HiSelective™ H_2S Medium Kit (powder form) K022

H_2S Test Medium is recommended for the simultaneous detection of *Salmonella, Vibrio, Citrobacter* species and *Escherichia coli* from water samples.

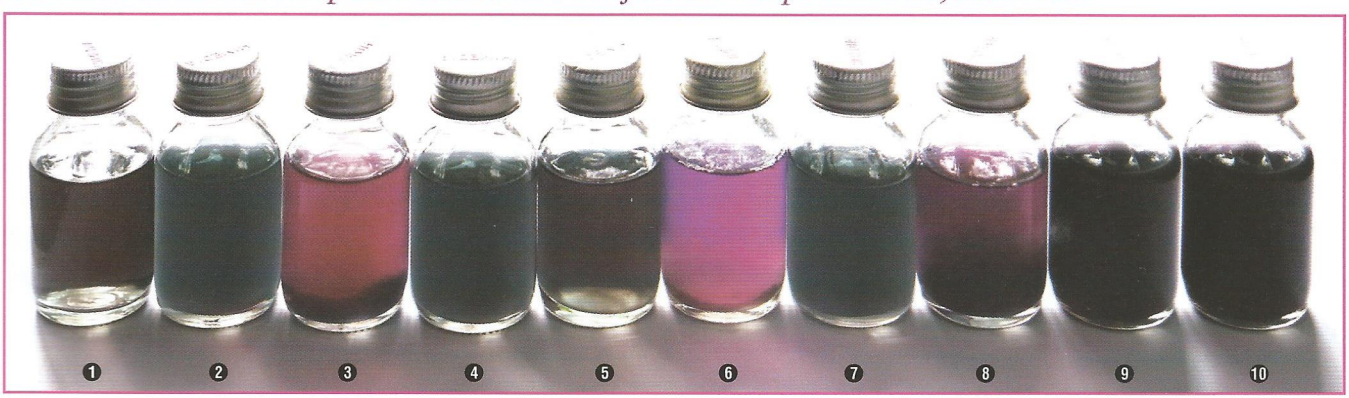
Introduction :

Supplies of drinking water contaminated with sewage or other excreted matter from man and animals may cause diseases like typhoid fever, cholera, campylobacteriosis, amoebiasis and helminthiasis. In the interests of public health, drinking water supplies should be tested to confirm the absence of contamination. Trying to detect the presence of all the different types of water-borne pathogens is laborious and impractical. A practical approach is to test the supply for the presence of faecal indicator bacteria.

The significance of various coliform organisms in water has been and is a subject of considerable study. Collectively, the coliforms are referred to as indicator organisms. The genera *Enterobacter, Klebsiella, Citrobacter* and *Escherichia* usually are represented in the majority of isolations made from raw and treated municipal water supplies.

One purpose of drinking water and wastewater treatment is to reduce the numbers of viable organisms to acceptable levels and to remove or inactivate all pathogens causing human disease. Water contamination and disease transmission may result from over-loaded sanitary waste disposal and potable water treatment systems. Outbreaks of gastroenteritis, pharyngo-conjunctivitis, folliculitis, otitis and pneumonia are associated with recreational activities like swimming, boating etc. Environmental Microbiological examinations are conducted to monitor compliance of the environment, to trouble shoot problems in treatment plants and distribution systems and in support of epidemiological investigations of disease outbreaks.

Kit contains sterile bottles with powder medium. Fill 20 ml of test water sample in the bottle, and incubate.



1. Control
2. *E. coli*
3. *Vibrio* species
4. *Shigella* species
5. *Streptococcus* species
6. *Enterobacter* species
7. *Pseudomona* species
8. *Klebsiella* species
9. *Salmonella* species
10. *Citrobacter* species

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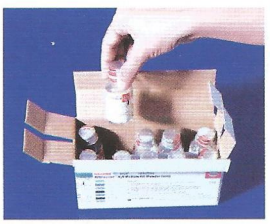
Systematic Diagram

① K022 HiSelective™ H_2S Medium Kit (Powder form)

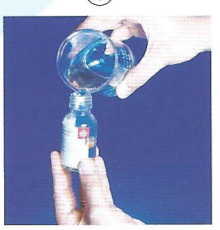
② 

Open the box and remove a bottle for testing

③ Remove the seal and loosen the cap by unscrewing in the anticlockwise direction

④ 

Remove the cap

⑤ 

Fill the bottle with water sample upto the arrow mark

⑥ 

Incubate the bottle at 32 - 35°C for 24 - 48 hours

After incubation medium colour remains bluish green with no haziness and no blackening

WATER IS POTABLE

1. *E. coli*
2. *Vibrio* species
3. *Shigella* species
4. *Streptococcus* species
5. *Enterobacter* species
6. *Pseudomonas* species
7. *Klebsiella* species
8. *Salmonella* species
9. *Citrobacter* species

WATER IS NOT POTABLE

Principle and Interpretation :

HiSelective H_2S Medium is a modification of the medium developed by Manja et al (1) for the simultaneous detection of *Salmonella, Vibrio, Citrobacter* species and *Escherichia coli* from water samples.

It has been reported that human faecal contamination is one of the main causes of water-borne diseases. In 1993, WHO (2) has therefore recommended regular testing of drinking water for thermotolerant coliforms and *Salmonella* species to ensure its complete absence. The frequent testing of drinking water in remote areas, as well as in developing countries, is rather difficult to achieve. *Salmonella* species associated with enteric fevers and other diseases are usually present in small numbers, compared to coliforms. *Vibrio cholerae* is the causative agent of cholera which is potentially a fatal diarrheal disease. *Citrobacter freundii* is often confused with *Escherichia* and *Salmonella*, however it is hydrogen sulphide positive unlike *Escherichia* and lacks the pathogenicity of *Salmonella*. Townsend, 1992 (3) has demonstrated the lack of correlation between coliform bacteria and the presence of *Salmonella* species in water, particularly in the tropics and subtropics. In Western Australia, 30% of all *Salmonella* isolations from water have occurred in the absence of indicator bacteria (4). The absence of *Escherichia coli* in *Salmonella* contaminated water is more often in the tropics. However, analysis of *Salmonella* using the culture methods is a four stage process involving pre-enrichment, selective enrichment, biochemical identification and confirmation by serological method. Thus, it is a very lengthy process which requires at least four days for completion. This kit provides faster results, in just 24 hours. Incubation upto 48 hours may be required before discarding negative bottles.

The medium contains casein enzymic hydrolysate which is a source of nitrogen. Ferric ammonium citrate and sodium thiosulphate are reduced by certain species of enteric organisms to produce H_2S, which turns medium black. The indicator mix in the medium is very sensitive to pH changes caused due to fermentation of sucrose. Bile salt inhibits the growth of accompanying microflora.

Directions :

- Fill vial with water upto arrow level. Swirl to dissolve the powder completely. Incubate at 35-37°C for 24-48 hours.
- Observe for turbidity with or without change of colour of the medium.
- If medium shows turbidity with blue / bluish purple or black colour, water is not fit for drinking. Black colour with turbidity of medium indicates presence of *Salmonella* or *Citrobacter* species, bluish green colour of medium with turbidity indicates *Escherichia coli*, bluish purple colour with turbidity indicates *Vibrio* species and dark purple colour with turbidity indicate presence of *Klebsiella* species.
- Add few drops of some disinfectant (i.e. Dettol, phenyl etc.) and discard the vial. Preferable to use the autoclave wherever the facility is available.

Quality Control :

Appearance of powder :

Light yellow to pink coloured, homogeneous, free flowing powder.

Appearance of solution :

Bluish green coloured, clear solution.

Cultural Response :

Cultural response is observed after an incubation at 35 - 37°C for 24 - 48 hours .

Control vial : Bluish green coloured, clear solution.

Organisms (ATCC)	Appearance of Medium following incubation	Colour appearance after growth
Klebsiella species	dark purple with turbidity	
Escherichia coli (25922)	bluish green with turbidity	
Enterobacter species	dark purple with turbidity	
Shigella species	bluish green with turbidity	
Citrobacter species	black with turbidity	
Streptococcus species	bluish green with turbidity	
Vibrio species	bluish purple with turbidity	
Pseudomonas species	bluish green with turbidity	
Salmonella species	black with turbidity	

References :

1. Manja K.S., Maurya M.S. and Rao K.M., 1982, A simple field test for the detection of faecal pollution in drinking water. Bulletin of the World Health Organisation, 60:797-801.
2. WHO, 2006, Guidelines for drinking water quality, Vol. 1 Recommendations, 1st Addendum to 3rd edition.
3. Townsend S.A., 1992, The relationships between *Salmonella* and faecal indicator bacteria concentrations in two pools in the Australia wet / dry tropics. Journal of Appl. Bacteriol. 73:182-188.
4. Peterson D.J., and Schorsch I., 1980, The microbiological surveillance of drinking water in Western Australia. WA Health Surveyor. 2 (June), 7-11.

Storage and Shelf-life :

Store below 30°C. It has shelf-life of 3 years.