



Coliform Bacteria in Water

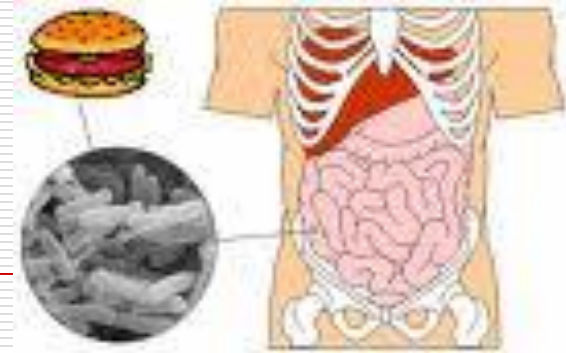


Water samples are first tested for ☐ Coliform Bacteria. This group of bacteria, normally found in the environment, is used as an indicator, to indicate the possible presence of Pathogens (disease causing organisms). Generally, when pathogens are present in drinking water, coliforms are present as well .

The **coliform** group is comprised of Gram-negative, ☐
nonspore-forming, rods, which ferment lactose to acid
and gas

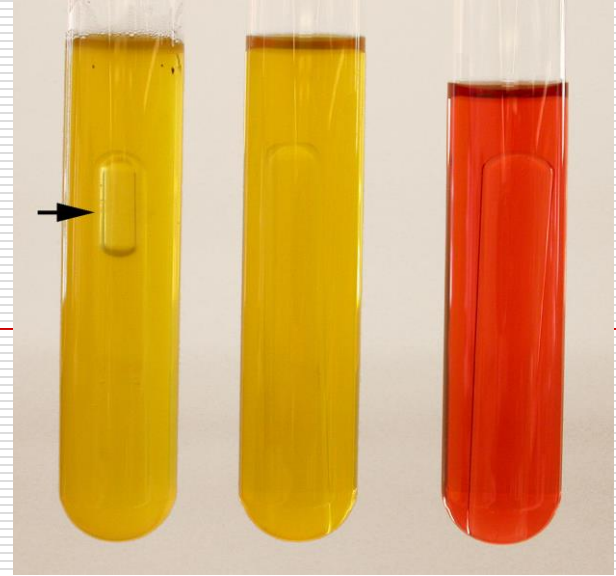
organisms in this group include *E. coli*, the ☐
only true fecal which is found only in fecal material from ☐
warm-blooded animals

The presence of this organism in a water is ☐
evidence of fecal contamination ☐



it impossible to test each water sample ☐
for each pathogenso it is easier to test
for the presence of
nonpathogenic intestinal organisms such
as
E. coli.

In this exercise, you will be testing ☐
water samples for the presence of
coliforms. There will be three ☐
principal tests: the **presumptive**,
confirmed and
completed tests (see flow-chart). ☐



The Presumptive Test ☐

In the presumptive test, a lactose ☐
broth tubes are inoculated with
amounts of the water sample to be ☐
tested.

Material: ☐

1. 3 tubes of lactose broth ☐

2. 10, 1.0 and 0.1 ml pipets ☐

3. Water samples ☐

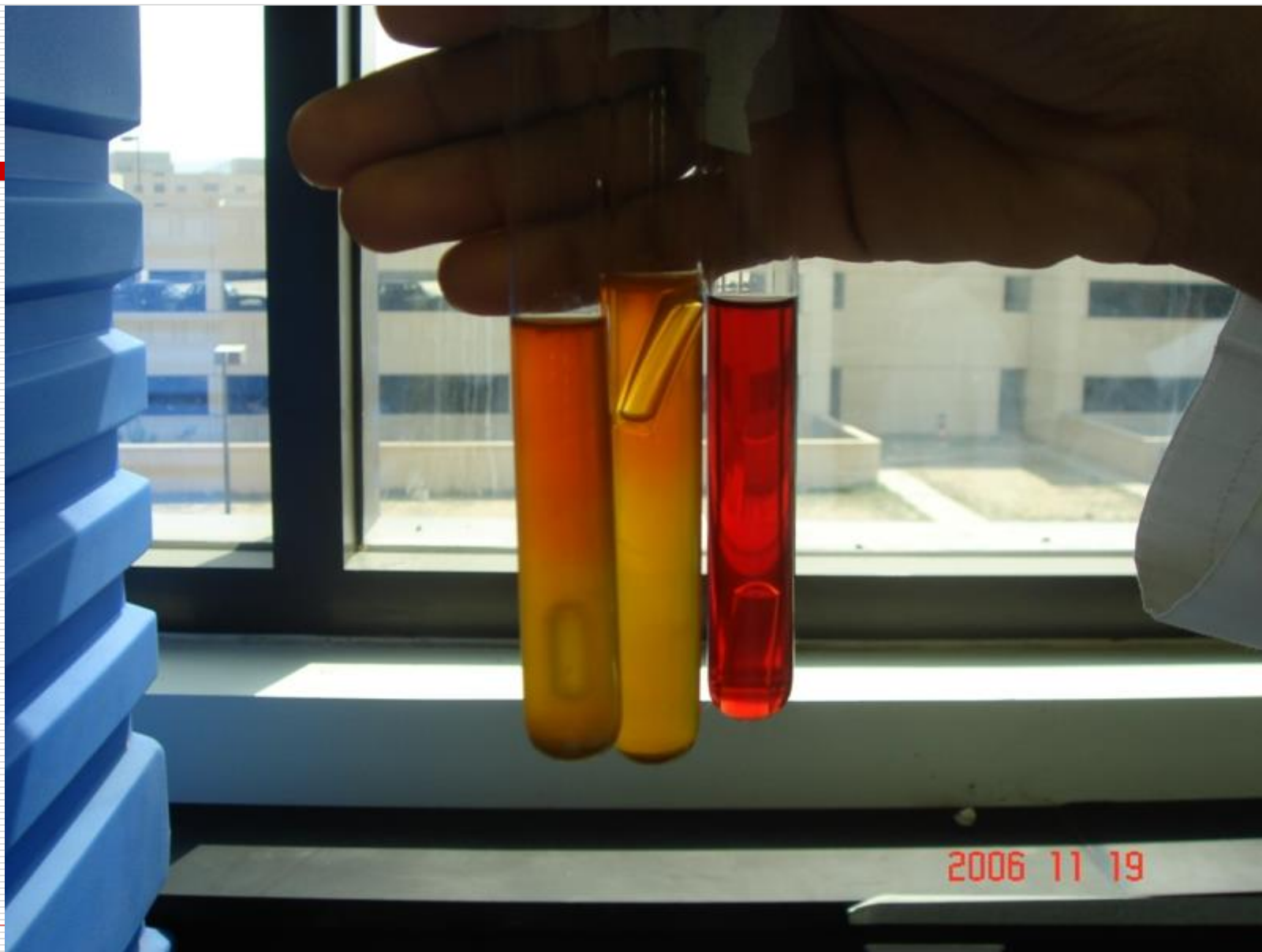
Procedure: (work in groups of four) ☐

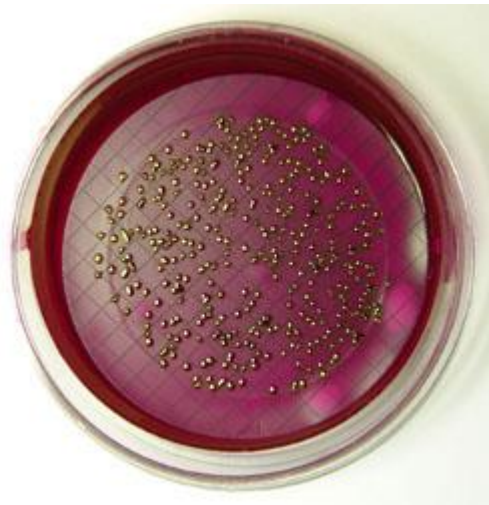
Presumptive Test ☐

1. Take a water sample (dilute) and inoculate three ☐
tubes of lactose broth with 10 ml, 1.0 ml and ☐
0.1 ml. ☐

2. Incubate all tubes at 37oC for **24 hours.** ☐

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1. Observe the number of tubes at each dilution that show acid, gas production in 24 hrs. Record results. ☐
 2. Reincubate for an additional **24 hours** at 37°C. ☐
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The Confirmed Test ☐

If any of the tubes inoculated with the water sample
produce gas, ☐
it is necessary to inoculate EMB (eosin methylene blue) ☐
agar plates
from a positive presumptive tube. The methylene blue ☐
in EMB agar inhibits Grampositive organisms and allows
the Gram-negative coliforms to grow

E. coli colonies are small and have a green metallic ☐

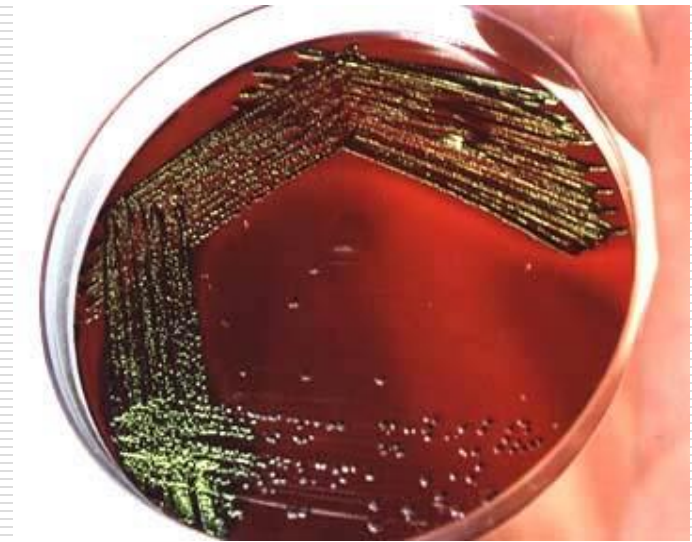
Material: ☐

- 1.** EMB agar plates ☐

Procedure: ☐

- 1.** Inoculate an EMB plate with material from a tube containing acid or gas.
 - 2.** Invert and incubate the plate at ☐
37°C for **24 hours**
-

1. Observe EMB agar plates. A ☐
positive confirmed test is indicated by
small
colonies green metallic sheen (*E. coli*). ☐
Record results





The Completed Test ☐

The completed test is using the ☐
organisms which grew on the
confirmed test media

These organisms are used to inoculate ☐
a tube of lactose broth After 24 hours
at 37°C

the lactose broth is checked for the ☐
production of gas.



Material: ☐

- 1.** Lactose broth tubes ☐

Procedure: ☐

- 1.** Inoculate a lactose broth tube with ☐
organisms from the
EMB plate. ☐
 - 2.** Incubate the broth tube at 37°C for **24** ☐
hours.
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- 1** in the lactose broth tube Check for ☐ gas production.
 - 2.** Make a Gram stain from the ☐ organisms
 - 3.** Record results.

