtration method is used to test large volume of liquid samples, including water and filterable beverages.

1.Pass the sample through a sterile membrane filter enclosed in a filtration assembly and attached to a vacuum source.

2.After filtering the sample, carefully remove the filter with sterile forceps and apply it to the surface of an agar plate or pad saturated with a broth medium. Avoid trapping air bubbles by using a rolling action. (The media used depends on the type of microorganism being tested.)

3.Invert plates and incubate under appropriate conditions.

4.Count colonies and calculate the most probable number.

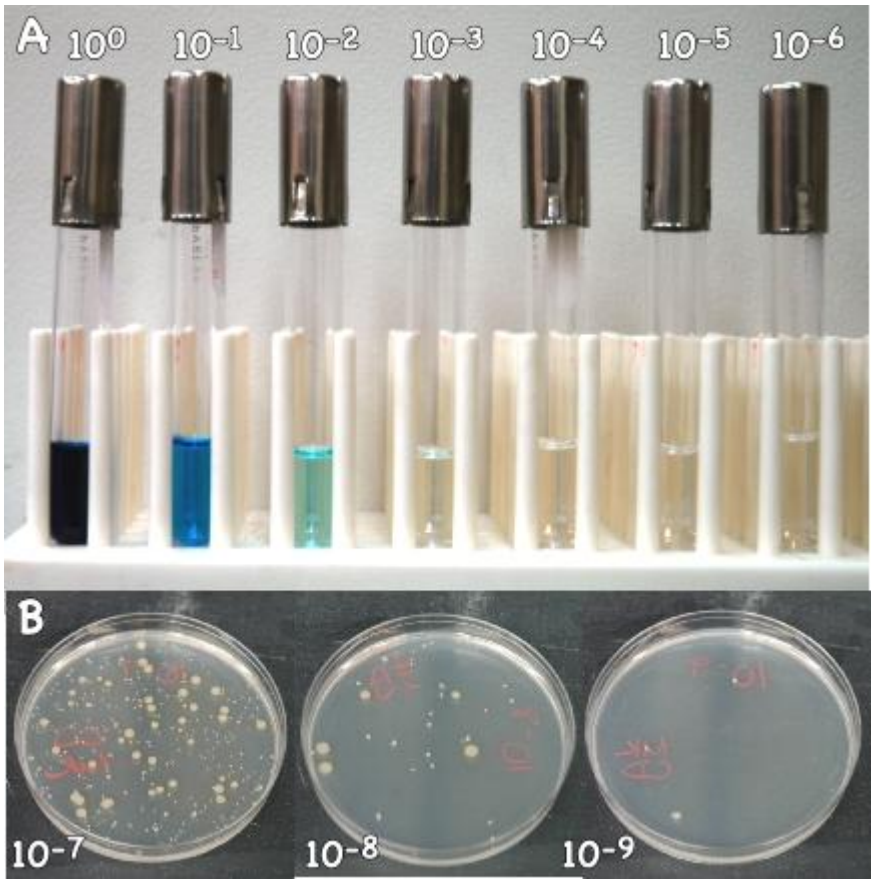
Viable plate counts

One of the most common methods of determining cell number is the viable plate count. A sample to be counted is diluted in a solution that will not harm the microbe, yet does not support its growth (so they do not grow during the analysis). In most cases a volume of liquid (or a portion of solid) from the sample is first diluted 10-fold into buffer and mixed thoroughly. In most cases, a 0.1-1.0 ml portion of this first dilution is then diluted a further 10-fold, giving a total dilution of 100-fold. This process is repeated until a concentration that is estimated to be about 1000 cells per ml is reached. In the spread-plate technique some of the highest dilutions (lowest bacterial density) are then taken and spread with a sterile glass rod onto a solid medium that will support the growth of the microbe. It is important that the liquid spread onto the plate soaks into the agar. This prevents left over liquid on the surface from causing colonies to run together and the need for dry

plates restricts the volume to 0.1 ml or less. A second method for counting viable bacteria is the pour plate technique, which consists of mixing a portion of the dilution with molten agar and pouring the mixture into a petri plate. In either case, sample dilution is high enough that individual cells are deposited on the agar and these give rise to colonies. By counting each colony, the total number of colony forming units (CFUs) on the plate is determined. By multiplying this count by the total dilution of the solution, it is possible to find the total number of CFUs in the original sample.

Figure 4-1 Dilution plating and viable plate counts

Nutrition
- Classification
- Metabolism
- Regulation of Metabolism
- Genomics and Genetics
- Archaeal Diversity
- Bacterial Diversity
- Animal Immunity
- Treatment and Prevention
- Bacterial Pathogens
- Viral Pathogens
- Host-Microbe Interactions
- Central Dogma
- Comments



(A) A demonstration of a decimal series of dilutions. The 10^0 sample is a concentrated solution of methylene blue. A 0.2 ml portion of this was added to 1.8 ml (1:9 ratio) of 0.85% saline to create the 1:10 dilution. After mixing, 0.2 ml of the 10^{-1} dilution was added to a second tube containing 1.8 ml to create the 10^{-2} dilution. This was continued to generate the dilution series.
(B) A series of pour plates demonstrating the appearance of a viable plate count. The 3 plates show a 10^{-7}, 10^{-8}, and 10^{-9} dilution of a natural sample. Note how the number of colony forming units decreases 10 fold between the plates.

One major disadvantage of the viable plate count is the assumption that each colony arises from one cell. In species where cells grow together in clusters, a gross underestimation of the true population results. One example of this are species of Staphylococcus, which is known to form clumps of microorganisms in solution. Each clump is therefore counted as one colony. This problem is why the term CFUs per ml is used instead ofâ bacteria per mlâ for the results of such an analysis. It is a constant reminder that one colony does not equal one cell. Great care must also be taking during dilution and plating to avoid errors. Even one error in dilution can have large effects on the final numbers. The rate at which bacteria give rise to an observable colony can also vary. If

too short an incubation time is used, some colonies may be missed. The temperature of incubation and medium conditions must also be optimized to achieve the largest colonies possible so that they are easily counted. Finally, this technique takes time. Depending on the organism, one day to several weeks might be necessary to determine the number of CFUs that were present when the experiment started. Such information may no longer be useful for many experiments.

Despite its shortcomings, the viable plate count is a popular method for determining cell number. The technique is sensitive and has the advantage of only counting living bacteria, which is often the important issue. Any concentration of microorganism can be easily counted, if the appropriate dilution is plated. It is even possible to concentrate a solution before counting, as is often done in water analysis, where bacterial populations are usually at low density. The equipment necessary for performing viable plate counts is readily available in any microbiology lab and is cheap in comparison to other methods. Finally, by using a selective medium it is possible to determine the number of bacteria of a certain class, even in mixed populations. These advantages have made viable plate counts a favorite of food, medical, aquatic and research laboratories for the routine determination of cell number.

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Microbiology and Bacteriology

The world of microbes

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2-4 Streak plates

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The streak plate method is a rapid and simple technique of mechanically diluting a relatively large concentration of microorganisms to a small, scattered population of cells. The goal is to obtain isolated colonies on a large part of the agar surface, so that desired species can then be brought into pure culture. Proper streaking of plates is an indispensable tool in microbiology. In most cases a closed inoculating loop is used for streaking plates. The wire loop should not be badly oxidized or pitted or it will fail to dilute the inoculum and will scratch the surface of the agar. Streak plates can be made from a broth culture, an agar slant or from an agar plate. It is sometimes convenient to suspend a bit of growth from a solid surface in sterile saline and use this as a source of inoculum. Resuspension of colonies or cultures grown on solid surfaces dilutes the culture and makes streak plating easier. A loopful of inoculum is transferred from the source and put on the agar surface. When using a large inoculum (a turbid culture or growth from a solid surface), a small spot is spread during the initial transfer. If the inoculum is from a lightly turbid suspension, the first phase of the streaking pattern is begun. Several basic patterns are illustrated in Figure 2-3. The three-phase streaking pattern is recommended for beginners because it is most likely to give satisfactory results with suspensions having a wide range of microbial density.

Books

The little Book module manual

Virtual Microbiology

Through the microscope: A look at all things small

Textbook Table of Contents

- Introduction & History
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- Bacterial Growth
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- Animal Immunity
- Treatment and Prevention
- Bacterial Pathogens
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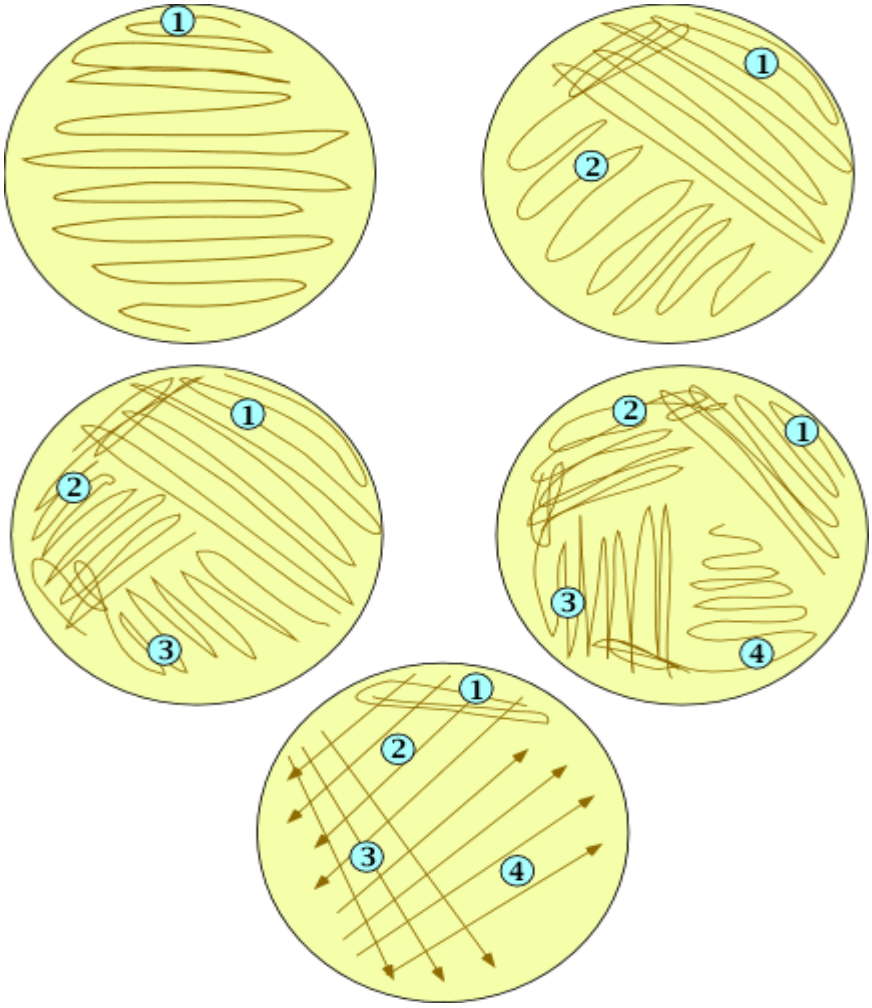
- Host-Microbe

Interactions
- Central Dogma
- Comments

Figure 2-3 Streaking patterns



There are a number of different methods for mechanically diluting microbes on a streak plate. The most common method is spreading microbes across a plate as shown in the first four figures. As the concentration of microbes increases so do the number of phases. Irrespective of the number of phases, loop is flamed between each one. The fifth plate shows an alternative method, where the streaks are not continuous, but are a series of parallel lines. foobar

Choosing a streaking pattern is a matter of individual preference and depends upon the number of microorganisms in the sample. Figure 2-3 demonstrates the most common patterns, but they

are not the only methods. The object of any streaking pattern is the continuous dilution of the inoculum to give many well isolated colonies. For multi-phase streaking it is crucial to flame the loop before starting the next phase. Note the slight overlap into the previous phase to pick up a small inoculum. To streak a plate...

1. Flame the loop to sterilize it and let cool.
2. Position the plate so that the spot of inoculum is nearest the hand not holding the loop (the opposite hand).
3. Lift the plate lid with the opposite hand; just enough to get the loop inside and touch the loop to the inoculum spot. It is often helpful to treat the inoculating loop as if it were a pencil - steadying the loop by resting the heel of the hand against the lab bench.
4. Move the loop back and forth across the spot and then gradually continue toward the center of the plate as you sweep back and forth. Use a very gentle and even pressure.
5. When creating each phase, do not worry about keeping each pass across the plate separate from previous ones.
6. When about 30% of the plate has been covered by the first streaking phase, remove the loop and flame sterilize it.
7. Repeat the above procedure for the second phase, but this time pick up some inoculum by crossing into the first phase 2-3 times and then not passing into it again (Figure 2-3).
8. Repeat as necessary for the third and fourth phases. After streaking the plate, flame sterilize the loop before setting it down.

Figure 2-4 demonstrates the technique of streak plates in a movie.

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Basic Pure Culture Techniques

1- Aseptic technique

Trying to study this **mixed population** is often difficult and in the tradition of the scientific method; researchers dissect a system and study each piece in isolation. For microorganisms this means separating the organisms and getting them into pure culture. **A pure culture** is defined as a growth of microorganisms (a culture) that contains one cell type. It is essential in microbiology to be able to obtain and preserve pure cultures. Over 100 years ago, **Robert Koch** devised methods to achieve this goal and the methods he developed are essentially still used today. The protocols used to maintain pure cultures are a major part of aseptic technique.

The goals of aseptic technique are two-fold. **The first objective is** to obtain pure cultures and secondly to prevent cross-contamination. Microorganisms in culture must not escape into the environment, and microbes in the environment must not get into the cultures we are studying. It is essential that aseptic technique be understood and practiced correctly. Contaminated cultures are worthless for diagnosis or for doing research on, because it is unclear what microbe is performing any action that is being observed.

Aseptic methods commonly used are flame sterilization, tube transfer, streak plates, spread plates and pour plates. Flame sterilization is an easy method to insure sterile transfer of a culture from a source to a growth medium. Tube transfer is useful for moving inocula from one tube to another. Mechanical dilution by making streak plates is the preferred method for obtaining a pure culture of a microorganism. Finally, spread plates and pour plates are common methods for enumerating microorganisms and are sometimes useful for obtaining isolated **colonies** .

2 - Flame sterilization and tube transfer

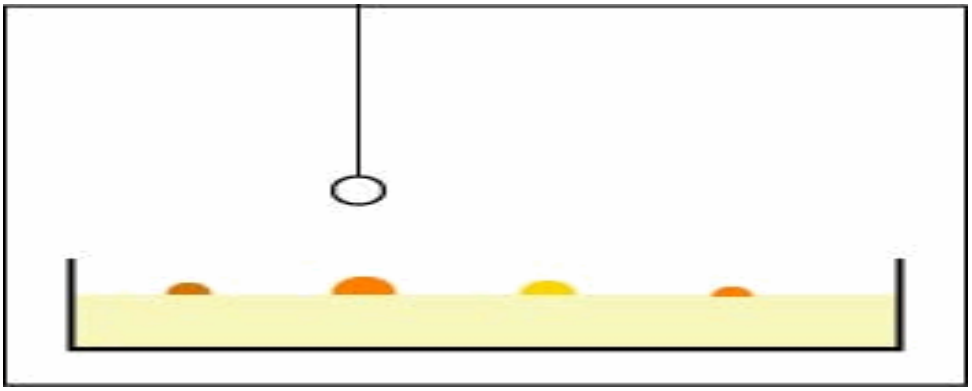
Flame sterilization is a very quick simple method of killing microorganisms on an inoculating loop or needle. The loop or needle is held **inside a flame for a few seconds** to bring it to redness and then **cooled**. Once cool, the loop or needle can be used for various culture manipulations. **Make sure that the area that contacts the culture is flamed to redness. Also, be patient and**

let the loop cool down, this usually takes about 15-30 seconds. Learning this technique is essential to everything else you do in microbiology.

Transfer of culture from agar plates to tubes, or from tube to tube, is a common, simple procedure. It is important to perform these transfers in a consistent and rapid manner. The following protocols have been found effective.

To transfer a culture from an agar plate to a broth or agar slant:

1. Place the Bunsen burner in front of you and assemble all necessary equipment with in arms reach. Position everything so that you will not burn yourself while trying to inoculate your tubes.
2. Label the tube of broth or agar to be inoculated with identifying marks. The culture, the date, and your initials for example. Place it in a rack in front of you.
3. Holding the inoculating loop handle, flame the entire wire to redness.
4. When the wire cools (about 15-30 seconds) remove the lid of the plate with your other hand and obtain an inoculum by removing a small portion of the surface growth on the agar plate. In most cases you will be picking an isolated colony. Choose a well isolated one. Do not dig into the agar. Replace the lid of the plate immediately.



5. Hold the tube to be inoculated with the free hand. Remove the cotton plug or cap of the tube with the little finger of the hand holding the needle holder. If a cotton-plugged tube is used, the mouth of the tube should be passed briefly through the flame to singe off dust and lint particles. (Dust or lint may fall into the tube and contaminate the medium.)
6. Introduce the inoculum into the tube, and streak gently the surface of the agar medium in the tube.
7. When inoculating a tube of broth, rub the wire against the glass just above the fluid level and then tip the tube slightly to wash the inoculum into the broth. The wire should not be rattled against the sides of the tube to shake an inoculum into the broth; this is unnecessary and may create a dangerous aerosol.
8. Replace the cap or plug (the latter after reflaming the mouth of the tube).
9. Flame the inoculating wire again to redness, slowly to avoid spattering. Put the loop holder down after the wire cools.

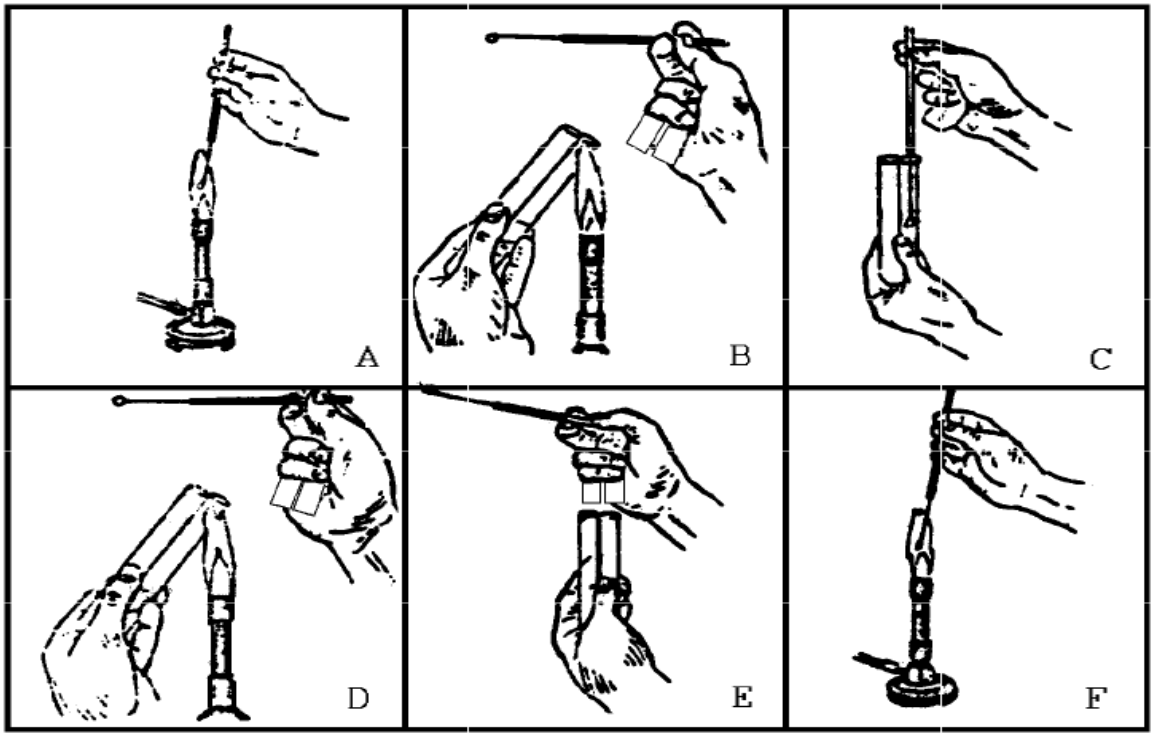
The standard method for transferring microbes from one medium to another. Each of the steps is described in the text.

In tube to tube transfers by loop or straight wire, both the tube containing the inoculum source and the tube to be inoculated are usually in the hand at the same time.

The tubes are positioned in the hand as shown in the Fig B-E. Plugs and caps can be loosened by twisting them.

1. The needle holder is taken in the other hand and the wire flamed and allowed to cool (Fig. A).
2. The plugs or caps are removed with the last two fingers of the hand holding the inoculating wire leaving the thumb and index finger free to hold and to manipulate the loop holder with the second finger as a guide and support. Flame the tops of the tubes.
3. Immerse the inoculating wire into the broth culture or scrape the wire across a portion of surface growth on an agar slant to obtain inoculum. Make the transfer from one tube to the other (Fig. C).
4. Flame the tubes (Fig. D).
5. Return the plugs or caps to the tubes (Fig. E).
6. Flame the inoculating wire to sterilize it (Fig. F).

An easy procedure which prevents hand fatigue and the danger of dropping the tubes is illustrated in the following Figure:



3. Making a medium - You try it

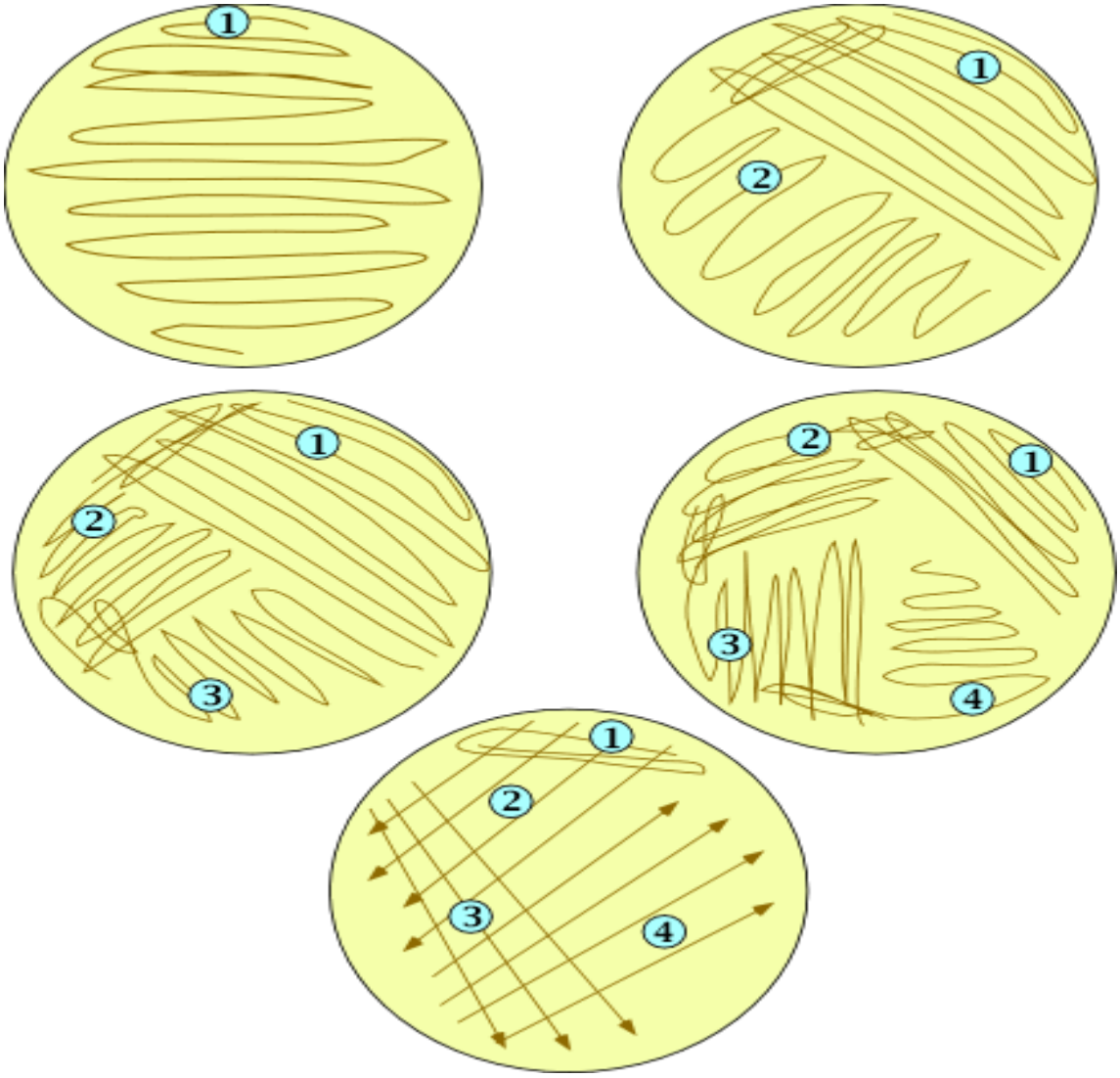
Making medium is as simple as cooking and a crude medium can be made in almost any kitchen with a few utensils and a source of heat. Below is described the production of a chicken broth **medium** that will grow many common microorganisms. Simply boiling a medium once, may kill most vegetative cells, but does not kill endospores. However, autoclaving is carried out usually (some time autoclaving is not necessary as mentied in preparation procedure) to ensure that all spores have been killed.

1. Add 250 ml (or the desired quantity needed) of reagent grade water into a glass container or some other vessel that can stand boiling water. The container should be something you can cover. Glass bottles **that can stand boiling** or canning jars work well.
2. To this water add, the defined amount of the stock broth medium, and stir until dissolved. Cover loosely so that steam can escape, but dust and dirt cannot enter.
3. Heat in already heated water bath until the media just begins to boil, adjust the pH if it is necessary and according to the directions from the manufacturer.

4. Autoclave the broth medium under pressure, temperature for the time defined by he manufacturer.
5. Place the medium in a warm place overnight to insure sterilization (no turbidity).

4- Streak plates

The streak plate method is a rapid and simple technique of mechanically diluting a relatively large concentration of microorganisms to a small, scattered population of cells. The goal is to obtain isolated colonies on a large part of the agar surface, so that desired species can then be brought into pure culture. Proper streaking of plates is an indispensable tool in microbiology. In most cases a closed inoculating loop is used for streaking plates. The wire loop should not be badly oxidized or pitted or it will fail to dilute the inoculum and will scratch the surface of the agar. Streak plates can be made from a broth culture, an agar slant or from an agar plate. It is sometimes convenient to suspend a bit of growth from a solid surface in sterile saline and use this as a source of inoculum. Resuspension of colonies or cultures grown on solid surfaces dilutes the culture and makes streak plating easier. A loopful of inoculum is transferred from the source and put on the agar surface. When using a large inoculum (a turbid culture or growth from a solid surface), a small spot is spread during the initial transfer. If the inoculum is from a lightly turbid suspension, the first phase of the streaking pattern is begun. Several basic patterns are illustrated in **the** following figure. The three-phase streaking pattern is recommended for beginners because it is most likely to give satisfactory results with suspensions having a wide range of microbial density.



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Choosing a streaking pattern is a matter of individual preference and depends upon the number of microorganisms in the sample. Last Figure demonstrates the most common patterns, but they are

not the only methods. The object of any streaking pattern is the continuous dilution of the inoculum to give many well isolated colonies. For multi-phase streaking it is crucial to flame the loop before starting the next phase. Note the slight overlap into the previous phase to pick up a small inoculum. To streak a plate:

1. Flame the loop to sterilize it and let cool.
2. Position the plate so that the spot of inoculum is nearest the hand not holding the loop (the opposite hand).
3. Lift the plate lid with the opposite hand; just enough to get the loop inside and touch the loop to the inoculum spot. It is often helpful to treat the inoculating loop as if it were a pencil - steadying the loop by resting the heel of the hand against the lab bench.
4. Move the loop back and forth across the spot and then gradually continue toward the center of the plate as you sweep back and forth. Use a very gentle and even pressure.
5. When creating each phase, do not worry about keeping each pass across the plate separate from previous ones.
6. When about 30% of the plate has been covered by the first streaking phase, remove the loop and flame sterilize it.
7. Repeat the above procedure for the second phase, but this time pick up some inoculum by crossing into the first phase 2-3 times and then not passing into it again.
8. Repeat as necessary for the third and fourth phases. After streaking the plate, flame sterilize the loop before setting it down.

5- Spread plates and dilution plating

An absolute requirement for a microbiologist is to be able to determine the concentration of microorganisms in a given sample. Various particle-counting devices, spectrophotometric methods and microscopic techniques have been used to count cells. However, one drawback to these methods is that they count dead as well as living cells. The most common method of enumerating viable cells is the plate-count method. Diluting microorganisms and placing them into **petri plates** (or plates) for incubation is another essential technique for working with microorganisms.This method suffers from some problems. First, only those organisms which can grow on the medium, and at the temperature and atmospheric conditions of incubation, will divide

and develop into colonies. Second, each colony may not represent the progeny from one cell, as two or more cells (those in clusters, chains or otherwise close to one another) can give rise to one colony. For these reasons the counts obtained from the plate-count method are given as the number of colony-forming units (CFU's) per ml (or gram) rather than the number of cells per ml (or gram). Despite these drawbacks, the plate-count method is a powerful means by which concentrations of viable organisms may be estimated. Also, if it is desirable to count a specific subgroup of microorganisms in a sample, selective media or special incubation conditions can often be used to encourage the growth of only this class of organism. As microbial quantitation involves the use of **pipettes** (or **micropipettes**) in preparing dilutions and inoculating plates, the beginning microbiologist must become familiar with their use.

Due to the possibility of ingesting pathogens and toxic liquids, mouth-pipetting is forbidden in the laboratory! Pipettes are filled and subsequently emptied by the use of propipettes or other pipette bulb. Pay close attention to the demonstration of their use.

Important Safety Consideration: When fitting the pipette and pipette bulb together, use very gentle pressure!! Do not jam these items together! (Force is usually not the answer, a good general rule to live by in this lab and in life for that matter.) The glass pipette will probably break and possibly cause severe injury. Handle the pipette only at the top inch or so.

For volumes of 5 ml of less, micropipettes are often the tool of choice. Instruments are available that are capable of dispensing 5 ml all the way to less than 1 µl (one one-millionth of a liter). Micropipettes have made it possible to miniaturize many experiments and greatly decrease the cost of running them. They are also easy to use and can dispense volumes quickly, increasing the number of experiments that can be performed in a set amount of time. Micropipettes are the tool of choice for small volumes.

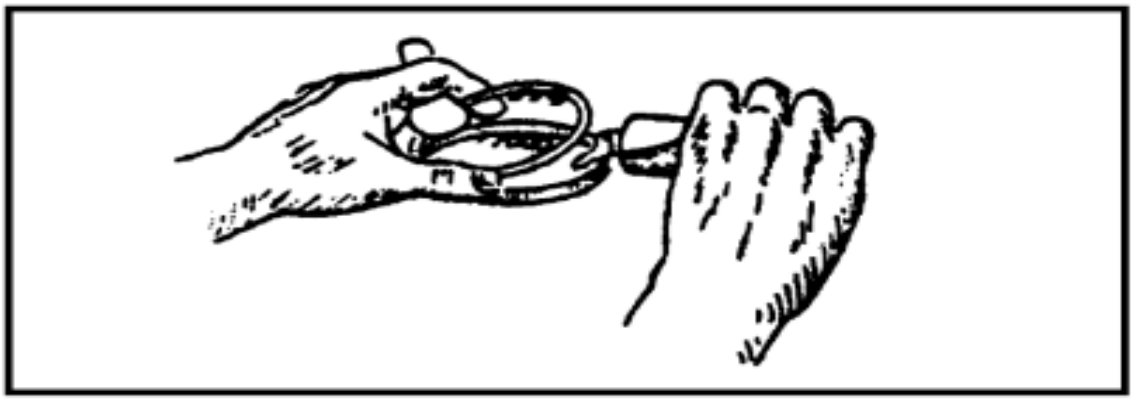
---

2 - 6 Pour Plates

A practical and common laboratory technique used in isolating pure cultures or enumerating the living microorganisms in water, milk, foods, and other materials is the pour plate technique.

To aseptically transfer liquid into a pour plate, raise one side of a Petri plate lid only just enough to allow access of the sample (from a tube or pipette). Transfer a known amount of the sample to the dish and cover immediately with the lid. Then pour 15-20 ml of sterile agar culture medium which has been melted and cooled to 45-50°C into the plate as shown in **Figure 2-5** [13]. The inoculum and medium are mixed by gentle rotation ten times in one direction and ten times in the other direction. The agar must be allowed to solidify completely before the plates are inverted for incubation. After incubation both surface and subsurface colonies will be observed.

Figure 2-5 Pour plates



Pour plates allow the addition of larger amounts of liquid (1-5 ml) to an agar dish. The sample is added to the bottom of a sterile Petri plate. Molten agar is then added to the plate aseptically. It is important to only open the cover enough to allow the pouring of the agar. This prevent contamination from the environment.

Figure 2-6 [14] is a movie demonstrating the pour plate technique.

Links

[1] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayarticle&art_id=26

[2] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayglossary#colonies

[3] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=1&chap_number=2

[4] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=2&chap_number=2

[5] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayglossary#tyndalization

[6] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=3&chap_number=2

[7] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=3&chap_number=2

[8] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=4&chap_number=2
[9] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayglossary#petri%20plates
[10] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayarticle&art_id=101
[11] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayglossary#pipettes
[12] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayglossary#micropipettes
[13] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=5&chap_number=2
[14] http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=3&fig_number=6&chap_number=2

Microbiology and Bacteriology

The world of microbes

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4-6 Direct particle counts

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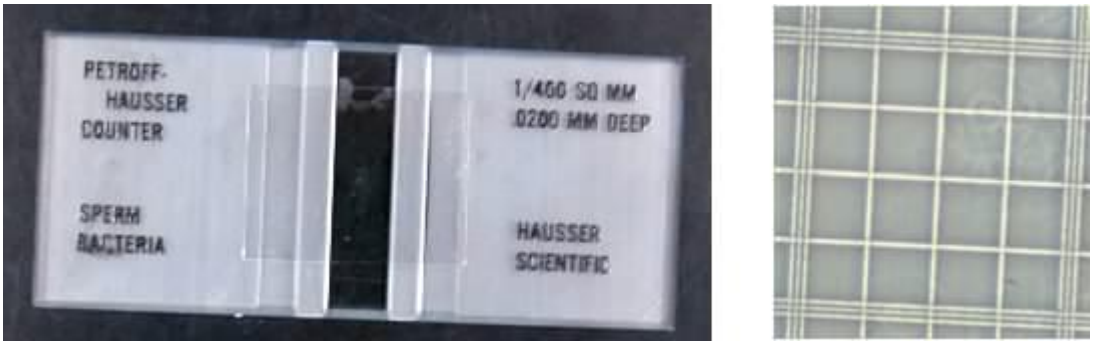
Counting chambers

The most direct method of counting microorganism is by the use of a microscope and a slide with special chambers of known volume. These slides allow the counting of a small number of cells in a small volume and extrapolating the result to determine the population. An example of such a device is shown in Figure 4-10. A culture is placed on the slide marked with precise grids. The number of cells present in each grid is counted and an average determined. Conversion using a formula gives the number of cells per milliliter in the culture. This method is rapid, a result can be known in just a few minutes, and is easy to perform. However, it is impossible to distinguish living cells from dead ones. If this distinction is important, direct microscopic counts are not the solution. Finally, cultures containing less than 1 million cells per ml are actually too dilute for direct counts since there will be too few cells in the very small volume that is actually examined under the microscope for an accurate count

Figure 4-10 The Petroff-Hauser counting chamber



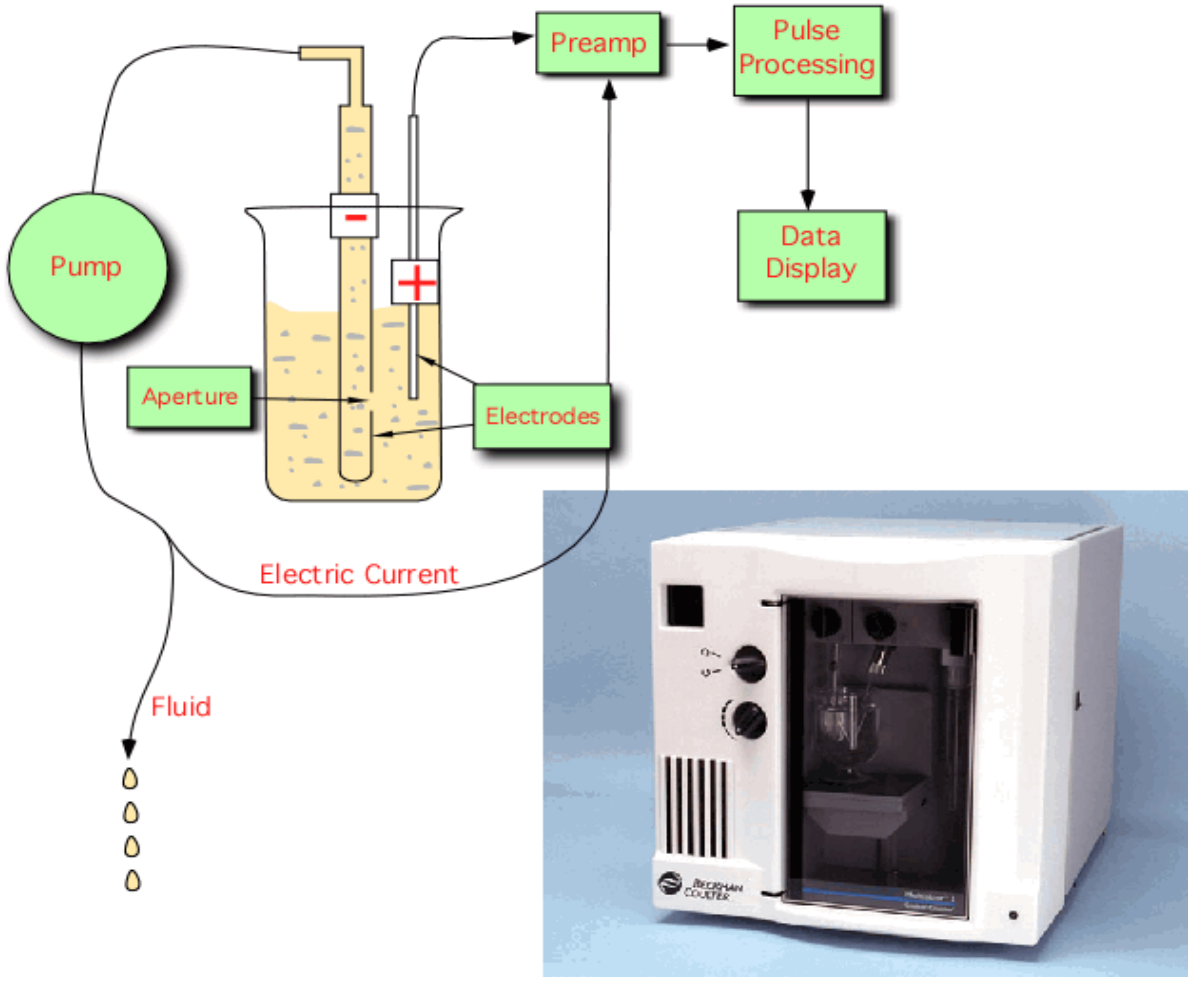
The center of the slide contains a precisely machined grid, with each square (1/20 mm x 1/20 mm) having a known area. The coverslip also rests above the slide a known distance (typcially 1/50 of a mm). Since each of these dimenstions is known, it is possible to calculate the number of cells in each square and pluggin it into a formula. On the left of the figure is a photograph of a Petroff-Hauser slide. On the right is a grid at 100 x magnification showing the size of the squares.

a picture of the petroff-hauser cell counter and then what it looks like on a slide.

Electronic particle counters

Electronic particle counters are useful if the number of bacteria in a sample needs to be counted on a routine basis. The method is based on the property that nonconductive particles, such as bacteria, will cause a disruption in an electric field as they pass through it. A Coulter counter is a type of electronic particle counter in which there is a small opening between electrodes through which suspended particles pass, see Figure 4-11. In this sensing zone, each particle displaces its own volume of electrolyte, causing a current pulse. The pulse is noted and recorded as one particle count. By precisely controlling the rate at which solution passes through the opening, it is possible to get exact, reproducible counts at a rate of up to several thousand bacteria per second. Coulter counters are highly dependent upon particle size and those dependent upon changes in current are near their detectable limits with microorganisms. Particle counters that use light diffraction as a means of sizing and counting particles are also manufactured and can detect particle less than 1 µm in diameter.

Figure 4-11 The coulter counter



A picture of a Coulter Counter. The diagram at the right demonstrates the opening that the microbes must pass through during counting. When microbes pass through the aperture the electrical potential across the electrodes is distrubes, which the dataprocessing system records as a count. In the lower right is an actual picture of a Multisizer™ 3 by Beckmann instruments that uses the Coulter counter priciple.

The advantage of this method is the simplicity of its operation and it reproducibility. As in microscopic counts, the machine cannot distinguish between living or dead cells or even between dust and bacteria. Any reasonably sized particle in the solution will be counted. There is also the expense of buying the counter, which can cost many thousands of dollars.

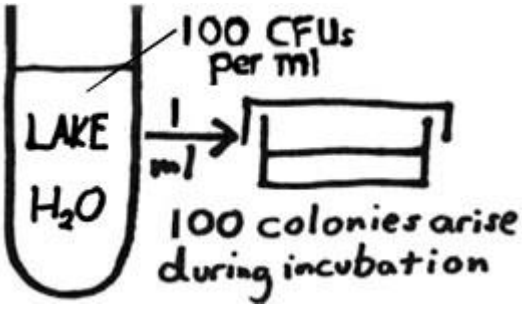
Dilution Theory – Page 1:

Dilution Plating

John L's Bacteriology Pages >
Selected General Topics > Dilution Theory:

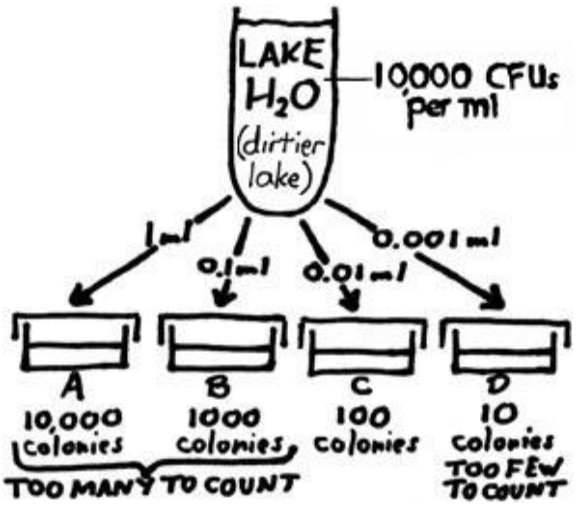
Dilution Theory per se:
• Page 1 – Dilution Plating
• Page 2 – More Dilution Plating
• Page 3 – The MPN Method

Supplementary Pages:
• Five-Tube MPN Table
• Practice Set 1 (Plating)
• Practice Set 2 (Plating&MPN)



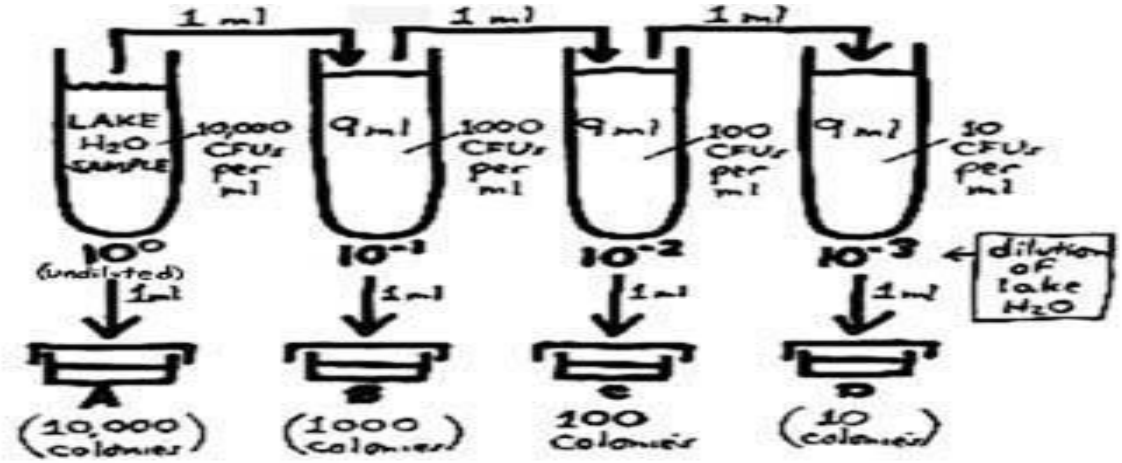
In **quantitative microbiology**, we are concerned with determining the **concentration of colony-forming units (CFUs) in our sample** – i.e., the number of CFUs per ml or per gram of the sample. For example, if we were to plate out **one ml** of a lake water sample and then – after incubation of the plates – find that **100 colonies** have arisen, we would then conclude that there were **100 CFUs per ml** of the lake water.

More realistically (as with most of our area lakes), the concentration of CFUs in the water could have been considerably greater. Counting the colonies on a plate inoculated with one ml of water may be impossible. **We would like to have "countable" plates – containing between 30 and 300 colonies.** If fewer than 30, we run into greater statistical inaccuracy. If greater than 300, the colonies would be tedious to count and also would tend to run together.



We may try to plate out smaller and smaller amounts, as in the example shown at right. With this new lake water sample, a countable plate (Plate C: 100 colonies) is achieved with the inoculation of 0.01 ml of sample. Figuring out the number of CFUs per ml of the sample would go like this: Whatever the **number of CFUs in the inoculum** of Plate C (**one-hundreth of a ml** of the sample), there would be **one hundred times as many CFUs in one ml** of the sample. So, if 100 CFUs are determined to be in the 0.01 ml inoculum, then 100 X 100 CFUs would be present in one ml; the final answer is **10,000 CFUs/ml** of the sample.

Two drawbacks to this procedure: It is difficult with our equipment to dispense amounts smaller than 0.1 ml. Also, the smaller the amount tested, the less representative it is of the sample.



So we now get into "dilution theory" to accomplish the equivalent of plating out succeedingly smaller amounts of sample. Making serial decimal dilutions (i.e., successive **1/10 dilutions**, each made by **adding one part of inoculum to 9 parts of diluent**) and inoculating one ml into each of the plates, we can construct a plating procedure (shown at right) that is **equivalent to the above**.

Illustrating how the concentration of CFUs decreases according to how these cell suspensions are diluted: We determined above that the **sample contains 10,000 CFUs per ml**. Taking out one ml and inoculating it into a 9 ml dilution blank (the second tube) would put the **10,000 CFUs into a total of 10 ml** which is equivalent to **1000 CFUs per ml of the 1/10 dilution of the sample**. The density of CFUs continues to decrease ten-fold with each subsequent dilution.

Counting 100 colonies in Plate C, note how we can work back to a concentration of 10,000 CFUs per ml of the sample. So, by inoculating 1 ml of a 10^{-2} dilution into the plate which is subsequently counted, we are theoretically doing the equivalent of plating 10^{-2} ml (i.e., 0.01 ml) of the lake water sample. (**Scientific notation** is reviewed here.)

IF **100 colonies** arise from plating **one ml** of a **1/100 dilution** of the lake water,
THEN there were **10,000 CFUs** (from **100 X 100**) per **one ml** of the **undiluted** water sample.

Compare the solution just obtained with the previous solution where Plate C was inoculated with 0.01 ml of sample:

IF **100 colonies** arise from plating **0.01 ml** of the water sample,
THEN there were **10,000 CFUs per one ml** of the water sample.

SOME MORE EXAMPLES:

I. For **Bacteriology 102** students, the above setup is applicable to Experiment 1, Period 2 where we plated one ml of a 10^{-2} (i.e., 1/100) dilution of lake water. In Period 3 – after

incubation of the plates – if we were to find 75 colonies on our plate, we could determine the CFUs per ml of the sample (at the time of Period 2) as follows:

IF **75 colonies** arise from plating **one ml** of a **1/100 dilution** of the lake water, THEN there were **7,500 CFUs** (**from 75 X 100**) per **one ml** of the **undiluted** water sample.

II. As we used a 10^{-4} (i.e., 1/10,000) dilution of **soil** in the same experiment, we could figure out the CFUs per gram of the soil the same way, remembering that **we consider milliliters and grams to be equivalent**:

IF **75 colonies** arise from plating **one ml** of a **1/10,000 dilution** of the soil, THEN there were **750,000 CFUs** (from **75 X 10,000**) **per one gram** of the **undiluted** soil sample.

III. **Consider this problem, the likes of which we often give in quizzes and on problem sets: Five ml** (not one!) of an undiluted spring water sample were added to a petri dish to which 15 ml of melted Plate Count Agar were then added. After mixing, the plate was allowed to solidify and then was incubated appropriately. After incubation, **50 colonies** were counted. How many CFUs were present **per one ml** of the original, undiluted spring water sample?

Here's the solution (and note the careful use of correct terminology): If 50 colonies arise from plating 5 ml of the sample, then there were 10 CFUs per one ml of the sample.

In working through this problem, consider the special bit of extraneous information thrown in – i.e., the amount of medium in the plate. Wouldn't you expect the same answer if you used a different amount of melted Plate Count Agar, say 20 or 25 ml? So, you

should still wind up with the answer being 10 CFUs per ml of the water sample.

On occasion, we unfortunately see an answer of more CFUs in one ml then there would have been in 5 ml! This can happen if one does not think through the setup of the problem and then treats the addition of sample to medium as a dilution. On the next page is the same problem worked out with our formulas.

LAB 3: OBTAINING PURE CULTURES FROM A MIXED POPULATION

DISCUSSION

As stated in Lab 2, microorganisms exist in nature as mixed populations. However, to study microorganisms in the laboratory we must have them in the form of a pure culture, that is, one in which all organisms are descendants of the same organism.

Two major steps are involved in obtaining pure cultures from a mixed population:

1. First, the mixture must be diluted until the various **individual microorganisms become separated** far enough apart on an agar surface that after incubation they form visible **colonies isolated from the colonies of other microorganisms**. This plate is called an **isolation plate**.

2. Then, an isolated colony can be aseptically **"picked off"** the isolation plate (see Fig. 1) and **transferred** to new sterile medium (see Fig. 3). After incubation, all organisms in the new culture will be descendants of the same organism, that is, a **pure culture**.

Animation showing a portion of a single colony being "picked off."

A. STREAK PLATE METHOD OF ISOLATION

The most common way of separating bacterial cells on the agar surface to obtain isolated colonies is the streak plate method we used in Lab 2 to inoculate a petri plate. It provides a simple and rapid method of diluting the sample by mechanical means. As the loop is streaked across the agar surface, more and more bacteria are rubbed off until individual separated organisms are deposited on the agar. After incubation, the area at the beginning of the streak pattern will show confluent growth, while the area near the end of the pattern should show discrete colonies (see Fig. 2).

B. THE POUR PLATE AND SPIN PLATE METHODS OF ISOLATION

Another method of separating bacteria is the pour plate method. With the **pour plate method**, the bacteria are mixed with melted agar until evenly distributed and separated throughout the liquid. The melted agar is then poured into an empty plate and allowed to solidify. After incubation, discrete bacterial colonies can then be found growing both on the agar and in the agar.

The **spin plate method** involves diluting the bacterial sample in tubes of sterile water, saline, or broth. Small samples of the diluted bacteria are then pipetted onto the surface of agar plates. A sterile, bent-glass rod is then used to spread the bacteria evenly over the entire agar surface (see Fig. 4) in order to see isolated colonies (see Fig. 5). In Lab 4 we will use this technique as part of the plate count method of enumerating bacteria.

C. USE OF SPECIALIZED MEDIA

To supplement mechanical techniques of isolation such as the streak plate method, many **special-purpose media** are available to the microbiologist to aid in the isolation and identification of specific microorganisms. These special purpose media fall into four groups: selective media, differential media, enrichment media, and combination selective and differential media.

1. Selective media

A selective medium has agents added which will **inhibit the growth of one group of organisms while permitting the growth of another**. For example, **Columbia CNA agar** has the antibiotics colistin and nalidixic acid added which inhibit the growth of gram-negative bacteria but not the growth of gram-positives. It is, therefore, said to be selective for gram-positive organisms, and would be useful in separating a mixture of gram-positive and gram-negative bacteria.

2. Differential media

A differential medium contains additives that **cause an observable color change in the medium when a particular chemical reaction occurs**. They are useful in differentiating bacteria according to some biochemical characteristic. In other words, **they indicate whether or not a certain organism can carry out a specific biochemical reaction** during its normal metabolism. Many such media will be used in future labs to aid in the identification of microorganisms.

3. Enrichment media

An enrichment medium contains additives that **enhance the growth of certain organisms**. This is useful when the organism you wish to culture is present in relatively small numbers compared to the other organisms growing in the mixture.

4. Combination selective and differential media

A combination selective and differential medium **permits the growth of one group of organisms while inhibiting the growth of another**. In addition, it differentiates those organisms that grow based on whether they can **carry out particular chemical reactions**. For example, Eosin Methylene Blue (EMB) agar is selective for gram-negative bacteria. The dyes eosin Y and methylene blue found in the medium inhibit the growth of gram-positive bacteria but not the growth of gram-negatives. In addition, it is useful in differentiating the various gram-negative enteric bacilli belonging to the bacterial family Enterobacteriaceae (see Labs 12 & 13). The appearance of typical members of this bacterial family on EMB agar is as follows:

- Escherichia coli: large, blue-black colonies with a green metallic sheen
- Enterobacter and Klebsiella: large, mucoid, pink to purple colonies with no metallic sheen
- Salmonella and Shigella and Proteus: large, colorless colonies
- Shigella: colorless to pink colonies

The color changes in the colonies are a result of bacterial fermentation of the sugar lactose while colorless colonies indicate lactose non-fermenters. Fermentation reactions will be discussed in more detail in Lab 8.

There are literally hundreds of special-purpose media available to the microbiologist. Today we will combine both a mechanical isolation technique (the streak plate) with selective and selective-differential media to obtain pure cultures from a mixture of bacteria. In future labs, such as 12 - 16, which deal with the isolation and identification of pathogenic bacteria, we will use many additional special-purpose media.

MEDIA

One plate of each of the following media: Trypticase Soy agar, Columbia CNA agar, and EMB agar.

ORGANISMS

A broth culture containing a mixture of one of the following gram-positive bacteria and one of the following gram-negative bacteria:

- Possible gram-positive bacteria:
  - Micrococcus luteus. A gram-positive coccus with a tetrad or a sarcina arrangement; produces circular, convex colonies with a yellow, water-insoluble pigment on Trypticase Soy agar.
    - **Micrococcus luteus growing on TSA**
    - **Close up of Micrococcus luteus growing on TSA**
  - Staphylococcus epidermidis. A gram-positive coccus with a staphylococcus arrangement; produces circular, convex, non-pigmented colonies on Trypticase Soy agar.
    - **Staphylococcus epidermidis growing on TSA**
    - **Close up of Staphylococcus epidermidis growing on TSA**
- Possible gram-negative bacteria:
  - Escherichia coli. A gram-negative bacillus; produces irregular, raised, non-pigmented colonies on Trypticase Soy agar.
    - **Escherichia coli growing on TSA**
  - Enterobacter aerogenes. A gram-negative bacillus; produces irregular raised, non-pigmented, possibly mucoid colonies on Trypticase Soy agar.
    - **Enterobacter aerogenes growing on TSA**

During the next three labs you will attempt to obtain pure cultures of each organism in your mixture and determine which two bacteria you have. **Today** you will try to separate the bacteria in the mixture in order to obtain isolated colonies; **next lab** you will identify the two bacteria in your mixture and pick off single isolated colonies of each of the two bacteria in order to get a pure

culture of each. The **following lab** you will prepare microscopy slides of each of the two pure cultures to determine if they are indeed pure.

PROCEDURE (to be done in pairs)

1. First attempt to obtain isolated colonies of the two organisms in your mixture by using mechanical methods on an all-purpose growth medium, Trypticase Soy agar. Streak the mixture on a plate of Trypticase Soy agar using one of the two streaking patterns illustrated in Lab 2, Fig. 4 and Fig. 5.

Animation showing how to streak an agar plate for isolation.

2. Streak the same mixture for isolation (see Fig. 4 and Fig. 5) on a plate of Columbia CNA agar (selective for gram-positive bacteria).

- **Micrococcus luteus** growing on Columbia CNA agar.
- **Staphylococcus epidermidis** growing on Columbia CNA agar.

3. Streak the same mixture for isolation (see Fig. 4 and Fig. 5) on a plate of EMB agar (selective for gram-negative bacteria and differential for certain members of the bacterial family Enterobacteriaceae).

- **Escherichia coli** growing on EMB agar.
- **Enterobacter aerogenes** growing on EMB agar.

4. Incubate the three plates **at 37°C** until the next lab period.

Return to Menu for Lab 3

RESULTS

1. Observe isolated colonies on the plates of Trypticase Soy agar, Columbia CNA agar, and EMB agar. Record your observations and conclusions.

Trypticase Soy agar	
Observations	
Conclusions	
Columbia CNA agar	
Observations	
Conclusions	
EMB agar	
Observations	
Conclusions	

2. Using any of the three plates, **pick off a** single isolated colony **of each of the two organisms in your original mixture and aseptically transfer them to separate plates of Trypticase Soy agar** (see Fig. 3). When picking off single colonies, **remove the top portion of the colony without touching the agar surface itself** to avoid picking up any inhibited bacteria from the surface of the agar. Use your regular plate-streaking pattern to inoculate these plates and incubate **at 37°C** until the next lab period. These will be your pure cultures for Lab 5 (Direct and Indirect stains).

Animation showing a portion of a single colony being "picked off."

Return to Menu for Lab 3

PERFORMANCE OBJECTIVES FOR LAB 3

After completing this lab, the student will be able to complete the following objectives:

DISCUSSION

1. Given a mixture of a gram-positive and a gram-negative bacterium and plates of of Columbia CNA, EMB, and Trypticase Soy agar, describe the steps you would take to eventually obtain pure cultures of each organism.

2. Define: selective medium, differential medium, enrichment medium, and combination selective-differential medium.

3. State the usefulness of Columbia CNA agar and EMB agar.

4. Describe how each of the following would appear when grown on EMB agar:

a. Escherichia coli
b. Enterobacter aerogenes
c. Salmonella

PROCEDURE

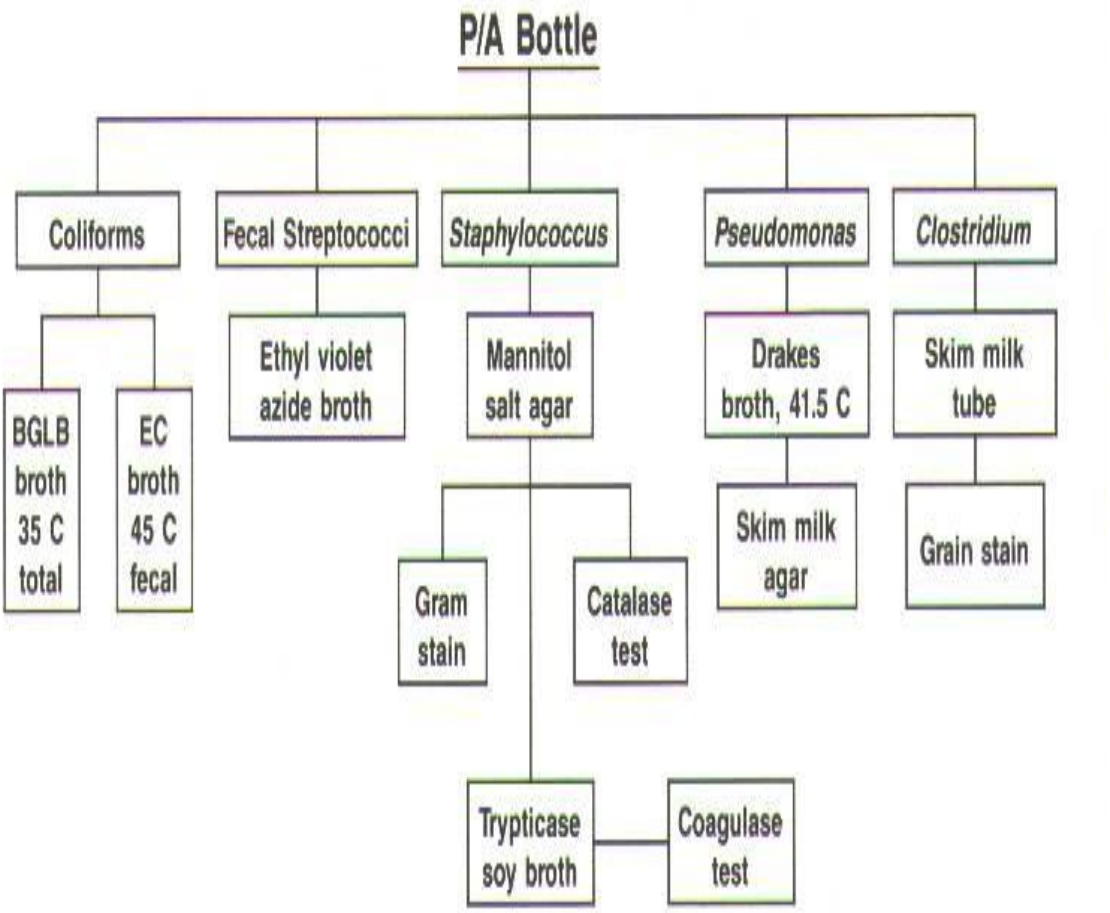
1. Using the streak plate method of isolation, obtain isolated colonies from a mixture of microorganisms.

2. Pick off isolated colonies of microorganisms growing on a streak plate and aseptically transfer them to sterile media to obtain pure cultures.

RESULTS

1. When given a plate of Columbia CNA agar or EMB agar showing discrete colonies, correctly interpret the results.

FIGURE 9:


P/A Bottle
Coliforms
Fecal Streptococci
Staphylococcus
Pseudomonas
Clostridium
BGLB broth 35 C total
EC broth 45 C fecal
Ethyl violet azide broth
Mannitol salt agar
Drakes broth, 41.5 C
Skim milk tube
Gram stain
Catalase test
Skim milk agar
Grain stain
Trypticase soy broth
Coagulase test

Pseudomonas Aeruginosa
By Murali Putty, EMLab™ Analyst

Pseudomonas aeruginosa is a gram-negative, aerobic rod measuring 0.5 to 0.8 µm belonging to the bacterial family Pseudomonadaceae. Like other Pseudomonads, P. aeruginosa secretes a variety of pigments, including pyocyanin (blue-green), fluorescein (yellow-green and fluorescent), and pyorubin (red-brown). P. aeruginosa is often preliminarily identified by its pearlescent appearance and grape-like odor in vitro.

Pseudomonas aeruginosa is widespread in nature, inhabiting soil, water, plants and animals (including humans). Identification of P. aeruginosa often includes identifying the production of both pyocyanin and fluorescein. Its optimum temperature for growth is 37°C, and it is able to grow at temperatures as high as 42°C. Pseudomonas aeruginosa has very simple nutritional requirements. It is often observed "growing in distilled water" which is evidence of its minimal nutritional needs. It is tolerant to a wide variety of physical conditions. P. aeruginosa is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon utilizing microorganism (or "HUM bug"), causing microbial corrosion. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics. These natural properties of the bacterium undoubtedly contribute to its ecological success.

Figure 1: Pseudomonas aeruginosa on Trypic Soy Agar (TSA).

Pseudomonas aeruginosa is an opportunistic pathogen, causing urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. Pseudomonas aeruginosa is primarily a nosocomial pathogen; its infection is a serious problem in patients hospitalized with cancer, cystic fibrosis and burns. The case fatality rate in these patients is 50 percent. According to the Centers for Disease Control (CDC), the overall incidence of P. aeruginosa infections in the US hospitals averages 0.4 percent (4 per 1000 discharges), and the bacterium is the fourth most commonly isolated nosocomial pathogen accounting for 10.1 percent of all hospital-acquired infections. The most commonly encountered infection outside hospitals is otitis externa ("Swimmer's ear"), of which P. aeruginosa causes 35-70%. They also cause eye infections ranging from perulent conjunctivitis to iridocyclitis, keratitis and iritis, corneal ulcer and panophthamitis. It is also a cause of "hot-tub rash" (dermatitis), caused by lack of proper, periodic attention to water quality.

Figure 2: Pseudomonas aeruginosa in Pseudomonas Isolation Agar.

P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance, which is large, smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence.

Like most environmental bacteria, P. aerugionosa lives predominantly in slime-enclosed biofilms adherent to available surface from which it periodically releases (free-swimming) cells. Most Pseudomonas infections are both invasive and toxinogenic. The ultimate Pseudomonas infection may be seen as composed of three distinct stages: (1) bacterial attachment and colonization; (2) local invasion; (3)

disseminated systemic disease. The slime-protected cells of P. aeruginosa produce remarkably few clinical symptoms while they are in the process of progressively colonizing inert surfaces and tissue surfaces within the host. The lipopolysaccharide layer helps the cell adhere to host tissues and prevents leukocytes from ingesting and lysing the organism. Lipases and exotoxins then procede to destroy host cell tissue which then leads to the complications associated with infection.

Only a few antibiotics are effective against Pseudomonas, including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains. The best way to reduce the spread of P. aeruginosa is to use good aseptic technique especially on hospital instruments and when in contact with patients. The combination of gentamicin and carbenicillin is frequently used to treat severe Pseudomonas infections.

References:

1. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover & R.H.Yolken (eds) 1995. Manual of Clinical Microbiology, 6th edition, pp. 510-515, ASM, Washington, D.C.

2. Baltch, A.L and R. P. Smith (eds) (1994) Pseudomonas Aeruginosa: Infections and Treatment. Marcel Dekker, Inc., New York, NY.

3. Wikipedia

4. Pseudomonas Genome Database

5. Todar's Online Textbook of Bacteriology

6. Pseudomonas Aeruginosa Infections

7. Topics in Infectious Diseases Newsletter: August 2001

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Appendix B: Medium Formulations & Preparation

Asparagine/Acetamide Broth: Media for *P. aeruginosa* by MPN

Formulation of Asparagine Broth

Asparagine, DL	3.0g
Dipotassium hydrogen phosphate	1.0g
Magnesium sulfate, heptahydrate	0.5g
Distilled water	1L

Preparation

Dissolve the ingredients in distilled water and adjust pH to 6.9 to 7.2. Sterilize.

Formulation of Acetamide Broth

Acetamide	10.0g
Sodium chloride	5.0g
Dipotassium hydrogen phosphate	1.39g
Potassium dihydrogen phosphate	0.73g
Magnesium sulfate, heptahydrate	0.5g
Phenol red	0.012g
Distilled water	1L

Preparation

Dissolve the ingredients in distilled water and adjust pH to 6.9 to 7.2. Sterilize.

m-E/Esculin Iron Agar: Media for Enterococci

Formulation of m-E

Peptone	10.0g
Sodium chloride	15.0g
Esculin	1.0g
Yeast extract	30.0g
Actidione	0.050g
Sodium azide	0.150g
Agar	15.0g
Distilled water	1L
Additional Ingredients	
Nalidixic acid	0.21g
Triphenyl tetrazolium chloride	0.15g

Preparation

Dissolve original ingredients in distilled water and autoclave for 15 minutes at 121°C. Add additional ingredients and adjust pH to 7.1 ±0.1. Pour 3.5 mL into 50 mm plates.

Formulation of Esculin Iron Agar

Esculin	1.0g
Ferric citrate	0.5g
Agar	15.0g
Distilled water	1L

Preparation

Adjust pH to 7.1 ± 0.1. Autoclave at 121°C for 15 minutes. Pour in 3.5 mL amounts into 50 mm plates.

m-PA: Medium for *Pseudomonas aeruginosa* by MF

Formulation

	Levin's	Standard Methods
L-lysine hydrochloride	5.0g	5.0g
Sodium chloride	5.0g	5.0g
Yeast extract	2.0g	2.0g
Xylose	2.5g	1.25g
Sodium thiosulfate	6.8g	5.0g
Sucrose	12.5g	12.5g
Lactose	12.5g	12.5g
Phenol red	0.08g	0.08g
Ferric ammonium citrate	0.8g	0.8g
Magnesium sulfate, heptahydrate	0	1.5g
Agar	15.0g	15.0g
Distilled water	1L	1L
Antibiotic		
Sulfapyridine (Nutritional Biochemicals)		0.176g
Kanamycin(Bristol-Myers)		0.0085g
Nalidixic acid(Cal Biochemicals)		0.037g
Actidione(Upjohn)		0.15g

Preparation

Dissolve all ingredients except antibiotics in distilled water. Autoclave at 121°C for 15 minutes. Cool to 55 to 60°C. Adjust pH to 7.1 ± 0.1 for *Standard Methods* formulation. Adjust pH to 6.5 for Levin's formulation. Add the antibiotics and mix. Pour 3 mL into 12 by 50 mm plates.

m-T7: Medium for total and fecal coliforms

Formulation

Protease Peptone no. 3 (Difco)	5.0g
Yeast extract	3.0g
Lactose	20.0g
Tergitol 7 (25% solution)	0.4 mL
Polyoxyethylene ether W-1	5.0g
Bromthymol blue	0.1g
Bromcresol purple	0.1g
Agar	15.0g
Distilled water	1L

Preparation

Dissolve all ingredients in distilled water. Autoclave at 121°C for 15 minutes. Adjust the pH to 7.4±0.2. Selectivity can be enhanced with the aseptic addition of 0.1mg per liter of penicillin g after autoclaving.

m-TEC: Medium for enumerating *E. coli*

Formulation

Protease peptone No. 3 (Difco)	5.0g
Yeast extract	3.0g
Lactose	10.0g
Sodium chloride	7.5g
Dipotassium hydrogen phosphate	3.3g
Potassium dihydrogen phosphate	1.0g
Sodium lauryl sulfate	0.2g
Sodium desoxycholate	0.1g
Bromcresol purple	0.08g
Agar	15.0g
Distilled water	1L

Preparation

Dissolve ingredients by stirring. Sterilize by autoclaving at 121°C for 15 minutes. Pour 4 mL into 10 by 50 mm plates. The pH of the medium is 7.3 ± 0.1.

m-TMM: Medium for total coliforms and *E. coli*

Formulation

Tryptone	5.0g
Yeast extract	2.5g
Lactose	10.0g
Bromcresol purple	0.080g
Dipotassium phosphate	3.3g
Potassium phosphate	1.0g
MUG (4-methylumbelliferyl -ß-D-glucuronide)	0.100g
Monesin (in 10 mL of 95% ethanol)	0.038g
Tergitol 7	0.25 mL
Agar	15.0g
Distilled water	1L

Preparation

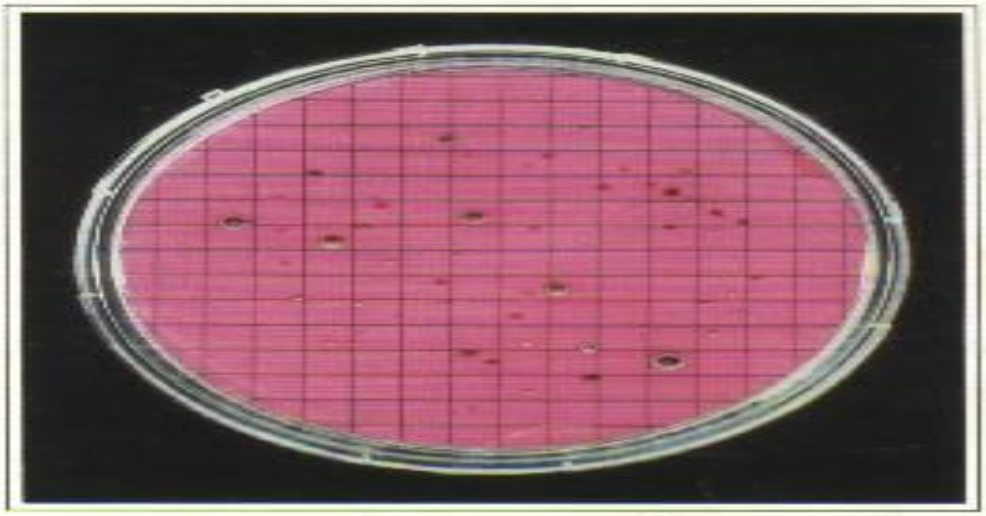
Dissolve the ingredients in distilled water. Autoclave for 15 minutes at 121°C. The final pH is 7.2. Pour 3 mL into sterilized 47 mm plates.

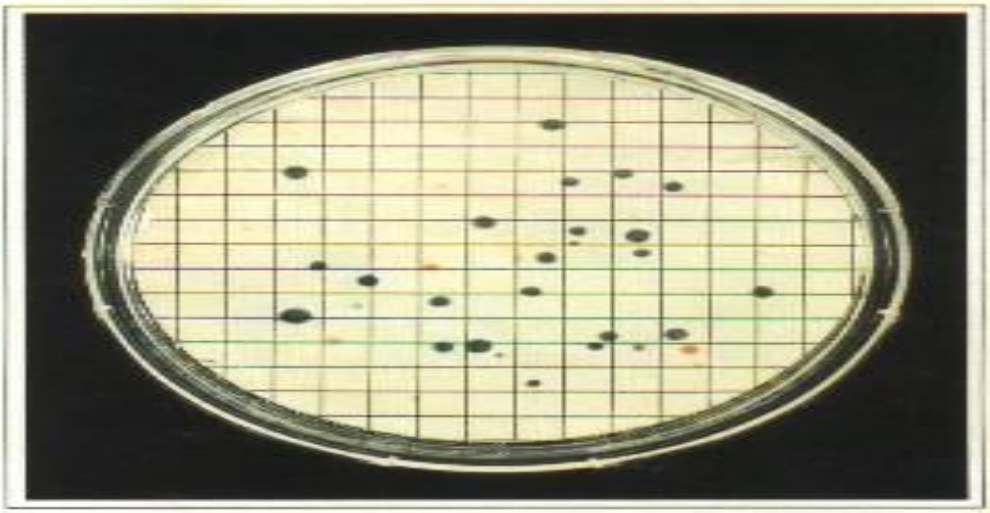
TABLE 5:

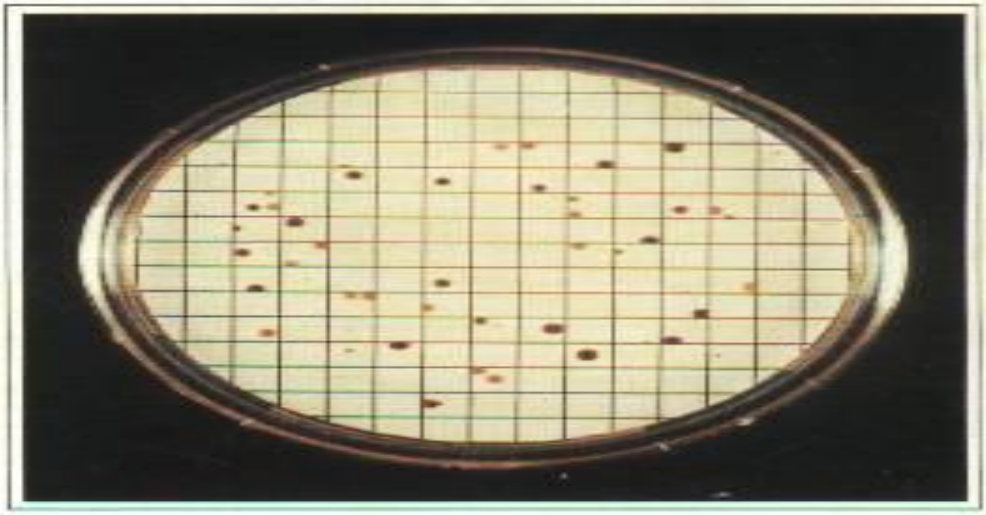
Suggested Sample Volumes for Membrane Filter Fecal Coliform Test*

	Volume to be Filtered mL						
Water Source	100	50	10	1	0.1	0.01	0.001
Lakes, reservoirs	X	X					
Wells, springs	X	X					
Water supply intake		X	X	X			
Natural bathing waters		X	X	X			
Sewage treatment plant, secondary effluent			X	X	X		
Farm ponds, rivers				X	X	X	
Storm water runoff				X	X	X	
Raw municipal sewage					X	X	X
Feedlot runoff					X	X	X

*Standard Methods for the Examination of Water and Wastewater, 16th ed., page 898







Membrane Filtration Method: Fecal Coliforms

BIOL/CSES 4644

Virginia Polytechnic Institute and State University

Introduction

The membrane filter (MF) technique is highly reproductible, can be used to test relatively large volumes of sample, and yields numerical results more rapidly than the multiple-tube procedure. The membrane filter technique is extremely useful in monitoring drinking water and a variety of natural waters. However, the MF technique has limitations, particularly when testing waters with high turbidity or noncoliform (background) bacteria. For such waters or when the membrane filter technique has not been used previously, it is desirable to conduct parallel tests with the multiple-tube fermentation technique to demonstrate applicability and comparability.

As related to the membrane filter technique, the coliform group may be defined as comprising all aerobic and many facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that develop a red colony with a metallic sheen within 24h at 35^{o}C on an Endo-type medium containing lactose. Some members of the total coliform group may produce a dark red or nucleated colony without a metallic sheen. When verified these are classified as atypical coliform colonies. When purified cultures of coliform bacteria are tested they produce a negative cytochrome oxidase (CO) and positive Beta-galactosidase (ONPG) reaction. Generally, all red, pink, blue, white, or colorless colonies lacking sheen are considered non-coliforms by this technique.

Fecal Coliform Membrane Filter Procedure

Fecal coliform bacterial densities may be determined either by the multiple-tube procedure or by a membrane filter (MF) technique. If the MF procedure is used for chlorinated effluents, demonstrate that it gives comparable information to that obtained by the multiple-tube test before accepting it as an alternative. The MF procedure uses an enriched lactose medium and incubation temperature of 44.5 +/- 0.2^{o}C for selectivity and gives 93% accuracy in differentiating between coliforms found in the feces of warm-blooded animals and those from other environmental sources. Because incubation temperature is critical, submerge waterproofed (plastic bag enclosures) MF cultures in a water bath for incubation at the elevated temperature or use an appropriate, accurate solid heat sink incubator. Alternatively, use an equivalent incubator that will hold the 44.5^{o}C temperature within 0.2^{o}C (throughout the chamber), over a 24-h period, while located in an environment of ambient air temperatures ranging from 5^{o}C to 35^{o}C.

1. Materials and Culture Medium

a. M-FC medium: The need for uniformity dictates the use of dehydrated media. Never prepare media from basic ingredients when suitable dehydrated media are available. Follow manufacturer's directions for rehydration. Commercially prepared media in liquid form (sterile ampule or other) also may be used if known to give equivalent results.

b. Culture Dishes: Use tight-fitting plastic dishes because the MF cultures are submerged in a water bath during incubation. Enclose groups of fecal coliform cultures is plastic bags or seal individual dishes with waterproof (freezer) tape to prevent leakage during submersion.

c. Incubator: The specificity of the fecal coliform test is related directly to the incubation temperature. Static air incubation may be a problem in some types of incubators because of potential heat layering within the chamber and the slow recovery of temperature each time the incubator is opened during daily operations. To meet the need for greater temperature control use a water bath, a heat-sink incubator, or a properly designed and constructed incubator giving equivalent results. A temperature tolerance of 44.5 +/- 0.2^{o}C can be obtained with most types of water baths that also are equipped with a gable top for the reduction of heat and water losses. A

circulating water bath is excellent but may not be essential to this test if the maximum permissible variation of 0.2^{o}C in temperature can be maintained with other equipment.

2. Procedure

a. Selection of sample size: Use sample volumes that will yield counts between 20 and 60 fecal coliform colonies per membrane. When the bacterial density of the sample is unknown, filter several decimal volumes to establish fecal coliform density. Estimate volume expected to yield a countable membrane and select two additional quantities representing one-tenth and ten times this volume, respectively.

b. Filtration of sample: Follow the same procedure and precautions as described above.

c. Preparation of culture dish: Place a sterile absorbent pad in each culture dish and pipet approximately 2 mL M-FC medium, prepared as directed above, to saturate pad. Carefully remove any excess liquid from the culture dish. Place prepared filter on medium-impregnated pad.

As a substrate substitution for the nutrient-saturated absorbent pad, add 1.5% agar to M-FC broth.

d. Incubation: Place prepared cultures in waterproof plastic bags or seal petri dishes, submerge in water bath, and incubate for 24 +/- 2h at 44.5 +/- 0.2^{o}C. Anchor dishes below the water surface to maintain critical temperature requirements. Place all prepared cultures in the water bath within 30 min after filtration. Alternatively, use an appropriate, accurate solid heat sink or equivalent incubator.

e. Counting: Colonies produced by fecal coliform bacteria on M-FC medium are various shades of blue. Pale yellow colonies may be atypical E.coli; verify for gas production in mannitol at 44.5^{o}C. Nonfecal coliform colonies are gray to cream-colored. Normally, few nonfecal coliform colonies will be observed on M-FC medium because of selective action of the elevated temperature and addition of rosolic acid salt reagent. Elevating the temperature to 45.0 +/- 0.2^{o}C may be useful in eliminating environmental Klebsiella from the fecal coliform population. Count

colonies with a low power (10-15 magnifications) binocular wide-field dissecting microscope or other optical device.

3. Calculation of Fecal Coliform Density

Compute the density from the sample quantities that produced MF counts within the desired range of 20 to 60 fecal coliform colonies. This colony density range is more restrictive than the 20 to 80 total coliform range because of larger colony size on M-FC medium. Record densities as fecal coliforms per 100 mL.

Compute the count by the following equation:

(total) Coliform colonies/100mL = (coliform colonies counted x 100) / (mL sample filtered)

For verified coliform counts, adjust the initial count based upon the positive verificationpercentage and report as "verified coliform count per 100 mL."

Percentage verified coliforms =
(number of verified colonies) / (total number of coliform colonies subjected to verification) x 100.

The plate in the upper left shows typical dark blue fecal coliform colonies on a membrane filter after incubation on M-FC agar. The plate in the lower center (purple) is M-FC agar before use, and the plate in the upper right (blue) is a control plate streaked with an E.coli culture.

4. Bibliography

Geldreich, E.E., H.F.Clark, C.B.Huff and L.C.Best. 1965. Fecal-coliform-organism medium for the membrane filter technique.J.Amer. Water Works Assoc. 57:208.

Green, B.L., W. Litsky and K.J.Sladek. 1980. Evalutation of membrane filter methods for enumeration of faecal coliforms from marine waters. Mar. Environ. Res. 67:267

Greenberg, A.E., L.S. Clesceri, and A.D.Eaton(eds). 1992. Standard Methods for the Examination of Water and Wastewater. Amer.Public Health Assoc. Washington, D.C.

Sartory, D.P. 1980. Membrane filtration faecal coliform determinations with unmodified and modified M-FC medium. Water SA 6:113.

Rychert, R.C. and G.R.Stephenson. 1981. Atypical Escherichia coli in streams. Appl. Environ. Microbiol. 41:1276.

Pagel, J.E., A.A.Qureshi, D.M.Young and L.T.Vlassoff. 1982. Comparison of four membrane filter methods for fecal coliform enumeration. Appl. Environ. Microbiol. 43:787.

Return to Water Testing Webpage.

Drinking Water System for the Children's Dormito

Mae Nam Khun, Thailand

Background

With the dormitory and septic system complete, the Seattle University EWB club returned to Mae Nam Khun in August 2007 to install a drinking water treatment system. Although the treatment system was not part of the original plan, it was a logical step for a

holistic project solution. This was particularly evident when the 2006 EWB-team witnessed large amounts of sediment in the water supply after a recent storm.

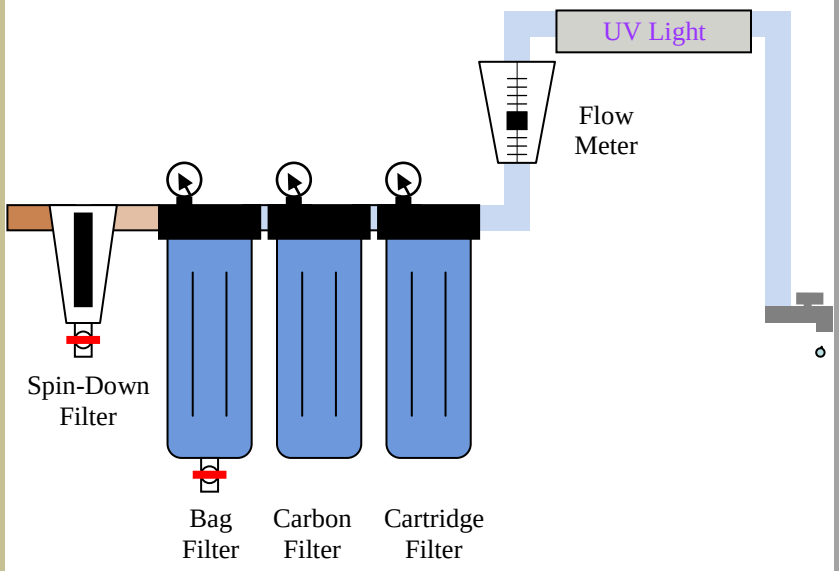
Previous investigations in MNK by the California Polytechnic State University at San Luis Obispo found that a 50,000 gallon water tank situated approximately 300 feet in elevation above the dormitory area was the source for the water distribution system. The existence of pressurized water and electrical power at the site provided good conditions for a modernized yet sustainable drinking water treatment system.

The System

In March 2007, SU-EWB partnered with the Society of Environmental Engineers and Scientists (SEES is an SU student chapter of the Water Environment Federation) to construct a prototype water treatment system which was demonstrated at the SU Student Center on Earth Day 2007.

The system begins with a spin-down sediment filter which removes particles that are 100-microns in diameter or larger. This filter is self-cleaning as trapped particles can be washed away with a turn of a valve. Next, a washable, nylon filter bag removes particles larger than 50-microns in diameter. This is followed by an activated carbon cartridge (similar to a Brita® filter) and finally a washable, 5-micron,

pleated-cellulose cartridge filter which is a required treatment step prior to disinfection with ultraviolet light. In the event that distribution system pressure is lost, water can be supplied to the system by a backup submersible pump that can be immersed in a nearby stream.

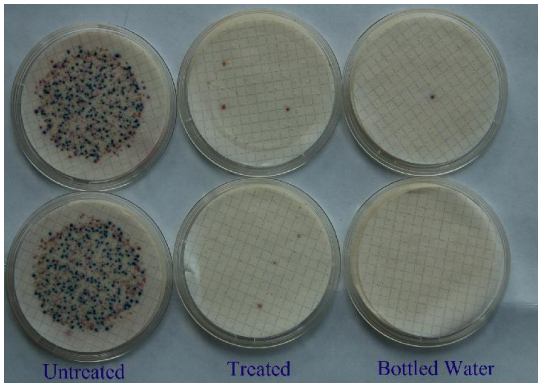


Implementation

In August 2007, an SU-EWB team consisting of faculty (Phil Thompson, Wes Lauer, Pierre Gehlen) and students (Ryan Daudistel ’08, Chris Stoll ’08) installed the system in MNK with the help of Bangkok resident and SU alumnus Akharint

"Nok" Khuhapinant ('03) and two Chiang Mai University environmental engineering students Vorakorn "Nook" Somkarnsmai and Wanawan "Nan" Pragot. Although the team took all of the necessary supplies with them, they purchased a UV system from L'Analytic Water Work LTD, a local supplier in Chiang Mai (3 hours from MNK). This was done when it was discovered that spare bulbs that had been shipped by courier from the U.S. had been destroyed in transit. The 'shipping experiment' revealed that the annual replacement of the UV bulb would have to be done with local materials, so a local system was installed.

Upon installation, the system was tested for E. Coli and general coliform bacteria using membrane filter test kits. The group also tested the water from a slow sand filter that had been installed 5-months earlier and was being used for drinking water. After 48 hours of incubation, the testing revealed that there were approximately 100 E. Coli per 100-ml sample from the sand filter but the new system had improved the drinking water to near bottled water quality!



The system has a maximum flow rate of 21,600 liters per day. The capital costs for this configuration was about $1000. We estimate the annual operation cost for bulb and cartridge replacement to be $500 or less which would result in a maximum 30-year present value of $9,600 or 0.007 cents (0.0025 baht) per liter of water produced.

Plans for the Future

We hope to get more accurate annual O/M costs by collecting life cycle data through a water system monitoring project with CMU's Environmental Engineering Department. With the guidance of Dr. Patiroop Polchan, CMU students Nan and Nook will continue quarterly biological testing of the system, and they will train additional students who will succeed them upon graduation in June 2008. In addition to monitoring water quality, these students will assist in gathering life cycle information for system components as well as water-borne illness data from the local medical clinic.

In addition, Ms. Lawan Khumyahd from L'Analytic Water Work LTD was a tremendous resource for supplying water system components, and she expressed a desire to help provide materials for replacement or for new EWB water projects in Thailand.

Over the 2007-2008 school year, the EWB and SEES clubs will be making modifications to the system, including the addition solar power and hand pumping. This would enable the system to be a stand-alone unit capable of treating surface water in areas without pressurized water systems or power.

Project Sponsors

This project was sponsored in part by the Associated Students of Seattle University, The Endowed Mission Fund, The College of Science and Engineering and by our generous annual donors.

COLIFORM IN DRINKING WATER

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Membrane Filtration

File "Drinking_water.htm" was last modified on 01 Jun 2008.
This page has been accessed times since 13 August 2001.

tap water (left) has no coliform "filtered water" is on the right...

7/20/89, rvsd 6 August 1993, 23 July '95, 22 July '97, 19 July 98, 30 June 99, 17 July 00, 9 Aug 00

Modified from: Standard Methods for the Examination of Water and Wastewater, 14th Ed, (1975). pages 928-935.

Students can test the drinking water from their homes, school, etc using this protocol. The health standard for drinking water allows no more than 5 coliform per hundred mL. For this reason, bacteria from 250 mL will be collected on a membrane filter, and grown on top of m Endo medium. Coliform bacteria appear as red colonies on this medium. More than 13 colonies with this assay does not meet health standards.
One student in 2001 tested her tap water before and after filtration through a water "purification" device. The image shows two plates , the one on the left is tap water, the plate on the right is water filtered through the filter. Clearly the filter is contributing bacterial load to the water. These devices must be regularly changed and maintained in order to prevent such contamination.

EQUIPMENT AND SUPPLIES:	**millipore filtering apparatus:**
Sterile 250 mL capped bottles (1/student) sterile 47 mm petri dishes (1 per student) sterile 47 mm memb. filters, 0.45 µm pores sterile 47 mm millipore pads vacuum pump 3 vacuum hoses joined with "T" joint 2 strong hose clamps 1000 mL side arm filter flask m-Endo Broth MF powder sterilized repipet in 250 mL bottle	sintered glass platform in #8 stopper glass cylinder, 300 mL capacity 800 mL beaker with 400 mL EtOH 150 mL beaker with 100 mL EtOH triangle-tipped Tongs forceps with bent tipped blade Bunsen burner protective eye wear

MAKE THESE TWO ILLUSTRATIONS WITH LISTED FEATURES LABELED:
(pay close attention to the location of the valves)

	Vacuum Filtering Apparatus:	**Plate Ready for Incubation:**
apparatus:  note the labeled valves: 	vacuum pump on/off power switch main vacuum line main vacuum line clamp T joint relief valve clamp filter flask #8 stopper screen platform membrane filter glass cylinder, 300 mL cylinder clamp	(exploded view) 50 mm petri dish top 47 mm membrane filter 47 mm pad with 2 mL m-Endo MF 50 mm petri dish bottom

I. COLLECTION OF WATER (Collect the same day as performance of assay):

	Determine the precise name of your water district, record it in your notebook. Run tap water until it is cold (to clear out pipes, at least a minute or so). Fill sterile 250 mL capped bottle with water, rinse several times, finally fill to neck, cap securely, maintaining sterility .

II. PREPARATION OF 50 mL OF MEDIUM (for 20 assays):

	a: For up to 20 determinations, weight out **2.4 g m-Endo Broth MF** powder into 150 mL beaker. b: Add **49 mL dH_20** and **1 mL EtOH**. c: Bring to boil, remove from heat immediately. Using sterile technique, pour into sterilized repipet vessel. Securely screw down repipet to container. Clamp to ring stand for stability.

III. PREPARATION OF PAD:

sterile pad in petri dish: 	add 2.0 mL m-Endo Broth	Label 47 mm top of sterile petri dish with initials, seat number, date & source. Flame off EtOH from bent blade-tipped forceps, pick up sterile pad, place in bottom of sterile petri dish , replace cover. Repipet **2.0 mL m-Endo Broth** sterilely onto pad , replace lid, keep bottom down.

IV. SET UP PLATE: Note: Wear safety goggles, tie back hair, keep flammable materials away from flame.

inadequate head space: 	close relief valve: 	**PRELIMINARY:** Check for adequate head space in the filter flask to contain the water you are about to filter. If inadequate, empty out the flask into the sink, and replace the support platform. If necessary, re-sterilize surface of support platform with EtOH, turn on vacuum briefly to dry, then tightly clamp the main vacuum line. Also, apply vacuum to the platform to remove any water or alcohol remaining from the previous filtrations by closing the relief valve, opening the main valve, then closing the main valve.

open relief valve: 	tongs in alcohol: 	Once the platform is dry: **SET UP MEMBRANE FILTER**: Flame off EtOH from blade forceps
flame off alcohol 	pick up sterile membrane 	pick up sterile membrane filter , (discard the blue spacer discs)
apply to platform 	center the membrane: 	center it on screen platform . Open main vacuum line to hold membrane in place (turn on pump if not on yet).

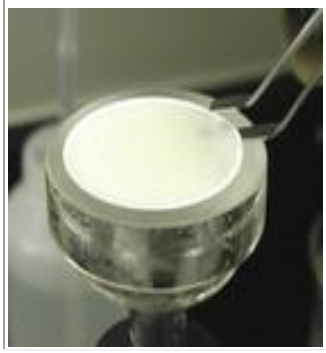
V. THE DANGEROUS STEP: STERILIZING AND FLAMING THE CYLINDER

grasp with fingers, then tongs: 	touch off excess alcohol 	**STERILIZE THE CYLINDER (Dangerous step)**: Ensure that all surfaces of the glass cylinder are immersed in 95% EtOH , pick up with fingers (touch outside of the cylinder only), invert. Grasp cylinder upside down with triangle-tipped tongs (Ekco, for instance) and allow excess EtOH to drip into 800 mL beaker, touching off the last drops on a paper towel.
Pass through flame to ingnite, hold away from people and beakers of alcohol! 	Maybe ignited too soon? 	CAREFULLY flame off the EtOH away from EtOH beakers and other students). It will flame up fairly high, but should burn off in a few seconds. Pass quickly through the flame once more to ensure that all of the EtOH has been removed.
	flaming stopped, grasp with fingers	Grasp the outside of the sterilized cylinder with your fingers. (It should not be too hot if you touched off the EtOH before flaming.)

		
		**SET UP FILTER APPARATUS**: Place sterile cylinder centered over the membrane filter and support platform. Clamp in place with spring clamp , vacuum still on.

VI. FILTERING THE WATER AND APPLYING MEMBRANE TO PREPARED PAD:

Pour water into apparatus 		**FILTER WATER THROUGH**: Pour your 250 mL water sample into the cylinder , monitoring that it is not leaking at the clamped joint .
the vacuum draws it through 	empty all 250 mL into cylinder 	The vacuum will draw the water through, and all bacteria which may be in the 250 mL sample will be trapped on the surface of the membrane filter.
unclamp cylinder 	remove cylinder 	When all of the water has been drawn through, unclamp and remove the cylinder , place it carefully back in the EtOH, top down. (Do not let it drop into the beaker.)

slip tweezers under membrane 	gently lift membrane 	**RELEASE VACUUM ON MEMBRANE FILTER** : Clamp the main vacuum line shut and open the relief valve to release the vacuum in the flask. With sterile, EtOH-free blade forceps, gently lift the edge of the membrane filter and remove from the screen platform . (Caution: the membrane filter is brittle.)
transfer to prepared dish 	slide over edge to drop onto pad 	**TRANSFER MEMBRANE FILTER TO PREPARED PAD** , avoiding bubbles by lowering from one side first. Rest it on far edge of petri dish, slowly pull it across the edge down toward you until . . .
do not let it rest on edge 	centered membrane on pad 	. . . it drops down onto the pad. If done properly, it will be centered on the pad . Ensure that the membrane is completely flat on the pad.

VII. INCUBATION AND CALCULATION OF RESULTS

	INCUBATE the plate without inverting (pad-side down) at 35C for 24 hours. **COUNT THE COLONIES**: Record the total number of colonies, and the number of coliform (red colonies) . Divide by 2.5 to yield the number of bacteria per 100 mL. According to national health standards for drinking water, the number of coliform/100 mL should not exceed 5. Most chlorinated tap water will have no bacteria in 250 mL, as seen on the left membrane in the first of the nest set of pictures. Enter your data in this sequence and format into your notebook, and then into the spreadsheet for the class table in the computer Desk No. Initials Source in detail (water district) Coliform/100 mL
tap water vs. "filtered" water  Williamsburg OH water	**TOP:** tap water and "filtered" water from the same home. Note that filters must be changed regularly, or else they become "nesting" places for bacteria. **MIDDLE:** The two water samples shown are both heavily contaminated. **BOTTOM:** For years, Williamsburg had problems with their water, as can be seen in the 8/14/00 sample. We even had a village official tell a student it was against the law for her bring in a sample of her own water to test...


1529 Redoak Dr. Loveland, OH 45140
Clermont County 250mL

Wilkes University
Center for Environmental Quality
Environmental Engineering and
Earth Sciences
Fecal Coliform and Bacterial Testing

Total and Fecal Coliform Bacteria Testing

Fecal COLIFORM BACTERIA

The total and fecal coliform bacteria test is a primary indicator of "potability" , suitability for consumption, of drinking water. It measures the concentration of total coliform bacteria associated with the possible presence of disease causing organisms.

Sources of Fecal Coliform
Potential Health Hazards
Fecal Colifrom Testing
Treatment

SOURCE of Fecal Coliform:

Coliform bacteria are a natural part of the microbiology of the intestinal tract of warm blooded mammals, including man. Coliform bacteria can also be found in soil, other animals, insects, etc. The total coliform group is relatively easy to culture in the lab, and therefore, has been selected as the primary indicator bacteria for the presence of disease causing organisms.

Potential Health Hazards:

Coliform bacteria are not pathogenic (disease causing) organisms, and are only mildly infectious. For this reason these bacteria are relatively safe to work with in the laboratory. If large numbers of coliforms are found in water, there is a high probability that other pathogenic bacteria or organisms, such as Giardia and Cryptosporidium, may be present. The PADEP requires public drinking water supplies to demonstrate the absence of total coliform per 100 mls (about 4 oz) of drinking water. At this time, there are no regulations governing individual water wells. It is up to the private well owner to have his or her water tested.

TESTING:

Approved tests for total coliform bacteria include the membrane filter, multiple tube fermentation, MPN and MMO-MUG ("Colilert") methods. The membrane filter method uses a fine porosity filter which can retain bacteria. The filter is placed in a petri (culture) dish on a pad with growth enrichment media (mEndo) and is incubated for 24 hrs at 35 degrees C. Individual bacteria cells which collect on the filter grow into dome-shaped colonies. The coliform bacteria have a gold-green sheen, and are counted directly from the dish. Since some other bacteria may develop a similar color, a confirmation test using more specific media is required. The confirmation procedure requires an additional 24 to 48 hrs to complete the test for suspected positive total coliform tests.

The MPN (most probable number) method uses a test tube full of media with a smaller inverted test tube inside which captures carbon dioxide gas released from the growth of coliform bacteria. A series of dilutions and replicates are set up, and those producing gas in 24 hrs at 35 degrees C

are counted. A statistical analysis is used to determine the most probable number of bacteria cells present.

Our laboratory uses a number of techniques including the membrane filter method. The sample should be collected in a specially prepared, sterile whirl pack bag for the test to be valid. The bags contain a small amount of sodium thiosulfate to remove any chlorine present, and have been sterilized. Sample collection should be done very carefully and directly into the bottle from the tap to avoid contamination of the bottle from hands or a transfer vessel such as a cup. The sample should be kept cool and delivered to the lab within 6 to 24 hrs for analysis. Total coliform bacteria testing is a relatively inexpensive when compared to the cost for the determination of the concentration or presence of viruses, Giardia, or Cryptosporidium.

TREATMENT:

Bacteria are removed by disinfection and/or filtration. Filtration alone may not be completely effective, but can improve the performance of disinfectants by removing sediment that can shelter the bacteria. Methods of adding chlorine to water include solution feeders for dry chlorine or liquid chlorine or by feeding gas chlorine directly from 100, 150, or 2000 lb. cylinders. Gas chlorination is recommended only for larger systems that can support the services of a trained water treatment plant operator. Chlorine is normally dosed to a concentration sufficient to maintain a free residual of at least 0.2 parts per million (PPM).

Other disinfectants include iodine, ozone, ultraviolet light, and physical methods such as boiling or steam sterilization. Chlorination is still the most common disinfection method in the United States, although recent concerns have been raised about the reaction of chlorine with organic matter in water. Such a reaction can result in the formation of trihalomethanes, which are suspect carcinogenic compounds. For most individual water supply systems, the most common form of treatment is ultraviolet disinfection. For information on water testing, please visit our Homeowner Outreach Webpage.

New Ozone Web Page

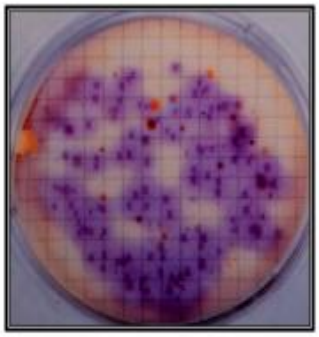
For More information about the Environmental Quality Center, please contact:

Attn: Mr. Brian Oram, Professional Geologist (PG)

Laboratory Director

Analytical Methods

E. coli colonies remain yellow after the urease test.



Yellow colonies that are not E. coli turn pink to purple after the urease test.

mTEC agar method for Escherichia coli: U.S. Environmental Protection Agency Method 1103.1.

Updated October 2007

The mTEC agar method is a two-step membrane-filtration method for detection of Escherichia coli (E. coli). This method can be done in the field or laboratory.

THEORY:

mTEC agar plates are incubated at 35°C for 2 hours followed by incubation at 44.5°C for 22-24 hours. The mTEC agar contains selective and differential agents. Sodium lauryl sulfate and sodium desoxycholate inhibit Gram + cocci and endospore-forming bacteria. Brom-cresol purple and brom-phenol red inhibit nontarget bacteria and cause a color change from purple to yellow when lactose is utilized. Lactose is utilized on mTEC by E. coli and other thermotolerant coliforms.

The mTEC method includes a second step—transfer of the membrane to another substrate for identification of E. coli. After incubation, filter membranes containing yellow colonies are transferred to pads saturated with urea-phenol solution. After 15-20 minutes of incubation on the urea substrate, colonies that are not E. coli turn from yellow to purple. This color change is caused by the breakdown of urea by the enzyme urease and a subsequent increase in pH. E. coli is urease negative, and colonies of E. coli remain yellow, yellow-brown, or yellow-green.

USE:

The mTEC method is recommended for use in monitoring fresh, estuarine, and marine surface waters. It was specifically developed to be used as a measure of recreational water quality.

MEDIA:

The mTEC medium is available commercially in the dehydrated form from Hardy Diagnostics (800/266-

2222, Cat C7741 (500 g)). To prepare urea-phenol substrate, ingredients can be purchased from Fisher Scientific (800/766-7000): phenol red (Cat AC41724-0050 (5 g)) and urea (Cat U15-500 (500 g)). (See preparation instructions (Appendix F)).

Pre-poured plates can be purchased in lots of 20 plates from Fisher Scientific (Cat BD215126) or VWR by special order. Other quantities and plate sizes are available from these manufacturers by special order.

Use phosphate buffered dilution water and 0.45 mm membrane filters. Buffer can be purchased from Hardy Diagnostics (Cat D699 (99mL) or Cat U193 (500mL)). (See buffer preparation (Appendix M))

Pall Gelman Microbiological Media

Wide variety of sterile ampule media for common microbiological analyses in clear glass or plastic ampules. Premeasured for maximum efficiency and convenience. 2mL ampules are a practical time-saving aid for culturing microorganisms on membrane filters. Ready to use; no dilutions necessary.

For water analyses, use MF-Endo Broth or M-FC Broth. Total coliform colonies grown using MF-Endo Broth are deep reddish-purple with a metallic green sheen; fecal coliforms grown using M-FC Broth are royal blue. For beverage quality control applications, use M-TGE Broth for total bacteria, or M-Green YM Broth for yeasts and molds. Meet Membrane Filter (MF) Technique Standard Methods requirements with 2mL ampules (requirements referenced in Standard Methods for the Examination of Water and Wastewater, 1992 APHA, AWWA, WEF, 20th Edition).

2mL wide-mouth glass ampules

Cat.No.	Name	Use	PK
629-68100	M-FC Broth	Fecal coliforms	$36.49 - PK of 20
629-68101	M-FC Broth w/ Rosolic Acid	Fecal coliforms	$36.49 - PK of 20
629-68102	MF-Endo Broth	Total coliforms	$36.49 - PK of 20

2mL plastic ampules

Cat.No.	Name	Use	PK
629-4302	M-FC Broth w/ Rosolic Acid	Fecal coliforms	$64.09 - PK of 50
629-68105	MF-Endo Broth	Total coliforms	$64.08 - PK of 50
629-68106	M-TGE Broth	Total bacteria	$64.09 - PK of 50
629-68107	M-Green YM Broth	Yeast and mold	$64.09 - PK of 50

Total Display Time: 2.39 sec.

Water Quality Testing

How to Do It Simply, Inexpensively and Accurately

BETA VERSION

Summary: A set of downloadable files which will help you learn how to do water testing as simply and inexpensively as possible.

On this page:

- Who needs this information?
- What the packet covers
- System requirements
- Excerpts

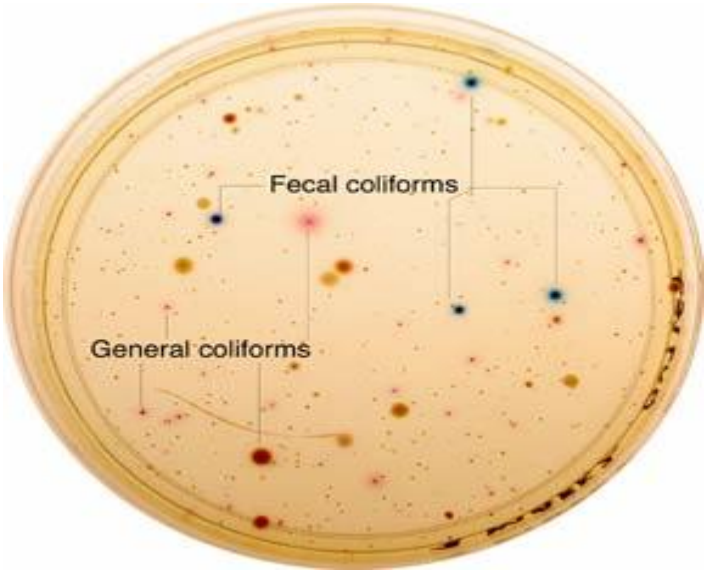
Village women in Mexico monitoring wells for nitrate contamination.

Testing village drinking water with Coliscan Membrane Filtration and Hach presence/ absence tests. The coliform levels from the drinking water well were consistently lower than the purchased bottled water, and frequently zero coloforms per 100 ml, which is a lower level than that frequently found in bottled drinking water in the US.

Testing a spring for electrical conductivity/ Total dissolved solids.

Tap water on right has no general coliforms. Same water after passing through a reverse/ osmosis drinking water filter (left) is positive for general coliforms. It's about to be tested with a UV light to see if it also has fecal colforms (it didn't).

River water in background has 20,000 coliforms per 100 ml, 500 times more than is typically considered safe for swimming. Amazingly, the water from the hand dug shallow well in the foreground had zero.



Photograph of a plate from a local swimming hole showing general and fecal coliform bacteria concentrations (sample size is 5 ml).

Who needs this information?

This information should be especially useful for lay people who have taken an interest in the quality of natural waters or a water system, including water quality activists, surfers, managers of small water systems (particularly those attempting to meet the onerous new clean water act requirements for surface water supplies), rural homeowners, development workers, aid workers and other water guardians who want to learn to test water or improve their existing testing program.

The suppliers of water quality testing supplies seem to assume that users are well-versed in all applicable lab techniques. They also seem to assume that users don't care how much the tests cost, or how much trash is generated.

This packet is designed to give your water quality testing effort a boost by sharing what we've learned about doing cheap, materials-efficient tests that help give an accurate picture of what is going on.

These tests are somewhat, but not too technically involved. If you think you could do high school chemistry lab experiments well if you did them over and over, you'll be fine. Detail orientation and persistence are the keys.

What the packet covers

- How to estimate water flow
- How to test for electrical conductivity (total dissolved solids, or TDS)
- How to test for turbidity (suspended solids, or SS)
- How to test for general and fecal coliform bacteria
- Sources for recommended equipment and materials
- Editable field data entry forms
- Editable computer data entry/ analysis forms
- Examples of hundreds of water samples, showing how they were coded, described, plated, and counted

The examples include samples showing the quality of natural waters, including:

- Oceans, beaches, lagoons, estuaries, surf breaks, and swimming holes
- Beaches
- Rain, tree canopy drip, natural surface runoff
- Groundwater
- Springs, seeps, creeks and rivers
- Natural pools and swimming holes
- Floodwaters

They also include samples from water and wastewater systems, including:

- Spring boxes, raw water pipes, treated water pipes
- Tank inlets, outlets
- The effect of ozone treatment
- Wells
- Ornamental fountains and pools
- Chlorinated water
- Roof runoff
- Harvested rainwater
- Road runoff
- Reverse osmosis tap water
- Raw sewage
- Clarified septic tank effluent
- Constructed wetland effluent
- Greywater

Author: Art Ludwig. Oasis Design, June 2004. 8.5x11, 34 pages, 7 tables. $14.95 for three file set (3.21 mb).

You can download files (or have them e mailed to you) immediately after processing your order.

All files have a 30 day unconditional money back guarantee. If you are not satisfied with the file for any reason, just let us know and your credit charge will be refunded in full (there is no shipping/handling on files).

System and software requirements

All the information is in PDF format and can be viewed/printed with Adobe Acrobat' free reader (download).

Microsoft Word is required to use the editable version of the instructions. Microsoft excel and some idea of how to use it are required to use the editable version of the data entry spreadsheets.

At present there isn't any interpretation of the examples.

This item is new and still somewhat ragged around the edges. We're considering it a "public beta," until we hear back that it is working well for people.

If we'd found it at the start of our water testing program, it would have saved us thousands of dollars of time. Please let us know if you've found it highly useful, incomprehensible, or whatever, using our Feedback page.

Excerpts

Equipment and materials

General and fecal coliform testing materials

Do you need an incubator?

In order to get:

- any results in cold conditions
- best results in hot conditions

- reliable results from the PA tests

you need an incubator. A lab incubator costs several hundred dollars. However, an inexpensive incubator for baby chickens works, if you've got the patience to set the temperature carefully and you keep it away from temperature swings and extremes.

hovabator 1602n incubator weight 4lbs 7.5 x18 x18 $33.95 912 236-0651 fax 234-9978.

A lightweight styrofoam incubator. It has it's own adjustable thermostat and provision for humidifying the interior, which can help keep petri dishes from drying out in arid conditions.

General and fecal coliform levels from 20 to 20,000 per 100 ml¼A cheap and easy test

Use Coliscan® Easygel®.

Coliform Easygel (28001) - Coliform growth medium- 10 tests/set $13.50

Coliscan media have a refrigerated shelf life of 6 months. Frozen, they last a year or more. At room temperature, a couple weeks.

You also need:
1 mL Dropper - # DRP01 Dropper, sterile/individually wrapped -Price: $0.12 ea
or
3 mL Dropper - # DRP03 Dropper, sterile/individually wrapped - Price: $0.14 ea

See Micrology Labs contact info at bottom of next listing.

General and fecal coliform levels from 1 to 100 per 100ml-A somewhat more difficult and time consuming test

Use Coliscan® Membrane filtration kit.

The apparatus is more involved, the materials are still cheap.

Coliscan MF Water Monitoring Kit - # CMFK2:

The kit comes complete. Kit includes: 1 Filtering apparatus, 1 Syringe with hose (vacuum device), 2 Coliscan MF bottles, 20 membrane filters, 20 dishes w/ absorbent pad, 20 3 mL Dropper, 5 filter support pads, Instruction and interpretation guide. $39.50

Coliscan media have a refrigerated shelf life of 6 months. Frozen, they last a year or more. At room temperature, a couple weeks.

1 888 easygel 327-9435 http://www.micrologylabs.com/

Micrology labs is a small operation; you can get people who really know what they are talking about on the phone.

General and fecal coliform levels, presence/ absence in up to 100ml

Presence/Absence Test, with MUG, disposable, twelve pack

Product #: 2401612, $ 40.30

In order to check for fecal coliforms, you also need a UV lamp: Hand-held, battery-operated, long-wave UV lamp. Uses four AA batteries not included.

Product #: 2584600, $ 18.85

800 227-4224 http://www.hach.com/

Electrical conductivity, TDS and Temperature

I like the DiST®5 handheld EC/TDS/Temp meter. It has these featues:

- Adjustable TDS ratio
- Temperature in °C and °F
- Completely waterproof, can be fully immersed in water

- Easy-to-read Custom Dual-level LCD
- Temperature Compensation (BETA B adjustable from 0.0 to 2.4)
- Replaceable Electrode
- Stability Indicator
- Battery Level Indicator
- Automatic calibration
- Auto shut-off

HI98311: DiST® supplied complete with protective cap, 4 x 1.5V batteries and instructions.... $78.00

http://www.automatedaquariums.com/h_98311.htm
Automated Aquarium Systems,™ Inc.
545 South Pacific Street
Tustin, CA 92780 USA
email: sales@automatedaquariums.com
phone/fax: (714) 669-1196

Turbidity

If you have to have EPA reportability, this is the cheapest solution I know of:

2100P Portable Turbidimeter
Features

- Lab Quality Results in a Portable Unit
- Range: 0 to 1000 NTU
- Selectable signal averaging mode compensates for fluctuations in readings caused by movement of large particles in the light path
- Pre-programmed calibration procedure, with microprocessor-controlled adjustment of calibration curve. No potentiometers to adjust
- Electronic zeroing: compensates for electronic and optical offsets. No manual adjustments are required

- Direct digital readout in NTU
- Meets or exceeds USEPA method 180.1 criteria
- Comes with six sample cells, 4 sealed vials of StablCal Primary Standards (<0.1, 20, 100, and 800 NTU), Secondary Gelex Standards, silicone oil and oiling cloth
- Two-year warranty

Product #: 4650000 $ 837.00
800 227-4224 http://www.hach.com/

Air travel with water quality testing materials

Important note:If you're travelling by air with your water test equipment, be sure to take MSDS sheets and a convincing story about how you are a water guardian, not a bioterrorist.

MSDS's are readily obtainable from the manufacturers.

See also:

Fecal coliform measurements
Water test results- Maruata
Slow sand filters
Sewers & water quality
Eco village house
Maruata at the Crossroads (book)
Wild Water Wisdom (article)
Understanding water class
Water central

Customer Satisfaction Survey

WATER ANALYSIS

Water supplies have to be constantly monitored for a variety of materials----bacteria, nitrates, pesticides, metals, etc. In this lab, you will analyze water for bacteria, and, in particular, an indicator group of bacteria called the **coliforms**. Although you will be looking at the total counts of **aerobic** bacteria in the water sample that YOU BRING TO LAB, the coliform bacteria are the critical test organisms which are found in improperly treated water or water that has fecal contamination. They have been used as such for most of the 20th century and still today.

Coliforms are bacteria that are naturally occurring in animals and in the environment: they are indicators of other potentially harmful microorganisms in drinking water. They are all gram negative bacteria that ferment lactose, and are non-sporeforming. **FECAL coliforms**, exemplified by **E. coli**, indicate water contaminated with animal or human waste, i.e. feces. Microbes in the fecal-contaminated water may cause food-borne illness that has short-term symptoms—nausea, diarrhea, vomiting—or if severe enough may cause death. This is a real problem in the immunocompromised, immunodepressed, and babies and children.

METHODS OF EVALUATING FOR BACTERIA

Membrane filter technique: Filtering 100 ml of water through a millipore filter with holes smaller than the bacteria causes the bacteria to be trapped on top of the filter. The filter pad is then placed on special coliform media which allows a colfirm count to be done.
Most probable number: The water sample is diluted and inoculated into a variety of specialized media tubes. The MPN is determined with the help of a standard chart, based on the number of tests that have turned positive.

The results are given as number of coliforms per 100 ml of water. If coliforms are present, the lab will generally recommend that a second sample be analyzed. If the number of coliforms was over 30, a second sample is essentially useless.

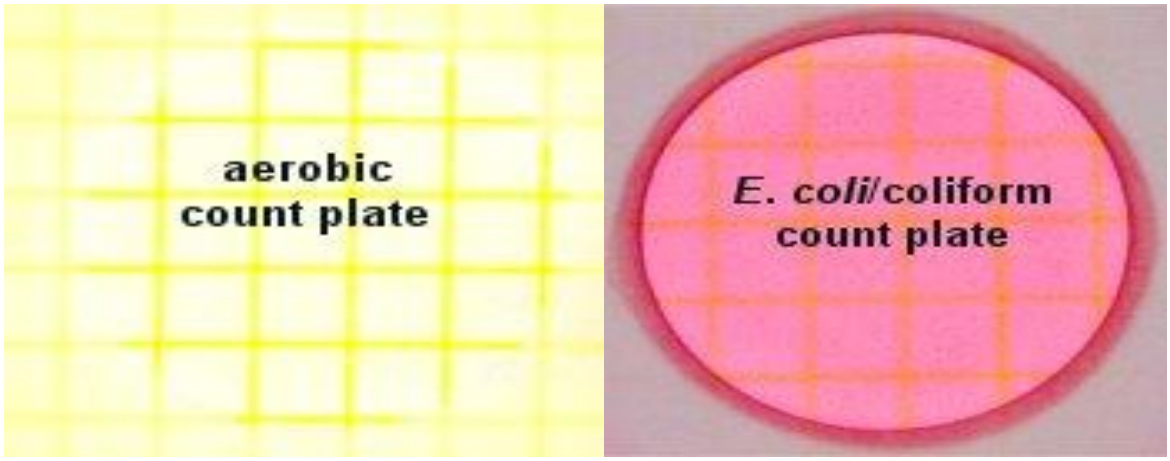
Possible results:

One or more coliform bacteria/100 ml = "does not meet the bacteriological standard purity" specific counts

too numerous to count (TNTC)

confluent growth

This lab incorporates a newer method of performing counts on bacteria, called Petrifilms from 3M corporation. There is more information—-interpretation and photos—-on these materials at **the 3M website**. Since the aerobic counts include all coliforms, the water sample will be diluted out. When the water sample is added to the dehydrated med, the water-soluble gel will rehydrate, forming a thin media plate of sorts.



OBJECTIVES:

Identify coliform bacteria using 3M Petrifilms.

Differentiate between coliforms and fecal coliforms.

Analyze water samples for bacterial counts.

MATERIALS NEEDED: per table

water sample

1 coliform/E. coli count petrifilm

2 aerobic count petrifilms
1 ml pipettes
scissors
2-99 ml phosphate dilution containers
humidified container to incubate petrifilm plates

THE PROCEDURE:

1. Bring water sample to class, preferably a rather dirty sample—not tap water.
2. Prepare a dilution of the water sample by taking a 1ml aliquot and placing it into a 99ml phosphate buffer solution. This is the 1/100 dilution.
3. Using a fresh pipette, prepare a 1/10,000 dilution by taking a 1ml aliquot and placing it into another 99ml phosphate buffer solution.
4. Be sure the containers are shaken well.
5. Place the Petrifilm flat on the table. Lift top film.
6. THE AEROBIC COUNT PLATE:

Place 1 ml of each dilution in the center of 2 petrifilm plates (already labeled with 1/100 and 1/10,000).

7. THE COLIFORM COUNT PLATE

Place 1 ml of the original water sample in the center of the petrifilm plate.

8. Release the top film and allow it to drop.
9. Using the small plastic spreader, ridge side DOWN, place it over the inoculum. Gently apply pressure on spreader to distribute the sample over the circular area. Do not rotate or twist the spreader.
10. Remove the spreader and wait at least 1 minute for the gel to form.
11. Incubate the plates with clear film side up in a humidifed container (1 for the entire class). You may stack the petrifilms.
12. Incubate the petrifilms at 30 degrees C for 48 hours.

INTERPRETATION:

1. Count the colonies on a Quebec colony counter or other magnified light source (with clear films down).
2. Refer to the INTERPRETATION GUIDE in print form or at the 3M website given above.

3. After counting the clear film covers can be lifted, and the colonies can be used for testing or staining.

AEROBIC COUNT PLATE:
There is a red indicator dye in the media gel that colors the colonies. Count all red colonies of any size or intensity of red.

COLIFORM COUNT PLATE:
The pH indicator violet red is incorporated into the gel, along with bile which inhibits gram positive bacteria. In addition, there is an indicator of the enzyme glucuronidase which will turn blue if the bacterium makes the enzyme. You may also see carbon dioxide gas bubbles between the film and the bottom of the petrifilm. Most **E. coli** make both the glucuronidase and CO_2, and those colonies will be blue or blue-red. Non-fecal coliforms will be red colonies.

QUESTIONS:

1. What criteria are used to define the coliform group?

2. Why is there a dye added to the coliform petrifilms?

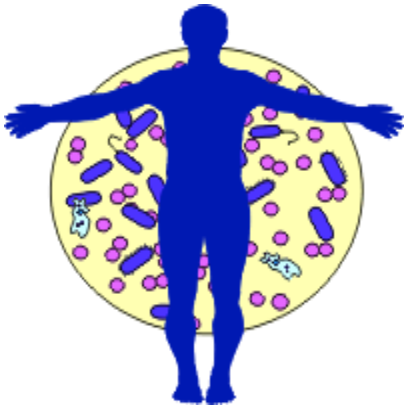
3. Why are coliforms used as indicator organisms for water impurity?

City of Boulder/USGS Water Quality Monitoring

General Information on Fecal Coliform

by Sheila Murphy

TOTAL AND FECAL COLIFORM BACTERIA



The coliform bacteria group consists of several genera of bacteria belonging to the family enterobacteriaceae. These mostly harmless bacteria live in soil, water, and the digestive system of animals. Fecal coliform bacteria, which belong to this group, are present in large numbers in the feces and intestinal tracts of humans and other warm-blooded animals, and can enter water bodies from human and animal waste. If a large number of fecal coliform bacteria (over 200 colonies/100 milliliters (ml) of water sample) are found in water, it is possible that pathogenic (disease- or illness-causing) organisms are also present in the water. Fecal coliform by themselves are usually* not pathogenic; they are indicator organisms, which means they may indicate the presence of other pathogenic bacteria. Pathogens are typically present in such small amounts it is impractical monitor them directly.

Swimming in waters with high levels of fecal coliform bacteria increases the chance of developing illness (fever, nausea or stomach cramps) from pathogens entering the body through the mouth, nose, ears, or cuts in the skin. Diseases and illnesses that can be contracted in water with high fecal coliform counts include typhoid fever, hepatitis, gastroenteritis, dysentery and ear infections. Fecal coliform, like other bacteria, can usually be killed by boiling water or by treating it with chlorine. Washing thoroughly with soap after contact with contaminated water can also help prevent infections.

Fecal coliform, like other bacteria, can usually be killed by boiling water or by treating it with chlorine. Washing thoroughly with soap after contact with contaminated water can also help prevent infections.

Measurement of Fecal Coliform

Bacteria are single-celled organisms that can only be seen with the aid of a very powerful microscope. However, coliform bacteria form colonies as they multiply, which may grow large enough to be seen. By growing and counting colonies of coliform bacteria from a sample of water, it is possible to determine approximately how many bacteria were originally present.

There are several ways coliform bacteria are grown and measured. Methods commonly used include the most probable number (MPN) method and the membrane filter (MF) method.

In the MPN method, a "presumptive test" is performed first. A series of fermentation tubes that contain lauryl tryptose broth are inoculated with the water sample and incubated for 24 hours at 35 □□C. Fermentation tubes are arranged in 3 or more rows, with 5 or 10 tubes per row, with varying dilutions of the samples in the tubes. The fermentation tube contains an inverted tube to trap gases that are produced by the coliform bacteria. After 24 hours, the fermentation tube is examined for gas production. If there is no gas production, the samples are incubated for another 24 hours and reexamined. If gas production is observed by the end of 48 hours, the presumptive test is positive; coliform bacteria are present in the sample. A "confirmed test" is then performed to determine if fecal coliform bacteria are present. For the confirmed test, some of the content of the fermentation tube is transferred with a sterile loop to a fermentation tube containing another broth. The sample is incubated in a water bath at 44.5 □□C for 24 hours. Gas production in the fermentation tube after 24 hours is considered a positive reaction, indicating fecal coliform. Based on which dilutions showed positive for coliform and/or fecal coliform, a table of most probable numbers is used to estimate the coliform content of the sample. The results are reported as most probable number (MPN) of coliform per 100 ml (American Public Health Association, 1998).

The MF method is more rapid than the MPN method, but the results are not as reliable for samples that contain many non-coliform bacteria, high turbidity, and/or toxic substances such as metals or phenols. The water sample is filtered through a sterile membrane filter. The filter is transferred to a sterile petri dish and placed on a nutrient pad saturated with broth. The plates are inverted, placed in watertight plastic bags, and incubated in a water bath at 44.5 degrees C for 24 hours. Colonies produced by fecal coliform bacteria are blue, and are counted using a microscope or magnifying lens. The fecal coliform density is recorded as the number of organisms per 100 ml.

Sometimes the unit of colony producing units per 100 milliliters of water (CPU/100 ml) is used; this is equal to the number of organisms per 100 ml.

Factors Affecting Fecal Coliform

Wastewater and Septic System Effluent

Fecal coliform is present in human waste, so the bacteria goes down the drains in our houses and businesses, and can enter streams from illegal or leaky sanitary sewer connections, poorly functioning septic systems, and poorly functioning wastewater treatment plant (WWTPs) effluent.

Animal Waste

A significant amount of fecal coliform is released in the wastes produced by animals. This can be a serious problem in waters near cattle feedlots, hog farms, dairies, and barnyards that have poor animal keeping practices and waste is not properly contained. In urban areas, fecal coliform can be contributed to surface water by dog, cat, raccoon, and human waste when it is carried into storm drains, creeks, and lakes during storms.

Sediment Load

High amounts of sediment are often related to high concentrations of pathogenic bacteria. The bacteria can attach to sediment particles, escaping invertebrate predators (Murdoch and Cheo, 1996). Fast-running water can carry more sediment, so higher levels of bacteria can occur during high runoff events. Bacteria are much more abundant on soils than in water.

Temperature

Bacteria grow faster at higher temperatures. The growth rate slows drastically at very low temperatures.

Nutrients

High levels of nutrients can increase the growth rate of bacteria.

Water Quality Standards Regarding Fecal Coliform

The U.S. Environmental Protection Agency (EPA) requires all drinking water systems to monitor for total coliforms in distribution systems. The EPA states that no more than 5.0% of samples can test positive for total coliform in a month. (For water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive). Every sample that has total coliforms must be analyzed for fecal coliforms. There cannot be any fecal coliforms in drinking water (U.S. EPA Office of Water current drinking water standards).

For treated drinking water, Colorado Department of Public Health and Environment Water Quality Control Division (CDPHE-WQCD) regulations are similar to those of U.S. EPA; there cannot be any fecal coliform in treated drinking water (see CDPHE-WQCD Primary Drinking Water Regulations for more information).

For domestic water supply, CDPHE-WQCD regulations state that fecal coliform count shall not exceed 2000 fecal coliforms per 100 ml (based on geometric mean of representative samples) (see Reg. 31, Basic Standards and Methodologies for Surface Water).

 CDPHE-WQCD regulations state that waters used for Class 1 primary contact (including such activities as swimming, rafting, and kayaking) should not have fecal coliform counts above 200 fecal coliforms per 100 ml. Waters used for Class 2 secondary contact (non-primary contact waters, including, but not limited to, fishing and other streamside or lakeside recreation) should not have fecal coliform counts above 2000 fecal coliforms per 100 ml.

Other Information About Fecal Coliform

The fecal coliform group includes all of the rod-shaped bacteria that are non-sporeforming, Gram-Negative , lactose-fermenting in 24 hours at 44.5 □□C, and which can grow with or without oxygen.

Fecal coliform is a type of fecal bacteria. Another type of fecal bacteria is Fecal Streptococcus . Fecal Streptococcus is a group of bacteria normally present in large numbers in the intestinal tracts of warm-blooded animals other than humans.

For information on how drinking water is treated to remove bacteria, see The City of Boulder's water quality fact sheet .

*Some strains of Escherichia coli , which are a type of fecal coliform, can cause intestinal illness. One such strain is E. coli O157:H7, which is found in the digestive tract of cattle. For more information on E.coli O157:H7 and other pathogenic bacteria, see the U.S. FDA Center for Food Safety & Applied Nutrition's "Bad Bug Book" .

Select here for a list references used in the preparation of this information
Select here for general information about other water quality parameters.
Select here for interpretation of Temperature data in the Boulder Creek Watershed

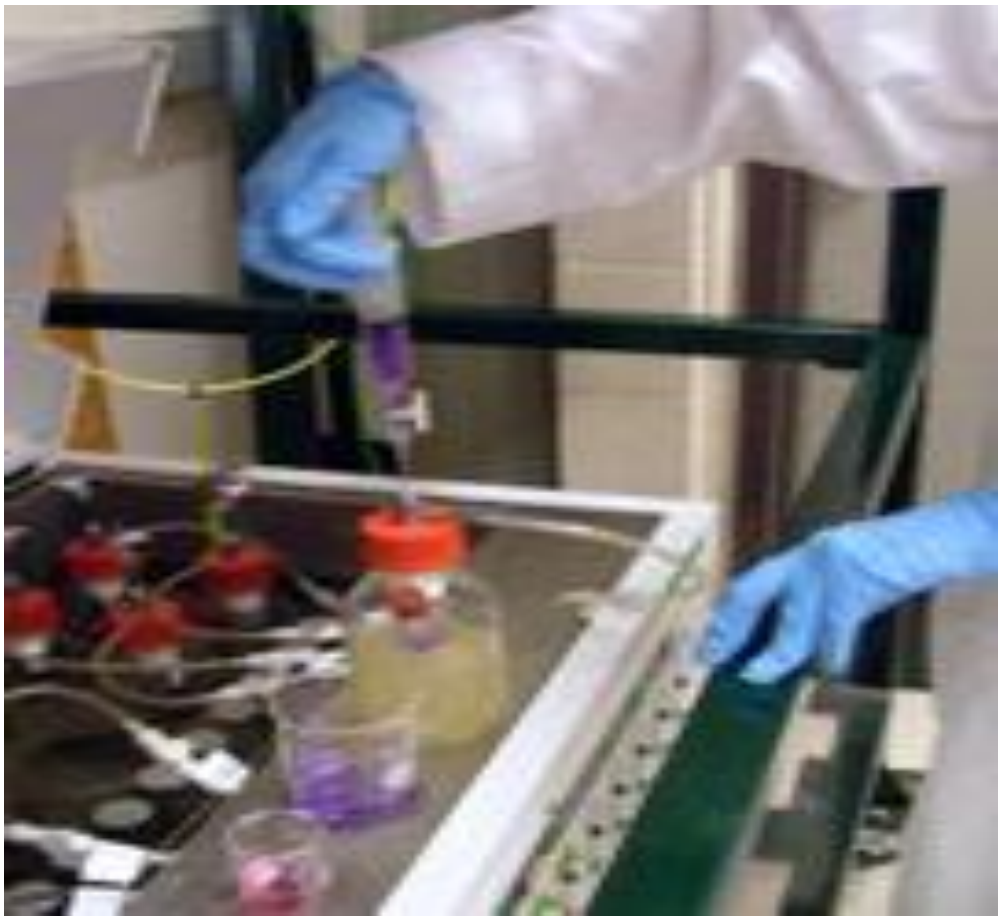
Jump to main content.

Pathogen Equivalency Committee (PEC)

Contact Us **Search:** All EPA This Area Go

- You are here: EPA Home
- Research & Development
- Risk Management Research
- Land
- PEC
- QAPP

Quality Assurance Project Plan (QAPP)



Maintaining a respirometer that measures a sample's oxygen usage.

Table of Contents

- Introduction
- Elements: Analytical Methods Common to Most QAPPs Prepared for Equivalency Recommendations
- Design - Goals and Objectives, Scale, and Scope
- Elements: Quality Assurance & Quality Control Measures
- Design - Pointers on Select Details
- Resources

Introduction

In reviewing Process to Further Reduce Pathogens and Process to Significantly Reduce Pathogens equivalency applications, the Pathogen Equivalency Committee (PEC) will verify that the results submitted in support of a process are statistically significant and were acquired taking into account appropriate quality assurance and quality control measures. (See Tip box below.) In some cases, the PEC may conduct on-site reviews.

A quality assurance project plan (QAPP) should be developed before beginning testing so that the desired quality in sample collection, laboratory analysis, data validation and reporting, and documentation and record keeping is achieved and maintained. A QAPP is a written document that provides a blueprint for the entire project and each specific task to ensure that the project produces reliable data that can be used to meet the project's overall objectives and goals.

TIP

Applicants are now **required** to prepare and submit a quality assurance project plan for the PEC to review **prior to** conducting any research in support of their application for equivalency. Very few exceptions to this requirement will be granted. PEC review and approval of an applicant's project plan prior to data collection will save time and money in the long run by ensuring that the proper data is collected in an appropriate manner and unnecessary data collection is eliminated.

The information required in the application for equivalency recommendation is largely drawn from the QAPP requirements. The extensive overlap between the QAPP and the application is evident in the combined Completeness Checklist below that the PEC uses to evaluate both documents. For this reason, careful preparation of a QAPP will serve as a good head start on the application itself. To assist the applicant in developing such a plan, QAPP Guidelines for Applied Research Projects are provided below. This guidance document contains information on requirements for the project description and objectives, project organization, experimental approach, sampling procedures, testing and measurement protocols, Quality Assurance/Quality Control checks, data reporting, data reduction, data validation, assessments, and references.

An anaerobic digester.

Some basic information on sampling procedures and analytical methods which may be useful in the development of a QAPP are summarized on this page and discussed in greater detail in Chapter 9 (PDF) (11 pp, 601 KB) of EPA/625/R-92/013. A list of quality management tools, including references and training to assist with the development of a QAPP can be found on the EPA's Quality System Web site. The Uniform Federal Policy for Quality Assurance Project Plans (EPA/505/B-04/900A) is another document you may wish to reference. This very thorough document is the result of collaboration between EPA, DoD, and DOE to standardize QAPP requirements and definitions. In addition to consulting these publications, applicants are encouraged to contact a Quality Assurance/Quality Control expert, statistician, or the PEC as they develop their testing plan, to discuss the quality assurance project plan and proposed sampling techniques.

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Elements: Analytical Methods Common to Most QAPPs Prepared for Equivalency Recommendations

Fecal Coliform (either EPA method is preferred)

- EPA Method 1680 (PDF) (51 pp, 1.11 MB): Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Laurel-Tryptose Broth (LTB) and EC Medium
- EPA Method 1681 (PDF) (50 pp, 889 KB): Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A-1 Medium
- Standard Methods for the Examination of Water and Wastewater Methods (18th ed.) 9221 E: Multiple-Tube Fermentation Technique Fecal Coliform Procedure, used in conjunction with Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix F: Sample Preparation Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A-1 Medium (PDF) (4 pp, 366 KB) (EPA/625/R-92/013) Revised July 2003
- Standard Methods for the Examination of Water and Wastewater Methods (18th ed.) 9222 D: Membrane Filter Technique Fecal Coliform Procedure, used in conjunction with Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix F: Sample Preparation Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A-1 Medium (PDF) (4 pp, 366 KB) (EPA/625/R-92/013) Revised July 2003 (acceptable for PSRP equivalencies only)

Salmonella spp.

- EPA Method 1682 (PDF) (48 pp, 1 MB): Salmonella in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium
- EPA/625/R-92/013, Appendix G (PDF) (9 pp, 425 KB): Kenner and Clark (1974) Analytical Method for Salmonella sp. Bacteria, used in conjunction with Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix F: Sample Preparation for Fecal Coliform Tests and Salmonella sp. Analysis (PDF) (4 pp, 366 KB) (EPA/625/R-92/013) Revised July 2003

Enteric Viruses

- Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix H: Method for the Recovery and Assay of Total Culturable Viruses from Sludge (PDF) (16 pp, 355 KB) (EPA/625/R-92/013) Revised July 2003
- **Viable Helminth Ova**

- Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix I: Test Method for Detecting, Enumerating, and Determining the Viability of Ascaris Ova in Sludge (PDF) (7 pp, 510 KB) (EPA/625/R-92/013) Revised July 2003

Percent Total and Volatile Solids (either method is acceptable)

- EPA Method 1684: Total, Fixed, and Volatile Solids in Water, Solids, and Biosolids
- Standard Methods for the Examination of Water and Wastewater Methods 2540 B & E: Total Solids Dried at 103 - 105°C and Fixed and Volatile Solids Ignited at 500°C or Method 2540 G: Total, Fixed, and Volatile Solids in Solid and Semisolid Samples

Optional and Surrogate Indicator Organisms

Specific methods for the analysis of optional and surrogate indicator organisms are not mandatory unlike the organisms used for microbial compliance monitoring of biosolids whose methods (as listed above) are specified by 40 CFR 503.8. However, acceptable protocols for analysis of microbial indicator organisms and specific pathogenic microorganisms have been developed for water, wastewater, soils, foods and other matrixes which have been incorporated into compendiums of industry standard assays or defined as Agency approved methods. Specifically, the references listed below identify methods which may be useful for analysis of biosolids. Caution should be used when selecting and using methods for alternate or surrogate indicator organisms since none of these methods have been subject to multi-laboratory validation studies for sewage sludge or biosolids.

- American Public Health Association, Standard Methods for the Examination of Water and Wastewater 21st Edition. 2005. Washington D.C.

- American Public Health Association, Compendium of Methods for the Microbiological Examination of Foods 4th Edition. 2001. Washington D.C.
- U.S. Food and Drug Administration, Bacteriological Analytical Manual 8th Edition. 1998. Washington D.C.
- U.S. Environmental Protection Agency, EPA Microbiology Home Page. 2007.

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Design - Goals and Objectives, Scale, and Scope

Goals and Objectives

Performing membrane filtration for microbial enumeration.

The goals and objectives of a QAPP prepared for the purposes of supporting an equivalency application will have the same standard components. The goal of such a QAPP will be to support

the equivalency of the process in question to a process to further (or significantly) reduce pathogens on a site-specific or national basis.

The objectives will vary depending on the type of equivalency because the criteria for verifying efficiency differ between a process to significantly reduce pathogens and a process to further reduce pathogens. (See the Equivalency Criteria page.) However, regardless of the equivalency type, the data should be defensible in demonstrating that the process is consistently capable of pathogen reduction on par with accepted processes.

Using a pipette to make accurate volumetric measurements.

Scale

Typically, to receive an equivalency recommendation, laboratory work is performed to establish the boundary conditions of all key process variables, and then pilot or full-scale testing is performed to demonstrate successful scale-up. The required elements of a QAPP can be quite different depending on scale. If laboratory-scale testing is to be used, the applicant may find it easier to divide their research into two phases and carry out the work under separate QAPPs, one for the laboratory-scale work and one for the scale-up work.

Planning a pilot-scale study includes some special considerations if one of its goals is to gather data for scaling up the process to a plant scale. The pilot unit should be truly representative of a full-scale operation. (See definitions of scale.) The conditions of the pilot-scale operation should be no more severe than those expected of the full-scale operation. These conditions will likely include for example, degree of mixing, nature of the flow (batch vs. flow though units and degree of short-circuiting in flow through units), vessel sizing, and proportions of the chemicals used. Any substantial departure in process parameters between the pilot-scale and the full-scale systems that has the potential to reduce the effectiveness of the process will invalidate any approvals given and will require a retest at the new condition.

Scope

A-1 broth inoculation tubes from a multiple tube fermentation analysis for fecal coliform density.

As discussed in the Basic Information web page, whether the goal is a site-specific or a national equivalency will also play a role in the overall QAPP design. For site-specific equivalencies work only needs to be performed on sludge collected from one location. For a national equivalency, however, the work must be repeated with significantly varying sludges. This would entail a combination of laboratory studies on a wide variety of sludges followed by scale-up testing using at least one sludge/location. Or if preferred, a mobile pilot-unit could be constructed and used for all testing, eliminating the need for laboratory studies.

Note that the pilot unit must be a true pilot-scale of the final full-scale system for the equivalency recommendation to apply to the full-scale and not just the pilot scale. (See the pilot-scale definition for more discussion on what is considered a true pilot-scale.)

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Elements: Quality Assurance & Quality Control Measures

- **Quality Assurance Measures** refer to protocols or activities undertaken to assure the reliability of the data collected. These measures generally apply widely to the project as a whole. Examples of quality assurance measures include, but are not limited to the following:
    - Holding times for microbial samples are generally 24 hours or less. A provision for checking holding times and consequences of holding time exceedances should be included.
    - Sample representativeness with respect to sampling and handling procedures can be assured through the use of duplicate sampling. An acceptable range of relative percent difference between a sample and its duplicate (typically 20%) should be set. Data falling outside this range is invalid.
    - Sample representativeness with respect to sample processing and analysis can be assured through replicate analysis. An acceptable range of relative standard deviation among replicate analyzes (typically 10%) should be set. Data falling outside this range is invalid.
    - Calibration and maintenance procedures, schedules, and standards (if applicable) must be specified for all equipment used through the study. For example, temperatures of refrigerators and incubators used should be verified with independent thermometers on a regular basis. Calibration of pH probes should be performed using appropriate standard solution ranges, etc.
- **Quality Control Measures** refer to actions included to assure that defined standards are met in the analysis of data. These measures are generally analyte or method specific and are often defined within the method procedure itself. Not all types of controls are necessary for every analyte. Examples of quality control measures that should be incorporated in a QAPP designed to support process equivalency include, but are not limited to the following:
    - Method blanks to ensure the workspace, handling procedures, and reagents are free from contamination. For example, processing a sample of reagent-grade water along with normal samples when measuring percent total solids. This method blank may not be zero, but should be insignificant compared to the actual samples

(e.g., % solids of the blank should be less than 10% of the lowest measured sample) or the entire data set will be invalid. Some method blanks may be a simple positive or negative. For example, incubation of one MPN tube without inoculation (media control) and one MPN tube after inoculation with pure dilution water when measuring fecal coliform.

- Positive and negative controls establish that the method is working as designed. For these controls, something known to produce the appropriate effect is to be added. For example, inoculating MPN tubes with pure cultures of E. coli and Enterobacter spp. would be a positive and negative control, respectively, for fecal coliform measurement.
- Matrix Spikes are necessary for methods that are known to have low or inconsistent percent recoveries. This includes helminth (Ascaris) ova and enteric viruses, which can have percent recoveries as low as 10-30%. In matrix spikes, known quantities of the analyte are added to the sample. After subtracting out the background level naturally present in the unspiked sample, a percent recovery can be calculated by dividing the measured value by the known spiked value. The matrix will affect the percent recovery so this test must be performed for the untreated and the finished product.

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Design - Pointers on Select Details

Proper project design and sampling techniques are of utmost importance to producing a successful QAPP. Though not all-inclusive, some important points to consider when planning your QAPP include:

Finished biosolids in a storage shelter.

- Accepted, state-of-the-art techniques for sampling and analyzing sludge should be used to ensure the quality of the data.
- The choice of sampling device should be appropriate for the physical characteristics of the sludge (viscosity and solids content).
- Effort must be made to minimize the possibility of sample contamination.
- The samples should be representative of the random and cyclic variation in sludge characteristics that occur during treatment. Representative samples can be obtained by compiling composite samples over volume, by ensuring that each grab sample or aliquot of a composite sample is as representative as possible of the total stream flow passing the sampling point, by establishing an appropriate frequency of sampling that accounts for variation, and by taking an appropriate number of samples to account for variation.
- A pair of input and output samples of non-batch systems can be drawn simultaneously. However, to ensure that measurements are independent, samples should not be taken on successive days. At least one estimated sludge retention time should separate each successive pair of input and output samples.
- If ambient conditions affect sludge microbial characteristics, sludge should be sampled after treatment under the least favorable conditions.
- Sampling, packaging, and shipping procedures should not alter the sludge character or quality.

- Laboratories providing analytical services should be experienced in the analysis of municipal sludges and biosolids and should be able to demonstrate compliance with analytical quality assurance protocols.
- Field verification and documentation by independent or third-party investigators is desirable.

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Resources

You will need Adobe Reader to view some of the files on this page. See EPA's PDF page to learn more.

Below are some resources to assist in the development of a successful and useful quality assurance project plan. Two example QAPPs and mock reviews of these QAPPs using the Completeness Checklist are provided. Although neither QAPP pertains to biosolids or, more specifically, the planning of a project to demonstrate Process to Further Reduce Pathogens or Process to Significantly Reduce Pathogens equivalency, the examples and mock reviews do illustrate the type of information that is required in a well-written QAPP.

QAPP Guidelines for Applied Research Projects (PDF) (5 pp, 110 KB) | **508-Compliant Version**
An annotated outline providing specific information on what to include in a well designed quality assurance project plan.

Completeness Checklist (DOC) (8 pp, 263 KB)
This checklist is used by the PEC to review submitted QAPPs and equivalency applications. It is provided to help applicants double-check that all required and applicable elements have been addressed in their QAPP/equivalency application before submittal.

Example QAPP - Biofilm Growth (DOC) (16 pp, 220 KB)
This example QAPP is entitled Growth rate of Biofilm Organisms in a Distribution System.

QAPP Checklist for Biofilm Growth (DOC) (8 pp, 251 KB)
A Completeness Checklist was filled out for the biofilm growth QAPP in a mock review showing the strengths and weaknesses.

Example QAPP - Particulate Nutrients (DOC) (19 pp, 236 KB)
This example QAPP is entitled Analysis of Particulate Bound Nutrients in Storm water.

QAPP Checklist for Particulate Nutrients (DOC) (9 pp, 252 KB)
A Completeness Checklist was filled out for the particulate nutrients QAPP in a mock review showing the strengths and weaknesses.

EPA's Quality System Web site
Additional resources to assist in the development of a quality assurance project plan can be found on this Web site.

Uniform Federal Policy for QAAPs
This document is a useful reference that provides detailed definitions of common QAPP elements.

You will need MS Word Viewer to view some of the files on this page. See About MS Word Viewer EXIT Disclaimer to learn more.

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وزارة الصحة والسكان
الوزير

بسم الله الرحمن الرحيم

قرار وزير الصحة والسكان
رقم (٨ ٥ ٤) لسنة ٢٠٠٧
بشأن الحدود القصوى للمعايير والمواصفات الواجب توافرها
فى المياه الصالحة للشرب والاستخدام المنزلى.

وزير الصحة والسكان :

بعد الاطلاع على القانون رقم ٢٧ لسنة ١٩٧٨ فى شأن تنظيم الموارد العامة للمياه اللازمة للشرب والاستعمال المنزلى.

وعلى قرار رئيس الجمهورية رقم ٢٧٠٣ لسنة ١٩٦٦ بانشاء اللجنة العليا للمياه.

وعلى قرار رئيس الجمهورية رقم ٢٤٢ لسنة ١٩٩٦ بتنظيم وزارة الصحة والسكان.

وعلى القرار الوزارى رقم ١٠٨ لسنة ١٩٩٥ بشأن المعايير والمواصفات الواجب توافرها فى المياه الصالحة للشرب والاستخدام المنزلى.

وعلى ما أوصت به اللجنة العليا للمياه بجلستها المنعقدة بتاريخ ٢٠٠٧/٥/٧.

وبناء على ما عرضته الادارة المركزية لشئون البيئة.

قـــــــــرر

مادة(١): تخضع المعايير والمواصفات الواجب توافرها فى المياه الصالحة للشرب والاستخدام المنزلى للحدود القصوى الموضحة قرين كل منها بالجداول المرفقة.

مادة(٢): تختص الإدارة المركزية للمعامل بوزارة الصحة والسكان وفروعها بالمحافظات بإجراء الفحوص والتحاليل الخاصة بالمياه المذكورة.

مادة(٣): يكون تنفيذ المعايير وخطط الرصد الذاتى والتفتيش الدورى وفقا لما هو وارد بالملاحق المرفقة بهذا القرار.

مادة(٤): ينشر هذا القرار فى الوقائع المصرية ويعمل به من اليوم التالى لتاريخ نشره، ويلغى كل ما يخالفه من قرارات.

وزير الصحة والسكان
أ.د. حاتم الجبلى

فى: ١٤ /١٠/٢٠٠٧

القاهرة: ٣ ش مجلس الشعب. ص.ب: ١١٥١٦ - ت: ٧٩٥٧٠٤٦ - ٧٩٤١٥٠٧(٠٢٠٢) - فاكس: ٧٩٥٣٩٦٦ (٠٢

رابعا : المعايير الميكروبيولوجيه : -

مسلسل	نوع الفحص	طريقة القياس المتبعة	الحد الأقصى المسموح به
أ	العدد الكلى للبكتريا	صب الأطباق poured plate method	- لا يزيد عن ٥٠ خلية / ١ سم٣ عند درجة حرارة ٣٧ درجة مئوية لمدة ٢٤ ساعة - لا يزيد عن٥٠ خلية / ١ سم٣ عند درجة حرارة ٢٢ درجة مئوية لمدة ٤٨ ساعة
ب	أدلة التلوث بكتيريا القولون الكلية TOTAL COLIFORM	" MF " أو " MPN "	- يجب ان تكون ٩٥ % من العينات التى يتم فحصها خلال العام خالية تماما من بكتيريا القولون حتى ١٠٠ سم٣ من العينة - كما يجب ألا تحتوى اى عينة من العينات على أكثر من ٢ خلية/ ١٠٠ سم ٣ على الا يتكرر ذلك فى عينتان متتاليتان من نفس المصدر
	بكتريا القولون البرازية " باسيل القولون النموذجى "		- يجب أن تكون جميع العينات خالية من باسيل القولون النموذجى .
	البكتريا السبحية البرازية		- يجب أن تكون جميع العينات خالية من الميكروب السبحى البرازى
ج	الفحص البيولوجى - عند فحص عينات المياه للطحالب - عند فحص عينات المياه ميكروسكوبيا		- يجب ألا يزيد نسبة الميكروسستين عن ١ ميكروجرام / لتر ويتم إجراء هذا التحليل فى حالة ظهور نمو مفاجئ للطحالب الخضراء المزرقة BLUE GRAEEN ALGAE أو وجود أعداد عالية منها . - يجب أن تكون خالية تماما من البروتوزوا الحية وجميع أطوار الديدان المسببة للأمراض

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ميكربيولوجيا المياه ـ معامل المحطات

Water Microbiology Plant Labratiores

Dr. Moustafa Atef Kenawy
Microbiologist

Lesson Plan- Presentation

Jan 2009

Deutsche Gesellschaft für Technische Zusammenarbeit - GTZ
Water and Wastewater Management Programme
GTZ Project No. 06.2006.3

خطة التدريس للدورة التدريبية فى مجال ميكروبيولوجيا مياه الشرب معامل المحطات

مقدمة إلى

الشركة القابضة لمياه الشرب و الصرف الصحى

Holding Company for Water and Wastewater

إدارة التدريب Training Unit

إعداد

البرنامج التدريبى ميكروبيولوجيا مياه الشرب
معامل المحطات

Moustafa Atef Kenawy
Microbiologist

Qena Laboratories Training Program
Water Microbiology
GTZ

يناير 2009

المحتويات

أولا: نظرة عامة على البرنامج التدريبى ميكروبيولوجيا المياه (معامل المحطات)

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2. المجموعة المستهدفة
3. عدد المتدربين
4. منهجية التدريب
5. مساعدات التدريب
6. مكان التدريب و طريقة الجلوس بجلسات التدريب

ثانيا: خطة التدريس بالدورة التدريبية ميكروبيولوجيا المياه

دورة(معامل المحطات)

1. أهداف الدورة
2. موضوعات الدورة
3. مدة الدورة
4. البرنامج الزمنى للدورة

أولا: نظرة عامة على البرنامج التدريبى ميكروبيولوجيا المياه (معامل المحطات)

<u>الهدف العام للدورة التدريبية</u>

اختبارات مياه الشرب لتحديد كفاءة المحطات المعالجة و المنتجة لمياه الشرب لصلاحيتها للاستهلاك الآدمى هو الوسيلة للتحقق من الصلاحية حتى لا يكون هناك مخاطر على صحة المستهلك. ومن الناحية البيولوجية يتم الاهتمام بمحتوى المياه من مسببات الأمراض. والبكتريولوجيا من الصعب أن يتم التحليل لجميع مسببات الأمراض لذلك يتم الكشف على ما يسمى بأدلة التلوث البرازى.

وعلى الرغم من أن محتوى المياه العام من البكتريا على صورة العدد الكلى للبكتريا قد يبدو الى أنه ليس له مدلول صحى الا أنه يعطى فكرة عن كفاءة عملية المعالجة ، وحالة نظم توزيع المياه. ولتأثيره على النجاح فى الكشف عن بكتريا القولون والتى هى أح د أدلة التلوث ولثبوت أهميته بالنسبة لفئات من المجتمع مثل كبار السن ومنخفضى المناعة لذلك أهتم بتقديره.

وكشافات التلوث البرازية مثل بكتريا القولون والبكتريا السبحية البرازية يعتمد النجاح فى الكشف عنها على اتباع الطرق الصحيحة فى الكشف عنها.

ولا يتأتى التقييم الص حيح الا باتباع الطرق القياسية فى الكشف عنها وتقدير عددها . ونظرا لأهمية ذلك فان الدورة الح الية تهتم بالتركيز على توضيح الطرق القياسية والاحتياطات اللازم مراعاتها للحصول على النتيجة الصحيحة . وسيتم استعراض الطرق المختلفة الممكن اتباعها وسنصل فى النهاية الى أنسب الطرق الممكن اتباعها لتوحيد طرق اختبار المياه على مستوى المعمل المركزي و معامل المحطات التى لها دور اساسى فى عمليات تقييم اداء محطات المعالجة و شبكات التوزيع.

1. **المجموعة المستهدفة**

الميكروبيولوجيين العاملين بمعامل المحطات لشركات المياه بقنا التابعة للشركة القابضة لمياه الشرب والصرف الصحى.

2. **عدد المتدربين**

يبلغ عدد المتدربين المقدر لحضور دورة ميكروبيولوجيا المياه 11 متدرب من شركات المياه بقنا.

3. **منهجية التدريب**

تعتمد منهجية التدريب بالدورة على عدة اسس يكون الهدف الرئيسى منها توصيل المعلومة بسهولة ويسر للمتدرب وكذلك ضمان المشاركة الفعالة من المتدربين أثناء جلسات التدريب والتأكد من الفهم الكامل لمحتويات وموضوعات الدورة والتدريب العملى والشخصى على الموضوعات التى ستتناولها الدورة.

هذا ويمكن تلخيص المنهجية المتبعة فيما يلى:

- **تقيم تحريرى:** لتحديد الموقف الفعلي للمعمل
- **المحاضرات:** التى يلقيها المدرب ذا الخبرة بهدف توصيل أحدث المعلومات على صورة نظرية وعملية والتأكد من التطبيق العملى بطريقة صحيحة وعلى أساس من الفهم مما يمكنه من تلاشى الأخطاء التى من الممكن أن تلعب دورا فى صحة النتائج التى يتحصل عليها والتى تهتم بجودة مياه الشرب.

- **الشرائح Power point:** التى تعرض أثناء الشرح لإبراز النقاط الرئيسية لكل موضوع فى تسلسل منطقى ولضمان وتثبيت المعلومة لدى المتدرب.

- **المناقشات المفتوحة:** ويديرها المدرب أو المحاضر وتتيح هذة المناقشات الفرصة لتبادل الأراء وتوجبه الأسئلة و الحصول على معلومات جديدة كما إنه يتم من خلالها نقل المعارف والخبرة العملية والنظرية من المدرب إلى المتدربين واصلاح لمفاهيم الغير صحيحة أو غير حديثة لدى المتدربين.

- **دراسة الحالات الواقعية:** وهى تفيد فى عرض المشاكل العملية التى يواجهها المتدربون أو التى سوف يواجهونها فى عملهم و أساليب التغلب عليها بالاسلوب العلمى الصحيح.

- **التدريب العملى:** والذى سيتاح بصورة فردية لكل متدرب باستخدام الطرق القياسية الحديثة لضمان الفهم التام والتطبيق الصحيح من المتدرب للمعلومات والطرق العملية التى تم تدريسها.

- **المراجع العلمية و الكودات و المواصفات:** يتم إعطاء المتدرب المراجع العلمية التى أعتمد عليها والتى يمكن الرجوع إليها لزيادة التعمق فى المجال وكذلك الإشارة ومناقشة الكود الخاص بتشغيل محطات معالجة مياه الشرب والمواصفات الحديثة الحاكمة والمعمول بها فى مصر وعلى المستوى الدولى فى مجال مياه الشرب.

- **فى نهاية الدورة يتم تقييم الحاضرين من خلال اختبار تحريرى فى مواد الدورة.**

4. <u>مساعدات التدريب</u>

- جهاز عرض الشرائح (Power Point Projector)
- سبورة بيضاء أو سبورة ورقية
- شاشات عرض.

5. <u>مكان التدريب و طريقة الجلوس بجلسات التدريب</u>

يجلس المتدربون وفى مواجهتهم المحاضر فى المنتصف وعلى يمينه جهاز الكمبيوتر لعرض الشرائح Power Point وشاشة العرض وعلى يساره السبورة البيضاء أو السبورة الورقية ويكون وضع كل من شاشة العرض والسبورة بحيث يسمح بسهولة الرؤية لجميع المتدربين.

وتقدر المساحة المطلوبة لقاعة التدريب بما لا يقل عن 5 × 7 مترا لتستوعب المتدربين والمدرب لتسمح بسهولة حركة المدرب وإمكانية وصولة لأماكن جلوس المتدربين. ويلزم أن تتوفر بالقاعة الإضاءة اللازمة والتهوية الكافية والأجهزة الصوتية المناسبة.

كما يلزم توفير معمل يتسع لعدد 10 – 12 متدرب (يمكن عمل 5 – 6 مجموعة من شخصين على الأكثر) والمعمل يكون مجهز بالامكانيات من أجهزة وأدوات، أما البيئات والكيماويات وبعض المستلزمات فسيقوم بتوفيرها هيئة gtz. تتم دورتان بتكرارها فى المعمل المركزى بمحطة الفسطاط بالقاهرة ودورتان بمكررها بالمعمل المركزى بشركة مياه البحيرة بدمنهور.

ثانيا: خطة التدريس بالدورة التدريبية

أ. المقاييس الميكروبيولوجية

محاضر: م مصطفى عاطف قناوي

تدريبات عملية: مساعد من هيئة العاملين بالمعمل الذى تتم فيه الدورة.

1 أهداف الدورة

*التعرف على البكتريا والصفات الخاصة بها

* انواع الاوساط و الاحتياجات الغذائية اللزمة لنمو البكتريا

*طرق التعقيم المختلفة

* كيفية تحضير الاوساط الغذائية المستخدمة في التحاليل البكتريولوجية وطرق الحفظ

* تحضير الزجاجيات و الادوات الخاصة بالتحاليل البكتريولوجية

* أسس طرق التحاليل البكتريولوجية للمياه الخام وتتبع كفاءة مراحل المعالجة ومياه الشرب فى الشبكات

* العد الكلى للبكتريا بطرقه المختلفة (الأطباق المصبوبة– أغشية الترشيح)

*طرق تقدير بكتريا القولون الكلية (الأنابيب المتعددة – أغشية الترشيح)

*طرق تقدير مجموعة بكتريا القولون البرازية (الأنابيب المتعددة – أغشية الترشيح).

* المواصفات المصرية لمياه الشرب.

2 موضوعات الدورة

- المقدمة وشرح الهدف من الدورة
- التعرف على البكتريا والصفات الخاصة بها
- انواع الاوساط و الاحتياجات الغذائية اللزمة لنمو البكتريا
- طرق التعقيم المختلفة

- كيفية تحضير الاوساط الغذائية المستخدمة في التحاليل البكتريولوجية وطرق الحفظ
- تحضير الزجاجيات و الادوات الخاصة بالتحاليل البكتريولوجية
- العدد الكلى للبكتريا الهتيروتروفية فى المياه مدلوله واستخداماته
- طريقة الأطباق المصبوبة لتقدير العدد الكلى للبكتريا الهتيروتروفية فى المياه
- طريقة الفرد على سطح الأطباق لتقدير العدد الكلى للبكتريا الهتيروتروفية فى المياه
- طريقة استخدام أغشية الترشيح لتقدير العدد الكلى للبكتريا الهتيروتروفية فى المياه
- كشافات التلوث
- تقدير بكتريا القولون الكلية (طريقة الأنابيب المتعددة وطريقة أغشية الترشيح)
- تقدير بكتريا القولون البرازية (طريقة الأنابيب المتعددة وطريقة أغشية الترشيح)

3 مدة الدورة

تستغرق الدورة مدة ستة أيام متواصلة و يبدأ العمل يوميا من الساعة الثامنة والنصف صباحا حتى الساعة الخامسة والنصف بعد الظهر، أى مدة تسع ساعات (بواقع ثلاث ساعات نظرى وخمس ساعات عملى) يوميا يتخللها ساعة لتناول المشروبات والغداء.

أ.4 البرنامج الزمنى للدورة

اليوم	الجلسة	التوقيت	الموضوع	المحتوى
اليوم الأول	التسجيل	8 – 8.30	إستقبال و تسجيل المشاركين فى الدورة	
	جلسة الإفتتاح	8.30 – 11.00	المقدمة والهدف	• مقدمة عامة • توزيع استمارات التعارف • التقيم المبدئي • أهداف الدورة • النتيجة المرجوة
	الجلسة الأولى	11.30 – 1.30	صفات البكتويا + عملي	• تعريف البكتيريا • الصفات المظهرية للبكتيريا • التركيب الخاص بها • مناقشة
	الجلسة الثانية	2.30 – 5.30	عملى	• غسيل و تحضير الدوات المعملية • تعقيم الادوات المعملية.

اليوم	الجلسة	التوقيت	الموضوع	المحتوى
اليوم الثانى	الجلسة الثالثة	11.00 – 8.30	الاوساط الغذائية	• انواع الاوساط الغذائية • طرق التحضير • طرق حفظ و تخزين الاوساط الغذائية
	الجلسة الرابعة	1.30 – 11.30	مياه المعامل + عملي	• طرق التحضير • طرق الحفظ و التخزين
	الجلسة الخامسة	5.30 – 2.30	عملى	• تحضير مياه المعامل • تحضير الوساط الغذائية • حفظ و تخزين الاوساط الغذائية
اليوم الثالث	الجلسة السادسة	11.00 – 8.30	طرق التعقيم المختلفة و تحضير الزجاجيات و الادوات	• التعقيم الجاف • التعقيم الرطب • التعقيم الكميائي • التعقيم بالترشيح
	الجلسة السابعة	1.30 – 11.30	البكتريا الهتيروتروفية + عملي	• تعريفه • مدلولها واستخداماتها- تقديرها بالطرق المختلفة
	الجلسة الثامنة	5.30 – 2.30	عملى	• تحضير العينات • زرع العينات • تقدير العدد الكلى للبكتريا فى نوعيات مياه مختلفة وبالطرق المختلفة.

اليوم	الجلسة	التوقيت	الموضوع	المحتوى
اليوم الرابع	الجلسة التاسعة	8.30 – 11.00-	أدلة التلوث بكتريا القولون الكلية	• شروط أدلة التلوث • تقدير مجموعة بكتريا القولون بالأنابيب المتعددة • تقدير كفاءة المحطات من خلال النتائج
	الجلسة العاشرة	11.30 – 2.00	طريقة أغشية الترشيح لتقدير بكتريا القولون + عملي	• شرح فلسفة الطريقة • طريقة اغشية الترشيح لتقدير مجموعة بكتريا القولون
	الجلسة الحادية عشر	2.30 -5.30	عملى	• تحضير البيئات اللازمة لتقدير مجموعة بكتريا القولون • تجهيز أنابيب البيئة لعمل اختبار تقديلا بكتريا القولون الكلية • تطبيق لطريقة الأنابيب المتعددة لتقدير بكتريا القولون الكلية (الاختبار المبدئى).
اليوم الخامس	الجلسة الثانية عشر	8,30-10.3	طرق تقدير بكتريا القولون البرازية بطريقة أغشية الترشيح	• طريقة أغشية الترشيح لتقدير بكتريا القولون البرازية • اختبارات verification

اليوم	الجلسة	التوقيت	الموضوع	المحتوى
	الجلسةالثالثة عشر	2.00 – 11.00	عملى	• تقدير بكتريا القولون البرازية من ناتج تقدير بكتريا القولون الكلية بطريقة الأنابيب المتعددة • الختبار التاكيدي لتقدير بكتريا القولون الكلية بطريقة الأنابيب المتعددة • الختبار التاكيدي لبكتريا السبحية البرازية
	الجلسة الرابعة عشر	5.30 – 3.00	عملى	• قراءة النتائج وتسجيلها ومناقشتها
اليوم السادس	الجلسة الثانية عشر	10.30-8,30	مناقشات	
	الجلسة الثالثة عشر	2.00- 11.00	الاختبار	
	الجلسة الرابعة عشر	4.30 – 3.00	عملى	• قراءة النتائج

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البكتيريا

- تصنيف الكائنات الحية تصنيف الكائنات الحية
- النواة
- البروتينات
- التراكيب الداخلية
- طرق النمو

الصفات المظهرية للخلية البكتيرية (المرفوليجية)

شكل وتجمع الخلايا البكتيرية.

١. البكتيريا المستديرة Spheres

- مفرد Coccus
- أزواج Diplococcus
- سلسة (سبحية) Streptococcus
- مجا ميع Tetrads
- عناقيد غير منتظمة تشبة عنقود العنب Staphylococcus
- تراكيب ذو ثلاثة أبعاد (مكعبات مكونة من ٨ خلايا او اكثر) Sarcina

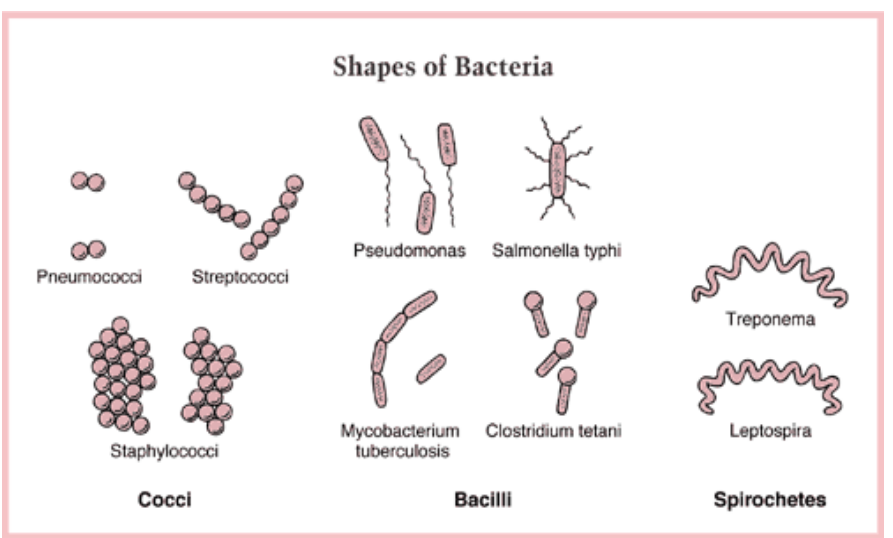
٢. البكتيريا العصوية Rod like

- مفرد Bacillus

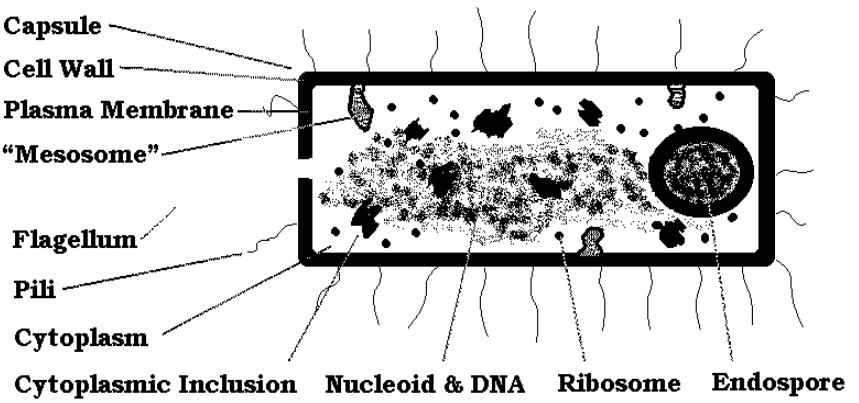
- أزواج Diplobacilli

- سلسة (سبحية) Streptobacilli

* البكتيريا الحلزونية Spiral
* ابلسبيروكيتات Spirochaetes
* الاكتينوميسيتات Actinomycetes
* الكوريني بكتيرات Coryne bacteria
* الميكوبكتيرات Myco bacteria
* البكتيرات الهلامية



التركيب الداخلى للخلية



حركة البكتيريا

-Gliding movement *الحركة الانزلاقية

-Flexion movement *الحركة الدورانية

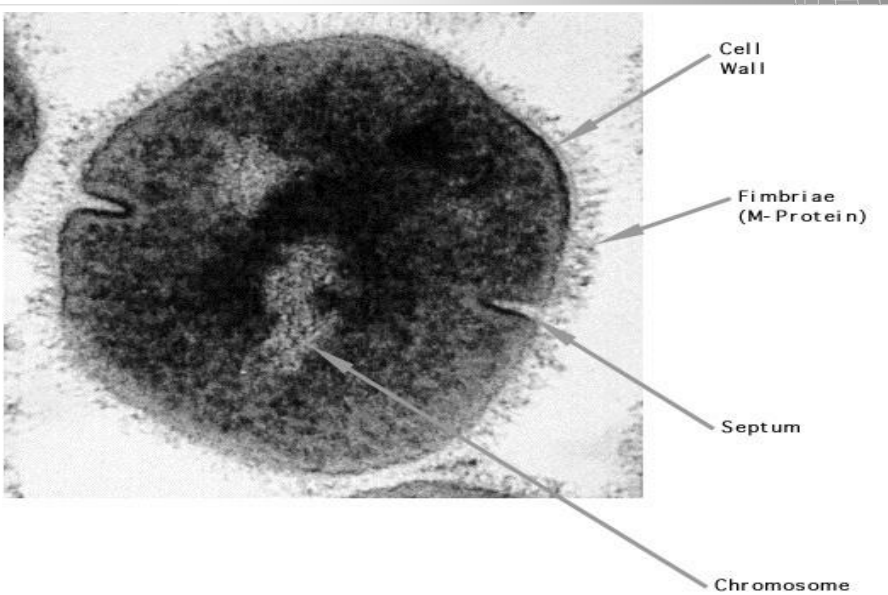
-Flagella *الحركة بالاسواط

النمو في البكتيريا :-

* الزيادة في تعداد الخلايا عن القدر الذي بدأت به المزرعة .

عملية التكاثر الخلوي:-

* إن نمو وانقسام الخلايا البكتيرية يمثل عملية دورية cyclical فكل خلية جديدة تتكون تصبح بدورها ذات قدرة على التكاثر .



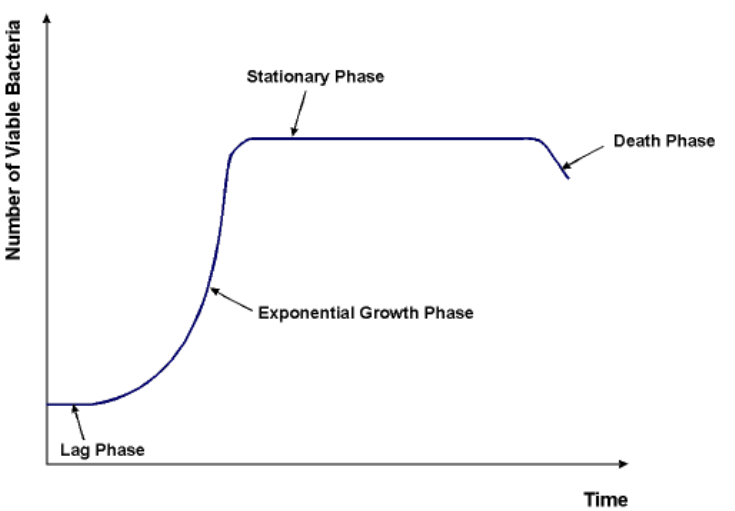
أطوار النمو والظروف التى يتاثر :-

١- طور الركود Lag phase

٢- طور النمو اللوغارتمي Log phase

٣- الطور الثابت Stationary phase

٤- طور تناقص النمو أو طور الموت The phase of decline or death



الاحتياجات الغذائية لا لبكتيريا

- الهيدروجين
- الأكسجين
- الكربون
- النيتروجين
- الأملاح
- بعض العناصر والمعادن

الظروف الفيزيائية التى تؤثر على نمو البكتريا

-الحرارة Temperature:-

- بكتريا محبة للبرودة Psychrophile
- بكتريا محبة للحراره المتوسطة Mesophiles
- بكتريا محبة للحراره المرتفعة Thermophiles

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تاثير درجات الحرارة المختلفة على نمو البكتريا

*درجة الحرارة لاقل نمو

Minimum growth temperature

*درجة الحرارة للنمو الامثل

Optimum growth temperature

*درجة الحرارة لاكثر نمو

Maximum growth temperature

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الاس الهيدروجيني pH

*معظم انواع البكتري تفضل النمو عند pH قريب من التعادل

*بعض انواع البكتريا تستطيع النمو عند pH منخفض acidophiles

*قد يصبح pH عامل محدد لنمو البكتيريا

الرطوبة

تاثر نسبة الرطوبة على نمو انواع من البكتيريا

الماء المعامل

الخصائص

*ماء مقطر أو Demineralized water

*Toxicity(فلوريد - الفضة - الرصاص -الكلور)

*يجب ان يكون خالي من التلوث بالمواد المغذية للبكتيريا

أنواع الأوساط الغذائية

*الأوساط الغذائية العامة (General Media)

*الأوساط الغذائية العازلة (Isolation Media)

*الأوساط الغذائية الاختيارية (Selective Media)

طرق التحضير الاوساط الغذائية

- الطرق العامة
- ضبط تفاعل التحضير
- تعقيم الاوساط الغذائية
- طرق حفظ الاوساط الغذائية
- تخزين الاوساط الغذائية

ضبط تفاعل التحضير

طرق التعقيم المختلفة

- التعقيم الجاف
- التعقيم الرطب
- التعقيم الكميائي
- التعقيم بالترشيح

البكتريا الهتيروتروفية

عد الأطباق الهتيروتروفيه

Heterotrophic Plate Count

(HPC)

ما هو الغرض من العد الطبقي؟

- مراقبة كفاءة عمليات معالجة المياه و التطهير.
- تقييم التغيرات فى نوعية المياه النهائية خلال التوزيع والتخزين، ونظافة نظام التوزيع.
- قياس استعادة النمو البكتيرى Re-growth أو مخاطر ما بعد النمو After-growth فى مياه الشرب المعالجة.

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•الأطباق المصبوبة Poured Plates

- بساطة وسهولة في اجراء التحاليل.
- تتسبب في الضرر ثانوى للبكتيريا نتيجية صب الاجار عند درجة حرارة مرتفعة ما بين ٤٣ – ٤٦ مئوية
- يتاثر معدل نمو البكتيريا نتيجة تواجدها في البيئة المائية الفقيرة و نقلها الى بيئة غنية.
- المستعمرات البكترية تكون مغمورة تحت سطح الاجار.
- ان أقصى حجم للعينة ممكن اختباره ١ملل.

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•الأطباق المفرودة Spread Plates Method

- أكثر صعوبة من طريقة PP .
- حجم العينة اصغر منت PP
- تحتاج الى تحضير الأطباق والادوات فى وقت سابق Consume more time.
- يلزم تدريب و مهارة عالية في اجراء التجربة.
- عدم استعمال البيئة عندما تكون كثيرة الرطوبة، أو اذا كانت قد فقدت كمية كبيرة من الرطوبة.

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•أغشية الترشيح Membrane Filter

- السماح بتحليل حجم من العينة أكبر من ١ ملل.
- الطريقة تسمح بعد تركيزات منخفضة من البكتريا فى مياه الشرب النهائية.
- طريقتى SPC , MF وباستخدام بيئتى m-HPC agar and R2A لعد البكتريا فى مياه الشرب ووجد أن طريقة PP تستخلص فقط ٨٠ % من البكتريا فى حين أن طريقة MF تستخلص ١٠٠% من البكتريا سواء باستعمال أى من بيئتى m-HPC or R2A

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استخدام مراقبة البكتريا الهتيروتروفية

عمليات المعالجة

- نسب الازالة بالنسبة للمياه الخام (صفر%)
- تخزين المياه الخام (٥٠ %)
- التجلط والترسيب (٦٢%)
- الترشيح الرملى السريع (٩٦%)
- التطهير (٩٩% أو أكثر).

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كشافات التلوث Indicator and indicator Bacteria

- من الصعب تحديد البكتريا المسببة للأمراض في الماء لصعوبة التحكم بها داخل المعمل.
- عدد البكتريا المسببة للأمراض بالنسبة للكائنات الأخرى قليل جدا (لذلك يجب أن يكون حجم العينة كبير).
- عند اختبار البكتريا المسببة للأمراض يتم استبدالها ببعض البكتريا المثالية التي تستخدم كدليل على جودة المياه (كاشف).

البكتريا المثالية التى تستخدم ككاشف على جودة المياه يجب أن تتوافر بها عدة صفات

- يجب أن تتواجد دائما في حالة وجود الكائنات المسببة للأمراض (كدليل على التلوث).
- ان ينعدم وجوده في المياه النظيفة الغير ملوثة.
- يجب أن تتواجد بكميات كبيرة في المياه الملوثة بالملوثات البرازية.
- يجب أن تكون ملائمتها للظروف البيئية و عمليات المعالجة مماثلة للبكتريا المسببة للأمراض.

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- يجب أن يتم تحديها بطريقة سهلة – بسيطة – غير مكلفة – و تبين نتائج دقيقة في وقت قصير .
- يجب أن تكون نسبتها بالمياه عالية بالنسبة للبكتريا الممرضة.
- يجب أن تكون ثابتة و غير مسببة للأمراض.
- يجب أن تكون مناسبة لكل أنواع مياه الشرب.

***من ذلك وجد أن Coliorm group من البكتريا المثالية للاستخدام ككاشف عن تلوث المياه.

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- **بكتريا القولون كدليل على التلوث**
  - Family : Enterobacteriaceae
  - الصفات : هوائية – لا هوائية اختيارية.
  - سالبة لصبغة جرام Gram negative
  - لا تكون جراثيم Non- spore forming
  - بكتريا خلاياها عصوية الشكل Rod – shaped
  - تحلل سكر اللاكتوز منتجة غاز و حمض في خلال ٤٨ ساعة عند درجة حرارة ٣٥ م.
  - تتواجد Coliform group في أمعاء الحيوانات.

** تستخدم مجموعة آل Coliform لتحديد مدى كفاءة عملية المعالجة وكذلك بالنسبة للشبكات

** كدليل على التلوث بالبكتيريا البرازية.

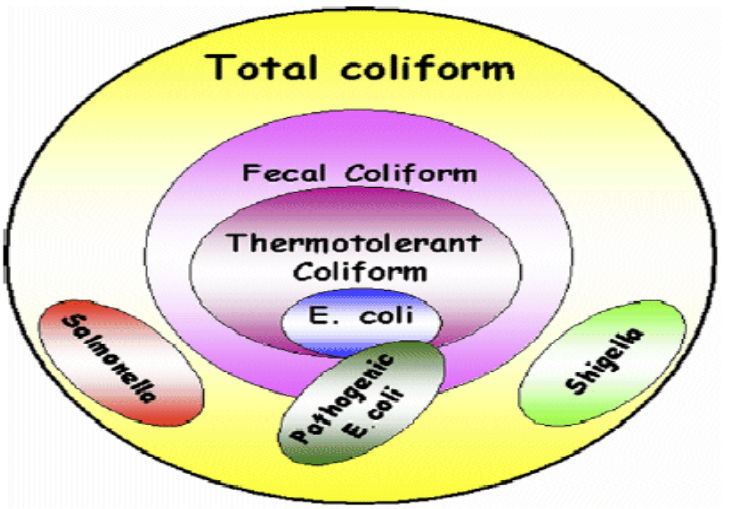
** عند خلو المياه المعالجة من Coliform Bacteria يدل ذلك على انخفاض البكتيريا المسببة للأمراض إلى اقل عدد لها

العيوب

*** Coliform Bacteria اقل مقاومة لعملية التعقيم من بعض الفيروسات و الحيوانات وحيدة الخلية المسببة للأمراض بأشكالها المختلفة

***ليس بالضرورة إذا وجدت الColiform Bacteria ان يوجد تلوث بال Fecal Bacteria

Fecal Coliforms and E.coli



البكتيريا القولونية البرازية

* هي جزء من المجموع الكلية Total coliform Bacteria

*E.Coli هي الجزء الأكبر من البكتيريا القولونية البرازية Fecal Coliform Bacteria

*يمكن تعين كلا من E.Coli و Fecal coliform في المعمل عن طريق قدرتهم على النمو في درجة حرارة ٤٤.٥ م.

*يعتبر كلا من E.Coli و Fecal coliform دلائل على وجود تلوث برازي افضل من ال Total coliform

*لا تستخدم E.Coli و Fecal coliform كدليل على كفاءة عملية المعالجة (على عكس ال Total Coliform) وذلك لان نسبة وجودها اقل من Total Coliform

*وكذلك طبقا لقاعدة Total coliform عندما تعطي العينات نتائج إيجابية لل Total Coliform group يجب ان يتم عمل اختبار لE.Coli و Fecal coliform

العد الاحتمالى لتقدير مجموعة بكتريا القولون بطريقة أنابيب التخمر المتعددة

* هذا الطريقة تعتمد على نظرية الاحتمالات وهى تقديرات لمتوسط كثافة البكتريا (القولون أو غيرها) فى العينة

* تسجل على صورة العدد الأكثر احتمالا.

* يستعمل ١٠ أنابيب مكررة تحتوى على ١٠ ملل أو٥ مكررات من أنابيب تحتوى على ٢٠ ملل.

طريقة التخمر القياسية لتقدير بكتريا القولون الكلية

Standard Total Coliform

Fermentation Technique

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Phase Presumptive المرحلة الافتراضية

* استعمل بيئة Lauryl tryptose فى المرحلة الافتراضية لاختبار الأنابيب المتعددة
* رتب الأنابيب فى صفوف من ٥ أو ١٠ أنابيب فى حامل أنابيب. عدد الصفوف.
* رج العينة أوالتخفيفات جيدا حوالى ٢٥ مرة.
* لقح كل انبوبة فى المجموعة بحجم متكرر من العينة . اخلط العينة مع البيئة بالهز برفق.
* حضن الأنابيب الملقحة أو الزجاجات عند ٣٥ ± ٥، درجة مئوية

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* بعد ٢٤ ساعة ± ٢ ساعة اختبر الأنابيب لوجود نمواو غاز (للايجابية)
* يجرى للأنابيب الموجبة الاختبار التأكيدى
* اذا لم يكن هناك غاز أو حامض ، أعد التحضين والكشف مرة أخرى فى نهاية ٤٨ ساعة ± ٣ ساعة.
* غياب تكون الغاز أو الحموضة فى نهاية التحضين يدل على النتيجة السالبة للاختبار.
* سجل النتيجة الموجبة واحتفظ بالأنابيب .

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المرحلة التأكيدية Confirmed phase

* استعمل بيئة مرق البريلينت جرين لاكتوز بيل للتخمر فى أنابيب للمرحلة التأكيدية فى أنابيب بها درهام.

*رج برفق الأنابيب الايجابية من المرحلة الافتراضية (حامض وغاز) لتعليق النمو من الكائنات فى الأنابيب.

* باستعمال لوب معقم (قطر فتحتها ٣ – ٣,٥ مم) انقل لوب واحدة أو أكثر من المزرعة من كل انبوبة ايجابية الى انبوبة بريلينت جرين.

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*حضن انابيب البريلينت جرين عند ٣٥ مئوية ± ٥، درجة

* تكون غاز فى انبوبة درهام بعد فترة تحضين (من ٢٤ ± ٢ ساعة) وخلال ٤٨ ساعة ± ٣ ساعة.

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طريقة بكتريا القولون البرازي Fecal Coliform

Procedure

* انابيب الاختبار الفرضى Presumptive test (LTB)
التى تظهر نتيجة ايجابية بان يكون فى أنابيب درهام أية كمية من الغازاو النمو خلال ٤٨ ساعة من التحضين يتم اجراء اختبار البكتريا القولونية البرازية.
* استخدم لوب قطر فتحتها ٣ – ٣,٥ مم لنقل النمو من كل انبوبة أو زجاجة موجبة.

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* حضن الأنابيب الملقحة (EC) فى حمام مائى عند ٤٤,٥ ± ٢ درجة مئوية لمدة ٢٤ ± ٢ ساعة.
* ضع كل أنابيب مرق EC الملقحة فى الحمام المائى خلال ٣٠ دقيقة من الانتهاء من حقنها. احرص على أن يكون فى الحمام المائى كمية من المياه تكفى لتغطية مستوى البيئة فى الأنابيب المحضنة.
* انتاج الغاز مع النمو فى بيئة مرق EC خلال ٢٤ +/- ٢ ساعة أو أقل يعتبر الاختبار موجب لبكتريا القولون البرازية.

طريقة أغشية الترشيح لمجموعة بكتريا القولون
Membrane Filter Technique of the Coliform Group

* البكتريا التى تنتج مستعمرات حمراء مع لمعة ذهبية (معدنية) Metallic sheen خلال٢٢-٢٤ ساعة من التحضين على ٣٥ ±٥، درجة مئوية على بيئة من نوع الاندو تعتبر عضومن مجموعة بكتريا القولون.
* اللمعة قد تغطى المستعمرة كلها أو قد تظهر على مركز المستعمرة فقط أو على الحواف .

*تحليل مياه الشرب بترشيح من ١٠٠ الى ١٠٠٠ ملل، أو بترشيح مكررات من عينات صغيرة الحجم مثل اثنان كلا ٥٠ ملل أو ٤ كلا ٢٥ ملل علي بيئة ليز اندو اجار.

*ضع من ٥الى ٧ملل من الوسط الغذائي في كل طبق ثم رشح ١٠٠ ملل من العينات وضع ورقة الترشيح ثم ضع بالحاضن للفترة المذكورة.

طريقة أغشية الترشيح لتقدير بكتريا القولون البرازية

Fecal Coliform Membrane Filter Procedure

* medium M-FC
* ضع ٥ الى ٧ ملل من البيئة m-FC في الاطباق.
* رشح ١٠٠ ملل من العينة ثم ضع ورقة التر شيح على الاطباق وحضن في الحمام المائي لمدة ٢٤ ±٢ ساعة عند ٤٤.٥ ±٢، درجة مؤية.
* العينة الاجابية تعطي درجات مختلفة من الازرق.

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