Climate and Abstraction Impacts in Atoll Environments (CAIA)

Assessment by USP to validate and improve the SPC field bacteriological water sampling and analysis methodology using E.Coli compact dry plate

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EUROPEAN UNION

Pacific Community
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USP
THE UNIVERSITY OF THE SOUTH PACIFIC

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Geoscience Division of the Pacific Community

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Abbreviations and symbols

APHA	-	American Public Health Association
CAIA	-	Climate and Abstraction Impacts in Atolls
E.coli	-	Escherichia coli
EPA	-	Environmental Protection Agency (US)
EU	-	European Union
GSD	-	GeoScience Division (SPC)
L	-	litres
ml or mL	-	milli-litres
P-A	-	presence-absence
RCT	-	residual chlorine test
SPC	-	Pacific Community
TC	-	Total Coliform
USP-IAS	-	University of the South Pacific's Institute of Applied Science
UV	-	ultra violet
WAF	-	Water Authority of Fiji

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- The microbiology team for their assistance during the field sampling, sample preparation and bacteria enumeration.
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1 Summary

As part of the Climate and Abstraction Impacts in Atoll environments (CAIA) Project, the Secretariat of the Pacific Community's GeoScience Division partnered with the University of the South Pacific's Institute of Applied Science to assess SPC's field bacteriological technique incorporating the E.coli (or EC) compact dry plate filtration membrane method. The purpose of this activity is to improve and validate SPC's field sampling and analysis techniques for microbiological analysis for use in remote settings in the Pacific.

While the microbiological field technique employed by SPC using the E.coli compact dry plate method is not the same level of accuracy as laboratory microbiological analysis; the field technique yields results with an acceptable level of accuracy suitable for making decisions regarding the potability of water from a microbiological perspective; and useful for guiding advice on categorising contamination level and appropriate remedial treatment.

The validation activity identified certain aspects of the methodology that could improve both the accuracy and consistency of results, which should be incorporated into an updated methodology. Additional attention is required in the sterilisation process of the sampling bottles, performed before use and when reusing the bottles. In the laboratory procedure, it was noted, that as the number of microbial colonies increased, differences between the SPC field results and the USP-IAS laboratory results became more marked. In general, SPC results for Total Coliform was consistent with the USP laboratory results. The E.coli results from the SPC methodology; however, yielded consistently higher counts than the USP laboratory results and this was attributed to errors in enumerating microbial colonies identified as E.coli. The use of a 10 ml or 1 ml sample dilution is recommended for when coliform load is expected to be high.

2 Introduction

The Climate and Abstraction Impacts in Atoll environments (CAIA) Project, is a partnership between the European Union, the University of the South Pacific's Institute of Applied Science (USP-IAS), and the GeoScience Division (GSD) of the Pacific Community (SPC). The project objective is to improve water security in atoll environments in the Pacific, supporting national, social, and economic development, and environmental protection into the future.

The specific objective of the CAIA Project is to develop practical, technical and management options, to improve water security and resilience of freshwater lenses under projected climate and abstraction scenarios for atoll island states.

This specific activity is to provide Pacific Island countries with a practical technique for microbial sampling and analysis in remote locations which can provide useful results regarding access to safe water for improved water security; and involved the assessment of SPC's water quality (microbiological)

field technique – the E.coli compact dry plate filtration membrane method[1] – against the USP-IAS accredited procedure and laboratory facility. This assessment was undertaken between April 18th and August 1st, 2016. See Annex 2 for a report from the USP-IAS on the assessment.

This report presents the background, methodology, results and recommendations from this work.

2.1 Purpose and scope

Partnering with USP-IAS provided an opportunity to get an independent assessment and review of the E.coli compact dry field technique as a fit-for-purpose approach to microbial sampling and analysis in remote islands in the Pacific, where proper laboratory facilities, procedures and guidelines are not always available.

The activities for this work entailed the:

1. assessment of sample bottles sterilisation procedures prior to sampling and determining the minimum concentration level of sanitisers required to effectively sterilise sampling bottles; and
2. the assessment and evaluation of the SPC E.coli field sampling and analysis techniques and accuracy of results.

2.2 Survey schedule and team

The laboratory and field activities in this work were undertaken by Aminisitai Loco and Anesh Kumar of the GSD-SPC, with guidance and support by SPC's Peter Sinclair; and the USP-IAS chemistry and microbiology teams, including Johann Poinapen, Usaia Dolodolotawake, Jaya Balram and Arun Pande.

The schedule of activities undertaken for the assessments is in Annex 1.

3 Background

Several water quality techniques had been used previously by SPC to ascertain the safety and potability of water sources in terms of bacteriological content. The majority of these bacteriological procedures, focus on (a) the presence or absence (P-A) assessment of E.coli and Total Coliform (TC); (b) H_2S producing bacteria as indicator of faecal contamination; and (c) the enumeration of Escherichia coli or E.coli[2] – this thermo-tolerant group of bacteria is evidence of recent faecal contamination and raises the risk in humans of contracting water-borne diseases (Mudaliar et al., 2008 and Mosley and Sharp, 2005).

[1] Henceforth referred by the shortened "E.coli compact dry" when not fully named

[2] Escherichia coli or E.coli is the scientific name for a bacterium that is commonly found in the lower intestine of warm blooded animals including humans. E.coli is a common water quality indicator and its presence in water indicates recent faecal contamination (WHO, 2004)

Detection of E.coli in the water supply should trigger further action like targeted sampling and/or treatment to maintain the safety and usability of any potable water source (Wisner and Adams, 2002 and WHO, 2004).

3.1 Review of bacteriological analysis techniques

With remote island locations and the current level of development in the Pacific, access to laboratory facilities suitable for microbiological analysis is very limited. Consequently, a number of proxy techniques for microbiological field assessment have been in use in the Pacific Island countries.

Techniques include, but are not limited to, the Hydrogen Sulphide (H_2S) test; the Collilert18 IDEXX procedures; and the E.coli compact dry plate filtration membrane method. These techniques are summarised in Table 1 with the full procedures presented in Annex 3.

Table 1. *Summary of microbiological assessment procedures used previously around the Pacific region.*

Technique	Procedure	Training required	Instrumentation	Accuracy
E.coli compact dry	A quantitative procedure using a petri-dish containing cultured media upon which a measured water sample, filtered through a 0.45 µm filter paper is placed and followed by a 18-24 hours incubation. (Key Diagnostic, 2012; and Centre for Disease Control and Prevention, 2010)	Rigorous training in sample preparation and quantification of E.coli and Total Coliform	E.coli compact dry plate, 5 ml/100 ml syringe, 0.45 µm filter paper, filter housing, sample bottles, tweezers, distilled water, incubator, bleach	• Provides quantifiable E.coli and TC count per 100 ml through specific colours for different colonies
Collilert 18 IDEXX	A presence and absence (P-A) system that involves adding a re-agent capsule to 100 ml of water and incubating samples for 18 - 24 hours. A UV light to determine the presence or absence of TC and E.coli (Ohio Water Microbiology Laboratory, 2013; and Centre for Disease Control and Prevention, 2010)	Basic training in Presence-Absence (P-A) test only, no quantification	Collilert reagents, sample bottles, UV lamp, incubator	• A dark yellow colour indicates the presence of TC and fluorescence under exposure to UV light indicates E.coli – this does not indicate level of contamination
Hydrogen Sulphide Test	A system that works on the generation of H_2S by faecal coliform indicator bacteria. This includes the addition of H_2S reagent in water followed by a 72 hours incubation (Manja et al., 1982; and Centre for Disease Control & Prevention, 2010)	Basic training in P-A test only, no quantification	Hydrogen Sulphide reagent, sample bottle, incubator	• Indicates presence of faecal coliform through H_2S-producing bacteria but potential of false positives is high due to naturally occurring sulphite reducing bacteria • The procedure does NOT show the level of contamination

3.2 The need for validation

There is a considerable amount of available information on the use and accuracy of the E.coli compact dry technique and its application for determination of E.coli and Total Coliform in agricultural and food products such as beef, chicken, pork liver, ham, raw tuna, pre-cut vegetables, tomato, yogurt, orange juice and soft drinks (Elllis and Meldrum, 2002; Key Diagnostics, 2016; Mizuochi and Kodaka, 2000; and Sato et al., 2001). This widespread usage in the food industry indicates the reliability, accuracy and reproducibility of the E.coli compact dry procedure. See box for product example from Hardy Diagnostics (2016).

PRODUCT DESCRIPTION FROM HARDY DIAGNOSTICS (2016)

Compact Dry™ E.coli is a ready-to-use chromogenic medium for performing *E.coli* and coliform counts that contains dehydrated culture media and a cold water-soluble gelling agent in a non-woven cloth matrix. The medium is instantly hydrated when inoculated with a sample, and capillary action diffuses the sample evenly over the matrix to form a gel within seconds. Compact Dry™ E.coli contains two chromogenic substrates, Magenta-Gal and X-Gluc, that yield a colored reaction when utilised and permit the differentiation of *E.coli* from other members of the coliform group.

The application of the E.coli compact dry technique for the assessment of microbial activity in water supply used for drinking in remote island locations, where the conditions for analysis may be outside the manufacturer's recommendations, requires some additional verification to ensure that the modified approach is sufficiently accurate to inform decisions on water quality maintenance.

Following from this, and as part of the CAIA Project's objective to develop practical tools to help improve water security in the Pacific region, a comparison of the E.coli compact dry and modified analysis techniques against accredited laboratory procedures and facilities was undertaken to test accuracy, reliability and comparability of SPC's E.coli compact dry methodology; and to ensure that it is fit for purpose. The USP-IAS laboratory, an accredited laboratory facility, was deemed the best organisation to undertake this validation work, with the focus of improvement to the technique, where warranted.

4 Methodology

The agreed methodology for the two (2) major components of this work is detailed in this section.

4.1 Assessment of sample bottle sterilisation procedure

To assess the SPC sterilisation procedure for sample bottles and determine if they are sufficiently cleaned so as to not influence end results, or had residual chlorine in sterilised bottles capable of inhibiting the growth of bacteria in sampled water. The assessment involved running a number of field sterilised sample bottles through the residual chlorine test (RCT), which entailed the following:

a. Soaking thirty (30) 250 ml screw-cap bottles and lids in 19 L of boiled water for 45 minutes in a sanitised esky.
b. Adding 810 ml of janola bleach, having 3% of sodium hypochlorite as the active ingredient.
c. Adequate stirring of the solution using a rod to ensure the bottles and lids are properly soaked in solution for more than 45 minutes.
d. Retrieving the bottles and lids and keeping them upside down in a basin to dry over night
e. Rinsing the bottles with either bottled water or de-ionised water – this was conducted to replicate SPC's field practice whereby the sample bottles are rinsed once by boiling water immediately after sterilisation and an additional 3 rinses are made with sample water before sample collection. This extended into testing other conditions where the bottles were randomly selected into 6 groups with different number of rinses undertaken.

Table 2. *Sample bottles sterilisation grouping.*

Number of bottles	Number of Rinses	Rinsing material
5	1	distilled water
5	3	bottled water
5	3	distilled water
2	4	distilled water
2	5	distilled water
2	6	distilled water

f. Conducting RCT using 25 ml of deionised water and using the Lovibond laboratory equipment and procedure and recording the results, simultaneously.

4.1.1 Further assessment of sterilisation procedure

Further assessment on the sterilisation procedure was conducted after a second phase of sampling conducted on 2nd June only at the Waimanu River. This was related to the suggestion of trialling a reduced concentration in sanitising solution to determine the minimum level of sanitation required to remove residual chlorine below the detectable limit while simultaneously sterilising the bottles. This involved the following:

a. Preparing 5 sanitising solutions consisting of boiled water and four solutions of janola bleach, having 3% sodium hypochlorite. The four bleach solutions were in the following volume to volume ratio of bleach to water: 0.5%, 1%, 2.5%, and 5%.
b. Stirring the solutions adequately to allow equal mixing and distribution.
c. Soaking the sample bottles for 45 minutes before they were rinsed 2 and 3 times with deionised water.
d. 2 sample bottles were not sterilised as control for Total Coliform test, conducted using USP-IAS procedures.
e. Conducting the RCT and recording the results.

f. The effectiveness of sterilisation was tested through the USP-IAS Total Coliform procedures.

4.2 Assessment of the SPC sampling and laboratory protocols and results

This involved the assessment of the SPC field sampling procedure; the observation of sample preparation and incubation practice in the laboratory; and the comparison of E.coli and Total Coliform counts with the USP-IAS laboratory test results on samples collected at the following four (4) agreed sites below:

1. Mr Usaia Dolodolotawake's rainwater tank located close to Wailase Creek;
2. a creek sample from Wailase Creek;
3. a river sample from the Water Authority Fiji, (WAF), Offtake at Waimanu River; and
4. a tap water sample from the USP-IAS campus.

Location of sampling sites are shown in Figure 1.

Figure 1. *The locations of the four (4) sampling sites comprising a creek and a rainwater sample from around the Wailase Creek area; a river sample from the Waimanu; and a tap water sample from the USP-IAS campus.*

SPC sample bottles were used in this phase. The expiry status of the SPC compact dry plates indicated that the time of the analysis the plates were approximately 6 months beyond their recommended use by date. As no plates were available which were within their recommended use-by date, the analysis was continued using a larger number of duplicate samples.

The usability of expired compact dry plates 6 months past their recommended use-by date was tested whereby three (3) samples at each site were collected and analysed by SPC and USP using some expired E.coli compact dry plates.

SPC's field technique (Annex 3) was assessed and tested against the USP-IAS accredited APHA methods AP 9222C and AP 9222G for Total Coliform and E.coli, respectively. The USP-IAS procedures required several dilutions to be done, with dilutions having a colony count of 20 – 80 selected for further determination.

4.2.1 Further assessment of sampling and laboratory procedures

A second phase for sampling and validation was conducted following a procedural error during the sample preparation, whereby all 50 ml plates exhibited no microbial growth after the incubation period. Repeated sampling was undertaken at the Waimanu WAF offtake location to address this error. Samples bottles from SPC and USP-IAS were used and each team followed their respective laboratory procedures and compared results for E.coli and Total Coliform counts afterwards.

Following the E.coli and Total Coliform laboratory procedure, further assessment of SPC's sterilisation procedure (in section 4.1.1) was conducted. This entailed the sterilisation of bottles in different concentration bleach solutions; and the strength of boiled water alone, as a sanitiser, was also tested. The sanitised sample bottles were filled with bottled water and tested for Total Coliform, whilst two (2) unsterilized sample bottles were used as control. The absence of coliform would suggest that the bottles were properly sanitised.

5 Results and discussion

5.1 Sanitising procedure assessment

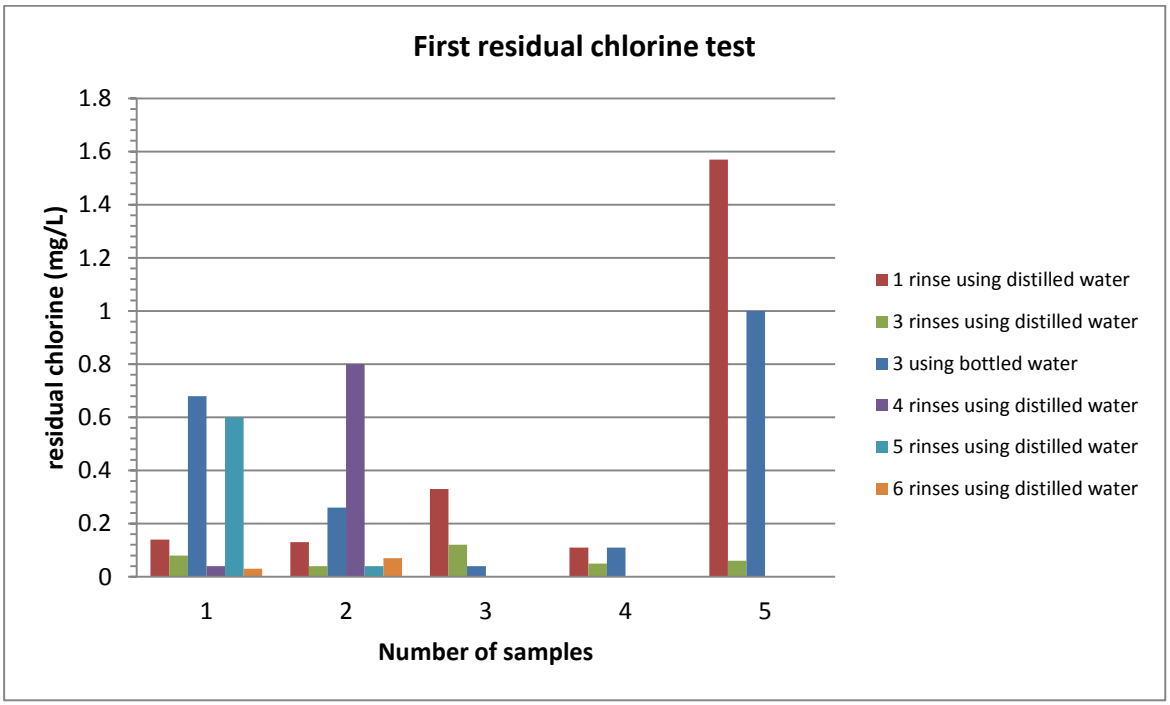

Figure 2. *Residual chlorine test results for differing rinse amounts.*

The residual chlorine test results shown in Figure 2 showed high variability in the amount of residual chlorine compared to the number of rinses with sterilised water. The highest values were recorded in Lot 1 (0.11 – 1.57) where only 1 rinse was undertaken and it is believed that the bleach was added direct to the esky container in which the bottles were already soaking in boiled distilled water. This is likely to result in the unequal distribution of sanitiser, despite stirring, where some bottles, particularly those towards the top of the esky, would be in contact with higher concentrations of chlorine. It would have been preferable to mix the sanitiser first with soaking water, allowing for equilibration of chlorine solution before soaking the bottles to allow equivalent chlorine contact.

It was also calculated that bleach solution had a chlorine concentration of 1,700 mg/L, which was deemed excessive. Super chlorination generally involves 10 times the recommended levels in drinking water, which is 0.5 – 1.0 mg/L (WHO, 2004). Thus, 17,000 mg/L free chlorine was excessive and would require significantly more rinsing to remove all residual chlorine. Based on the results it is recommended that the current sterilisation solution of 5% bleach be used. This requires no less than four (4) thorough rinses to effectively remove residual chlorine to undetectable levels. Further, it is suggested that with this concentration of *active ingredient* that the sample bottle be rinsed a minimum of four (4) times with the sample water prior to sample collection. Further testing using a reduced *sanitising* solution was undertaken to review its effectiveness at sterilisation and to optimise the effect of rinsing.

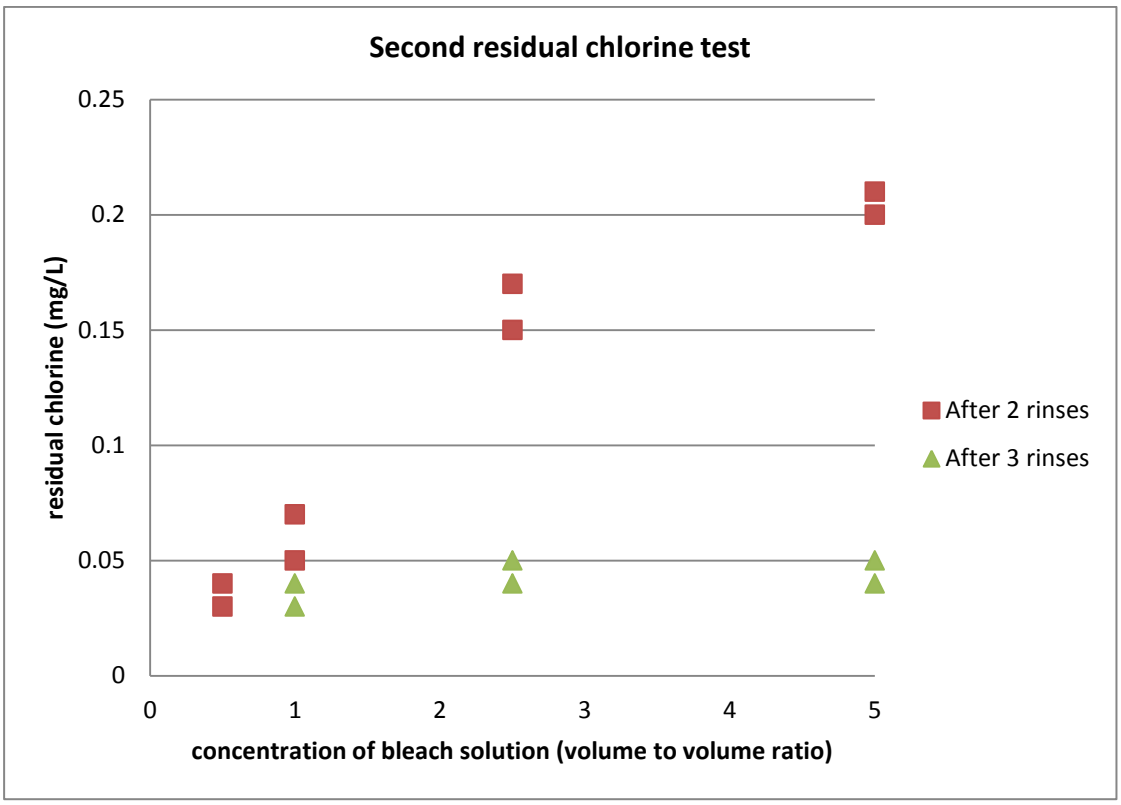

Figure 3. *Residual chlorine test results for different numbers of rinses.*

Table 3. *Total Coliform was tested for in 6 groups of sample bottles after the 2nd sampling phase. Unsterilized bottles were used as control. Bottles were sterilised using various concentrations of janola bleach, which has 3% sodium hypochlorite; and boiling water.*

Sample	Total Coliform cfu/100 ml
Control 1 (unsterilized)	88
Boiled water	4800
0.5% sanitised solution bottle	<1
1% sanitised solution bottle	<1
2.5% sanitised solution bottle	<1
5% sanitised solution bottle	<1

Figure 3 shows that the change in the method of adding sanitiser (i.e. adding sanitiser, mixed and allowed to equilibrate before soaking bottles) reduced number of rinsings required to remove residual chlorine. Most of the residual chlorine was removed after three (3) rinses. Having an additional three (3) rinses with sample water prior to sample collection will further help the rinsing process. Table 3 shows that adding bleach is required to properly sterilise the sampling bottles; and soaking in boiled water alone for 45 minutes was inadequate to remove all the coliform from the used bottles – this is evident in the Total Coliform detected, which could have resulted from residual contamination of sample bottles.

5.2 E.coli and Total Coliform procedure validation

Table 4. *E.coli and Total Coliform test results from USP-IAS and SPC procedures.*

Sample	USP-IAS		SPC			
	E.coli/100 ml	Total Coliform/100 ml	1ml E.coli/100 ml	1 ml Total Coliform/ 100 ml	50 ml E.coli/100 ml	50 ml Total Coliform/100 ml
Control		0	0	0	NA	NA
Control		0	0	0	NA	NA
Tap water A		0	0	0	NA	NA
Tap water B		0	0	0	NA	NA
Tap water C			0	0	NA	NA
Creek A	838	6700	300	8100	NA	NA
Creek B	0	7500	0	8600	NA	NA
Creek C			100	7900	NA	NA
River A	3500	7800	300	8500	NA	NA
River B	878	7900	100	7400	NA	NA
River C			300	5300	NA	NA
Rainwater A	1900	3100	0	700	NA	NA
Rainwater B	3200	3800	100	600	NA	NA
Rainwater C			100	1000	NA	NA

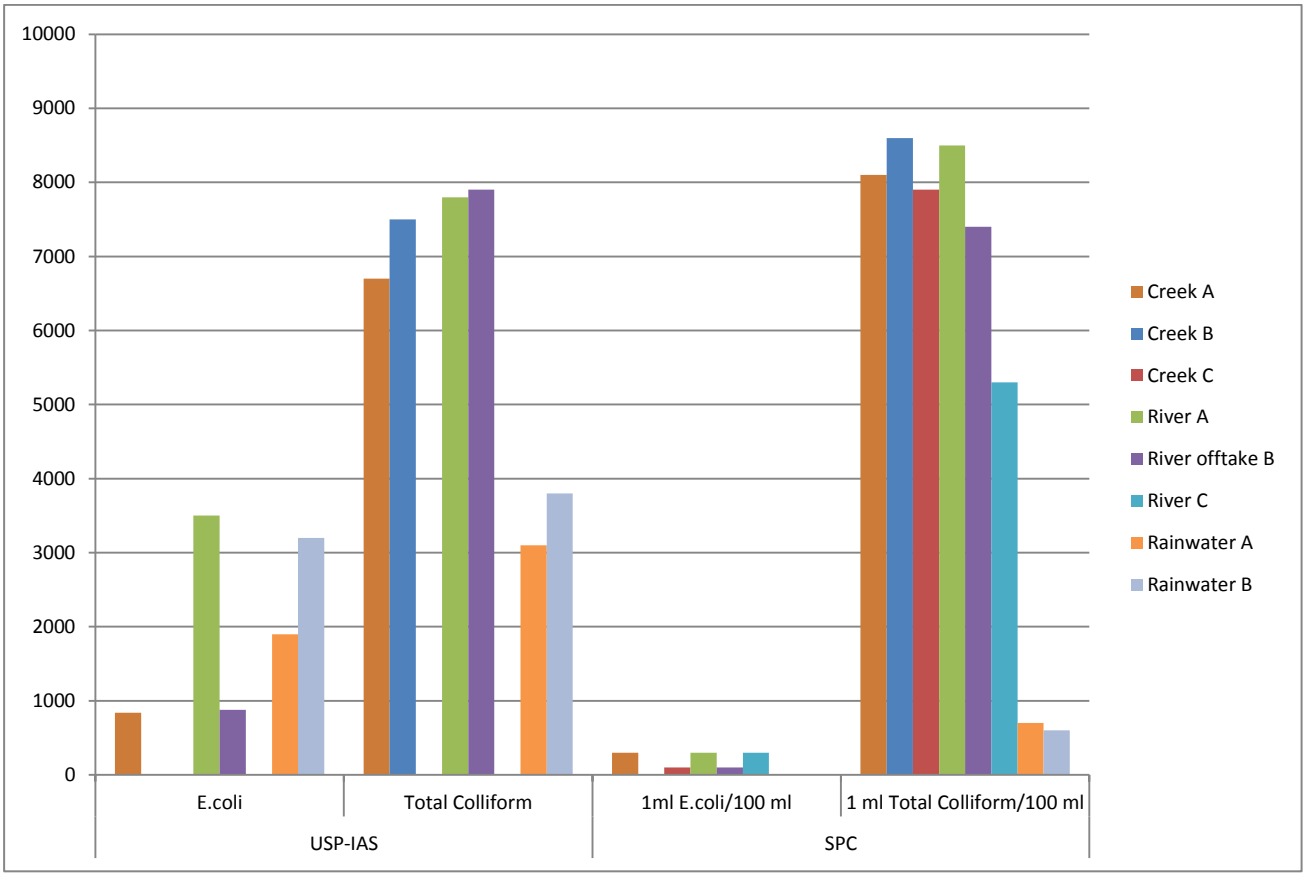

Figure 4. *Variability in USP-IAS and SPC bacteriological counts from the 1st sampling phase.*

5.3 Observations based on results

The results presented in Table 4 and Figure 4 showed the following:

1. Control samples (with zero bacteria loading) agree quite well for both USP-IAS and SPC Total Coliform and E.coli counts.
2. Total Coliform counts agree reasonably well with all samples except for rainwater, which showed significant difference. This was attributed to underestimation in the SPC counts.
3. E.coli results differ significantly; again with SPC results appearing to be underestimations. This is attributed to the Total Coliform load. E.coli results in low Total Coliform loads yield similar values between the two techniques; however, high Total Coliform loads are showing major differences in E.coli counts between the two approaches. It is suggested that the high Total Coliform load is creating a false positive or enhanced E.coli count results.
4. During the initial sampling, the SPC 50 ml plates did not show any bacteriological growth due to an error during sample preparation. The error was the non-addition of 1 ml of sterile water to rehydrate the agar plate prior to the placement of the sample-rinsed filter papers.
5. The three (3) samples tested by SPC produced consistent results suggesting that the compact plates remained usable, even though 6 months expired.

Table 5. *E.coli and Total Coliform results from USP-IAS and SPC procedures.*

Sample	USP-IAS		SPC			
	E.coli/100 ml	Total Coliform/100 ml	1 ml E.coli/100 ml	1 ml Total Coliform/100 ml	50 ml E.coli/100 ml	50 ml Total Coliform/100 ml
Control	<1	<1	0	0	0	0
Control	<1	<1	0	0	0	0
River 1	0	2700	100	2400	64	442
River 2	0	4100	0	4300	50	512
River 3	0	3400	100	3500	90	200
River 4	0	4300	0	2700	114	752

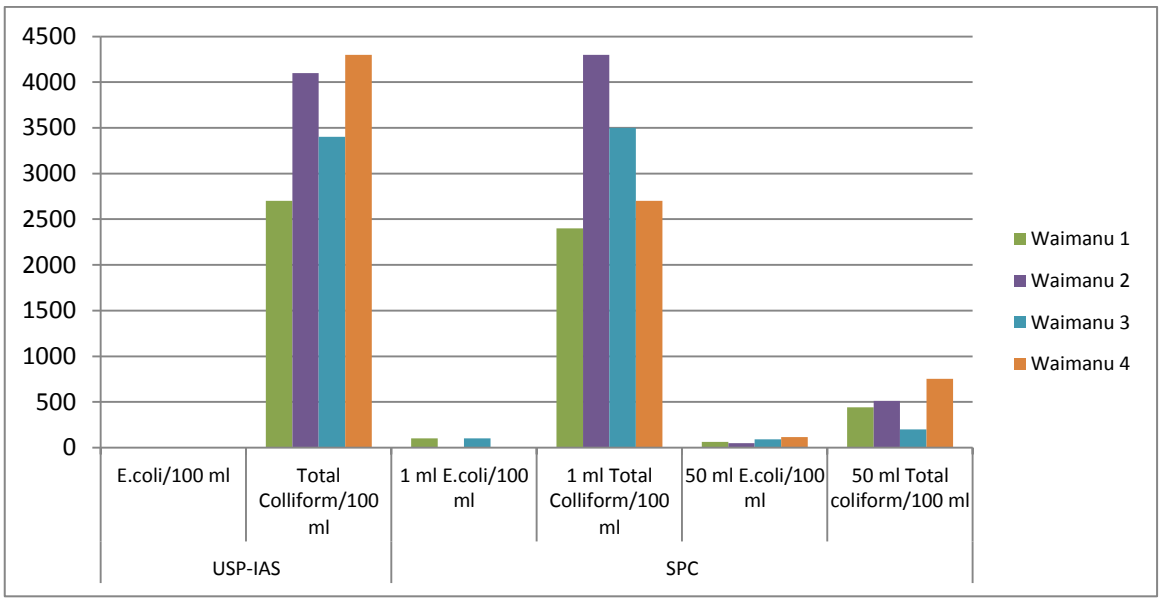

Figure 5. *USP-IAS and SPC bacteriological counts from the 2nd sampling phase.*

Results of the second validation tests as shown in Table 5 and Figure 5 suggest the following:

1. Total Coliform results for SPC and USP were in good agreement across a wider range of coliform/microbial counts. This agreement indicated that the bottles were satisfactorily sterilised and the field procedures for the E.coli compact dry technique compared favourably with the IAS laboratory technique.
2. E.coli counts where sample loadings were low to no coliform indicated good agreement of SPC with USP-IAS techniques. Where Total Coliform loadings were high, the E.coli results from the E.coli compact dry technique were very different from the USP-IAS results bringing into question over reliance on the E.coli compact dry technique. It is suggested that identification and enumeration of the colonies may be a factor in the greater differences in results from both techniques. The E.coli compact dry technique is suspected to be overestimating E.coli coliform counts.
3. The volume dilutions used in the analysis may also play a factor in the variability of results for E.coli; noting that the Total Coliform counts at 1 ml dilution indicated consistent and comparable results. The SPC E.coli results using the 50 ml volume yielded consistent values between 50 and 114 cfu/100 ml, which although slightly higher than the laboratory results of less than 1 suggests a similar categorisation of microbial activity relative to the water's potability. Similarly, the 1 ml sample volume generated counts of 1 colony indicates 100 cfu/100 ml. This variability suggests that high Total Coliform loads impact the ability of the E.coli compact dry plate to accurately distinguish bacterial colonies; however, erroneous counting by enumerators when colonies are denser cannot be discounted.
4. To address the incompatibility in E.coli results from the two techniques for an unexpected coliform load, it has been recommended that a 10 ml sample dilution should be useful, which may lower the coliform load on the agar plate and aid in a more accurate enumeration of different coliform units.

Due to discrepancies between the SPC and USP-IAS E.coli counts from the two (2) sampling results, USP IAS recommended that SPC undertakes at least one round of proficiency testing for potable water analysis to provide more confidence with the enumeration of colonies. This test program is undertaken by IAS for its chemical and microbiological accreditation (U. Dolodolotawake, 2017, personal communication, 16th January). IAS intends to accommodate an extra water sample from SPC during its testing schedule, with a charge of around $200.00. This sample will be sent to an independent laboratory in New Zealand for E.coli and Total Coliform analyses. SPC and USP will be required to collect and analyze additional samples using their respective bacteriological procedures – these results will be compared with the official results from New Zealand. The accuracy and reliability of the results will be based on mean and z-scores provided for all methods used.

6 Conclusions and recommendations

The validation work by USP-IAS allowed a useful assessment of the SPC's field microbial technique for use in remote island locations – identifying areas of improvement for increased confidence in, and accuracy of, the results.

The assessment of sample bottle sterilisation procedures concluded the following:

a. Sample bottles need to be cleansed using a sterilising agent as soaking in boiling water alone was confirmed to be inadequate.
b. Sanitising solution of concentrations in the range 0.5 – 5% worked well.
c. Pre-equilibrating the sanitising solution is important to improve free chlorine distribution in solution and thus providing more even chlorine contact on all bottles.
d. A post-sanitising soaking step is recommended to assist in the removal of free chlorine before sample collection and storage and thereby reducing rinsing frequency.

The assessment of the E.coli compact dry plate filtration membrane method confirmed the following:

a. The SPC and USP-IAS results worked well for Total Coliform but appear to indicate over reading of E.coli, particularly where the Total Coliform load is high.
b. Both the procedures were in agreement when both Total Coliform and E.coli were absent in the sample.
c. The 50 ml sample volume may lead to coliform overload on the compact dry plate and potentially cause errors in enumeration.

Recommendations to improve the SPC field technique are as follows:

For the sanitation procedure, it is recommended that:

1. 2.5 % sanitising solution (volume to volume ratio) is considered adequate to sterilise the samples bottles prior to sample collection.
2. Sanitising solution needs to be adequately stirred before sample bottles are soaked, to allow the solution to be equilibrated and so ensure equal distribution of free chlorine; and uniform chlorine contact with all sample bottles.

3. With a reduced concentration of bleach solution of 2.5%, three (3) rinses with boiled or distilled (immediately after soaking) and the (3) rinses with sample water will be required prior to sample collection. This should remove the residual chlorine below detectable limit.

For the E.coli and Total Coliform procedures and enumeration, it is suggested that:

1. the volume of sample dilution be reduced to 1 ml or 10 ml where there is likelihood of high coliform load in water sample; and that
2. SPC undertake a proficiency testing regime which can be arranged through USP-IAS.

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Annex 1: Schedule of activities

Table A1.1. *Schedule of activities for the assessment of the SPC field technique for bacteriological water sampling.*

Date	Activity	Duration
18/04/2016	• Conducting residual chlorine test at USP-IAS through following: ❖ Drying bottles overnight ❖ Tested and recorded residual chlorine ❖ Discussed results with USP-IAS team ❖ Proceeded with increasing the number of rinses ❖ Repeat residual chlorine test	1.5 days
21/04/2016	• Undertake field sampling and laboratory work through the following: ❖ Preparation of sample bottles through sterilisation procedure ❖ Carrying out of field sampling of rainwater, creek, river, and tap water at Wailase, Waimanu and USP using SPC bottles ❖ Usage of SPC's sample preparation procedure at USP-IAS lab ❖ Allowed 24 hrs incubation ❖ Enumerated E.coli and Total Coliform counts at USP-IAS ❖ Comparison of SPC results against USP-IAS results	2 days
1/06/2016	• After discussion of previous procedure and results, a 2nd validation phase was agreed. This included the following: ❖ Preparation of sample bottles ❖ Field sampling at Waimanu River ❖ Undertaking sample preparation procedure at the USP-IAS laboratory ❖ Allowed 24 hrs incubation ❖ Conduct E.coli and Total Coliform counting at USP-IAS ❖ Testing different concentrations of sanitation solution, namely boiled water, 0.5%, 1%, 2.5% and 5% of janola bleach ❖ Test for Total Coliform to determine the effectiveness of the different sanitation solutions	3 days

Annex 2: USP-IAS report and results

FACULTY OF SCIENCE TECHNOLOGY AND ENVIRONMENT
Institute of Applied Sciences

Validation of Sampling Procedures and the E.Coli Compact Dry Plate and Sampling and analysis of Bonriki Reserve Water

(SPC –CAIA Project)

Prepared by:

Institute of Applied Sciences

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The University of the South Pacific

1.0 INTRODUCTION

The Pacific Community (SPC) in partnership with the University of the South Pacific's Institute of Applied Sciences (IAS) laboratory collaborated to:

I. Validate SPC's water bacteriological technique which could later be applied to field situations, specifically small island states that lack substantive water testing facilities. In this partnership IAS will; A) investigate SPC's sampling methods including sample bottle preparations and then B), carry out a comparison of E.Coli results of SPC and IAS methods using the same lot of samples. Further studies (C) of work done in A &B were also undertaken (variation)
and

II. Collect, analyze and together with SPC, categorize Bonriki Water Reserve, South Tarawa, Kiribati. In this partnership IAS will; A) prepare all the necessary sampling gear inclusive of sample bottles; B), carry out the sampling process in Tarawa, Kiribati; C) ensure proper storage and preservation of samples on-route to the laboratory ; D) transport samples back to IAS laboratory, Fiji, including taking care of border requirements pertaining to water sample trans-border movement; E) analyses of the water samples for parameters listed in 2.2) and in association with SPC undertake a hydro classification of ions using stiff plots and piper diagram and G) provide a written report on the results for all the chemical analyte tested.

Part I:

VALIDATION OF SAMPLIMG TECHNIQUE AND THE COMPACT DRY E.COLI METHOD

2.0 METHOD VALIDATION

2.1 Sampling/ sampling bottles preparation

2.1.1 Methodology

(This procedure was conducted by SPC staff at IAS lab and was observed by assigned IAS laboratory staff.)

Thirty new 250mL polypropylene bottles with lids were brought into the IAS laboratory in a sanitized esky. In the lab, the bottles were opened and replaced into the esky. Approximately 19L of boiling water was added and to this, 810mL of the sanitizer (Janola) was added to make a 5% (v/v) sanitation solution. A stirring rod was used to ensure bottles and lids were well soaked and left to stand for 45 minutes. Bottles and lids were removed and kept upside down in a basin to dry over-night.

The following rinsing regime was carried out the next day.

2.1.1.1 Sample Bottles Lot 1

Five bottles were randomly picked, labelled 1-5, each filled with 125mL distilled water, closed tightly and thoroughly shaken to remove any residual sanitizer. The 125mL rinsate was discarded and the bottles rinsed with a further 25ml of distilled water. The rinsate from this was retained for residual chlorine testing.

2.1.1.2 Sample Bottles Lot 2

Five more bottles (6-10) were chosen but bottles were rinsed 3x with 125mLs of distilled water and rinsate discarded. A final 25ml rinse was retained for residual chlorine testing.

2.1.1.3 Sample Bottles Lot 3

Two bottles 11-12 were treated as above but were rinsed out 4x.

2.1.1.4 Sample Bottles Lot 4

Two bottles 18-19 were treated as above but were rinsed out 5x.

2.1.1.5 Sample Bottles Lot 5

The last two bottles 20-21 were rinsed 5 times and treated as previous.

2.1.1.6 Sample Bottles Lot 6

Four bottles (13-17) were rinsed 3x with Aqua Safe Bottled water and a final 25mL rinsate retained for residual chlorine testing.

Chlorine analysis was carried out by IAS Staff using the DPD colorimetric kit (Lovibond Kit).

2.1.2 Sample collection

The sterile sample bottles were rinsed several times with sample prior to sample collection. All sample bottles were clearly labelled and samples were kept cool in an esky whilst being taken to the laboratory

2.2 Results:

Table 2.1: Residual Chlorine after X rinses

Bottle Number	Bottle Lot Number	Number of Rinses with Distilled Water	Number of Rinses with Aquasafe Bottled Water	Residual Chlorine mg/L
1	1	1	-	0.14
2				0.13
3				0.33
4				0.11
5				1.57
11	2	3	-	0.08
12				<0.05 (0.04)
13				0.12
14				0.05
15				0.06
16	3	4	-	<0.05 (0.04)
17				0.08
18	4	5	-	0.06
19				<0.05 (0.04)
20	5	6	-	<0.05 (0.03)
21				0.07
6	6	-	3	0.68
7				0.26
8				<0.05 (0.04)
9				0.11
10				1.00

2.3 Observations from Results

- It can be seen that there are cases of higher variability in amount of residual chlorine in some bottles of the same lot, significantly Lot 1 (0.11- 1.57) and Lot 6- Aqua Safe rinse (0.04 – 1.00). This could be attributed to the application of the sanitizer. In this instance the sanitizer was poured over the soaked bottles and then stirred. It would have been preferable to mix the sanitizer first with soaking water, allow for equilibration of chlorine and then soak bottles and lids. This allows for equivalent chlorine contact.
- It was calculated that chlorine concentration of the sanitizing solution was about 1700mg/L. This seems excessive. Super chlorination generally involves 10 times the recommended levels in drinking water (0.5 – 1.0). So a 10ppm solution or even a 100ppm one suffices. The 1700mg/L free chlorine seems excessive and will only mean more rinsing needs to be done before all residual chlorine are removed.
- The current sanitation procedure requires no less than 4 thorough rinses to effectively remove residual chlorine to undetectable level. It may be necessary to review the strength of sanitizing solution to optimize rinsing.
- May need to consider rinsing bottles with sample

3.0 EVALUATION OF SPC'S COMPACT DRY METHOD FOR E.COLI AGAINST IAS's APHA ACCREDITTED METHOD

3.1 Methodology

3.1.1 Samples:

Two sets of water samples were collected from five different sites. One of the sets was analyzed by SPC other by IAS

3.1.2 Analytical Method

For total coliform IAS used its accredited APHA method AP 9222C while method AP 9222G was used for E.coli. These membrane filtration methods require several dilutions be done and the dilution that has a colony count in the range 20-80 is chosen for further determination. SPC used their E.coli Compact Dry Plate Kit method.

Results for total coliform and E.coli obtained from the two different methods were compared.

3.2 Results

Table 3.1: Total Coliform and E.coli obtained from different methods

	IAS Micro Laboratory Results							SPC Result					
SPC Sample ID	**Total Coliform/ E.Coli**							**Total Coliform**			**E.Coli**		
	-1mL	1ml	10ml	100 ml	TC /100 mL	TC/100mL	E.coli/ 100mL	TC cfu/100mL			E.coli cfu/100mL		
											/ml	/50 ml	/100 ml
						Verified	Verified						
Aquasafe Control A				0	<1			0	0	<1	0	0	<1
Aqua Safe Control B				0	<1			0		<1	0		<1
US Tap Water A				0	<1			0	0	<1	0	0	<1
USP Tap Water B				0	<1			0		<1	0		<1
Waimanu WAF off take A	11	78	TNTC	TNTC	7800	7800	3500	85	0	8500	3	0	300
Waimanu WAF off take-B	16	79	TNTC	TNTC	7900	7900	878	74		7400	1		100
Field Sample								53		5300	3	0	300
Wailase Creek A	11	67	TNTC	TNTC	6700	6700	838	81		8100	3	0	300
Wailase Creek B	10	75	TNTC	TNTC	7500	7500	<1	86		8600	0		<1
Field Sample								79	0	7900	1		100
Rain Water Tank A	6	44	TNTC	TNTC	4400	3100	1900	7	0	700	0	0	<1
Rain Water Tank B	2	38	TNTC	TNTC	3800	3800	3200				1		100
Field Sample								6	0	600	0	0	<1

3.2.1 Observations from results from the two methods

- Control samples (zero loading of bacteria) agree quite well for both E.coli and total coliform
- Total coliform counts agreed to a good extent except for Rain Water Tank A
- E.coli results differ quite markedly except for Wailase Creek B (but there could be errors in these results since sample A had counts for both groups and sample A and B are both from the same source)
- The differences in the E.coli results needs further investigations especially with respect to total coliform load. E.coli results are same when there is no coliform while results differ markedly when there is a high coliform count. This may mean that a higher coliform load is creating a false positive/enhanced E.coli counts on the Compact dry plate
- The consistent zero E.coli count for the 50ml sample volume suggest that a systematic error has occurred on the plate as no growth was registered

3.2.2 Other comments

- Based on on-line EC Compact Dry brochures, plate is to be inverted during incubation. This is not mentioned in SPC's procedure and may need to be included. The impact on result though may not be that great.

4.0 FURTHER VALIDATION WORK

4.1 Introduction

Following the first round of activities, it was decided that immediate re-validation work is necessary. All activities in this exercise were carried out by both IAS and SPC staff.

4.2 Method Validation

4.2.1 Methodology

Sample bottles were provided by SPC and rinsed using the 5% sterilizing solution and rinsing regimes. The samples were analyzed for Total Coliform and *E.coli* using SPC's and IAS's respective methods.

4.2.2 Sampling:

Samples were collected from the WAF Waimanu uptake. Four samples were uplifted and these were halved to made up samples 1 for both IAS and SPC (IAS 1, SPC 1) etc. This ensured that both groups were analyzing the same samples. Four samples in total were collected (SPC/ IAS 1- 4). A further two samples used as control were collected using IAS bottles.

4.3 RESULTS

Table 4.1

Results for Total Coliform and E.coli for both IAS laboratory and SPC

	IAS						SPC Result						
SPC Sample ID	**Total Coliform**					***E.coli***	**SPC Sample ID**	**Total Coliform**			***E.coli***		
	-1mL	**1 mL**	**10 mL**	**TC/ 100mL**	**TC/ 100mL**	***E.coli*/ 100mL**		**TC cfu/100mL**			***E.coli* cfu/100mL**		
								1mL	**50mL**	**cfu/ 100mL**	***1mL***	***50mL***	***cfu/100 mL***
					Verified	Verified							
1. C1 IAS Bottle	6	73	TNTC	7300	3200	<1							
2. C2 IAS Bottle	10	67	TNTC	6700	1700	<1							
3. IAS 1	5	71	TNTC	7100	2700	<1	SPC 1	24	442	2400	1	32	64
4. IAS 2	10	66	TNTC	6600	4100	<1	SPC 2	43	512	4300	0	25	50
5.IAS 3	8	67	TNTC	6700	3400	<1	SPC 3	35	200	3500	1	45	90
6.IAS 4	6	78	TNTC	7800	4300	<1	SPC 4	27	752	2700	0	57	114

4.4 Discussions

From Table 4.1, it can be seen that total coliform for both groups were in good agreement (except sample 4). The agreement in these results indicated that bottles were satisfactorily prepared and both methods worked well for total coliform. The control sample also had total coliform counts quite similar to those observed in the samples. The closeness of these results indicated that the bacterial count obtained by both the groups have a high degree of acceptability.

The E.coli results however were different. IAS samples including control had non-detects while SPC had a consistent range of values.

Of interest though was the sample volumes used for the essays. Both groups used one mL of sample for total coliform and results agreed quite well. For E.coli however, IAS used one mL while SPC used 50ml for its

determination (instead of 1ml). In their 1mL sample run, two of their samples had non-detects while the other two had 1 cfu/mL E.coli.

The consistent E.coli results (64, 50, 90, 114 cfu/100mL) obtained by SPC seem to raise the question whether a high total coliform load (as in results) impacts on the ability of the compact dry method to accurately differentiate bacterial colonies. Further, there is a possibility that the enumerator will err in counting if colonies were dense. It was noted that for samples (bottled water) that had no coliforms, both methods worked equally well, no E.coli was detected.

It is suggested that the volume used for total coliform be used for E.coli enumeration as well. Further, the use of 10mls sample volume as suggested by the method should be explored.

For a more conclusive outcome, SPC is strongly recommended to participate in at least one round of proficiency testing of potable water. This can be arranged through IAS.

4.5 Conclusion

- The E.Coli Compact Dry Plate Kit worked well for total coliform but seem to have problems with E.coli when there is a high total coliform load
- The kit worked well (in agreement with IAS's results) when both total coliforms and E.coli were absent
- The 50mL sample volume used may lead to coliform overload on plate and potentially cause erroneous enumeration

4.6 Recommendations

- Volume of sample used to be reduced to 10mLs or 1mL where there is a likelihood of high coliform load.
- SPC must participate in a proficiency testing regime which can be arranged through IAS to minimize costs

4.7 SANITATION PROCEDURE VALIDATION

4.7.1 Introduction

Following on from initial work done, it was recommended that other rinsing regimes be trialed as it was felt that the 5% bleach solution currently used required several rounds of rinsing before residual chlorine from bleach was satisfactorily removed. These extra rounds of rinsing meant that more rinsing water needed to be taken along during sampling trips and time will be saved if number of rinses were reduced.

4.7.2 Methodology

Using boiled water, 2 liters of 0.5, 1.0, 2.5 and 5% bleach solutions were used to sanitize the bottles (those contaminated from the Waimanu samples). Two bottles were used for the different solutions. All bottles were separately soaked in distilled water before the rinsing process. The bottles were then rinsed with 125ml distilled water followed by a 25ml rinse which was used for residual chlorine determination.

Two sample bottles were soaked in boiling water only while a further two were not cleaned at all to act as control. The presence of coliforms in these bottles (control) would indicate that the bottles were actually contaminated before sanitizing.

After the rinsing, the bottles were filled with bottled water (which was assumed to have no coliform) and these were tested for total coliform using IAS standard method. The absence of coliform would indicate that the bottles were properly sanitized.

4.7.3 Results

Results of sanitizing process showed that with the change in the method of adding the sanitizer (sanitizer added, allowed to equilibrate and then bottles soaked) and the new added soaking step had resulted in reduced rinsing frequency. Most of the residual chlorine has been removed after three rinses. Further, sample bottles will be rinsed with samples prior to sample collection which will further help the rinsing process.

Table 4.2: Residual Chlorine of Various Sanitizer Concentrations after X Rinses

	Residual Chlorine (mg/L) after	
% Sanitation Solution	**2 Rinses**	**3 Rinses**
0.5 Bottle 1	0.03	
0.5 Bottle 2	0.04	
1.0 Bottle 1	0.07	0.03
1.0 Bottle 2	0.05	0.04
2.5 bottle 1	0.17	0.05
2.5 bottle 2	0.15	0.04
5.0 Bottle 1	0.20	0.04
5.0 Bottle 2	0.21	0.05

Table 4.3: Results for Total Coliform to test Cleaning Process

Results for Total Coliform					
Sample ID					
Sanitizer	**1mL**	**10mL**	**100mL**	**TC/100mL**	**TC/100mL(verified)**
Control 1	0	19	88	88	<1
Control 2	4	19	61	61	<1
Boiled Water Sample 1	4	8	50	<1	<1
Boiled Water Sample 2	7	48	TNTC	4800	<1
0.5% Sample 1	0	0	14	<1	
0.5% Sample 1	0	0	17	<1	
1.0% Sample 1	0	0	0	<1	
1.0% Sample 2	0	0	0	<1	
2.5% Sample 1	0	0	0	<1	<1
2.5% Sample 2	0	0	0	<1	<1
5% Sample 1	0	0	0	<1	<1
5% Sample 2	0	0	0	<1	

From Table 4.3 it can be noted that soaking in boiled water for 45 minutes was inadequate as a sanitizing procedure. Results showed that after soaking, the sterile sample had total coliform which must have resulted from contamination from sample bottles.

Clearly, rinsing with various concentrations of sanitizer as in Table 4.3 satisfactorily removed all microbes from contaminated bottles. The control bottles showed counts which implied that the sanitized bottles were contaminated in the first place and the sanitizing process worked well.

From these results, it is recommended that a new soaking procedure be adopted with a 2.5% sanitizing solution. The bottles are to be rinsed 3 times with distilled water to remove residual chlorine and rinsing with samples before collection further aids this process.

4.7.4 Conclusions

- Sample bottles needs to be cleaned using a sterilizing agent. Soaking in boiling water alone seems inadequate
- Sanitizing solutions of concentration of 0.5 – 5% worked well
- Pre-equilibrating sanitizing solution is vital for equal free chlorine distribution in solution thus providing equivalent chlorine contact on all bottles
- A soaking step post sanitizing removes most chlorine before storage thus reducing subsequent rinsing frequency

4.7.5 Recommendations

- A 2.5% sanitizing solution to be used
- The solution to be prepared and equilibrate before soaking of bottles
- Soaking in clean (distilled) water immediately after above step
- Bottles to be rinse at most three times with distilled water and equivalent number of times with sample before sample collection.

PART II

TESTING OF BONRIKI WATER RESERVE WATER

5.0 METHODOLOGY

5.1 Sample bottles preparation

The required sample bottles were soaked overnight in 10% hydrochloric acid and then rinsed several times with deionized water. The bottles were stored in eskys prior to sample collection.

5.2 Sample Collection and Preservation

For Galleries, where there is continuous water extraction, samples were collected from service taps. The taps were allowed to flush out for about two minutes before sample collection begins. For monitoring wells, samples were collected at various depths and this was done by connecting specific lengths tubes to water pumps which then pumps water out. Again water was left to run before sample collection is undertaken. For sea water samples, collection was done using a bailer. The rainwater sample was obtained from clean water containers attached to the rain logger. In all occasions, sample bottles were rinsed a number of times with samples before actual sample collection.

Prior to the rinsing and collection processes, bottles were labelled with site location, time and date collected and name of collector. Once samples are collected, they are tightly closed and placed in eskys with cooling cubes in them.

Samples collected on day 1 were from Galleries 1,8,9,10,11,12,13,15,16,17,18,19 &20. Day 2 consists of samples from Galleries 2,4,5,6,7,21 &22, monitoring wells BN 27 (6 samples), BN2B (3 samples), BN2 (3

samples), BN34 (5 samples), BN22 (6 samples), 2 sea water samples from either side of the lagoon and a rainwater one from the rainfall logger.

More coolant was added to the eskys which were then tightly sealed for transportation to the IAS laboratory in Suva, Fiji. Samples were analyzed for the following parameters; sodium, calcium, magnesium, potassium, alkalinity, sulfate and chloride.

5.3 Analytical Method

5.3.1 Major Cations

The major cations (Na, K, Mg, and Ca.) were analyzed following method AP 2261. Here the samples were analyzed by flame Atomic Absorption Spectroscopy. External standards were used to quantify cations in samples. The uncertainty of measurement is about 15%.

Total Alkalinity determination was done using method AP2320 B. Here a known volume of sample was titrated against standardized hydrochloric acid and total alkalinity determined at alkalinity endpoint. The uncertainty of measurement is 4%. Similarly chloride determination also uses a titrimetric method where a sample aliquot was titrated against standardized silver nitrate with an uncertainty of 6%

Using method AP4500 SO_4^{2-} E, barium chloride was added to a known volume sample and sulfate was determined using a uv-vis spectrophotometer with external standards. The uncertainty of measurement is 11%.

6.0 Results:

Results are presented in Appendix 1

7.0 References

Standard Methods for the Examination of Water and Waste Water, 22nd Edition, 2012

E.coli Compact Dry Plate and Filtration Membrane Procedures (as provided by SPC).

Annex 3: SPC water quality procedures

(after Loco et al., 2015)

WATER QUALITY SAMPLING AND ANALYSIS

This annex provides various sampling procedures that had been used by SPC in remote island settings. It should be noted that the procedures will give some indications of E.coli presence but have variations in how tested samples are prepared and stored or incubated.

These suggested procedures have been tested previously in several Pacific Island countries, generating huge interest amongst governing authorities in relation to providing the level of water sources bacteriological contamination.

Note: It is also very important to note features such as water color and algal growth in wells, as this may indicate high nutrient levels in wells and may prompt E.coli sampling.

1 – Procedure for Collecting Source Water Samples for E.coli Analyses

Check to make sure the following materials are available before collecting sample:

1. Detergent to wash hands before sampling.
2. Sterile, non-toxic, glass or plastic container with a leak-proof lid. Container should be capable of holding 120-mL or 250-mL with ample headspace to facilitate mixing of sample by shaking prior to analysis (Nalgene polypropylene wide-mouth bottles).
3. Marker pens to label sample.

Collecting the Sample

1. Record the sample number, sample location, sampler's name, observations, and sampling date and time in a sampling log book.
2. Water taps used for sampling should be free of aerators, strainers, hose attachments, mixing type faucets, and purification devices. The service line should be cleared before sampling by maintaining a steady water flow for at least two minutes (until the water changes temperature).
3. Adjust the flow of water out of the tap or hose so the water will not splash out when it is collected into the sample container.
4. If there is not an inline tap that allows for the sampling of source water prior to treatment, samples should be collected as close to the intake as possible.
5. For wells, water samples should be collected close to the surface using a grab sampling technique. Samples may be collected manually by direct submersion of the bottle into the water or by using a grab sampling device, as simple as a metal pole with an adjustable clamp at one end that holds the sampling bottle in place. In case grab sampler is not available use clean container to get the water out of the well and fill the sampling bottle.
6. Using aseptic technique (i.e. sanitise tap, do not touch the inside of the sample container, etc.), fill the E.coli sample container, leaving at least 1 inch of head space. Do not expose an opened container any longer than necessary. Record the sample number, date and time of sample collection, sample location, and analysis requested on the sample container.

7. Immediately following sample collection, tighten the sample container lid, ensuring that you don't touch the inside of the bottle or the lid. Place the samples in box with ice to preserve the sample for analysis.
8. If the sample will be shipped off-site for analysis, and will not be shipped for several hours, place the sample container upright in a refrigerator to maintain the sample at a temperature of less than 10°C, but not freezing, prior to shipment. If a refrigerator is not available, wrap the sample with insulation such as bubble wrap or paper towels (to prevent freezing), place the sample in a ziplock bag, and place the bag containing the sample in the shipping cooler with wet ice or ice packs. Replace with fresh ice or ice packs immediately prior to shipment.

2 – Water Sample Collection – Standard Operating Procedure

Sampling from Well

1. Wash hands and dry with clean tissue.
2. Label the sample bottle appropriately, Date, Time, Household Number and Census Block Number.
3. Abstract well water with use of clean abstracting bucket/tin cans, ensuring that it does not touch the walls of the well.
4. Take sample from at least a depth of 30-40 cm.
5. Rinse the abstracting device with well water three times before taking sample and transferring to the sampling bottle.
6. With cleans hands carefully remove the cap of the sampling bottle, and fill it with 100 ml of sample.
7. Strongly shake the sample to dissolve sodium thiosulphate in the sample bottle. Securely close the bottle by screwing the cap back on the bottle.
8. Collect water in the bucket which has been rinsed three times with sample water.
9. Record salinity measurement and temperature.
10. Record any other relevant comments, on the groundwater physical sheet.

Sampling from Tap

1. Remove any attachments from the tap.
2. Open the tap and flush the system for at least a minute.
3. Wash hands and dry with tissue.
4. Label the sample bottle appropriately, Date, Time, Household Number and Census Block Number.
5. With clean hands carefully remove the cap of sampling bottle and fill it with 100 ml of tap water. Securely close the bottle by screwing the cap back on the sampling bottle.
6. Strongly shake the sample to dissolve sodium thiosulphate in the sample bottle.
7. Collect water in the bucket which has been rinsed three times with sample water.
8. Record salinity measurement and temperature.
9. Record any other relevant comments, on the physical sheet.

3 – E.Coli Sampling Procedure: Collilert 18 Presence and Absence Test Procedure

Presence/Absence Test Procedure

1. Carefully separate one Snap Pack from the strip, taking care not to accidentally open adjacent pack.
2. Tap the Snap Pack to ensure that all of the Collilert powder is in the bottom part of the pack.
3. Open one pack by snapping back the top at the score line.

Caution: Do not touch the opening of pack.

4. Add the reagent powder to the sample bottle.
5. Aseptically cap and seal the vessel.
6. Shake until the reagent powder dissolves.
7. Incubate for 24 hours at 35° ± 0.5°C. (Used Esky for incubation for this survey.)
8. Read the results at 24 hours.
9. Fluorescence indicates presence of E.coli.

Result Analysis

Upon incubation period, if no yellow colour is observed, the test is negative.

For samples with observed colour change –

- If the sample has a yellow colour, the presence of Total Coliform is confirmed. If colour is not uniform, mix by inversion then recheck.

- If yellow is observed, check for fluorescence, by placing a 6 watt 365 nm UV light within five inches of the sample in a dark environment, ensuring the light is facing towards the sample

4 – EC-compact dry plate Sampling Procedures

Standard Membrane Filtration Method

(Adapted from the USA Environmental Protection Agency (EPA) and American Public Health Association methods (APHA) (EPA, 2000; APHA, 1995)

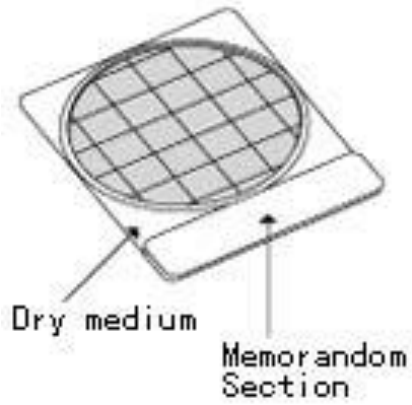
Samples: Collect water samples in sterile bottles (left in boiling water for 10 minutes and cooled) or bottles that have been rinsed three times at the site with well water, rainwater or water to be tested.

Storage: Store samples as cool as possible and in the dark, but not in a freezer. So either in a chilly bin or a bag with cool elements. Samples need to be processed within 12 hours of collection, make sure no melted water comes into contact with the sample.

Equipment: Need per sample, all sterile (boiled sterile, or three times rinsed with sterile/boiled water between samples):

3 X Compact Dry Plates (These plates contain dehydrated agar that allow the bacteria to grow once rehydrated)

1 X 50 ml sterile syringe
1 X 20/15/10 ml syringe
1 X sterile filter housing
2X GN6 Metricell gridded white filters (PallGellman)
Tweezers for filters (not sterile)
Bleach
Sterile/Boiled Water
1X 20/10 ml syringes



Suggested volumes: 1 ml, 10 ml and 50 ml for each sample.

The cleaner the water the more volume you need to filter, never more than 100 ml. If the water is heavily contaminated and you get more than 200 colonies on your 1 ml plate you will need to dilute the sample, e.g. 1 in 10 ml and then filter 1 ml of this (or even 1 in 100 ml). Just remember to add the dilution in your final calculation.

PROCEDURE

1. Field Sampling Procedure

a. Rinse sample bailer three times with sample water before rinsing sample bottles.
b. Fill bottle with sampled water
c. Label sample with water source details (e.g. W001 for well 1 and T001 for rainwater tank 1). Make sure that other relevant details, such as sample collection time, location name and well or tank owner's name is also recorded.
d. Place sample bottle carefully in a cooler box and close lid properly.
e. Samples should be analysed within 12 hours post-sample collection.

2. Pre-sampling procedure (sample bottles sterilisation)

a. Rinse and clean sample bottles thoroughly with clean water (preferably boiled water).

b. Rinse bottles in a covered bucket or cooler box with 5% bleach for at least 30 minutes.
c. Again, rinse bottles thoroughly in boiled water to clean and remove residual bleach.
d. Sanitise your hands with soap before taking the bottles out and drying them using tissue paper.
e. Clean storage container with bleach and boiled water prior to placing the sample bottles inside prior to field visit.

3. Lab Sampling Preparation

Set-up procedures

a. Make sure there is enough boiled water, sterile water and bleach to conduct the whole procedure.
b. Prepare the three sanitised containers for holding boiled water, sterile water and 5% bleach.
c. Prepare a 1 ml, 50 ml and filter housing (all adequately sanitised).
d. Prepare filter papers.
e. Prepare and sanitise a pair of tweezers for transferring filter papers to the filter housing and onto the re-hydrated plate.
f. Prepare and clean a table with bleach and boil water.
g. Prepare tissue papers for drying/cleaning any sample water on the table.
h. Prepare a bucket or container for storing all unused sample water and wastewater.

1 ml sample preparation

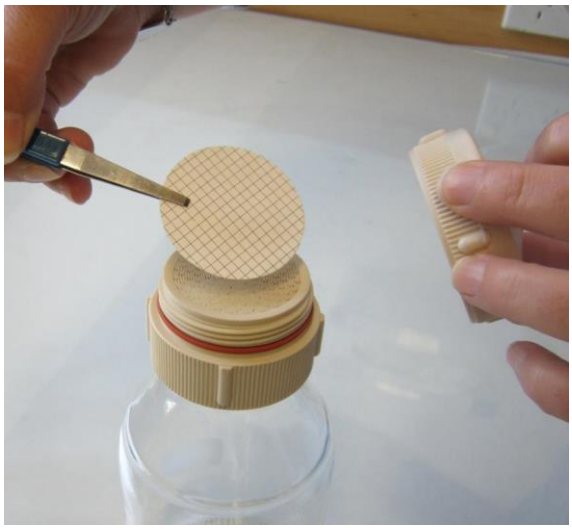
a. Label all 1 ml dry compact plates with sample details (e.g. sample number, team number etc).
b. Purge 1 ml syringe with boiled water before filling and pouring 1 ml sterile water onto the first plate for our 1 ml CONTROL.
c. Rinse the 1 ml syringe thoroughly with boiled water (3 times) to clean it before filling it up with sterile water to cool it down.
d. Fill the 1 ml syringe with sample water and the pour the sample into its labelled compact dry plate.
e. Repeat the above, until all samples have been prepared for 1 ml analysis.
f. Store all the rehydrated 1 ml plates in a cooler box and record the incubation start time.

50 ml sample preparation

a. Label all 50 ml dry compact plates with sample details (e.g. sample number, team number etc.), including the CONTROL.
b. All plates will need to be rehydrated with 1 ml sterile water prior to placement of sample-rinsed filter papers. (see below)

c. Purge 1 ml syringe with boiled water before filling and pouring 1 ml sterile water onto all labelled dry plates to rehydrate the agar.
d. Fill and rinse the 50 ml syringe with boiled water 3 times. The first rinse should be discharged into the wastewater bucket, while the next two rinses should be run through the filter housing to clean it. Make sure the housing is closed (hand-tight).

e. Rinse the 50 ml syringe now with sterile water and push water through the filter housing to cool both the syringe and filter housing, and at the same time keep filter housing sanitised.
f. Now rinse the 50 ml syringe with the sampled water 3 times. These rinses should be dumped in the wastewater bucket.
g. Sanitise pair of tweezers through 5% bleach and then sterile water. The latter is aimed at removing any residual bleach.
h. Get a filter paper using the sanitised tweezers and place it on the filter housing, place filter (grid side up) on the filter support and close the housing tightly carefully, make sure that the o-ring (orange) is in the correct place (Do not touch the inside of the housing) (see below).



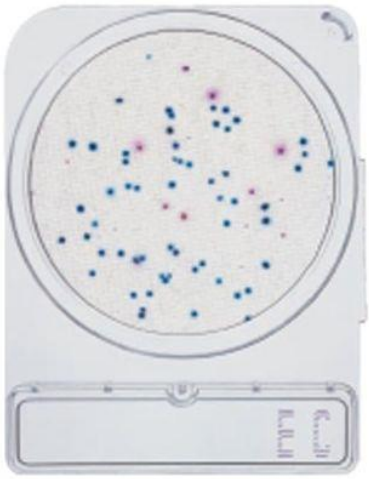
i. Sanitise the tweezers again in bleach and sterile water.

j. Fill the 50 ml syringe with the sample and run this through the filter housing (with the filter paper in). After emptying the syringe, push air through the filter housing (2 or 3 times) to remove any residual water from the filter housing.

k. Use the sanitised tweezers to transfer the filter paper from the housing to the re-hydrated plate.
l. Repeat steps d to e until all samples have been prepared for 50 ml analysis.
m. For the CONTROL, use sterile water, instead of sample water, and subsequent to thoroughly rinsing the syringe and filter housing with boiled and sterile water.
n. Store all the rehydrated 1 ml plates in a cooler box and record the incubation start time.

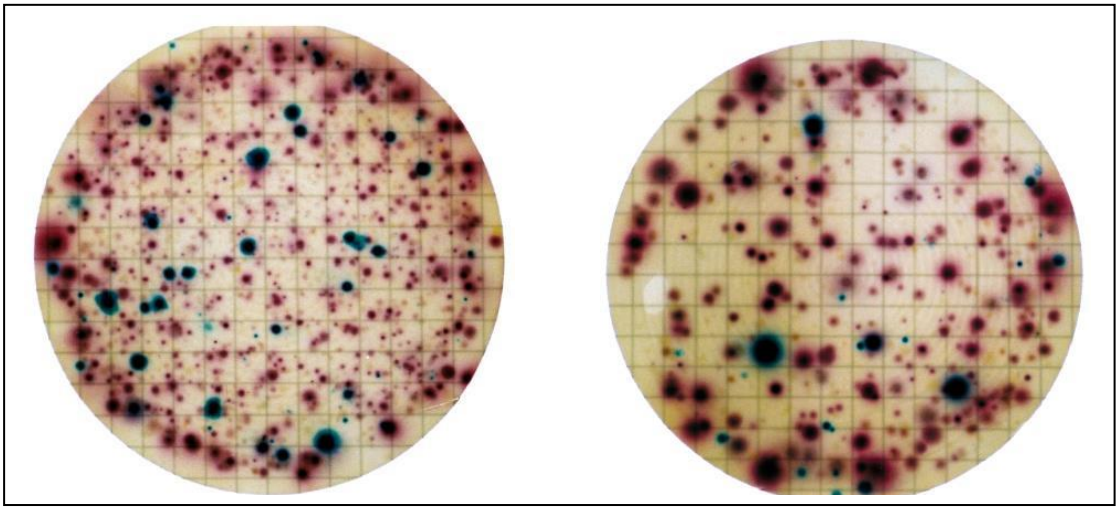
4. Post sampling procedure

a. Repeat pre-sampling routine to prepare for the next sampling phase



Counting Procedure:

Compact Dry plates: Count the number of red and blue colonies
The number of red and blue colonies = Total Coliforms
The number of blue colonies = E.coli
Or the filters on Compact Dry may look like this, again count the number of blue and red colonies.



Note: The left plate would not be counted as there are too many colonies to count (>200).

Count only plates that have between 20 - 200 colonies on them. If the count is too high count 10 squares randomly on the filter, work out the average per square and multiply by 100 to get a count per filter. This is not a good way of doing it but will give you an estimate from which you can decide what volume you should have filtered. Calculate all your final numbers as bacteria per 100 ml. If you get no growth on 50 ml the result is <1 per 50 ml, you would need to repeat this with 100 ml of new sample as the drinking water standard is 100 ml.

Cleaning of Equipment:

Clean the syringes and filter housing using 5% bleach and then 3 times with boiled water to remove any residual bleach. Then put a large pot of water on the stove to boil. Let this come to the boil and boil it for 10minutes to kill any pathogens in it. Then place the syringes and filter housings in it and boil it for a further 10minutes. Turn of the stove and let the syringes and filter housings cool down in the water with a lid on the pot and remove while they are still warm but as hot as to burn you and place them in a clean plastic container or zip lock bags.

Boiled Water:

Make fresh boiled water each night for the following day of sample processing. Bring a pot of water to the boil and boil continuously for 10 minutes, pour while still hot into a glass bottle, that you have previously cleaned with 5% bleach and rinsed 3 times with boiling water. Let the water cool overnight and use the next day for your sample analysis.

5 – H_2S Strip Method Procedures

Introduction:

The H_2S strip method is used as a field kit to assess the microbial quality of water. The H2S strip method detects hydrogen sulphide producing bacteria.

The H_2S strip method was developed for testing water. It is a simple, affordable, on site method, which has >85 % correlation when compared to faecal coliforms normally used to assess water quality. The method does not require expensive laboratory equipment such as filtration apparatus and incubators.

Methodology:

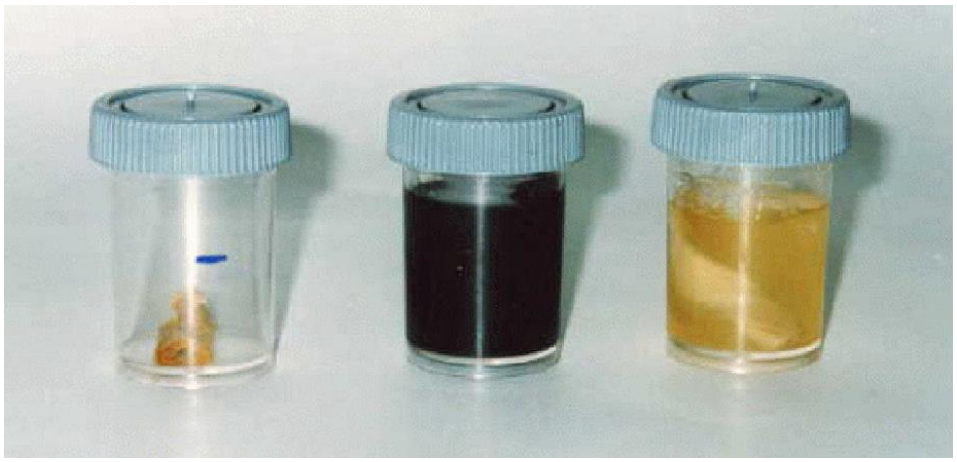
The H_2S (hydrogen sulphide) test was originally developed over 20 years ago, to detect in a volume of water, the production of H_2S by enteric bacteria associated with faecal contamination. The contamination is detected by formation of a black precipitate from the reaction of the H_2S with iron in the 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 faecal contamination of drinking water. A method for preparing and conducting the test is detailed as below.

Preparation of H_2S test containers

1. Any type of glass bottle or tube with a volume of between 20 ml to 200 ml, which has a heat resistant cap/lid, can be used. The bottles or tubes are first cleaned and calibrated to indicate a 10 ml, 20 ml or 100 ml volume, or any volume in-between – depending upon their size. Calibration is done with a graduated cylinder or other measuring device. A glass marking pencil, permanent ink pen or tape can be used to mark the desired volume.
2. The medium used in the test is prepared from the following chemicals, which are dissolved into distilled or dechlorinated tap water while stirring.
3. If not immediately used, the media can be sterilized and stored in a refrigerator until ready for use.
4. Taking tissue paper, filter paper, non-toxic paper towelling, absorbent pads used for membrane filtration, or any other type of absorbent material (coasters used in bars work well if no black ink is used), place a measured quantity of media onto the paper so that a pad or paper strip for a 10 ml test sample contains 0.5 ml of media, a 50 ml sample will use 1 ml of media and a 100 ml sample will require 2.5 ml of media (a pipette graduated at 0.1 ml intervals may be necessary for this). Alternatively, paper strips can be cut to a size that has absorbed 0.5 ml of media. If the sample to be tested is 10 ml, use one strip; if 50 ml, use two strips; and if 100 ml, use five strips. The next step is to dry the strips in an oven at about 55^{o}C. These reagent-impregnated strips can be stored dry (in an envelope or preferably a zip-locked bag) for several months – until ready for use. It is always advisable if the health authority supplies prepared strips to the community.
5. When ready to conduct the test, a strip or strips are introduced into the appropriate (clean) sample bottle, loosely capped and sterilised. This can be done with an autoclave or a simple pressure cooker for 15 minutes at 115^{o}C. It can also be done by steam (in a rice steamer) for about 30 minutes or in a hot air oven at about 120^{o}C for 60 minutes. The tubes or bottles are then allowed to cool and the caps or lids tightly sealed. The tubes or bottles should be stored in a dark place until ready for use.

Reading and interpreting results

6. After sampling, place all test samples in a dark place and incubate at room temperature for a total of three days. Every 12-18 hours examine the samples for changes in colour. The date and time of each observation is recorded on the report form and the observations are recorded as follows: (–) = no change; (+) = slight change, the paper strip or water has turned grey; (++) = the paper strip is partially black; (+++) = the strip and the water sample itself are noticeably black.
7. As noted above, a colour change indicates the presence of bacteria of faecal origin. The speed of the reaction will determine the density of organisms present; i.e. the quicker the reaction the higher the number of faecal organisms present. This can also be interpreted in terms of a risk factor. For example, no colour change until day three indicates a lesser risk than a (+++) change within 12 hours.



Empty sample bottle (left) positive result (middle) negative result (right)

3. Positive results would indicate that the water is contaminated and results of sanitary survey should be considered to determine the source of contaminant. For example, if the tank inlet is not screened and the results of the H_2S test are positive on the first day, the users should disinfect the water and the tank inlet should be screened.
4. Boil or treat the water until further results are obtained and you know the water is safe to drink.
5. If bottles are damaged do not use.

Sampling procedure:

8. At the time of sampling, label each container with a sample number. Also record the date, time and location of collection on the container with a glass marking pencil or permanent ink pen.
9. Flame the mouth of the tap nearest to the tank and let the water run freely for at least 30 seconds. Place the opened H_2S sample collection bottle under the tap and collect the pre-calibrated amount being careful not to contaminate the cap. It should be noted however that samples should not be collected from taps that are leaking and flaming the tap is not necessary if you are testing the quality of the water as it is actually consumed. Each day of sampling, a control is collected. This is a sample that is known to be uncontaminated, such as boiled water, commercially bottled water, or water treated with chlorine. The control sample is used as a benchmark to compare colour change in the test samples and to ensure that the sample bottles have been properly sterilised prior to use.

Note: There will be slight change in the colour of the sample to a pale yellow or light brown due to the colour of the reagent, which is normal (see above).

Annex 4: Selected photos

Figure A4.1. *Soaking sampling bottles in a sterilised esky prior to adding of bleach solution.*

Figure A4.2. *Bottles dried overnight after sterilisation process before randomly selected for residual chlorine test.*

Figure A4.3. *Lovibond laboratory kit and chlorine tablets used for measuring residual chlorine.*

Figure A4.4. *Preparing samples for chlorine test.*

Figure A4.5. *Recording of measured residual chlorine values.*

Figure A4.6. *Bottles labelled in groups and according to the number of rinses applied.*

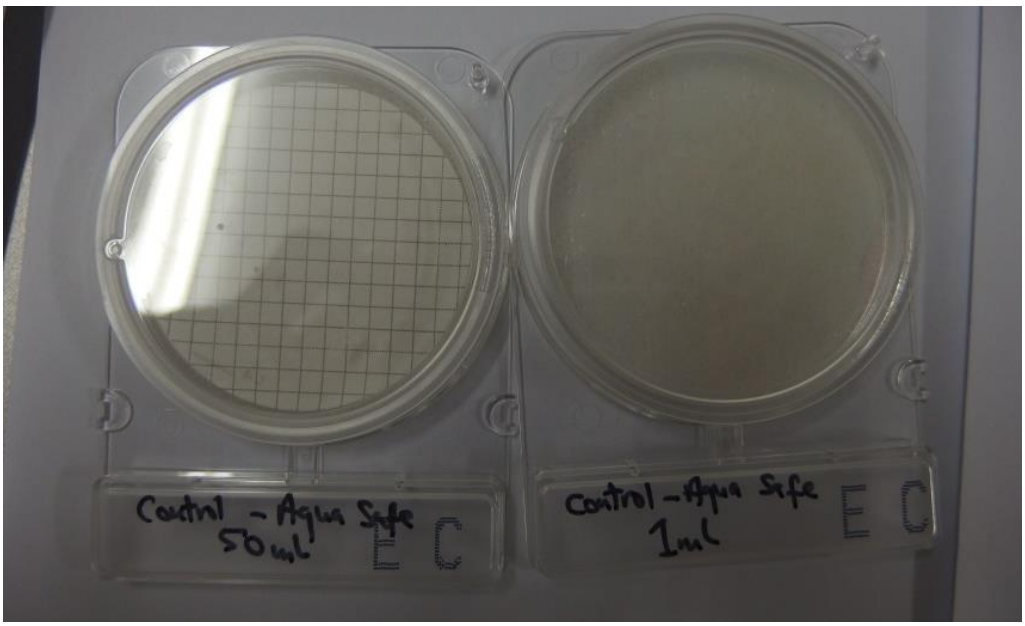


Figure A4.7. *Control from Aqua Safe bottled water used on the 1st phase. Note that 50 ml is showing blank as a result of procedural error.*

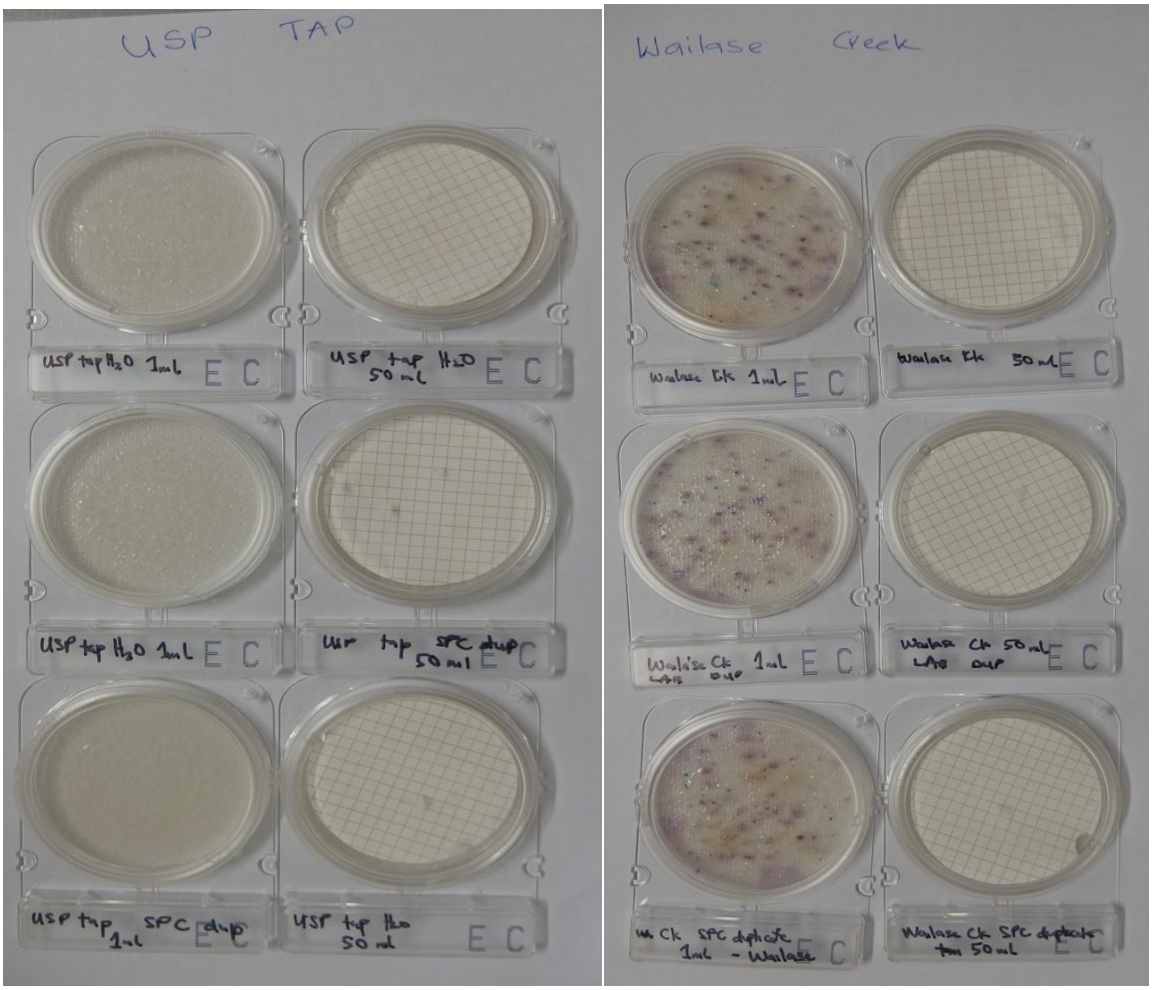


Figure A4.8. *SPC's E.coli and Total Coliform results from tap and creek water samples. Note the consistent blanks in 50 ml sample volume.*

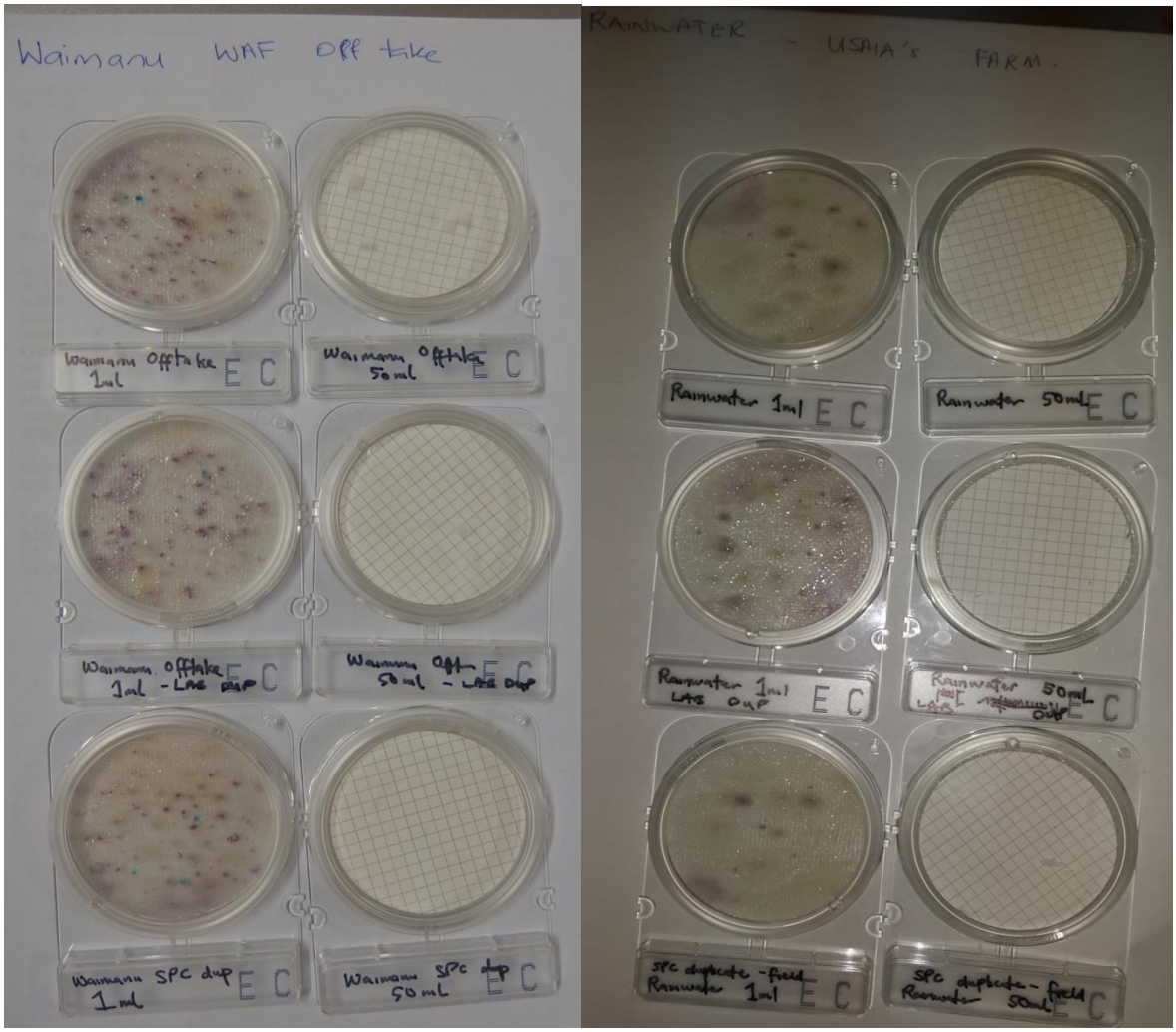


***Figure A4.9**. The SPC E.coli and Total Coliform results from river and rainwater samples. Note the consistent blanks in 50 ml sample volume.*

***Figure A4.10**. Conducting sample collection using USP-IAS sampling equipment.*

Figure A4.11. *Pouring sample water into SPC bottles.*

Figure A4.12. *Placement of SPC sample bottles in the sanitised esky.*

Figure A4.13. *Sterilised USP-IAS sampling bottles.*

Figure A4.14. *Filling USP-IAS sampling bottles on the 2nd field sampling trip to Waimanu River, with the WAF pumping station in the background.*

Figure A4.15. *SPC laboratory procedures ready to be undertaken at the USP-IAS microbiology lab.*

Figure A4.16. *Rinsing of sterile water and running through the filter housing as a sterilisation step.*

Figure A4.17. *Labelled basins having boiled water (in blue) and various concentrations of bleach solution (volume to volume percentage).*

Figure A4.18. *Preparing samples for 2nd residual chlorine test.*

Figure A4.19. *2nd phase of chlorine test.*

Figure A4.20. *E.coli and Total Coliform enumeration at USP-IAS lab.*

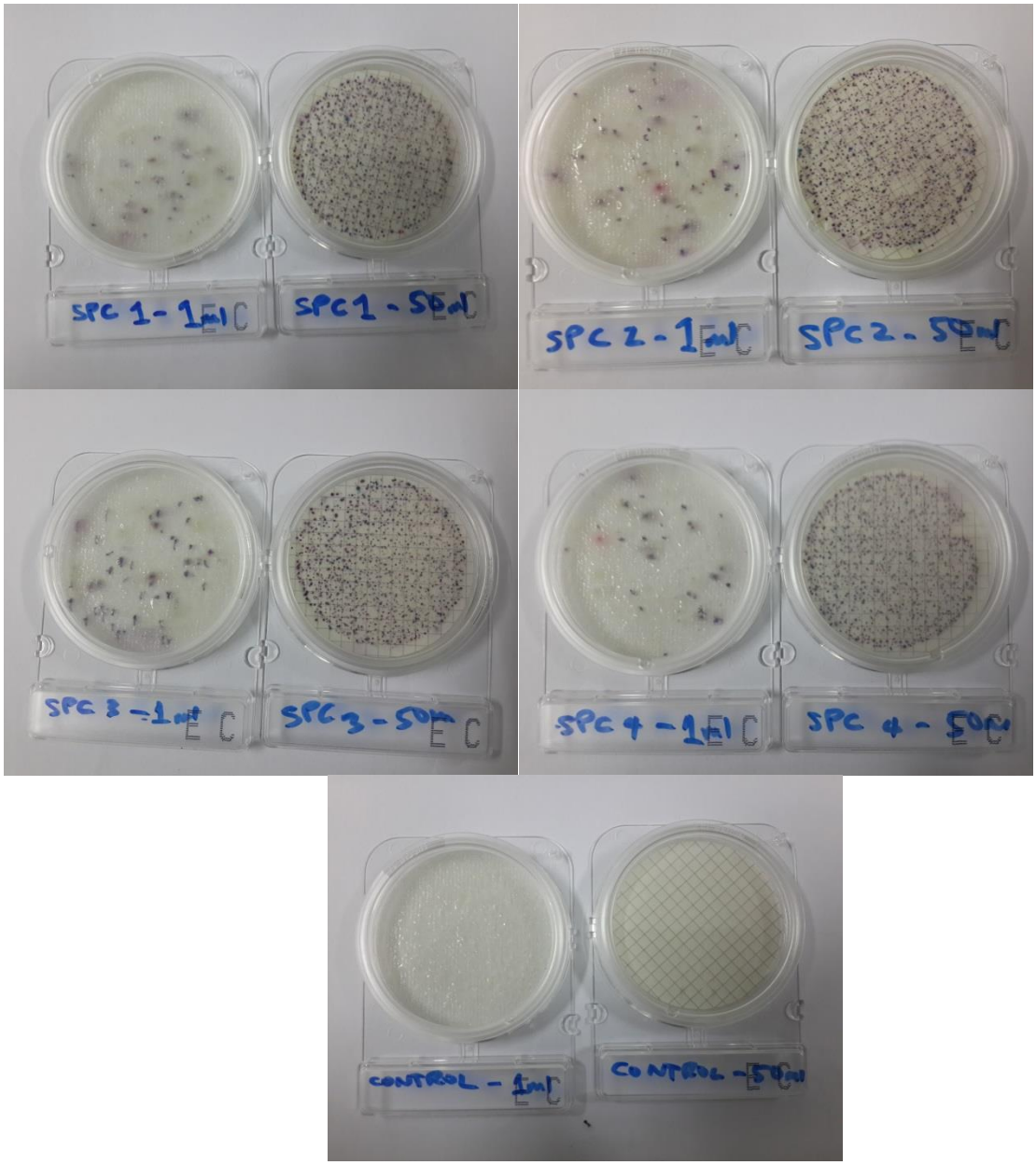


Figure A4.21. *Compact plate showing coliform growth in four (4) SPC samples collected on the 2nd sampling phase at Waimanu River. Note the improved results in 50 ml volume arising from the addition of 1 ml to rehydrate the agar plate before the placement of sample-rinsed filter paper. This step was missed in the 1st sampling phase.*

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