# Measurement of the quality of potable water by detection of $H_2S$ -producing bacteria; comparison with detection of fecal coliforms by the Millipore method in rural Nicaragua.

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## INTRODUCTION

For quality control of potable water in rural areas of Nicaragua as well as in the cities of Matagalpa, Jinotega and Madriz we have used the Millipore method of membrane filtration to measure fecal coliforms. The method has many advantages; it is a standard method, recognized world-wide as trustworthy. It can be used in the field as well as in large urban and small rural laboratories. During 16 years of use in Nicaragua, we have found it possible to train workers with a wide range of experience an education in its use and the results thus obtained have always trustworthy.

However the method does have disadvantages. In order to obtain trustworthy results it is necessary to incubate the samples an elevated temperature of exactly 44.5 ±0.2 ºC. Thus the method requires an expensive incubator and a reliable source of electricity, and the cost per test is relatively high.

Therefore, in order to complement the Millipore method to measure the quality of potable water we searched for a method that could be used in rural areas without the need for a laboratory nor a source of electricity. We considered various presence/ absence methods such as those that use the hydrolysis of MUG to detect fecal coliforms; however the majority suffered from two of the same disadvantages as the Millipore method: they required incubation at a precise temperature, and they were too costly.

However the method measuring the presence/ absence of $H_2S$-producing bacteria in water (Manja et al., 1982 ) apparently does not have these disadvantages; samples are incubated at ambient temperature, and the reagents are cheap, at more or less half the cost of other tests. Various published studies have shown that in samples of potable water from a variety of countries (especially tropical) an excellent correlation between the presence of fecal coliforms and of $H_2S$ -producing bacteria (Manja et al. (1982), Kromoredgo & Fujioa (1991), Kaspar et al. (1992), Grant & Ziel (1996)). The correlation is not perfect. One study showed that the correlation with total coliforms was better than with fecal coliforms (Grant & Ziel (1996)), and one study in Malaysia showed little correlation (Desmarchelier et al, 1992). However the method appeared promising for our use in rural Nicaragua. We thus undertook a study to confirm that in the water samples from rural communities in the departments of Matagalpa and Jinotega there was a correlation between the presence of fecal coliforms and of $H_2S$-producing bacteria equal to that shown by others and sufficient that the method could serve for control of the quality of potable water in rural Nicaragua.

## METHODS

**Millipore method for fecal coliforms:**
We use the membrane filtration method for enumeration of fecal coliforms described in "Standard Methods for the Examination of Water and Wastewater" published by the American Public Health Association and approved by the Committee on Standard Methods in 1991. The filters used were from Gelman or Millipore Corp. The growth medium was mFC from Difco.

**Method of detection of bacteria producing $H_2S$.**
To detect the presence of $H_2S$ -producing bacteria in water the growth- medium components shown in Table 1 are added, in dry or concentrated form, to a 100 ml sample of water (Manja et al. (1982), Grant & Ziel (1996)).

Table 1. Growth medium components for detection of $H_2S$ -producing bacteria in water

| **Component** | **grams/ 100 ml water** |
|---|---|
| Peptone | 2.0 |
| Potassium phosphate ($K_2HPO_4$) | 0.15 |
| Ferric Ammonium Citrate | 0.075 |
| Sodium Thiosulfate | 0.1 |
| Sodium Lauryl Sulfate | 0.01 |

The sample is incubated at a temperature between 25C and 35C for 14 hours. During the incubation bacteria produce $H_2S$ from the peptone and the sodium thiosulfate. The $H_2S$ reacts with the ferric ammonium citrate forming a black precipitate of iron sulfides (FeS, or $Fe_2S_3$).
We used the medium in the form of sterile dry powder in capsules, available from the Hach Co., Loveland, CO as "Pathoscreen P/A", Cat.# 26106-96 .

We modified the method of Manja *et al* by incubating the samples in 118 ml "Whirlpak" sterile sample bags (Nasco, Fort Atkinson, WI; Cat# B1062) instead of sterile glass bottles. The entire apparatus is contained in a plastic box of approximately 40 x 20 x 20 centimeters, adapted from a 16-inch tool box (see Figure 1). There is a wire rack which can hold up to 15 Whirlpak bags (Fisher Scientific, cat# ) a thermometer, and containers for cotton swabs, alcohol and the capsules of "Pathoscreen P/A" growth medium.

**Figure 1; Apparatus used for detection in water of bacteria producing $H_2S$.**

After marking the 'Whirlpak' bag so as to identify the sample and the time of sampling, a sample of water is taken according to the norms of "Standard Methods for the Examination of Water and Wastewater ", filling the bag to the 100 ml line. The bag was placed, still open, into the wire rack. A capsule of medium and the blade of a knife or scissors were sterilized with an alcohol-soaked cotton swab, the capsule was cut open and the dry growth medium was poured into the water sample. The bag was carefully closed, without the usual whirling motion, and left in the wire rack at ambient temperature with the box lid closed for 24 to 30 hours. The temperature in the box was checked occasionally to ensure that it was between 25º and 35º C. If the temperature was below 25ºC incubation was continued for at least 30 hours. If it threatened to rise above 35º C any number of means, could be used to cool the samples, including placing the box in the shade or wrapping it in a wet towel.
In Figure 2 are shown examples of typical samples after incubation.
The results from these samples would be recorded as follows:

Negative ( - ) No growth, sample still clear and yellow (e.g. Samples D & E)
Negative ( OTB ) Turbidity, perhaps some change of color, but not completely black (e.g. Sample C).
Positive ( + ) Medium completely black, odor of $H_2S$ (e.g. Samples A & B)

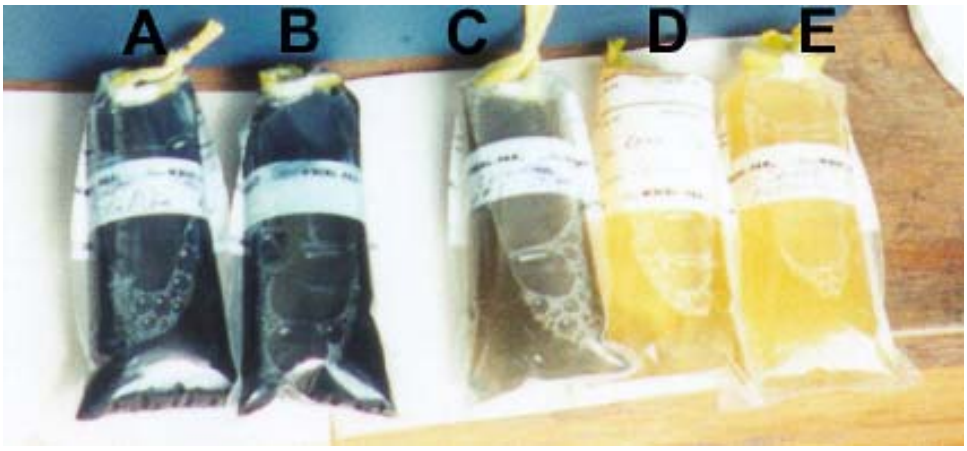


**Figure 2. Typical water samples after incubation to detect bacteria producing $H_2S$.**

**Design of the study.**

We obtained 215 samples from water systems of a number of rural communities in the Departments of Matagalpa and Jinotega, Nicaragua. The types of systems sampled are shown in Table 2. From each point of sampling we obtained two identical samples. To one was added immediately the Pathoscreen P/A medium to begin the test for detection of $H_2S$-producing bacteria. The other was placed in an insulated box, without ice, and carried to a laboratory where it was tested by the Millipore method to measure fecal coliforms. After reading and recording the results the two tests were compared using the statistical program "JMP" .

**Table 2. Summary of water systems sampled in this study.**

| Type of system | Samples total | Chlorinated Samples | Samples with zero Fecal coliforms /100 ml | | Fecal coliforms /100 ml Average/ sample |
|---|---|---|---|---|---|
| | | | # samples | % of total | |
| Deep well drilled by machine | 95 | 4 | 71 | 75 | 34 |
| Hand-dug well | 7 | 0 | 1 | 14 | 80 |
| Gravity systems: | | | | | |
| Open source | 41 | 10 | 8 | 20 | 22 |
| Closed source | 69 | 0 | 32 | 46 | 14 |
| Others | 3 | 0 | 1 | 33 | |
| | | | | | |
| Total | 215 | 14 | 113 | 53 | 26 |

## RESULTS

A summary of the systems sampled is in Table 2. They are as far as possible representative of those that we work with in the rural areas of Nicaragua and include in those classified as "Others" a river and two household water containers.

According to the membrane filtration test, 113 (53%) of the 215 samples had zero fecal coliforms, while 100 (47%) had no $H_2S$-producing bacteria. In Table 3 the results of the study are shown as a Contingency Table in which the results of both the fecal coliform test and of the detection of $H_2S$ -producing bacteria are represented only as either absence (zero) or presence (more than zero).
The two tests agreed exactly in 86% of the samples and from the statistical analysis the probability that the agreement occurred by chance was less than 0.01%. It is important to point out that where there was not agreement the majority (22 of 30, 10% of total samples) were " false positives", that is, samples that showed the presence of $H_2S$ -producing bacteria but with zero fecal coliforms. Only 3.7% of the total number of samples tested as " false negatives ", that is, negative for the presence of $H_2S$-producing bacteria but positive for fecal coliforms.

**Table 3. Comparison of the two tests for fecal contamination of potable water by means of a Contingency Table.**

| | | $H_2S$-producing bacteria | | |
|---|---|---|---|---|
| | Number of samples (% of Total) | **presence** | **absence** | Total (% of Total) |
| **Fecal coliforms, Millipore Method** | **presence** | 93 (43.3%) | 8 (3.7%) | 101 (47%) |
| | **absence** | 22 (10.2%) | 67 (42.8%) | 114 (53%) |
| | Total (% of Total) | 115 (53.5%) | 100 (46.5%) | 215 (100%) |

| **Test** | $\chi^2$ | **Prob** > $\chi^2$ |
|---|---|---|
| Likelihood ratio | 129 | <.0001 |
| Pearson | 114 | <.0001 |
| Fisher's exact test | -- | <.0001 |

This analysis does not take into account the concentration of fecal coliforms in each sample and how this would affect correlation between the two tests. Thus we repeated

the analysis using logistic regression, shown in Figure 3. The estimate of the correlation was not significantly different, at 87%.

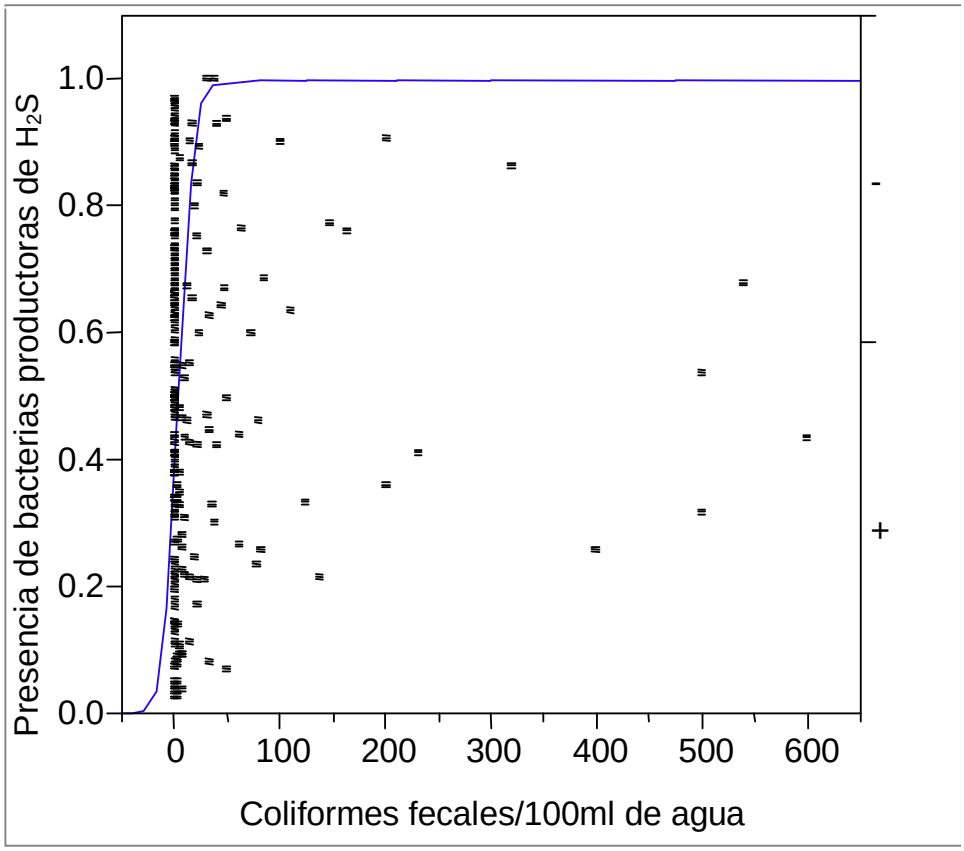


**Figure 3. Comparison of the two tests for fecal contamination of potable water by logistic regression.**

## CONCLUSIONS

This study confirms that in the rural water supplies of Matagalpa and Jinotega there is a strong correlation between the presence of fecal coliforms and of $H_2S$-producing bacteria equal to that found in studies in other countries (Manja et al. (1982), Kromoredgo y Fujioa (1991), Kaspar et al. (1992), Grant y Ziel (1996)). An analysis of the data by two methods showed that a strong, statistically significant correlation existed between the two methods; the strength of the correlation was 86%. Where there was not agreement between the two tests the majority were false positives; only 3.7% of the total number of samples tested for presence/ absence of H2S -producing bacteria resulted as false negatives.

Detection of $H_2S$ -producing bacteria cannot completely substitute for measurement of fecal coliforms by the Millipore method as a standard method for control of quality of potable water supplies. However the test can certainly play an important role in public health work. In rural areas where there is no laboratory the method can serve very well as an alternative test to estimate the quality of potable water , a test which is simple, relatively cheap and very reliable.

## References


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