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# Evaluation of the $H_2S$ Method for Detection of Fecal Contamination of Drinking Water

**Water, Sanitation and Health**
**Department of Protection and the Human Environment**
**World Health Organization**
**Geneva**

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# Evaluation of the $H_2S$ Method for Detection of Fecal Contamination of Drinking Water

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# FOREWORD

Around 2.2 million die of basic hygiene related diseases, like diarrhoea, every year. The great majority are children in developing countries. Interventions in hygiene, sanitation and water supply make proven contributors to controlling this disease burden. For decades, universal access to safe water and sanitation has been promoted as an essential step in reducing this preventable disease burden

Nevertheless the target of "universal access" to improved water sources and basic sanitation remains elusive. The "Millenium Declaration" established the lesser but still ambitious goal of halving the proportion of people without access to safe water by 2015.

The provision of drinking water of acceptable microbiological quality and low infectious disease risk requires a number of essential elements within a Water Safety Plan. Within any water safety plan emphasis is placed on controlling and detecting fecal contamination of drinking water and its sources. Traditionally, this measure of fecal contamination has been a bacterium or group of bacteria considered indicative of fecal contamination. The measurement of such indicator bacteria of fecal contamination requires trained analysts, media and other supporting materials and facilities available only in a microbiology laboratory or the use of a water microbiology field analysis kit.

Lack of access to laboratories or field analysis kits is an obstacle to the provision of microbiologically safe drinking water to many communities and people worldwide. In an effort to overcome this problem, a number of alternative indicators and tests to detect fecal contamination of drinking water have been proposed and developed. Some of these proposed fecal indicators and their tests are simple, low cost and do not require a microbiology laboratory or bacteriological field test kit. Some of these simple, low cost fecal indicator tests have come into use in actual drinking water supply practice. Prominent among these is the so-called hydrogen sulfide or $H_2S$ test, which is intended to detect or quantify hydrogen sulfide-producing bacteria, considered to be associated with fecal contamination.

The purpose of this report is to review the basis of the hydrogen sulfide test as a measure of fecal contamination of drinking water and the available scientific and empirical evidence for and against the test as a valid, useful and reliable measure of fecal contamination and drinking water quality. The report addresses the fundamental microbiological considerations of the test, including its chemical and biochemical basis, what organisms it detects and how it detects and quantifies them and the reported experiences with its practical application to assessing water quality.

In developing this report many sources of data and supporting information were generously provided by developers and users of the test and others who also have attempted to modify, improve, validate and apply it. We are grateful to these many individual and organizations for their assistance. In particular, we gratefully thank the following for providing reports and other supporting information:
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UNICEF, New Delhi

Ms. Bettina Genthe, Division of Water Environment and Forestry, CSIR, Stellenbosch, South Africa

This report has been prepared as part of a programme of activity towards the updating of WHO's Guidelines for Drinking-water Quality. Following a process of development and review it is released in draft form.

This document represents "work in progress" and further information concerning the $H_2S$ test and experience with its application would be welcome. Such information should be forwarded to:

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# 1. INTRODUCTION AND BACKGROUND

This report critically reviews the scientific basis, validity, available data and other evidence for and against $H_2S$ tests as measures or indicators of fecal contamination in drinking water. The test was originally developed to detect in a volume of water the production of $H_2S$ by enteric bacteria associated with fecal contamination by the formation of a black precipitate from the reaction of the $H_2S$ with iron in the medium. This relatively simple, low cost test has been studied, modified in various ways, tested and used to some extent in many parts of the world as an indicator of fecal contamination of drinking water.

Since the initial report of the $H_2S$ test 20 years ago (Manja et al., 1982), many versions or modifications of it have been described in the published literature. Hence, there are now many versions of the $H_2S$ test and these differ in medium composition, the preparation of the medium and supporting materials, test format and sample volumes, incubation time, incubation temperature and scoring of results. The test is not standardized worldwide and only some versions of it have been subjected to collaborative testing or comparison with other bacteriological tests for fecal contamination. The plethora of different $H_2S$ test versions and the variety of ways they have been evaluated in laboratory and filed studies make it difficult to compare them. The essential criteria of any test to detect and quantify fecal contamination of drinking water and other waters are used as the basis for evaluating the validity and performance of various versions of the $H_2S$ test and the quality of the data available for evaluation and validation purposes.

## 2. FECAL INDICATOR DETECTION AND TESTING IN DRINKING WATER: CONTEXT, PURPOSES, NEEDS AND CRITERIA

### 2.1 Drinking Water Safety Plans and Detecting fecal indicators in drinking water

An essential goal for the provision of safe drinking water is that it be essentially free of (at low risk of containing) disease-causing microorganisms. Since the beginning of the 20th century, the detection of fecal indicator bacteria in drinking water has been used as the basis of criteria, guidelines and standards for acceptable limits of fecal contamination and as the basis for judging or predicting the possible presence or absence of pathogenic (disease-causing) microorganisms. The WHO Guidelines for Drinking-water Quality (GDWQ) and many other authorities continue to support the use of bacterial indicator levels and their measurement as a basis for judging and verifying drinking water quality. However, such fecal indicator analysis of drinking water as a measure of end-product quality and determinant of microbial disease risk is only one of many measures and activities in an overall system for providing safe drinking water. In the updating of the WHO GDWQ, the goal of providing safe drinking water will be promoted through the development and use of a Water Safety Plan. This plan includes risk assessment, water system assessment and process control that examines all aspects of drinking water from its source, through treatment and distribution (or collection and storage) to the consumer. It uses a management plan that incorporates Hazard Analysis-Critical Control Points (HACCP). In such a plan the measurement of fecal indicator presence or levels in water is only one of several management tools and not always among the most critical ones for process control or overall collection, production and delivery of drinking water of acceptable microbial quality. Nevertheless, measures or indicators of water quality, particularly those measuring or indicating fecal contamination, are useful if not essential tools in the provision of safe drinking water. The ability to easily, rapidly and affordably detect fecal contamination in drinking water is still a desirable goal and worthy endeavor in the overall effort to provide microbiologically safe (low risk) drinking water.

### 2.2 Purposes and Needs for Detecting Indicators of Fecal Contamination in Drinking Water

Determining the microbial quality of drinking water by measuring the presence, absence or concentrations of indicator bacteria continues to be widely practiced worldwide to: (1) meet water quality standards and guidelines, (2) to determine source water quality, treatment system efficacy and distribution system integrity, and (3) to inform Water Safety Plans, risk assessments and management systems. In some countries and regions and for international commerce (commercial bottled water) and transportation (airplane, rail and other travel conveyances), such analysis of drinking water may be required by law or governance.

In addition to these purposes and needs, measuring the microbial quality of water for presence of fecal contamination can be and is now being done for other beneficial purposes. One such purpose is for community involvement and empowerment in the provision, management and monitoring of drinking water, including its sources and treatment. Great efforts are being made to encourage local participation in the

provision of safe drinking water and in the oversight or monitoring of its provision by other responsible parties (governments, privatized water companies, water supply contractors, water vendors, etc). The ability to test drinking water for fecal contamination is a powerful and empowering tool for these purposes.

Another useful purpose for measuring the microbial quality of water is educational. Teaching people about the microbial quality of water and the fundamentals of germ theory within the context of education and outreach programs for water, sanitation and hygiene at the individual, household, community and regional levels is a continuing and long-term goal in the global health initiative. In delivering these educational messages the availability of simple, practical, accessible and affordable tests for fecal contamination of drinking water are extremely useful and potentially powerful tools. In some situations the best tests to accomplish these goals are those that are the simplest to use, understand, visualize and interpret. This is because such tests can be widely disseminated both directly by the primary educators and then subsequently via communications within households, families, schools and communities and by other means (educational materials such as leaflets, signs and labels). For these purposes the $H_2S$ tests and other simple and affordable tests have great value and even greater potential use for drinking water supply management and health education in the water and sanitation sectors.

However, meeting these needs and purposes is served only if the tests are reliable and provide the correct information. If they are unreliable and if they provide incorrect information (false positives and false negatives), their potential uses and benefits will be undermined. Therefore, $H_2S$ tests and other simple tests to detect and quantify fecal contamination of drinking water must be evaluated and judged on the basis of their reliability and predictability as well as their accessibility, practicality and affordability.

### 2.3 Criteria for detecting fecal contamination of drinking water using indicators

For more than 100 years, bacteriological tests have been used to detect fecal contamination of drinking water, other waters and other media, such as wastewater and foods. During this time, there has been an evolution in the bacterial indicators used and the articulation of the criteria for an ideal or reliable indicator of fecal contamination in drinking water and other waters (Olivieri, 1983; Sinton et al., 1998). The current criteria of an ideal or preferred indicator of fecal contamination have been defined and stated by WHO and other authorities. According to these authorities the essential criteria of a fecal indicator are the following (WHO, 2002):

- The indicator should be absent in unpolluted water and present when the source of pathogenic microorganisms of concern (fecal contamination) is present.
- The indicator should be present in greater numbers than the pathogenic microorganisms.
- The indicator should respond to natural environmental conditions and water treatment processes in a manner similar to the pathogens of concern.
- The indicator should be easy to isolate, identify and enumerate.
- The test should be inexpensive thereby permitting numerous analyses to be taken.

- The indicator should not be a pathogenic microorganism (to minimise the health risk to analysts).

Authorities have identified at least one additional criterion for indicators of fecal contamination (as distinct from indicators of other sources of microbial contamination) (Olivieri, 1983). This fecal indicator criterion is:

- The indicator should not multiply in the environment

The rationale for this last criterion is that the presence and concentration of fecal indicators should be in proportion to the level of fecal contamination. Hence, microbial proliferation in the environment could result in the microbe being present at high concentrations when no fecal contamination (and its pathogens) or very low levels of fecal contamination are actually present.
Therefore, any indicator of fecal contamination of drinking water and its sources is best judged according to these essential criteria and perhaps other criteria that may be relevant for or specific to a particular indicator and type of test for it.

## 2.4 Microbiological presence-absence tests and their use in detecting and quantifying fecal contamination

In addition to the criteria described above, some microbial tests for fecal contamination of water are based on the presence (P) or absence (A) of the microbial indicator in a specified volume of water, a so-called P-A test. In the application of P-A tests to detecting bacterial indicators of fecal contamination in drinking water the tested sample volume is 100 ml. According to some standards and guidelines, the fecal indicator is expected or required to be absent (A) in all of (zero tolerance) or most of (e.g., 95%) the 100-ml sample volumes successively tested over time. In other (i.e. non – P/A) formats of fecal indicator analysis of water, the concentration of the target microbe is determined by using multiple and varying sample volumes, each of which is scored as positive or negative for the test microbe. These data are then used to estimate microbial concentration by a quantal method typically the Most Probable Number (a maximum likelihood analysis method). Alternatively, the water is analyzed for the fecal indicator microbe or microbe group by an enumerative method in which the concentration of bacteria per unit volume of water can be expressed as colony forming units (CFU) per unit volume.

The use of the P-A concept and P-A tests for fecal indicator bacteria, primarily coliforms, fecal coliforms and *E. coli*, has a history of development that goes back more than four decades (Clark, 1968). Considerable effort in the form of expert analysis and judgement went into the development and implementation of P-A tests for these microbes in drinking water. Much of this effort included consideration of the wealth of available historical data on the occurrence of these indicator bacteria in municipal drinking water, based on the frequency of positive results (fecal indicator presence) in 100-ml volumes of drinking water and the acceptability (or risk) of drinking waters based on these observed frequencies. These analyses led to current guidelines and standards for the microbial quality of drinking water based on positive P-A test results. There appears to be no corresponding analyses and expert judgement that went into the development of $H_2S$ test as a P-A test. Instead, results of P-A $H_2S$ tests have been compared to results for various fecal indicator microbe tests to

determine the extent to which the results give the same outcome when scored as positive or negative results. In the initial report of the $H_2S$ test by Manja et al. (1982) the test was applied to various drinking water samples of several cities in India. Water samples containing 10 or more coliform bacteria by MPN were subjected to the new $H_2S$ test using a 20-ml sample volume in a P-A format. On this basis, positive $H_2S$ tests were considered unsatisfactory as drinking water because they contained 10 or more total coliforms and were positive for $H_2S$. Since then, others have compared $H_2S$ tests to tests for fecal indictor bacteria using either these or other criteria.

The use of this comparative approach in determining the validity of the $H_2S$ test has never been subject to review of its scientific merit and validity. Considering the differences in the target bacteria being detected, absent any consideration of pathogen presence in water, and without formal efforts to determine how well they fulfill the essential criteria of an ideal or acceptable indicator of fecal contamination, the validity of $H_2S$ tests, the meaning and reliability of interpretation of their results, and their ability to predict microbial health risks is a matter of concern. Because the basis of the WHO GDWQ are now becoming risk-based, the absence of a microbial risk basis for $H_2S$ tests raises concerns about their validity and interpretation in judging the acceptability of drinking water quality. Hence, the application of P-A criteria or various quantitative criteria to $H_2S$ tests as fecal indicator tests is an important but still unresolved issue. There are no fully articulated public health or risk-based criteria for specifying the volume of water to be tested in a P-A test format or the acceptable (or unacceptable) concentrations of $H_2S$ bacteria as measured in a quantitative test. In this report, the use of P-A tests and criteria for $H_2S$ tests will be considered in terms of the validity and reliability of this test format to detect and quality fecal contamination in drinking water and its sources.

## 2.5 Advantages, disadvantages and limitations of tests for bacterial indicators of fecal contamination

Fecal indicator bacteria generally are present in much higher numbers than the frank waterborne pathogens of fecal origin, they are easier to detect relatively rapidly by standard culture methods and the costs of analysis are far less than the costs of analyses for pathogens. A number of well-developed and extensively tested methods are widely available for the detection of various fecal indicator bacteria such as thermotolerant (fecal) coliforms and *E. coli* in drinking water and its sources. The historical basis, uses and interpretations of these tests are described in detail elsewhere (WHO, 2002). Suffice it to say that testing for these bacterial indicators of fecal contamination of drinking water is still encouraged and widely used worldwide.

Despite their advantages and value, the use of bacterial indicators of fecal contamination and the methods for them have limitations. In addition, because of the previously noted lack of accessibility or availability in many settings in many parts of the world, it has become apparent that there are other limitations to and problems with the use of the usual bacterial indicators of fecal contamination of drinking water (thermotolerant coliforms and *E. coli*). It has been well documented that waters considered bacteriologically safe (less than 1 bacterial fecal indicator per 100 ml), can contain sufficient pathogenic enteric viruses and protozoans to cause disease outbreaks (Berry and Noton, 1976; Craun and Gunn, 1979; MacKenzie et al., 1994). Other fecal indicator microbes, such as enterococci, spores of *Clostridium perfringens*

and coliphages, can be detected in drinking water when the usual coliform bacteria (total or thermotolerant) or *E. coli* are not detectable. Furthermore, there is some evidence that coliforms possibly including *E. coli* can proliferate in tropical and sub-tropical waters. Warmer water temperatures may contribute to the growth of coliforms, thermotolerant coliforms and *E. coli* and the greater survival of some enteric bacteria, notably *Salmonella,* compared to coliforms (Hazen, 1988; Iverson and Fleay, 1991; Jimenez et al., 1989; Townsend, 1992). For these reasons, coliforms, thermotolerant coliforms and *E. coli* are not ideal indicators of fecal contamination and alternative indicators of fecal contamination continue to be considered, evaluated and applied (Sinton, 1998; Sobsey, 2001; WHO 2002).

## 2.6 Needs for and benefits of alternative tests to detect fecal contamination of drinking water

The requirements for laboratory resources or field analysis kits for standard bacteriological tests for fecal contamination of drinking water are major barriers to their accessibility in many parts of the world. The need for sterilized bacteriological materials (media, sample bottles, sterile diluent, culture tubes, bottle or plates, membrane filters, pipettes or other volumetric dispensing devices, etc), controlled temperature incubators, the required use of aseptic technique by trained individuals, and relatively high costs make it difficult, impractical or impossible to perform these tests in many places. The resources and infrastructure are simply not available to allow for routine bacteriological testing of drinking water using the standardized methods for fecal indicator bacteria analysis.

The lack of availability of standard bacteriological tests for drinking water quality highlights the great need for a rapid, simple, inexpensive test for the microbial quality of drinking water. This need is especially great for small community and household water supplies that lack access to and can not afford conventional bacteriological testing of drinking water. On-site testing using portable equipment and use of simplified tests, such as the $H_2S$ tests, may both contribute to overcoming these constraints.

$H_2S$ tests deserve evaluation as accessible alternatives to conventional bacteriological tests for fecal contamination of drinking water. Therefore, the potential merits and beneficial uses of $H_2S$ tests deserve consideration, as does the determination of their reliability and predictability in detecting fecal contamination of drinking water. Key issues to be addressed are whether $H_2S$ tests are sufficiently reliable and adequately developed as tests of fecal contamination of drinking water to be recommended for widespread and routine use, and if, so what caveats and cautions should be applied and under what conditions.

This report addresses the key aspects of $H_2S$ tests as indicators of fecal contamination of drinking water. The aspects to be addressed are as follows:

- what organisms the tests actually measure;
- the extent to which test measurements are or are not indicative of fecal contamination; and under what conditions;
- the basis for and likelihood of false positive and negative results;

- the current state of the methodology with respect to reliability, uniformity, practicality, availability and cost and
- the extent to which the tests fulfill the ideal criteria of an indicator of fecal contamination; and recommendations for future actions and their directions.

## 3. BASIS AND HISTORICAL DEVELOPMENT $H_2S$ TESTS

In 1982 Manja et al. reported the development of a simple method for detecting evidence of fecal contamination in drinking water. The test was intended to meet the "…need for a simple, reliable field test for use by village public health workers" to detect fecal contamination in drinking water. They observed that the presence of coliform bacteria in drinking water was consistently associated with organisms that produce hydrogen sulfide ($H_2S$). The test is based on the readily observable formation of an iron sulfide precipitate on a paper strip (or in the water sample liquid) in a bottle or test tube, as a result of the reaction of $H_2S$ with iron. The test is intended to detect bacteria associated with fecal contamination due to the activity of these microorganisms in reducing organic sulfur to the sulfide oxidation state (as $H_2S$ gas) which then reacts rapidly with iron to form a black, iron sulfide precipitate (Allen and Geldreich, 1975). The advantage of the method is its simplicity, low cost and ability to be performed in the absence of a typical microbiology laboratory or field laboratory test kit. Tubes or other containers holding the test materials are prepared in a central laboratory to be used in the field by minimally trained personnel.

Over the last two decades, various investigators have tested this method and various modifications of it in different tropic and temperate regions, including Indonesia, Peru, Paraguay, and Chile, Nepal, and South Africa (Ratto et. al., 1989; Kromoredjo and Fujioka, 1991, Kaspar et al., 1992; Castillo et. al., 1994; Venkobachar et al., 1994; Martins et. al., 1997; Rijal and Fujioka, 1998; Genthe and Franck, 1999), and compared it to traditional bacterial indicators of fecal contamination of water. As will be described in more detail in a later section of this report, the results of these studies generally indicate that the method gives results comparable to the test for traditional bacterial indicators of fecal contamination and is sometimes superior to these test in detecting fecal contamination, based on other criteria for evidence of fecal contamination. Furthermore, some studies indicate method worked well as a presumptive test for the detection of *Salmonella* (Gawthorne et. al., 1996).

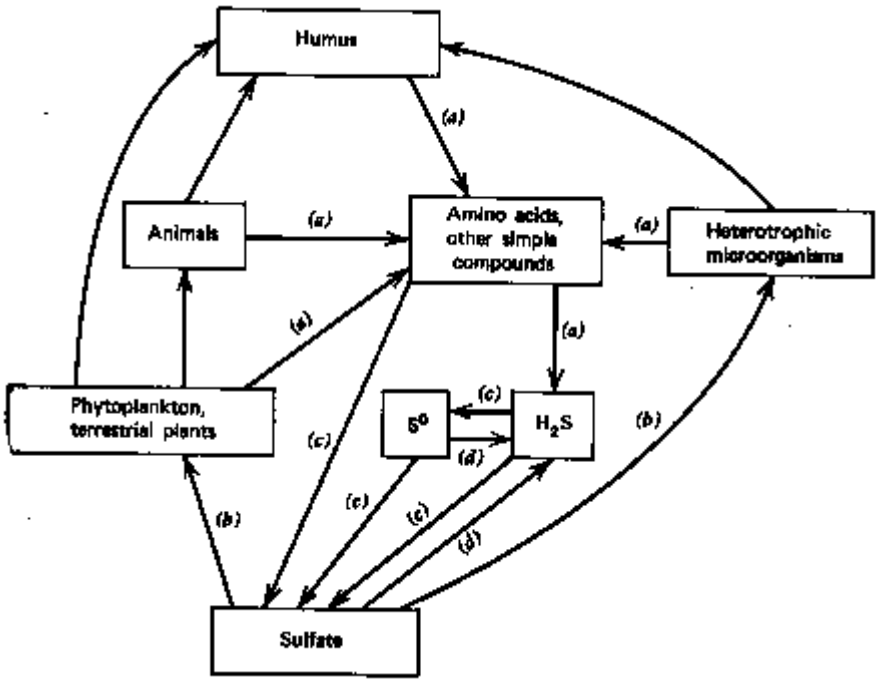
### 3.1 What $H_2S$ Tests Measure and How They Measure It

The $H_2S$ method also, commonly called the paper strip method, and the various modifications of it do not consistently measure the presence of either total coliform bacteria, specific groups of fecal bacteria (e.g., fecal coliforms) or a specific fecal bacterium (*E. coli*). The test is based on measuring bacteria that produce hydrogen sulfide under the test conditions employed. However, some coliform bacteria (e.g., *Citrobacter* spp.), some other enteric bacteria (e.g., *Clostridium perfringens*) as well as many other types of bacteria produce $H_2S$. The test measures the production (actually, the presence) of $H_2S$ by its reaction with iron to form an insoluble, black precipitate of iron sulfide. Given the low solubility product of iron sulfide, the test can detect even small amounts of sulfide formation or presence. Any source of $H_2S$ in the sample can lead to a positive result. Sulfides also can be formed by abiotic chemical reactions. Many different bacteria, from a variety of habitats, including many of enteric origin, can release sulfide from proteins, amino acids and other reduced sulfur compounds by reduction reactions. Therefore, there are many possible sources of a positive result in the $H_2S$ test.

In evaluations of the $H_2S$ test, several investigators have attempted to identify by speciation, the bacteria present in positive $H_2S$ tests. Castillo et al., (1994) found a large variety of bacteria, primarily various *Enterobacteriaceae* and *Clostridium perfringens*, in samples giving positive reactions in the $H_2S$ test: *Enterobacter, c*lostridia, *Klebsiella, Escherichia, Salmonella, Acinetobacter, Aeromonas, Morganella*. Ratto et al. (1989) found *Citrobacter* to be a common organism in positive $H_2S$ tests. This suggests that while the test organisms many not be all coliforms they are organisms typically associated with the intestinal tracts of warm-blooded animals. Because some of these microbes may arise from fecal contamination of non-human origin, the test is not specific for human fecal contamination. In many settings domestic and agricultural animals and humans live in intimate contact and therefore, distinguishing the contributions of human and animal sources to fecal contamination of water is not possible and perhaps not necessary. Because animal fecal contamination also contains a variety of human pathogens, such contamination also poses risks to human health if present in drinking water. Although the intent of $H_2S$ tests is to detect bacteria associated with fecal contamination, there is considerable concern that the test also may detect bacteria not associated with fecal contamination and its attendant pathogens. Therefore, an examination of the sources, sinks and transformation of sulfur and the role of microbes in its cycling is important to understanding the applicability of this test.

### 3.2 Hydrogen Sulfide and the Biogeochemistry of Sulfur

**The sulfur cycle and the role of $H_2S$ and bacteria in it.** Sulfur is one of the ten most abundant elements on earth and its various elemental, oxidized and reduced forms is driven by a biogeochemical cycle, the sulfur cycle, involving bacteria and other microbes. The key pathways and constituents of this biogeochemical cycle are shown in Figure 1.



As shown in Figure 1, hydrogen sulfide is a key compound in the sulfur cycle and one of the most abundant forms of sulfur in the environment. Four fundamental types of reactions are involved in the sulfur cycle: (a) mineralization or decomposition of organic sulfur (from living cells or of synthetic origin), (b) microbial assimilation of simple sulfur compounds into biomass, (c) oxidation of elemental sulfur and inorganic compounds such as sulfides and thiosulfate and (d) reduction of sulfate and other anions to sulfide. $H_2S$ is a direct intermediate in three of these reactions: mineralization, sulfur oxidation and sulfate reduction, all of which can be mediated by various microbes.

Of particular interest for $H_2S$ production are the reductive sulfur reactions mediated by various anaerobic bacteria and the decomposition reactions on sulfur amino acids and other forms of sulfur in biomass. As shown in Table 1, elemental sulfur can be anaerobically reduced by bacteria growing on acetate, such as *Desulfuromonas acetoxidans*, which occurs in anaerobic sediments rich in sulfide and elemental sulfur. Sulfate reduction occurs in many anaerobic environments by the activity of *Desulfovibrio*, other strict anaerobes and by some *Bacillus* and *Pseudomonas* species. $H_2S$ also is produced by sulfur respiration with molecular hydrogen, and this reaction occurs in surface and subsurface geohydrothermal environments (e.g., hot springs, subsurface and submarine hydrothermal vents, etc.). $H_2S$ also is produced by mineralization or decomposition of amino acids and other organic forms of sulfur in biomass. This is a widespread phenomenon in many environments and is produced by many different kinds of bacteria.

**Table 1. Microbial sources of hydrogen sulfide in water and other environmental media**

| Reaction | Conditions | Typical Locations | Bacteria | Comments |
|---|---|---|---|---|
| Reduction of elemental sulfur | Anaerobic | Anaerobic sediments | *Desulfuromonas acetoxidans* | Occurs in fresh waters |
| Sulfur respiration with molecular hydrogen | Anaerobic | Submarine hydrothermal vents | Thermophilic archaebacteria | Occurs in fresh waters |
| Sulfate reduction | Anaerobic | Many: water logged soils, sediments and other anaerobic conditions where sulfate is present | *Desulfovibrio, Desulfomonile, Desulfovibrio, Desulfobacter, Desulfuromonas*and others, including some *Bacillus* and *Pseudomonas* species | Occurs in fresh waters under a wide range of conditions |
| Decomposition of organic sulfur in biomass | Anaerobic or aerobic | Many but wherever there are dead plants and animals | Many; includes desulfhydration of cystiene to yield $H_2S$, ammonia and pyruvic acid | Occurs in fresh waters under a wide range of conditions |

As indicated above, many bacteria can produce $H_2S$ in water and in media (soils, sediments, etc.) in contact with and containing water under a variety of environmental conditions unrelated to the presence of fecal contamination of water. Therefore, it is

possible that false positive results for fecal contamination in $H_2S$ tests can come from a number of sources, including the presence of sulfides of non-biological origin and from the activities of microbes of non-fecal origin. Because of these possibilities, care must be taken in interpreting positive results from $H_2S$ tests. The water source and its environmental conditions, especially geohydrological conditions, must be considered carefully in order to reliably interpret a positive result as being indicative of fecal contamination.

Table 2 represents an attempt to list and summarize the properties of bacteria capable of producing $H_2S$. This list is, which was compiled in part from the 8$^{th}$ edition Bergey's Manual of Determinative Microbiology, is not comprehensive because hydrogen sulfide production information is not available for all genera or species within genera (Buchanan and Gibbons, 1974). It is also clear that these cultured organisms represent a very small fraction of all the microorganisms that occur in natural waters, soil and in association with plants and animals. They may represent as little as 1% of the total bacterial population. Because it is well known and generally accepted that only a small portion of the bacteria in water and other media have actually been isolated and characterized, it is likely that many other $H_2S$ producers exist that have not been described (Altas and Bartha, 1993). Indeed, new $H_2S$ producers continue to be discovered, characterized and taxonomized (Fukui et al., 1999).

The list in Table 2 is based on genera that contain members that can produce hydrogen sulfide. In most cases not all species in a genera have this ability. However, it makes little sense to expand the list to species because there is still great confusion about species names and relationships within genera. Furthermore, there continue to be issues in bacterial taxonomy based on classifications using phenotypic criteria and those using genetic (nucleotide sequence) data. Additionally, only a small fraction of the species in most genera has been described. Despite these limitations in the criteria for listing them, it is clear that many organisms have the potential to give a positive response in the $H_2S$ test.

## Table 2. Microorganisms capable of producing hydrogen sulfide

| Name | Common Source or Habitat | Pathogens | Capable of Giving Positive Test[1] |
|---|---|---|---|
| Phototrophic Bacteria Gram negative | | | |
| Rhodospirillum | Water- strict anaerobic | None reported | Unlikely- slow growth |
| Myxobacteria Gram negative | | | |
| Flexibacteria | Water, soil | None reported | Possible |
| Simonsiella | Mammal oral saprophytes | None reported | Possible |
| Alysiella | Mammal oral saprophytes | None reported | Possible |
| **Budding or Appendaged- Gram negative** | | | |
| Hypomicrobium | Soil, water | None reported | Unlikely- slow growth |
| **Spirochaetes and Spirals- Gram negative** | | | |
| Treponema | Animals | Several | Unlikely- fastidious |
| Spirillum | Fresh and salt water | None reported | Possible |
| Campylobacter | Man and animals | Several | Possible |
| **Gram negative Rod and Cocci** | | | |
| Xanthomonas | Soil, water | Plant pathogens | Possible |
| Agrobacterium | Soil, plant tissues | Plant saprophytes and pathogens | Possible |
| Halobacterium- Archebacteria | Water | None reported | Not possible - require 12% NaCl |
| Halococcus- Archebacteria | Water | None reported | Not possible – require 12% NaCl |
| Brucella | Animal bodies | Many human and animal | Unlikely- require $CO_2$ to grow |
| Francisella | Water | Human and animal parasites | Unlikely- fastidious |
| **Gram negative Facultative Rods- Enterics** | | | |
| Edwardsiella | Water, human & animal intestines | Possible | Likely + |
| Citrobacter | Water, food, animal feces, urine | None reported | Likely + |
| Salmonella | Water, food, animals | Many pathogens | Likely + |
| Proteus | Soil, water, animal feces | Possible, none reported | Likely + |
| Yersinia | Human and animal intestines | Yes | Likely + |
| Klebsiella | Widely distributed | Unlikely | Likely + |
| Erwinia | Plant | Plant pathogens | Unlikely- rarely in water |
| Aeromonas | Animals | Possible | Likely + |
| Zymomonas | Beer, fermenting fruit | None reported | Possible |
| Flavobacterium | Water | Rodent pathogen | Possible- $H_2S$ rare |
| Pasteurella | Animals | Many animal pathogens | Unlikely- fastidious |
| Actinobacillus | Animal membranes and tissues | Animal pathogens | Likely + |
| Cardiobacterium | Human nose and throat | Possible | Unlikely- fastidious |
| Streptobacillus | Mammal intestines | Animal parasites and pathogens | Unlikely- fastidious |
| **Gram negative Anaerobic** | | | |
| Bacteroides | Intestinal, oral cavity animals | Rumen bacteria- unlikely | Unlikely- fastidious |

[1] Fastidious means microbes require blood, serum, sterols, etc; slow growth = not able to produce response in the incubation period of the test; no substrates means the needed nutrients are not in the test medium

| | | | |
|---|---|---|---|
| Fusobacterium | Animal mucus membranes | Several | Likely + but rare in water |
| Desulfovibrio | Soil, water, sediment | None reported | Unlikely- no substrates |
| | | | |
| Desulfomonas | Human intestinal tract | None reported | Unlikely – fastidious |
| Desulfobacter | Soil, water, sediment | | Possible |
| Desulfococcus | Soil, water, sediment | None reported | Possible |
| Desulfuromonas | Soil, water, sediment | | Possible |
| Desulfosarcina | Soil, water, sediment | | Possible |
| Butyrivibrio | Rumen | None reported | Unlikely- fastidious |
| Selenomonas | Animal intestines | None reported | Possible |
| **Gram negative Cocci** | | | |
| Neisseria | Animals- many tissues | Many | Unlikely- fastidious |
| Veillonella | Mouth, intestinal track of animals | Possible | Unlikely- slow growth |
| Megasphaera | Rumen, sheep intestine | None reported | Unlikely- slow growth |
| **Gram positive Cocci** | | | |
| Staphylococcus | Human skin, membranes, air, dust | Many | Likely + |
| Peptococcus | Animals- mostly humans | Possible | Likely + |
| Peptostreptococcus | Animal respiratory and UG tract | Several | Possible |
| | | | |
| **Gram positive Endospore Formers** | | | |
| Clostridium | Soil, water, sediment | Some | Possible |
| Desulfotomaculum | Soil, water | None reported | Unlikely- no substrates |
| **Gram positive Non-Spore Formers** | | | |
| Erysipelothrix | Soil, water, fish, animals | Some | Likely + |
| **Actinomycetes- Gram positive** | | | |
| Eubacterium | Cavities of man and animals | Some | Possible |
| Actinomycetes | Soil, water | Both man and animals | Unlikely- slow growth |
| Arachnia | Soil, humans, animals | Some | Likely + |
| Rothia | Soil, animals | None reported | Unlikely- slow growth |
| Actinoplanes | Plants, soil, animal skin | None reported | Unlikely- slow growth |
| Planobispora | Soil | None reported | Possible |
| Dactylosporangium | Soil | None reported | Possible |
| Streptoverticillium | Soil- antibiotic producers | None reported | Unlikely- slow growth |
| Thermomonospora | Soil, animal wastes | None reported | Unlikely- Thermophilic |
| **Mycoplasmas Gram negative** | | | |
| Mycoplasma | Parasites of man | Several | Unlikely- fastidious |

It is highly likely that almost any water will contain a mixture of organisms of both fecal and non-fecal origin that can, under some set of conditions, give a positive result in $H_2S$ tests. There appears to be no reasonable way to preclude all of them from being the sources of false positive reactions. Organisms of fecal origin tend to grow more rapidly than many environmental isolates and are therefore favored under the incubation conditions of the test. The longer incubation times required for the sulfate reducers to be important and for most non-fecal heterotrophs to grow would argue for care to be exercised to keep the incubation times as short as possible, probably no more than 48 hours. However, even shorter incubation times may not prevent the growth and detection of these bacteria in $H_2S$ tests, as will be further documented below. Additional suggestions are made below for modifications to the $H_2S$ media and incubation procedures that may reduce the likelihood of false positive results from environmental $H_2S$-forming organisms.

### 3.3 Sulfate reducing bacteria and $H_2S$ tests

**Hydrogen sulfide producing bacteria**

To adequately evaluate the usefulness of $H_2S$ tests for fecal contamination it is necessary to examine which bacteria are likely to be important because they produce hydrogen sulfide. As shown in Table 2, many bacteria are capable of producing hydrogen sulfide from organic materials. Some of these are unique to or strongly associated with fecal contamination and many others are not. A major group of environmental bacteria producing $H_2S$ is the sulfate reducing bacteria group. Sulfate reducing bacteria (SRB) are key players in the global sulfur cycle. They represent a heterogeneous group of Bacteria and Archaea physiologically unified by their ability to perform dissimilatory sulfate reduction for energy-generating processes. In contrast to assimilatory sulfate reduction the use of sulfate as electron acceptor and its reduction to hydrogen sulfide is restricted to this group. These bacteria are ubiquitous and occur in a variety of habitats, including marine and freshwaters and their sediments, soils, biofilms, microbial mats, intestinal contents, termite guts, walls of "black smokers" and in association with marine worms. Based on 16S rRNA sequences the SRB can be phylogenetically divided into five distinct lineages: (1) Gram-negative mesophilic SRB (delta-Proteobacteria), (2) Gram-positive sporeforming SRP (Low G+C Gram-positive Bacteria), (3) thermophilic bacterial SRB (Nitrospira pyhlum), (4) thermophilic bacterial SRB (Thermodesulfobacterium group), and (5) thermophilic archaeal SRB (Euryarchaeota).

It might be assumed that anaerobic sulfate reducers are unlikely to be able to produce a positive reaction in this test. Sulfate reducers occur in freshwater environments, but they are rarely present in high numbers and usually are associated with the sediments and with geothermal and hydrothermal environments (Widdel, 1988). However, at least some SRBs are microaerophilic and not strictly anaerobic, and these bacteria are now known to be capable of degrading a wide variety of substrates, including saturated hydrocarbons and a variety of aromatic substrates. If the concentrations of sulfate in freshwater are very low, there will be little substrate for these bacteria to use (Wetzel, 2001). However, where sulfate concentrations in water are high, such as geothermal environments, sulfate-reducing bacteria could give a positive results in $H_2S$ tests. It might be assumed that because sulfate reducers do not metabolize complex organic compounds, such as those included as substrates in the test medium, instead requiring short chain organic acids, and other products of fermentation, they would not grow and give positive results in $H_2S$ tests (Kelly and Smith, 1990, Widdel, 1988). For a positive reaction to occur there would need to be time the system to become highly anaerobic, time for fermentative bacteria to produce these products and then allow for the growth of the sulfate reducers. It might be assumed that these conditions are not likely to be achieved in the incubation times typically employed in $H_2S$ tests (1-2 days). However, it is likely that they could be achieved in longer in incubation times of 5 to 7 days, which have been employed in some versions of $H_2S$ tests (Widdel, 1988).

**Studies on non-fecal, environmental bacteria in an $H_2S$ test**

Experiments were conducted in the laboratory of Mark Sobsey to determine if mixed populations of sulfate reducing bacteria (SRBs) would give a positive result in an $H_2S$ test. Sixteen samples of SRBs were obtained from the microbial ecology laboratory of Terry Hazen, Lawrence Berkeley Laboratory. The bacteria were soil isolates from a pristine geothermal region in Kanchatka, Russia, where studies are being done to isolate bacteria of potential value for commercial biogeochemical enterprises. These bacteria were grown in SIM medium and then tested using a commercial $H_2S$ test, the HACH Pathoscreen Field Kit, which is designed to detect hydrogen sulfide-producing enteric bacteria. The HACH test cultures were incubated at 29° to 32°, and examined after 24 hours and 48 hours for darkening of the medium or formation of a black precipitate. Of the sixteen isolates, four produced hydrogen sulfide by the HACH $H_2S$ assay, which is a false positive rate of 25%. These results suggest that $H_2S$ tests may give false positive results for fecal contamination by give positive results for $H_2S$-producing bacteria (primarily sulfate reducing bacteria) unrelated to and not originating from fecal contamination.

### 3.4 Other possible limitations or sources of misinterpretation in the $H_2S$ test

Another issue to consider in relation to $H_2S$ tests is the use of thiosulfate and cystine in the medium as a possible dechlorinating agent. Thiosulfate, like sulfate, could serve as a source of sulfur for microbial reduction and $H_2S$ production, and cystine can be degraded, releasing $H_2S$. The same considerations discussed for sulfate reducers also apply to these obligate anaerobes that degrade amino acids ands other carbon compounds containing sulfur.

Only some of the research conducted to date on the $H_2S$ test has addressed groundwater specifically, and when it has, apparently false positive results have been observed (Kaspar et al., 1992). In ground waters, particularly those contaminated with human or animal wastes, fecal or otherwise, or those containing reduced sulfur from natural or anthropogenic sources, there is a high potential for anaerobic aquifers and the formation of sulfides by bacteria of non-human or non-animal origin. In many rural areas small-scale industry, animal husbandry, and human dwellings are all contiguous, which offers the potential for sulfide formation from sediment-derived degradation of organic wastes from these sources, only some of which are fecal sources.. The rapid reaction of the iron with sulfide already present in a water sample could produce a darkening in an $H_2S$ test almost immediately upon addition of the sample. For this reason, it is very important that the test procedure include visual checking for a quick or early positive reaction, after perhaps a few minutes to one-hour of incubation. A positive result so quickly is likely to mean that the sample already contained sulfides. Such a result is not readily interpretable as either positive or negative for fecal contamination because it is not useable as evidence of microbially mediated $H_2S$ activity likely to be associated with fecal bacteria.

Because a black precipitate is the experimental end point of the $H_2S$ test, there may be concern for formation of other dark colored metal salts from constituents present in a water sample. The Handbook of Chemistry and Physics identifies relatively few iron salts that are black besides FeS. Some of the oxides of iron can form black precipitates but they are unlikely to form in water samples as a result of microbial activity and should be visible immediately at the onset of the test if present. Other metals can react with sulfide to produce a black or dark precipitate, but such precipitates, if produced after a time period consistent with microbial activity also would be evidence of $H_2S$ production.

**Microbially induced corrosion as a source of $H_2S$-producing bacteria in water**

Another concern with the $H_2S$ test and its susceptibility to detecting organisms of non-fecal origin is microbially-induced corrosion of iron, steel and other metals associated with water sources,

treatment systems, conveyances and storage facilities. Microbially-induced metal corrosion is caused by a number of naturally occurring bacteria and fungi in microbial communities that include sulfate reducing bacteria, acid producing bacteria and other types of bacteria involved in the corrosion process (Pope and Morris, 1995; Yasushi, 1998). Microbially induced corrosion as a source of $H_2S$ and $H_2S$ producing (sulfate reducing) bacteria is a widespread problem in drinking water supplies. In terms of vulnerability, small, rural groundwater supplies may be at particular risk because of their construction and materials, the presence of iron, steel and perhaps other metals at air-water interfaces, and the presence of risers that are particularly prone to bacterial colonization and corrosion (Tyrell et al., 1996).

### 3.5 $H_2S$ Test Procedures: Media, Formats and Test Conditions

In the initial development of the $H_2S$ test by Manja et al. (1982) the test was applied to various drinking water samples of several cities in India . Water samples containing 10 or more coliform bacteria by MPN were subjected to the new $H_2S$ test using a 20-ml sample volume in a P-A format. On this basis positive $H_2S$ tests were considered unsatisfactory as drinking water. Since this original description of the $H_2S$ test, several investigators have reported modifications of the test intended to improve its performance. Such modifications have included: test medium, medium preparation (dried at elevated temperature, lyophilized, autoclaved only, etc.) sample volume (20 ml,, 100 ml, etc.), paper use, paper type and paper size to which the medium is absorbed, incubation times and temperatures, and test formats (presence-absence, quantitative MPN and membrane filter enumeration). The $H_2S$ test conditions used in the original study and some of the various modifications reported in the literature are summarized in Table 3 below, including some information on how they were tested.

## Table 3. Comparisons of Media, Materials and Methods for the $H_2S$ Test in Different Reported Studies

| Medium Composition | Paper and Size | Preparation | Dried?/ Conditions | Format/Use Procedures | Samples Tested and Other Tests | Comments | Reference |
|---|---|---|---|---|---|---|---|
| 1) 20g peptone, 1.5 g dipotassium hydrogen phosphate, 0.75g ferric ammonium citrate, 1g sodium thiosulfate, 1 ml Teepol, 50 ml water | Tissue paper, 80 $cm^2$, folded | Absorb 1 ml, place in bottle; sterilize | Yes/50°C | P-A, 20 ml sample; Ambient (30-37°C) incubation; 12-18 hrs. | S and G, MPN coliform tests for samples with 10 or more TC/100 ml | First reported development and use of the $H_2S$ test | Manja et al., 1982 |
| 2) Ditto 1) above | Ditto 1) above | Ditto 1) above | Ditto 1) above | P-A, 20-ml sample; incubate at 22 and 35°C | Potable water samples, Lima, Peru | Compared to MPN and P-A tests for coliforms and fecal coliforms | Ratto et al., 1989 |
| 3) Ditto 1) above | Ditto 1 above | Ditto 1 above | Not reported | P-A; 20-ml sample; Ambient 26-30oC incubation; 12-15 hrs. | S (Rx and Cl2); Colilert and LTB-MUG | Compared to *E. coli* tests in municipal water | Kromoredjo and Fujioka, 1991 |
| 4) Ditto 1) above, except specified only certain peptones | None | 2 ml medium in bottle; sterilize | No/lyophilize instead of heat, after autoclaving | P-A, 20 ml sample; Ambient (22-37oC) | S , G and mineral waters; compare to TC and FC MPN tests | Slightly modified medium and its preparation and test format (no tissue paper) | Kaspar et al., 1992 |
| 5) Per Liter: 400 g peptone, 30 g $K_2HPO_4$, 15 g ferric ammonium citrate, 20 g sodium thiosulfate, 20 mL Teepol and 0.25 g/L L-cystine, pH = 6.9 | Same as above | 1 microliter added to folded tissue paper, dried, added to bottle, autoclaved | Yes/at 50°C for 3-4 hours, after autoclaving | Q, 5 bottles of 20-ml sample volumes each | Various, S and G, sometimes diluted; MPN coliforms and fecal coliform tests | Improved test using cystine in medium and a paper strip in a quantitative, 5-bottle (MPN) format | Venkobachar et al., 1994 |
| 6) Ditto 1) above | Not reported | Not reported | Not reported | P-A, 100 ml | Various treated and untreated waters; total coliforms and coliphages | Applied to Chilean waters; isolated and speciated bacteria from positive $H_2S$ tests | Castillo et al., 1994 |
| 7) Ditto 1) above | Paper towel, 80 $cm^2$, folded | Ditto 1) above | No | P-A, 20 ml sample, | Distilled water seeded with Salmonella | Optimized as presumptive *Salmonella* Test | Gawthorne et al., 1996 |
| 8) Ditto 1) above | Not reported | Not reported | Not reported | P-A, 100 ml | Various S and G; total coliform bacteria and coliphages | Tested for comparative detection in treated and untreated Chilean drinking waters | Martins et al., 1997 |
| 9) Ditto 5) above | Paper towel 50 $cm^2$ | 1 ml aliquots added to folded paper towel, autoclaved; placed in sterile plastic 40-ml sample bottles | No | P-A, 20 ml sample | Various S and G; Total and fecal coliforms by MF methods | Tested for sensitivity and specificity with pos. and neg. controls and in presence of high levels of other bacteria; also field tested | Genthe and Franck, 1999 |
| 10) Ditto 1) above | Ditto 1) above | Ditto 1) above | Ditto 1) above | P-A, 20 ml | Ground waters | Isolated and speciated bacteria from pos. samples | Naraju and Sastri, 1999 |
| 11) Tested 3 media: ditto 1) above; ditto 1) above + 0.125g; - ditto 1) above+5 g yeast extract and only 15g peptone | same as above | Add 5 ml medium, place in bottle, autoclave | No | P-A, 100 ml sample, multiple temperatures from 0-47°C, incubate up to 5 days | Feces diluted in sterile distilled water; 100-ml $H_2S$ test vs. fecal coliforms | optimize medium, incubation time and temperature on samples of feces, but not water | Pillai et al., 1999 |
| 12) 10 g peptone, 1.5 g dipotassium hydrogen phosphate, 1.5 g ferric | None | Prepare agar medium, autoclave and | No | Filter sample thru 47-mm diameter membrane filter; place on agar | Cistern, ground and stream waters; total and fecal coliforms and *E. coli* | Compared $H_2S$ MPN to $H_2S$ MF to TC and E. coli. | Rijal et al., 2000 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ammonium citrate, 1 g sodium thiosulfate,, 15 g agar and 1 L deionized water, pH 7 | | pour into 60x15 mm plates | | medium; incubate anaerobically at RT (25-30°C) , 24 hrs. | | | |
| 13) ditto 1) above; ditto 1) above, except 15g peptone and 1g yeast extract; "1above except 250mg L-cystiene, "1)above except 15g peptone and 250mg L-cystiene | "1)above | "1)above; or single-strength powder media, radiation sterilization; no tissue | Yes for original medium/60°C for 2 days. | P-A, 20 ml sample; 35oC; 18, 24 and 48 hour incubations times | | Compare original liquid and commercial spray dried media on Citrobacter and Salmonella | Manja et al, 2001, 2001 |

Abbreviations: G = groundwater, S = surface water, P-A = presence-absence test, Q = quantitative test, TC = total coliforms, FC = fecal or thermotolerant coliforms, Ec = *E. coli*

As shown by the data summarized in Table 3 above, various modifications of the $H_2S$ test have been reported. Besides the use of different modifications of the original test in the various studies, the modifications were developed and evaluated using different samples in the various studies. Some studies used diluted feces, others used laboratory cultures of specific bacteria, such as *Salmonella* and others used field samples of water of varying quality.

Pillai et al. (1999) used feces diluted in distilled water to evaluate medium composition, incubation time, incubation temperature and fecal coliform bacteria concentration to optimize conditions for a 100-ml sample volume $H_2S$ test. They found that lower concentrations of bacteria required longer incubation times and higher temperatures for $H_2S$ detection, results did not require an incubator if room temperature was between 20-44$^oC$, and the presence of L-cystine in the medium improved detection. The test was not applied to field samples of water.

Gawthorne et al. (1996) evaluated the $H_2S$ test for *Salmonella* detection using four species grown in the laboratory and then seeded into water. They found that detection of as little 5 CFU/100 ml was possible, longer incubation times (48 hours) increased detection of low *Salmonella* levels, and the presence of other bacteria has no effect on *Salmonella* detection. The $H_2S$ test was recommended as a presumptive test for *Salmonella* in drinking water in conjunction with coliform testing.

Venkobachar and colleagues (1994) incorporated cystine into the $H_2S$ medium and compared the original $H_2S$ test to the one with the modified medium containing cystine using different water sources. Correlation analyses indicated that the cystine-modified test was more sensitive and less time-consuming than the original test.

Rijal and colleagues (2001) developed and evaluated two modifications of the $H_2S$ test: (1) a MPN version using replicate sample volumes of 1, 10 and 100 ml and an enumerative version for $H_2S$ colonies on membrane filters in using an agar medium. When both $H_2S$ tests were compared to each other and to coliforms and *E. coli* in rainwater cisterns of drinking water, both $H_2S$ methods gave results comparable to *E. coli*.

In studies by Manja et al. (2001) the following media were compared for the $H_2S$ test: (1) original $H_2S$ medium, (2) original medium with 250mg L-cystiene, (3) original medium with decreased peptone at 15g and added yeast extract at 2 g, and (4) medium 3 with 250 mg L-cystiene and a the lower peptone concentration of 15 g Based on detecting low levels of *Citrobacter freundii* and *Salmonella typhimurium* type strains seeded into sterile distilled water at about 5 CFU per sample, medium formulation 3 (original medium plus 250 mg/L cystiene) was judged to give the best results based on the numbers of positive samples obtained. In addition, the commercial powder form of the medium gave better results than the strip medium (liquid medium applied to paper and dried in the lab). It was concluded that as few as 1 *Salmonella* was detectable in 20 ml of sample.

As summarized in Table 3, the results of several studies indicate that various forms of $H_2S$ test have been evaluated and are being used. The various forms of the $H_2S$ test differ in the following ways: the medium and its preparation procedures, media format (dried onto paper strip, use as powder, and agar medium), test format (presence-absence, MPN, and membrane filter), sample volumes, incubation times and incubation temperatures. If the results of these studies are taken together, it appears that the

addition of cystine or cystiene, longer incubation times (24-48 hours) and incubation temperatures in the range of 25-35°C give the best results in terms of detecting low levels of $H_2S$ producing bacteria. However, there has been no systematic comparison of the various forms of $H_2S$ tests used by different investigators and no effort to achieve a standard test procedure. Until recently, all of the $H_2S$ tests required the use of media formulated from scratch and applied to paper strips manually. Therefore, the test media or materials were was not readily available from commercial sources. In India, there have been efforts to have the medium made commercially and to implement performance criteria for the commercially prepared medium. In the United States of America one Company has marketed an $H_2S$ test kit for use by small labs and consumers. However, this commercial test is probably too complex, too inaccessible and too costly to be used in the developing world in response to the constraints in section 2.6. Research and development studies have been done in India by UNICEF (United Nations Children's Fund)-India and its partners (Rajiv Gandhi National Drinking Water Mission, Department of Drinking Water Supply, Ministry of Rural Development, Government of India) to develop, evaluate and disseminate the specifications for a $H_2S$ test and field kit for use in drinking water (Manja et al., 2001). The test is not advocated as a replacement for conventional coliform and other bacteriological testing. It is recommended for use by community workers to monitor water supply sources. At the present time there remain considerable obstacles to the widespread use of the $H_2S$ tests because of their lack of uniformity and lack of availability in a ready-to-use form. Greater efforts to determine the optimum properties for and test conditions of $H_2S$ tests are recommended. Also recommended are further efforts to evaluate their validity, reliability and predictability as fecal indicators before widespread production, dissemination and use of either commercial or made-from-scratch $H_2S$ tests.

### 3.6 Comparison of $H_2S$ Tests to Other Tests for Detecting Fecal Contamination of Water

In evaluating the performance of the $H_2S$ test for detecting bacteriological evidence of fecal contamination of water, it is necessary to have a basis or reference point ("benchmark") for determining efficacy. In some studies the basis for determining efficacy has been the use of sterile water seeded with known quantities of specific $H_2S$-producing bacteria or mixed populations of $H_2S$ bacteria in the form of diluted feces. However, such testing does not address the variability of water matrices in terms of their chemical and microbiological quality. Testing of natural waters containing fecal contamination also is necessary. Therefore, many evaluations of the $H_2S$ test have been based on performance comparisons with other, conventional bacteriological tests for fecal contamination of drinking water when applied concurrently to field samples of water. Tests for fecal contamination of water against which the $H_2S$ test has been compared includes: coliforms, thermotolerant (fecal) coliforms, *E. coli*, *Clostridium perfringens*, *Salmonella* and coliphages. Because none of these other tests are ideal at detecting fecal contamination of water, the results of such comparisons are open to interpretation. However, most investigators assume that if the $H_2S$ test gives positive results similar to or greater than the reference test, its performance is acceptable. The results of a number of comparative studies of the $H_2S$ test against other bacteriological tests for fecal contamination are summarized in Table 4 below.

**Table 4. Comparisons of $H_2S$ and Standard Bacteriological Tests Applied to Various Samples**

| Water Samples | Bacterial Indicator(s) Compared to $H_2S$ test | Bacterial Results Suitable/Unsuitable | $H_2S$ test Results Suitable/Unsuitable | Agree-ment, % | % Disagreement | Reference |
|---|---|---|---|---|---|---|
| Various S and G Waters; India | Coliforms (+ *E. coli* in one set of samples) | 332/337 (coliforms) | 332/337 | 88.3 | 13% and 13% | Manja et al., 1982 |
| Tap water, Peru | Total and fecal coliforms | 11/9 (TC); 11/9 FC | 11/9 | 95 | 5% | Ratto et al., 1989 |
| Tap (Rx S), Banjarmasin, Indonesia | Coliforms and *E. coli* | 1/24 Colilert 3/24 LTB-MUG | 2/24 | 96 | 4 | Kromoredjo and Fujioka, 1991 |
| Various S and G, India | Total and fecal coliform MPN and original H2S test | Not Reported; see % agreement and disagreement | Nor reported; see % agreement and disagreement | TC: 89 | 11 | Venkobachar et al., 1992 |
| | | | | FC: 91 | 9 | |
| | | | | Orig H2S: 94 | 6 | |
| Undisinfected water, Chile | | 49/170 (TC) | 36-40/179-183* | 90-92 | 10-12% | Castillo et al., 1994 |
| Disinfected water, Chile | | 290/113 | 254-278/125-149* | 71-77% | 23-29% | Castillo et al., 1994 |
| Raw waters, Chile | | 42/12 | 42/12 | 100 | 0 | Martins et al., 1997 |
| Treated water, Chile | | 50/4 | 43/9 | 81 | 19 | Martins et al., 1997 |
| Mysore, India | Not Done | Not Done | 14/37 | No Data | No data | Nagaraju and Sastri, 1999 |
| Various, South Africa | Total and fecal coliform MF | 189/224 | 150/263 | 82% | 18% | Genthe and Franck, 1999 |
| Feces diluted in distilled water | Fecal coliform | Tested but reported results not quantified | Tested but reported results not quantified | Not reported | Not reported | Pillai et al., 1999 |
| Cistern rainwater | | 0/25 (TC), 8/17 *(E. coli*) | 8/17 $H_2S$ agar 9/16 $H_2S$ MPN | 68 TC 100 *E. coli* | 0 TC 32 *E. coli* | Rijal et al., 2000 |
| Municipal drinking water supplies | Fecal coliform MPN | 294/392 | 296/390 | 90% (92% for (+)ive; 88% for (-)ive | 10% | Manja et al., 2001 |

As shown in Table 4, when applied to water samples having 10 or more total coliform MPN per 100-ml, Manja et al. (1982) scored the same numbers of samples suitable and suitable by both MPN coliform tests (#10/100 ml = suitable >10/100 ml = unsuitable) and the $H_2S$ test (not black in 20 ml = suitable and black in 20 ml = unsuitable). When the water samples were divided into ranges of

coliform concentrations, the $H_2S$ test gave positive reactions for all samples with 41 or more coliforms per 100 ml, 25/34 $H_2S$ positive for samples with 21-40 coliforms per 100 ml, and 37/44 positive with 11-20 coliforms per 100 ml. $H_2S$ positive-samples contained *Citrobacter freundii* (23 samples), *Salmonella* species (6 samples), *Proteus mirabilis* (2 samples), *Arizona* species (2 samples) , Klebsiella (1 sample) and *E. coli* (3 sample). Only 1 type of $H_2S$-producing organisms was isolated from each separate sample and the methods of isolation were not specified. No tests were done for the presence of absence of other, environmental $H_2S$-producing bacteria. No specific tests were done to determine the presence of viral, bacterial or parasitic pathogens, although *Salmonella* were detected in some samples. The authors considered the test reliable, simple to perform and useful for screening purposes where resources, time, manpower and laboratory facilities are limited.

Ratto et al. (1989) evaluated the original $H_2S$ test at incubation temperatures of 22 and 35°C and compared it to MPN and P-A total coliform (TC) and fecal coliform (FC) tests on 20 potable water samples from Lima, Peru. The frequency of positive (unsuitable) samples was similar but not identical for all tests: 9/20 by P-A, 9/20 by $H_2S$ at 35°C, 6/20 by $H_2S$ at 22°C, 8/20 by TC MPN and 6/20 by FC MPN. It was concluded that the $H_2S$ test was an equally or more sensitive test than TC and FC tests and was an ideal procedure for isolated water supplies where laboratory facilities do not exist.

Kaspar et al. (1992) evaluated a modified version of the original $H_2S$ test (no tissue paper and lyophilizing rather than heat drying of the medium) and applied it to 101 water samples. They concluded that the test was not suitable for control of surface water and dug well water due to the frequent presence of non-fecal (total) coliforms presumed to arise from degradation of plant tissues and poikilothermic animals. Dug wells nearly always gave positive results in the $H_2S$ test and in the coliform test, but not fecal coliforms. They concluded that the test was useful for qualitative screening of piped or treated water systems. However, it was concluded that positive $H_2S$ test results must be confirmed by standard bacteriological tests. The test was considered valuable as an educational and motivational tool for improved water sanitation, because of the color change and foul smell from positive samples.

Venkobachar et al. (1994) developed a modified $H_2S$ test that included cystine in the medium and was used in an MPN test with five 20-ml samples. The modified test reduced the test time from 23 to 17 hours, was more sensitive than the original $H_2S$ test, and was well correlated with total coliform (89%) and fecal coliform (91%) tests when applied to 101 water samples. It was concluded to be simple, requiring little laboratory support and well suited for routine quality assessment of rural water sources.

Sivaborvorn (1988) tested 705 samples from a variety of waters in Thailand (shallow and deep wells, rainwater, pond water) by the original $H_2S$ test and by coliform MPN. Based on agreement between a positive $H_2S$ test and 10 MPN/100 ml as a coliform or fecal coliform positive, the two tests agreed 85% and 88% of the time, respectively. It was concluded that the $H_2S$ test can be used to screen water for fecal contamination in the field where laboratory facilities are limited.

Castillo et al. (1994) reported that for 622 water samples tested by the $H_2S$ and coliform tests, 168 samples were positive by both tests and 179 samples were negative by both tests. The $H_2S$ test produced about 10% more positive samples than the coliform test but included samples that were positive for *Clostridium* spp. The $H_2S$ test gave similar results at both 32 and 35°C, indicating that temperatures in this range are not critical. Bacteria detected from $H_2S$ positive samples included

*Klebsiella* spp., *Enterobacter* spp., *E. coli*, *Citrobacter* spp., *Aeromonas* spp., *Clostridium* spp., *Hafnia* spp., *Salmonella* spp., *Acinetobacter* spp., *Morganella* spp.. It was concluded that the simplicity and low cost of the H2S test was applicable to tropical and subtropical potable waters.

In studies of 54 complete conventionally treated drinking waters and their corresponding raw source waters Martins et al. (1997) found 100% agreement between total coliform and $H_2S$ results for raw waters and 81% agreement for treated waters (Table 3). The $H_2S$ test was modified by increasing the sample size to 100 ml. In treated waters more samples were positive by the $H_2S$ test (9) than by the coliform test (7), which was attributed to the presence of *Clostridium perfringens* in the $H_2S$-positive but TC-negative samples. In treated waters the $H_2S$ and TC results were significantly positively correlated ($P < 0.0001$) Spearman rank correlation test) but in raw waters they were significantly negatively correlated ($P = 0.0008$). The authors concluded that the $H_2S$ test was a suitable indicator of potable water quality and treatment and provided greater protection than the total coliform test.

Nagaraju and Sastri (1999) tested ground water wells of Mysore city, India for $H_2S$ bacteria using the methods of Manja et al. (1982) and 24-hour incubation at 37°C. 37 of 51 ground water samples were positive. From these $H_2S$-positive samples the following 63 bacteria were isolated: *Proteus mirabilis* (19), *Proteus vulgaris* (14), *Citrobacter freundi* (13), *Salmonella* spp. (8), *Klebsiella* pneumoniae (5) and *Klebsiella ozaene* (4) were isolated.

Genthe and Franck (1999) evaluated the $H_2S$ test of Venkobachar et al. (1994) for specificity, sensitivity and interference by non-target bacteria using seeded positive and negative samples and reported favorable results. When applied to 413 water samples from various sources, including ground and surface water, the $H_2S$ test showed 82% and 86% agreement with fecal coliform results when applied to higher quality (so-called level 1) waters with test incubation temperatures of 35 and 22°C, respectively. It was concluded that the $H_2S$ test was sensitive and correlated with traditional indicator bacteria, especially fecal coliforms. The test was considered useful for on-site field use, light, easy to use and portable. The test was recommended for use in addition to current water quality indicators of microbial quality, and especially where testing would otherwise not normally be done.

Pillai et al. (1999) evaluated various modifications of $H_2S$ tests for detection of fecal contamination using 100-ml volumes of feces diluted in distilled water to contain different levels of fecal coliform bacteria. The presence of cystine in the medium and higher incubation temperatures 28-44°C vs. 22°C) improved detection, with lower levels fecal contamination (fewer fecal coliforms) detected faster.

Rijal et al. (2000) compared two versions of the $H_2S$ test, a paper strip MPN and a membrane filter enumeration on agar medium, to each other and to the occurrence of total coliforms and *E. coli* in samples of cistern rainwater, ground water and stream water. Similar detection of bacterial contamination was achieved by the MPN, MF version of the $H_2S$ test and *E. coli*, although total coliforms were detected in more samples than were either *E. coli* or $H_2S$ bacteria. The $H_2S$ test was compared to total and fecal coliform and *E. coli* tests to determine efficacy of a solar disinfection system. Similar results for indicator reductions were achieved by all fecal indicator tests used. The authors concluded that the $H_2S$ test was a reliable method to measure the hygienic quality of water.

Manja et al. (2001) compared the $H_2S$ test (with cystiene in the medium, different sample volumes, different incubation times and incubation at different temperatures) to MPN tests for coliforms for

detecting fecal contamination in 686 water samples in India. The $H_2S$ test gave results comparable to the MPN test (not significantly different), with concordance in 620 (90%) samples, negative $H_2S$ test and positive MPN test (false negative) in 34 samples (4.9%), and positive $H_2S$ test and negative MPN test (false positive) in 32 samples (4.7%). However, 21 of 23 "false positive" (negative coliform MPN) samples had coliforms in $H_2S$ bottles. Agreement of $H_2S$-positive and coliform- positive samples increased from 91% at 48 hours to 95% at 72 hours. The $H_2S$ test results were comparable (not significantly different) for sample volumes of 20, 55 and 100 ml. Positive $H_2S$ results were generally obtained in 18-48 hours of incubation at 25-44$^oC$. Use of an incubation temperature below 25$^oC$ was not recommended.

As shown in Table 4, The $H_2S$ method has been extensively studied by a number of investigators in different parts of the world. Such studies include evaluations of the original method, studies on modifications of the method and field testing, usually with side-by-side comparison to other water quality tests. In some of these comparison studies the data are limited or have not been subjected to rigorous statistical analysis. However, the results of most studies suggest that the $H_2S$ method detects fecally contaminated water with about the same frequency and magnitude as the traditional methods to which it was compared. In general, the sensitivity of the $H_2S$ test appears about the same as other tests for fecal contamination of water, although as already noted, this aspect of the test has not been rigorously tested in some of the reported studies. Testing conditions and format, sample size, incubation temperature and incubation time influences test sensitivity. Because these conditions have differed among the different studies reported in the literature, it is difficult to make consistent comparisons and draw overall conclusions. However when comparisons with other methods of detecting fecal contamination were done, the $H_2S$ method appeared to have sensitivity similar to the other methods, based on finding contaminated samples.

In most comparative studies there were always samples that yield positive results for other microbiological tests and negative $H_2S$ tests, and vice versa. However, such results are not unexpected. For one, the various tests measure different things and do not always employ the same sample volumes. Furthermore, when the levels of microbial contamination are low, it is statistically possible for one sample volume to contain bacteria of interest and for another to not contain them. Where study data were subjected to statistic analysis most studies found high associations (e.g., correlation) between fecal indicator bacteria (e.g., E. coli) and positive $H_2S$ results. Given the previously discussed ability of a large variety of heterotrophic bacteria to produce a positive $H_2S$ test and therefore a false positive the observed correlations suggest that in most natural and treated waters the majority of the $H_2S$ producers come from organisms associated with the human or animal digestive tract. A false positive is less likely to lead to a risk of disease because it would result in the suspect water either not being used or subject to additional testing.

Of great concern with the $H_2S$ test as with other fecal indicator tests is the potential for false negatives; that is, not detecting fecal contamination when it is present. In this case the test does not identify water that is unsafe and the water could be consumed, leading to pathogen ingestion and to disease. The method, as with the various bacteriological tests, does not detect viruses or parasites. Testing of drinking water for the many viruses and parasites of concern is still impractical and unaffordable and still not done on a routine basis in most countries and regions. However, the $H_2S$ test detects bacteria other than coliforms that are associated with fecal contamination, including *Clostridium perfringens*. *Clostridium perfringens* is one of the more resistant indicators of fecal contamination and can be found in drinking waters when no coliforms can be found. Therefore, it is possible for the $H_2S$ test to give a

positive result when fecal contamination is present even if no coliforms are present. Such findings have been observed in some comparative studies between the $H_2S$ test and other bacteriological tests.

### 3.7 Determination if $H_2S$ Tests Meet the Criteria of an Ideal or Preferred Indicator of Fecal Contamination

In Table 5 below are listed the essential criteria of an ideal or preferred indicator of fecal contamination of drinking water and other waters and the extent to which these criteria were addressed and fulfilled in previously published studies on the $H_2S$ test as a method to detect fecal contamination. It is apparent from this compilation of data that most of the key criteria for fecal indicators of water quality were not investigated in the studies reported in the literature to date. This lack of data on the extent to which $H_2S$ tests fulfill the essential criteria of an indicator is a major concern. This is because the test has been in existence for two decades, it has been repeatedly modified, tested and field applied in many parts of the world, it is now widely promoted by some scientists and other authorities, and yet it has never been subjected to critical testing for its ability to fulfill or meet the essential criteria of a fecal indicator of drinking water quality.

## Table 5. Fecal Indicator Criteria for Fecal Contamination and The Extent to Which they are Met in Reported Studies of H2S Tests

| Reference | Absent in non-fecally contaminated water | Present in fecally contaminated water | Outnumber pathogens | Detects Non-patho-genic Bacteria | Respond to environ-mental condi-tions like patho-gens | Respond to treatment like pathogens | Ease of Use | Cost (Inex-pen-sive?) | No envir-onmen-Tal multi-Plica-Tiom |
|---|---|---|---|---|---|---|---|---|---|
| Manja et al., 1982 | T/ by comparison to other indicators of fecal contamination | T/ by comparison to other indicators of fe | NT | T/Some detected are non-pathogenic | NT | NT | NT | NT | NT/ M for some |
| Ratto et al., 1989 | T/Yes, by comparison to other indicators of fecal contamination | T/Yes, By comparison to other indicators of fecal contamination | NT | T/Some detected are non-pathogenic | NT | NT | NT | NT | NT |
| Kromoredjo and Fujioka, 1991 | T, Yes, by comparison to other indicators of fecal contamination | T/ by comparison to other indicators of fecal contamination | NT | NT | NT | NT | NT | T/ by cost compariso n | NT |
| Kaspar et al., 1992 | T/ by comparison to other indicators of fecal contamination | T/Yes, by comparison to other indicators of fecal contamination | NT | T/Some are | NT | NT | T/ Yes* | NT | NT/ |
| Venkobachar, 1994 | T/Yes, by comparison to other indicators of fecal contamination | T/Yes, by comparison to other indicators of fecal contamination | NT | T/Some detect-ed are non-pathogenic bacteria | NT | NT | T/ Yes | NT | NT |
| Castillo et al., 1994 | T/Yes, by comparison to other indicators of fecal contamination | T/Yes, by comparison to other indicators of fecal contamination | NT | NT | NT | NT | NT | NT | NT |
| Gawthorne et al., 1997 | NT/ Lab studies on seeded water | T/Yes, by comparison to levels of Salmonella in seeded waters | NT | NT | NT | NT | NT | NT | NT |
| Martins et al., 1997 | T/Yes, by comparison to other indicators of fecal contamination | T/Yes, By comparison to other indicators of fecal contamination | NT | NT | NT | Y, Indirect T; results compared for Rx'd and UnRx'ed water | NT | NT | NT |
| Genthe and Franck, 1999 | T/ Yes, by comparison to other indicators of fecal contamination | T/ Yes, by comparison to other indicators of fecal contamination | NT | NT | NT | NT | T/ Yes | T/ Yes, by cost compariso n | NT |
| Nagaraju and Sastri, 1999 | NT | NT | NT | T/ Yes | T/ Yes | NY | Yes | NT | NT |
| Rijal et al., 2000 | T/yes, by comparison to other indicators of fecal contamination | T/yes, by comparison to other indicators of fecal contamination | NT | T/Some detected are non-pathogenic | NT | T/ Yes, compared $H_2S$, TC and FC tests on solar pasteurized waters | NT | NT | NY |
| Manja et al., 2001 | T/ Yes, comparison to other indicators of fecal contamination | T/ Yes, by comparison to other indicators of fecal contamination | NT | NT | NT | NT | NT | NT | NT |

Abbreviations: T = tested; NT = not tested by objective or specified measures or methods

### 3.8 Potential modifications of the $H_2S$ test to improve specificity for $H_2S$-producing bacteria of fecal origin

There are several modifications to the $H_2S$ test that could be considered in an effort to make it more specific for organisms of fecal origin and to reduce the probability of organisms of non-fecal origin giving a positive response. These modifications fall into two main categories: modifications of the medium itself and modifications of the incubation conditions.

The inclusion of bile salts such as sodium deoxycholate or taurocholate in the medium is a common method for inhibiting the growth of many microorganisms. These surfactants can cause the lysis of sensitive cells. Because human and animal intestinal flora has to tolerate these materials in the intestine they tend to be less sensitive to them. Indeed, the use of bile salts is common in bacteriological media to detect Enterobacteriaeceae. Archebacteria and many eubacteria are sensitive, while *Salmonella* and *E. coli* are not (Gerhardt et al., 1994; Kamekura et al., 1988). Gram negative organisms are generally insensitive, while gram positives other than Group D streptococci are sensitive. Therefore, the inclusion of bile salts at 0.5% would eliminate the bacilli, the archebacteria and most other soil organisms and prevent them from producing a positive result. However, this modification also may decrease the detection of *Clostridium perfringens*, which can be of fecal origin and is detectable by current versions of the medium. The extent to which the addition of a bile salt would inhibit *C. perfringens* detection by its $H_2S$ production is uncertain and would have to be investigated.

Several modifications of the incubation procedure are suggested in an effort to increase specificity for bacteria of fecal origin. One modification to consider is aeration of the medium by vigorous shaking prior to incubation in an effort to make the system aerobic. This would slow or preclude the growth of many anaerobic or microaerophilic organisms, some of which (e.g., the sulfate reducing bacteria) may give false positives. Additionally, elevated incubation temperatures would be advantageous in reducing the growth of some soil and water organisms of natural origin. Some of these environmental bacteria generally do not grow as well at temperature above $30^o$ C, in contrast to bacteria of human or animal origins. However, elevated incubation temperature may not be an effective way to control the growth of thermotolerant or thermophilic bacteria of natural origin, such as those from geohydrothermal environments because these bacteria are adapted to higher temperatures. Furthermore, higher incubation temperatures such as $35^o$ C will make the test potentially more difficult to apply in the field and in settings other than microbiology laboratories because an incubator may be required.

### 3.9 Costs of $H_2S$ Tests

It was not possible to find detailed breakdowns of estimated costs for $H_2S$ tests, but all reports indicate lower costs than conventional bacteriological tests for fecal contamination. Several investigators have listed the costs of $H_2S$ tests and compared them to the costs of standard bacteriological tests for fecal contamination of water.

Kromoredjo and Fujioka (1991) reported that the cost to analyze one sample by a 5-tube MPN test was: US$6.50 by commercially available, defined substrate

technology test, US$1.60 by fecal coliform test using lauryl tryptose broth (LTB) plus 4-methylumbelliferryl-Beta-D-glucuronide (MUG) and $US0.62 using the $H_2S$ test. They noted that the relative costs for the commercially available tests would be even higher because its high shipping costs were not included and the tubs would be discarded after their use, while the shipping costs for LTB-MUG fecal coliform and $H_2S$ tests would be comparatively low and the tubes used in these tests would be reused up to five times. Kaspar et al. (1992) indicated that the cost of the $H_2S$ test was much lower than the costs of common microbiological tests, such as those for coliforms, but detailed costs estimates and comparisons were not provided. However, they indicated that the costs of materials for an $H_2S$ test were about US$0.25 per unit. Genthe and Franck (1999) stated that the cost of the $H_2S$ test was inexpensive. They listed the approximate costs of the H2S test at <5.00 South African Rand or ZAR <US$0.44). The estimated costs of materials for the membrane filter (MF) fecal coliform test were about 7.00 ZAR or about US$0.61 and for the defined substrate technology tests, they were about 30-50 ZAR or US$2.60-4.35). The costs for the $H_2S$ test did not include material preparation time and the costs for the fecal coliform MF test did not include media preparation and carrying our filtration.

Overall, these data indicate that the $H_2S$ test costs are relatively low compared to those for standard bacteriological tests, either prepared in one's lab or commercially purchased as ready to use. However, a more formal analysis of $H_2S$ test costs listing all of the cost elements and methods of cost calculation is recommended. It should be recalled that the costs of analyses may represent a small fraction of total costs if staff time and travel costs are taken into account.

# 4. SUMMARY AND CONCLUSIONS

The $H_2S$ method in various modifications has been tested in many places in different waters and produced results reported as indicating it to be a reasonable approach for testing treated and untreated waters for fecal contamination. It offers advantages including low cost (estimated at 20% of the cost of coliform assays), simplicity and ease of application to environmental samples.

It has not been suggested as a replacement for other testing procedures for fecal contamination of water.

Because it has not been adequately tested in regions with temperate and cold climates nothing can be said about its applicability in those regions.

Because it offers the potential for testing water in places where other testing methods are not feasible, its promotion, dissemination and use have been encouraged by many developers and evaluators. However, as is apparent from the review and analyses presented here, $H_2S$ tests have not been evaluated and judged according to the generally accepted criteria of an indicator of fecal contamination, except perhaps indirectly and by comparison. That is, no systematic efforts have been made to determine directly if $H_2S$ tests fulfill the essential criteria for an indicator of fecal contamination in treated and untreated drinking water and its sources. Instead, previous studies have attempted to validate and evaluate the test against on the basis of the detection of established fecal indicator bacteria and certain pathogens, notably *Salmonella*, in experimentally seeded and in field samples of treated and untreated water. If there is adequate agreement (correlation, concordance, etc.) in the classification or quality determination of water based on a standard or generally accepted fecal indicator and an $H_2S$ test, the $H_2S$ test is taken to be an acceptable indicator of fecal contamination. In some studies these measures of agreement have been further supported by the isolation and identification of bacteria of fecal origin from $H_2S$-positive cultures. The criteria for determining agreement between outcomes for $H_2S$ and fecal indicator bacteria tests in comparison studies are not uniform among the various studies reported and often have not been explicitly stated. In some studies these comparisons are supported by statistical analyses for correlations or other associations and in other studies they are not. In some studies, the comparative criteria include efforts to determine sensitivity (lower limit of detection) and specificity (ability to detect specific bacteria or bacterial groups). In all of the reported studies, no efforts have been made to determine if the $H_2S$ tests detect non-fecal bacteria capable of producing hydrogen sulfide, such as sulfate reducing bacteria. A preliminary study conducted in the laboratory of one of us (Mark Sobsey) showed that a standard, commercially available $H_2S$ test detects sulfate-reducing bacteria of non-fecal origin in 25% of the samples tested.

Because of these deficiencies, it is not possible to widely and unequivocally recommend $H_2S$ tests for the determination of fecal contamination in drinking water. There remain too many uncertainties about the reliability, specificity and sensitivity of the test for detecting fecal contamination of drinking water and its sources.

Despite this lack of formal analytical support for and validation or verification of $H_2S$ tests, there are many studies reporting relatively good agreement in the classification

of drinking waters as suitable or unsuitable based on the results of $H_2S$ tests in comparison with fecal indicator bacteria tests. Hence, there is substantial empirical evidence showing that $H_2S$ tests and fecal indicator bacteria tests may provide information on the suitability or unsuitability of drinking water with respect to fecal contamination. For this reason, there are good reasons to support the further investigation and use of $H_2S$ tests under certain circumstances and in certain settings. In particular, if the alternative to $H_2S$ testing is no water quality testing at all for fecal contamination, the $H_2S$ test is recommended for use, with caution. The caution concerns possible false positive results due to $H_2S$ presence or formation in water from sources other than fecal contamination. In addition, $H_2S$ testing also is recommended with caution for educational and motivational purposes to promote water sanitation and hygiene education in outreach and dissemination programs. Again, it must be established or verified that the test will give correct results with respect to water classification as suitable or unsuitable when applied to the treated or untreated drinking waters being tested.

Before its adoption for widespread use standardization of the $H_2S$ test procedure as well as conditions and precautions for its use and interpretation based on an improved evidence base will be necessary. The studies conducted to date have used a variety of different media compositions, test strips in some cases, general darkening of the medium in others and even membrane filters and agar media in yet others. A variety of different incubation times and temperatures have also been used. A number of investigators have addressed incubation time and temperature issues that can result in false negatives if the appropriate choices are not made (temperatures too low or incubation times too short). Some have suggested the addition of cystine or cysteine to the medium and supplementing the medium with yeast extract and deoxycholate. There is likely to be merit in many of these suggestions.

Empirically, the test appears to detect mostly organisms of fecal origin (human or animal), and therefore, it may have value. There is no reasonable way, however, to preclude a false positive in samples containing other $H_2S$ producers, nor is there an easy and expedient way to determine what those organisms may be. The ability to form $H_2S$ is too widely distributed within the microbial world for it to be an unequivocal test of fecal contamination in water. It also cannot discriminate between organisms of human or animal origin, as long as they produce $H_2S$. Most of the problems likely to be encountered with $H_2S$ tests are false positives rather than false negatives. Such misclassification of drinking water errs on the side of safety. However, it can result in the rejection of water that is acceptable with respect to fecal contamination, and it may prompt efforts to seek or provide alternative or further improved drinking waters. Such efforts in effect increase the costs of providing safe water. If a false positive leads to more testing or the rejection for use of the water for drinking, than alternative sources must be sought and this may be more expensive.

Inadequate attention has been given to the use and reliability of the method for testing ground waters. In many parts of the developing world ground water is the only source of drinking water. In groundwater, there is the strong possibility of sulfides being present due to natural geohydrological sources and to anthropogenic impacts other than fecal contamination, both of which are false positive results. The use of the $H_2S$ test in ground waters needs to be further assessed, as does modification of the

procedure, as suggested above, to allow determination of false positives due to sulfide and non-fecal sulfide-producing bacteria in the sample water.

**In assessing the applicability of the $H_2S$ test in presence/absence format, the limitations of P/A testing should be recalled. P/A testing was developed for and is applicable where most tests provide a negative result. Where a significant proportion of tests provide a positive reaction quantitative testing is preferred in order to determine relative health risk and therefore relative priority of need for correction, such as by improved or greater treatment or by finding a higher quality source water for supply.**

**Water quality testing alone is an inadequate response to the challenges of ensuring water safety. Occasional tests conducted on a water supply may provide a false sense of security as water quality can vary widely and rapidly. For these reasons water quality testing should be accompanied by verification of the state of the source or supply, for instance, by sanitary inspection as described in the WHO Guidelines for Drinking-water Quality Volume 3. As previously indicated, end-product testing is now only one of several key management tools in the provision of safe drinking water under the forthcoming Guidelines from Drinking Water Quality. The development, implementation and use of a Water Safety Plan that considers the quality and overall management of drinking water from source to consumer is the goal for all water supplies. In such a plan, now including hazard analysis-critical control points (HACCP), endproduct testing is not a critical control point (CCP).**

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