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Canada

Evaluation of the *Aquasure Pro 3000™* Single Test Precision Portable Incubator Technology (STEPPI™)

used to conduct
Presence-Absence Tests for Total Coliforms
and E. coli in Drinking Water

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EXECUTIVE SUMMARY

Regular testing of water supplies is fundamental in maintaining and improving water quality in rural Canada. However, many individuals and remote communities find it difficult to collect and transport water samples to a laboratory for testing. New products and technologies, which allow rapid onsite screening for microorganisms in water supplies, may simplify and thereby, encourage more frequent testing. To gain acceptance, these new products and technologies must be reviewed by trained personnel and information regarding their benefits and limitations must be transferred to the general public.

To address the concerns of rural Canadians who rely on groundwater as their principle source of potable water, the Prairie Farm Rehabilitation Administration (PFRA) created the Sustainable Water Well Initiative (SWWI). One aspect of this initiative includes working with rural communities, private industry, provincial agencies and researchers to develop and evaluate new technologies related to sustaining and protecting groundwater supplies.

Under the Sustainable Water Well Initiative, PFRA and the Saskatchewan Department of Health, performed an evaluation of the Pro 3000™ Single Test Precision Portable Incubator (STEPPI™) Technology. This technology was developed by Aquasure™ Technologies Inc., to allow onsite testing for coliform bacteria (i.e. Total Coliforms and E. coli bacteria) in drinking water, using standard Presence-Absence (P-A) methods. Two proto-type incubators and P-A test reagent, developed by Environmental Biodetection Products Inc. (EBPI), were provided by Aquasure™ Technologies Inc. for the evaluation. Water samples are simultaneously tested for Total Coliforms and Escherichia coliforms (E. coli) using EBPIÊs P-A reagent.

Results from this evaluation indicate that the Aquasure Pro 3000™ incubators and the P-A reagent developed by EBPI provide a reliable method for *screening* water for Total Coliforms and E. coli bacteria. In addition, this evaluation demonstrated a number of benefits which portable P-A test instruments, such as the Aquasure Pro 3000™, can provide to individual well owners, small communities and field researchers who require only a few bacteriological tests at any given time.
These benefits include:

- **Shorter time periods between sampling and test results.**

Laboratories generally require 2 to 3 days to test and process results. With the P-A/Aquasure Pro 3000™ technology, samples can be tested onsite at ambient temperatures between 5°C to 35°C, and therefore, test results can be obtained 24 to 28 hours following sample collection. The reduced time period between sampling and analysis will allow consumers of private water supplies and rural water treatment operators to respond quickly to treatment equipment failures. In addition, onsite testing will benefit remote communities or individuals that may normally require several days to transport water samples to a laboratory for testing.

- **Convenient and simple operating procedures with no laboratory facilities required.** No special skills are required to operate the Aquasure Pro 3000™ incubators. Once a P-A reagent has been added to the samples, they can immediately be placed into the incubator units. This removes the difficulties associated with preserving and transporting samples to a laboratory.

- **P-A test results are generally easy to interpret.** When using the P-A reagent, developed by EBPI, water samples change from a yellow colour to blue after 24 hours incubation at 35°C if coliform bacteria are present. Water samples that are positive for coliform bacteria will also turn a brilliant fluorescent blue when exposed to UV light, if E. coli are present.

When using P-A tests, to screen drinking water supplies, the following factors must be considered:

1. P-A tests for coliform bacteria are not designed to measure general bacterial populations. Portable technologies, such as the Aquasure Pro 3000™, and presence-absence tests provide a quick method for *screening* drinking water for coliform bacteria. However, standard membrane filtration methods are more effective at identifying problems with a treatment system as these methods will show evidence of high non-coliform bacterial populations.

2. Any positive results which occur using a commercial P-A test must be confirmed by submitting additional samples to the provincial health laboratory or an accredited laboratory certified to perform bacteriological tests on drinking water.

3. Instructions provided by the P-A reagent manufacturer must be followed carefully. If incubation times are extended past the recommended time periods or water samples are handled improperly, false positives may occur.

4. Initially, operators of the P-A/Aquasure Pro 3000™ unit may require the use of a colour chart or a reference bottle to interpret E. coli test results. Some reflectance of the UV light can occur on test jars that are negative for E. coli. Initially, operators may confuse this reflectance with a positive E. coli test result. This potential problem is removed once the operator gains experience and observes a true fluorescence (i.e. positive E. coli) response.

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1.0 INTRODUCTION

Wherever people, livestock and water supply converge to a common location there is a risk of faecal contamination of the water supply. Rural farm wells are particularly at risk since they are often managed with an "install and forget" attitude. However, when properly constructed, operated and monitored, water wells can provide a long-term supply of safe drinking water.

Regular bacteriological testing for coliform bacteria (i.e. Total Coliforms and E. coli) is generally accepted as the best method for monitoring a water supply for faecal contamination. Coliforms are indicator organisms whose presence is evidence that the water may have been polluted with the faeces of humans or warm-blooded animals.

Although many rural Canadians realize the importance of bacteriological testing, there are a number of factors that discourage individual well owners and remote communities that rely on groundwater supplies from regularly testing their water. These individuals and communities find it difficult to transport water samples from their well to a laboratory for testing within the recommended time periods. Others are uncertain of sampling and handling procedures, which can lead to erroneous test results.

Portable technologies and quick methods for screening drinking water onsite for the Presence-Absence (P-A) of coliform bacteria may simplify bacteriological testing and thereby encourage more frequent testing. Frequent testing reduces the risk of drinking contaminated water. Rapid onsite testing may be particularly useful when a failure in treatment technology is suspected.

Federal and Provincial Health Departments strongly recommend that positive results obtained from P-A test kits be confirmed by a laboratory. However, they recognize that technologies developed to screen drinking water onsite could enhance the testing of drinking water by municipalities, other providers of drinking water and by private well owners. Certain P-A methods for Total Coliform and E. coli testing were accepted in May of 2000 by the Northwest Territories Department of Health and Social Services as alternates for reporting bacteriological quality of drinking water (Northern Territories Water and Waste Association, 2000). The Ontario Ministry of Environment and Energy (MOEE) and Ministry of Health (MOH) recently agreed that commercially available P-A tests may be used at municipal laboratories, private laboratories or water works facilities to screen samples routinely collected from community water supplies (Ontario Ministry of Environment and Energy, 1997).

Groundwater research may also benefit from onsite P-A testing. Due to the location of field research sites, it is often difficult to collect, transport and test water samples within the required time period for bacteriological testing. Project activities may be delayed while researchers wait for test results from a laboratory. Onsite P-A testing offers the possibility of shorter time periods between sampling and test results. The simplicity and portability of these tests may lead to more comprehensive research.

2.0 PROJECT BACKGROUND

In April, 2001, PFRA in cooperation with the Saskatchewan Department of Health and Aquasure™ Technologies Inc., began an evaluation of Aquasure's Pro 3000™ (Single Test Precision Portable Incubator - STEPPI™ technology). This patent pending technology was recently developed by Aquasure™ Technologies Inc. of Toronto, Ontario to allow onsite P-A testing for bacteria indicative of faecal contamination (i.e. Total Coliforms and E. coli) in drinking water. On April 25, 2001, Ron Emburgh and Ravi Kanipayor from Aquasure™ Technologies Inc. provided a presentation on the Aquasure Pro 3000™ - STEPPI™ technology at PFRA headquarters in Regina to representatives from Saskatchewan Health and PFRA who agreed to co-operatively evaluate this innovative technology.

This current evaluation was undertaken as part of the Sustainable Water Well Initiative (SWWI), which was created by the Prairie Farm Rehabilitation Administration (PFRA) in 1996, to address water well problems experienced by rural residents across the Canadian Prairies. The goal of this initiative is to work with rural communities, private industry, provincial agencies and researchers to provide improved knowledge on methods used to diagnose, prevent and treat well problems. One aspect of this work is to develop and evaluate new technologies related to sustaining and protecting groundwater supplies.

Two proto-type incubators, Aquasure's Aqualert™ test kit containing a chromogenic reagent developed by EBPI (Environmental Biodetection Products Inc.) and a UV light were provided by Aquasure™ for the STEPPI™ technology evaluation. The reagent permits rapid simultaneous detection of Total Coliforms based on the development of a visual blue colour during incubation, and E. coli detection based on the appearance of a blue colour and fluorescence.

The main objectives of this evaluation were as follows:

1. Identify qualities of the Aquasure Pro 3000™ technology that may enhance or encourage more frequent testing of drinking water by rural well owners and remote communities.

2. Determine the benefits of this technology to field research projects.

3. Identify factors that may impact the P-A test results when using the Aquasure Pro 3000™ - STEPPI™ technology and the chromogenic reagent provided by EBPI, and provide recommendations on addressing these impacts.

The following sections provide a summary of the Aquasure Pro 3000™ - STEPPI™ technology evaluation procedures and results. Also provided is some background information on indicator organisms and a brief description on bacteriological testing for indicator organisms used to assess the quality of a water source.

3.0 BACTERIOLOGICAL ANALYSIS OF DRINKING WATER

The microbial quality of drinking water is based on testing for indicator organisms. An indicator organism is described as a microorganism whose presence is evidence that the water has been polluted with faeces of humans or warm-blooded animals. Pathogenic microorganisms causing enteric diseases in humans originate from faecal discharges of diseased persons and animals. Consequently, water contaminated from faecal pollution is considered to be potentially harmful. Various species of viruses, bacteria, and protozoans can be pathogenic. However, drinking water is not tested for pathogens because the tests are difficult to perform, unreliable and, for some pathogenic microorganisms, impossible to perform (Viessman and Hammer, 1993). Therefore, tests for indicator organisms are used to determine if a water source has been contaminated.

The concept of indicator organisms was introduced in 1892 and is now the basis for most microbiological quality standards in water. Indicator organisms should meet the following criteria:

1. may be present in all types of water
2. are present when pathogens are present and are present in higher numbers
3. have a better survival rate in water than pathogens
4. must be easy to analyse

Presently, no organism or group of organisms satisfies all the criteria for an indicator: However, the coliform group satisfies most of the requirements. Unfortunately, the absence of Coliforms does not effectively predict the absence of non-bacterial pathogens, such as Giardia, Cryptosporidium or viruses.

3.1 Total Coliforms

Tests for coliform bacteria are routinely used by Provincial Health Departments to screen drinking water for faecal contamination. Nonpathogenic Faecal Coliform bacteria, that reside in the intestinal tract of humans and animals are excreted in large numbers in faeces. Hence, coliform bacteria are *indicator* organisms of faecal contamination and the *possible* presence of pathogens.

In laboratory testing, the term 'Total Coliforms' refers to coliform bacteria from faeces, soil, or other origin. The United States Environmental Protection Agency (USEPA) describes Total Coliforms as any bacteria from faeces, soil, or other origin that grow in a lactose (i.e. milk sugar) broth, producing acid and gas at 35 °C or 95 °F within 48 hours of incubation.

Some genera of coliform bacteria found in water and soil are not of faecal origin, but grow and reproduce on organic matter outside the intestines of humans and animals.

These coliforms indicate neither faecal contamination nor the possible presence of pathogens. However, the presence of excess Total Coliforms in drinking water is an indication that treatment is inadequate or that the distribution system is experiencing regrowth or infiltration (Health Canada, 1996). The Environmental Analysis Report, distributed by the Saskatchewan Provincial Health Laboratory (Prov. Lab) to private water supply users, states that "the only acceptable result for coliform bacteria is NIL". This document also states that, "the presence of any coliform bacteria suggests your water is unacceptable to drink".

3.2 Faecal Coliforms

When Total Coliforms are detected in a *public* water supply, further tests for Faecal Coliforms are performed. 'Faecal Coliforms' refers to coliform bacteria from human or warm blooded animal faeces. These coliforms have traditionally been described as any bacteria, including E. coli, that grow in a lactose broth, producing acid and gas after 22-26 hours of incubation at the elevated temperature of 44.5 ºC. Many laboratories are moving towards testing for E. coli, which is a more specific group of Faecal Coliforms. E. coli testing provides results for determining if a water source has been contaminated by faecal matter, since this group of bacteria grow in the intestine of both man and animal.

Testing for faecal contamination is particularly important on farms or in farming communities, where livestock are raised near water supplies, as there is a significant level of infectious disease transmission between animals and people. "It has been recognized that certain strains of E. coli have a significant asymptomatic prevalence in cattle herds and that exposure through poor manure handling practices is a large concern" (Don Lush, 2001).

3.3 Background Bacteria

The Canadian Drinking Water Guidelines suggest that in addition to routine analyses for coliform bacteria, all drinking water supplies should be routinely analyzed for general or background bacterial populations. These Guidelines state that excessive concentrations of general bacterial populations may hinder the recovery of coliform bacteria and thereby prevent the detection of potential threat to public health (Health Canada, 1996).

Studies performed under SWWI show that many water wells, both municipal and private, contain large densities of naturally-occurring bacteria. Problems associated with these large densities of bacteria are of greatest concern to rural Canadians, as many of their water supplies are not treated for bacterial contamination.

Large populations of background bacteria in untreated water supplies can hinder the recovery of coliform bacteria when using standard membrane filtration test methods. Water samples tested for Total Coliforms, by the Prov. Lab, will often be reported as Not Ascertainable (NA) (i.e. greater than 200 colonies of background bacteria per 100 ml of

water). These results occur when large quantities of background bacteria present in a water sample cause the test media to become overgrown with a variety of bacterial colonies. In these cases, it is difficult to determine if coliform bacteria are present.

The Municipal Drinking Water Quality Objectives developed by Saskatchewan Environment and Resource Management (SERM) suggest that no treated water should contain more than 200 colonies of background bacteria per 100 ml of sample. When a treated Municipal water sample is "Not Ascertainable" it suggests that there is a problem with the treatment system, and therefore, the Prov. Lab advises against drinking the water. For private water supplies, the Prov. Lab Environmental Analysis Report states, "a Not Ascertainable result suggests your water is unacceptable to drink". Remedial action is recommended to reduce the level of background bacteria in the water supply. Subsequent retesting is also recommended.

3.4 Summary of Analytical Methods Employed by Provincial Laboratory for the Determination of Coliform Bacteria in Water

3.4.1 Total Coliform Bacteria by Membrane Filtration

Drinking water and natural water samples are analyzed by the Provincial Laboratory using the standard total coliform membrane filter procedure as recommended in *Standard Methods for the Examination of Water and Wastewater*.

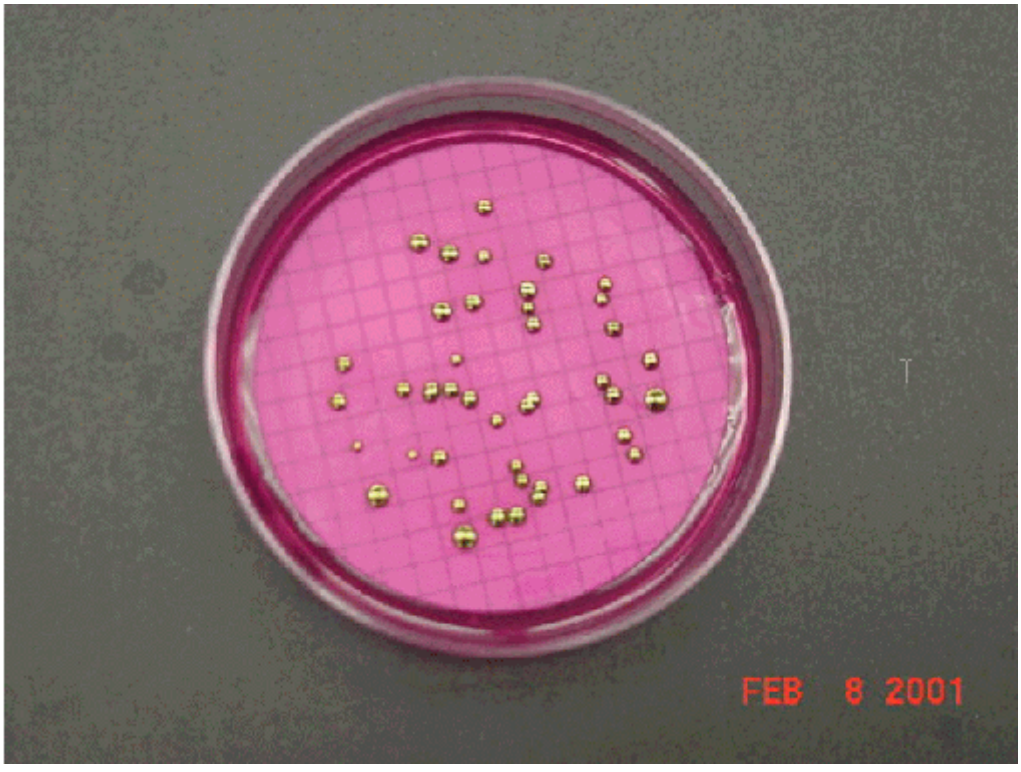

Photo 1: Positive total coliform plate

In this procedure, a 100 mL portion of the water sample is filtered through a 0.45 micron membrane filter. The filter is then placed onto an M-Endo agar medium and incubated at 35 $\pm$ 0.5 ^{N}C for 24 $\pm$ 2 hours. Bacteria that produce a red colony with a golden metallic sheen are considered to be coliform bacteria (see Photo 1).

The membrane filtration procedure has been utilized for many years in water testing laboratories throughout the world. While it is highly reliable and reproducible, it does have its limitations. High levels of suspended particulate matter may inhibit bacterial growth or restrict the volume of water that can be filtered. Water samples with high levels of non-coliform bacteria are also problematic. *Standard Methods for the Examination of Water and Wastewater* recommends the use of the chromogenic substrate coliform test procedure for such samples. Photo 2 shows an example of an M-Endo plate for a water sample containing a high level of non-coliform bacteria. These non-coliform bacteria may suppress growth of coliform organisms and in some cases mask the presence of the target organisms.

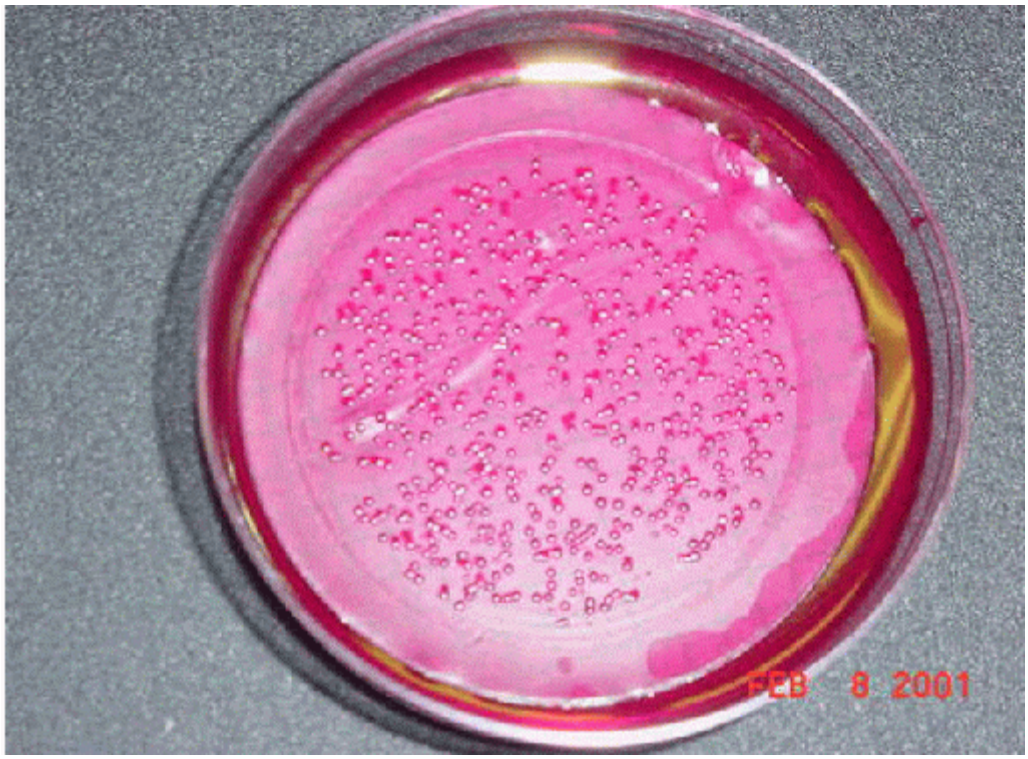

Photo 2: M-Endo plate for water sample containing high level of non-coliform bacteria (>200 background organisms).

3.4.2 E. Coli by MUG Presence/Absence

The analytical procedure for the detection of E. coli in drinking water samples is based upon EPA Method 1104, *Detection of Escherichia Coli in Drinking Water by the EC Medium with MUG Tube Procedure*. In this procedure, cultured colonies are swabbed from the total coliform membrane filter. The swab is used to inoculate a tube

containing EC broth with 4-methylumbelliferyl-β-D-glucuronide (EC + MUG). The inoculated broth is incubated at 44.5 ± 0.2 °C for 24 ± 2 hours. The tube is examined under UV light (366 nm) and the observation of bright blue
fluorescence is indicative of the presence of E. coli. A positive E. coli result is shown in Photo 3. The Provincial Laboratory currently swabs any total coliform plate that contains colonies of coliforms or greater than 200 colonies of background bacteria.

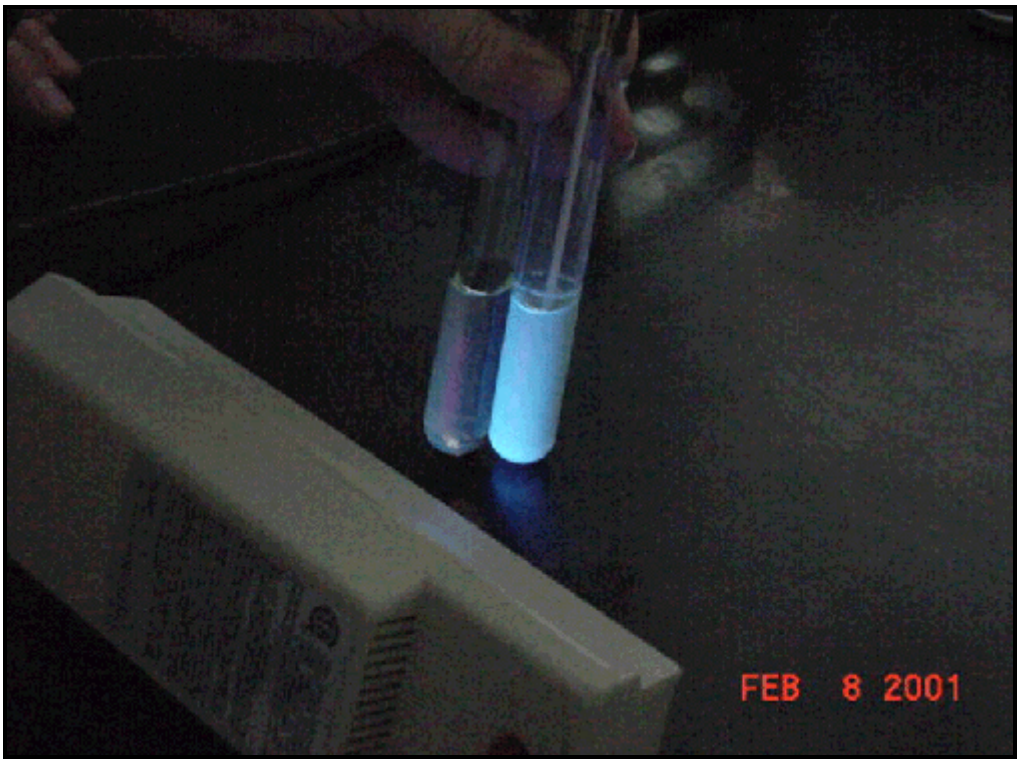

Photo 3: Tubes of inoculated EC + MUG medium.
The tube on the left is for a negative control while the tube on the right is from a positive control containing E. coli.

3.5 Presence-Absence testing for Total Coliforms and E. coli

Presence-Absence (P-A) methods are qualitative tests that indicate only the presence or absence of organisms, not the number of organisms. Although P-A tests for Total Coliforms and Escherichia coli (E. Coli) have been used for many years by provincial laboratories for screening drinking water, a number of these test products are now commercially available. They offer an effective method for screening drinking water when assurance of zero coliform organisms is required.

The coliform and E. Coli detection principles, upon which commercially available P-A methods are based, are presented in "Standard Methods for the Examination of Water and Wastewater". Commercial P-A tests rely on incubation at a constant temperature for

reliable test results. In the P-A Coliform test, 100 ml of water sample is added to a bottle containing P-A broth (i.e. reagent). The sample bottle is then incubated at 35 (+/ - 0.5) °C for 24 to 48 hours. Currently, most incubators are designed for laboratory use and for batch sampling.

P-A methods may prove very useful in confirming the presence or absence of Total Coliform bacteria in water samples that are Not Ascertainable, using standard Provincial Health Laboratory methods, due to large densities of background bacteria. Method 9221 F of Standard Methods for Examination of Water and Wastewater, 20th Edition, states "comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could over-grow coliform colonies and cause problems in detection". *However, standard membrane filtration methods used by Prov. Labs are more effective at identifying problems with a treatment system as these methods will show evidence of high non-coliform bacterial populations*. P-A tests for coliform bacteria are not designed to measure general bacterial populations.

3.5.1 The Aquasure Pro 3000™ - STEPPI™ Technology

The Aquasure Pro 3000™ - STEPPI™ technology includes the Aquasure Pro 3000™ portable incubator, a test kit and a small UV light. The "Aqualert™" test kit, contains a sterile 100 ml disposable test bottle with reagent and chlorine remover. The bottles are uniquely designed to fit over a heating element inside the incubator. Photo 4 shows the proto-type incubators used in this evaluation.

Photo 4: Aquasure Pro 3000™ Incubator and UV light

The Aquasure Pro 3000™ incubator unit is ideally designed for individual well owners and small communities that require only a few bacteriological tests at any given time. This technology utilizes a linear heating system to incubate individual water samples at a constant temperature of 35 (+/- 0.5) °C for the required incubation period. It is a compact, portable unit with a software driven,

microprocessor which monitors the operation of the unit for precision control of the water temperature. Quality control functions are also built in to ensure the validity of the test results.

Unlike most incubators, water samples, in the Aquasure Pro 3000™, are heated directly and the water temperature is monitored directly. Most laboratory incubators heat the air inside the incubator chamber which, in turn, heats the sample. Heating the water directly ensures that the sample reaches optimum temperature quickly and maintains a constant temperature during the entire incubation period. The vacuum lid insulates the water sample so that the unit can be used inside or outside at temperatures between 5 °C to 35°C. Indicator lights are used to warn of any power disruption and to indicate if the temperature of the water sample has fallen outside the accepted range (Emburgh and Kanipayor, 2001).

3.5.2 Presence-Absence Test Reagent

A chromogenic reagent developed by EBPI was used during the evaluation of the Aquasure Pro 3000™ - STEPPI™ technology. This P-A reagent permits rapid simultaneous detection of Total Coliforms and E. coli, using the chromogenic compound 5-bromo-4-chloro-3-indolyl-*B*-D-galactopyranoside (X-Gal) and the fluorogenic compound 4-methylumbelliferyl-*B*-D-glucuronide (MUG) in a single medium. After incubation at 35 °C for 24 to 28 hours, the development of a blue colour in an initially light yellow coloured solution, indicates the presence of total coliforms; fluorescence at 365 nm in the same vessel demonstrates the presence of E. coli (Manafi and Rosmann, 2001).

Using the P-A reagent developed by EBPI, water samples are simultaneously tested for Total Coliforms based on detection of the enzyme βετα-D-galactosidase and for E. coli based on the detection of the enzyme βετα-D-glucuronidase. With this method, coliforms are defined as all bacteria which produce the enzyme βετα-D-galactosidase, which cleaves the chromogenic substrate X-Gal. The production of this enzyme by coliform bacteria causes the culture media to change from yellow to a blue colour. The presence of at least one viable coliform bacterium in the solution is sufficient to produce a visible blue colour at 35 °C within 24 hours. E. coli are defined as coliform bacteria that produce the enzyme βετα-D-glucuronidase, which cleaves the fluorogenic substrate MUG, resulting in the appearance of a brilliant blue colour (fluorescence) when grown in the medium for 24 to 28 hours at 35°C. If the water sample contains E. coli, the culture media will fluoresce when exposed to UV light (Don Lush, 2001).

The following photos illustrate positive and negative test results for Total Coliforms and E. coli, using the Aquasure Pro 3000™ - STEPPI™ technology and

the P-A reagent developed by EBPI. Photo 5 is a picture of two different water samples in the Aqualert™ test jars following 24 hours incubation. The jar on the right illustrates

Photo 5: Total Coliform test results

a water sample, plus reagent after 24 hours incubation, that does not contain coliform bacteria. The yellow colour is caused by the reagent. The jar on the left shows how a water sample may appear after 24 hours incubation when it contains coliform bacteria. The coliforms in this water sample produced a blue colour. However, mixed with

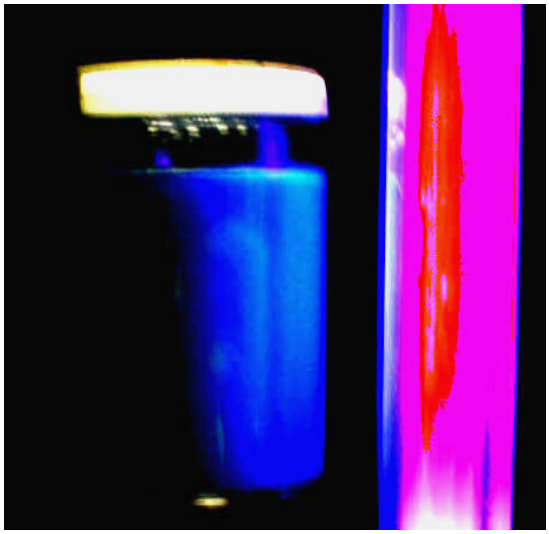

Photo 6: Fluorescence caused by E. coli bacteria

residual yellow from the reagent it is visible as a dark blue-green. Photo 6 illustrates the fluorescence caused by E. coli in a water sample following 24 hours incubation. The object on the right in Photo 6 is a small UV light.

4.0 EVALUATION PROCEDURE AND RESULTS

Evaluation of the STEPPI™ technology began on April 27, 2001 following a demonstration by representatives from Aquasure™ Technologies Inc. Two Aquasure Pro 3000™ units, sample bottles, reagent and a UV light were provided by Aquasure™ for the evaluation. The incubators were set up at PFRA's "Technology Adaptation Facility" in Regina, Saskatchewan.

In total, 21 water samples were tested using the Aquasure Pro 3000™ units. For the initial demonstration, 3 of the 21 samples were provided by the Prov. Lab. Coliform bacteria were added to these water samples before the demonstration. Four of the 21 samples were sterile distilled water samples prepared at PFRA's facility. The sterile samples acted as blanks and were used to ensure the media had not become contaminated during the test period. Fourteen of the water samples were collected from PFRA's Caledonia research site at various times over the test period. Comparative tests, between standard methods performed at the Provincial Health Laboratory and the standard P-A method performed at PFRA's facility using the Aquasure Pro 3000™ units, were conducted on 13 of the water samples collected from Caledonia. These comparative tests were used to confirm the P-A test results.

The 4 sterile distilled water samples were negative for both Total Coliforms and E. coli. The 3 water samples provided by the Provincial Health Lab were spiked with the following coliform bacteria: 1) Enterobacter aerogenes, 2) E. coli and 3) E. coli and Enterobacter aerogenes.

Results of the P-A tests using the Aquasure Pro 3000™ technology were as follows:

1. Positive for Total Coliforms, negative for E. coli
2. Positive for both Total Coliforms and E. coli
3. Positive for both Total Coliforms and E. coli.

Some reflectance of the UV light occurred on the test jar that was negative for E. coli, but positive for Total Coliforms. Although this reflectance is quite different then the brilliant fluorescent blue of a water sample containing E. coli, Aquasure Pro 3000™ unit operators may confuse this reflectance with a positive result when using P-A chromogenic reagents. This problem can be overcome with the aid of a colour chart, by using a non-reflective bottle or by using a reference bottle.

4.1 Sample Collection

Water samples collected by PFRA staff, from wells located on the Caledonia research site, were collected over a period of 2 weeks. During this time period, 7 trips were made to the research site and two untreated water samples were collected during each trip. Water samples were collected directly from various wells at the site, using sterile disposable bailers. Each water sample was split to allow testing at both PFRA's facility and the Provincial Health Lab.

One of each split sample was packed in ice and delivered to the Prov. Lab on the day of collection. The remaining split samples, also packed in ice, were delivered to PFRA's facility. Testing procedures used at both test locations are outlined in Appendix A.

4.2 Comparative Test Results

Comparative tests, between standard methods used at the Provincial Health Laboratory and the standard P-A method used with the Aquasure Pro 3000™ units, were performed on 13 of the water samples collected from Caledonia. Total Coliform test results are summarized in Table 1 (see appendix A for specific test results).

Table 1: Comparative Test Results

Test Results	Provincial Lab Results	P-A/Aquasure Pro 3000™ Test Results
Negative for T. Coliforms/E. coli	5	10
Not Ascertainable for T. Coliforms	6	1
Positive for T. Coliforms	2	2

The P-A test results using the Aquasure Pro 3000™ technology demonstrated consistency with the Saskatchewan Provincial Health Laboratory test results. Both test methods showed that 11 of the 13 comparative water samples were negative or not ascertainable (NA) for Total Coliforms. In addition, 2 of the 13 comparative water samples were positive for both Total Coliforms and E. coli, using both methods.

The NA results suggest that these water samples may contain high levels of background bacteria. Results from the Prov. Lab tests indicate that six of the comparative water samples were Not Ascertainable for Total Coliforms. One of these samples was also NA using the Presence-Absence method. Don Lush from EBPI states that, "in some cases, if the sample does contain high numbers of non-coliform bacteria, the media may give a weak blue/green colour... if any blue green colour is produced the sample should be verified through standard membrane filtration testing". Alternate test methods are required to determine general or background bacterial populations, as P-A tests for coliform bacteria are not designed to measure general bacterial populations.

P-A tests may provide an effective way to confirm the absence of coliform bacteria in water samples that are Not Ascertainable using Provincial Lab methods. Of the six water samples that were Not Ascertainable using Prov. Lab methods, only one was Not Ascertainable using the P-A reagent with the Aquasure Pro 3000™ technology. The remaining 5 samples showed negative

Total Coliform test results using the P-A method. Method 9221 F, of Standard Methods for Examination of Water and Wastewater, 20th Edition states, "comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could over-grow coliform colonies and cause problems in detection".

4.3 Comparative Test Variations

A number of comparative tests were used to identify factors that may impact P-A test results when using the Aquasure Pro 3000™ technology. To investigate how variations in sample preservation and storage may affect test results, four water samples collected from Caledonia, were tested before and after extended periods of storage at room temperature. Other samples were incubated for extended periods to determine the impact of altering test procedures. In addition, untreated water samples were used for the comparative tests to investigate the impacts that background bacteria may have on test results.

4.3.1 Effects of Extended Storage Before Testing

Improper sample preservation and storage may lead to erroneous test results. Ideally, tests for Total Coliforms and E. coli should begin within 24 to 48 hours following collection and water samples should be kept at 4°C until testing begins. Unfortunately, many well owners do not or are unable to follow this procedure.

Due to time constraints, only 4 water samples were used to investigate how altering sample preservation and storage procedures may impact the P-A test results. Samples 9-B and 9-Q were split to allow comparative testing before and after storage at room temperature for @ 24 hours and water samples 10-B and 10-Q were split to allow testing before and after storage at room temperature for @ 48 hours.

Comparative tests were conducted on a portion of samples 9-B and 9-Q shortly after collection. Comparative tests were also conducted on the remaining portion of both samples, after storage at room temperature for 24 hours. Total Coliform test results for these samples are provided in Table 2.

Table 2: Samples 9-B and 9-Q Total Coliform Test Results

Test Used	Sample 9-B Domestic Well Test Results		Sample 9-Q Piez C4 Test Results	
	Before Storage	After 24 hrs Storage	Before Storage	After 24 hrs Storage
P-A/Aquasure Pro 3000™	neg	neg	neg	neg
Provincial Lab Test	neg	neg	NA	NA

Storage at room temperature for 24 hours did not change the Prov. Lab or the P-A test results for Total Coliforms. Both test methods showed that Sample 9-B was negative for coliforms before and after storage. Sample 9-Q was also negative both before and after storage using the P-A method and NA before and after storage using the Prov. Lab method.

Comparative tests were conducted on a portion of samples 10-B and 10-Q before storage. The remaining portion of both samples were stored at room temperature for 48 hours and then tested using only the P-A method with the Aquasure Pro 3000™ technology. Prior to performing the comparative tests, sample 10-B was spiked with E. coli by staff at the Prov. Lab. This was done to determine if extended storage may cause a false negative when testing for Total Coliforms. Total Coliform test results are provided in Table 3.

Table 3: Samples 10-B and 10-Q Total Coliform Test Results

Test used	Sample 10-B Spiked Sample Test Results		Sample 10-Q Piez C4 Test Results	
	Before Storage	After 48 hrs Storage	Before Storage	After 48 hrs Storage
P-A/Aquasure Pro 3000™	pos	pos	neg	neg
Provincial Lab Test	pos	not tested	NA	not tested

Storage at room temperature for 48 hours did not change the P-A test results for Total Coliforms. Sample 10-B was positive for Total Coliforms and E. coli both before and after storage. Sample 10-Q was negative both before and after storage.

4.3.2 Effects of Extended Incubation Time

In 1997, the Ontario Ministry of Environment and Energy released a guidance document entitled, "Sample Collection and the Use of Commercial Presence-Absence Tests for the Bacteriological Analysis of Drinking Water". This document states that the performance of P-A products may be enhanced by extending incubation to at least 48 hours. The document adds that extended incubation may allow recovery of coliforms which have been damaged by exposure to disinfectants used to treat drinking water.

Extending incubation times may allow recovery of stressed coliforms. However, large populations of non-coliform bacteria may produce false positives if the incubation time is extended. Don Lush, from EBPI, states that, "large densities of some non-coliform species of the genera Aeromonas and Pseudomonas, which possess beta galactosidase activity, may cause false positive test results. These

bacteria are suppressed in EBPI's test methodology and generally will not produce a positive response within 28 hours at 35 °C (unless they are at densities of greater than 10,000cfu/ml sample). Hence, false positives seldom occur within 28 hours at 35 °C, unless bacterial densities are high."

To examine the effects of extended incubation on the P-A method used in this evaluation, three of the comparative water samples (samples 7-B, 8-B and 8-Q) were examined following 24 hours in the Aquasure Pro 3000™ incubators and then returned to the incubators. Following 43 hours in the incubators sample 7-B was re-examined. Samples 8-B and 8-Q were examined following 24 hours incubation and then returned to the incubators. Following 64.5 hours incubation these samples were re-examined. The P-A/Aquasure Pro 3000™ test results for Total Coliforms are shown in Table 4.

Table 4: Samples 7-B, 8-B and 8-Q Total Coliform Test Results Before and After Extended Incubation

Sample	**Sample 7-B Piez C13**		**Sample 8-B Piez C4**		**Sample 8-Q Piez C6**	
Incubation Time	**at 24 hrs.**	**at 43 hrs.**	**at 24 hrs.**	**at 64.5 hrs.**	**at 24 hrs.**	**at 64.5 hrs.**
P-A/Aquasure Pro 3000™ Results	neg	pos	NA	pos	neg	neg
Provincial Lab Test Results	NA	not tested	NA	not tested	NA	not tested

The P-A test results for Total Coliforms were affected by extending incubation times. Sample 7-B was negative for Total Coliforms after 24 hours incubation. However, following extended incubation the sample gave a positive result for Total Coliforms. Total Coliform test results for sample 8-B were unclear (NA) after 24 hours as the sample was a very light green in colour. After 64.5 hours of incubation the sample was positive for Total Coliforms. Sample 8-Q was negative for Total Coliforms both before and after extended incubation. These results underscore the importance of following proper test procedures. Although this P-A test method requires incubation of the water sample at 35 °C for 24 hours, false positives seldom occur within 28 hours. Therefore, when using this test method, water samples placed in the Aquasure Pro 3000™ incubators must be examined after 24 hours of incubation and before 28 hours of incubation has elapsed.

The change in test results with extended incubation was likely caused by high levels of non-coliform background bacteria. The three water samples identified in Table 4 likely contained high levels of background bacteria as these samples

were untreated and each of the samples were not ascertainable for Total Coliform bacteria when tested by the Prov. Lab. None of these samples contained E. coli bacteria. Although non-coliform beta galactosidase activity is suppressed in EBPI's P-A test methodology, extended incubation and high levels of background bacteria may have caused the change in test results. The positive test results that occurred in samples 7-B and 8-B, after extended incubation, were not caused by chlorine stressed coliforms as the water samples were not exposed to a disinfectant.

5.0 CONCLUSIONS

This evaluation of the Aquasure Pro 3000™ - STEPPI™ technology using a chromogenic presence-absence reagent, was performed to: 1) identify qualities of the technology that may enhance or encourage more frequent testing of drinking water 2) determine the benefits of the technology to field research projects 3) identify factors that may impact P-A/Aquasure Pro 3000™ test results. The following conclusions are based on the findings of this evaluation.

1. The Aquasure Pro 3000™ - STEPPI™ technology and the P-A reagent developed by EBPI provide a reliable method for screening water for Total Coliforms and E. coli bacteria. Aquasure Pro 3000™ test results demonstrated consistency with current Saskatchewan Provincial Health Laboratory test results. Both test methods showed that 11 of the 13 comparative water samples were negative or not ascertainable for Total Coliforms. Two of the 13 comparative water samples were positive for Total Coliforms and E. coli using both test methods.

2. The simplicity and portability of P-A testing using the Aquasure Pro 3000™ - STEPPI™ technology should encourage more frequent testing of drinking water. Special skills are not required to operate this technology and no sample preservation is required if samples are immediately placed in the portable incubators.

3. Test results are obtained quickly using P-A tests and the Aquasure Pro 3000™ - STEPPI™ technology. When the incubators are set up onsite, test results can be observed within 24 to 28 hours of sample collection. Rapid, onsite testing may be particularly useful when a failure in treatment technology is suspected. The reduced time between sampling and analysis will allow water treatment operators to quickly respond to adverse conditions. Onsite testing will also benefit remote communities or individuals that may normally require several days to transport water samples to a laboratory for testing.

4. Shorter time periods between sampling and test results provide definite benefits to field research projects. Field research often involves collecting multiple samples from one or more locations over several days. In these situations it is difficult to get samples into a lab within an acceptable time frame. In addition, laboratories generally require 2 to 3 days to test and process results.

With portable P-A test instruments, such as the Aquasure Pro 3000™, samples can be tested onsite and results obtained within 24 hours. Therefore, project activities, which are based on immediate test results can proceed more quickly.

5. In general, the P-A test results were easy to interpret. All but one of the water samples tested in this evaluation either remained yellow (negative for Total Coliforms) or turned a dark blue-green colour (positive for Total Coliforms) within the 24 hour incubation period. Only one sample was Not Ascertainable (light green) after 24 hours in the STEPPI™ incubator. This sample likely contained high levels of background bacteria as it was also Not Ascertainable for Total Coliforms using the Prov. Lab methods. Don Lush from EBPI suggests that if any blue-green colour is observed the sample should be verified through standard membrane filtration testing.

6. Interpretation of E. coli tests require some previous understanding of how a positive P-A test result will appear. Some reflectance of the UV light occurred in the water samples that were negative for E. coli but positive for Total Coliforms. Although this reflectance is quite different then the brilliant fluorescent blue of a water sample containing E. coli, initially, STEPPI™ test operators may confuse this reflectance with a positive result. This potential problem is removed when the operator gains experience and has seen a true fluorescence response.

7. Further testing is required to determine the effects of storage on test results. Storing water samples at room temperature before testing did not affect the P-A/Aquasure Pro 3000™ test results. However, due to the time constraints of this evaluation only 4 samples were tested after extended storage.

8. When using EBPI's P-A test method, water samples placed in the Aquasure Pro 3000™ incubators must be examined before 28 hours of incubation has elapsed. EBPI states that, "unless general bacterial densities are greater than 10,000 cfu/ml sample, false positives seldom occur within 28 hours incubation at 35 °C." However, extended incubation (> 28 hours) can affect the P-A/Aquasure Pro 3000™ Total Coliform test results. During this evaluation, three water samples were incubated for extended periods. Test results from two of these samples changed from negative, for Total Coliforms at 24 hours, to positive when incubation times were extended past the suggested 28 hours. To avoid false positive results, incubation times must not extend past 28 hours. To avoid false negative results, samples must be incubated for a minimum of 24 hours at 35 °C.

6.0 REFERENCES

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APPENDIX A

Evaluation of the Aquasure Pro 3000™
Single Test Precision Portable Incubator Technology (STEPPI™)

Presence-Absence Test Procedures

Evaluation of the STEPPI™ technology began on April 27, 2001. Two Aquasure Pro 3000™ incubation units, sample bottles, presence-absence reagent and a UV light were provided by Aquasure™ for the evaluation. The reagent, developed by Environmental Biodetection Products Inc. (EBPI), is a chromogenic reagent which permits simultaneous detection of Total Coliforms and E. coli following 24 to 28 hours incubation.

In total, 21 water samples were tested using the Aquasure Pro 3000™ units which were set up at PFRA's "Technology Adaptation Facility" in Regina. Three of the 21 samples were provided by the Prov. Lab. for the initial demonstration. Coliform bacteria were added to these 3 water samples before the demonstration. Four of the 21 samples were sterilized distilled water prepared at PFRA's facility. The sterile samples acted as blanks and were used to ensure the media had not become contaminated throughout the test period. Fourteen of the water samples were collected from PFRA's Caledonia research site at various times over the test period. Comparative tests, between standard methods performed at the Provincial Health Laboratory and the standard P-A method performed at PFRA's facility using the Aquasure Pro 3000™ units, were run on 13 of the water samples collected from Caledonia. These comparative tests were used to confirm the P-A test results.

Water samples collected by PFRA staff, from wells located on the Caledonia research site, were split to allow testing at both PFRA's facility and the Provincial Health Lab. One of each split sample was packed in ice and delivered to the Prov. Lab on the day of collection. The remaining split samples, also packed in ice, were delivered to PFRA's Technology Adaptation Facility. The preparations and procedures used at PFRA's facility to test the water samples for Total Coliforms and E. coli are outlined in the attached data collection sheet.

Test procedures used by PFRA staff during this evaluation may differ slightly from procedures that will be followed by future Aquasure Pro 3000™ operators. Disposable sterile test jars were not available at the time of this evaluation therefore the test jars used in this evaluation required sterilization before each test. In the future operators using the Aquasure Pro 3000™ incubator units and the Aqualert™ test kits will be provided with sterile disposable test bottles, reagent and chlorine remover. Representatives from Aquasure Technology Inc. provided the following procedures for use with the Aquasure Pro 3000™ incubator units and the disposable test jars:

Onsite Presence Absence Test Procedure for Total Coliforms Using the Aquasure Pro 3000™

1) Remove the tamper proof seal and twist open the specimen bottle cap
2) Fill the bottle with the water for testing up to the 100 ml mark
3) Add the reagent (if not already in the bottle) and close the bottle with the cap firmly
4) Swirl gently to dissolve the reagent
5) Place the sample bottle in the Aquasure Pro 3000™ unit, close the lid
6) Turn the power on
7) Observe the colour change of the test water (test result) after the 24 hour incubation time (the time may vary with different reagents)
8) If colour change is not distinct after 24 hours then incubate for an additional 4 hours (do not exceed 28 hours)
9) Record results

STEPPI™ TEST #_____

Sample ID:_____

Sample collected by:_____ **Collection time and date:**_____

Sample tested by:_____ **Testing time and date:**_____

Objectives:_____

_____

Comments:_____

_____

_____

STEPPI™ Test Preparation and Procedures

_____ Tape removed from lids and washed thoroughly with jars in hot, soapy water

_____ Disinfect jars and lids in chlorine solution (100 mL of 12% Sodium Hypochlorite added to 1000 mL of distilled water) for at least two hours (Start time: _____ End time: _____)

_____ Distilled water (1000 mL) boiled at least 20 minutes (Boiling time: _____ to _____)

_____ Samples removed from fridge approximately one half hour before testing (Time of removal: _____)

_____ Jars and lids rinsed with boiled, distilled water

_____ Jars and lids set on counter to air dry

_____ Holes in lids covered with electrical tape

_____ Chromogenic Reagent media added to jars with sterile medicine spoon (1.7 grams added to jar on scale)

_____ 100 mL of collected sample water added to jars with sterile syringe

_____ Lids snapped tightly on jars (Two hole on shorter jar Q, one hole on taller jar B)

_____ Jars placed in incubator unit (shorter jar in unit Q, taller jar in unit B)

Incubator Unit B:_____

Switched on ____

Start time _____

Red light on _____

Green light on ____

Comment:_____

_____

Incubator Unit Q:_____

Switched on ____

Start time _____

Red light on ____

Green light on ____

Comment:_____

_____

STEPPI™ TEST RESULTS	Total Coliforms (Negative)		Total Coliforms (Positive)		E. coli (Negative)		E. coli (Positive)		Evaluation of Results Time / Date
	Maxwell	Provincial	Maxwell	Provincial	Maxwell	Provincial	Maxwell	Provincial	
Incubator B									
Incubator Q									

Comments:_____

_____

_____

Single Test Precision Portable Incubator Technology (STEPPI) Evaluation - Test Results

		Collected		Date Tested		Total Coliform		E-Coli		Comments
Test	Sample ID	From	Date	PFRA	Prov. Lab	PFRA	Prov. Lab	PFRA	Prov. Lab	
1	B (E-aergenes)	Prov. Lab	25/04/01	25/04/01		pos		neg		
	Q (E-coli)	Prov. Lab	25/04/01	25/04/01		pos		pos		
2	B (Sterile Water)	Maxwell	27/04/01	27/04/01		neg		NR		
	Q (E-coli/E-aergenes)	Prov. Lab	25/04/01	27/04/01		pos		pos		
										Samples 3B and 3Q incubated at maxwell
3	B (Sterile Water)	Maxwell	30/04/01	30/04/01		neg		NR		for extended period.
	Q (Piez C-8)	Caledonia	24/04/01	30/04/01		neg		NR		checked at 24 hrs - Negative for Coliforms
										checked at 42 hrs - Negative for Coliforms
4	B (Piez C-8)	Caledonia	01/05/01	02/05/01		neg		NR		
	Q (Piez C-8)	Caledonia	01/05/01	02/05/01	02/05/01	neg	neg	NR	NR	
5	B (Piez C-4)	Caledonia	03/05/01	04/05/01	04/05/01	neg	NA	NR	neg	
	Q (House Well)	Caledonia	03/05/01	04/05/01	04/05/01	neg	neg	NR	NR	
6	B (Piez C-11)	Caledonia	08/05/01	08/05/01	09/05/01	neg	neg	NR	NR	
	Q (Piez C-13)	Caledonia	08/05/01	08/05/01	09/05/01	neg	neg	NR	NR	
										7B incubated at Maxwell for extended period
7	B (Piez C-13)	Caledonia	09/05/01	09/05/01	09/05/01	neg	NA	NR	neg	checked at 24 hrs - Negative for Coliforms
	Q (Wet Well)	Caledonia	09/05/01	09/05/01	09/05/01	pos	pos	pos	pos	checked at 43 hrs - Dark green
8	B (Piez C-4)	Caledonia	11/05/01	11/05/01	11/05/01	*unknown	NA	neg		*At 24 hrs sample 8-B was N-A as
	Q (Piez C-6)	Caledonia	11/05/01	11/05/01	11/05/01	neg	NA	NR		sample was a light green in color.
										At 64.5 hrs incubation 8-B was dark green
9	B (House Well)	Caledonia	14/05/01	14/05/01	14/05/01	neg	neg	NR	NR	At 64.5 hrs incubation 8-Q remained negative
				15/05/01	15/05/01	neg	neg	NR	NR	Both samples, 9B and 9Q, were split into 4
	Q (Piez C-4)	Caledonia	14/05/01	14/05/01	14/05/01	neg	NA	NR	neg	to permit testing (at Maxwell and Prov Lab)
				15/05/01	15/05/01	neg	NA	NR	neg	before and after standing at room temp for 24 hrs.
10	B (House Well)	Caledonia	15/05/01	17/05/01	16/05/01	*pos	pos	pos	pos	*Both Samples, 10B and 10Q, taken to Prov Lab
				19/05/01		pos		pos		where one sample was spiked with E. coli
	Q (Piez C-4)	Caledonia	15/05/01	17/05/01	16/05/01	neg	NA	NR	neg	Samples were tested before and after
				19/05/01		neg		NR		standing at room temp for 48 hrs.
										The 48 hr samples were tested at Maxwell only.
11	B (Sterile water/reagentB)	Maxwell	18/05/01	18/05/01		neg		NR		
	Q (Sterile water/reagentA)	Maxwell	18/05/01	18/05/01		neg		NR		

pos - positive test result **neg** - negative test result ***NA** - Not Ascertainable ***NR** - Test not required