Bacteriological Examination of Water

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Water examination

Physical examination

Chemical examination

Microbiological examination

Each gram of human feces contains approximately 10 billion of pathogenic bacteria, such as *Salmonella*, associated with gastroenteritis.

In addition, feces may contain pathogenic viruses, protozoa and parasites, these organisms could cause disease. When testing drinking water for contamination,

Health authorities therefore use the presence of more easily detected fecal bacteria as indicators of the presence of fecal contamination

Indicator Bacteria

Group of microorganisms use to reflect the quality and safety of water.

- **Coliform and Fecal coliform bacteria**
- ***Enterococcus* and *Streptococcus faecalis***
- ***Pseudomonas aeruginosa***
- ***Staphylococcus spp***
- ***Clostridium***
- ***Other bacteria (Salmonella & Shigella)***

Criteria for selecting an indicator of fecal contamination in water

(1) They are normally not present in water or soil

(2) They are relatively easy to identify,

(3) They survive a *little* longer in water than enteric pathogens

(4) Must be associated with feces

(5) Test should be less complex than test for pathogen

Table 7.1 Waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Persistence in water supplies[a]	Resistance to chlorine[b]	Relative infectivity[c]	Important animal source
Bacteria					
Burkholderia pseudomallei	Low	May multiply	Low	Low	No
Campylobacter jejuni, C. coli	High	Moderate	Low	Moderate	Yes
Escherichia coli – Pathogenic[d]	High	Moderate	Low	Low	Yes
E. coli – Enterohaemorrhagic	High	Moderate	Low	High	Yes
Legionella spp.	High	Multiply	Low	Moderate	No
Non-tuberculous mycobacteria	Low	Multiply	High	Low	No
Pseudomonas aeruginosa[e]	Moderate	May multiply	Moderate	Low	No
Salmonella typhi	High	Moderate	Low	Low	No
Other salmonellae	High	May multiply	Low	Low	Yes
Shigella spp.	High	Short	Low	Moderate	No
Vibrio cholerae	High	Short	Low	Low	No
Yersinia enterocolitica	High	Long	Low	Low	Yes

- The presences of these microorganisms in water implies that water may contain pathogen or un safe for drinking

- **The presence *E. coli*, in water and often Enterococci, is direct evidence of fecal contamination from warm-blooded organisms.**
- **Their presence indicates the possible presence of pathogens**

- **A few strains of *E. coli* are pathogenic, such as *E. coli* O157:H7, but most strains are not.**

Total Coliforms

The total coliform group

Belongs to the family Enterobacteriaceae and includes the aerobic and facultative anaerobic, gram-negative,

non-spore-forming, rod-shaped bacteria that ferment lactose with gas production within 48 hours at 35oC (APHA, 1998).

Total coliforms include *Escherichia coli, Enterobacter, Klebsiella,* and *Citrobacter.*

Fecal coliforms

- Fecal coliforms are thermotolerant bacteria that include all coliforms that can ferment lactose at 44.5 ^{o}C.
- The fecal coliform group comprises bacteria such as *Escherichia coli* or *Klebsiella pneumonae*.
- The presence of fecal coliforms indicates the presence of fecal material from warm-blooded.

Examination test

- **Qualitative test**
- Determination the presence or absence of coliforms, By **Most Probable Number** (MPN) of coliforms present in 100 ml of water

Examination test

- **Presumptive Test**
- **Confirmed Test**
- **Completed Test**

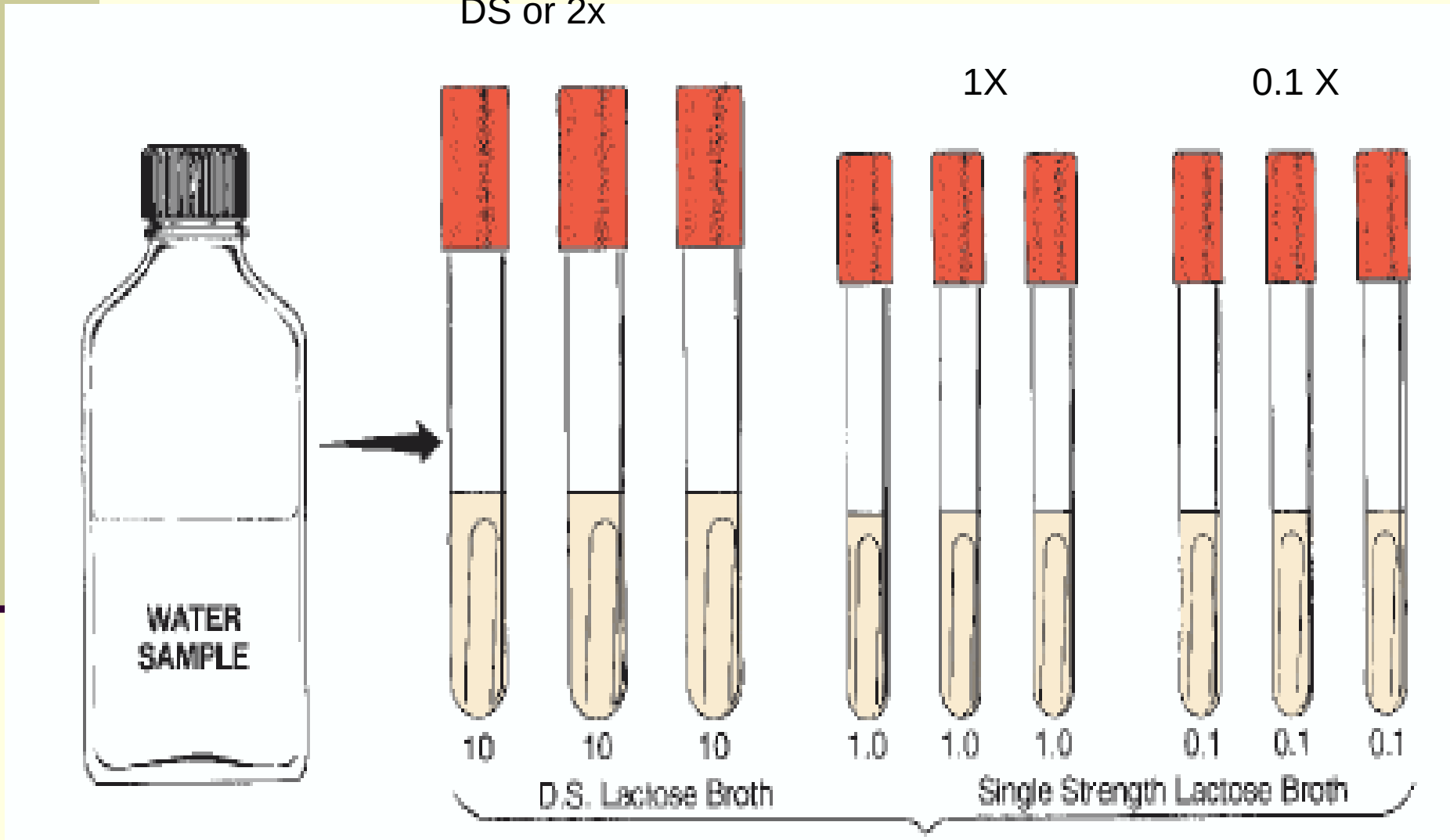
Presumptive Test

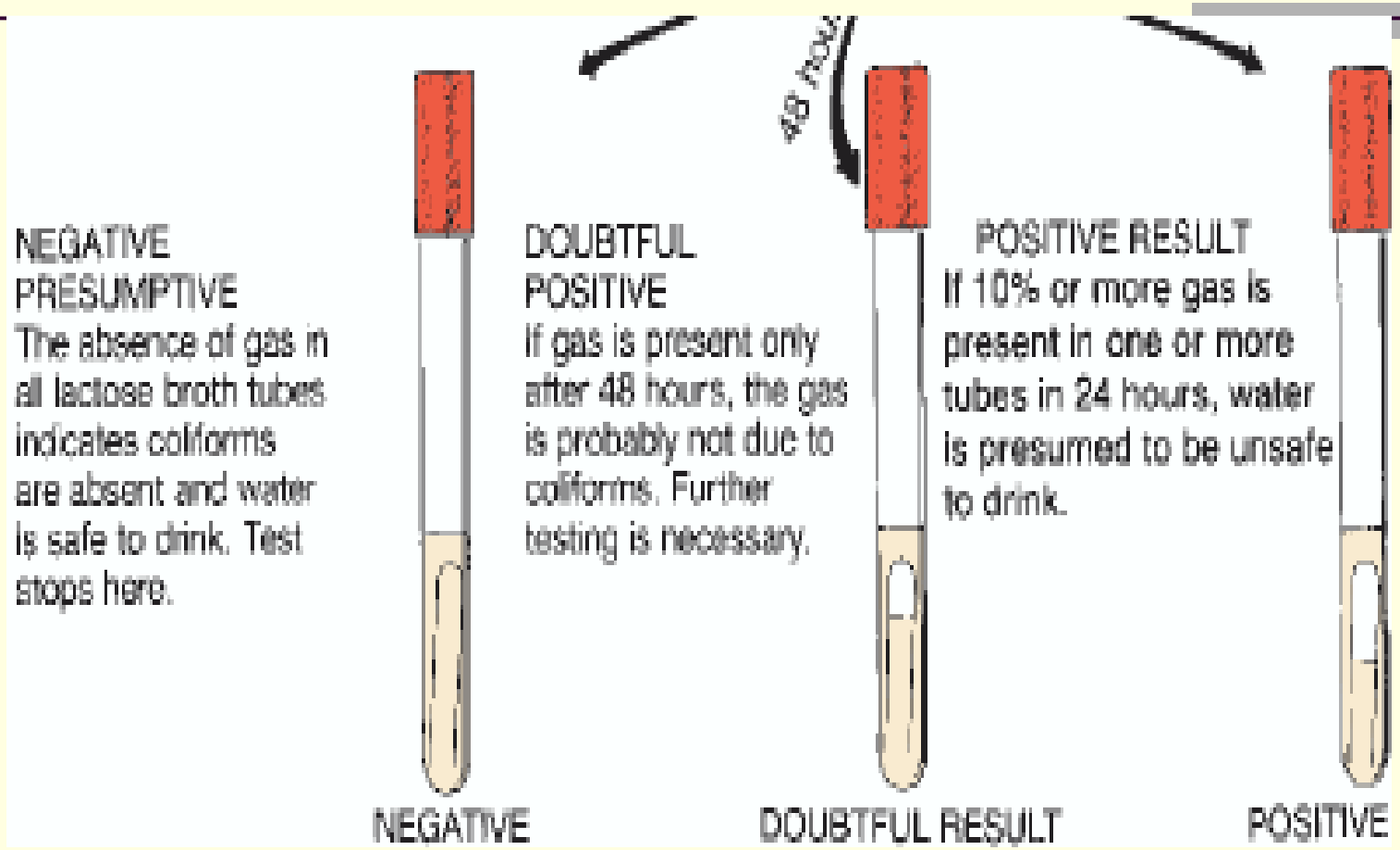
In the presumptive test a series of 9 or 15 tubes of Medium are inoculated with measured amounts of water to see if the water contains any lactose-fermenting bacteria that produce gas.

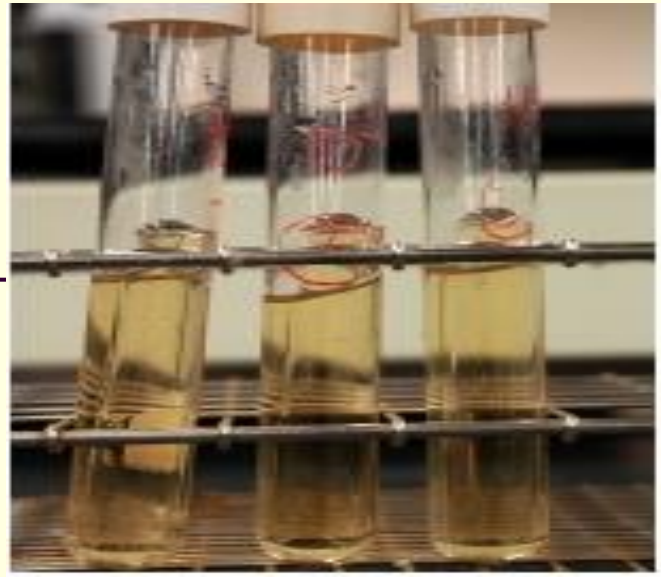
Media used

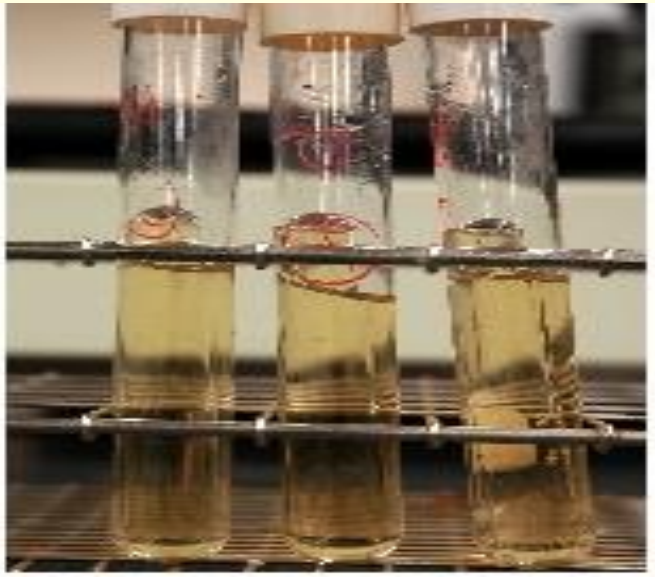
- ***E. coli* broth**
- **Lactose broth**
- MacConky broth
- Brilliant Green Bile Broth

Presumptive test

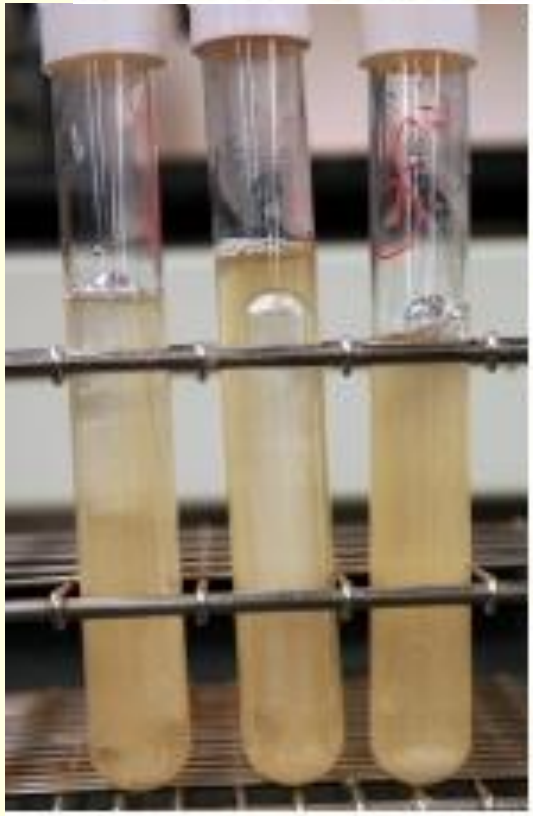


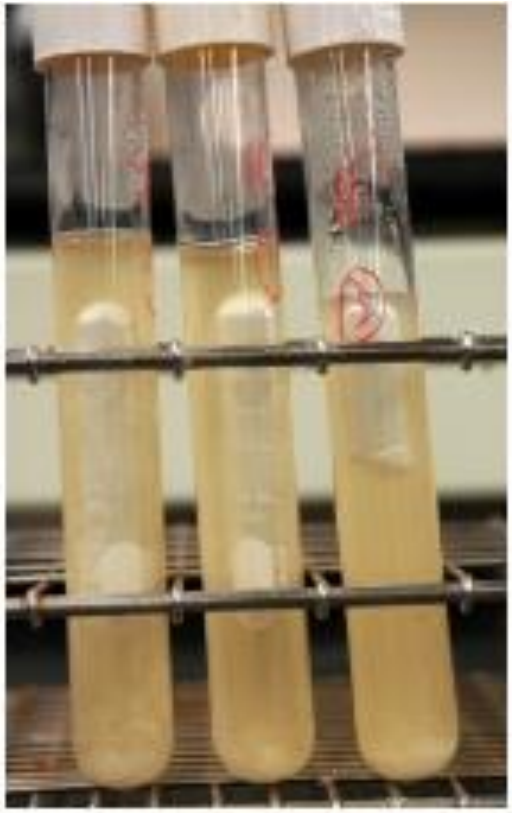

NEGATIVE
PRESUMPTIVE
The absence of gas in all lactose broth tubes indicates coliforms are absent and water is safe to drink. Test stops here.
DOUBTFUL
POSITIVE
If gas is present only after 48 hours, the gas is probably not due to coliforms. Further testing is necessary.
POSITIVE RESULT
If 10% or more gas is present in one or more tubes in 24 hours, water is presumed to be unsafe to drink.
NEGATIVE
DOUBTFUL RESULT
POSITIVE

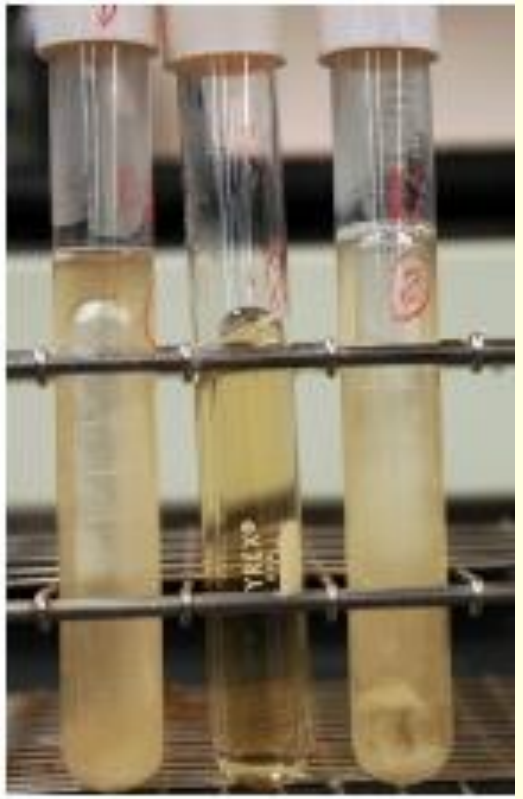




A







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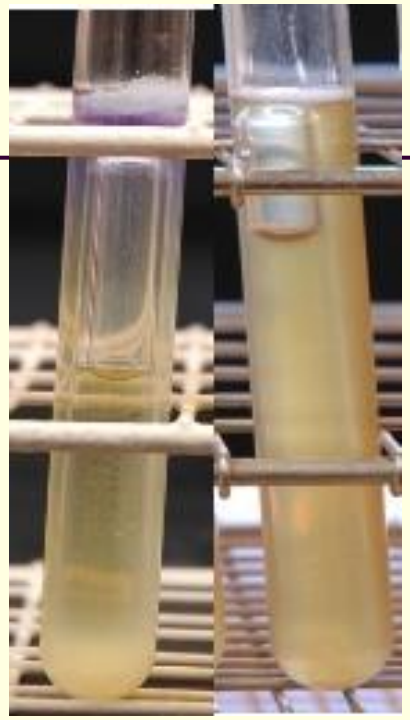
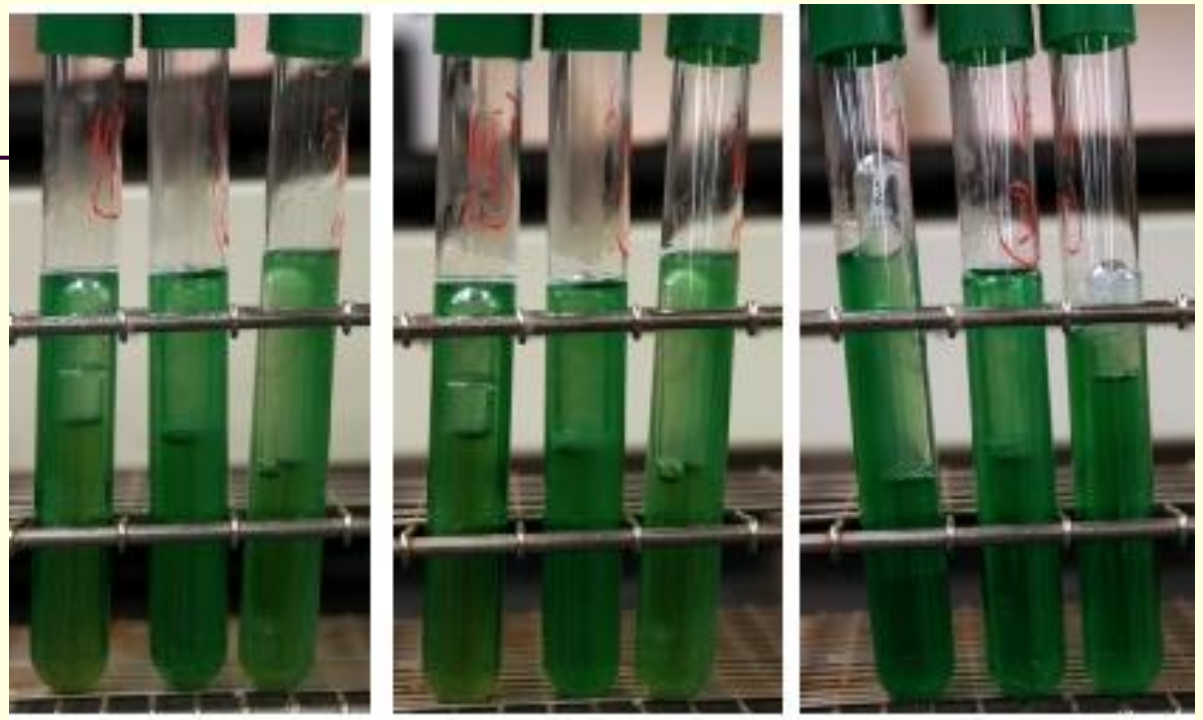


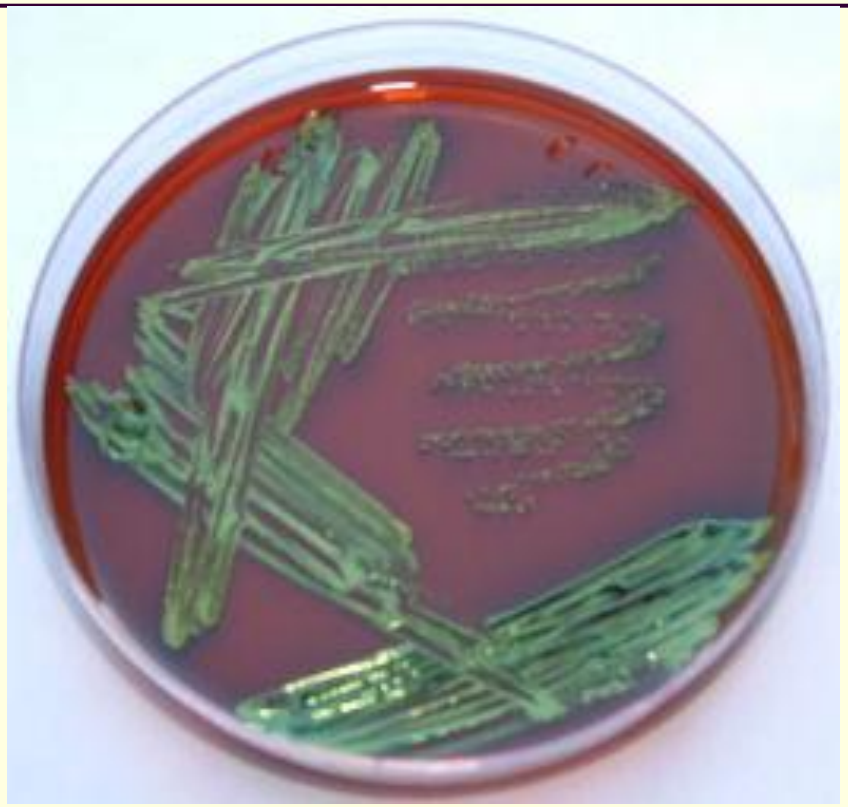
Lactose broth



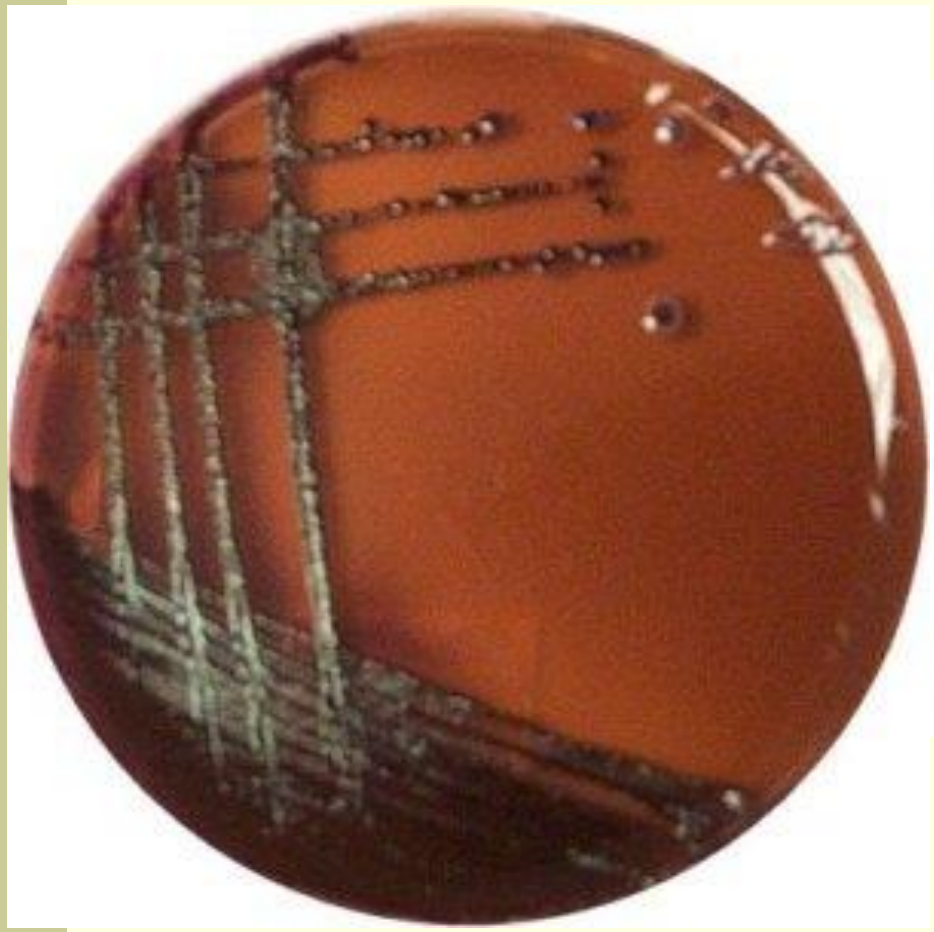
BGBB

NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF			MPN INDEX	95 PERCENT CONFIDENCE LIMITS	
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each	per 100 ml	LOWER	UPPER
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

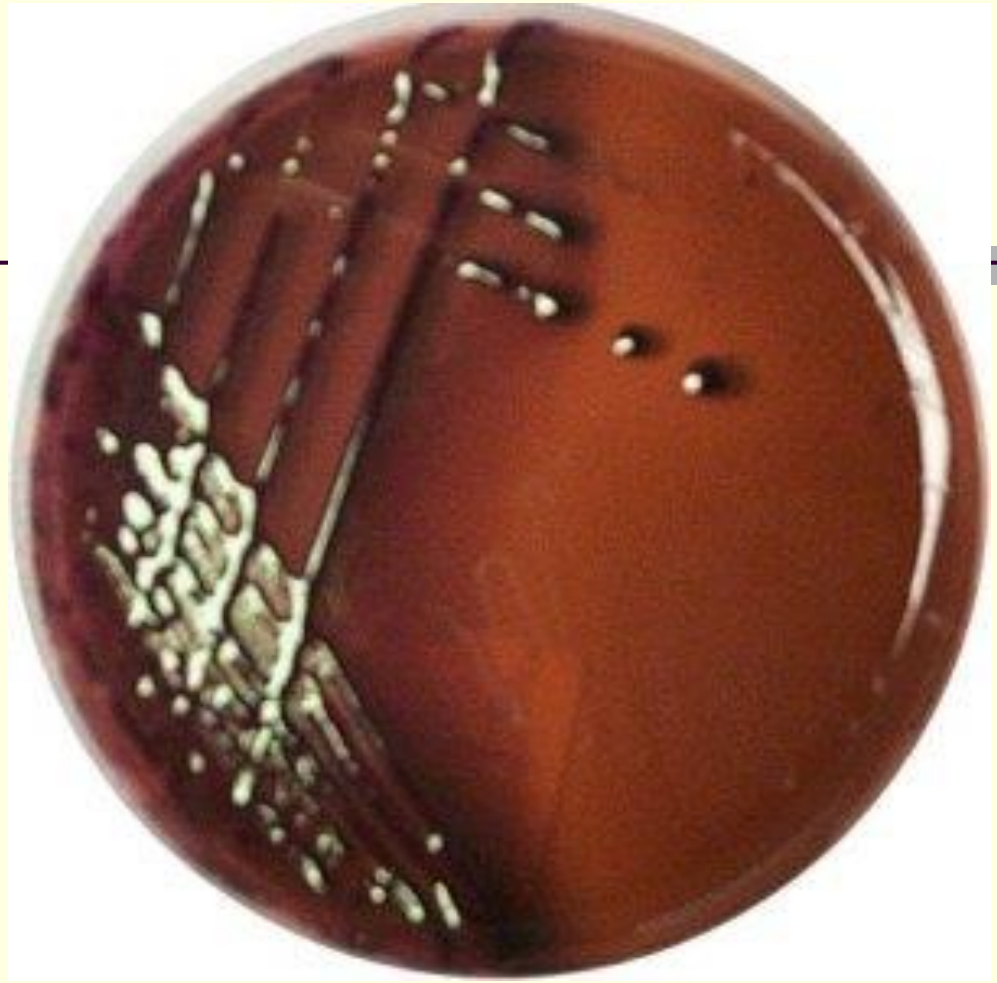
Confirmatory test



***E. coli* on EMB**

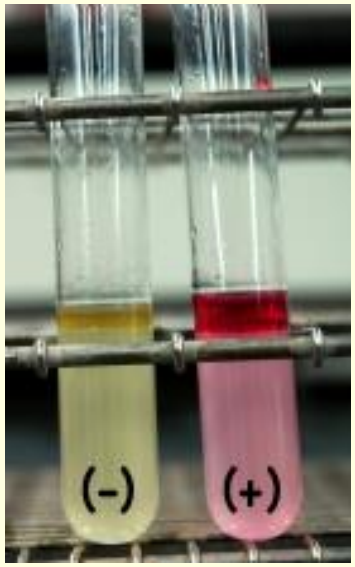


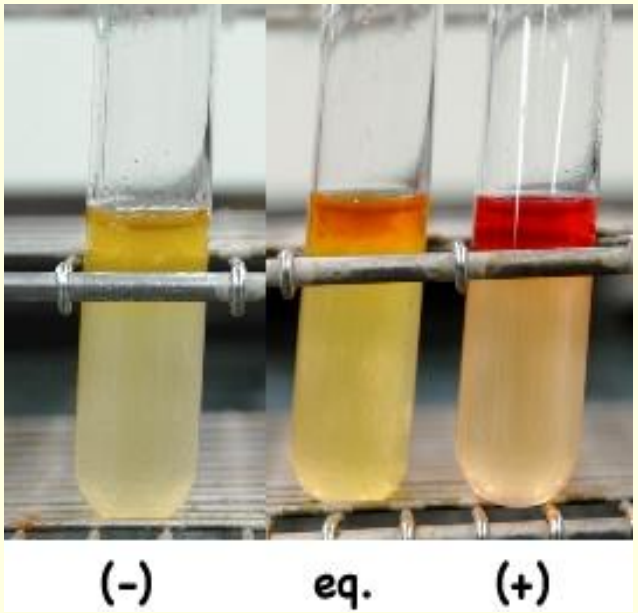
Enterobacter aerogenes Growing on EMB, colonies are less darksurrounded by a wide, light mucoid



Klebsiella which ferment lactose but accumulate less acid, with dark colonies

Complete test





Fecal Coliforms that produce acid and gas from lactose at 44.5± 0.2°C within 24±2h, also known as faecal coliforms due to their role as faecal indicators.

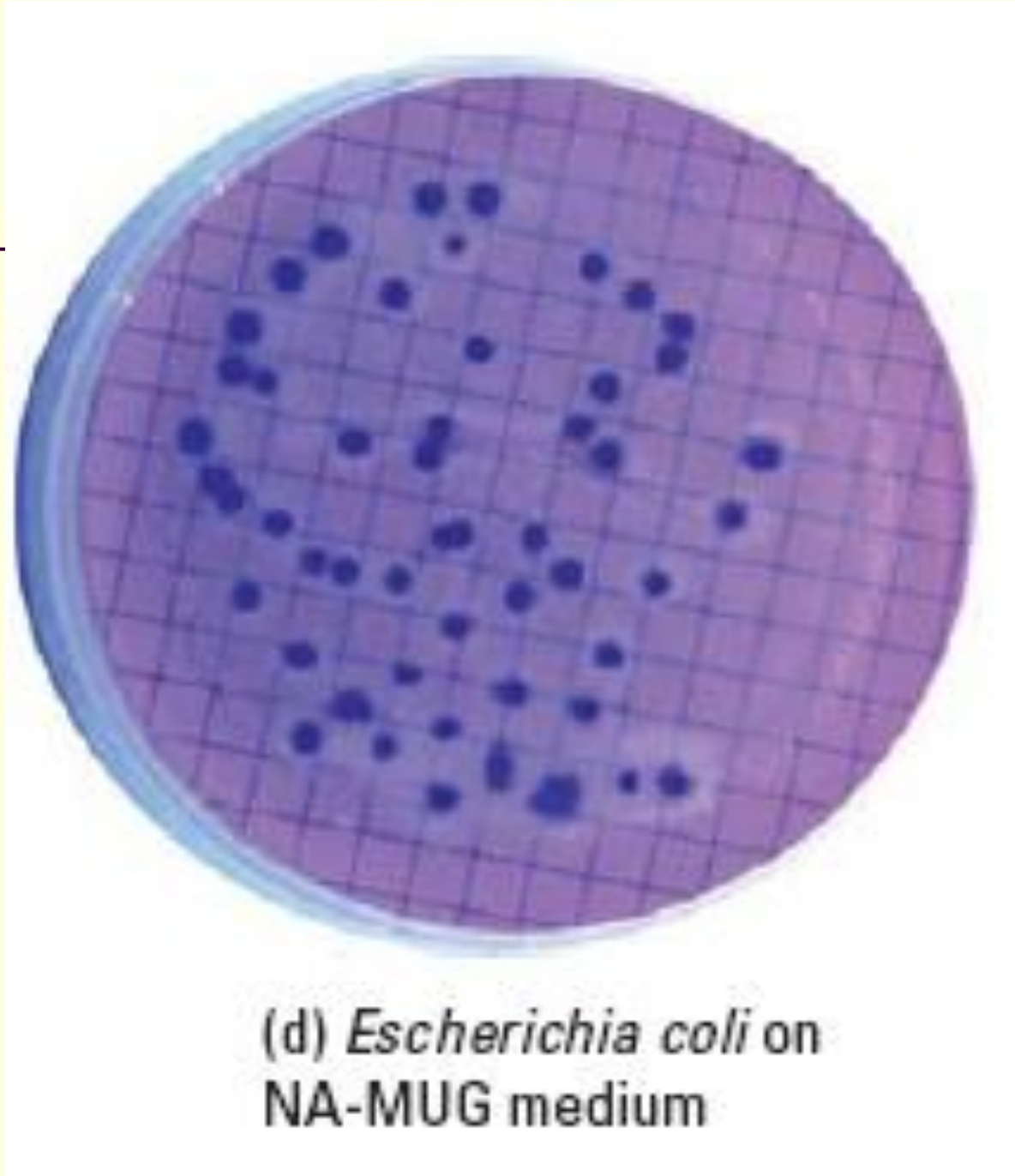
Some Rapid Methods for Coliform Detection

Enzymatic assays provide an alternative approach for rapid and sensitive detection of total coliforms

In most tests, the detection of total coliforms is based on the ß-galactosidase activity.

The enzyme substrates used are chromogenic substrates such as **ONPG** (o-nitrophenyl-b-D-galactopyranoside), **CPRG** (chlorophenol red-b-D galactopyranoside), **X-GAL** (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside).

- Fluorogenic substrates are also used and include 4-methylumbelliferone-D-galactoside (**MUGA)** or fluorescein-di—D-galactopyranoside (**FDG)**



(d) *Escherichia coli* on NA-MUG medium

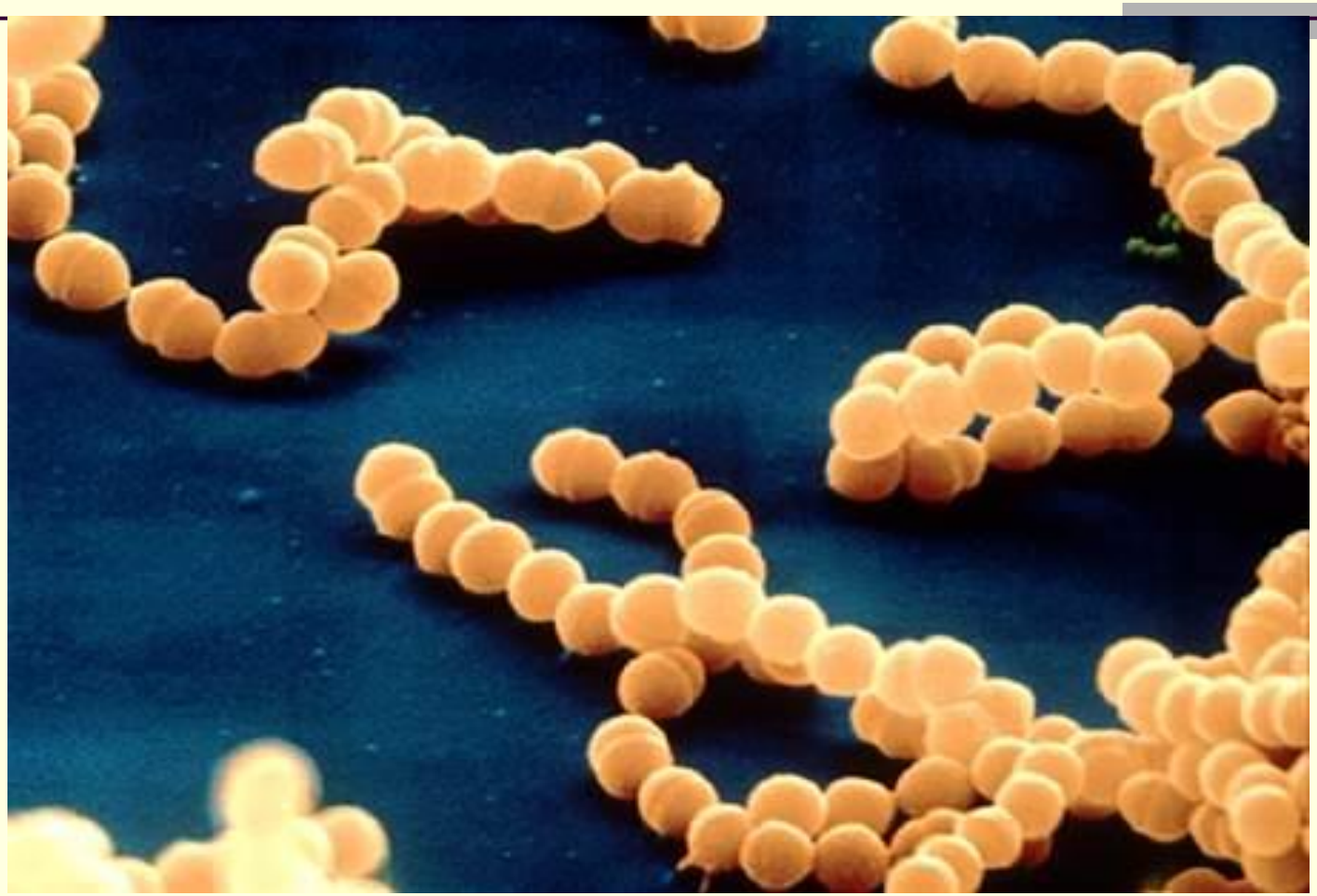
Faecal streptococci (FS):

Gram-positive, catalase-negative cocci from selective media

(e.g. azide dextrose broth or m Enterococcus agar)

- *S. faecalis*
- *S. faecium*
- *S. durans*
- *S. bovis*
- *S.equinus*

Streptococcus faecalis



Fecal streptococci

Azide Dextrose Broth

Approximate Formula* Per Liter

Beef Extract ..... 4.5 g

Pancreatic Digest of Casein ..... 7.5 g

Proteose Peptone No. 3 .....7.5 g

Dextrose ..... 7.5 g

Sodium Chloride ..... 7.5 g

Sodium Azide .. .. 0.2 g

Bromo cresol purple 0.032g

Presumptive test

Staphylococcs aureus

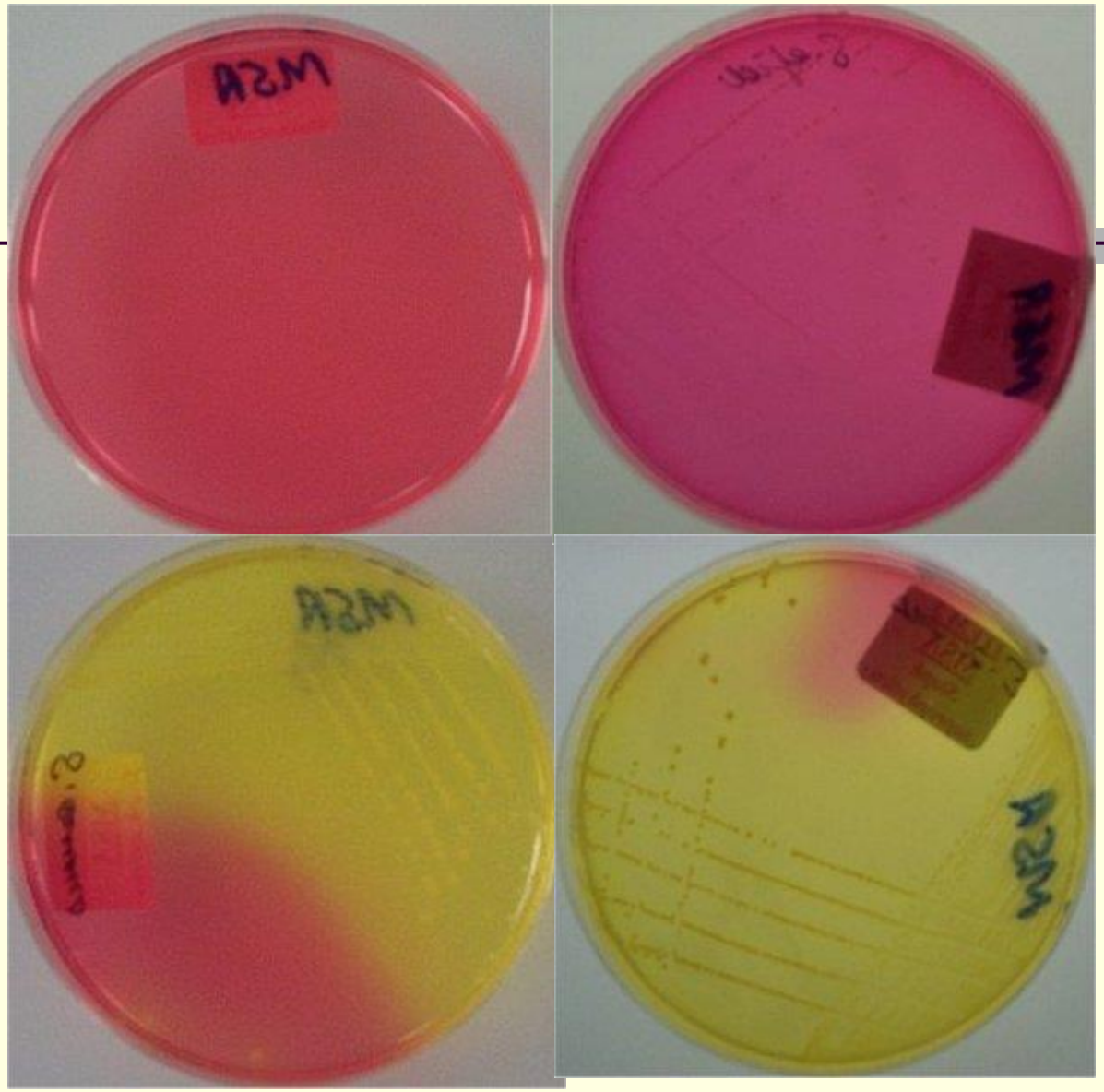
Media Used

- TSA with Pyruvic acid and NaCl
- MSA Mannitol Salt Agar
- **BAIRD-PARKER AGAR BASE**

Mannitol Salt Agar

- Proteose Peptone ..... 10.0 g
- Beef Extract..... ..... 1.0 g
- D-Mannitol ..... 10.0 g
- Sodium Chloride ..... 75.0 g
- Agar ..... 15.0 g
- Phenol Red ..... 25.0 mg

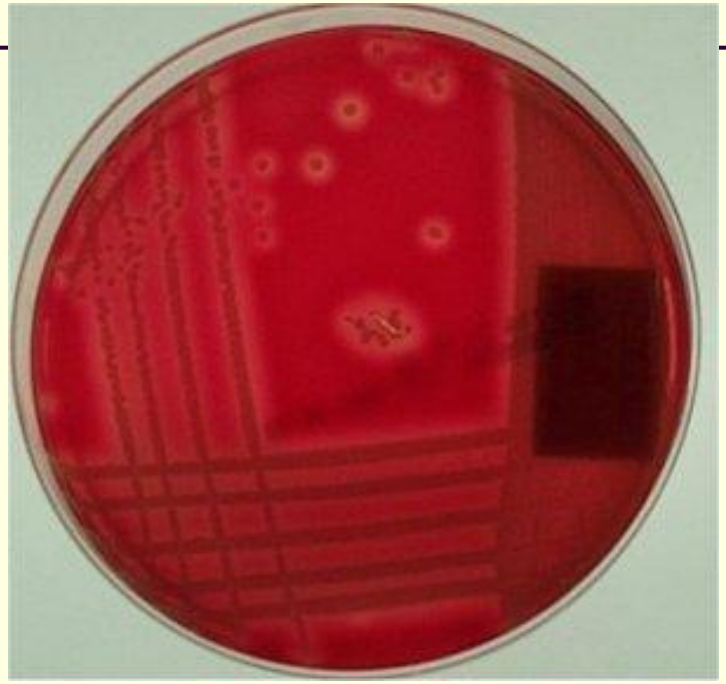
***Staphylococcs aureus on* MSA**



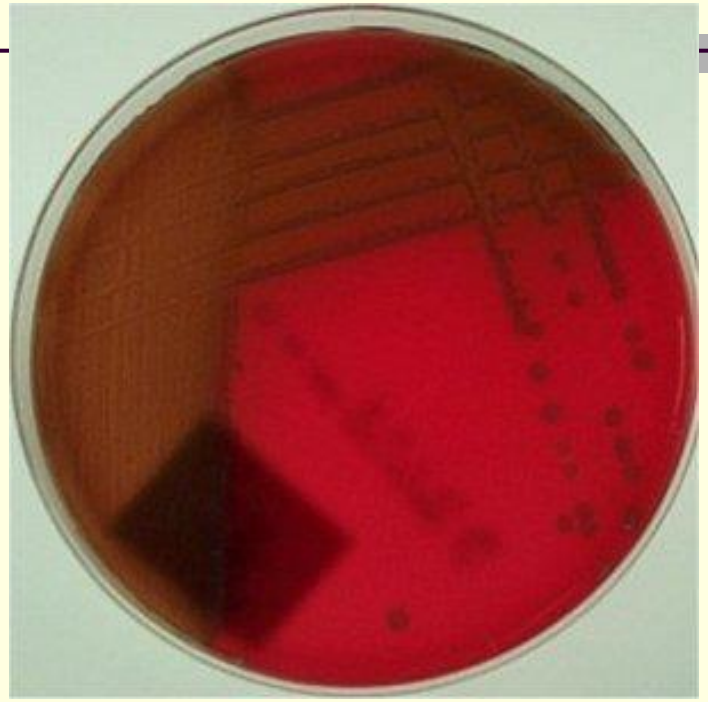
***Staphylococcs spp on* MSA**

Confirmed test

Complete test



Staphylococcs aureus



S. epidermises

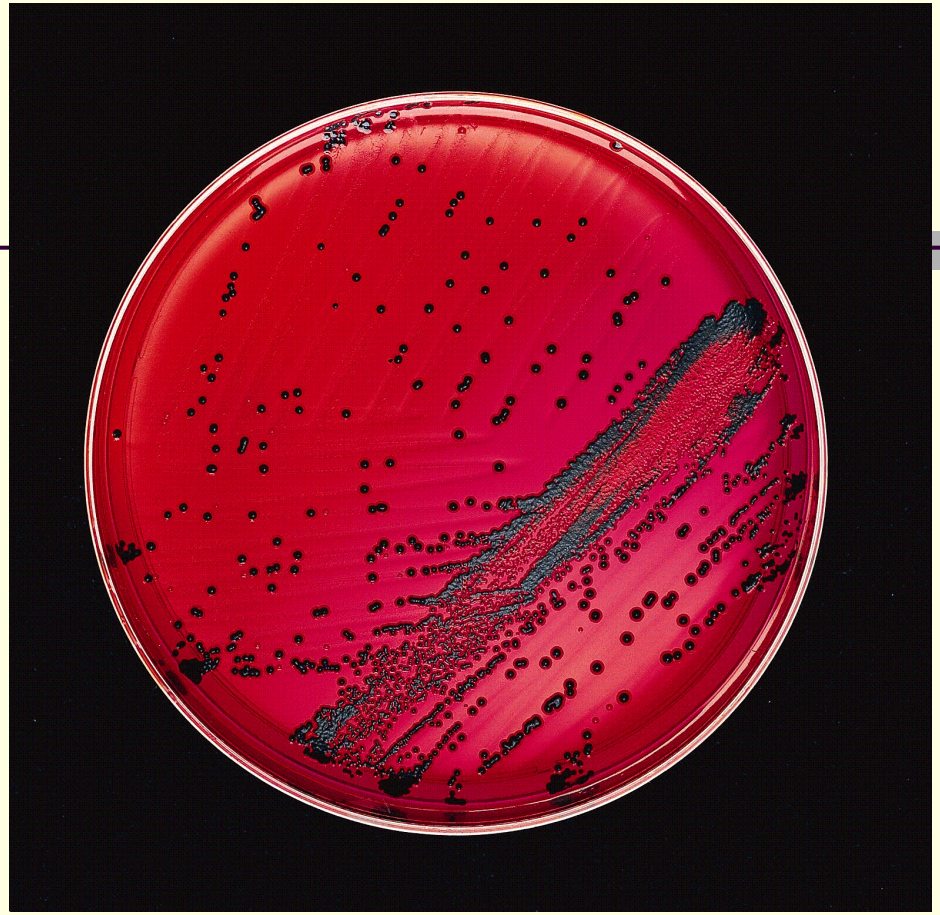
S. saprophitics

Salmonella spp.

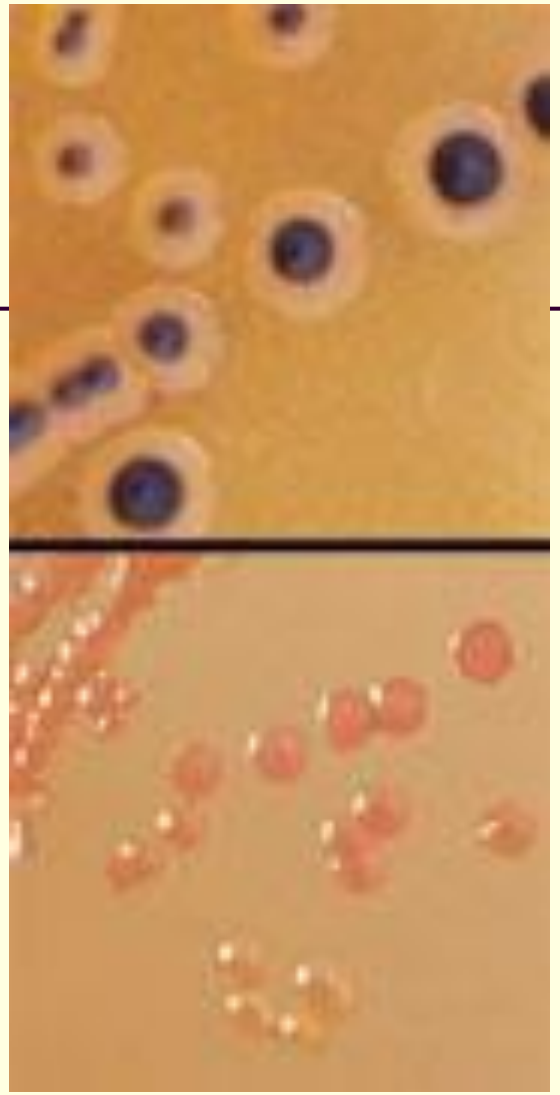
- belong to the family Enterobacteriaceae. They are motile, Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation.

Media Used

- XLD
- SS Agar
- Selenite F Broth
- Bismuth Sulfite Agar



Salmonella on XLD Agar



Salmonella and *Shigella on SS Agar*

Clostridium perfringens

- ***Clostridium perfringens*** **is an obligate anaerobic enteric bacterium.**

- **Its spores are generally more tolerant to environmental effects than other traditional entericindicators.**

- ***C. perfringens*** **has been suggested as an alternative bacterial indicator of fecal pollution because it is primarily associated with human wastes, and it is widely distributed in feces, sewage, and polluted waters.**

Media Used

- Fluid Thioglycollate Medium
- **Typical Composition (g/litre)**

  Peptone from casein 15.0; yeast extract 5.0; D(+) glucose 5.5; L-cystine 0.5; sodium chloride 2.5; sodium thioglycollate 0.5;

نتائج التحليل الميكربيولوجى لعينات مياه الشرب بشبكة مياه جامعة أسيوط

م	مصدر المياه	التحليل البكتريولوجى				
		الهـوائـــى				اللاهوائى
		Viable Count	Mac broth		.Mac Agar	E.M.B	Colysirs
			S.S	D.S			
١	بئر ١ المساكن الغربية	-ve	-ve	-ve	-ve	-ve	-ve
٢	بئر ٢ المساكن الغربية	+ve nonPath	-ve	-ve	-ve	-ve	-ve
٣	الخزان العالى – المساكن الغربية	-ve	-ve	-ve	-ve	-ve	-ve
٤	بيارة السحب للمحطة النقابى	-ve	-ve	-ve	-ve	-ve	-ve
٥	مخرج المحطة النقالى الغربية	-ve	-ve	-ve	-ve	-ve	-ve
٦	بئر الهندسة	+venon Path	-ve	-ve	-ve	-ve	-ve
٧	بئر البوابة البحرية	-ve	-ve	-ve	-ve	-ve	-ve
٨	بئر الاستاد الرياضى	+vePath	+ve	+ve	+ve	+ve	-ve
٩	بئر مزرعة الانتاج الحيوانى	+vepath	+ve	+ve	+ve	+ve	+ve
١٠	صنبور مزرعة الانتاج الحيوانى	+vePath	+ve	+ve	+ve	+ve	+ve
١١	كلية العلوم	-ve	-ve	-ve	-ve	-ve	-ve
١٢	كلية الزراعة	-ve	-ve	-ve	-ve	-ve	-ve
١٣	كلية الطب البيطرى	-ve	-ve	-ve	-ve	-ve	-ve
١٤	كلية الهندسة	-ve	-ve	-ve	-ve	-ve	-ve
١٥	المستشفى الجامعى	-ve	-ve	-ve	-ve	-ve	+ve
١٦	المدينة طلاب	-ve	-ve	-ve	-ve	-ve	-ve
١٧	المدينة طالبات	-ve	-ve	-ve	-ve	-ve	-ve
١٨	المسكن الشرقية	-ve	-ve	-ve	-ve	-ve	-ve
١٩	المساكن الغربية	-ve	-ve	-ve	-ve	-ve	-ve
٢٠	المستشفى البيطرى	-ve	-ve	-ve	-ve	-ve	-ve
٢١	استراحة أعضاء هيئة التدريس	-ve	-ve	-ve	-ve	-ve	-ve
	المسموح به عالمياً	-ve	-ve	-ve	-ve	-ve	-ve

نتائج الفحص البكتريولوجى لمصادر المياه المختلفة بمنطقة عرب المدابغ

أسم الموقع	العدد الكلى Viable Count	Mac.Conkey broth		Mac Conkey Agar	E.M.B
		S.S	D.S		
مدخل محطة المعالجة	Path ++	+Ve	+Ve	+Ve	+Ve
مدخل محطة المعالجة مختلط	Path ++	+Ve	+Ve	+Ve	+Ve
مخرج المحطة المعالجة قبل الكلور	Path ++	+Ve	+Ve	+Ve	+Ve
الترعة المجاورة لمحطة المعالجة ١,٥كم	Path ++	+Ve	+Ve	+Ve	+Ve
طلمبة خاصة بقرية (أبو القاسم)	grouth	سالب	سالب	سالب	سالب
ترعة الصرف المدابغ عند منقباد	Path ++	+Ve	+Ve	+Ve	+Ve
ترعة نجع حمادى (الزنار)	Path ++	+Ve	+Ve	+Ve	+Ve
مدخل محطة تنقية مياه الشرب (على ترعة الزنار)	Path +	+Ve	+Ve	+Ve	+Ve
مياه خارجية من المحطة السابقة	سالب	سالب	سالب	سالب	سالب
بداية ترعة المدابغ	Path ++	+Ve	+Ve	+Ve	+Ve
ترعة المدابغ على بعد ٤كم	Path ++	+Ve	+Ve	+Ve	+Ve
ستشفى علوان بئر رقم (٢)	سالب	سالب	سالب	سالب	سالب
مستشفى علوان بئر رقم (٣)	سالب	سالب	سالب	سالب	سالب
مستشفى علوان (مياه المخرج)	سالب	سالب	سالب	سالب	سالب
علـــــــــــوان	Path ++	+Ve	+Ve	+Ve	+Ve
ترعة نجع حمادى (علوان)	Path ++	+Ve	+Ve	+Ve	+Ve

و الحمد لله رب العالمين