# Universidad Nacional Mayor de San Marcos

(Universidad del Perú, DECANA DE AMERICA)

IDRC PROJECT: WATER QUALITY CONTROL LATIN-AMERICA (PERU)

CENTRE FILE : 3P - 85 - 0006 - 03 /6.1

Evaluation of the Coliphage Procedure and the Presence/Absence Test as Simple Rapid Economical Methods For Screening Potable Water Sources and Potable Water Supplies in Perú.

## FINAL REPORT

**CLEIBA Institute**
**Centro Latinoamericano de Enseñanza e Investigación de Bacteriología Alimentaria**

LIMA - PERU

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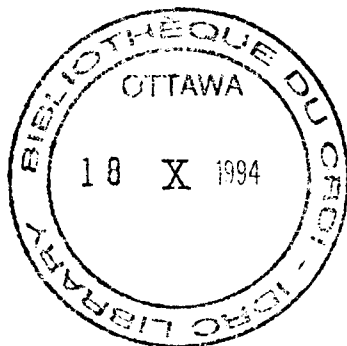
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# ABSTRACT

Different methods were compared for the detection of total coliforms, fecal coliforms and coliphages analyzing per triplicate 80 samples of raw water and 160 of treated water. The techniques used for raw water were: the coliphage detection, the conventional MPN using EC medium versus A1 Broth at 44.5ºC and the filtration membrane procedures with the M-FC Gelman and M-FC-Iso-Grid membranes. Each technique was evaluated and the results were expressed in 100 ml of water. The coliphage technique was positive in all river water samples. We found the relation coliphages/fecal coliforms 1:10 and different values for the other kind of waters examined.

In 36% of spring and well water samples where low number of fecal coliforms were found, no coliphages were detected. The A1 test showed similar results to the EC medium regarding selectivity and specificity. The filtration membrane technique gave similar data between the two systems compared, but both systems produced different level of recuperation of fecal coliforms in relation with the MPN test.

In 160 samples of treated water (110 of drinking water and 50 of bottled water) the P/A, H2S and coliphages tests were tried. In drinking water 42% of the samples showed one or more indicator of microbial contamination and 18% were positive only to the P/A test. Comparing the results for fecal coliforms, the P/A produced 18.2% positive samples; the H2S test produced 16.4% and the MPN test only produced 11.8% positive samples. Therefore the P/A test is the most sensitive technique.

In the coliphages detection in treated water 34% of the samples were positive within the range 1 to 57 PFU/100 ml and 18% of the samples were positive for coliphages and negative for total and fecal coliforms.

1.   INTRODUCTION

Perú as many other countries is committed with the International
Decade for Drinking Water Supply and Security.

In the latinoamerican countries the Health Institutions have assumed
the responsability for the surveillance of the quality of the water  because
this activity has been traditionally related to the public drinking water
supply.

Perú has three different geographical regions: Coast, Highlands  and
Tropical Jungle and embraces 25 departments, for administrative purposes. Due
to the fact that Perú's territory is highly irregular the basic services  for
security are deficient. A large percentage of the rural and suburban   areas
lack drinking water and sewage systems.This factor determines the high
incidence of infant mortality due to infectious diseases that use water as  a
vehicle.

An analysis made by the Ministry of Housing related to these services
indicates, that the high rate of the national urban population growth   is
determined by: the demografic increase and the large migration from rural
areas. This causes the build up of poor housing communities around   the
perimeter of the big cities, which lack all kind of services.  This human
settlements are called "growing villages" (pueblos jóvenes).

The water supply and security services have a mixed administration.
SENAPA is the national authority for water supply and its responsability  is
to coordinate the activities of the cities outside the capital city.

It has the control of 53 treatment plants. Nineteen of them  have
laboratories with basic equipment for physical, chemical and microbiological
analysis.

Lima, the capital city, has its own water authority (SEDAPAL).  This
entity is encharged of the surveillance of the quality of the drinking water
and sewage facilities. Covers a population of about 7 million people. Has two
laboratories, one in its headquearters and the other in the water   treatment

plant. According to peruvian legislation, the Health Ministry establishes the necessities regarding the protection of the water resources, desinfection, treatment for supply and water sampling. The new administration encharges the Basic Rural Sanity Direction (DISABAR) the rural area service. This Direction has the control of the natural water resources.

According to peruvian legislation, the surveillance and quality control of waters for all the twenty millions of inhabitants that Peru has nowadays (65% lacated in cities), is responsability of the Health Ministry through its Technical Directorate of the Environment (DITESA). And its Departmental Units in coordination with the regional hospitals.

Until 1984 did not existed a Committee responsable for the development, evaluation and adoption of standard analytical methods. Therefore, the few operative laboratories analyzed different parameters, expressed their results in various ways and applied different limits. At this moment the WHO guidelines have been adopted and are being implemented througout the country.

A WHO report dated may 1987 says: "The existing health service and water authority laboratories in Peru are both poorly structured and equipped nonetheless the Ministry of Health does have a basic, contralised laboratory service within its Technical Directorate of the Environment (DITESA). It provides a unified Central Reference Laboratory fully equipped for microbiological, inorganic, organic and organoleptic analytical functions. Initially, as a decade objetive, it has been recommended that DITESA should also develop health regional laboratories and support surveillance using basic portable field test kits in the provincial towns and cities".

At this moment DITESA is undergoing a Surveillance Program of water under assignment by the Overseas Development Administration of the gobernment of the United Kingdow through DELAGUA Ltd. (Public Consultanst), CEPIS/PAHO/WHO - Lima.

For the monitoring of water in the rural area water testing kits are been used, name brand MILLIPORE and DELAGUA. This last portable laboratory kit is equipped to do physical, chemical and microbiological tests such as pH, residual chlorine, turbidity and conductivity.

Regarding the microbiological procedures, the water quality standards are based on the coliform test as the national standard requests. But the bacteriological analysis of water is very little done througout the country. This is due to the lack of qualified technical and administrative personnel as well as to the lack of laboratory facilities. Only in the large cities and the capital city this activity is done in some extent.

An important factor in the development and maintenance of a safe water supply is the ability to assess quickly and economically the microbiological quality of the potable water and their sources.

This research study was undertaken, through the financial and consultant support provided by the International Development Research Centre (IDRC), Otawa, Canada, to evaluate the use of coliphage as an indicator of the sanitary quality of the source water for potable water upplies. The final goal of this research was to develop a classification system for the potable water source based on coliphage counts and sanitary services of the sites.

According to the specific objetives, it was evaluated potable water supplies, both bottled and tap using most probable number bacteriological procedures of the country plus all of the following tests, the P/A test, the $H_2S$ paper srip test and coliphage counts. For raw water, to compare rutine APHA, MPN, fecal coliforms MF of two membrane filter procedures (QA hydrophobic square membrane and Gelman membrane) and APHA A1 Broth, to evaluate the relationship between coliphage and fecal coliform and design criteia for classifying water source in Peru.

The results of these studies are presented

2.    MATERIALS AND METHODS.

2.1   Four E.coli strains frequently isolated from Peruvian waters, E.coli

CLEIBA$_1$ and E.coli CLEIBA$_2$ and Brasilian waters, E.coli 2262-4   and

E.coli 28767-7 were compared to E.coli C (ATCC 13706) in 27 water sample

study for their sensitivity and selectivity as potencial coliphage hosts

for the Peruvian study.

2.2   Eighty water samples, collected in triplicate, from  rivers, springs and

wells from which drinking water is obtained were tested for:

(A) coliphage concentration, following the revised method in     "A

simplified method for coliphage detection in natural waters" by ISBISTER,

SIMONS, SCOTT and KITCHEN, using the addition of 0.08 ml of 1% 2,3,5

triphenyl tetrazolium-chloride.

(B) fecal coliforms by (1) MPN technique using LST Broth 35ºC, BGB.   2%

35ºC, and EC medium 44.5ºC; (2) A$_1$ Broth at 44.5ºC (APHA Standard

method); (3) MF techniques using M-FC agar at 44.5ºC and Gelman GN-6

0.45 micron membrane filter and (4) QA square grid MF technique   at

44.5ºC using hydrophobic square gridded membrane filters developed  by

SHARPE (1981) and marketed as ISO-GRID Method (QA Laboratories,Toronto,

Canada) also used with MFC agar at 44.5ºC.

2.3   Twenty water samples per triplicate were tested to identify the  fecal

coliforms from MPN technique EC medium and A1  broth as well as on the

membranes Gelman and Iso-grid using the IMVIC test, oxidase procedure,

lysine decarboxilase test and ornithine decarboxilase test.

2.4   Eighty water samples per triplicate were tested for evaluation of  the

two membrane procedures in the detection of fecal coliforms.

2.5   110 potable water samples that were collected from distribution  lines

and wells subjected to chlorination were tested by the P/A  test CLARK

et al, 1962) as detailed in APHA Standard Method, Section 908 E.

Positive tests were confirmed for total coliforms, fecal coliforms,

Pseudomonas, aeruginosa, Clostridium perfringens, Aeromonas, fecal

streptococci, and <u>Staphylococcus aureus</u>.

2.6 The above 110 potable water samples were also tested using the Hydrogen Sulphide paper strip technique as detailed in the "Simplified Test for the Detection of Fecal Pollution in Drinking Water" by HAZBUN and PARKER.

Positive samples were confirmed for coliforms, fecal coliforms,<u>Salmonella</u>, <u>Proteus</u> and <u>Clostridium</u>.

2.7 The above 110 potable water samples were also tested by total and fecal coliforms tests (APHA Standard Methods, 1985) using the five-tube MPN procedure with lauryl tryptose broth and billiant green lactose bile broth for total coliform and confirmtion in EC broth for fecal coliforms.

2.8 50 potable water samples were tested for Coliphage test as detailed in point 2.1 (A), but using 100 ml of water sample, 100 ml of media and plates 150 x20 mm.

2.9 50 bottled drinking water samples were examined, 25 with gas and 25 without gas for P/A test, H2S test, total and fecal Coliforms and HPC.

2.10 Statistical Methodology: Several non parametric statistics methods were applied to evaluate the association among bacteriological tests which can be found in HOLLANDER and WOLFE (1973).

## 3. RESULTS

Table 1    presents the incidence of total coliforms, fecal coliforms and coliphage in raw water based on triplicate samples.

Table 2    presents percentage of positive samples of Fecal Coliforms versus Coliphages.

Table 3    presents percentage of isolation of E.coli, other coliforms and non coliforms in EC medium, A1 Broth, M-FC (Gelman and Iso-Grid membranes) in order to evaluate the specifity of these media in the detection of fecal coliform.

Table 4    presents percentage of isolation of E. coli, other coliforms and non coliforms by the two membrane filtration procedures in order to evaluate its efficiency in the dtection of fecal coliforms.

Table 5    presents Spearman's Rank Correlation Matrix.

Table 6    presents Sum Ranks Associated with each Fecal Coliform Test.

Table 7    presents Absolute Differences between the Sum Ranks of Each Pair of Fecal Coliform tests.

Table 8    presents results of potable water samples, positive by one or more bacterial indicator test.

Table 9    presents results of bacterial and coliphage tests in potable water samples collected from distribution lines.

Table 10   presents results of bottled water samples, positive by one or more bacterial indicator test.

Table 11   presents McNemar statistics for comparing P/A test with H2S test.

Table 12   presents McNemar statistics for comparing MPN test for TC and FC with P/A H2S tests.

Table 13   presents McNemar statistics for comparing coliphage test with the P/A, H2S, TC and FC tests.

Figure 1   presents Box plots for the distribution of bacteriological data in Raw Water.

Figure 2    presents Contingency tables for P/A and $H_2S$ tests in different

            drinking water types.

Figure 3    presents Box plots for the ln HPC and ln TC.

Figure 4    presents Contingency tables for the P/A test and the MPN tests for

            TC and FC.

Figure 5    presents Contingency tables for the H2S test and the MPN tests for

            TC and FC.

Figure 6    presents Contingency tables for the Coliphage test and the P/A,

            H2S, TC and FC tests.

4.    DISCUSSION

4.1.  Coliphage tests

With no background data on the specificity of South American strains of E,coli to act as coliphage hosts, it was decided to evaluate and compare several commonly isolated South American strains of E.coli for their ability to act as universal hosts. The four E.coli strains selected CLEIBA$_1$ and CLEIBA$_2$ (Peru) and 2262-4 and 28767-7 (Brazil) were evaluated in several different natural water.

The E.coli strains 2262-4 and 28767-4 were only evaluated in two water samples as these samples produced coliphage plaques of 4960 and 2185 per 100 mL with the E.coli C and no plaques with hosts 2262-4 and 28767-4.

CLEIBA,E.coli host strain produced a mean plaque count of 1882 compared to a mean plaque count of 5722 for the E.coli C host in Rimac River and well water samples during the period 10-8-86 to 29-8-86. Water samples tested using CLEIBA$_2$ E.coli host produced a mean coliphage plaque count of 2095 compared to 2130 for the E.coli C host (16 samples). However, when only September data are compared mean plaque count for CLEIBA$_2$ was 2293 compared to the E.coli C mean count of 1005. The difference between these counts was due to a single sample collected from the Rimac River on 25-9-86 which produced 750 plaques on E.coli C and 9500 plaques on CLEIBA$_2$. However, based on the overall results from all comparisons and the recommendations of APHA (1985), ASTM (1982) and the work of WETZEL et al. (1982), it was decided to continue the rest of the research using E. coli C as the host strain .

## 4.2 Raw water

The results presented belong to the analysis of 80 samples per triplicate, 48 samples come from rivers. One of them, Rimac river, provides water to the treatment Plant that supplies the city of Lima with drinking water. The other 4 rivers provide water for agricultural purpose.

In the rural areas the water supply is from wells and/or springs. 23 samples from well water and 9 from springs water have been analyzed. The rural well are not protected, from external contamination.

### 4.2.1 Comparison of the conventional MPN (EC) techniques with $A_1$ and MFC in the fecal coliform detection.

In table 1 are displayed the results of mean, maximum and minimun values obtained with over all data for TC, FC(EC), FC($A_1$) FC(M-FC GELMAN and ISO-GRID) and coliphage.

Regarding the results obtained through the filtration membrane procedure, was found that FC(MFC-G) presents only 54% of efectivites vs FC(EC) and 57% vs FC(A1) for all kinds of water evaluated.

This low values for the filtration membrane procedure is in agreement with the work of JACOB and COLAB. (1986). They found that the membrane technique detected 64% of total coliform vs 82% detected by the MPN test.

We found even lower values. The reason for the low tecnique sensitivity could be the existence of injured coliform cells that are not counted or that the M-FC selective media inhibits the injured coliform cells.

Table 2 are displayed results of the identification of coliforms and we can see that the EC media recuperats 89.1% of E.coli and A1 media 86.8% both give close values. The recuperation of other coliforms bacteria (Klebsiella, Enterobacter, Citrobacter) and non-coliform (Aeromonas and others) is also low.

When the membrane were evaluated for selectibity with regard to the E.coli recuperation, The M-FC Gelman detected 92% with respect to the

EC and A1 recuperation and the MFC IsoGrid, 84%.Was found that Klebsiella has a high interference in the E.coli isolation 22% for M-FC Iso Grid technique.

Fig. 1 gives the box plots of various microbiological data (ln scale)and shows symetric distribution for the total and fecal coliform data. The plots show that total coliform data have the least spread while that of the coliphage have the highest apread.

From the plots the fecal coliform tests can be grouped into two groups with the tests within each group being similar. One group consists of the EC and A1 MPN broth tests and the other includes the GELMAN membrane filter and ISO-GRID MF tests.

Table 5 gives the Spearman's Rank correlation matrix for the bacteriological techniques and turbidity.

The results indicate significant association between bacteriological test and turbidity .

All bacteriological tests are positively and highly ($P \ll 0.01$) correlated The correlation value indicate that EC and $A_1$ test produce similar results and the same is true for the membrane filter and Iso Grid tests. Regarding A1 media we can also observe that gives good values for the fecal coliform detection, with the advantage of a shorter analysis time (24 hours).

Fecal coliform tests were further evaluated using Freedman's rank sum test for the two way lay out with water samples representing the level of the first factor and the four fecal coliform test representing the level of the second factor.The observed value test is 118.544 which is highly significant ( $P \ll 0.01$) when compared the critical values of the chi-square distribution on 3 degrees of freedom.

The sum of the ranks associated with each test is given in table 6 which show that the EC MPN broth test produced the highest estimate of the fecal coliforms population and the Gelman membrane filter procedure produced

the lowest estimate of the population.To evaluate these differences

further, table 7 gives the absolute differences in the sum of the ranks

for each pair of tests and the results of performing the multiple

comparison tests which are asociated with the Freedman's test. The

results show that there are no significant differences between the EC

and A1 techniques or between the Gelman membrane filter and ISO-GRID

membrane filter tests.

4.2.2 Comparison of the specificity of the two membranes.

When compared the effectivity of the 2 membranes through fecal coliform

numeration, was observed that they were similar (Table Nº 3).

For the determination of the specificity of the 2 membranes in the

E.coli recuperation, 848 strains isolated from M-FC Gelman and 876

strains isolated from M-FC- Iso-Grid were assayed. Table Nº 3 shows that

Gelman recovery 80.4% of E.coli and Iso-Grid 67.3%. Klebsiella is found

in a considerable percentage: 9.1% for Gelman and 20.8% for Iso-Grid,

other coliforms and non-coliforms are found in a low percentage.

MFC-IsoGrid detected lower percentage of E.coli in comparison with the

other tests . Trough Wilcoxon signed rank test. we analized data obtained

in both membranes. The observed values of the test is T= 191. It means

that MF Gelman test identified higher number of E.coli than MF Iso-

Grid test ( $p < 0.001$ ).

TOBIN and DUTKA (1977) found significant difference in 9 types of

filtration membranes studied.These differences were due to many factors:

conformation of the membrane pore, liquid flux, presence of heavy metals

in the membranes and the type of membrane sterilization.

We shall point out that for this work the Gelman membranes were

sterilized by autoclave while the Iso-Grid membranes came in individual

sterilized containers.

Other observations on the handling of the membranes are:

i) Other colonies than E.coli such as Citrobacter sp. and Klebsiella sp.

also grow and develop blue color but less intense, it is always necessary to

confirm the presumptive presence of E.coli. This increases the analysis time.

ii) With regard to the counting of the colonies in the membrane we

found that the Gelman membrane has a limitation because the number of surface

colonies that can be counted ranged from 80 to 100. The Iso-Grid membrane has

cells where each of the unities that form colonies are located. This makes

the counting procedure easier and allows a high counting range up to 1,600

CFU/membrane .

Summarizing, the filtration membrane method is quick and easy but

based on the data so far obtained it would be necessary to do a bacterial

injury study to increase its efficiency.

4.1.3 Comparison between the conventional MPN method (EC) with the coliphage

detection test.

In rivers, the coliphage test gives values with a decimal reduction

(90% of the population) in comparison to the number of fecal coliform

obtained through the MPN (EC) technique. The relation between coliphages/

fecal coliforms is 1:10. In springs, the reduction is of 87% and the

relation is 1:7.7 and in wells the reduction is of 72% and the relation is

1:3.6. (Table Nº 1).

Has been observed that it is posible to obtain a direct relation

between coliphages/fecal coliforms when the number of fecal coliforms is

lower than $10^3$/100 ml. When the number of FC is higher there is no relation

and the number of coliphages is uncomprehensive.

In Table Nº 4 is observed that 100% of the river water samples are

coliphage positive. In well and spring water the percentages are lower:47%

and 44%, respectively.

From 23 well water samples analyzed 9 were negative for coliphage

but positive for fecal coliforms. These samples had low levels of FC (0.7 -

19/100 ml). The same is observed in spring water where 3 samples were

negative for coliphages and had low levels of FC (0.6 - 400/100 ml).These

results could be an account of the small quantigy of sample taken for analysis or to the retention of the phages by the soil.

In the comparison between EC and Coliphage test we got a good correlation 0.877, which is highly significant ( P $\ll$ 0.01) Table 5.

Fig. 1 gives the boxplots of coliphage of some microbiological tests and we can observed that coliphage have the highest spread.

Summarizing, if it were true that statistical analysis showed positive and significant ( p $\ll$ 0.01) correlation in raw water, however, with well water and spring water these is not direct correlations.

4.3.    Drinking water

4.3.1. Potable water

110 samples of drinking water collected from distribution lines and wells which are subjected to chlorination were examined. The P/A test and $H_2S$ test were comapred with the MPN procedure for total and fecal coliforms. For the first 60 samples we have done the heterotrophic plate count (HPC) and for the last 50 samples coliphage detection.

From the 110 drinking water samples 64 were negative for the P/A test, H2S test and MPN for total and fecal coliforms. In Tables Nº 8 and 9 are displayed the results obtained in the 46 positive samples ( 41.8%) for one or more indicator of microbial contamination. Only 12 samples (10%) were positive to the P/A test, 20 samples ( 18.2%) were positive for the P/A and MPN tests and only 5 samples (4.5%) were positive for the H2S test. The HPC range varied from 5 to 8.5 x $10^3$ ufc/ml.

4.3.2. Bottled waters.

50 bottled water samples were analyzed. 25 of them with gas: 10 in 450 ml glass bottles, 8 in siphon glass bottles and 10 in 2 liters plastic bottles; 25 samples without gas, all in 20 liters plastic bottle dispenser.

Table Nº 10 presents the results obtained in bottled waters (8 with gas and 14 without gas). 32% from bottled waters with gas had microbial contamination: 7 samples (28%) contained <u>Pseudomonas aeruginosa</u> and 5 (20%)

total and fecal coliforms tested by the P/A method. No coliform presence was detected by the MPN technique in any of the samples assayed. The range of HPC varied from 30 to 6.5 x $10^2$ ufc/ml with a mean value of 2.5 x $10^3$ ufc/ml.

The bottled water samples without gas were positive in 56% for one or more indicator of bacterial contamination: 10 samples (40%) contained Pseudomonas aeruginosa, 8 samples (32%) total coliforms, 2 samples (8%) fecal coliforms by the P/A test, 5 samples (20%) were positive to the H2S test (isolating Citrobacter freundii, Klebsiella spp., E.coli, Pseudomonas aeruginosa)The HPC values found varied from 1.1 x $10^2$ to 1.6 x $10^4$ ufc/ml with a mean value of 2.3 x $10^3$ ufc/ml.

Comparison of the conventional MPN method versus the P/A test and H2S Test for the detection of total and fecal coliforms.

Figure 2 displays the set of contingency tables which sumarize the information available about P/A and H2S tests and their association for different types of drinking.water.These tables show that the P/A test is more likely to produce positive results than the H2S test and this appears to be consistent for all drinking water types.The contingency tables for all the data show that out of 160 samples, 54 were positive on the basis of P/A test and only 19 and 20 were positive using the H2S test at 22º and 35ºC, respectively. Table 11 gives the observed values of the McNemar statistic test. The results show that the P/A test produces more significant positive results than H2S test for all drinking water types.

Figure 3 gives the box plots for the ln HPC and ln TC for different entries to the Contingency Tables when there are sufficient data for representing the box plot. For bottled waters the median log HPC is 7.3 when the presence absence test is positive and the H2S is negative. This can be compared to the median of 4.1 when both tests are negative. One major feature is that the box plot corresponding -+ class shows more spread than that corresponding -- to class.Similar conclusions can be reached for distribution system I and for the data from the wells.

The box plots for total coliforms on the other hand are significantly

different in the case of the class ++ from all the other classes. This perhaps indicates that when both tests are positive they are indicative of the presence of coliform bacteria.

The contingency tables ( Fig. 4) show the association between the P/A test and the fecal and total coliform MPN tests.These tables show that a positive and or negative result using MPN is always associated with a positive and or negative result. for P/A test, but the P/A test is more able to detected the presence of fecal and total coliform in the water samples than the MPN tests. McNemar statistics for comparing the P/A test with the TC and FC tests are 29.1212 and 40, respectively, which are significant at << 1% level. This provides strong evidence for the superiority of the P/A test when compared to TC and FC test in detecting the presence of coliforms in the water.

The contingency tables ( Fic. 5) show that the $H_2S$ test produces results very similar to MPN technique for TC and FC at 0.10 significant level, except at 35ºC, in which TC has a tendency to give more positive values than H2S (0.02 significant level).

These results indicate that the P/A test is more sensitive than the MPN test for the total and fecal coliforms detection in this kind of water. Also the $H_2S$ test gives a recovery percentage of coliforms similar to the one obtained by the MPN test for TC determination.

JACOBS and colab. (1966) in a comparative study of techniques for total coliforms detection in water systems also found a higher sensitivity for the P/A test ( 88%) against 82% for the MPN test and 64% for the filtration membrane procedure.

Coliphages detection in drinking water samples.

Of the 50 water samples examined 17 resulted positive (34%) within the range < 1 to 57 PFU/100 ml with a mean value of 11.6 PFU/100 ml (Table Nº 9). It was possible to detect small number of PFU in large volumes of water (100 ml) because the APHA technique sensitivity was increased using 5 times the quantity of culture media and larger Petri dishes (150 x 20 mm).

GRABOW and COUBROUGH (1986) described a practical single agar-layer plaque assay for directly testing 100 ml samples of water . They found that it was more sensitive, reliable and accurate than various other methods and proved rapid,simple and economic.

EL - ABAGY, DUTKA and KAWEL (1988) found coliphages present in drinking water samples that were negative for total and fecal coliforms.

SIM and DUTKA (1987) stated that drinking water free from coliforms can carry pathogenic microorganisms. Many water samples free from coliforms contain various coliphages concentrations, this indicates that the water has had an inadequate treatment and thus enteric human virus could also survive this treatment process.

5. CONCLUSIONS

On the basis of all the data generated inthis study the following conclusions were reached:

1. There is a significant correlation among coliphage and other fecal coliform indicators, but is same case the results of well water and spring water were contradictory, so more detaild studies should be done in these waters.

2. The MPN procedure for the detection of fecal coliforms using the A-1 broth presents good sensitivity specificity and also good correlation with conventional methodology, so it can be used in place of MPN conventional methodology for FC determination giving rapid answer (24 hs.).

3. In the detection of fecal coliforms using the membrane filtration technique, M-FC Gelman and M-FC Iso-Grid, it was found that these systems present good correlation with conventional methodology but MFC Iso-Grid detecte lower percentage of E.coli .

4. The P/A test is more able to detecte the presence of fecal and total coliform than the MPN tests and H2S test for all drinking water types.

   Also the P/A test has been found be less costly (materials and manpower) then traditional Peruvian bacteriological water quality testing procedure.

5. The $H_2S$ test showed the same sensibity as the MPN test for TC determination, but less sensibility for FC determination. Of all the procedures evaluate the H2S test is the simplest to perform and is the least costly (material and manpower). Based on these bottled water and potable water studies, it appears to be equally safe/ hazardous to drink bottled water with or without gas and water from Lima potable water distribution systems.

6. In drinking water the coliphage test produces similar results to P/A and
   TC MPN test drinking water testing procedure. It also produces more
   statistical significant than H2S test and FC test, but was necessary  to
   use 5 times more the quantity of the culture media and water sample (100
   mL) as indicated in the basic technique, this added media made the
   procedure more expensive.

## 6. RECOMMENDATIONS

1. Based on the results obtained in the detection of coliphages in raw waters it would be interesting to make a comparative study of coliphages versus coliforms. This study could use the same numeration system for both indicators, that is the MPN technique. Could be perfomed for the evaluation of surface waters (springs) and rural well waters due to the fact that using the plate count method the results so far obtained were not equivalent.

2. A study of rural potable water supplies employing the H2S and P/A tests should be initiated to fill our knowledge gap in this area.

3. Evaluate the 10 tube- MPN technique for coliphages in drinking water and also use this procedure in Recommendation # 2.

4. Perform a bacterial injury study in fecal coliforms during the membrane filtration procedure.

5. To try and understand the lack of sensitivity of the MF technique in Peruvian waters, it would be interesting to carry out a study to understand whether injured fecal coliforms are not enumerated by the MF procedure or are they injured by the HF procedure and thus not counted.

## ACKNOWLEDGEMENTS

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Table Nº 1

Incidence of Fecal Coliforms in Raw Water

| Parameters | Rivers | | Springs | | Wells | |
|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range |
| Total Coliforms[1] | $3.4 \times 10^4$ | $3 \times 10^2 - 7 \times 10^5$ | $2.9 \times 10^3$ | $3 - 1.6 \times 10^4$ | $1.7 \times 10^3$ | $4 - 1.1 \times 10^4$ |
| Fecal Coliform (EC)[1] | $2.0 \times 10^4$ | $70 - 2.6 \times 10^5$ | $1.4 \times 10^3$ | $<2 - 8 \times 10^3$ | $1.1 \times 10^3$ | $<2 - 5.7 \times 10^4$ |
| Fecal Coliform ($A_1$)[1] | $1.7 \times 10^4$ | $90 - 2.4 \times 10^5$ | $1.9 \times 10^3$ | $<2 - 9 \times 10^3$ | $8.4 \times 10^2$ | $<1 - 2.2 \times 10^4$ |
| Fecal Coliform[2] (M-FC Gelman) | $1.0 \times 10^4$ | $63 - 2.4 \times 10^4$ | $7.3 \times 10^2$ | $<1 - 4.6 \times 10^3$ | $6.8 \times 10^2$ | $<1 - 2.5 \times 10^3$ |
| Fecal Coliform[2] (M-FC Iso-Grid) | $1.1 \times 10^4$ | $80 - 3.8 \times 10^4$ | $7.1 \times 10^2$ | $<1 - 4.4 \times 10^3$ | $6.2 \times 10^2$ | $<1 - 2.9 \times 10^3$ |
| Coliphage[3] | $2.0 \times 10^3$ | $5 - 1.5 \times 10^4$ | $1.9 \times 10^2$ | $5 - 1.6 \times 10^3$ | $3.1 \times 10^2$ | $<5 - 1.4 \times 10^3$ |
| Total samples | 48 | | 9 | | 23 | |

1    MPN/100 mL

2    CFU/100 mL

3    PFU/100 mL

Table 2.

Presence of E.coli, other coliforms and non coliforms in EC medium, A1 Broth, M-FC (Gelman membrane) and M-FC (Iso-Grid membrane)

(Expressed in %)

| BACT. ID / CULTURE MEDIA / WATER SOURCE | E.coli | | | | Klebsiella | | | | Enterobacter | | | | Citrobacter | | | | Aeromonas | | | | Others | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E.C | A1 | MFC $G^1$ | MFC $I^2$ | E.C | A1 | MFC $G^1$ | MFC $I^2$ | E.C | A1 | MFC $G^1$ | MFC $I^2$ | E.C | A1 | MFC $G^1$ | MFC $I^2$ | E.C | A1 | MFC $G^1$ | MFC $I^2$ | E.C | A1 | MFC $G^1$ | MFC $I^2$ |
| Rivers | 94.2 | 89.3 | 84.3 | 88.9 | 3.0 | 8.3 | 4.9 | 7.1 | 1.6 | 1.0 | 3.5 | 3.0 | 1.0 | 0.3 | 5.3 | - | - | 0.3 | 0.8 | 0.8 | - | 0.6 | 0.4 | 0.4 |
| Springs | 94.8 | 83.8 | 88.3 | 70.4 | 5.1 | 14.5 | 6.9 | 22.7 | - | 1.6 | 2.3 | 6.8 | - | - | 2.3 | - | - | - | - | - | - | - | - | - |
| Wells | 81.9 | 84.6 | 75.0 | 47.3 | 14.5 | 14.5 | 19.2 | 33.9 | 3.5 | 0.4 | 2.8 | 14.2 | - | 0.4 | 0.9 | 4.4 | - | - | 0.9 | - | - | - | 0.9 | - |
| Total | 89.1 | 86.8 | 82.2 | 74.6 | 8.0 | 11.4 | 9.1 | 16.7 | 2.3 | 0.6 | 3.2 | 6.7 | 0.4 | 0.3 | 3.7 | 1.3 | - | 0.3 | 0.8 | 0.5 | - | 0.3 | 0.8 | 0.5 |

1 Gelman membrane

2 Iso-Grid membrane

Table 3. Presence of E.coli, other coliforms and non coliforms in the two membrane filtration procedure.

(Expressed in %)

| Microorganism | M-FC GELMAN Nº | % | M-FC. ISO-GRID Nº | % |
|---|---|---|---|---|
| E.coli | 683 | 80.4 | 589 | 67.3 |
| Enterobacter sp. | 28 | 3.2 | 45 | 5.1 |
| Klebsiella sp. | 78 | 9.1 | 178 | 20.3 |
| Citrobacter sp. | 40 | 4.7 | 44 | 5.0 |
| Aeromonas sp. | 10 | 1.1 | 10 | 1.1 |
| Lact (-) | 1 | 0.1 | 4 | 0.4 |
| Not identified | 8 | 0.9 | 6 | 0.6 |
| Total | 848 | | 876 | |

Table 4. Percentage of positive samples of
Fecal Coliforms vs Coliphages

| Sources Nº of samples | Fecal coliforms | | Coliphages | |
|---|---|---|---|---|
| | NºPositive samples | % | NºPositive samples | % |
| River (48) | 48 | 100 | 48 | 100 |
| Well (23) | 20 | 86 | 11 | 47 |
| Spring (9) | 7 | 77 | 4 | 44 |

Table 5. Spearman's Rank Correlation Matrix.

|  | Turbidity | TC | EC | A-1 | Gelman MF | IsoGrid MF |
|---|---|---|---|---|---|---|
| TC | .610 | | | | | |
| EC | .576 | .937 | | | | |
| A-1 | .577 | .908 | .955 | | | |
| Gelman | .515 | .784 | .834 | .850 | | |
| Iso | .574 | .802 | .851 | .853 | .951 | |
| Coliphage | .750 | .869 | .877 | .874 | .795 | .802 |

Correlation are significant at the << 0.01 level

Table 6. Sum Ranks Associated with Each Fecal Coliform test

|  | Fecal coliform test | | | |
|---|---|---|---|---|
|  | EC MPN | A1 MPN | Gelman MF | Iso-Grid MF |
| Sum Ranks | 592.5 | 564.5 | 379.0 | 384.0 |

Table 7. Absolute Differences between the Sam Ranks of Each Pair of Fecal Coliform Tests.

.

|  | Fecal Coliform Tests | | | |
|---|---|---|---|---|
|  | EC MPN | A1 MPN | Gelman MF | Iso-Grid MF |
| EC |  | 28 | 213.5[**] | 208.5[**] |
| A1 |  |  | 185.5[**] | 180.5[**] |
| Gelman |  |  |  | 5 |

[**] Values are significant at the $\propto$ 1% level.

Table 8. POTABLE WATER SAMPLES, positive by one or more bacterial indicator tests.

| Water Source | Free Residual Chlorine mg/L | P/A Test/100 mL | | | | | | | H₂S Test/ 100 mL | | | | MPN/ 100 mL | | HPC8 /mL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TC[1] | FC2 | FS3 | Cl.p[4] | P.a[5] | S.a[6] | Aer[7] | + or neg. 22°C | + or neg. 35°C | Bacteria 22°C | identified 35°C | TC | FC | |
| Distribution system | 0.10 | P | P | A | A | A | A | A | | | | | 2 | <2 | 210 |
| Distribution system | 0.25 | P | P | A | A | A | A | P | + | + | Aeromonas | Citrobacter P.aeruginosa Aeromonas | | | 200 |
| Distribution system | 0.0 | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 298 |
| Distribution system | 0.0 | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 195 |
| Distribution system | 0.1 | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 6200 |
| Distribution system | 0.1 | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 7200 |
| Well | 0.0 | P | P | A | A | A | A | A | - | - | | | 130 | <2 | 3500 |
| Distribution system | 0.0 | P | A | A | A | P | A | A | - | - | | | <2 | <2 | 298 |
| Well | 0.0 | P | A | A | A | A | A | A | - | - | | | 130 | <2 | 3500 |
| Distribution system | 0.0 | P | A | A | A | P | A | A | - | - | | | <2 | <2 | 298 |
| Well | 0.0 | P | A | A | A | A | A | A | - | - | | | >1600 | 1600 | 8500 |
| Well | 0.1 | P | A | A | A | A | A | A | - | - | | | <2 | <2 | 11 |
| Distribution system | 0.25 | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 13 |
| Distribution system | 0.25 | A | A | A | A | A | A | A | + | + | Citro‑ bacter | Citrobacter Clostridium | <2 | <2 | 11 |

TC[1]   - total coliforms

FC2    - fecal coliforms

FS[3]   - fecal streptococci

Cl.p[4]  - Clostridium perfringens

P.a[5]  - Pseudomonas aeruginosa

S.a[6]  - Staphylococcus aureus

Aer[7]  - Aeromonas

HPC8   - heterotrophic plate count

TABLE 9. Results of bacterial and coliphage tests on potable water samples collected from distribution lines.

| Water Source | Free Residual Chlorine mg/L | P/A Test/100 mL | | | | | | | H2S Test + or neg | | Bacteria Identified | | TC FC MPN /100 mL | | Coliphage PFU[8] /100 mL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TC[1] | FC[2] | FS[3] | C.p.[4] | P.a[5] | S.a.[6] | Aero[7] | 22°C | 35°C | 22°C | 35°C | TC | FC | |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Citrobacter | Citrobacter, E.coli | <2 | <2 | <1 |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Citrobacter | Citrobacter, E.coli | 4 | 4 | 1 |
| Well | 0.0 | P | P | A | A | A | A | A | - | + | Not confirmed | Citrobacter, E.coli | 12 | 9 | <1 |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Citrobacter | Citrobacter, E.coli | 2 | 2 | <1 |
| Well | 0.0 | P | P | A | A | P | A | A | - | + | Not confirmed | Citrobacter, E.coli | 21 | 13 | 13 |
| Well | 0.0 | A | A | A | A | A | A | A | + | + | Not confirmed | Citrobacter, E.coli | <2 | <2 | 8 |
| Well | 0.0 | P | P | A | A | A | A | A | - | + | Citrobacter | Citrobacter, E.coli | 4 | <2 | 15 |
| Well | 0.0 | A | A | A | A | A | A | A | + | + | Not confirmed | Citrobacter, E.coli | <2 | <2 | 9 |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Not confirmed | Citrobacter, E.coli | 2 | 2 | 5 |
| Well | 0.0 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 2 |
| Distribution system | 0.0 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 1 |
| Distribution system | 0.0 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 12 |
| Distribution system | 0.1 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 3 |
| Distribution system | 0.1 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 57 |
| Distribution system | 0.1 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 1 |
| Distribution system | 0.0 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 4 |
| Distribution system | 0.0 | A | A | A | A | A | A | A | - | - | | | <2 | <2 | 14 |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Citrobacter, E.coli | Citrobacter, E.coli | 21 | 21 | 9 |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Citrobacter, E.coli | Citrobacter, E.coli | 26 | 6 | 2 |

TC[1] - total coliforms  
FC[2] - fecal coliforms  
FS[3] - fecal streptococci  
C.p.[4] - Clostridium perfringens  

P.a.[5] - Pseudomonas aeruginosa  
S.a.[6] - Staphylococcus aureus  
Aero[7] - Aeromonas spp  
PFU[8] - plaque forming units

TABLE 9. Cont. a. Results of bacterial and coliphage tests on potable water samples collected from distribution lines.

| Water Source | Residual Chlorine mg/L | P/A Test/100 mL | | | | | | | H2S Test | | | | TC MPN /100 mL | FC /100 mL | Coliphage PFU8 /100 mL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TC1 | FC2 | FS3 | C.p.4 | P.a.5 | S.a.6 | Aero 7 | + or neg 22°C | 35°C | Bacteria Identified 22°C | 35°C | | | |
| Distribution System | 0.5 | A | A | A | A | A | A | A | - | - | | | 2 | < 2 | < 1 |
| Well | 0.0 | P | A | A | A | A | A | A | + | + | Citrobacter | Citrobacter | 26 | 4 | < 1 |
| Well | 0.0 | P | P | A | A | A | A | A | - | - | | | 2 | < 2 | < 1 |
| Well | 0.0 | P | P | A | A | A | A | A | + | + | Citrobacter | Citrobacter | 130 | 11 | < 1 |
| Distribution system | 0.0 | P | A | A | A | A | A | A | - | - | | | < 2 | < 2 | < 1 |
| Distribution system | 0.0 | P | A | A | A | P | A | A | - | - | | | 2 | 2 | < 1 |
| Well | 0.0 | A | A | A | A | A | A | A | ÷ | - | Pseudomonas | | < 2 | < 2 | < 1 |
| Well | 0.0 | A | A | A | A | A | A | A | + | - | Pseudomonas | | < 2 | < 2 | < 1 |
| Well | 0.0 | P | P | P | A | A | A | A | + | + | Citrobacter | Citrobacter | 26 | 9 | 21 |
| Well | 0.0 | P | P | A | A | A | A | A | - | - | | | 7 | 4 | < 1 |
| Distribution system | 0.25 | P | A | A | A | A | A | A | - | - | | | < 2 | < 2 | < 1 |
| Distribution system | 0.1 | P | A | A | A | A | A | A | - | - | | | 2 | < 2 | < 1 |
| Well | 0.0 | P | P | A | A | A | A | A | - | - | | | 30 | 8 | < 1 |

TC1     - total coliforms
FC2     - fecal coliforms
FS3     - fecal streptococci
C.p. 4  - Clostridium perfringens

P.a.5  - Pseudomonas aeruginosa
S.a.6  - Staphylococcus aureus
Aero7  - Aeromonas spp.
PFU8   - plaque forming units

Table 10. BOTTLED WATER SAMPLES positive by one or more bacterial indicator tests.

| Water Source | Free Residual Chlorine mg/L | P/A Test/100 mL TC[1] | FC[2] | FS[3] | Cl.p[4] | P.a[5] | S.a.[6] | Aer[7] | H2S Test/100 mL + or neg 22°C | 35°C | Bacteria Identified 22°C | 35°C | MPN/100 mL TC | FC | HPC[8] /mL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bottled water with gas | - | A | A | A | A | P | A | P | - | - | | | <2 | <2 | 5100 |
| Bottled water with gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 6200 |
| Bottled water with gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 6500 |
| Bottled water with gas | - | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 30 |
| Bottled water with gas | - | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 50 |
| Bottled water with gas | - | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 68 |
| Bottled water with gas | - | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 1520 |
| Bottled water with gas | - | P | P | A | A | P | A | A | - | - | | | <2 | <2 | 1480 |
| Bottled water no gas | - | P | A | A | A | A | A | A | + | + | Citrobacter Klebsiella | Citrobacter Klebsiella | <2 | <2 | 112 |
| Bottled water no gas | - | P | A | A | A | A | A | A | - | - | | | <2 | <2 | 105 |
| Bottled water no gas | - | P | P | P | A | P | A | A | + | + | E.coli Pseudomonas | Citrobacter E.coli Pseudomonas | 12 | 7 | 2140 |
| Bottled water no gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 520 |
| Bottled water no gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 350 |
| Bottled water no gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 2200 |
| Bottled water no gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 295 |
| Bottled water no gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 16000 |
| Bottled water no gas | - | P | P | A | A | A | A | A | - | - | | | <2 | <2 | 1300 |
| Bottled water no gas | - | P | A | A | A | A | A | A | - | - | | | <2 | <2 | 2400 |
| Bottled water no gas | - | A | A | A | A | P | A | A | - | - | | | <2 | <2 | 3600 |
| Bottled water no gas | - | P | A | A | A | P | A | A | + | + | Citrobacter | Citrobacter | 26 | <2 | 9500 |
| Bottled water no gas | - | P | A | A | A | P | A | A | + | . | Citrobacter | Citrobacter | 22 | 2 | 1200 |
| Bottled water no gas | - | P | A | A | A | P | A | A | + | + | Citrobacter | Citrobacter | 17 | <2 | 521 |

TC[1] - total coliforms  
FC[2] - fecal coliforms  
FS[3] - fecal streptococci  

Cl.p.[4] - Clostridium perfringens  
P.a.[5] - Pseudomonas aeruginosa  
S.a.[6] - Staphylococcus aureus  

Aer.[7] - Aeromonas  
HPC[8] - heterotrophic plate count.

Table 11 . Mc Nemar statistics for comparing P/A with $H_2S$ tests.

| Mc Nemar statistic | Bottled Water | | Distribution systems | | | Well | All Data |
|---|---|---|---|---|---|---|---|
| | Gas | No Gas | I | II | III | | |
| | 0* | 0* | 1* | 0 | 1* | 3** | 30.4211* |

\*    Significant at the 5% level

\*\*   Significant at the 7% level

Table 12. Mc Nemar statistics for comparing MPN test for TC and FC with
P/A and H$_2$S tests.

| Mc Nemar Statistic | P/A | H$_2$S 22ºC | 35ºC |
|---|---|---|---|
| TC | 29.1212*** | 11* | 8* |
| FC | 40 *** | 6* | 4* |

*      No significant at the 10% level

**     Significant at the 22 level

***    Significant at $\ll$ 1% level

Table 13. Mc Nemar statistics for comparing coliphage test with the P/A, $H_2S$, TC and FC tests.

| Mc Nemar statistic | P/A | $H_2S$ | | TC | FC |
|---|---|---|---|---|---|
| | | 22ºC | 35ºC | | |
| | 9∴ | 11∴∴ | 9∴∴∴ | 10∴ | 10∴∴∴ |

∴      No significant at  < 25% level

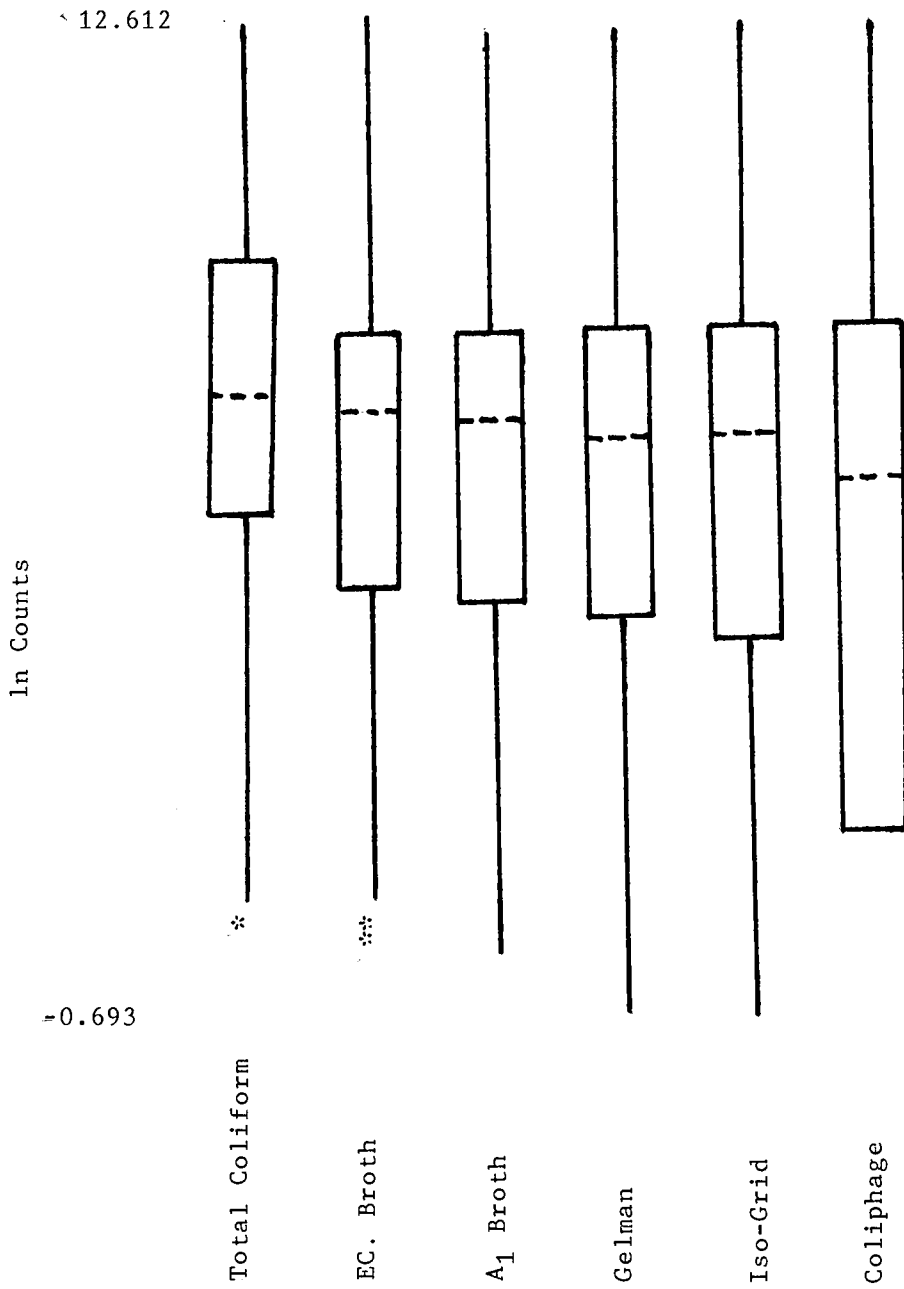∴∴     Significant at the 16% level

∴∴∴    Significant at the 22% level.

Figure 1. Box Plot for the distribution of bacteriological data in Raw Water.

Bottled water with gas

- H$_2$S +

| P/A | - | 17 | 0 | 17 |
|---|---|---|---|---|
|  | + | 8 | 0 | 8 |
|  |  | 25 | 0 | 25 |

Bottled water without gas

- H$_2$S +

| P/A | - | 11 | 0 | 11 |
|---|---|---|---|---|
|  | + | 9 | 5 | 14 |
|  |  | 20 | 5 | 25 |

Distribution system  I

- H$_2$S +

| P/A | - | 37 | 1 | 38 |
|---|---|---|---|---|
|  | + | 11 | 1 | 12 |
|  |  | 48 | 2 | 50 |

Distribution system II

- H$_2$S +

| P/A | - | 26 | 0 | 26 |
|---|---|---|---|---|
|  | + | 0 | 0 | 0 |
|  |  | 26 | 0 | 26 |

Distribution system I & II

- H$_2$S +

| P/A | - | 63 | 1 | 64 |
|---|---|---|---|---|
|  | + | 11 | 1 | 12 |
|  |  | 74 | 2 | 76 |

Wells

- H$_2$S +

| P/A | - | 13 | 1 | 14 |
|---|---|---|---|---|
|  | + | 8 | 12 | 20 |
|  |  | 21 | 13 | 34 |

All Data

- H$_2$S + 22ºC

| P/A | - | 102 | 4 | 106 |
|---|---|---|---|---|
|  | + | 39 | 15 | 54 |
|  |  | 141 | 19 | 160 |

All Data

- H$_2$0 + 35ºC

| P/A | - | 104 | 2 | 106 |
|---|---|---|---|---|
|  | + | 36 | 18 | 54 |
|  |  | 140 | 20 | 160 |

Figure 2. Contingency tables for P/A and H2S in different drinking water types.
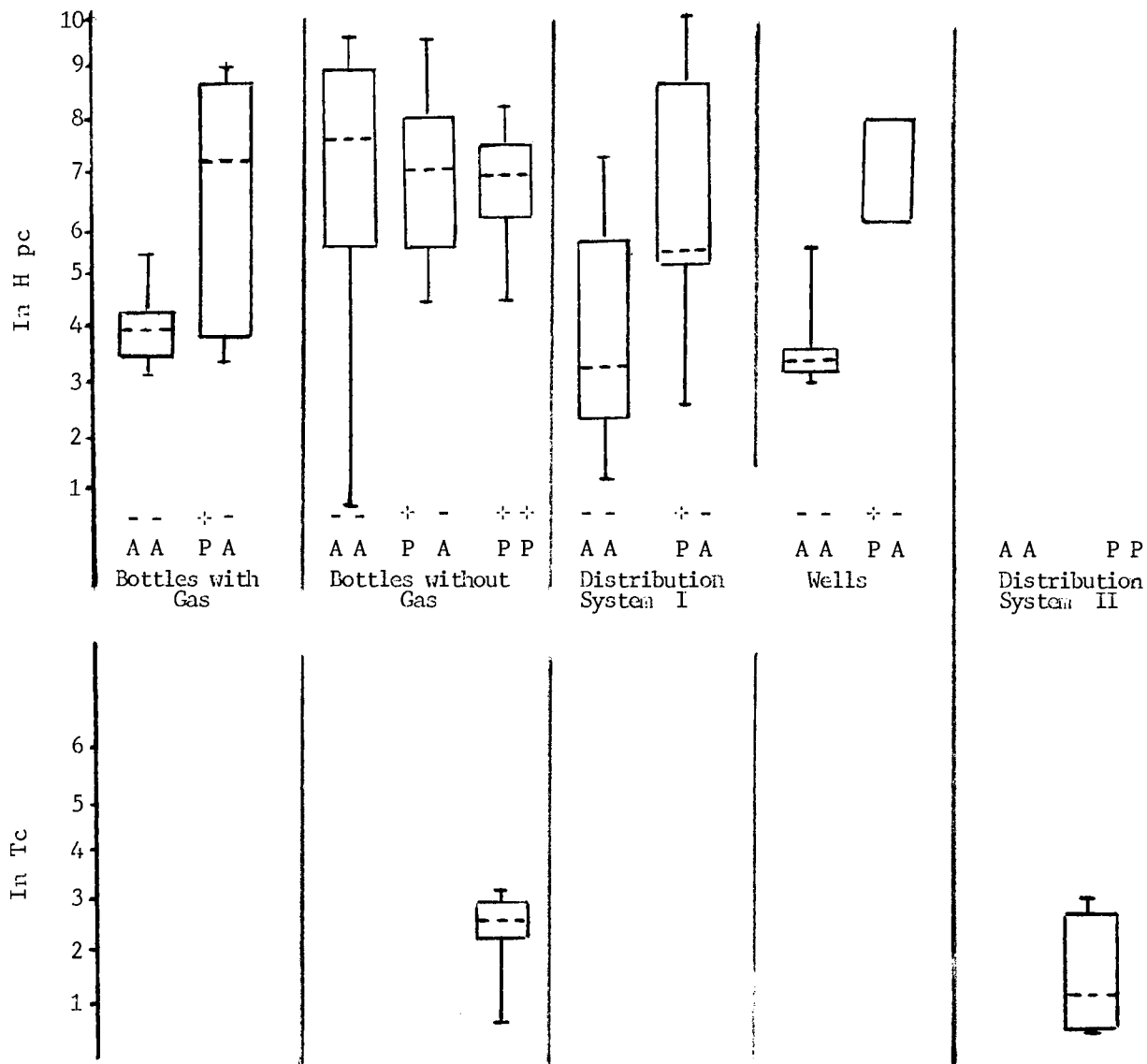
Figure 3: The Association between P/A, $H_2S$ and heterotrophic plate count tests in drinking water.

Figure 4.   Contingency tables for the P/A test and the MPN tests for

TC and FC.

### TC

| H$_2$S 22ºC | TC − | TC ÷ | |
|---|---|---|---|
| − | 130 | 11 | 141 |
| ÷ | 7 | 12 | 19 |
| | 137 | 23 | 160 |

### TC

| H$_2$S 35ºC | TC − | TC ÷ | |
|---|---|---|---|
| − | 132 | 8 | 140 |
| ÷ | 5 | 15 | 20 |
| | 137 | 23 | 160 |

### FC

| H$_2$S 22ºC | FC − | FC ÷ | |
|---|---|---|---|
| − | 135 | 6 | 141 |
| ÷ | 11 | 8 | 19 |
| | 146 | 14 | 160 |

### FC

| H$_2$S 35ºC | FC − | FC ÷ | |
|---|---|---|---|
| − | 136 | 4 | 140 |
| ÷ | 10 | 10 | 20 |
| | 146 | 14 | 160 |

Figure 5. Contingency tables for the H$_2$S test and the MPN tests for TC and FC.

Coliphage

**P/A**

|     | −  | ÷  |    |
|-----|----|----|----|
| −   | 22 | 9  | 31 |
| ÷   | 11 | 8  | 19 |
|     | 33 | 17 | 50 |

Coliphage

**H$_2$S 22ºC**

|     | −  | ÷  |    |
|-----|----|----|----|
| −   | 27 | 11 | 38 |
| ÷   | 6  | 6  | 12 |
|     | 33 | 17 | 50 |

Coliphage

**H$_2$S 35ºC**

|     | −  | ÷  |    |
|-----|----|----|----|
| −   | 28 | 9  | 37 |
| ÷   | 5  | 8  | 13 |
|     | 33 | 17 | 50 |

Coliphage

**TC**

|     | −  | ÷  |    |
|-----|----|----|----|
| −   | 24 | 10 | 34 |
| ÷   | 9  | 7  | 16 |
|     | 33 | 17 | 50 |

Coliphage

**FC**

|     | −  | ÷  |    |
|-----|----|----|----|
| −   | 27 | 10 | 37 |
| ÷   | 6  | 7  | 13 |
|     | 33 | 17 | 50 |

Figure 6. Contingency tables for the Coliphage test and the P/A, H$_2$S, TC and FC tests.

# SUMMARY