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Biochemical Test for Detecting Hydrogen Sulphide (H_2S) Producing Bacteria

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SUMMARY

Hydrogen sulphide (H_2S) production test is generally used to detect the production of the concerned gas by any organism. This test is basically useful to identify the bacteria under family Enterobacteriaceae. This group of bacteria produce H_2S via reduction of sulphur containing amino acids like methionine and cysteine, or of inorganic sulphur compounds like thiosulfates, sulphates or sulphites. The production of hydrogen sulphide can be determined by introducing heavy metal salt of either iron or lead as H_2S indicator in the nutrient culture medium containing cysteine and sodium thiosulfates as the sulphur-containing substrates. For this purpose, several types of media can be used like SIM (Sulphide Indole Motility), TIA (Triple Sugar Iron Agar), KIA (Kligler's Iron Agar) and lead acetate test, among which the last one is most sensitive to detect H_2S production. This is a colourless gas which after production, reacts with the metal salt incorporated and subsequently forms visible insoluble black ferrous sulphide precipitate.

INTRODUCTION

In 1982 Manja *et al.* developed a simple method for detection of evidence of faecal contamination in drinking water. The team made a keen observation that presence of coliform bacteria in drinking water are in consistent association of organisms which produce hydrogen sulphide (H_2S). Some microorganisms possess the trait which enables them to reduce sulphur-containing compounds to H_2S during their metabolism and this character is commonly exploited as an important test measure for the identification in laboratories. There are numerous methods used for detecting the production of H_2S by different microorganisms. These vary with sulphur sources as well as the metal salts used as indicators of H_2S formation. Due to the semisolid nature, lack of sucrose-like interfering carbohydrates, and with the use of peptonized iron as indicator, SIM is generally found to be more sensitive in the detection of H_2S than TSI and KIA. However, Lead acetate paper is almost 10 times more sensitive than other media. This test is used to determine whether the microbe reduces sulphur-containing compounds to sulphides to produce hydrogen sulphide gas. The hydrogen sulphide-positive organisms are *Enterobacter aerogenes, Shigella dysenteriae, Erwinia sp., Citrobacter freundii, Salmonella sp., Proteus mirabilis, Proteus vulgaris, Edwardsiella tarda*

Principle and procedure of detecting hydrogen sulphide (H_2S) producing bacteria:

A sulphur compound as substrate and an iron compound as indicator are incorporated in the test medium to examine the production of hydrogen sulphide. If the sulphur compound gets reduced by the bacterial strain, hydrogen sulphide is supposed to be produced. This test thus helps in determining whether the test-microbes are able to produce sulphides from sulphur-containing compounds via metabolic reduction. H_2S can be generated from certain bacteria by reducing sulphur-containing amino acids like methionine, cysteine, or via reduction of inorganic sulphurs such as thiosulphates, sulphates or sulphites during degradation of protein or during anaerobic respiration employing sulphur instead of oxygen. Both these cases result in H_2S production, which ultimately reacts with the iron compound present in the medium and precipitates as black coloured insoluble ferric sulphide. The black colour is the indication of H_2S being produced. The detection of H_2S produced by a microbial organism is mainly utilised to boost the identification of that specific organism. This test can be done using different types of media such as iron agars like Triple Sugar Iron (TSI) and Kligler's Iron Agar (KIA) media, SIM medium and Lead Acetate Paper. Composition of media are as follows.

Properties of Media and its composition required for H_2S detection:

The tests can be performed using any of the media available for this purpose namely Kligler's Iron Agar (KIA), Triple Sugar Iron (TSI), SIM medium and Lead Acetate Paper. Iron agars are suitable for detection of H_2S production by Enterobacter. Its presence is indicated by the ferric citrate added in the medium. Sulphide indole

motility (SIM) medium is composed of ferrous ammonium sulphate and sodium thiosulphate, together serving as the indicators of H_2S. Production of this gas is detected with black precipitate of ferrous sulphide, which is produced as a result of the reaction of ferrous ammonium sulphate with H_2S gas. Lead acetate paper test is a sensitive technique for detecting H2S production. The SIM Media compositions for 1000ml distilled water are, Peptone (30.0 g), Beef extract (3.0 g), Agar (3.0 g), Ferrous ammonium sulphate (0.2 g), Sodium thiosulphate (0.025 g). Final PH pf media should be maintained 7.3±0.2 (at 25°C). Likewise Lead acetate paper test media Composition are yeast extracts (5.0 g), Potassium phosphate, Ammonium phosphate, Magnesium sulphate (0.5 g each), Sodium chloride (0.2 g), Cysteine hydrochloride (0.1 g).

Procedure of detecting H_2S Producing bacteria

I. In Sulphide indole motility (SIM) medium

- The organism is aseptically inoculated into labelled tubes by means of stab inoculation.
- These inoculated tubes are incubated for 24-48 hours at 37°C.
- Look for the formation of black precipitate in the medium.

II. In Kligler's iron agar (KIA)

- Aseptic inoculation of test organism is done into KIA and is incubated overnight at appropriate temperature.
- Check for blackening of the medium.

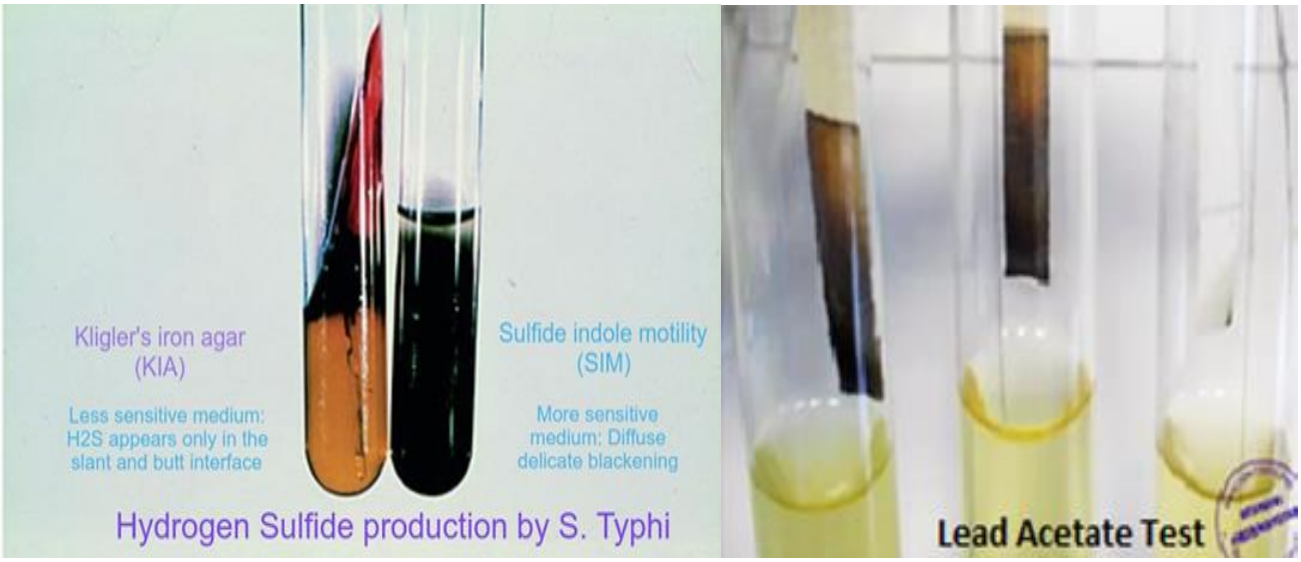
III. Triple Sugar Iron Agar (TSIA)

- Using aseptic technique, inoculate each experimental organism into its appropriately labelled tube by means of a stab-and-streak inoculation. (*Note: Do not fully tighten the screw cap*) then incubate for 18 to 24 hours at 37°C.
- Examine the colour of both the butt and slant of all agar slant cultures. Based on the observations, determine the type of reaction that has taken place (acid, alkaline, or none) and the carbohydrate that has been fermented (dextrose, lactose, and/or sucrose, all, or none) in each culture. Record the observations and results in the chart provided in the Lab Report.
- Examine all cultures for the presence or absence of blackening within the medium. Based on the observations, we can conclude whether each organism was capable of producing H_2S or not. Record the observations and results in the chart provided in the Lab Report.

IV. Lead Acetate Paper Test

- Aseptic inoculation is done in a tube containing 5 ml of sterile peptone water or nutrient broth with the test organism.
- Lead acetate strip is prepared by immersing strip of Whatman filter paper in 5% lead acetate solution, air drying and autoclaved.
- The paper strip is suspended in the neck of the tube above the medium held by a plug or screw cap and stopper tightly.
- The inoculated medium is incubated at 35-37°C, and examined daily for development of blackening in the lower part of the strip.

As there are various types of media with varying degrees of sensitivity available for detecting H_2S production, microbiologists can select a particular detection system as per their requirement and characteristics of test isolate. Like in case of lead acetate, being the most sensitive indicator in this regard, it can be used for detecting bacteria which produce only trace amounts of H_2S. It is to be noted that when incorporated in culture media, lead acetate might cause inhibition of the growth of many fastidious bacteria. So, instead of incorporating the lead acetate impregnated filter paper into the media, it should be draped under the cap of the culture tube while testing.



(**Source:** https://www.tgw1916.net/images/SAM_1009_H2S.jpg)

Fig 1: Left- H_2S production test performed in KIA and SIM media in bacterium *Salmonella typhi* (Tankeshwar, A.,2021). Right- H_2S production test performed with lead acetate strips

Observation:

The tubes are examined up to 14 days. In all the H_2S detecting systems, H_2S reacts forming a black lead sulphide discoloration of the media or strip. This end-point is usually insoluble, a heavy metal sulphide, producing black precipitate in the medium or in the filter paper strip. Because hydrogen ions must be present for H_2S formation, the blackening is first observed in test media where acid formation is maximum. So, results can be seen first along the inoculating line, within the depths of slanted media, or at the centres of colonies growing on agar surfaces. Positive results can be detected by blackening on the medium whereas in negative results no blackening on the medium is seen.

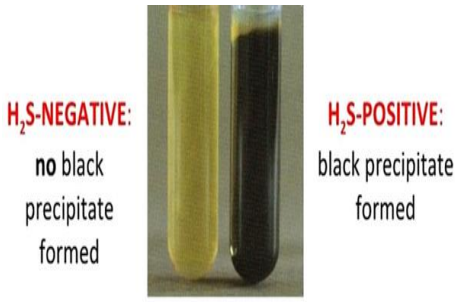


Fig 2- Endpoint result of H_2S production test (Left- Negative; Right- Positive) (Aryal, S., 2019)

Advantages

- Low-in-cost. All the materials used can be found locally.
- While performing the test it is not necessary to have access to laboratory or expensive equipment like autoclave or incubator. Only a simple balance to weigh the media, pipettes, and a method of sterilizing the kits are needed.
- Samples do not require to be stored under refrigeration.

Disadvantages

- For the organisms utilizing sucrose H2S production may be inhibited on TSI as sucrose is responsible for suppressing the enzyme mechanism that results in H2S production.
- Lead acetate being toxic to bacteria may inhibit the growth of the same. The media should not be allowed to touch the strip.

- It is recommended that biochemical, molecular, immunological, or mass spectrometry testing of bacteria should be performed on colonies obtained from pure culture for complete identification.

CONCLUSION

As H_2S detected to be present in one medium might not be detected in other medium due to difference in levels of sensitivity, it is important to know which test system has been used while interpreting identification charts. For that, SIM, TSIA or KIA tubes are usually used for the detection of hydrogen sulphide producing bacteria in diagnostic microbiology. Among these three major biochemical test media used in this regard, TSIA is least sensitive. It has been found that sucrose, if present in test medium, suppresses the production of H_2S. For this, lack of carbohydrates and use of peptonized iron as indicator make SIM a better test medium to detect H_2S production. This is more sensitive than TSIA and KIA.

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