Scholar Commons
USF University of South Florida

University of South Florida
Scholar Commons

Graduate Theses and Dissertations Graduate School

5-1-2014

Drinking Water in the Developing World: Sources of Fecal Contamination in Pitcher Pump Systems and Measurement Alternatives

Meghan Wahlstrom
University of South Florida, mawahlstr@gmail.com

Follow this and additional works at: http://scholarcommons.usf.edu/etd

Part of the Environmental Engineering Commons, Public Health Commons, and the Water Resource Management Commons

Scholar Commons Citation
Wahlstrom, Meghan, "Drinking Water in the Developing World: Sources of Fecal Contamination in Pitcher Pump Systems and Measurement Alternatives" (2014). *Graduate Theses and Dissertations.*
http://scholarcommons.usf.edu/etd/5146

This Thesis is brought to you for free and open access by the Graduate School at Scholar Commons. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Scholar Commons. For more information, please contact scholarcommons@usf.edu.

Drinking Water in the Developing World:

Sources of Fecal Contamination in Pitcher Pump Systems and Measurement Alternatives

by

Meghan Wahlstrom-Ramler

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Environmental Engineering
Department of Civil and Environmental Engineering
College of Engineering
University of South Florida

Co-Major Professor: Sarina J. Ergas, Ph.D.
Co- Major Professor: James R. Mihelcic, Ph.D.
Valerie J. Harwood, Ph.D.

Date of Approval:
March 25, 2014

Keywords: Groundwater, coliforms, microbial test kits, health, Millennium Development Goal

Copyright © 2014, Meghan Wahlstrom-Ramler

ACKNOWLEDGMENTS

First and foremost I would like to thank Dr. Sarina Ergas for serving as my major professor. Without her support and guidance this thesis would not be possible. I would also like to thank Dr. James Mihelcic and Dr. Valerie Harwood for serving on my thesis committee. Thank you to Jonathan Annis for requesting an MI student and allowing me the opportunity to serve in Madagascar and thank you to Mike MacCarthy for being a great mentor both in the field and back on campus. Thanks to Brad Akers for his help with data collection in Madagascar. A special thanks to Dr. Sarah Taylor for her insight into statistics and to Sarah Hayman for being willing to talk statistical procedures with me for hours on end. Additionally I would like to thank Marie Onnie Razifikalo, the Peace Corps Madagascar staff, and the community of Andemaka. Finally, a very heartfelt thanks to my both my Malagasy and U.S. families for their unwavering support.

This study was conducted as part of a partnership between the University of South Florida (Tampa) (USF) and RANO HamPivoatra (Water for Progress), a USAID-funded project in Madagascar implemented by a consortium led by the NGOs CRS and CARE.

This material is based upon work supported by the National Science Foundation under Grant No. DUE 0965743. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

TABLE OF CONTENTS

LIST OF TABLES iv

LIST OF FIGURES vii

ABSTRACT ix

CHAPTER 1: INTRODUCTION 1
1.1 Research Motivation 1
1.2 Background 4
1.2.1 Pitcher Pumps in Tamatave Madagascar 4
1.2.2 The Need for Water Quality Testing 5
1.3 Objectives 6

CHAPTER 2: EVALUATION OF THE EFFECT OF PITCHER PUMP SYSTEM DEPTH ON WATER QUALITY AND IDENTIFICATION OF FACTORS LEADING TO SOURCE WATER CONTAMINATION 8
2.1 Introduction 8
2.2 Literature Review 10
2.2.1 Improved Water Sources and Contamination 10
2.2.2 Measuring Microbial Risk 11
2.2.3 Sources and Pathways of Contamination 13
2.2.4 Removal Mechanisms 18
2.2.4.1 Physical Removal Mechanisms 19
2.2.4.2 Die-off 21
2.3 Materials and Methods 23
2.3.1 Study Site 24
2.3.2 Phase I 26
2.3.2.1 Household Visits 26
2.3.2.2 Sample Collection 29
2.3.2.3 Sample Analysis 31
2.3.2.4 Measuring Well Depth 33
2.3.3 Phase II 34
2.3.3.1 Site Description 34
2.3.3.2 Monitoring Well Installation and Development 35
2.3.3.3 Sample Collection 38
2.3.3.4 Sample Analysis 40
2.3.4 Statistical Analysis 40
2.4 Results and Discussion 43
2.4.1 Phase I General Survey Results 43
2.4.2 Confirmation of Depth 46

2.4.3 Phase I Sampling Results ... 46
2.4.3.1 Thermotolerant Coliform Levels as a Function of Depth ... 49
2.4.3.2 Thermotolerant Coliform Levels as a Function of Neighborhood ... 52
2.4.3.3 Thermotolerant Coliform Levels as a Function of Priming ... 52
2.4.3.4 Thermotolerant Coliform Levels as a Function of Repairs ... 56
2.4.3.5 Thermotolerant Coliform Levels as a Function of Distance ... 56
2.4.3.6 Thermotolerant Coliform Levels as a Function of Number of Users ... 57
2.4.2 Phase II ... 58
2.5 Conclusions and Recommendations ... 60
2.5.1 Recommendations to Tamatave ... 61
2.5.2 Recommendations for Future Research ... 62

CHAPTER 3: EVALUATION OF ALTERNATIVE METHODS FOR DETECTION AND ENUMERATION OF FECAL INDICATOR BACTERIA IN DEVELOPING COUNTRIES ... 64
3.1 Introduction ... 64
3.2 Materials and Methods ... 66
3.2.1 Water Samples ... 66
3.2.2 Incubation Temperatures ... 66
3.2.3 FIB Enumeration by Standard MF ... 67
3.2.4 FIB Enumeration by Colilert ... 67
3.2.5 FIB Enumeration by Coliscan-MF ... 67
3.2.6 FIB Enumeration by Modified 3-M ... 68
3.2.7 Confirmation of Identification as *E.coli* ... 68
3.2.8 Effect of Experience on Results ... 69
3.2.9 Statistical Analysis ... 70
3.2.10 Economic Analysis ... 70
3.3 Results ... 70
3.3.1 FIB Kit Performance at Varying Temperatures ... 70
3.3.2 Confirmation of Identification as *E. coli* ... 71
3.3.3 Effect of Analyst Experience ... 72
3.3.4 Cost Comparison ... 73
3.4 Discussion ... 73
3.5 Conclusions ... 76

CHAPTER 4: OVERALL CONCLUSIONS AND RECOMMENDATIONS ... 82

REFERENCES ... 84

APPENDICES ... 95
Appendix A: IRB Correspondence ... 96
Appendix B: Relationship Between Inside and Outside Pipe Diameters ... 97
Appendix C: Copy of Survey Given to Pitcher Pump System Users in Tamatave ... 98
Appendix D: Survey Results from Pitcher Pump Users in Tamatave ... 100
Appendix E: Phase I Thermotolerant Coliform Sampling Results ... 119
Appendix F: Phase II Thermotolerant Coliform Sampling Results ... 121

Appendix G: Raw Data from the Investigation into Effect of Alternative Temperatures on Three FIB Test Kits 122
Appendix H: Raw Data from Volunteer Experiments Testing the FIB Kits 129
Appendix I: Permission to Use Tables and Figures 137

LIST OF TABLES

Table 1: Typical range of hydraulic conductivity (m/d) based on soil type 17

Table 2: Number of surveys conducted in each of the seven neighborhoods visited 28

Table 3: Number of water quality samples collected in six of the seven survey neighborhoods 30

Table 4: Explanation of how the data were broken up into categories for use in the statistical analysis of the results 42

Table 5: Summary of selected results from the general surveys 44

Table 6: Depths of the Pitcher Pump systems as reported by the household vs. measured depths 46

Table 7: Thermotolerant coliform risk levels at varying well depths; n = 56 51

Table 8: Thermotolerant coliform risk levels in the different neighborhoods; n = 56 52

Table 9: Thermotolerant coliform risk level as a function of priming; n = 44 55

Table 10: Thermotolerant coliform risk level as a function of the frequency of repairs; n = 39 56

Table 11: Thermotolerant coliform risk level as a function of distance from on-site sanitation systems; n = 49 57

Table 12: Standard temperature, substrates, and associated color changes with the kits tested in this research 78

Table 13: Percentage of false positive and false negative results for *E. coli* at varying temperatures 78

Table 14: Ranking of test kits using *E. coli* results only 79

Table 15: FIB concentrations produced by inexperienced volunteers (I) and expert (E) for each method 79

Table 16: FIB concentrations prepared by the expert (E) and then counted by the inexperienced volunteers (I) for each method 80

Table 17: Economic analysis, electricity requirements, and rankings by volunteers 81

Table B.1: Inside and outside pipe diameters ... 97

Table D.1: Results from the survey questions asked during household visits in Tamatave ... 100

Table D.2: Cost of the Pitcher Pump systems after the year 2000 ... 116

Table D.3: Distance between the Pitcher Pump system and on-site sanitation ... 117

Table E.1: Thermotolerant coliform sampling results from Phase I ... 119

Table F.1: Thermotolerant coliform sampling results from Phase II ... 121

Table G.1: Raw data from standard methods - mTec ... 122

Table G.2: Raw data from standard methods - mEndo ... 122

Table G.3: Raw data from Coliscan MF at 22.0°C ... 123

Table G.4: Raw data from Coliscan MF at 35.0°C. ... 124

Table G.5: Raw data from Coliscan MF at 44.5°C ... 125

Table G.6: Raw data from Colilert at 22.0°C ... 126

Table G.7: Raw data from Colilert at 35.0°C ... 126

Table G.8: Raw data from Colilert at 44.5°C ... 126

Table G.9: Raw data from modified 3-M at 22.0°C ... 127

Table G.10: Raw data from modified 3-M at 35.0°C ... 127

Table G.11: Raw data from modified 3-M at 44.5°C ... 128

Table H.1: Raw data from when volunteers prepared the plates – standard methods ... 129

Table H.2: Raw data from when volunteers prepared the plates – Coliscan MF ... 130

Table H.3: Raw data from when volunteers prepared the plates – Colilert ... 131

Table H.4: Raw data from when volunteers prepared the plates – modified 3-M ... 132

Table H.5: Raw data from when volunteers counted the plates – standard methods ... 133

Table H.6: Raw data from when volunteers counted the plates – Colilert ... 134

Table H.7: Raw data from when volunteers counted the plates – Coliscan MF ... 135

Table H.8: Raw data from when volunteers counted the plates – modified 3-M 136

LIST OF FIGURES

Figure 1: Map indicating the progress of individual countries towards MDG Target 7C 3

Figure 2: Schematic of the Pitcher Pump system 4

Figure 3: Relationship between thermotolerant coliform counts and well depth, Tamatave and Foulpointe... 9

Figure 4: Pathways of microbial contamination.. 14

Figure 5: Common forms of on-site sanitation facilities found in Tamatave, Madagascar 15

Figure 6: Map of Madagascar with the study site highlighted 25

Figure 7: A map of Tamatave with the neighborhoods highlighted where samples were collected 28

Figure 8: Collecting a sample to be used for the analysis of thermotolerant coliforms........................... 31

Figure 9: Sample processing equipment 32

Figure 10: Process used to measure the depth of the wells 33

Figure 11: Location of the pilot study conducted during Phase II 35

Figure 12: Base of a monitoring well.. 36

Figure 13: Materials and processes used to install the monitoring wells 37

Figure 14: The Malagasy research assistant developing the monitoring wells 38

Figure 15: Bailer constructed to sample the monitoring wells 39

Figure 16: The three main drinking water sources as reported by the survey members along with the reported treatment methods from households that received their cooking/drinking water from the Pitcher Pump system some or all of the time 43

Figure 17: Relationship between the depth of the well and cost 45

Figure 18: Percentage of samples that fell in the different drinking water risk categories 47

Figure 19: Map of Tamatave with the neighborhoods and sample locations highlighted 48

Figure 20: Log_{10} thermotolerant coliform concentrations as a function of the depth of the wells 50

Figure 21: Change in thermotolerant coliform concentration (cfu/100 ml) as depth of the monitoring wells increased 59

Figure 22: Total coliform concentrations determined by standard MF (line) and FIB test kits at varying temperatures 77

Figure 23: *E. coli* concentrations determined by standard MF (line) and test kits at varying temperatures 77

ABSTRACT

It has been reported that globally we have achieved Millennium Development Goal (MDG) Target 7C, to halve the proportion of the population without access to safe drinking water; however, there is a major flaw with this statement. While Target 7C calls for access to 'safe' drinking water, what is actually being measured and reported is access to an 'improved' water source. The World Health Organization (WHO) maintains that they must use this proxy measure because the methods for water quality testing are too expensive and logistically complicated, but by doing so, they may be over reporting safe water coverage.

This was shown to be true in Tamatave, Madagascar, where thermotolerant coliforms were detected in water from a type of 'improved' source, the Pitcher Pump system. This research looked at several parameters - Pitcher Pump system depth, sampling neighborhood, requirement of pump priming, frequency that the system was repaired, distance from on-site sanitation, and number of users – to see if they were influencing water quality. Of all the parameters tested, only priming was found to be significantly associated with the levels of thermotolerant coliforms detected (Fisher exact test $p = 0.03$). Using a Mann-Whitney U test, it was shown that the median thermotolerant coliform concentration was significantly higher in primed wells (41.3 cfu/100 ml) than unprimed wells (3.5) ($p = 0.01$ cfu/100 ml).

A pilot study was conducted to look at only the effect of depth and to determine if a depth could be identified that could provide safe drinking water. The result of the pilot study showed that, while thermotolerant coliform concentration did decrease with increasing depth, even at the deepest well of 9.4 m, levels were still above 100 cfu/100 ml.

Additional research was conducted to investigate the performance and cost of three test kits for both total coliform and *Escherichia coli* quantification for water quality analysis in developing countries. IDEXX Colilert Quanti-trays® (Colilert), Micrology Laboratories Coliscan® Membrane Filtration tests (Coliscan MF) and a modified method for 3-M Petrifilm™ Coliform/*E. coli* plates (modified 3-M) were compared with standard membrane filtration (standard MF) methods under a range of incubation temperature conditions (22.0, 35.0 and 44.5°C). Each test method was also performed by inexperienced volunteers, with the results compared to those of an experienced technician. At non-standard temperatures, Coliscan MF proved to be the most accurate when compared to standard methods, with a significant difference with only total coliforms at 44.5°C. Modified 3-M had the poorest correlation with standard MF over the range of temperatures tested, with significant differences noted for all the temperatures except for *E. coli* at 44.5°C. Inexperienced university volunteers found Colilert easiest to use, but Coliscan MF produced *E. coli* results that were most similar to the experts. Coliscan MF was found to have the overall best performance and lowest cost in this study; however, it did produce high numbers of false positive results.

CHAPTER 1: INTRODUCTION

1.1 Research Motivation

Diarrheal disease, which is generally caused by lack of access to safe drinking water and sanitation, kills approximately 1.5 million children each year (UNICEF/WHO, 2009). It is the second leading cause of death for children under 5 in the developing world and accounts for more deaths than malaria, measles, and AIDS combined (UNICEF/WHO, 2009). In Madagascar specifically, where much of the field research described in this thesis took place, approximately 22.5% of the deaths of children under 5 were attributed to this diarrheal disease (Black et al., 2010).

Concern about diarrheal disease isn't just related to mortality. There is also concern about how it can affect quality of life. Persistent diarrheal disease in early childhood has been linked to problems later in life, such as stunting (Moore et al., 2001; Assis et al., 2005; Black et al., 2008), impaired fitness (Guerrant et al., 1999), cognitive impairment (Guerrant et al., 1999; Niehaus et al., 2002) and lower school performance (Lorntz et al., 2006).

The economic impact of diarrheal disease is also significant. One of the most common forms of treatment is Oral Rehydration Therapy (ORT) which, in Madagascar, can cost around US$0.57 per packet. If you assume 4.4 cases per person per year (using the average from the cases per child per year presented in the WHO African Region and extending that to adults [Kosek et al., 2003]), three episodes per case, and 6.7 people per household (personal observation), diarrheal disease can cost a family approximately US$50.41 each year. This is of course an extreme underestimation of the economic burden because it does not take into account the costs of transportation to the clinic, doctor visits, or loss of wages (estimated at two lost working days per episode [Hutton & Haller, 2004]). With 92% of the

Malagasy population living below US$2 per day, this is an economic burden that many families cannot bear (The World Bank, 2013).

Worldwide, the disability adjusted life years (DALYs) attributed to diarrheal disease has been reported to range from approximately 60.7 million to 100 million DALYs (Hutton & Haller, 2004; Murray, 2012). DALYs are the sum of years lost to disability (YLD) and years of potential life lost due to fatal conditions (YPLL) (Guerrant et al., 2002). While access to safe drinking water will not eliminate all the cases of diarrheal disease, Esrey et al. (1991) estimates that a 15% reduction could be achieved by improving water quality and a 20% reduction could be achieved by increasing water quantity. In addition, improved access to safe drinking water can “improve health and education outcomes, and contribute to reduced poverty and sustainable development as a whole” (UNICEF, 2006). Stated in a different way, a reduction in the incidence of diarrheal disease is an important piece in the overall development of a country.

Lack of access to safe drinking water is enough of a concern that the United Nations (UN) included it in their 2010 MDGs. It is specifically referred to in Target 7C, which has the goal to reduce the proportion of people without sustainable access to safe drinking water and sanitation facilities by half by the year 2015, using 1990 as a starting point (UN, 2013). Progress towards the goal is reported by the Joint Monitoring Programme for Water Supply and Sanitation (JMP), a consortium of the WHO and the United Nations International Children’s Emergency Fund (UNICEF). Due to the difficulty of performing water quality tests in the developing world, a proxy indicator has been established to determine if a person has access to safe drinking water. This indicator measures the percent of the population using an improved water source (UNICEF/WHO, 2012). An improved water source is a structure which, by the nature of its construction, protects its water from outside contamination (UNICEF/WHO, 2012). These structures include piped water to homes, public standpipes, boreholes, protected dug wells, and protected springs (Mihelcic et al., 2009).

In regards to access to drinking water, it is being reported that globally we have achieved Target 7C. The JMP reports that, as of 2011, only an estimated 11% of the population (approximately 780 million people) was still using unimproved water sources as compared to the estimated 24% in 1990 (UNICEF/WHO, 2013). However, while the target has been met globally, if things are broken down country by country, it is clear that huge disparities still exist (Figure 1). As Figure 1 shows, much of Africa, as well as parts of Central Asia and Oceania, are not currently on track to meet the MDGs. In Madagascar specifically, where some of the research for this thesis took place, as of 2011, only around 78% of the urban and 34% of the rural populations were using an improved water source (WHO/UNICEF, 2013).

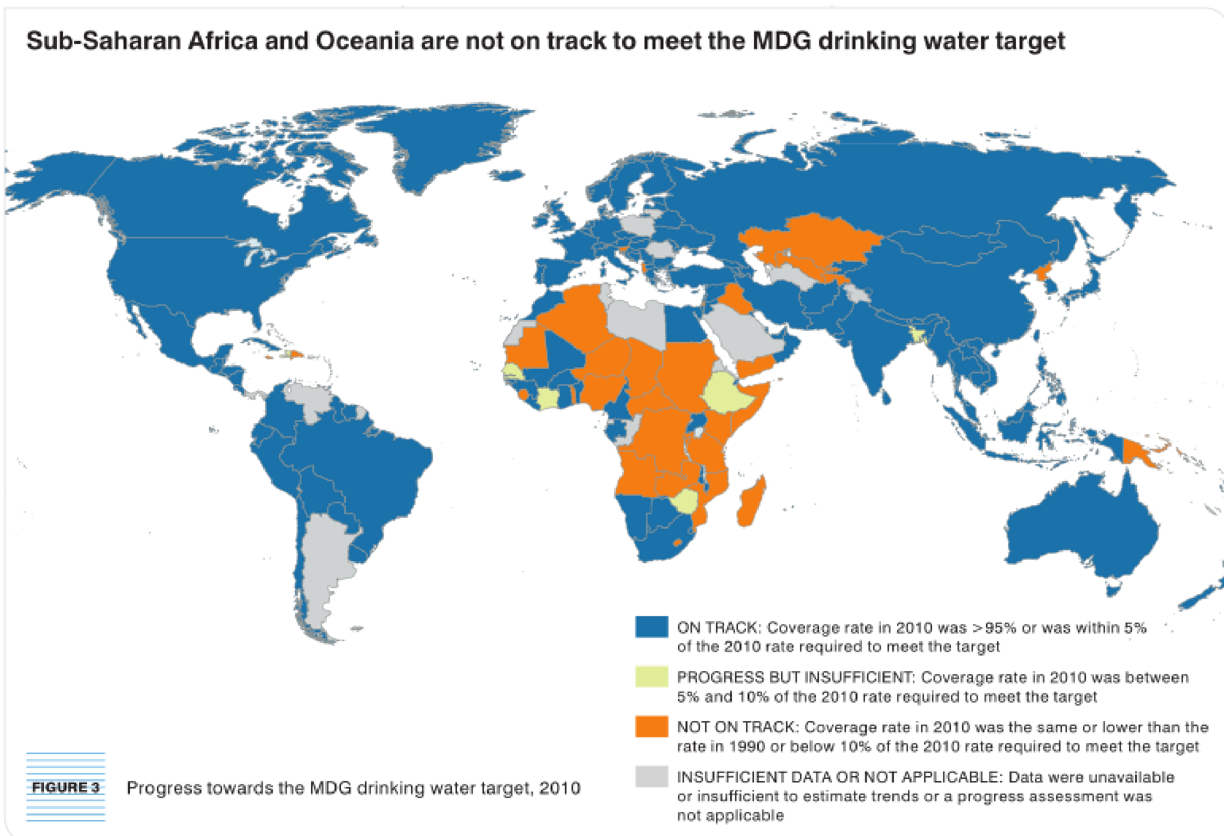


Figure 1: Map indicating the progress of individual countries towards MDG Target 7C. Much of Africa, as well as some parts of Oceania and Central Asia, are currently not making sufficient progress (reprinted with permission from UNICEF/WHO, 2012).

1.2 Background

1.2.1 Pitcher Pumps in Tamatave Madagascar

In Tamatave, Madagascar people are working to improve their access to safe drinking water through the use of a self-supply system[1] called a Pitcher Pump. The Pitcher Pump, or *Pompe Tany* as it's called in Malagasy (the language of Madagascar), consists of a suction pump attached to a manually drilled well. The well is installed by first drilling down to near the shallow water table and then inserting the well casing, including the well screen and point, into the hole and hammering down into the water table. Once the pipe is in place, the pump head is attached directly to the well casing. The well pipe and wellpoint are made of galvanized iron, and the brass well screen is welded on with lead (Pb)-containing solder. The pump head is made of mild, galvanized, or stainless steel and contains two check valves made of leather and commonly weighted with Pb (Figure 2).

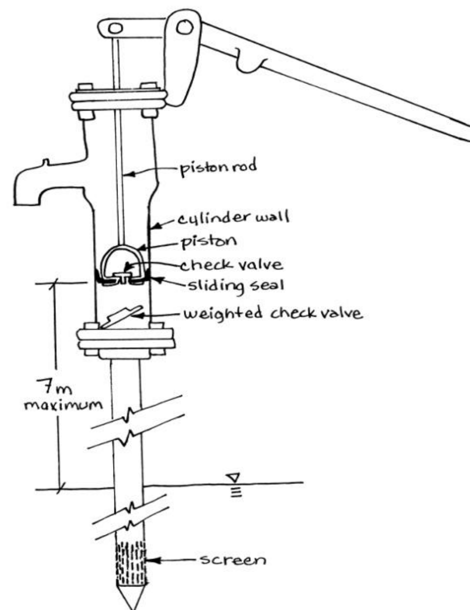


Figure 2: Schematic of the Pitcher Pump system (Reprinted from Mihelcic et al., 2009, with permission from Linda D. Phillips)

---

[1] [1] Self-supply is typically a low-cost technology which is used to extract shallow groundwater or to collect rainwater. The driving force behind this movement is through user investment and the users' interest to increase their access to an affordable and convenient water supply. An important component of this movement is the fact that the households cover the full cost of the systems (MacCarthy et al., 2013a).

It is generally accepted that Pitcher Pump systems are limited to a maximum lift (top of the water table to pump head valves) of 7 m (Baumann, 2011). Current research by Katherine Marshall, a student in the University of South Florida (USF) Peace Corps Master's International (PCMI) program, however, indicates that greater lifts can potentially be achieved. She has found that the distance is dependent on temperature and elevation, and can possibly reach 8.8 to 10.1 m (Marshall, 2014). Field evidence from Madagascar matches these values.

The Pitcher Pump system was introduced to Madagascar in the 1960s by a French expatriate. Approximately 50 small businesses are producing this technology in Tamatave and an estimated 9,000 pumps are currently in use throughout the city. Pumps generally cost around US$35-100, with much of the cost dependent on the depth that the well pipe is installed (MacCarthy et al., 2013a). In a 2011 survey, MacCarthy et al. (2013a) found that 100% of the 53 households interviewed purchased the pumps themselves without any subsidy.

Despite the success of the Pitcher Pump systems in Madagascar, some concerns remain. While Pitcher Pumps are considered an 'improved' drinking water technology, this does not guarantee that the water they produce is safe for consumption without treatment. In a preliminary sampling event conducted in 2011, 45.1% (23 of 51) of the systems sampled had thermotolerant coliform levels above 10 cfu/100 ml (MacCarthy et al., 2013a). This is important because the Malagasy standard for drinking water is no more than 10 cfu/100 ml of thermotolerant (fecal) coliforms (PAEPAR, 2005). This means that many of the Pitcher Pump systems did not produce water that is considered safe to drink.

1.2.2 The Need for Water Quality Testing

The detected contamination of the groundwater provided by the Pitcher Pump systems highlights a major flaw associated with MDG Target 7C. While it aims to improve access to safe drinking water, there are no guidelines related to the actual chemical or microbial quality of the water. Instead, as stated previously, it uses the proxy measure of 'improved' water source and equates that to 'safe'

drinking water. Safe drinking water is water that has acceptable levels of microbial and chemical contamination (see WHO, 2011 for these guidelines). While 'improved' water sources genearlly offer more protection than unimproved sources, it is not a guarantee that the water they provide will be safe for consumption without further treatment. For example, the JMP in 2010 estimated that only 11% of the global population was using unimproved water sources, yet, during that same year, Onda et al. (2012) estimated that 28% of the global population was using unsafe water. The difference between these two estimates shows that 'improved' doesn't always mean 'safe'. This is discussed further in Section 2.2.1. The UN acknowledges that proxy measures are used because the microbial and chemical testing of water is expensive and logistically complicated (UNICEF/WHO, 2012). This indicates that there is a need for "rapid, reliable, and cost effective ways of measuring water quality locally" (UNICEF/WHO, 2010).

The standard method for determining the quality of drinking water is to test for fecal indicator bacteria (FIB), specifically total and thermotolerant coliforms, in a laboratory using a membrane filtration method (USEPA, 2002; APHA, 2012). The use of these organisms will be discussed in more detail in Section 2.2.2. These standard methods require laboratory equipment and materials that are not always available to, or within the budgets of, local non-governmental organizations (NGOs) and local health ministries working in the area. It is therefore crucial to identify possible substitute methods that can produce reliable and accurate results using low-cost equipment.

1.3 Objectives

The overall goal of this research was to identify specific conditions that were having a negative impact on the quality of water produced by Pitcher Pump systems in Tamatave, Madagascar. The site is a sandy, urban, developing world city. A secondary goal was to identify alternative ways to test for FIB when working in resource limited areas and with a limited budget. Two specific objectives were carried out to achieve this goal.

The first objective was to investigate if the depth at which a Pitcher Pump system was installed had any effect on the microbial quality of the water it produced. A secondary objective was to identify any factors that could be leading to source water contamination. This work was done in two phases in Tamatave, Madagascar. Phase I consisted of a mass sampling event of 61 wells spread out over six neighborhoods. For Phase II, a pilot study was performed at one of the sites chosen from Phase I. To accomplish this pilot study, four monitoring wells were installed at depths of 6.5, 7.7, 8.7 and 9.4 m bgs, respectively.

The second objective was to compare several simple, inexpensive, portable test kits for FIB testing to see if they could be substituted for standard methods in the field. This work was done in two phases at the USF campus. Phase I consisted of analyzing the cost and performance of three different test kits, IDEXX Colilert Quanti-trays® (Colilert), Micrology Laboratories, Coliscan® Membrane Filtration (Coliscan MF) tests and 3-M Petrifilm™ Coliform/*E. coli* at standard (35°C) and non-standard (22°C and 44.5°C) temperatures. Phase II consisted of studying the effect of analyst experience on the performance of the three test kits.

CHAPTER 2: EVALUATION OF THE EFFECT OF PITCHER PUMP SYSTEM DEPTH ON WATER QUALITY AND IDENTIFICATION OF FACTORS LEADING TO SOURCE WATER CONTAMINATION

2.1 Introduction

Work for this portion of the thesis took place in Tamatave, Madagascar and was a follow up to a study conducted on unsubsidized self-supply in eastern Madagascar (see MacCarthy et al., 2013a). Briefly, water quality analysis was performed on 51 Pitcher Pump systems in both Tamatave, and the neighboring town of Foulpointe. Results of this analysis showed that 72.5% (37 of 51) of the Pitcher Pump systems contained some level thermotolerant coliforms, with 41.5% (23 of 51) of the samples above 10 cfu/100 ml. This is important because, as stated previously, the Malagasy drinking water standard states that the level of thermotolerant coliforms in drinking water should be less than, or equal to, 10 cfu/100 ml (PAEPAER, 2005). This indicated that the groundwater was being contaminated by leaching from on-site sanitation facilities or from surface infiltration. A possible relationship between the depth of the Pitcher Pump system and water quality was observed. As Figure 3 shows, the deeper wells (>7 m bgs) in the study area contained lower thermotolerant coliform counts than the shallower wells (<7 m bgs) (Figure 3). Specifically, none of the wells deeper than 7 m showed thermotolerant coliform concentrations above 10 cfu/100 ml. This was a potentially important finding, because it suggested that safe drinking water (i.e. thermotolerant coliform concentrations of less than 10 cfu/100 ml) might be achieved by increasing the depth at which the Pitcher Pump systems draw water.

However, the cost of a Pitcher Pump system is heavily influenced by the depth at which the well is installed (i.e. the cost increase as the depth increases). Therefore, in order to keep the cost down, Pitcher Pump systems are generally only installed 1-2 m below the dry season water table. As these are unsubsidized systems often times purchased by individual families, this low cost is very important. This

research further investigated the effect of the installation depth of a Pitcher Pump system, on water quality in an urban, developing country context. Additional factors such as the neighborhood where the samples were collected, whether or not the pumps required priming, the frequency of repairs, distance from on-site sanitation, and number of households using the system, were considered also, to see if they had any influence on the water quality.

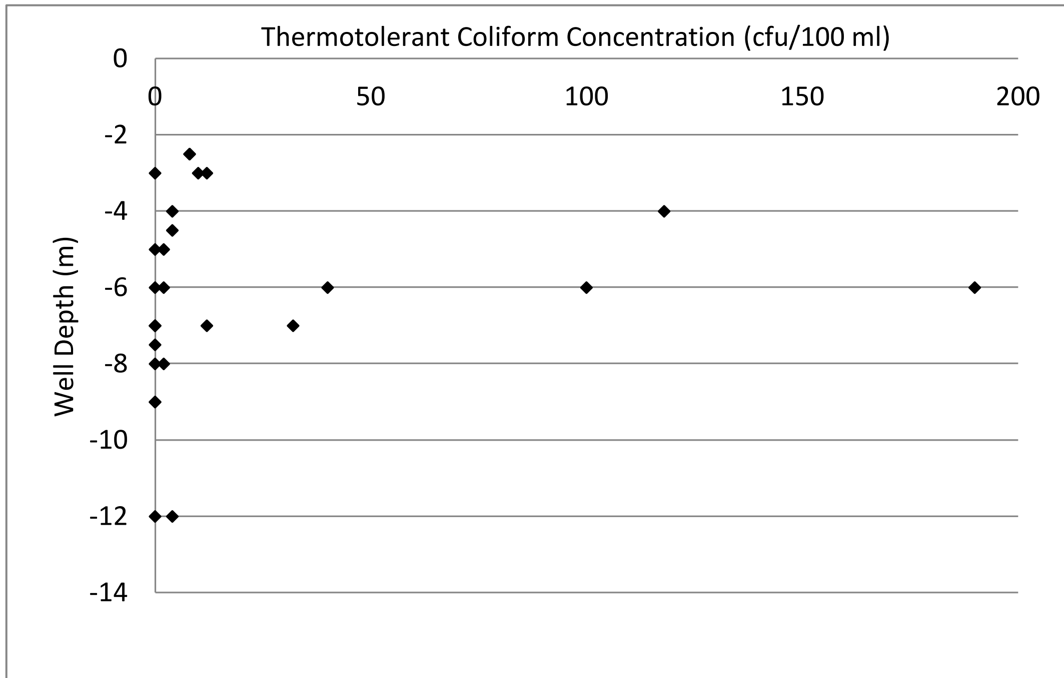


Figure 3: Relationship between thermotolerant coliform counts and well depth, Tamatave and Foulpointe. Results suggest that deeper wells (i.e. greater than 7 m bgs) may provide a higher quality of water. Data were collected and analyzed by USF Global Health doctoral student, James McKnight during a 2011 trip to Tamatave (Mihelcic, J. and MacCarthy, M. memo to CRS and CARE, 28 May, 2013).

This study was conducted as a partnership between USF and RANO HamPivotra (HP) (Water for Progress). Rano HP is a USAID (United States Agency for International Development) funded project in Madagascar implemented by a consortium led by the NGOs CRS (Catholic Relief Services) and CARE (Cooperative for Assistance and Relief Everywhere). Research investigating the potential for lead (Pb) leaching from these same Pitcher Pump systems was conducted at the same time as this investigation by a fellow USF PCMI student, D. Brad Akers (Akers, 2014).

2.2 Literature Review

2.2.1 Improved Water Sources and Contamination

As stated in Section 1.1, WHO and UNICEF use the designation 'access to improved water source' to mean access to safe drinking water. They use this designation because it is assumed that, due to the superior design and/or quality of construction, water supplied by an improved source is safe for consumption without further treatment (WHO/UNICEF, 2012). In contrast, the WHO and UNICEF assume that water from an unimproved source is automatically contaminated with either microbial or chemical pollutants. The extent to which the assumed lack of contamination of an improved source is actually true was tested when WHO and UNICEF implemented a Rapid Assessment of Drinking-Water Quality (RADWQ) pilot study in five countries to measure the quality of water from 'improved' sources. The five countries in which the study took place were Ethiopia, Jordan, Nicaragua, Nigeria, and Tajikistan (Aldana, 2010; Aliev et al., 2010; Ince et al., 2010; Properzi, 2010; Tadesse et al., 2010; Bain et al., 2012). Based on the results of the RADWQ, the JMP estimate of the percentage of the population with access to clean drinking water decreased by 11% in Ethiopia, 16% in Nicaragua, 15% in Nigeria, and 7% in Tajikistan, with Jordan showing only a slight reduction (Bain et al., 2012). The majority of the decrease was due to microbial contamination, with a small amount attributed to chemical contamination. Contamination also varied widely amongst the types of water sources tested. For example, in Nicaragua only 10.1% of the samples collected from public piped water sources were contaminated with thermotolerant coliforms, whereas 60.9% of community water systems, 54.3% of borehole/tubewells, and 80.7% of the protected wells were contaminated (Aldana, 2010).

Onda et al. (2012) used the same RADWQ data to calculate the percentage of the global population using unsafe drinking water. The authors estimated that, rather than the 11% (780 million people) stated by the JMP, the real percentage was closer to 28% (1.8 billion people). They then added in sanitary risk assessment scores to calculate the number of people who receive water from a source

that had an elevated risk of contamination. A sanitary risk score is calculated by answering ten yes or no questions related to the study site. If a “yes” is answered then that means there is a risk present, and a score of one is assigned. “No” means the risk is not present and results in a score of zero for that question. A final score of 0-3 is considered low risk, 3-5 is medium risk, 6-8 is high risk, and 9-10 is very high risk (MacDonald et al., 2005). Based on the sanitary risk scores, they estimated that an additional 18% of the global population (1.2 billion people) was receiving water from sources or systems that presented significant sanitary risks (based on having greater than two of the common sanitary risks). This number could go as high as 3 billion if they used the most stringent guidelines of zero cfu/100 ml of fecal coliforms and low sanitary risk score. While these are by no means exact numbers, it does highlight the dangers of classifying a water source as safe based only on the manner of their design.

2.2.2 Measuring Microbial Risk

Fecal matter is known to carry a number of pathogens, most notably viruses, bacteria, protozoa, and helminths (worms) (Lewis et al., 1980). One gram of feces can contain as many as 10 million viruses, 1 million bacteria, 1,000 parasite cysts and 100 parasite eggs (Jenkins, 2005). When looking at possible contaminants of groundwater from fecal matter, protozoa and helminths, which are larger in size (>25 µm), tend to be filtered out fairly quickly in the soil, and are therefore generally not a concern (Lewis et al., 1980). Viruses and bacteria are much smaller and have more of a potential to impact a drinking water source (0.01 - 0.25 µm and 2.0 - 2.5 µm, respectively) (Lewis et al., 1980). Some bacteria and viruses found in fecal matter include Hepatitis A and E, Astrovirus, Calcivirus, Rotaviruses, Norwalk-type virus, Coxsackieviruses, Echoviruses, *Campylbacter jejuni*, various strains of *E. coli*, *Salmonella typhi*, *Shgellae spp.*, and *Vibrio cholerae* O1(BGS, 2002). When testing the quality of a water source, it would be costly and time consuming to test for all the types of pathogens listed above; therefore, the use of an indicator organism is generally recommended. The EPA requires that these organisms provide “evidence of the presence or absence of a pathogenic organism surviving under similar physical, chemical, and

nutrient conditions" (EPA, 2013). The EPA listed the following criteria for an ideal indicator organism (EPA, 2013):

1) Be easily detected using simple laboratory tests.
2) Generally not be present in unpolluted waters.
3) Appear in concentrations that can be correlated with the extent of contamination.
4) Have a die-off rate that is not faster than the die-off rate of the pathogens of concern.

Total and thermotolerant coliforms are the most common indicator organisms. Of the thermotolerant coliforms, *E. coli*, which is found in 94-100% of human feces, is the most used (Tallon et al., 2005). However, a number of studies have shown weak or limited correlation between the presence of thermotolerant coliforms and *E. coli* and incidents of diarrheal disease (Jensen et al., 2004; Brown et al., 2008). When a strong correlation has been observed, it has generally been with *E. coli* at levels greater than 10^3 cfu or MPN/100 ml (Moe et al., 1991). Additionally, some studies have shown that these organisms might have the ability to survive and, in some cases, even multiply in tropical freshwater and soils (Carrillo et al., 1985; Lopez-Torres et al., 1987; Fujioka et al., 1988, 1999; Harding & Fujioka, 1991; Byappanahalli & Fujioka, 1998, 2004; Solo-Gabriele et al., 2000; Desmarais et al., 2002). For example, Fujioka et al. (1988) found high concentrations of thermotolerant coliforms in the soils and waters of Hawaii, away from human settlement. They argue that this contamination could not have come from animal deposits alone, because that would require large, consistent amounts of feces, yet no large animals are found in Hawaii. In another study, Byappanahalli and Fujioka (1998) found that *E. coli* and thermotolerant coliforms had the ability to multiply in sterilized soils spiked with primary-treated sewage. They also found that these organisms could grow on unsterilized soils but simple nutrients needed to be added first, indicating that other organisms were outcompeting the *E. coli* and thermotolerant coliforms when resources were limited. This last observation was noted in a later study by the same authors, though in this study they stated that the *E. coli* was robust enough to establish

itself as a minor population in the soil, despite the competition with the native microflora (Byappanahalli & Fujioka, 2004).

Despite this, *E. coli* is the preferred FIB of the WHO (WHO, 2011). When it is not possible to test for *E. coli*, thermotolerant coliform (sometimes referred to as fecal coliforms) have been determined to be an acceptable alternative. Usually, thermotolerant coliforms are most consistently *E. coli* (94-96.8%), but they can be other species as well such as *Klebsiella, Enterobacter,* and *Citrobacter* (Tallon et al., 2005).

2.2.3 Sources and Pathways of Contamination

As previous studies showed, despite their more advanced design, improved water sources still have the potential to become contaminated. This is especially true in areas where people get their drinking water from shallow wells, which are more at risk of being affected by the chemical and/or microbial contamination found at [or just below] the ground surface (Melian et al., 1999). Two main pathways by which fecal matter can enter a drinking water source are localized and aquifer pathways (BGS, 2002) (Figure 4).

Localized pathways come about through poor design and construction of the groundwater extraction system. This can allow for the direct entry of surficial sources of fecal matter into the water source (BGS, 2002). Examples of these sources of fecal matter are livestock, land application of organic wastes, on-site wastewater treatment, and solid waste landfill sites (Hyndes et al., 2012). Based on my experience in Madagascar, specifically at my Peace Corps site, open defecation should also be included on this list.

Localized pathways have been identified as a main pathway for contamination in several published studies. The most common reasons for the existence of this route are, improper design/construction (Howard et al., 2003; Cronin et al., 2006; Hynds et al., 2014), poor well maintenance (Gelinas et al., 1996; Howard et al., 2003; Cronin et al., 2006; Godfrey et al., 2006; Gonzales et al., 2008),

poorly sealed annulus (Godfrey et al., 2006), absent, cracked, or improperly constructed aprons (Godfrey et al., 2006; Escamilla et al., 2013; Oluwasanya, 2013; Hynds et al., 2014), and poor drainage away from the well head (Godfrey, et al., 2006; Oluwasanya, 2013). Briefly, aprons are impermeable surfaces that are constructed around a water point in order to protect the water from surface contamination infiltrating directly next to the structure (Skinner, 2012). However, the presence or absence of protective structures doesn't necessarily always correlate to higher levels of contamination. For example, van Geen et al. (2011) did not find any systematic difference in the detection of *E. coli* between tubewells with or without a concrete apron, though they did state that the pathway shouldn't be ruled out. Localized pathways can be easily avoided by proper design and construction of the water supply system as well as with proper maintenance (BGS, 2002).

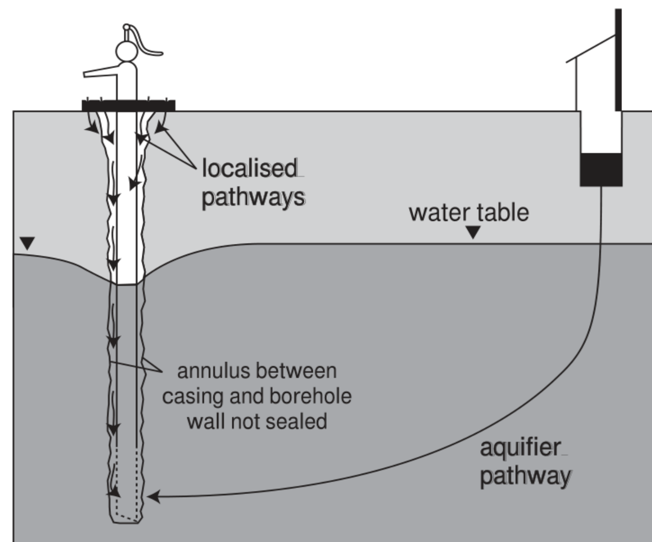


Figure 4: Pathways of microbial contamination. The two main pathways in which contamination can enter a drinking water extraction system are localized and aquifer. (Reproduced from BGS, 2002).

With an aquifer pathway, microorganisms leach out the base of a sanitation system and travel through the subsurface until they reach the drinking water extraction point (BGS, 2002). In Tamatave, Madagascar, common types of on-site sanitation facilities are simple pit latrines, simple pit latrines lined with metal drums, pour-flush latrines, and septic systems (Practica, 2012) (Figure 5a, b, and c).

Figure 5: Common forms of on-site sanitation facilities found in Tamatave, Madagascar. a) Simple pit latrine (picture taken by the D. Brad Akers); b) simple pit latrine lined with a metal drum (picture taken by the author); c) pour-flush latrine (picture taken the author)

It is much harder to determine if the water at the extraction point is being contaminated via the aquifer pathway because there is no visible evidence of broken or missing components like there is with localized pathways; however, through observations, tracer studies, and data analysis, many authors have linked on-site sanitation systems to the impaired groundwater quality measurements at their study site (see Graham & Polizzoto, 2013 for an extensive literature review). Some example studies are shown here. Using groundwater flow coupled with microbial and chemical analysis, Dzwario et al. (2006) concluded that pit latrines were contaminating the groundwater up to 25 m away in the Marondera district of Zimbabwe. The great distance can possibly be attributed to a sandy soil and thin unsaturated zone below the base of the pit. How those components can lead to greater groundwater contamination

is explored further below. Using physiochemical grouping and bivariate risk factor analysis, Hynds et al. (2014) found that septic tank setback/gradient was an important factor contributing to the frequency in which wells were contaminated in Ireland. As the lateral distance from the septic system increased, the likelihood of contamination decreased. Other factors associated with localized pathways were noted, but septic tank setback was the only thing consistent across the study groups. Using a combination of epidemiological, microbiological, and hydrogeological methods, a leaky septic tank system was found to be the primary cause of a groundwater-related Norovirus outbreak in Wisconsin (Borchardt et al., 2010). Pujari et al. (2011) tested physical and chemical parameters and concluded that on-site sanitation facilities at their study sites in India were affecting the quality of groundwater at a site with shallow wells where the subsurface was hard rock.

The degree to which a sanitation system will potentially contaminate groundwater largely depends on the soil and subsurface hydrology, though other factors such as distance between the two structures will play a role as well. The actual removal mechanisms will be discussed further in Section 2.2.4 but, in general, whether or not a drinking water source will become contaminated is determined by how quickly the pathogens reach that point. This rate of transport is a function of soil type and local hydrology, and can be broken up into two zones, saturated and unsaturated (BGS, 2002). Pathogen movement in the unsaturated zone has been found to be much slower than in the saturated zone (Cave & Kolsky, 1999). For example, common reported values for flow through the unsaturated zone generally do not exceed 0.2 to 0.3 m/d, whereas flow through the saturated zone is generally on the order of 2 m/d or less, but has been found to reach 10 to 100 m/d (Lewis et al., 1980; Cave & Kolsky, 1999; BGS, 2002; MacDonald et al., 2005). The differences in flow rates are related to how the pathogens move through the subsurface. In the unsaturated zone, they move downward with the infiltrating water through interconnected saturated pores via gravity, as well as along the soil particle surface (Lewis et al., 1980). In the saturated zone, the pathogens move mainly with the groundwater through fully saturated

pores (Lewis et al., 1980) (see Figure 4). The much slower travel times in the unsaturated zone highlight its importance to microbial die-off (described in Section 2.2.4.2). As Lewis et al. (1980) stated, “the unsaturated zone is the most important line of defense against fecal pollution of aquifers.” In order to achieve the greatest amount of time in this zone, sanitation pits should be kept well above the water table (Cave & Kolsky, 1999). There are various recommendations for what this distance should be. In Madagascar, the guideline is a minimum of 2 m between the base of the pit and the water table in sandy soils. Other sources say a minimum of 1.5 m for basic pit and VIP latrines, and 3 m for a pour flush latrine (Mihelcic et al., 2009). MacDonald et al. (2005) on the other hand, calls for separation based on specific soil type. The authors state that, in order to be protective of groundwater, less than 5 m is required in fine silt, sand, and clay; a minimum of 5-10 m is needed in weathered basement and medium sand; and, greater than 10 m is needed in coarse sands and gravel, sandstones, limestones, and fractured rock.

As stated previously, soil type will influence the rate at which the effluent and groundwater travels through the soil. In general, movement through fine grained soils is slower than through coarse grained. For example, Banerjee (2011) noted that chemical pollution traveled a maximum of only 2.1 m in 10 days in clayey silty soil, but in gravel-sand soils that distance increased to 10.2 m for that same time period. MacDonald et al. (2005) offers the following hydraulic conductivity values for different soil types:

Table 1: Typical range of hydraulic conductivity (m/d) based on soil type. (Adapted with permission from MacDonald et al., 2005)

Subsurface	**Typical hydraulic conductivity (m/d)**
Silt	0.01-0.1
Fine silty sand	0.1-10
Weathered basement (not fractured)	0.01-10
Medium sand	10-100
Gravel	100-1000
Fractured rocks	Difficult to characterized but 10s to 100s

The effects of localized and aquifer pathways can be compounded by increased population density (Howard et al., 2003; Nsubuga et al., 2004; van Geen et al., 2011; Escamilla et al., 2013; Wright et al., 2013). For example, Escamilla et al. (2013) found that population count and *E. coli* were significantly correlated ($p<0.05$) and that as latrine and population count increased, so did the frequency at which *E. coli* was detected. They also calculated that, for every additional unsanitary latrine added within approximately 40 m of a well, the *E. coli* detection frequency increased by 1.5%. Nsubuga et al. (2004) found that protected springs in high-density settlements showed higher levels of both chemical and biological pollution, which they linked to the presence of more pit-latrines, animals, and other wastes. Pit latrine density was significantly correlated with levels of chloride and nitrates at a study site in Kenya, suggesting that the higher population densities were leading to increased groundwater contamination (Wright et al., 2013). When less densely populated areas have shown higher levels of contamination then densely populated areas, it is usually related to a difference in the type of source water. For example, Musa et al. (1999) noted higher levels of contamination in rural communities than in urban communities, but the water sources varied dramatically between the two. In the rural areas, people were using either water from the river or from ponded rainwater. In contrast, those in the urban settings were receiving piped water that had been treated, or water from a tubewell.

2.2.4 Removal Mechanisms

The subsurface has long been used as a means for purifying water contaminated with human and/or animal waste (Lewis et al., 1980). Its remediation capability is the reasoning behind drainfields and slow sand filtration. This section focuses specifically on the aquifer pathways of contamination and the ways that contamination can be mitigated before it has the chance to reach the drinking water extraction point. The risk that on-site sanitation presents can vary drastically from site to site depending on the subsurface conditions. The actual removal of pathogens is a function of physical removal and die-off.

2.2.4.1 Physical Removal Mechanisms

Physical removal mechanisms include filtration (sometimes called straining) and adsorption. Most prior research hasn't separated these two mechanisms so they will be discussed together here. Filtration is the physical removal of pathogens as they move through the soils (Bitton & Gerba, 1994). Fine grained soils such as silts, clays, and fine sands have been found to be more effective than coarse grained soils at filtering out pathogens in the subsurface (Karathanasis et al., 2007); though, in general, filtration only becomes significant when the average size of the pathogen is greater than 5% of the average pore size (Ginn et al., 2002).

More so than within the subsurface, greater removal of pathogens has been found at the infiltration surface (Lewis et al., 1980). In slow sand filtration this is known as the Schmutzdek layer and accounts for 98% of the removal of pathogens with a size of 1-60 µm (AWWA, 1990, as reported in Mihelcic et al., 2009). In studies related to pathogen removal from on-site sanitation systems, this layer is often called the "clogging zone". Three main processes are thought to contribute to pore clogging (BGS, 2002):

1) The blockage of pores by solids that are filtered directly out of the effluent.
2) The accumulation of the biomass from the growth of the accumulated microorganisms.
3) The production of slimes by certain bacteria.

The phenomenon of the clogging zone was demonstrated during a field experiment conducted in Alabama that was designed to study the effects of groundwater pollution from a borehole latrine (Caldwell & Parr, 1937). The authors noted that *Balantidium coli* was detected at distances up to 10 m (35 ft) away from the newly installed latrines in the beginning of the experiment but then after the first 3 months, the flow was greatly restricted. Within 7 months, movement of the bacteria was limited to the area just around the pit. The same observation was made in parallel investigations (Caldwell, 1937, 1938a and 1938b). Another example of the clogged zone was noted in a study by Ziebell et al. (1975) (as

reported in BGS, 2002) who found that bacterial populations were reduced drastically 30 cm after the clogged zone. When looking at the establishment of the clogged zone, Caldwell (1937) noted that it takes longer in a pit latrine than with a bored latrine, which she attributed to the greater volume per depth of penetration in the pit latrine.

Adsorption is another process by which pathogens can be immobilized in the subsurface and involves the attachment of the organism to the soil surface (Bitton & Gerba, 1994). While more effective on smaller bacteria than large, it is a process that still must be considered (Bitton & Gerba, 1994). Under most natural pH conditions, both the pathogens and soil surfaces have negative charges and, therefore, there is a tendency for the two to repel each other (BGS, 2002). Some soils, such as allophonic and pumice sand, however, are fairly good at removing bacteria because they have an affinity for the negatively charged particles (Pang, 2009). Adsorption generally increases as clay content increases because of the large surface area per volume that the clay particles have, although some of the noted removal could also be from filtration (Lewis et al., 1980). Adsorption also increases as the cation concentration increases and increases as the pH decreases (Lewis et al., 1980). One very important point is the fact that adsorption is reversible. Often after heavy rainfall, the microbes that were adsorbed are released, which is one reason the levels of microorganism detected in the water source generally increases after it rains (BGS, 2002).

Despite their increased capacity for filtration and adsorption, clayey and silty soils may not always be the most desirable for pathogen removal. This is due to their tendency to form macropores and their large moisture holding capacity (Pang, 2009). Ultimately, Pang (2009) concluded that allophanic soils, pumice sand, fine sand, and highly weathered aquifer rocks were the most effective in removing pathogens and structured clayey soil, stony soils, coarse gravel aquifers, fractured rocks, and karst limestones were the least effective.

2.2.4.2 Die-off

Survival time of fecal bacteria varies widely between species and is influenced heavily by environmental conditions (Lewis et al., 1980). MacDonald et al. (2005) recommends a minimum residence time of 25 days for these organisms in the unsaturated zone in order to be protective of the groundwater; however, much longer times have been noted. Rudolfs et al., (1950) provides a thorough literature review on the survival time of several pathogens and reports a range of several days to several hundred days, depending on study conditions. The rate of die-off for a pathogen is a function of, among other things, moisture content, temperature, pH, organic content, and predation.

Of all the factors listed, moisture content is one of the most influential. Survival time generally increases as moisture content increases (Beard, 1940; Kibby et al., 1978; BGS, 2002). For example, Beard (1940) found that survival time of *Eberthella typhosus* was longer during the rainy season than the dry season across all soil types tested, except for peat. A similar observation was made during an investigation into the survival of *Streptococcus faecalis* in the soil under different moisture and temperature conditions. The time it took to achieve a 95% reduction was greater under moist conditions (e.g. 53 days at 25°C) than dry (e.g. 9 days at 25°C), and this was consistent across all temperatures tested (Kibby et al., 1978). Survival time is also greater in soils with a higher moisture retention capacity. In the same experiment, dry season survival was greater in soils with a higher moisture retention capacity. For example, *E. typhosus* was still detected at 21 days in adobe soil, but died off sometime between two and seven days in sand

The other dominant factor in determining the rate at which pathogens will die-off is temperature. In general, increased temperature results in decreased survival time (McFeters & Stuart, 1972; Kibby et al., 1978; Reddy et al., 1981). Kibby et al. (1978) found that *S. faecalis* survival times were greater at lower temperatures across all moisture conditions. For example, when the soil was at field conditions, the time required for a 95% reduction was approximately 60 days at 4°C, 43 days at 10°C, 38

days at 25°C, and 16 days at 37°C. Using data collected from other studies, Reddy et al. (1981) calculated that the die-off rate of various types of bacteria nearly doubles with each 10°C rise in temperature, specifically when looking at a range of temperatures between 5 and 30°C. McFeters and Stuart (1972) found that the amount of time that it took to achieve a 50% reduction in *E. coli* populations decreased as the temperature increased. The biggest effect they noted was at temperatures between 5 and 15°C. Above 15°C, an increase in temperature also reduced survival time, but the change in survival time was not as significant. One exception to this was noted in a study by Jiang et al. (2002). When studying the survival time of *E. coli* D157:H7 in manure-amended soils, survival time increased as the temperature increased.

Soil pH will also affect the survival time. McFeters and Stuart (1972) studied the effect of pH on the survival time of *E. coli*. The authors found that survival was greatest when the soils were near neutral (pH 6-7) and decreased away from that range. Reddy et al. (1981) concluded the same thing through a literature review. Lewis et al. (1980) states that acidic soils (pH 3-5) will result in lower survival times of pathogens.

Organic content within the soil has also been found to have an effect on the survival time of the various pathogens. Increased pathogen survival times, as well as the potential for some re-growth, have been found in soils containing higher levels of organic matter (Lewis et al., 1980; Desmarais et al., 2002). For example, *E. typhosa* was found to have increased survival times (upwards of approximately 400 days) when added to the soil as a broth culture (Rudolfs et al., 1950). It is thought that this was due to the availability of nutrients within the growth media. Increased survival may not be just related to nutrients, however, but also the fact that organic matter is better at retaining moisture (Tate, 1978). In addition, soluble organics can sometimes compete with the pathogens for adsorption sites causing a decrease in the adsorption of pathogens and sometimes even the release of those pathogens already adsorbed (Lewis et al., 1980).

Natural predators in the soil have also been found to cause a reduction in the bacterial population (Tate, 1978; Byappanahalli & Fujioka, 1998, 2004; Sørensen et al., 1999; Jiang et al., 2002). The effect of predation was noted in a study by Jiang et al. (2002), who observed that *E. coli* survived longer in manure-amended soil that had been autoclaved as compared to un-autoclaved soil. The authors concluded that this was likely due to the removal of the natural predators within the soil. When adding *E. coli* to a muck soil, Tate (1978) noted a six-fold increase in the natural protozoa population which was associated with a decrease in the *E. coli* population. Sørensen et al. (1999), noticed a similar inverse relationship when they increased the amount of *E. coli* K12 being added to the soil. When the authors added a eukaryotic inhibitor which killed the indigenous eukaryotic organism, the survival of *E. coli* increased.

2.3 Materials and Methods

The study site, which is detailed in Section 2.3.1, was the urban city of Tamatave, Madagascar. As stated previously, the objective of the research was to investigate if the depth of the Pitcher Pump systems had an effect on the microbial water quality. A secondary objective was to identify factors that could be leading to source water contamination. In order to achieve this, data were collected into two phases.

Data for Phase I were collected in December, 2012 in seven different neighborhoods throughout the city of Tamatave. Techniques used to collect the information consisted of household surveys, observations of water and sanitation infrastructure, and analysis of water quality for thermotolerant coliforms. More detail on Phase I can be found in Section 2.3.2.

Data for Phase II were collected in January and April, 2013 at one specific site in Tamatave chosen during Phase I. Techniques used to collect the data consisted of the installation of four monitoring wells at varying depths and analysis of water quality for thermotolerant coliforms. More detail on Phase II can be found in Section 2.3.3.

The majority of the data collected during Phases I and II were analyzed upon return to the USF campus. Data were evaluated using a variety of non-parametric and parametric tests to analyze nominal, ordinal, and scalar data. More detail on data analysis can be found in Section 2.3.4.

2.3.1 Study Site

Tamatave is the capital of the Atsinanana (east) region of Madagascar and is the chief seaport of the country (Figure 6). The estimated population of Tamatave in 2013 was calculated to be approximately 280,000 people (MacCarthy et al., 2013a), with the main ethnic group in the region being Betsimsaraka (Davies, 2008). The economic growth rate in Madagascar has been slow (approximately 1.9%) owning mainly to a coup that occurred in 2009, which left the country in turmoil and foreign investors wary (CIA, 2014a). In comparison, the growth rate of the economy before the crisis was approximately 5% per year. This reduction in growth rate is thought to have led to a loss of US$8 billion from the Malagasy economy (The World Bank, 2013). A new president, Hery Martial Rakotoarimanana Rajaonarimampiania, was elected in 2013; however, it is too soon to know what effect he will have on the economy (CIA, 2014a).

While there is no specific rainy season, the wetter months are from December to March and the dryer months are from September to November (Davies, 2008). Average yearly rainfall is around 2,000 to 3,000 mm/yr (Davies, 2008). Observations by the author during the installation of monitoring wells indicate that the soil profile consists of medium grained, subangular sand to at least 4.2 m bgs (Unified Soil Classification System [USCS] = SM). After that point, soil classification was no longer feasible. Depth to groundwater was measured in December, 2012 and indicated that the water table is shallow. The median depth was 4.1 m, with a range from 3.2 to 9.0 m bgs. This is similar to what was found by Rakotondrainibe (2005), who reported that groundwater was generally between 2-3 m bgs. Hydraulic conductivity in fine and medium grained ranges from 0.1 to 10 and 10 to 100 m/d, respectively (MacDonald et al., 2005). As the sands tended more to the medium grain size, the hydraulic conductivity

is probably on the lower portion of the medium sand grain range (10-50 m/d). Attempts were made in the field to calculate the recharge rate of the monitoring wells installed during Phase II, but these attempts were not successful.

Figure 6: Map of Madagascar with the study site highlighted (adapted from CIA, 2014b).

As stated in Section 1.2.1, there are an estimated 9,000 Pitcher Pump systems currently in use in Tamatave, serving 170,000 people (MacCarthy et al., 2013a). Again, MacCarthy et al. (2013a) detected contamination above the Malagasy drinking water standard in 45.1% of the 51 Pitcher Pump systems tested. If you apply that percentage to the total number of Pitcher Pump systems, and assume that 75% of the households drink water from these sources (MacCarthy et al., 2013a), an estimated 57,503 people could be consuming water that is considered unsafe by Malagasy standard. Piped water is available throughout the city and is maintained by JIRAMA, who supplies water and electricity to some areas of Madagascar. Sampling conducted by James McKnight and Michael MacCarthy, as well as by the author, indicated that JIRAMA water was free of thermotolerant coliforms. During household interviews conducted as part of the 2011 investigation by Michael MacCarthy, many homeowners expressed interest in becoming connected to the piped water supply but, at an estimated minimum cost of US$215 (based on 2013 prices), this was out of their price range (MacCarthy et al., 2013a). Public taps are

located throughout the neighborhoods and people are able to purchase water by the bucket. Prices are around US$0.03 per 20-L jerry can or US$0.02 per 10-L bucket; however, this is still too cost prohibitive for many households (Ranaritsera, personal communication to D. Brad Akers, 2012).

On-site sanitation facilities could be one possible source of groundwater contamination. Tamatave is an urban city with around 97% of the population using latrines as their means of excreta removal (Practica, 2012). This translates to approximately 21,900 latrines (Practica, 2012). While the recommended minimum distance between the on-site sanitation system and water source in Tamatave is 10 meters (PAEPAR, 2005), it is often not possible to achieve this due to lack of space. Other possible sources of pollution, such as chickens, open defecation, and garbage piles/pits, can also be found on or near properties with Pitcher Pump systems. These and many more factors could be leading to the contamination of the wells in the area.

2.3.2 Phase I

Phase I was implemented for several reasons. The first was to gain a general understanding of the quality of water provided by the Pitcher Pump systems. Second was to expand on the observation made by Michael MacCarthy and James McKnight about the relationship between depth and water quality, with the ultimate goal of determining if there was a well depth that would consistently provide water with thermotolerant coliform concentration below 10 cfu/100 ml. The final goal was to identify specific factors that could impact the water quality. As stated previously, data were obtained using surveys, observations, and water quality measurements.

2.3.2.1 Household Visits

Surveys were developed by D. Brad Akers with the intent of collecting basic quantitative and qualitative data regarding household demographics, water usage and treatment, sanitation infrastructure, and the Pitcher Pump systems (Akers, 2014). The Institutional Review Board (IRB) administered by the Human Research Protection Program (HRPP) was not involved in the development

of the survey. This was based on the fact that initial research by Michael MacCarthy was submitted to IRB and was ultimately determined to not be human subject research. This meant that it did not require IRB approval. Upon return to the USF campus, concerns were raised that some of the questions asked during this investigation regarding water usage and treatment could be construed as data collected on "human subjects". The IRB was consulted and permission was given to use most of the data collected (see Appendix A for e-mail correspondence). Data which were deemed unacceptable by the IRB were neither included in the analysis nor reported anywhere in this thesis.

Malagasy culture dictates that permission be given by the local leader (knows as *Chef du Fokontany*) in order to perform any work in the areas under his control. Therefore, upon entering each new neighborhood, that specific *Chef du Fokontany* was consulted to his gain approval of our study. Once permission was given, a first site was identified. The only requirement of the first site was that it had a functioning Pitcher Pump system. Subsequent sites were identified using a 'modified snowball method'. The original snowball method involves using sample members to provide names of potential next sample members (Everitt & Skrondall, 2010). However, this proved to be problematic as the sample member would often provide the name of a neighbor. This led to households with very similar parameters which was unacceptable because this study required wells of varying depths. In addition, houses were visited along with D. Brad Akers who had his own set of criteria which needed to be met (different pump age, manufacturer, etc.). The 'snowball method' was adapted in such a way that some sites were skipped in order to meet the requirements of differing parameters.

In total, 53 households were interviewed in seven different neighborhoods, specifically Managarivotra Sud (purple), Mangarivotra Nord (blue), Andranomadio (red), Ambalakisoa (green), Ankirihiry (orange), Antanambao Veriery (maroon), and Tanambao V (not shown) (Figure 7 and Table 2). Tanambao V was left off Figure 7 because the surveys conducted there were part of the lead leaching study by fellow USF PCMI student D. Brad Akers only, and no water samples were collected. Some of the

survey answers provided by those three households were used in general data analysis (Section 2.4.1). The surveys were implemented with the help of a local Malagasy research assistant, Marie Onnie Razifikalo (Onnie). Onnie had assisted with the previous research conducted by Michael MacCarthy, and was thus familiar with the overall project goals. The surveys were conducted in Malagasy with the answers translated back to English after they were complete. Observational data and water samples were collected by both the author and D. Brad Akers. Water sample collection procedures are discussed in more detail in Section 2.3.2.2.

Table 2: Number of surveys conducted in each of the seven neighborhoods visited.

Neighborhood	Number of Surveys
Mangarivotra Nord	9
Ankirihiry	5
Ambalakisoa	12
Antanambao Veriery	2
Andranomadio	8
Tanambao V	3
Mangarivotra Sud	14

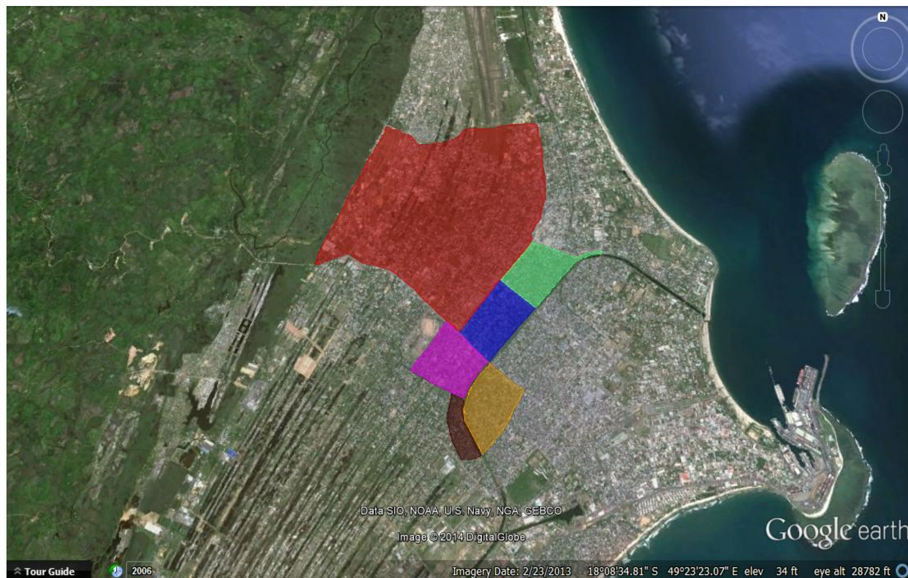


Figure 7: A map of Tamatave with the neighborhoods highlighted where samples were collected. The author has edited the GoogleEarth Map by adding in boxes to indicate the sample locations. Mangarivotra Sud = purple; Mangarivotra Nord = blue; Andranomadio = red; Ambalakisoa = green; Ankirihiry = orange; Antanambao Veriery = maroon ("Tamatave, Madagascar". MAP. SIO, NOAA, U.S. Navy, NGA, GEBCO, DigitalGlobe. 2014. GoogleEarth. Vers. 7.1.2.2041., Google, 2013).

Observational data were collected regarding the conditions of the Pitcher Pump systems and, if present, sanitation infrastructure. GPS coordinates of the Pitcher Pump systems and on-site sanitation facilities were collected using a Garmin eTrex Legend H handheld GPS device (Garmin Ltd, Olathe, KS) which was provided by the Peace Corps – Madagascar office. The coordinates were later used to determine the lateral separation between the Pitcher Pump system and on-site sanitation facility by converting the GPS coordinates into distance (in meters) using a coordinate distance calculator found online (Boulter, 1994). Lateral separation was also found by the author pacing the distance between the two structures and converting the paces into meters.

The depth of the Pitcher Pump system was recorded at each household with the depth generally being self reported by the person surveyed. In addition to reporting the depth, the owner was also asked to visually represent how long the pipe was before installation, either by indicating how tall the pipe was in reference to a tree or house, or by indicating where the base and top of the pipe were located on the ground prior to installation. If the owner could not provide a depth, provided two measurements that were not similar, or provided a depth that seemed unreasonable for the area, the interview was stopped and a new site was identified. Since the cost of the system was based partially on how deep the well was installed, it was assumed that the household had a general idea of the length of pipe installed. The actual depth at five Pitcher Pump systems was physically checked as well (Section 2.3.2.4).

2.3.2.2 Sample Collection

Water provided by the Pitcher Pump system was sampled and analyzed for the presence of thermotolerant coliforms. Samples were collected in six of the seven neighborhoods where surveys were conducted, with more systems sampled than surveys given. An additional seven Pitcher Pump systems were sampled in Mangarivotra Nord and an additional four in Mangarivotra Sud, for a total of 61 sample locations (Table 3).

Table 3: Number of water quality samples collected in six of the seven survey neighborhoods. There are seven more samples than surveys from Mangarivotra Nord and four more samples than surveys from Mangarivotra Sud.

Neighborhood	Number of Samples
Mangarivotra Nord	16
Ankirihiry	5
Ambalakisoa	12
Antanambao Veriery	2
Andranomadio	8
Mangarivotra Sud	18
Total	61

Prior to collecting the samples from the Pitcher Pump systems, approximately 2.5 well volumes were purged from the wells. Note that this is slightly below the three well volumes recommended by the EPA (Vail, 2013); however, systems were being used multiple times throughout the day, meaning that the water did not stay stagnant within the pipe for any long period of time. In addition, the well depths were almost always over reported by the owners. Well volume was calculated using the following formula:

$$V = \frac{\pi D_I^2}{4} * h * 1000 \quad (1)$$

where V is well volume (L), D_I is the inside diameter of the pipe (m), h is the height of water in the pipe (m), and 1,000 is the conversion factor from m^3 to L.

In order to obtain the inside diameter of the well, the circumference the pipe was first measured. Next that value was converted to the outside diameter. This relationship between the inside and outside diameter was found by measuring pipe that was available at the local hardware store (Appendix B).

Samples were collected in sterile, 350 ml glass jars, or, on two days, clean (but not sterilized) plastic PET bottles (Figure 8). Jars were rinsed twice with the sample water prior to collection. The jars were labeled with the sample ID, date, and time of collection. Bottles were capped and placed on ice until they could be brought back to the laboratory for analysis. All samples were analyzed within 30 hours (time requirement for *E. coli* testing) (EPA, 2009).

Figure 8: Collecting a sample to be used for the analysis of thermotolerant coliforms (picture taken by Michael MacCarthy).

2.3.2.3 Sample Analysis

Samples were analyzed using the Oxfam-DelAgua® Portable Water Testing Kit (DelAgua, Marlborough, UK) (Figure 9a). Due to the small batch of tests the incubator could handle at one time (16), the large number of Pitcher Pump systems that needed to be analyzed, and the limited time, tests were done in duplicate rather than triplicate. During one sampling day single samples were analyzed.

The growth medium, a lauryl sulphate broth, was prepared based on manufacturer's specifications for preparation of the culture medium in the field. The clean water used to make the broth was bottled water, specifically Evian. Several brands of bottled water were tested and Evian was the only one that had the correct pH (between 6.8 and 8.2). As no autoclave was available, the media was sterilized using a pressure cooker, where it was kept for 15 – 20 minutes at full pressure (approximately 15 psi). This preparation method was an alternative suggested by the manufacturer to be used when an autoclave is not available.

Tests were performed in accordance with the manufacturer's specifications. Except for a few occasions where smaller volumes were used, 100 mL of sample water was filtered through a 0.45 µm pore-size, 47 mm gridded membrane (Millipore, Billerica, MA) using a small, hand-operated filter provided by DelAgua (Figure 9b). Membranes were aseptically transferred to a 50 mm metal petri dish containing an absorbent pad that had been prepared with the growth media. Since methanol was not available, petri dishes and filters were sterilized after each use by submerging them in boiling water for 10 minutes.

Samples were incubated at approximately 44°C ±0.5°C for 16-18 hours, as per the manufacturer's recommendation. Colonies that produced a yellow color and were between 1-3 mm in diameter were counted positive for thermotolerant coliforms. When counting the plates, DelAgua recommends 200 colonies as the upper limit for what can be accurately counted. Above that, the results are to be considered too-numerous-to-count (TNTC). When this occurred, the plates were assigned the value of 200 cfu/100 ml for statistical analysis purposes.

Figure 9: Sample processing equipment. (a) DelAgua incubator portable incubator and metal petri dishes (picture taken by the author); (b) Laboratory set up within the house in Tamateve. Photo is showing the handheld filter apparatus, sample collection containers, metal petri dishes and absorbent pads (picture taken by Brad Akers).

2.3.2.4 Measuring Well Depth

Actual depths of the wells were measured at only five locations in the Mangarivotra Sud neighborhood with the help of a local Malagasy technician. Depth was checked by lowering a string with attached weight (either a nut or bolt) into the pipe until the weight hit the bottom of the well (Figure 10a). The string was marked where it exited the well head and then the entire string was removed. The length of the string from the bolt to the mark was measured (Figure 10b). Depth was calculated by subtracting the height of the above ground portion of the Pitcher Pump system from the measured depth.

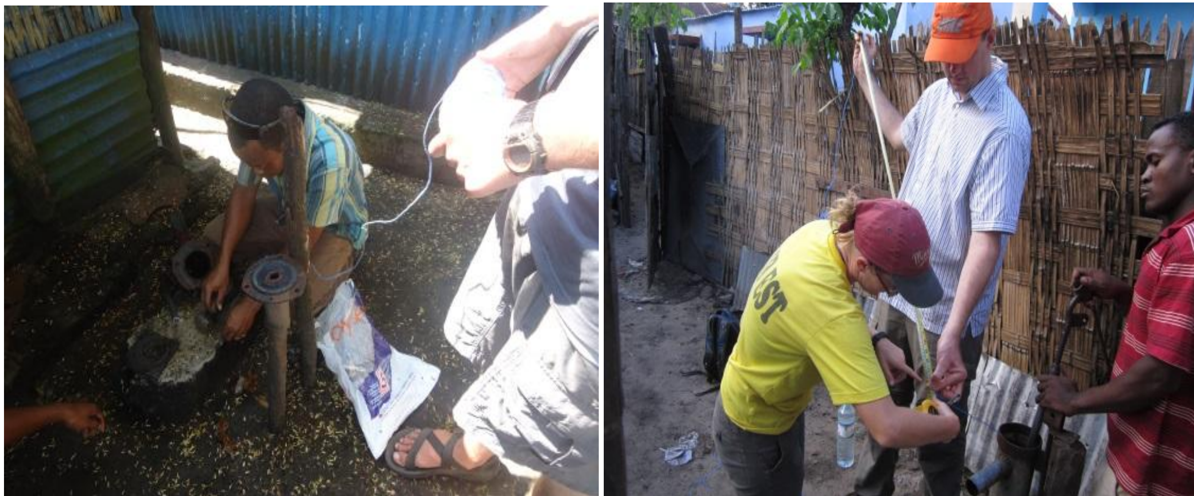


Figure 10: Process used to measure the depth of the wells. (a) Measuring the depth of the well by lowering a string with a weight to the bottom of the well. The local Malagasy technician (pictured on the left) is preparing a replacement weighted leather check valve to be installed after the measurement is complete (picture taken by the author); (b) Measuring the length of the rope (picture taken by D. Brad Akers).

The small amount of measured depths is a function of the pump conditions. In many cases, the bolts were too rusty to allow for the removal of the pump head in the limited amount of time available. In other cases, the pipe diameter was too small to allow a measuring device into it. Finally, a new leather check valve was installed at each Pitcher Pump system where the depth was measured. This cost was another limiting factor to the number of well depths that could be checked.

2.3.3 Phase II

Phase II was implemented to collect data only on the effect that well depth had on the concentration of thermotolerant coliforms found in the groundwater. The study was done based the fact that the results from Phase I indicated potential confounding factors, which made it difficult to determine the effect of depth. Four monitoring wells were installed at depths of 6.5, 7.7, 8.7, and 9.4 m bgs, respectively.

2.3.3.1 Site Description

A pilot study was conducted at one of the sites in the Mangarivotra Sud neighborhood and was chosen from the list of sites sampled during Phase I (Figure 11). The site was chosen for several different reasons.

1) Permission was able to be obtained from the household to conduct a study at their property. As part of the agreement with the homeowner, the house received a new Pitcher Pump system upon completion of the study.
2) Their property was fenced in to protect the monitoring wells.
3) Thermotolerant coliforms measured from Phase I were in the high risk zone (average 115 cfu/100 ml). Risk levels will be discussed in Section 2.4.3.
4) There was adequate space around the existing Pitcher Pump system for the installation of four monitoring wells.
5) The lateral separation between the existing Pitcher Pump system and on-site sanitation system was small (approximately 6 m).
6) There was a thin unsaturated zone below the base of the pit (noted between 0.8 and non-existent in January and April, respectively).
7) Groundwater flow direction was suspected to be from the on-site sanitation system to the Pitcher Pump system. Flow direction was determined based on site observations and GoogleEarth.

Figure 11: Location of the pilot study conducted during Phase II. The author has edited the GoogleEarth map by adding in a dot and textbox to indicate where the pilot study was conducted. ("Tamatave, Madagascar". MAP. SIO, NOAA, U.S. Navy, NGA, GEBCO, DigitalGlobe .2014. GoogleEarth. Vers. 7.1.2.2041., Google, 2013).

2.3.3.2 Monitoring Well Installation and Development

Monitoring wells were installed over the course of three days in December, 2013. Well construction and installation was performed by a local Malagasy technician, with the help of two assistants. Based on parallel research by D. Brad Akers, which suggested that unacceptable levels of lead (>10 µg/l) were leaching from the Pitcher Pump systems (Akers, 2014), an alternative well design was used. Due to the absence of a well head, the only components of concern were the brass well screen and Pb-containing solder. To eliminate these pieces, the monitoring well was constructed using a 32 mm (outer diameter [OD]) PVC pipe. Slots were cut near the base of the pipe to allow water to enter the system (Figure 12a). A polyester cloth sock acted as the well screen and thus no lead components were present (Figure 12b).

Figure 12: Base of a monitoring well. (a) Slots were cut into the base of the monitoring well to allow water to enter (picture taken by the author) (b) A polyester cloth was used as a filter (picture taken by the author)

The PVC pipe was not strong enough to handle the installation process (physically hammering the top of the well to drive the pipe into the ground) so an outer casing made of 40 mm [OD] galvanized iron (GI) was used. Slots were also cut into the GI pipe but no filter was added (Figure 13a). To install the well, the technician first manually drilled down close to the water table (Figure 13b). Soil cuttings from this step were used to classify the soil type using USCS. At around 4.2 m bgs, the auger was removed and the GI outer casing containing the PCV pipe was placed in the hole. The entire system was driven into the ground by the technician until the specified depth was reached (Figure13c). Marks were made on the pipe to show where to stop. This process was repeated three more times to install the remaining monitoring wells. Final well depths were 6.5, 7.7, 8.7, and 9.4 m bgs, respectively (Figure 13d). Only four monitoring wells were installed due to sample processing limitations. Water samples from the Pitcher Pump system and monitoring wells were to be performed in triplicate (15 tests) and the incubator used could only handle 16 tests at one time. In order to be able to collect and analyze all the samples on the same day, the limit was four monitoring wells. A starting depth of 6.5 m for monitoring well installation was chosen because it was just below the reported depths of several of the surrounding Pitcher Pump systems (6 m).

Figure 13: Materials and processes used to install the monitoring wells. (a) The GI outer casing (top) and agar(bottom) (picture taken by the author); (b) The technician's assistant drilling down near the water table (picture taken by the author); (c) The technician hammering the well into place (picture taken by the author); (d) Four completed monitoring wells with their corresponding depths noted (picture taken by the author).

After the monitoring wells were installed, they were developed with the help of the local Malagasy research assistant, Onnie (Figure 14). Development was done to restore the natural hydraulic properties that were damaged during monitoring well installation and to allow the water to flow more freely into the well (Driscoll, 1986). The development technique used was based on EMAS methods and was suggested by Michael MacCarthy (see MacCarthy et al., 2013b for a description of the technology).

Water was removed using a long pipe with a marble check valve. The pipe was placed inside the well and raised and lowered quickly to force the water up and out the top of the monitoring well. In between water removal, the well was periodically surged. This was accomplished using a pipe with a rubber "washer" attached to the outside to create a seal within the monitoring well. As the pipe was lifted up, the water was pulled into the well and when the pipe was pushed down, the water was forced back out into the surrounding subsurface. At least 40-L of water were removed and development continued until the extracted water was clear.

Figure 14: The Malagasy research assistant developing the monitoring wells. Development was done using a technique based on EMAS methods (picture taken by Michael MacCarthy).

2.3.3.3 Sample Collection

The monitoring wells were sampled twice in the beginning of January and three times in April. The low number of sampling days was due to unforeseen events which limited access to the sample processing equipment in April. Samples were collected using bailers, which were constructed by the author (Figure 15a, b). To construct the bailers, a 21 mm ID/25mm OD PVC pipe was used as the casing for the bailer. At the base, a small piece of 15mm ID/20mm OD PCV pipe was placed inside the larger pipe to provide a seat that a marble could sit on. The marble was used to create a seal that let water in when the bailer was lowered but would not allow water to flow back out when it was raised. The upward motion of the marble was restricted by a nail. Four bailers were made, one for each monitoring

well. For the samples collected in January and for one of the samples in April, the same bailer was used on the same one well each time. For the last two sampling events, the same bailer had to be used for both the 6.5 and 7.7 m deep wells because one of the bailers broke. The bailers were cleaned between wells.

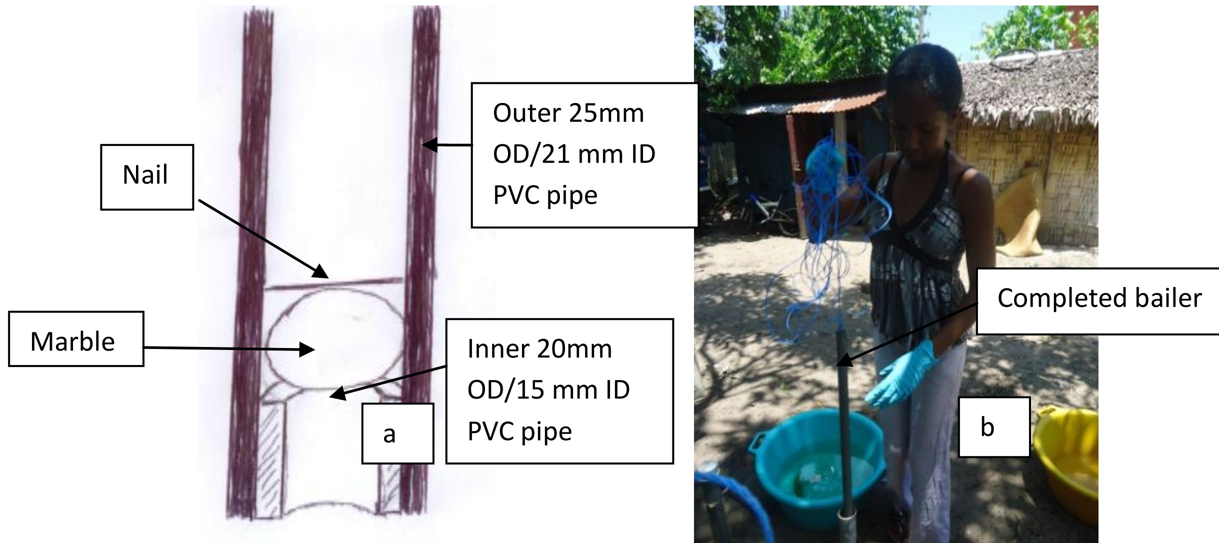


Figure 15: Bailer constructed to sample the monitoring wells. (a) A sketch showing the different components of the bailer which was used to collect samples from the monitoring wells (drawn by the author). (b) The bailer being used by the local research assistant to collect a sample (picture taken by the author).

The depth of the water table was measured in the 6.5 m deep well prior to sample collection. The depth was measured in the same way the well depths were in Section 2.3.2.4 but, rather than lowering the weight to the bottom of the well, it was stopped once it hit the water table. The measuring device was washed with soapy water and rinsed well with bottled water before it was used.

Prior to sample collection, three well volumes were purged (Vail, 2013). Samples were collected in sterile, 350 ml glass jars. The jars were rinsed twice with the sample water prior to collection. Jars were labeled with the sample ID, date, and time of collection. Samples were capped and place on ice until they could be brought back to the laboratory for analysis. All samples were analyzed within 30 hours (EPA, 2009).

2.3.3.4 Sample Analysis

Samples were analyzed using the same growth media and most of the same materials used during Phase I. For Phase II, analyses were performed in triplicate with volumes ranging from 25 to 100 ml. The same growth media from Phase I was used but, rather than the DelAgua incubator, a WagTech Portalab® Portable Water Testing Kit (WagTech) (WagTech WTD, UK) was used. Note that the medium and incubation temperature used in the kit is the same as in the DelAgua kit, so the method of analysis was the same.

There were two issues with the sample analysis in April. On April 5, 2012, despite being calibrated, the WagTech incubator ran at an elevated temperature of 48°C. As a result, there was no colony growth, except for the plates associated with the 8.7 m deep well. On the third day of sampling, April 7, 2012, the battery to the incubator ran out. WagTech is rechargeable but the power had been out in the city of Tamatave for the previous several days and, therefore, the incubator was not able to be charged.

2.3.4 Statistical Analysis

SPSS version 22 software (IBM, Armonk, NY) was used for statistical analysis of the data from Phase I. Data were analyzed using mainly non-parametric tests because the data violated the assumption of normality, which has been found to be common when looking at water resources data (Helsel & Hirsh,2002). For descriptive analysis, the median, average, and standard deviation were calculated. Fisher's exact test, Pearson's product-moment correlation (Pearson's correlation) (r), Spearman's rank order correlation (Spearman's correlation) (r_s), and Mann-Whitney U tests were employed for further data analysis. To run some of these tests, data had to be broken into categories (Table 4).

Fisher's exact test was employed to determine if there was an association between two categorical variables. Fisher's exact test is similar to the much more commonly used Pearson's Chi-

squared test for independence but is applied to situations when cells have an expected count of less than five. Historically this test was only used on 2 x 2 contingency tables because the method of calculation becomes too difficult to do by hand when the tables become larger. Due to advances in computer programming, however, it can now be used on N x M tables (Mehta & Patel, 1983). Using Fisher's exact test, thermotolerant coliform risk categories were compared to well depth, whether or not the pumps needed to be primed, frequency of repair, and neighborhood. The null hypothesis assumes that there is no association between the categories and is rejected if $p < 0.05$.

Pearson's correlation (r) is used with normally distributed data with the purpose of measuring the strength of the linear association between two variables. It was used to determine if there was a relationship between the depth of the Pitcher Pump system and cost. The resulting r is a value between -1 and +1. When the value is positive, there is a positive association between the two variables. When the r value is negative, there is a negative association. The closer the r value is to +1 or -1, the stronger the association. The null hypothesis is that there is no association between the two variables and is rejected if $p < 0.05$.

Spearman's correlation (r_s) is used to measure the strength and direction of the association between two variables. It was used to determine if there was a relationship between the number of users and the level of thermotolerant coliforms detected. For the purpose of this thesis, Spearman's correlation was used to measure the association between scalar and ordinal data. As with Pearson's correlation, the resulting r_s is a number between -1 and +1. The closer to -1 or +1 the r_s value is, the stronger the association. The null hypothesis assumes no relationship between the variables and this assumption is rejected if $p < 0.05$.

Mann-Whitney U test was used to determine if there was a difference between two groups when looking at a single, scalar variable. It was used to further investigate the relationship between pump priming (groups) and thermotolerant coliform concentration (scalar variable). Depending on how

the data are distributed, this test will indicate whether there is a difference in the "distributions" or a difference in the "medians" in the two groups. The similarity of the distribution is determined by visual inspection of the data plotted on a 'population pyramid'. The null hypothesis is that the distributions or medians are similar and is rejected if $p<0.05$.

Due to the limited number of sampling events with useable result ($n = 3$), it was not possible to perform any statistical analysis on the data collected during Phase I. The data from the sampling events were plotted on a graph in Excel and observations were made on the visual analysis of the resulting trends.

Table 4: Explanation of how the data were broken up into categories for use in the statistical analysis of the results. The main parameters are given along with their corresponding categories. An explanation of the values are given for the data contained in each of the categories. Categories were used when analyzing data using the Fisher's exact test, Spearman's rank order correlation, and Mann-Whitney U test.

Parameter	Category	Value
Thermotolerant Coliform Concentration (cfu/100 ml)	Compliance	0 -10 cfu/100 ml
	Intermediate Risk	11-100 cfu/100 ml
	High Risk	>100 cfu/100 ml
Pitcher Pump System Depth	Shallow	0-4 m
	Medium	4.1-8 m
	Deep	≥8.1 m
Pump Priming	Yes	Pumps required priming
	No	Pumps did not require priming
Frequency of Repairs	Monthly or more	Repairs were made monthly or more frequently
	1.1 to 5.9 months	Repairs were made less often than monthly but more often than semiannually
	6 to 11.9 months	Repairs were made less often then semiannually but more often than yearly
	Yearly or less	Repairs were made yearly or less frequently
Neighborhood	Mangarivotra Nord	Samples were collected in Mangarivotra Nord
	Ankirihiry	Samples were collected in Ankirihiry
	Ambalakisoa	Samples were collected in Ambalakisoa
	Antanambao Veriery	Samples were collected in Antanambao Veriery
	Andranomadio	Samples were collected in Andranomadio
	Mangarivotra Sud	Samples were collected in Mangarivotra Sud
Lateral separation between on-site sanitation and Pitcher Pump system	≤10 m	On-site sanitation systems were found within 10m of the Pitcher Pump systems
	>10 m	On-site sanitation systems were not found within 10m of the Pitcher Pump systems

2.4 Results and Discussion

2.4.1 Phase I General Survey Results

A total of 53 households were interviewed from seven different neighborhoods. Select data are shown in Table 5, with a copy of the survey in Appendix C. A full summary of the results can be found in Appendix D. As stated in Section 2.3.2.1, the surveys were conducted with the help of a local Malagasy translator, Onnie

Of the 53 households interviewed, 43.4% (23) obtained their drinking water exclusively from the Pitcher Pumps, 13.2% (7) used both Pitcher Pumps and the municipal water source (JIRAMA), and 43.4% (23) used exclusively JIRAMA (Figure 16). Of the 30 households that collected their drinking water from Pitcher Pumps some or all of the time, 63.3% (19) reported boiling as their treatment method, 3.3% (1) reported using solar disinfection (SODIS) and 3.3% (1) stated they both boiled their water and added chlorine. The remaining nine (30%) households reported that they did not treat their water (Figure 16).

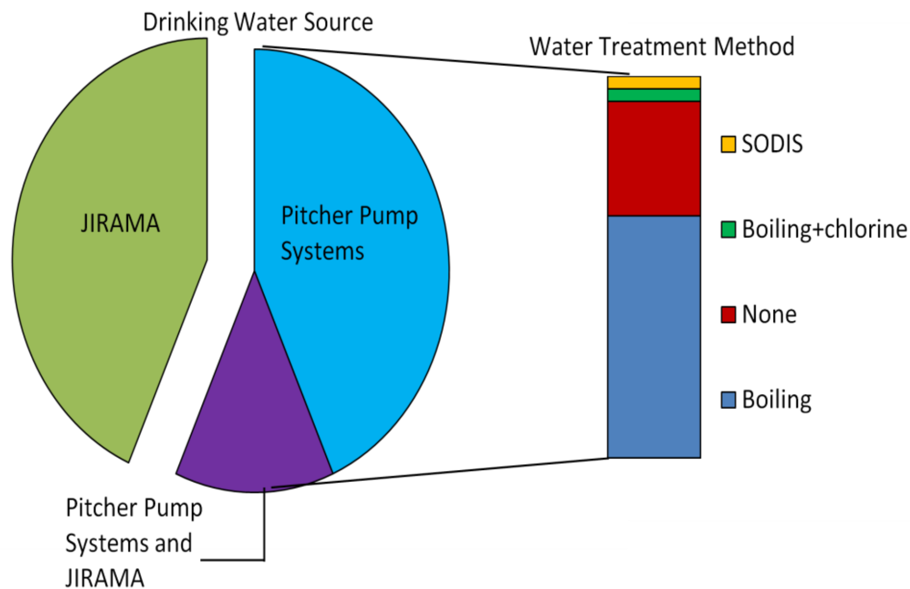


Figure 16: The three main drinking water sources as reported by the survey members along with the reported treatment methods from households that received their cooking/drinking water from the Pitcher Pump system some or all of the time.

Table 5: Summary of selected results from the general surveys. Results shown here are broken up by neighborhood.

		Drinking Water Source			Treat				Average	
Neighborhood	**Number of Interviews**	**P.P. Only**	**JIRAMA + P.P.**	**JIRAMA Only**	**Yes**	**No**	**Average Depth (m) (range)**	**Cost[b,c] ($US)**	**Age (Years)**	**% of Houses with Latrines**
Mangarivotra Nord[a]	9	5	1	3	4	5	6.2[a] (3.5-7.6)	$29	9.7	88.7
Ankirihiry	5	2	0	3	1	4	5.5 (3.7-7.2)	$21	9.2	100
Ambalakisoa	12	6	3	3	6	6	6.9 (3.5-9.5)	$60	13.5	66.7
Antanambao Veriery	2	2	0	0	1	1	12.6 (12.6-12.7)	$138	<1	100
Andranomadio	8	0	0	8	0	8	3.9 (2.5-5.6)	$33	6.4	100
Mangarivotra Sud[a]	14	7	2	5	7	7	6.1[a] (3.5-10.7)	$52	11.6	85.7
Antanamboa V	3	1	1	1	2	1	8.5 (7-10)	$66	12.0	100
Overall	*53*	*23*	*7*	*23*	*21*	*32*	*6.3 (2.5-12.7)*	*$51*	*10.0*	*81.1*

[a]more samples and depths were collected then interviews given
[b]costs were obtained in Malagasy Ariary and were converted to US$ using the conversion rate of US$1 = MGA$2,200
[c]only costs for wells installed during and after 2000 were use

The average reported depth of the Pitcher Pump systems over all the neighborhoods was 6.3 m, with a range from 2.5 to 12.7 m. In general, the wells were most shallow in Andranomadio (3.9 m) and deepest in Antanamboa Veriery (12.6 m). The median cost of a Pitcher Pump system installed after the year 2000 was approximately US$46 (Appendix D) and was correlated with the depth of the installation (Pearson's correlation r = 0.57; p = 0.001) (Figure 17). The ages of the wells ranged from less than 1 year old to over 30 years, with an average of around 10 years. No aprons or annular seals were noted at any of the Pitcher Pump systems studied.

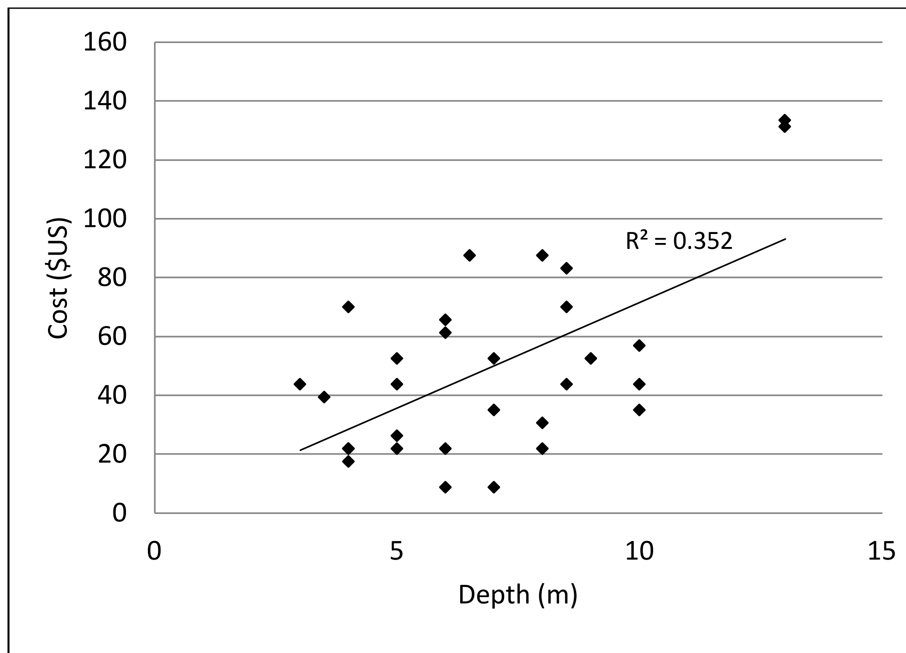


Figure 17: Relationship between the depth of the well and cost. In general, as the depth of the well increases, so does the cost (Pearson's r = 0.57; p = 0.001).

Of the households surveyed, 81.8% had some sort of on-site sanitation system. The average lateral separation between the Pitcher Pump system and on-site sanitation was approximately 9.4 m with a range of 2.0 – 22.9 m. The average reported depth of the sanitation pit was 2.1 m with a range from 1 to 4 m. In December, 2012, the median depth of the groundwater table in Mangarivotra Sud was 3.9 m with a range from 3.2 to 9 m bgs. The significance of these results will be discussed in more detail in the following sections.

2.4.2 Confirmation of Depth

Depths of the Pitcher Pump Systems were measured at five properties in Mangarivotra Sud to see how the actual depth compared with the depth reported by the owner. This comparison was important to know for data analysis purposes because, when analyzing the effect of depth on water quality, mostly homeowner reported values were used. If it was shown that the homeowners were drastically off, then the analysis would be inaccurate. The results are shown in Table 6.

Table 6: Depths of the Pitcher Pump systems as reported by the household vs. measured depths

Location	Reported Pipe Length (m)	Confirmed Pipe Length (m)	Difference (m)
Mangarivotra Sud.3	13	11.2	-1.8
Mangarivotra Sud.5	5	4.0	-1
Mangarivotra Sud.7	6.5	5.9	-0.6
Mangarivotra Sud.9	7	7.3	+0.3
Mangarivotra Sud.12	5-6	5.4	NA

In most instances the confirmed pipe length was less than the depth reported by the homeowner. This could be due to at least two reasons. The first could be that the technicians estimated the pipe length rather than actually measuring it prior to installation. The second could be that the technicians reported pipe lengths that were slightly greater than what was used, since the amount they are paid is based partly on how deep the Pitcher Pump systems are installed (i.e. the length of the pipe used). Based on these results it was decided to group the wells into shallow (0-4 m bgs), medium (4.1 to 8 m bgs), and deep (<8.1 m bgs) categories.

2.4.3 Phase I Sampling Results

A total of 61 Pitcher Pump systems were sampled over six of the neighborhoods; however, due to improper labeling of two samples, Ambalakisoa 5 and 12 could not be used. A full summary of the sampling results can be found in Appendix E. Of the 59 samples remaining, 55.9% (33) were in compliance with the Malagasy guideline of ≤10 cfu/100 ml (compliance), 22.0% (13) were in the intermediate risk to human health category of 11-100 cfu/100 ml (intermediate risk), and 22.0% (13) were in the high risk to human health category of >100 cfu/100 ml (high risk) (Figure 18). The

intermediate and high risk categories are based on risk levels presented by the WHO (WHO, 1997). These three risk categories are used in the following sections during data analysis.

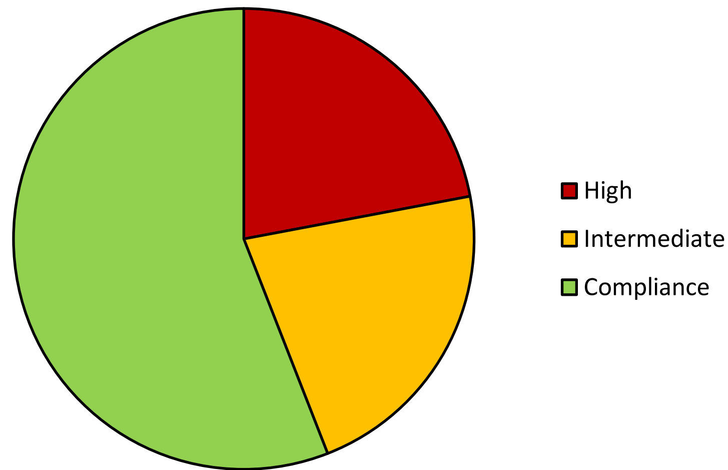


Figure 18: Percentage of samples that fell in the different drinking water risk categories. Compliance = 0-10 cfu/100 ml; Intermediate = 11-100 cfu/100 ml; High = >100 ml

The fact that almost half (26 of 59; 44.1%) of the samples tested from the Pitcher Pump systems were above the Malagasy drinking water standard is a concern because 30% (9 of 30) of the households who obtained their drinking water from these systems reported that they did not treat their water prior to consumption. In addition, while not observed specifically in Tamatave, during her two years as a Peace Corps Volunteer, the author noted many cases where households were incorrectly boiling their water. Households were either merely heating it up or not allowing for a full minute of a rolling boil as recommended by the WHO (WHO, 1997).

A graphical representation of the sample locations is shown in Figure 19. The colors of the markers correspond with the risk level detected from the water sampled there. Green dots represent pumps that were in compliance with the Malagasy standard, orange represents 'intermediate risk', and red represents 'high risk'. As the figure shows, there is no discernible geographic pattern as to how the thermotolerant coliforms are distributed. Contamination is widespread, with pumps that were in compliance located next to pumps with intermediate or high risk.

Figure 19: Map of Tamatave with the neighborhoods and sample locations highlighted. The author modified the GoogleEarth map by 1) adding in shaded areas for the neighborhoods worked in and 2) adding points were samples were located ("Tamatave, Madagascar". MAP. DigitalGlobe. 2014. GoogleEarth. Vers. 7.1.2.2041., Google, 2013).

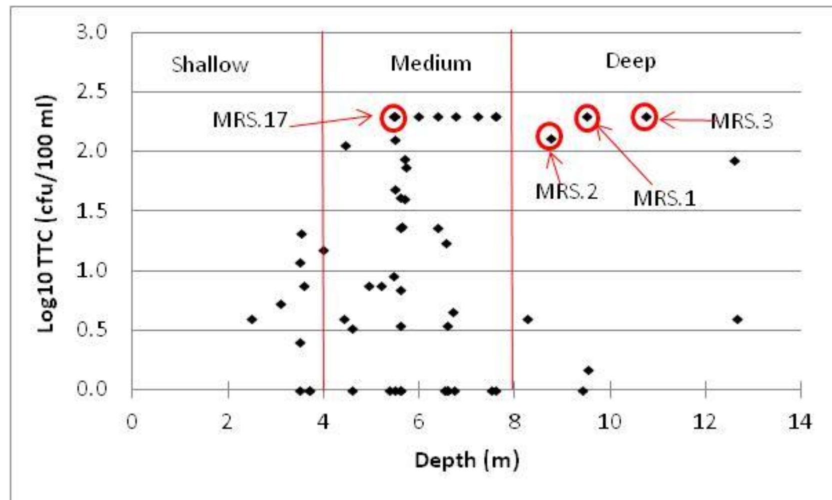
Some of the characteristics of Tamatave lend itself to increased aquifer vulnerability which, in turn, can lead to widespread contamination. These characteristics are high population density, sandy soils, and a thin unsaturated zone. As discussed in Section 2.2.3, increased population density has been shown to lead to increased groundwater contamination in some situations (Howard et al., 2003; Nsubuga et al., 2004; van Geen et al., 2011; Escamilla et al., 2013; Wright et al., 2013). The thin unsaturated zone also presents a risk. As stated in Section 2.4.1, average depth of pit latrines at the study site was 2.1 m, and the median depth to the groundwater table was 3.9 m. This means that that the average thickness of the unsaturated zone below the base of the pit was approximately 1.8 m. While this is close to the Malagasy standard of 2 m (PAEPAR, 2005), it is possible that it is still not sufficient, especially considering soil type. For example, as stated previously, MacDonald et al. (2005) recommends a minimum of 5-10 m of unsaturated zone in sandy soils to be protective of groundwater quality. Finally, the fine to medium grained sandy soils presents another risk due to the relatively high hydraulic conductivity typically observed in these soil types (leaning towards the lower end of 10 to 100 m/d

[MacDonald et al., 2005]), which allows for the rapid transport of pathogens. Coupled with the close proximity of the on-site sanitation facility to the drinking water extraction point, there may not be sufficient time for complete die-off of the pathogens.

In the following sections the thermotolerant coliform results are explored further. As stated previously, thermotolerant coliform results were broken up into the three risk categories (compliance, intermediate, and high) and were first analyzed in relation to well depth (Section 2.4.3.1). Based on those results, it was determined that further analysis of additional parameters was required. The additional parameters were neighborhood, whether or not priming was required, frequency of repairs, distance between the Pitcher Pump system and sanitation system, and number of users.

2.4.3.1 Thermotolerant Coliform Levels as a Function of Depth

The distribution of thermotolerant coliforms as a function of depth is shown in Figure 20. Lines have been added to show the cut off between the shallow, medium, and deep ranges of the wells. Similar to the results found in 2011 by Michael MacCarthy and James McKnight, the medium depth pumps showed the highest levels of contamination. Unlike the previous sampling event, thermotolerant coliform levels above 10 cfu/100 ml were noted at depths greater than 7 meters. Four locations have been highlighted in Figure 20 and are discussed in further detail. Mangarivotra Sud 1 and 2 had reported well depths of 8.8 and 9.5 m, respectively, and both had thermotolerant coliforms concentrations at levels greater than 100 cfu/100 ml. While these neighboring pumps are technically deep, just to the east, the ground drops down into a canal. It is suspected that the well screens are just below the elevation of the canal and some canal water could be drawn into the system during pumping with little chance for attenuation. Surface water runoff from the area flows into the canal and it's reasonable to assume that the water there is heavily contaminated; however, no measurements were made of the thermotolerant coliform concentration in the canal. Based on these observations, the results from Mangarivotra Sud 1 and 2 were removed from further analysis.



[a]TTC = Thermotolerant coliforms

Figure 20: Log_{10} thermotolerant coliform concentrations as a function of the depth of the wells. Lines have been added in to delineate the shallow (0-4m), medium (4.1-8 m), and deep wells (>8.1 m). Three of the four highlight wells were not used in further statistical analysis due to some site specific conditions; n = 59.

The results from Mangarivotra Sud 17 were also removed from data analysis. Water quality measurements were collected from this pump several times and consistantly returned extremely high levels of contamination. While samples from some of the other Pitcher Pump systems produced results that were above the upper counting limit of 200 colonies per plate, the plates were at least somewhat readable. In contrast, samples from Mangarivotra Sud 17 had to be performed using volumes of 5 ml or less to produce seperated colonies. When run at the lower volumes, it was found that the thermotolerant coliform concentraion was approximately 4,290 cfu/100 ml, which was well above the results found at any of the other locations. A possible source of the contamination is a pipe suspected to be discharging waste from a neighboring property. Due to limited time and materials, this was never confirmed. It should be noted the the interviewee at this household reported getting drinking water from the community taps provided by JIRAMA and was not drinking the water from the Pitcher Pump systems.

Finally, Mangarivota Sud 3 is called out on the graph, but has been kept in the data set and is used in further analysis. The confirmed depth of the pipe was 10.7 m bgs (11.2 m total minus the portion

above the ground) and the thermotolerant coliform concentration was >200 cfu/100 ml. The owners reported that they recently had the pipe removed and shortened, only to subsequently have it removed and re-lengthened. This could have led to outside contamination entering the Pitcher Pump system but, since the owners could not provide a date for this work nor the name of the technician, the results were retained for further analysis.

A Fisher's exact test was conducted to determine if there was a relationship between the shallow, medium, and deep wells and the risk levels of thermotolerant coliforms (compliance, intermediate, and high) detected. The number of Pitcher Pump systems in each category are shown in Table 7, along with the median and average thermotolerant coliform concentrations. The results of the test indicated that there was no stastically significant association between the depth of the Pitcher Pump system and risk level of the sample collected there (p = 0.59). Average thermotolerant coliform concentration in the shallow wells were lower than those in the medium and deep wells (6.9 cfu/100 ml as compared to 33.1 and 75.6 cfu/100 ml, respectively); however, the median concentrations were similar between all three depth categories. The lack of identifiable association between the depth of the Pitcher Pump systems and the corresponding health risk level could potentially be attributed to the presence of confounding factors, some of which are discussed in further detail in the following sections. In addition, the sandy soil and shallow aquifer could potentially render the groundwater vulnerable, allowing for deeper penetration of the pathogens.

Table 7: Thermotolerant coliform risk levels at varying well depths (Fisher's exact test = 3.00; p = 0.59); n = 56. Standard deviation is shown in the parentheses

Pump Depth	**Thermotolerant Coliform Risk Level**			**Median TTC (cfu/100 ml)**	**Average TTC[a] (cfu/100 ml)**
	Compliance	**Intermediate**	**High**		
Shallow	7	3	0	4.7	6.9 (±6.9)
Medium	22	9	9	7.5	51.8 (±75.6)
Deep	4	1	1	4.0	49.3[b] (±33.1)

[a]TTC = Thermotolerant Coliforms

[b]average thermotolerant coliform concentration is being heavily influenced by Mangarivotra Sud.3. When that value is removed the average concentration reduces to 19 cfu/100 ml.

2.4.3.2 Thermotolerant Coliform Levels as a Function of Neighborhood

Results from the neighborhoods in which the samples were collected were analyzed using a Fisher's exact test. The goal was to determine whether location (based on neighborhood) had an effect on the levels of thermotolerant coliforms detected there. The number of Pitcher Pump systems in each neighborhood according to risk category is shown in Table 8, along with the median and average thermotolerant coliform concentrations. Fisher's exact test did not find a statistically significant association between the neighborhood in which the sample was collected and the risk level ($p = 0.61$). When considering that all the sample locations were contained within a 1.2 km radius, it is likely this was due to similar characteristics within the neighborhoods, for example, soil type, groundwater table elevation, and population density.

Table 8: Thermotolerant coliform risk levels in the different neighborhoods (Fisher's Exact = 8.15; $p = 0.61$); $n = 56$. Standard deviation is shown in the parentheses.

Neighborhood	Thermotolerant Coliform Risk Level			Median TTC[a] (cfu/100 ml)	Average TTC[a] (cfu/100 ml)
	Compliance	Intermediate	High		
Mangarivotra Nord	8	5	3	12.0	45.7 (±69.7)
Ankirihiry	3	1	1	7.5	57.3 (±85.4)
Ambalakisoa	8	1	1	1.0	23.0 (±62.5)
Andranomadio	5	3	0	1.0	11.3 (±13.3)
Antanamboa Veriery	1	1	0	4.0	44.8 (±57.6)
Mangarivotra Sud	8	2	5	9.0	67.1 (±88.1)

[a]TTC = Thermotolerant coliforms

2.4.3.3 Thermotolerant Coliforms Levels as a Function of Priming

A Fisher's exact test was conducted to determine whether there was a relationship between priming the pump and the risk level of the water produced. Priming entails adding water to the pump head prior to use when there is a leak in the system. In the case of the Pitcher Pump systems in Tamatave, this was most often related to an inadequate seal caused by faulty leather valves. The number of Pitcher Pump systems in each risk category according to whether or not they require priming is shown in Table 9, along with the median and average thermotolerant coliform concentrations. There was a statistically significant association between the requirement of priming and risk level detected (p =

0.03); however, Fisher's exact test could not say what that association was. For that, a Mann-Whitney U test was used. The Mann-Whitney U test was performed to see if there was a difference in the median thermotolerant coliform values between the two categories, primed and un-primed. The difference in medians could be found because distribution patterns of the thermotolerant coliforms were similar between the two categories, as assessed by visual inspection. The median scores for thermotolerant coliforms in unprimed wells (3.5 cfu/100 ml) were statistically significantly lower than the median scores in the primed wells (41.3 cfu/100 ml) ($U = 108.5$, $z = -2.58$, $p = 0.01$).

Similar results were found in a previous study by Guillemin et al. (1991). The authors studied boreholes in rural Burkina Faso and found a statistically significant association between the quality of water provided by the borehole and pump priming ($p<0.001$). In contrast, van Geen et al. (2011) did not find a statistically significant link between the contamination of tubewells in two cities in Bangladesh and pump priming. Other studies suggest priming as a way for contamination to enter the system, but provide no statistical analysis of the data to test this association (Macdonald et al., 1999;Ahmed et al., 2002). For example, Macdonald et al. (1999) and Ahmed et al. (2002), studied tubewells in the same two cities in Bangladesh. The Macdonald et al. (1999) paper proposed that the microbial contamination detected in the systems was not due to pathogen transport via the aquifer pathway because travel time/distance was too great. In addition, wells at one site, Keraniganj, were significantly more contaminated then wells at the other site, Dattapara, despite the fact that the average depth of the Keraniganj wells was greater than those in Dattapara (54 m and 15 m, respectively). The authors suggested that microbial contamination was entering the system either through inadequate or deteriorated well-headworks or through pump priming. Ahmed et al. (2002) reported more detailed information regarding the study and provided the results of a sanitary risk assessment. The authors concluded that although no one factor had a consistent relationship with poor water quality, priming was a likely pathway for contamination.

The increase in contamination associated with primed wells could be a function of the water added to the well head. At many of the households interviewed, previously purged water was used to prime the pump. These pumps are being used on a fairly consistent basis so the priming water will rarely sit for more than 24 hours before being used. It is important to note that no samples were collected or analyzed from the water used for priming due to time constraints and the limited number of samples that could be tested per day. In general, however, water quality has been found to decrease after collection, sometimes significantly (Khairy et al., 1982; Mølbak et al., 1989; Sandiford et al., 1989; Musa et al., 1999; Roberts et al., 2001; Wright et al., 2004). Wright et al. (2004) performed a meta-analysis of 57 studies which measured coliform counts (*E. coli*, thermotolerant coliforms, and total coliforms) in water both at the source and stored in the home. Their results indicated that approximately half of the studies showed a significant decrease in water quality after the water was collected and in no cases did the quality significantly improve after collection. An increase in the level of contamination after collection was also noted in a study conducted in Sierra Leone (Clasen & Bastable, 2003). The authors collected samples from 20 drinking water sources, 17 of which were rehabilitated by Oxfam, and from the stored water at 100 households (five from each source). They found that the mean levels of thermotolerant coliforms at the rehabilitated and non-rehabilitated sources (0.23 and 407 cfu/100 ml, respectively) were less than that of the same water stored in the home (244 and 882 cfu/100 ml, respectively). In Liberia, stored water was significantly more likely to be contaminated with enterobacteria than water collected at the tap ($p = 0.025$, X^2) (Molbak et al., 1989). When looking at stored water from a study in Sudan, concentrations of thermotolerant coliforms were considerably higher in the stored water than in the water at the tap or wells (Musa et al., 1999).

Contamination appears to be rapid and can increase over time. In a case study at a refugee camp in Malawi, Roberts et al. (2001) found that most of the water sources sampled (29 of 41; 71%) had concentrations of thermotolerant coliforms less than 1 cfu/100 ml. Right after collection, however, that

concentration increased. In standard buckets for example, the level of thermotolerant coliforms detected immediately after collection was just below 150 cfu/100 ml. Concentrations continued to rise until 4 hours after collection when, at that point, the concentrations began to drop. Even at the last time sampled (6 hours after collection), however, concentrations of thermotolerant coliforms were still above 100 cfu/100 ml. When the authors conducted an additional study, they found that the reduction in thermotolerant coliform concentration between 4 to 6 hours was likely due to settling because when they shook the water in the bucket and then collected the sample, the concentrations increased. Looking at water collected from taps in Egypt, Khairy et al. (1982) also found that the concentration of parasites (*Entamoeba.histolytica, Entamoeba coli, and Giardia*) increased over time (up to at least 12 hours), however, they also found that the prevalence rate did not change (i.e. no new stored water samples were becoming contaminated). This likely contamination of water after collection means that, by priming the systems, the user was potentially adding contaminated water to the pump head. Even though the water is almost immediately flushed out, thermotolerant coliforms can remain behind by attaching themselves to pump head components (Ferguson et al., 2014).

These results indicate that a reduction in the thermotolerant coliform concentration in the Pitcher Pump systems might be achieved by ensuring that the leather valves seal properly. This could mean frequent repairs to the system, possibly every few months. The cost to repair the top valve in 2011 was around US$2.27 – 3.64 and the cost to repair the bottom valve was around US$3.18 – 4.55 (prices obtained by Michael MacCarthy). These prices include both the cost of the leather and the labor involved. It is uncertain if this is a cost that the households can bear.

Table 9: Thermotolerant coliform risk level as a function of priming (Fisher's exact = 7.07; p = 0.03); n = 44. Standard deviation is shown in the parentheses

Prime	**Thermotolerant Coliform Risk Level**			**Median TTC[a] (cfu/100 ml)**	**Average TTC[a] cfu/100 ml**
	Compliance	**Intermediate**	**High**		
Yes	5	5	4	41.3	70.9 (±20.9)
No	23	3	4	3.5	29.9 (±63.5)

[a]TTC = Thermotolerant coliforms

2.4.3.4 Thermotolerant Coliform Levels as a Function of Repairs

A Fisher's exact test was conducted to determine whether there was an association between the frequency of repairs made to the Pitcher Pump systems and the risk level of the water produced. The most common repair made to the Pitcher Pump system was the replacement of the leather check valves (41 of 48 repairs; 84.4%), followed by repairs to the pipe (3 of 48; 6.3%), and finally repairs to the pump screen or lever (both 1 of 48; 4.2%). The number of Pitcher Pump systems in each repair category is shown according to the corresponding risk level in Table 10, along with the median and average thermotolerant coliform concentrations. There was no statistically significant association between the frequencies of repair and the risk level (p = 0.09).

With the exception of the Pitcher Pump systems that received repairs once a month or more frequently, the average and median thermotolerant coliform concentrations decreased as the frequency of repairs decreased. When pumps are being repaired, parts are exposed to outside elements; this presents a chance for the introduction of more bacteria. The less frequent the repairs, the less frequent the chance for outside contamination, which may be the reason for the lower levels of thermotolerant coliforms.

Table 10: Thermotolerant coliform risk level as a function of the frequency of repairs (Fisher's exact test = 8.32; p = 0.16); n = 39. Standard deviation is shown in the parentheses

Frequency of Repairs	**Thermotolerant Coliform Risk Level**			**Median TTC[a] (cfu/100 ml)**	**Average TTC[a] (cfu/100 ml)**
	Compliance	**Intermediate**	**High**		
Monthly or more	5	3	0	4.3	17.1(±26.8)
1.1 to 5.9 months	7	1	5	7.5	73.6(±93.9)
6 to 11.9 months	7	3	1	5.8	36.9 (±63.4)
Yearly or less	6	0	1	0.0	29.6 (±75.2)

[a]TTC = Thermotolerant coliforms

2.4.3.5 Thermotolerant Coliform Levels as a Function of Distance

As stated in Section 2.4.1, the average distance between the sanitation facility and Pitcher Pump systems was 9.4 m, with a range of 2.0 to 22.9 m. A summary of the distances can be found in Appendix D. As a reference, the Malagasy standard for lateral separation between these two systems is 10 m

(PAEPAR, 2005). A Fisher's exact test was conducted in order to determine if there was a relationship between whether or not a sanitation facility was within 10 m of the Pitcher Pump system and the associated risk level of the water produced. The number of Pitcher Pump systems in each distance and risk category is shown in Table 11, along with the median and average thermotolerant coliform concentrations. There was no statistically significant association between risk level and the presence or absence of an on-site sanitation system within 10 m of the Pitcher Pump system ($p = 0.62$). Furthermore, the presence of intermediate and high risk samples in both groups indicates that the current standard of 10 m lateral separation might not be sufficient. While removal of pathogens over short distances has been noted (Caldwell, 1983; Baars, 1957), some studies done in similar conditions (i.e. sandy soils and high water table) have determined that latrines can cause elevated levels of FIB at more than 30 m away (Sangodoiyn, 1994; Murka et al., 2012).

Table 11: Thermotolerant coliform risk level as a function of distance from on-site sanitation systems (Fisher's = 0.07; $p = 0.62$); $n = 49$. Standard deviation is shown in the parentheses.

Distance	Thermotolerant Coliform Risk Level			Median TTC[a] (cfu/100 ml)	Average TTC[a] cfu/100 ml
	Compliance	Intermediate	High		
<10 m	15	8	6	7.5	50.8 (±74.9)
>10 m	12	5	3	7.3	43.0 (±70.9)

[a]TTC = Thermotolerant coliforms

2.4.3.6 Thermotolerant Coliform Levels as a Function of the Number of Users

A Pitcher Pump system is generally purchased by one family and then used by several surrounding households. The average number of households using a single Pitcher Pump system was 4.8 with a range from 1-16. Spearman's correlation was run to assess the association between the number of households using the system and thermotolerant coliform risk category. Bacteria, which have been shown to grow on the surfaces of the soil particles, can sometimes break loose when water is pumped through an aquifer (Bitton & Gerba, 1994). It was thought that the higher the number of users, the more chance the pathogens would have to break off. In addition, a higher number of users leads to more of a chance of contamination from surficial sources. The result of the analysis showed no real significant

correlation between the two variables (r_s = -0.08; p = 0.59) which is similar to what was found by Escamilla et al. (2013). In contrast, Sandiford (1989) found a relationship between the two variables with an increase in thermotolerant coliform contamination associated with an increase in the number of users. It should be noted that they were looking at unprotected sources and the difference in the overall population density of the two villages they worked may have played a role.

2.4.2 Phase II

As stated in Sections 2.3.3.3 and 2.3.3.4, limited access to sampling equipment and fluctuating incubator temperatures led to a smaller data set than planned for Phase II. While it does prevent firm conclusions from being presented, general trends can be assessed. Results from the samples collected on January 9th and 11th, 2013 and April 6th, 2013 are shown in Figure 21. The full results can be found in Appendix F. Note that the results from the 8.7 m deep monitoring well from the April 6, 2013 sampling event are not shown. This is because at some time between January and April, someone removed the well cap and put dirt down into the well, resulting in the inability to collect a clear sample. In addition, the results from the existing Pitcher Pump system are also not shown. This was because of two reasons. First, the actual depth of the existing system could not be measured. Second, due to sample processing and handling problems, results were not able to be obtained on two of the sampling occasions. In general, the thermotolerant coliform concentration decreased as the depth increased, but at no point was it below the Malagasy standard of 10 cfu/100 ml.

Results from the January 9th and April 11th sampling events are fairly similar, but the samples collected on January 11th produced results that were much higher. Heavy rains, which have been shown to lead to an increase of thermotolerant coliforms in the groundwater (Barrell & Rowland, 1979), occurred on January 10th and continued into the morning of January 11th. The increase in contamination after a rain fall event is likely due to two factors. First, rainfall may have mobilized bacteria in the subsurface. Second, the rains may have led to the flushing of surficial source of fecal matter into the

wells (Barrell & Rowland, 1979). The lack of any type of concrete apron or annular seal makes the monitoring wells vulnerable to contamination via the localized pathway. With such a limited data set, however, it is hard to draw any real conclusion.

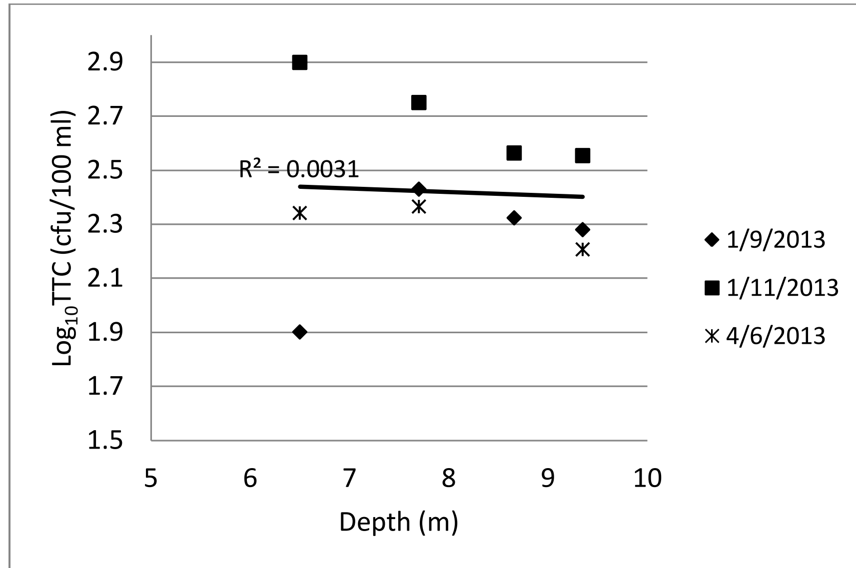


Figure 21: Change in thermotolerant coliform concentration (cfu/100 ml) as depth of the monitoring wells increased.

The depth at which microbial contamination in the subsurface is detected varies widely between studies, with some finding great removal within a few cm of the infiltration site, while others have detected contamination in wells or pumps several tens of meters or greater below the ground surface (Baars, 1957; Banerjee, 2011; Isikwue et al., 2011; Gonzales, 2008). The deeper contamination could be caused by localized pathways of contamination. The results from this study show levels of above 100 cfu/100 ml, even at 9.4 m and it is unclear at what depth the groundwater would meet Malagasy drinking water standards. Conditions at this site render the aquifer particularly vulnerable. The groundwater is shallow, with depths measured at 3.8 and 2.9 m bgs in January and April, respectively. If the depth of the pit latrine really is 3 m, as was reported by the household, then the unsatured zone beneath the base of the pit is thin (between 0.8 m and nonexistent). That, coupled with the fine to medium sandy soil present, indicates the potential for fairly rapid transport of the pathogens with little

chance for attenuation before reaching the groundwater extraction point. Based on these results, and the results from Phase I, no depth was found that would consistently produce water that was safe for consumption without further treatment.

2.5 Conclusions and Recommendations

There were two main objectives to this research. The first was to determine if there was a link between microbial quality of water and the depth at which the Pitcher Pump systems were installed, specifically to see if a well depth could be identified that would consistently provide safe drinking water (≤10 cfu/100 ml). Second was to identify other factors that could be leading to source water contamination.

In regards to the effect the depth, the results from Phase I did not indicate any statistically significant relationship between the depth of the Pitcher Pump system (shallow, medium, or deep) and the levels of thermotolerant coliforms detected (compliance, intermediate, or high) ($p = 0.59$). In addition, no depth could be identified that would consistently provide water that met the Malagasy drinking water standard which is thermotolerant coliform levels of less than or equal to 10 cfu/100 ml. If there was an effect associated with depth, it is likely that this link was masked by many other confounding factors. When controlling for these other factors, as was done in Phase II, it appears that there is an inverse relationship between depth and thermotolerant coliform concentration. That is, as the well depth increased, the concentration of thermotolerant coliforms decreased; however, with the limited data set ($n = 3$), only general trends can be commented on. No real conclusion based on statistical analysis can be assigned. When assessing the results from Phase II, it appears that even in the deepest monitoring well of 9.4 m, thermotolerant coliform concentrations are still above 100 cfu/100 ml. This means that the water was not safe for human consumption without some type of additional treatment method. The increase in the level of thermotolerant coliforms in all the monitoring wells after

a day and a half of heavy rainfall suggests that surficial sources of contamination might be having a greater effect on the water quality that previously thought.

When looking at other factors that could be leading to the fecal contamination detected in the various Pitcher Pump systems, only pump priming had a statistically significant association with the levels of thermotolerant coliforms that the pumps produced ($p = 0.03$). Specifically, the median thermotolerant coliform concentration in the wells which required priming (41.3 cfu/100 ml) was significantly higher than in those that did not require priming (3.5 cfu/100 ml). The reason for this significant difference is likely due to the fact that households are putting contaminated water back into the system to prime the pumps prior. The lack of correlation between other factors identified and the levels of thermotolerant coliforms detected in the various Pitcher Pump systems indicates that the pathways of contamination are very complex and interrelated making it hard to isolate only one specific factor. The high population density, sandy soils, shallow groundwater table and lack of protection around the wells make the Pitcher Pumps in Tamatave particularly vulnerable to contamination.

2.5.1 Recommendations to Tamatave

Water quality data from Phase I showed that 44.1% (26 of 59) of the Pitcher Pump systems do not supply water that is considered safe for human consumption by the Malagasy standard without further treatment. From the general survey, it was identified that 30% (9 of 30) of the households interviewed did not treat their water prior to consumption. Furthermore, those that reported boiling as a treatment method (66.7%, 20 of 30) might not be doing so properly. Taking into consideration those factors, it is recommended that an educational campaign be put in place to promote proper boiling practices (i.e. boiling for 1 min). It is believed that this could be a successful campaign because it would fit into pre-existing Malagasy cultural practices. In general, the Malagasy drink what is called ranon'apango which is a kind of rice tea. After the rice is cooked, most of it is taken out of the pot and served for people to eat, but a little is left behind. The rice that is left behind is put back into the fire and

burned. Once it becomes burned, water is added into the pot and then heated up prior to being served. Since the water is already being heated, and in some cases even brought to a boil, promoting boiling as a water treatment method would not require introducing a new behavior, which has been proven to be very difficult to do, but rather would require people to adjust an already existing practice. In addition, proper boiling should not add much of an economic burden to the household as they are already using fuel (wood or charcoal) to cook their food and heat the water for ranon'apango. Proper boiling would only add a few minutes onto their food preparation process. It is important to note here that it is not being recommended that people stop using their Pitcher Pump systems. These systems allow people increase their water quantity which has been shown to have a greater impact on the reduction of cases of morbidity related to diarrheal disease than water quality. Specifically, Esrey et al. (1991) estimates that a 20% reduction in diarrheal morbidity can come from access to water of adequate quantity as compared to the only 15% reduction from access to water of adequate quality. When people have an increased water supply they are able to use some of that water for hygiene purposes.

2.5.2 Recommendations for Future Research

Based on the results of the investigation, it is not recommended that the effect of well depth be studied further. While the results from Phase II did show a general trend that suggested a decrease in thermotolerant coliform concentration with an increase in well depth, even at 9.4 m this decrease was not significant. Furthermore, several of the deep wells in Phase I produced water that was in the intermediate to high risk category even at 10.7 and 12.6 m. Theoretically, as long as the distance between the water table and check valve is no more than seven to ten meters, the Pitcher Pump systems can operate as deep as the technicians can physically install them. It is possible then that even deeper depths could be reached that may provide safe drinking water, however, to do so might be cost prohibitive. As stated previously, the cost of the Pitcher Pump system is based partially on how deep it is installed, with an additional US$5-7 per meter of depth. Adding on several meters to a pipe length may

make these systems unaffordable to the homeowner. Furthermore, as the depth increases, the difficulty of installing the well also increases. This could potentially cause the technicians to raise their prices, making the systems even more unaffordable.

With that in mind, it is recommended that a study be conducted to look at the impact of a properly constructed concrete apron around the base of the well. The lack of correlation between depth and thermotolerant coliform risk level from Phase I and the increase in thermotolerant coliforms after a rainfall event in Phase II indicate that the Pitcher Pump systems may be impacted by surficial contamination as a greater degree than originally thought.

An additional study should also be conducted to further investigate the effect of pump priming. It is suggested at several wells be identified that currently require priming and produce water with contain thermotolerant coliforms in the intermediate to high risk categories. These wells could be repaired so that priming is no longer required and then samples be taken for some amount of time afterward to see if the thermotolerant coliform concentrations decrease.

CHAPTER 3: EVALUATION OF ALTERNATIVE METHODS FOR DETECTION AND ENUMERATION OF FECAL INDICATOR BACTERIA IN DEVELOPING COUNTRIES

The work presented in Chapter 3 is from research conducted at the USF campus during the spring of 2011, and the following is a paper based on this research. The paper has recently been re-submitted to the *Journal of Water and Health* but has not yet been accepted at the time of this thesis publication.

3.1 Introduction

Waterborne diseases are responsible for approximately 1.5 million deaths of children under 5 each year (UNICEF/WHO, 2009), highlighting the need for reliable and accurate methods to test the microbial quality of drinking water. Thermotolerant coliforms and *E. coli* are often used as FIB, because they are found in high concentrations in the gastrointestinal tracts of warm blooded of animals and are the preferred FIB for assessing drinking water quality by the WHO (WHO, 2011). However, the equipment, materials, sterile conditions, electricity and specialized training needed to perform FIB testing using standard membrane filtration (MF) methods (e.g. USEPA, 2002; APHA, 2012) are not always available. In addition, community health workers in non-governmental organizations (NGOs) and local health ministries in developing countries often operate on limited budgets and under non-ideal conditions making the need for simple, inexpensive tests crucial.

A number of easy-to use, inexpensive, portable test kits have been developed for FIB enumeration. However, to the author's knowledge limited rigorous testing has been conducted on the performance of FIB test kits at varying temperatures, especially in the lower 20°Cs, or on their reliability when used by technicians with limited training. Those working in developing countries must often rely on alternative means to incubate samples when electricity is lacking. Based on our informal survey of

development practitioners, alternative incubation methods include ambient-temperature incubation, placing petri dishes behind a refrigerator, using chemical or electric heating pads or incandescent lamps, solar incubators, and human or animal body heat. Brown et al. (2011) compared water quality data from both Cambodia and the Dominican Republic using 3-M Petrifilm plates and Colilert with standard MF under both standard and ambient temperature incubation conditions (26 to >32 °C). Differences between paired samples incubated at ambient and standard temperatures were found to be within the range of intra-sample variability for each method. Their results suggest that, for the range of temperatures tested, ambient temperature incubation is a useful alternative for enumerating FIB when using these methods if no incubator is available.

This research investigated the cost and performance of IDEXX Colilert Quanti-trays® 2000 (Colilert), Micrology Laboratories, Coliscan® Membrane Filtration (Coliscan MF) tests and 3-M Petrifilm™ Coliform/*E. coli* plates (modified 3-M) for enumerating FIB in drinking water sources in developing countries. Variables investigated included incubation temperature and analyst experience. MUG broth was used to confirm the accuracy of *E. coli* identification. Additional tests were conducted to determine the ease of use and accuracy of the kits when tests were performed by inexperienced personnel. Economic analysis included the major equipment and supplies required for each kit.

Although a simple, low cost presence/absence test for bacteria that produce hydrogen sulfide (H_2S) is available and is widely used in developing countries (Manja et al., 1982), the H_2S method was not evaluated in this study because it targets a different group of microorganisms (see Wright et al., 2012 for recent meta-analysis of studies and the accuracy and specificity of this test). There are also field kits, such as those produced by DelAuga (Marlborough, UK)**,** which use a lauryl sulfate broth media for the enumeration of thermotolerant coliforms and provide a portable, rechargeable incubator. These methods were not evaluated in this study either, as the kits are fairly expensive and may be out of reach for some international development workers and local health ministries.

3.2 Materials and Methods

A summary of the test kits evaluated in this study, including their standard incubation temperatures, substrates, and color changes for general coliforms and *E. coli* enumeration, is given in Table 12. Tests were performed in triplicate during the spring of 2011 on natural surface water seeded with primary sewage effluent and incubated at 22.0, 35.0 and 44.5°C. An initial round of testing produced colonies that were too numerous to count (TNTC) based on the manufacturers' specifications and therefore could not be used when determining the accuracy of the kits compared to standard MF; however, they were used to determine the percentage of false positives and false negatives. University student volunteers were provided with a short training session and illustrated users' manuals created in house. After using the test kits, the volunteers completed a survey, ranking the tests from easiest to most difficult and providing additional comments to the researchers.

3.2.1 Water Samples

Surface water samples were collected in sterile, 100 ml plastic bottles from a stormwater detention pond on the University of South Florida campus. The water was seeded with 1% volume by volume (V/V) of primary-treated municipal wastewater from the Howard F. Curren Advanced Wastewater Treatment Plant in Tampa, FL to simulate a surface water source contaminated with fecal material. The data presented here are from tests performed on a single sample of sewage contaminated surface water, rather than independent samples. All tests were performed on the same day to control for microbial growth/death over time and the sample was mixed after each triplicate test was plated to ensure adequate mixing.

3.2.2 Incubation Temperatures

FIB test kits were incubated at 22.0, 35.0, and 44.5°C. Samples maintained at 35.0 and 44.5°C were incubated for approximately 24 hours. Samples maintained at 22.0°C were incubated approximately 48 hours based on preliminary results showing more accurate results with a longer

incubation time at this temperature (data not shown). Note that the manufacturer's protocol for all kits was 24 hours of incubation at 35.0 °C (Table 12).

3.2.3 FIB Enumeration by Standard MF

Standard MF was used as the positive control. Seeded pond water volumes ranging from 0.05 to 0.5 ml were tested using *Standard Methods* (9222B) for enumeration of total coliforms (APHA, 2012) using 0.45 µm pore-size, 47 mm diameter gridded membranes (Millipore, Billerica, MA). Colonies with a green metallic sheen on mEndo agar (Difco, Franklin Lakes, NJ) were counted as total coliforms. Seeded pond water volumes ranging from 1 to 10 ml were used for EPA Method 1103.1 for enumeration of *E. coli* (USEPA 2002). Note that there was a modification to the Standard Methods as a water bath was not used. Colonies that retained a yellow to yellow/brown color after the aseptic transfer of the membranes from mTEC agar (Difco laboratories, Franklin Lakes, NJ) to a urea substrate (urea 2g/ 100ml; phenol red 0.01g/100 ml; Fisher Scientific, Pittsburgh, PA) were counted as *E. coli*.

3.2.4 FIB Enumeration by Colilert

Tests were performed in accordance with the manufacturer's specifications. Most probable number (MPN) estimates of total coliform and *E. coli* concentrations were obtained by diluting the seeded pond water by 1:100 and 1:200, with sterile deionized water prior to testing, based on the manufacturers' specifications. Wells with a yellow color were counted as positive for total coliforms. Wells that were both yellow and fluoresced under a 366 nm ultraviolet light were counted as positive for *E. coli*. MPN estimates were calculated using tables supplied by the manufacturer.

3.2.5 FIB Enumeration by Coliscan-MF

Tests were performed in accordance with the manufacturer's specifications. Seeded pond water volumes ranging from 0.1 to 7 ml were filtered through 0.45 µm, 47 mm gridded membranes (Millipore, Billerica, MA) using a small, hand-operated filter funnel and vacuum pump (Micrology Laboratories, Goshen, IN). Membranes were aseptically transferred to a 50 mm dish containing an absorbent pad that

had been prepared with Coliscan MF media as per manufacture's specifications. Colonies that produced a red color were counted as positive for general coliforms and colonies that produced a blue were counted as positive for *E. coli*. The sum of the blue and red colonies gave the total coliform count.

3.2.6 FIB Enumeration by Modified 3-M

The 3-M plates used in this study were only able to test 1 ml of sample per plate and are therefore not sensitive enough for use with water of drinking water quality. A modification of the 3-M tests (modified 3-M) was developed in consultation with a 3-M employee (Amann, E., Global Marketing Manager, 3-M Company, personal communication, 2011). Recently 3-M developed a kit specifically for water quality testing, the 3M™ Petrifilm™ Aqua Plate, but this was not used in this experiment as it was not available at the time. Seeded pond water volumes ranging from 0.1 to10 ml were filtered through 0.45 µm, 47 mm gridded membranes (Millipore, Billerica, MA) using the small, hand-operated filter funnel and vacuum pump described above (Micrology Laboratories, Goshen, IN). The membranes were aseptically transferred to 3-M Petrifilm plates that had been prepared by pipetting 1 ml of sterile deionized water onto the center of the bottom film. Colonies that produced a red color were counted as positive for general coliforms and colonies that produced a blue color were counted as positive for *E. coli*. The sum of the blue and red colonies gave the total coliform count. For a colony to be counted positive for either total coliforms or *E. coli,* there should be an associated gas bubble. However, the membranes made it difficult to confirm the presence of a gas bubble in the area of color change and it was sometimes difficult to distinguish the colonies from the background.

3.2.7 Confirmation of Identification as *E. coli*

The percentage of false-positive and false-negative results for *E. coli* produced by each test kit at the various temperatures tested was determined following *Standard Methods* (APHA ,2012; Method 9221F). False positive rates are also provided for standard MF. False negatives for standard MF were not calculated because total coliforms and *E. coli* cannot be differentiated on mEndo medium, and atypical

colonies on mTEC medium (which could be considered non-*E. coli* coliforms) were not observed. Total coliform (other than *E. coli*) and *E. coli* colonies were picked from the standard MF, Coliscan-MF and modified 3-M plates using a sterile wire loop and transferred to a well in a 96-well plate containing EC-MUG broth (Difco, Franklin Lakes, NJ). For the Colilert method, the back of a well was pierced using a sterile needle and a small amount of the liquid was removed using a sterile wire loop and transferred to the well plate. A total of 44 presumed total coliform and 44 presumed *E. coli* colonies or samples were taken from each test type at each temperature to have a representative sample, assuming a Poisson distribution (Gotelli & Ellison 2004), except for standard MF where only 40 colonies were used. Plates were incubated for 24 hours at 44.5°C. Wells that fluoresced were considered positive for *E. coli*. A false-positive result was considered to be a colony or well that tested positive for *E. coli* in a test kit but did not fluoresce in MUG broth, while a false-negative result was considered to a colony or well that tested negative for *E. coli* from a test kit (a general coliform) but did fluoresce in MUG broth. The percent false positive or false negative for each kit was determined by expressing the number of false positive or false negatives over the total number of colonies tested (44 or 40).

3.2.8 Effect of Experience on Results

Twelve students were recruited from a University of South Florida course in Sustainable Development. Most of the students had no prior formal training in the test methods used. The students were aware of the research goals and the water source, but did not have any knowledge of prior test results from this water source. Groups of 2-3 students were given a short demonstration, and an illustrated user's manual was provided at each workstation (contact the corresponding author for a copy of these manuals). Each group performed standard MF and Colilert in triplicate on the same water sample. Coliscan MF and modified 3-M were performed by four of the five groups (two of the four groups performed the Coliscan in duplicate rather than triplicate) using the same water sample as the standard MF and Colilert tests. Tests were incubated at 35.0°C, with the exception of standard MF for *E*

coli which was incubated at 44.5°C. Tests were conducted in parallel by one of the authors of this paper (Meghan Wahlstrom-Ramler) for comparison. Each student also counted colonies or positive wells on plates and trays that had been prepared the previous day by the experienced technician. Students completed a survey, ranking the methods and providing additional comments to the researchers

3.2.9 Statistical Analysis

As stated previously, tests were performed in triplicate at a range of volumes or dilutions at 22.0, 35.0 and 44.5°C. Only the plates containing colonies which fell in the range of the manufacturers' recommendations were used. Two-way analysis of variance (ANOVA) with bonferonni post tests was used to compare the *E. coli* and total coliform concentrations from the different test methods and temperatures. Statistical analyses were conducted using GraphPad Prism Software, version 5.02 for Windows (San Diego California, USA), at an alpha level of 0.05. In some cases, the sample size from the volunteers was too small to draw conclusions based on a statistical analysis of the data. As it is important to understand the effect that analyst experience has on the accuracy of the test kits, means and standard deviations for these results were calculated and are included in this paper.

3.2.10 Economic Analysis

Major equipment (pumps, filtering equipment, sealer) and supply (chemicals, plates, test kits) costs were estimated using 2013 prices from US laboratory supply companies (Fisher Scientific, Pittsburgh PA; Hach Co., Loveland, CO; Sigma-Aldrich Co., St. Louis, MO). No costs for incubators, labor or electrical power were included. Costs were estimated for the initial materials (ex. pumps and sealer) and for the consumables needed to perform 100 tests.

3.3 Results

3.3.1 FIB Kit Performance at Varying Temperatures

The results of FIB testing at varying temperature are shown in Figures 22 and 23. The full results can be found in Appendix G. Note that the low number of replicate samples and the small sample size

may have contributed to the high standard deviations observed with some of the kits. At both standard temperature (35.0 °C) and at the lower temperature of 22.0 °C, Colilert and Coliscan MF were comparable to standard MF for both total coliforms and *E. coli*, while significant differences were observed with the modified 3-M method. It should be noted that the *E. coli* colony counts for Colilert and modified 3-M at 22.0 and 35.0°C were low (below the manufactures' recommended lower limits), which could have skewed the results. When incubated at 44.5°C, both Coliscan and modified 3-M were comparable to standard MF for *E. coli* but not for total coliforms. At 44.5°C the majority of the growth was *E.* coli with very low to no general coliform growth. Colilert tests incubated at 44.5°C showed no color change or fluorescence in any wells. However, in our prior experimental runs, incubation of Colilert at 44.5°C did produce wells with color changes and that fluoresced. In a study submitted to USAID in 2007 (PATH, 2007), the researchers looked at the number of different strains of fecal coliforms that grew at standard and non-standard temperatures for different test kits. The authors found that the number of independent strains of coliforms was greatly reduced with incubation at 45°C. It is possible then that the strain of *E. coli* contained in the sewage sample for this test was unable to grow at the elevated temperature of 44.5°C.

3.3.2 Confirmation of Identification as *E. coli*.

In prior studies, the different test kits have been shown to produce false positive (Watkins et al., 1988; Fricker et al*.,* 1997; Umble et al., 1999; Yakub et al*.*, 2002; Vail et al., 2003; Buckalew et al., 2006; Medhurst et al., 2007; Sercu et al., 2011) and false negative (Watkins et al,. 1988; Clark et al*.,* 1991; Umble et al., 1999; Medhurst et al*.,* 2007) results that can lead to the misrepresentation of water quality. The percentages of false-positive and false-negative *E. coli* results from each test kit at each temperature are shown in Table 13.

At 35.0 °C, modified 3-M and standard MF produced the highest percentage of false positive colonies (20.5%) and Coliscan-MF produced the highest percentage of false negatives (25.0%). With the

lengthier incubation at 22.0 °C, Coliscan-MF produced the highest percentage of false-positives (31.8%) and modified 3-M kits produced the highest percentage of false-negatives (50.0%). With incubation at 44.5 °C, Coliscan-MF and modified 3-M produced the greatest percentage of false positives (18.2%). Colilert produced the greatest percentage of false negatives (22.7%).

3.3.3 Effect of Analyst Experience

With regard to ease of use (Table 14), the majority of the volunteers indicated that Colilert was the easiest to use, while no significant differences were observed in their rankings of the other kits. However, comments by the volunteers indicated that they found the modified 3-M plates difficult to count and that the use of the hand-operated filtration unit decreased the ease of use for both modified 3-M and Coliscan MF. Full results from the volunteer experiments can be found in Appendix H.

As discussed previously, the sample size was too small to carry out statistical analysis of the differences between the volunteer and expert results. The results presented here are based on whether the volunteer's results fell within $\pm$ 1 standard deviation of the expert's results. When the volunteers prepared the plates, the results were similar to those of the expert (Table 15). When volunteers counted plates and trays that were prepared by the expert, their counts for *E. coli* were different for two of the standard MF plates, one of the Colilert plates, and one of the modified 3-M plates (Table 16). For general coliforms there was a difference between the expert and volunteer plates for one of the standard MF plates and two of the modified 3-M plates. The general coliform counts for the modified 3-M plates were fairly high, which may have made them difficult to interpret.

Note that these tests were performed by highly educated volunteers who were only involved with sample processing and counting. Therefore, the degree of inconsistency between expert and non-experts is likely to be lower than real world results where sample collection and storage by non-experts may result in increased risk of sample contamination and improper storage time and temperature conditions.

3.3.4 Cost Comparison

The materials cost (Table 17) shows that the Colilert tests have the most expensive non-consumables ($3,795) as compared to the other three (approximately $9 for Coliscan-MF and modified 3-M and $16 for standard MF). Colilert was also the most expensive when looking at the consumables cost per 100 tests ($1,329), followed by modified 3-M ($888), then Coliscan-MF ($834).

3.4 Discussion

With a significant amount of morbidity and mortality cause by waterborne illnesses in the developing world, there is a need to ensure that high quality water is used for consumption and personal hygiene. In the developing world, however, access to the costly equipment and supplies, reliable electricity and qualified personnel for conducting specialized tests for FIB is often limited. Considering these constraints this research evaluated the performance of three different FIB test kits to investigate how they might perform in situations where resources are limited. Each of the kits were ranked from best (1) to worst (3) based on their performance for each of the parameters tested as well as cost (Table 14). Based on the unweighted means, Coliscan-MF scored the best (1.4), followed by Colilert (1.9), and finally modified 3-M (2.7). However, a major concern with Coliscan-MF was the high false positive results (Table 13), which could misrepresent high quality water as contaminated.

The Colilert kits produced results that were similar to standard MF for both total coliforms and *E. coli* when the trays were incubated at 35.0 °C and 22.0°C. Similar results for *E. coli* have been observed by other researchers when comparing Colilert defined substrates to MF techniques at standard temperatures (Clark et al., 1991; Fricker et al., 1997; Yakub et al., 2003; Buckalew et al., 2008) and Colilert is approved by the USEPA for testing water of drinking water quality (U.S. Federal Register, 1989; U.S. Federal Register, 1992). At 44.5 °C no results were produced. Over the range of temperatures, false positive *E. coli* results for Colilert were low (4.6 – 6.8%) and fell within the range of 3.0-10.7% observed by Yakub et al. (2003), when looking at surface water. Sercu et al. (2011) found a higher range

of 14-23% while Fricker et al. (1997) observed a 0% false positive rate. False-negatives for Colilert (22.7-25.0%) were higher than those reported by Clark et al. (1991), who reported false negatives of 19%, when looking at untreated waters. Volunteers found Colilert to be the easiest to use and to count (Table 14); however, there was a fairly large degree of variability between the volunteers when they were counting the plates for general coliforms (Table 16). This high degree of variability could be due to the fact that there were no reference plates to show the degree of color change required and that counting was done over several hours. While samples were refrigerated when possible between groups additional color changes could have occurred over those hours. There were possible differences when counting the *E. coli* concentrations for one of the tests. As with total coliforms, this could be cause by the extended length of time over which the results were counted. The results of the volunteer analysis highlight the fact that proper training is critical even with an easy-to-use method such as the Colilert Quantitrays. The main drawbacks of Colilert were the high cost, the need for a UV light, and the need for a large bulky sealing device that requires electricity.

The Coliscan MF kits produced results that were similar to standard MF for both total coliforms and *E. coli* when the trays were incubated at 22.0 and 35.0 °C and *E. coli* at 44.5°C. This is similar to the results presented in Umble et al. (1999) who found that Coliscan MF produced results similar to standard MF at standard temperatures. Incubation of Coliscan MF tests at 44.5°C produced general coliform results that were significantly different than standard MF. At 35.0°C false-positives were 4.6% and were similar to the 7.7% found by Watkins et al. (1988) (when looking at secondary wastewater effluent only) and 3.8% by Umble et al. (1999), but lower than the 30% observed by Medhurst et al. (2007). Once outside the standard temperature of 35.0°C, the false positives increased (31.8% at 22.0°C and 18.2% at 44.5°C). False negatives at 35.0°C were relatively high (25%). In comparison, Watkins et al. (1988) found a false negative rate of 0% (when looking at secondary wastewater effluent only) and Umble et al. (1999) found 0.8%. False-negatives at non-standard temperatures were lower than at

standards (20.5% at 22.0°C and 6.8% at 44.5°C). Volunteers were able to produce *E. coli* results that were similar to the experts with Coliscan MF; however, they reported that it was difficult to use, most likely because of the hand-operated filtration apparatus. When counting the pre-prepared plates, no differences were noted for either general coliforms or *E. coli*. The Coliscan MF kits had the lowest cost of any of the kits tested. One drawback to the Coliscan MF method is that it requires a special media, which is used to prepare the absorbent pad. This media should ideally be refrigerated. However; as long as that media is kept in a dark location which is not prone to heat up excessively, it can be kept at room temperature for up to two months (Roth, J., Micrology Laboratories, Personal Communication, March 28, 2013).

Modified 3-M kits produced total coliform and *E. coli* results that were significantly different than standard MF at 35.0°C and 22.0°C and total coliform results at 44.5°C. In contrast, Vail et al. (2003) found no consistent difference between paired measurements made with Petrifilm and mTec; however, they were not using the modified method. The percentage of false negatives at 22.0°C was the highest of any test (50.0%). The filter paper added made the color changes and appearance of gas bubbles not as readily apparent as with the non-modified version of the test which may have led to the high false negative rate. When performed by the volunteers no differences were noted with the *E. coli* concentrations produced between the volunteers and the expert. The general coliform colonies were too numerous to count. When counting the plates, differences were noted in one of the three tests for *E. coli* and two of the three tests for general coliforms and volunteers noted that counting the colonies on the modified 3-M plates was difficult. For a colony to be considered positive for *E. coli* or coliforms on the 3-M plates there must be both a blue or red color, respectively and an associated gas bubble (Vail et al., 2003). While it is relatively easy to see the gas bubble on the plates without the membrane, the membrane makes the bubbles difficult to see. The background color also makes it difficult to identify total coliform colonies. As mentioned previously, modification of the test to incorporate a MF step using

the hand-operated filtration apparatus was needed to increase the sample size in order to analyze water of drinking water quality. With this in mind, our false positive/false negative results fall more in line with those of other authors. Vail et al. (2003) found that if no gas bubble was produced, the false-positive rate was between 53.8 and 71.4%, which was higher than the result we obtained (11.4-20.5%). In comparison, they found that the false positive rate was 0% if there was an associated gas bubble.

3.5 Conclusions

This study investigated the cost and performance of three FIB test kits at varying incubation temperatures and when used by inexperienced volunteers. WHO guidelines recommend *E. coli* as the preferred FIB for assessing water of drinking water quality (WHO 2008). Therefore, it is an important finding that *E. coli* results were not significantly different than standard MF for Coliscan and Colilert incubated at 22.0°C for 48 hours; however, more research is needed on the performance of FIB test kits at a broader range of incubation temperatures relevant to tropical and sub-tropical regions. When looking at an unweighted average of performance and cost metrics, Coliscan MF was a good method for *E. coli* enumeration at non-standard temperatures. Of the three kits it was the least expensive and was similar to standard MF when performed by an experienced technician. Results were produced that were comparable to the experts when both prepared and counted by the volunteers and had overall the lowest % of false negatives. Coliscan-MF also had the highest false positive rate at 22.0°C.

To our knowledge this is the first peer-reviewed study to report results of water quality testing using the modified 3-M method. Petrifilm plates are compact, lightweight, can be used with a hand-operated filtration apparatus and did not require a high capital investment or electricity; however, more work is needed to reduce the errors caused by the difficulty visualizing the associated gas bubbles.

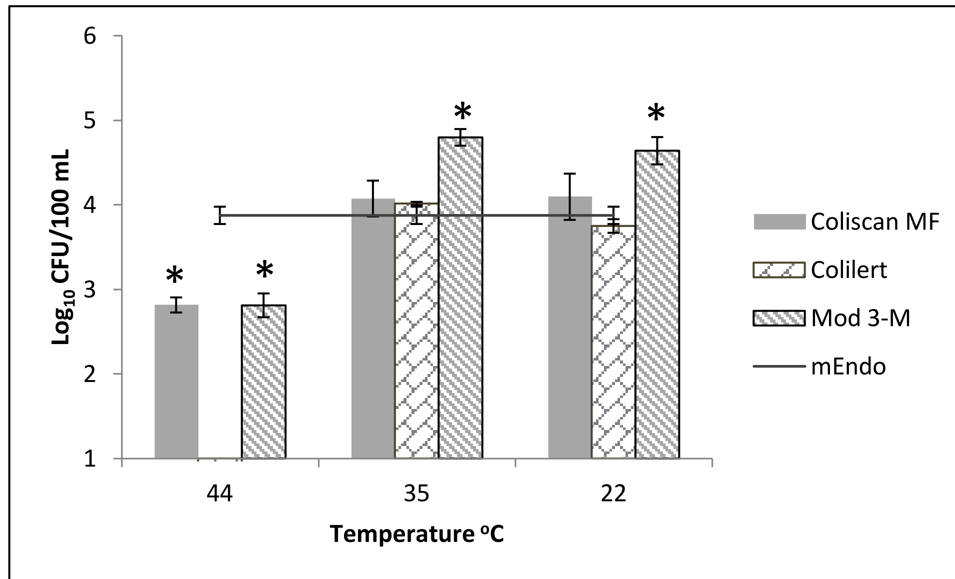


Figure 22: Total coliform concentrations determined by standard MF (line) and FIB test kits at varying temperatures. Error bars show ± one standard deviation from the mean. Asterisks (*) indicate results that were significantly different from standard MF (α = 0.05). Colilert tests were all below detection limits at 44.5°C.

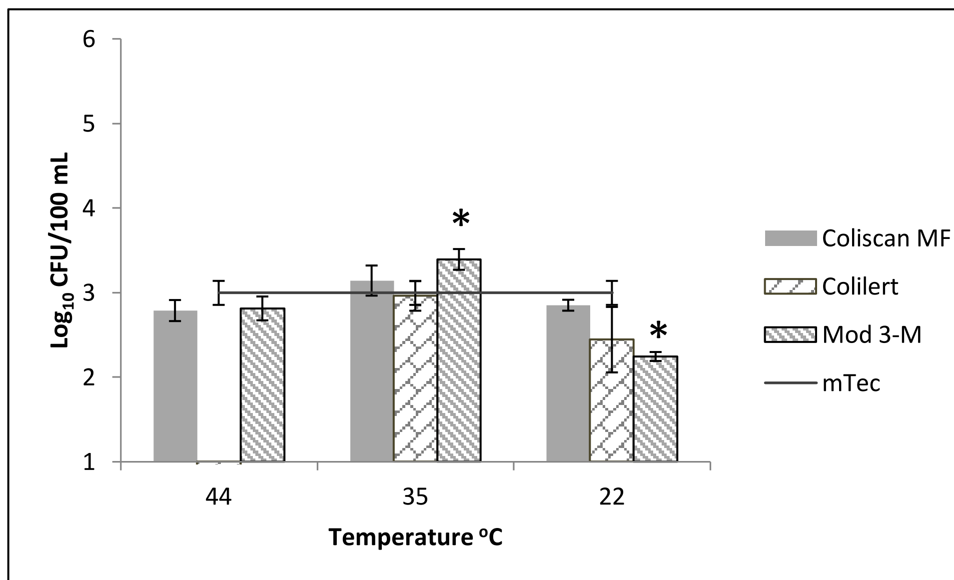


Figure 23: *E. coli* concentrations determined by standard MF (line) and test kits at varying temperatures. Error bars show ± one standard deviation from the mean. Asterisks (*) indicate results that were significantly different from standard MF (α = 0.05). Colilert tests were all below detection limits at 44.5°C.

Table 12: Standard temperature, substrates, and associated color changes with the kits tested in this research.

Field Kits	Standard Temp (°C)	General Coliforms		*Escherichia coli*	
		Substrate[a]	Color Change	Substrate[a]	Color Change
Colilert	35.0	ONPG	Yellow	MUG	Fluoresce
Coliscan - MF	35.0	RED-GAL®	Red	X-GLUC	Blue
Mod 3-M	35.0	Proprietary[b]	Red w/gas	Proprietary[b]	Blue w/gas

[a]ONPG = Ortho-nitrophenyl-β-D galactopyranoside, MUG = 4-methyl-umbelliferyl-β-D-glucuronide, Gal = 6-Chloro-3-Indolyl-β-D-galactoside, X-Gluc = 5-Bromo-4-Chloro-3-Indoyl- β-D-glucuronide (Olstadt et al., 2007)

[b]Substrates were not provided in manufacturers' protocols.

Table 13: Percentage of false positive and false negative results for *E. coli* at varying temperatures

	Temperature	False Positive	False Negative
mTEC	35.0/44.5	20.5%	NA
Colilert	22.0	4.6%	25.0%
	35.0	4.6%	22.7%
	44.5	6.8%	22.7%
Coliscan - MF	22.0	31.8%	20.5%
	35.0	4.6%	25.0%
	44.5	18.2%	6.8%
Modified 3-M	22.0	11.4%	50.0%
	35.0	20.5%	11.4%
	44.5	18.2%	11.4%

Table 14: Ranking of test kits using *E. coli* results only. Rankings go from best (1) to worst (3).

Method	Agreement with standard MF[a]	Cost	Ease of Use	Accuracy when performed by volunteers[b]	Accuracy when counted by volunteers	% false positive[a]	% false negative[a]	Average Score[c]
Colilert	2	3	1	2	2	1	2	1.9
Coliscan MF	1	1	2	1	1	3	1	1.4
Mod 3-M	3	2	3	3	3	2	3	2.7

[a] When looking at the performance of each kit over the range of temperatures, the ranking was based on the performance at each temperature with an emphasis placed on the results at 22°C

[b] As there were no significant difference noted between the plates prepared by the volunteers and that of the expert, ranking was based on the P-value

[c] The average score was found by adding each of the individual rankings for each criteria and dividing by the total number of criteria (7)

Table 15: FIB concentrations produced by inexperienced volunteers (I) and expert (E) for each method. Standard deviations are shown in parentheses. TC, total coliforms; EC, *E. coli.*

Method	TC prepared by volunteers CFU/100 mL		Within SD (Y/N)	EC prepared by volunteers CFU/100 mL		Within SD (Y/N)
	I	*E*		*I*	*E*	
Standard MF	40.1 (± 22.4)	57.0(± 8.5)	Y	3.6 (±2.4)	5(± 3.0)	Y
Colilert	395.0 (165.3)	248.1(NA)[a]	Y	2.3 (± 1.4)	3.4(1.6)	Y
Coliscan-MF	127 (± NA)[a]	84.5(± NA)[b]	NA	5.2 (± 4.0)	5.5(± 0.7)	Y
Mod 3-M	TNTC	TNTC	NA	6.1 (± 2.8)	11.3(± 6.1)	Y

[*]Represents values that were significantly different from the expert

[a] All but one of the plates prepared was TNTC

[b] Only two of the three plates produced countable results

Table 16: FIB concentrations prepared by the expert (E) and then counted by the inexperienced volunteers (I) for each method. Standard deviations are shown in parentheses. TC, total coliforms; EC, *E. coli*

Method	**TC counted by volunteers CFU/100 mL**		**Within SD (Y/N)**	**EC counted by volunteers CFU/100 mL**		**Within SD (Y/N)**
	I	***E***		***I***	***E***	
Standard MF	41.0 (± 18.5)	37	Y	53.6 (± 7.1)	61*	N
	27.8 (± 6.9)	30	Y	67.7 (± 11.4)	69	Y
	37.7 (± 8.6)	48*	N	40.2 (± 5.8)	34*	N
Colilert	132.0 (± 43.0)	107	Y	4.5 (± 1.8)	6.3	Y
	114.4 (± 37.7)	NA	NA	4.7 (± 0.8)	NA	NA
	134.7 (± 55.5)	105	Y	5.2(±0.6)	8*	Y
Coliscan-MF	65.9 (± 17.1)	60	Y	12.1 (± 7.7)	11	Y
	60.6 (±20.4)	46	Y	14.1 (±3.1)	16	Y
	71.3 (± 18.2)	72	Y	11.4 (±1.3)	11	Y
Mod 3-M	174.2 (±27.6)	145*	N	10 (± 0)	10	Y
	179.0 (± 22.9)	161	Y	10.6 (±1.1)	11	Y
	161.5 (±39.8)	121*	N	14.0 (±1.2)	17*	N

*Represents values that were not within a standard deviation of the expert

Table 17: Economic analysis, electricity requirements, and rankings by volunteers. Standard deviations for the rankings are shown in parentheses

Method	Non-consumables costs $US[a]	Consumables costs $US	Electricity needed?	Ease of counting[f]	Ease of use[f]
Standard MF	$15.80	$873.56[b]	Yes	2.6 (1.3)	2.4 (1.1)
Colilert	$3,794.50	$1,329.00[c]	Yes	1.3 (0.82)	1.7 (1.0)
Coliscan MF	$9.00	$834.31[d]	No	2.6 (0.92)	3 (1.1)
Modified 3-M	$9.00	$888.20[e]	No	2.9 (0.99)	2.9 (0.92)

[a]includes only a sealer, UV lamp, and 250 ml beaker for Colilert method, a portable filter apparatus and 2, 100 ml beakers for standard MF, and a portable filter apparatus for the Coliscan MF and Modified 3-M methods. No incubators, autoclaves, or biosafety cabinets are included.

[b]includes mTec and mEndo agar, urea substrate, phenol red, membrane filters, ethanol, and petri dishes with absorbant pads

[c]from the cost of a 100 test pack which includes the Colilert media and Quanti-Tray as well as a 100 pack of 100 ml disposable pipettes

[d]from the cost of a 100 test pack which includes the coliscan medium, membrane filters, and petri dishes with absorbent pads and a 100 pack of 100 mL disposable pipettes

[e]includes the price of 2, 50 pack 3-M test kits , a 100 pack of disposable 100 ml pipettes, and a 100 pack of membrane filters

[f]1 = easiest, 4 = most difficult

CHAPTER 4: OVERALL CONCLUSIONS AND RECOMMENDATIONS

Research for this thesis consisted of two separate yet related topics. Below are general conclusions from each of the studies. For a more detailed summary, please refer to Sections 2.5 and 3.5. The first study involved thermotolerant coliform contamination of Pitcher Pump systems in Tamatave, Madagascar. The following is a summary of the conclusions and recommendations from that investigation:

1) Thermotolerant coliform results indicated that 55.9% (33 of 59) of the Pitcher Pump systems were in compliance with the Malagasy standard (≤10 cfu/100 ml), 22.0% (13 of 59) produced water that posed on intermediate risk to human health, and 22% (13 of 59) produced water that posed a high risk to human health.
2) No depth could be found that would consistently provide safe drinking water and no statistically significant link was found between the depth and water quality.
3) Results from the Phase II pilot study indicated a general tread of a decrease in thermotolerant coliform concentration with an increase in well depth but not enough data were gathered to draw any conclusive conclusions. Furthermore, even at the deepest depth of 9.4 m bgs, the thermotolerant coliform levels were still well above 10 cfu/100 ml.
4) There was a statistically significant association between whether or not a pump required priming and the risk level of the water produced ($p = 0.03$) and the median thermotolerant coliform concentration in primed wells (41.3 cfu/100 ml) was significantly higher than those of the unprimed wells (3.5 cfu/100 ml).

5) Note that this study does not recommend that households using Pitcher Pumps in Tamatave switch to another water source; however, it is recommended that an educational campaign be put in place to promote boiling as a water treatment option.
6) Investigate what effect the presence of a concrete apron has on the levels of thermotolerant coliforms detected in the Pitcher Pump systems.
7) Further investigate whether maintenance of the wells to reduce the need for pump priming will improve the water quality.

The second was an investigation into alternative methods to test for FIB. In regards to the investigation into alternative methods for the enumeration of fecal indicator bacteria, the following conclusions were made:

1) When looking at an unweighted average of performance metrics, Colisan MF performed the best. Its results most approximated those of standard methods at both standard and non-standard temperatures, was performed and counted most accurately when used by inexperienced volunteers, and was the least expensive. However, it did produce the greatest percentage of false positive results.
2) Modified 3-M was the least accurate and this is potentially due to the difficulty associated with the filter paper.
3) It is recommended that further testing be conducted on the performance of FIB test kits at a broader range of incubation temperatures relevant to tropical and sub-tropical regions.

REFERENCES

Ahmed, K.M., Khandkar, Z.Z., Lawrence, A.R., Macdonald, D.M.J., & Islam, M.S. (2002*). Appendix A: An investigation of the impact of on-site sanitation on the quality of groundwater supplies in two per-urban areas of Dhaka, Bangladesh*. In: Assessing Risk to Groundwater from On-site Sanitation: Scientific Review and Case Studies. Keyworth, UK: British Geological Survey, 37-67. Retrieved from http://r4d.dfid.gov.uk/pdf/outputs/r68692.

Akers, D.B. (2014). *Lead (Pb) contamination of water drawn from pitcher pumps in eastern Madagascar* (Master's thesis). University of South Florida, Tampa, FL.

Aldana, J.M. (2010). *Rapid assessment of drinking-water quality in the Republic of Nicaragua: country report of the pilot project implementation in 2004-2005*. Geneva, Switzerland: World Health Organization (WHO).

Aliev. S., Shodmonov P, Babakhanova N., & Schmoll O. (2010). *Rapid assessment of drinking-water quality in the Republic of Tajikistan: country report of the pilot project implementation in 2004–2005*. Geneva, Switzerland: World Health Organization (WHO).

APHA. (2012). *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association APHA-AWWA-WEF, Washington, DC.

Assis, A.M.O., Barreto, M.L., Santos, L.M.P., Fiaccone, R., & da Silva Gomes, G.S. (2005). Growth faltering in childhood related to diarrhea: A longitudinal community based study. *European Journal of Clinical Nutrition*, *59*(11), 1317-1323.

Baars, J.K. (1957). Travel of pollution, and purification en route, in sandy soils. *Bulletin of the World Health Organization*, *16*(4), 727.

Bain, R.E., Gundry, S.W., Wright, J.A., Yang, H., Pedley, S., & Bartram, J.K. (2012). Accounting for water quality in monitoring access to safe drinking-water as part of the Millennium Development Goals: lessons from five countries. *Bulletin of the World Health Organization*, 90(3), 228-235.

Banerjee, G. (2011). Underground pollution travel from leach pits of on-site sanitation facilities: a case study. *Clean Technology Environmental Policy,* 13(3), 489-497.

Barrell, R.A.E, & Rowland, M.G.M. (1979). The relationship between rainfall and well water pollution in a West African (Gambian) village. *Journal of Hygiene,* 83(1), 143-150.

Baumann, E. (2011). *Low Cost Handpumps Field Note 2001-3.* St Gallen, Switzerland: Rural Water Supply Network (RWSN)

Beard, P.J. (1940). Longevity of *Eberthalla Typhosus* in various soils. *American Journal of Public Health and Nations Health*, 30(9), 1077-1082.

Bitton, G., & Gerba, C. P. (1994). Microbial pollutants: Their survival and transport pattern to groundwater. In G. Bitton and C. Gerba (Eds.), *Groundwater Pollution Microbiology* Malabar, FL: Krieger Publishing Company.

Black, R.E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., De Onis, M., Ezzati, M., ... & Rivera, J. (2008). Maternal and child undernutrition: Global and regional exposures and health consequences. *The Lancet*, *371*(9608), 243-260.

Black, R.E., Cousens, S., Johnson, H.L, Lawn, J. E., Rudan, I., Bassani, D. G., ... Mathers, C. (2010). Global, regional, and national causes of child mortality in 2008: A systematic analysis. *The Lancet*, 375(9730), 1969-1987.

BGS (British Geological Survey). (2002). *Assessing the risk to groundwater from on-site sanitation: scientific review and case studies.* (Publication No. CR/02/079N). Keyworth, UK: British Geological Survey. Retrieved from http://r4d.dfid.gov.uk/pdf/outputs/r68692.pdf

Borchardt, M.A., Bradbury, K.R., Alexander, E.C. Jr., Kolberg, R.J., Archer, J.R., Braatz, L.A., … & Spencer, S.K. (2010). Norovirus outbreak caused by a new septic system in a dolomite aquifer. *Groundwater*, 49(1), 85-97.

Boulter, J. (1993). *GPS coordinate distance calculator*. Retrieved from http://boulter.com/gps/distance/.

Brown, J., Stauber, C., Murpy, J.L., Khan, A., Mu, T., Elliott, M., & Sobsey, M.D. (2011). Ambient-temperature incubation for the field detection of *Escherichia coli* in drinking water. *Journal of Applied Microbiology,* 110, 915-923.

Brown, J.M., Proum, S., & Sobsey, M.D. (2008). *Escherichia coli* in household drinking water and diarrheal disease risk: evidence from Cambodia. *Water Science & Technology,* 58(4), 757-763.

Buckalew, D.W., Hartman, L.J., Grimsley, G.A., Martin, A.E., & Register, K.M. (2006). A long-term study comparing membrane filtration with Colilert® defined substrates in detecting fecal coliforms and *Escherichia coli* in natural waters. *Journal of Environmental Management,* 80, 191-197.

Byappanahalli, M. & Fujioka, R. (2004). Indigenous soil bacteria and low moisture may limit but allow faecal bacteria to multiply and become a minor population in tropical soils. *Water Science and Technology*, 50(1), 27-32.

Byappanahalli, M.N. & Fujioka, R.S. (1998). Evidence that tropical soil environments can support the growth of *Escherichia coli*. *Water, Science, and Technology*, 38(12), 171-174.

Caldwell, E. L. (1937). Pollution flow from pit latrines when an impervious stratum closely underlies the flow. *Journal of Infectious Diseases*, 61(3), 270-288.

Caldwell, E.L., & Parr, L.W. (1937). Ground water pollution and the bored hole latrine. *Journal of Infectious Diseases*, *61*(2), 148-183.

Caldwell, E.L. (1938a). Pollution flow from a pit latrine when permeable soils of considerable depth exist below the pit. *Journal of Infectious Diseases*, 62(3), 225-258.

Caldwell, E.L. (1938b). Studies of subsoil pollution in relation to possible contamination of the ground water from human excreta deposited in experimental latrines. *Journal of Infectious Diseases*, 62(3), 272-292.

Carrillo, M., Estrada, E., & Hazen, T.C. (1985). Survival and enumeration of the fecal indicators *Bifidobacterium adolscentis* and *Escherichia coli* in a tropical rain forest watershed. *Applied and Environmental Microbiology*, 50(2), 468-476.

Cave, B., & Kolsky, P. (1999). *Groundwater, latrines and health well study no. 163*. London School of Hygiene and Tropical Medicine. Leicestershire, UK: WEDC, Loughborough University. Retrieved from http://www.lboro.ac.uk/well/resources/well-studies/summaries-htm/task0163.htm

Central Intelligence Agency (CIA). (2014a). Madagascar. *The World Factbook.* Retrieved from https://www.cia.gov/library/publications/the-world-factbook/geos/ma.html

Central Intelligence Agency (CIA). (2014b). CIA Maps: Madagascar (2014). In *The World Factbook.* Retrieved from https://www.cia.gov/library/publications/the-world-factbook/geos/ma.html

Clark, D.L., Milner, B.B., Stewart, M.H., Wolfe, R.L., & Olson, B.H. (1991). Comparative study of commercial 4-methylumbelliferyl-β-D-glucuronide preparations with the *Standard Methods* membrane filtration fecal coliform test for the detection of *Escherichia coli* in water samples. *Applied Environmental Microbiology,* 57(5), 1528-1534.

Clasen, T.F. & Bastable, A. (2003). Faecal contamination of drinking water during collection and household storage: The need to extend protection to the point of use. *Journal of Water and Health*, 1(3), 109-115.

Cronin, A.A., Breslin, N., Gibson, J., & Pedley, S. (2006). Monitoring source and domestic water quality in parallel with sanitary risk identification in Northern Mozambique to prioritise protection interventions. *Journal of Water and Health*, 4(3), 333-345.

Davies, J. (2008). Chapter 14: Hydrogeology. In: *British Geological Survey, United States Geological Survey and Cabinet GLW Conseil, 2008* Repulbique de Madagascar Ministere de L'Energie et des Mines (MEM/SG/DG/UCP/PRGM); Projet de Gouvernance des Ressources Minerales (PGRM): British Geological Survey.

Desmarais, T.R., Solo-Gabriel, H.M., & Palmer, C.J. (2002). Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Applied and Environmental Microbiology*, 68(3), 1165-1172.

Driscoll, F.G. (1986). *Groundwater and wells* (2nd ed.). St. Paul, MN: Johnson Division.

Dzwairo, B., Hoko, Z., Love, D., & Guzha, E. (2006). Assessment of the impacts of pit latrines on groundwater quality in rural areas: A case study from Marondera district, Zimbabwe. *Physics and Chemistry of the Earth, Parts A/B/C*, *31*(15), 779-788.

EPA. (2009). *Analytical methods approved for drinking water compliance monitoring under the total coliform rule*. Retrieved from http://www.epa.gov/ogwdw/methods/pdfs/methods/methods_tcr.pdf

EPA. (2013, June 20) *National Beach Guidance and required Performance Criteria – Appendix 1C1: Indicator Organisms*. Retrieved from: http://water.epa.gov/grants_funding/beachgrants/app1c.cfm

Escamilla, V., Knappett, P. S., Yunus, M., Streatfield, P. K., & Emch, M. (2013). Influence of latrine proximity and type on tubewell water quality and diarrheal disease in Bangladesh. *Annals of the Association of American Geographers*, 103(2), 299-308.

Esrey, S. A., Potash, J. B., Roberts, L., & Shiff, C. (1991). Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm, infection, schistosomiasis, and trachoma. *Bulletin of the World Health Organization*, 69(5), 609-621.

Everitt, B., & Skrondal, A. (2002). *The Cambridge dictionary of statistics* (Vol. 106). Cambridge, UK: Cambridge University Press.

Ferguson, A.S., Layton, A.C., Mailloux, B.J., Culligan, P.J., Williams, D.E., Smartt, A.E.,… & van Geen, A. (2012). Comparison of fecal indicators with pathogenic bacteria and rotavirus in groundwater. *Science of the Total Environment*, 431, 314-32.

Fricker, E.J., Illingworth, K.S. & Fricker, C.R. (1997). Use of two formulations of Colilert and Quantitray™ for assessment of the bacteriological quality of water. *Water Resources,* 31(10), 2495-2499.

Fujioka, R., Slan-Denton, C., Borja, M., Castro, J., & Morphew, K. (1999). Soil: The environmental source of *Escherichia coli* and Enterococci in Guam's streams. *Journal of Applied Microbiology Symposium Supplement*, 85, 83S-89S.

Fujioka, R.S., Tenno, K, & Kansako, S. (1988). Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. *Toxicity Assessment: An International Journal*, 3, 613-630.

Gelinas, Y., Randall, H., Robidous, L., & Schmit, J.P. (1996). Well water survey in two districts of Conakry (Republic of Guinea), and comparison with the piped city water. *Water Resources*, 30(9), 2017-2026.

Ginn, T. R., Wood, B. D., Nelson, K. E., Scheibe, T. D., Murphy, E. M., & Clement, T. P. (2002). Processes in microbial transport in the natural subsurface. *Advances in Water Resources*, *25*(8), 1017-1042.

Godfrey, S., Timo, F., & Smith, M. (2006). Microbial risk assessment and management of shallow groundwater sources in Lichinga, Mozambique. *Water and Environment Journal*, 20, 194-202.

Gonzales, T. R. (2008). The effects that well depth and wellhead protection have on bacterial contamination of private water wells in the Estes Park Valley, Colorado. *Journal of Environmental Health*, 71(5), 17-25.

Gotelli, N.J., & Ellison, A.M. (2004). *A primer for ecological statistics*. Sunderland, MA: Sinauer Associates Inc.

Graham, J.P., & Polizzotto, M.L. (2013). Pit latrines and their impacts on groundwater quality: A systematic review. *Environmental Health Perspectives*, 121(5), 521-530.

Guerrant, D.I., Moore, S.R., Lima, A.A., Patrick, P.D., Schorling, J.B., & Guerrant, R.L. (1999). Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. *The American Journal of Tropical Medicine and Hygiene*, 61(5), 707-713.

Guerrant, R.L., Kosek, M., Lima, A.A., Lorntz, B., & Guyatt, H.L. (2002). Updating the DALYs for diarrhoeal disease. *Trends in Parasitology*, 18(5), 191-193.

Guillemin, F., Henry, P., Uwechue, N., & Monjour, L. (1991). Faecal contamination of rural water supply in the Sahelian area. *Water Research*, 25(8), 923-927.

Hardina, C.M., & Fujioka, R.S. (1991). Soil: The environmental source of *Escherichia coli* and Enterococci in Hawaii's streams. *Environmental Toxicology and Water Quality: An International Journal*, 6, 185-195.

Helsel, D.R. & Hirsch, R.M. (2002). *Statistical methods in water resources.* In: U.S. Geological Survey Techniques of Water-Resources Investigations. USGS: Washington, DC. Retrieved from ://water.usgs.gov/pubs/twri/twri4a3/.

Howard, G., Pedley, S., Barrett, M., Nalubga, M., & Johal, K. (2003). Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda. *Water Research*, 37, 3421-3429.

Hutton, G., & Haller, L. (2004). *Evaluation of the costs and benefits of water and sanitation improvements at the global level* (WHO/SDE/WSH/04.04). Geneva, Switzerland: WHO Press. Retrieved from http://www.who.int/water_sanitation_health/wsh0404.pdf.

Hynds, P.D., Misstear, B.D, & Gill, L.W. (2012). Development of a microbial contamination susceptibility model for private domestic groundwater sources. *Water Resources Research*, 48 (12).

Hynds, Paul, Misstear, Bruce D., Gill, L.W., Murphy, & Heather M. (2014). Groundwater source contamination mechanisms: Physicochemical profile clustering, risk factor analysis and multivariate modeling. *Journal of Contaminant Hydrology*, Manuscript accepted for publication.

Ince, M., Bashier, D., Oni, O.O.O., Awe, E.O., Ogbechie, V., Korve, K., … & Kehinde, M. (2010). *Rapid assessment of drinking-water quality in the Federal Republic of Nigeria: Country report of the pilot project implementation in 2004-2005.* Geneva, Switzerland: World Health Organization (WHO).

Isikwue, M.O., Iorver, D., & Onoja, S.B. (2011). Effect of depth on microbial pollution of shallow wells in Makurdi Metropoilis, Benue State, Nigeria. *British Journal of Environment & Climate Change*, 1(3), 66-73.

Jenkins, J. C. (2005). *The humanure handbook: a guide to composting human manure* (3rd ed.). Grove City, PA: Joseph Jenkins, Inc.

Jensen, P.K., Jayasinghe, G., van der Hook, W., Cairncross, S., & Dalsgaard, A. (2004). Is there an association between bacteriological drinking water quality and childhood diarrhea in developing countries? *Tropical Medicine and International Health* 9(11), 1210-1215.

Jiang, X., Morgan, J., & Doyle, M.P. (2002). Fate of *Escherichia coli* O157:H7 in manure amended soils. *Applied Environmental Microbiology*, 68(5), 2605-2609.

Karathanasis, A., Mueller, T., Boone, B., & Thompson, Y. (2006). Effect of soil depth and texture on fecal bacteria removal from septic effluents. *Journal of Water and Health*, 4(3), 395-404.

Khairy, A.E.M., Sebaie, O.E., Aleya, A.G., & Attar, L.E. (1982). The sanitary condition of rural drinking water in a Nile Delta village: I. Parasitological assessment of 'zir' stored and direct tap water. *Journal of Hygiene*. 88(57), 57-61.

Kibbey, H.J., Hagedorn, C., & McCoy, E.L. (1978). Use of fecal streptococci as indicators of pollution in soils. *Applied and Environmental Microbiology,* 35(4), 711-717.

Kosek, M., Bern, C., & Guerrant, R. L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization*, 81(3), 197-204.

Lewis, W.J., Foster, S.S., & Drasar, B.S. (1980). *Risk of groundwater pollution by on-site sanitation in developing countries* (IRCWD Report No. 01/82). Duebendorf, Switzerland: International Reference Centre for Waste Disposal (IRCWD).

Lopez-Torres, A.J., Hazen, T.C., & Toranzos, G.A. (1987). Distribution and in situ survival and activity of *Klebsiella pneumonia* and *Escherichia coli* in a tropical rain forest watershed. *Current Microbiology*, 15, 213-218.

Lorntz, B., Soares, A. M., Moore, S. R., Pinkerton, R., Gansneder, B., Bovbjerg, V. E., ... & Guerrant, R. L. (2006). Early childhood diarrhea predicts impaired school performance. *The Pediatric Infectious Disease Journal*, 25(6), 513-520.

MacCarthy, M. F., Annis, J. E., & Mihelcic, J.R. (2013a). Unsubsidized self-supply in Eastern Madagascar. *Water Alternatives*, 6(3), 609-621.

MacCarthy, M.F., Buckingham, J.W., & Mihelcic, J.R. (2013b). *EMAS Household Water Supply Technologies in Bolivia.* St Gallen, Switzerland: Rural Water Supply Network (RWSN).

MacDonald, A., Davies, J., Calow, R., & Chilton, J. (2005). *Developing groundwater: a guide for rural water supply*. Bourton on Dunsmore, UK: Practical Action Publishing.

Macdonald, D., Ahmed, K.M., Islam, S., Lawrence, A., & Khandker, Z.Z. (1999). Pit latrines – a source of contamination in peri-urban Dhaka? *Waterlines*, 17(4), 6-8.

McFeters, G.A. & Stuart, D.G. (1972). Survival of coliform bacteria in natural waters: Field and laboratory studies with membrane-filter chambers. *Applied Microbiology*, 24(5), 805-811.

Manja K.S., Maurya M.S & Rao K.M. (1982). A simple field test for the detection of faecal pollution in drinking water. *Bulletin of the World Health Organization*, 60(5), 797–801.

Marshall, K. (2014). *Understanding the theory of suction pump operation.* [PowerPoint slides].

Medhurst, L.J., Vargas-Arias, M., Stec, E. & Banda, K. (2007*). Comparison of membrane filtration using M-Coliblue media with Coliscan Easygel for enumerating E. coli and total coliforms in Donaldson Run*. In: Proc. Virginia/West Virginia Water Research Symposium.

Mehta, C.R., & Patel, N. R. (1983). A network algorithm for performing Fisher's exact test in r x c contingency tables. *Journal of the American Statistical Association,* 78(382), 427-434.

Melian, R., Myrlian, N., Gouriev, A., Moraru, C., & Radstake, F. (1999). Groundwater quality and rural drinking-water supplies in the Republic of Moldova. *Hydrogeology Journal*, 7, 188-196.

Mihelcic, J.R., Myre, E.A., Fry, L.M., Phillips, L.D., & Barkdoll, B.D. (2009). *Field Guide in Environmental Engineering for Development Workers: Water, Sanitation, Indoor Air*. Reston, VA: American Society of Civil Engineers (ASCE) Press.

Moe, C.L., Sobsey, M.D., Samsa, G.P, & Mesolo, V. (1991). Bacterial indicators of risk of diarrhoeal disease from drinking-water in the Philippines. *Bulletin of the World Health Organization.* 69(3), 305-317.

Mølbak, K., Højlyng, N, Jepsen, S., & Gaarslev, K. (1989). Bacterial contamination of stored water and stored food: A potential source of diarrheal disease in West Africa. *Epidemiology and Infection*, 102(2), 309-316.

Moore, S.R., Lima, A.A.M., Conaway, M.R., Schorling, J.B., Soares, A.M., & Guerrant, R.L. (2001). Early childhood diarrhoea and helminthiases associate with long-term linear growth faltering. *International Journal of Epidemiology*, 30(6), 1457-1464.

Murray, C. J., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., ... & Bridgett, L. (2013). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, 380(9859), 2197-2223.

Muruka, C., Fagbamigbe, F.A., Muruka, A., Njuguna, J., Otieno, D.O., Onyando, J., ... & Wamalwa, D. (2012). The Relationship between Bacteriological Quality of Dug-Wells and Pit Latrine Siting in an Unplanned Peri-Urban Settlement: A Case Study of Langas–Eldoret Municipality, Western Kenya. *Public Health Research*, *2*(2), 32-36.

Musa, H.A., Shears, P., Kafi, S., & Elsabag, S.K. (1999). Water quality and public health in northern Sudan: A study of rural and peri-urban communities. *Journal of Applied Microbiology*, 87(5), 676-682.

Niehaus, M.D., Moore, S.R., Patrick, P.D., Derr, L.L., Lorntz, B., Lima, A.A., & Guerrant, R.L. (2002). Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. *American Journal of Tropical Medicine and Hygiene*, 66(5), 590-593.

Nsubuga, F.B., Kansiime, F., & Okot-Okumu, J. (2004). Pollution of protected springs in relation to high and low density settlements in Kampala—Uganda. *Physics and Chemistry of the Earth, Parts A/B/C*, 29(15), 1153-1159.

Oluwasanya, G. (2013). Qualitative risk assessment of self-supply hand-dug wells in Abeokuta, Nigeria: A water safety plan approach. *Waterlines*, 32(1), 36-49.

Olstadt, J., Schauer, J.J, Standridge, J., & Kluender, S. (2007). A comparison of ten USEPA approved total coliform/*E. coli* tests. *Journal of Water and Health*, 5(2), 267- 282.

Onda, K., LoBuglio, J., & Bartram, J. (2012). Global access to safe water: Accounting for water quality and the resulting impact on MDG progress. *International Journal of Environmental Research and Public Health*. 9, 880-894.

PAEPAR. Ministere de l'Energie et des Mines de Direction de l'Eau et de l'Assainissement, (2005). *Manuel de procedure pour la mise en place des projets eau et assainissement* (05-04/MEM/PAEPAR/BP/AEPG)

Pang, L. (2009). Microbial removal rates in subsurface media estimated from published studies of field experiments and large intact soil cores. *Journal of Environmental Quality*, *38*(4), 1531-1559.

PATH. (2007). *Efficacy of Standard Methods for Detection of Fecal Contamination at a Range of Non-Standard Temperatures*. Seattle, WA: PATH.

Practica Foundation. ONG PROTOS a Madagascar des ONG Saint Gabriel. (2005). *Assainissement des excretas dans la ville de Toamasina.*

Properzi, F. (2010). *Rapid assessment of drinking-water quality in the Hashemite Kingdom of Jordan: country report of the pilot project implementation in 2004-2005*. Geneva, Switzerland: World Health Organization (WHO).

Pujari, P.R., Padmakar, C., Labhasetwar, P K., Mahore, P., & Ganguly, A.K. (2012). Assessment of the impact of on-site sanitation systems on groundwater pollution in two diverse geological settings—a case study from India. *Environmental Monitoring and Assessment*, 184(1), 251-263.

Rakotondrainibe, J.H. (2005). *Les 8 zones hydrogeologiques et les principales napps aquifers de Madagascar*.

Reddy, K.R., Khaleel, R., & Overcash, M.R. (1981). Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. *Journal of Environmental Quality,* 10(3), 255-266.

Roberts, L., Chartier, Y., Chartier, O., Malenga, G., Toole, M., & Rodka, H. (2001). Keeping clean water clean at a Malawi refugee camp: a randomized intervention trial. *Bulletin of the World Health Organization*, 79(4), 280-287.

Rudolfs, W., Falk, L.L., & Ragotzkie, R.A. (1950). Literature review on the occurrence and survival of enteric, pathogenic, and relative organisms in soil, water, sewage, and sludges, and on vegetation. *Sewage and Industrial Wastes*, 22(10), 1261-1281.

Sandiford, P., Gorter, A.C., Davey Smith, G., & Pauw, J.P.C. (1989). Determinants of drinking water quality in rural Nicaragua. *Epidemiology and Infection*, *102*(03), 429-438.

Sangodoyin, A.Y. (1993). Considerations on contamination of groundwater by waste disposal systems in Nigeria. *Environmental Technology*, 14(10), 957-964.

Sercu B., Van De Werfhorst, L.C., Murray, J.L., & Holden, P.A. (2011). Cultivation-independent analysis of bacteria in IDEXX Quanti-Tray/2000 fecal indicator assays. *Applied Environmental Microbio*logy, 77(2), 627-633.

Skinner, B. (2012). *An engineer's guide to apron slabs for water points* [booklet]. Leicestershire, UK: WEDC, Loughborough University.

Solo-Gabriele, H.M., Wolfert, M.A., Desmarais, T.R., & Palmer, C.J. (2000). Source of *Escherichia coli* in a coastal subtropical environment. *Applied and Environmental Microbiology*, 66(1), 230-237.

Sørensen, S.J., Schyberg, T., & Rønn, R. (1999). Predation by protozoa on *Escherichia coli* K12 in soil and transfer of resistance plasmid RP4 to indigenous bacteria in soil. *Applied Soil Ecology*, 11(1), 79-90.

Tadesse, D., Desta, A., Geyid, A., Girma, W., Fisseha, S., & Schmoll, O. (2010). *Rapid assessment of drinking-water quality in the Federal Democratic Republic of Ethiopia: country report of the pilot project implementation in 2004-2005.* Geneva, Switzerland: World Health Organization (WHO).

Tallon, P., Magujna, B., Lofranco, C., & Leung, K.T. (2005). Microbial indicators of faecal contamination in water: A current perspective. *Water, Air, and Soil Pollution*, 166, 139-166.

Tate, R.L. (1978). Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. *Applied and Environmental Microbiology*, 35(5), 925-929.

The World Bank. (2013, June 5). Madagascar: Measuring the impact of the political crisis. Retrieved from http://www.worldbank.org/en/news/feature/2013/06/05/madagascar-measuring-the-impact-of-the-political-crisis

Umble, A.K., Kolo, L.E., Newvine, L.A., Roth, G.N. & Roth, J.N. (1999). Elkhart, Ind., tests an improved simplified membrane filtration method for *Escherichia coli* detection and enumeration. *Water Environmental Technology,* 11(4), 57-59.

UN (2013). The Millennium Development Goals Report 2013. New York, NY:United Nations. Retrieved from: http://www.un.org/millenniumgoals/pdf/report-2013/mdg-report-2013-english.pdf

UNICEF. (2006). *Progress for children: a report card on water and sanitation #5.* New York, NY:UNICEF.

UNICEF/WHO. (2009). *Diarrhoea: Why children and still dying and what can be done*. (UNICEF ISBN 978-92-806-4462-3). Geneva, Switzerland: World Health Organization (WHO). Retrieved from http://whqlibdoc.who.int/publications/2009/9789241598415_eng.pdf?ua=1

UNICEF/WHO. (2010). *Progress on Sanitation and Drinking-water: 2010 update.* Geneva, Switzerland: World Health Organization:WHO Retrieved from http://www.unicef.org/media/files/JMPreport2012.pdf

UNICEF/WHO. (2012). *Progress on drinking water and sanitation: 2012 update*. Geneva, Switzerland: World Health Organization:WHO. Retrieved from http://www.unicef.org/media/files/JMPreport2012.pdf

UNICEF/WHO. (2013). *Progress on sanitation and drinking water. 2013 updated*. Geneva, Switzerland: World Health Organization:WHO. Retrieved from http://www.wssinfo.org/fileadmin/user_upload/resources/JMPreport2013.pdf

U.S. Federal Register. (1989). *National primary drinking water regulations; total coliforms (including fecal coliforms and E. coli); final rule*. Federal Register 54(124), 27544-57568.

U.S. Federal Register. (1992). *National primary drinking water regulations: analytical techniques, coliform bacteria, final rule*. Federal Register, 57, 24744-24746.

USEPA. (2002). *Method 1103.1: Escherichia coli (E. coli) in water by membrane filtration using membrane-thermotolerant Escherichia coli agar (mTEC)*. Publication EPA-821-R-02-020, EPA Office of Water (4303T), Washington DC, DC: USEPA.

Vail, J. (2013). *Groundwater sampling*. (Publication No. SESDPRO-301-R3). Retrieved from EPA Region 4 website: http://www.epa.gov/region4/sesd/fbqstp/Groundwater-Sampling.pdf

Vail, J.H., Morgan, R., Merino, C.R., Gonzales, F., Miller, R & Ram, L.J. (2003). Enumeration of Waterborne *Escherichia coli* with Petrifilm Plates: Comparison to Standard Methods. *Journal of Environmental Quality,* 32, 368-373.

van Geen, A., Ahmed, K. M., Akita, Y., Alam, M. J., Culligan, P. J., Emch, M., ..., & Yunus, M. (2011). Fecal contamination of shallow tubewells in Bangladesh inversely related to arsenic. *Environmental Science and Technology*, 45(4), 1199-1205.

Watkins, W.D., Rippey, S.R., Clavet, C.R., Kelley-Reitz, D.J. & Burkhardt, W., III. (1988). Novel compound for identifying *Escherichia coli*. *Applied Environmental Microbio*logy, 54(7), 1874-1875.
WHO. (1997). *Guidelines for drinking water quality, second edition: Volume 3, Surveillance and control of community supplies*. Geneva, Switzerland: WHO Press.

WHO. (2011). *Guidelines for drinking water quality, fourth edition*. Geneva, Switzerland: WHO Press.

WHO/UNICEF. (2012). *Joint Monitoring Programme for Water Supply and Sanitation: Estimates for the use of improved drinking-water sources Updated March 2012*. Geneva, Switzerland: WHO Press.

Wright, J., Gundry, S., & Conroy, R. (2004). Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. *Tropical Medicine and International Health*, *9*(1), 106-117.

Wright, J.A., Cronin, A., Okotto-Okotto, J., Yang, H., Pedley, S., & Gundry, S.W. (2013). A spatial analysis of pit latrine density and groundwater source contamination. *Environmental Monitoring and Assessment,* 185(5), 4261-4272.

Wright, J.A., Yang, H., Walker, K., Pedley, S., Elliott, J. & Gundry, S.W. (2012). The H2S test versus standard indicator bacteria tests for faecal contamination of water: Systematic review and meta-analysis. *Tropical Medicine International Health,* 17(1), 94-105.

Yakub, G.P., Castric, D.A., Stadterman-Knauer, K.L., Tobin, M.J., Blazina, M., Heineman, T.N., ... & Frazier, L. (2002). Evaluation of Colilert and Enterolert defined substrate methodology for wastewater applications. *Water Environmental Research,* 74, 131-135.

Ziebell, W.A., Nero, D.H., Deininger, J.F., & McCoy, E. (1974*). Use of bacteria in assessing waste treatment and soil disposal systems*. In: Proceedings of the National Home Sewage Disposal Symposium of the American Society of Agricultural Engineers

APPENDICES

Appendix A: IRB Correspondence

Hi Meghan,

Thank you for your email. I vaguely remember the work Mike was doing and remember it being specific to the pump and not to individuals which is why we determined he did not require IRB review/oversight. However, in reviewing your questionnaire, it appears that you were asking questions specific to the individual (i.e., how often does your family see an incident of diarrhea) and recording identifiable data (names and GPS coordinates) and therefore, this is human subjects research that required IRBreview/oversight. The IRB does not conduct retrospective review of research; however, upon speaking with the IRB Chairperson, we have determined that if you only use data from the questions below (see highlighted section), this is not human subjects research.

Cheryl

Cheryl L. Byers, MHA, CIP

Assistant Vice President for Research Compliance

Research Integrity Officer

University of South Florida

From: Meghan Wahlstrom [mailto:mawahlstr@gmail.com]
Sent: Wednesday, February 19, 2014 9:08 AM
To: Byers, Cheryl
Subject: IRB Question

Hi Meghan,
It was the questions you sent:
- how they used the water collected from the Pitcher Pumps (i.e cooking, washing, drinking, etc.),
- the method they used (if any) to treat their water,
- the number of families who used the pump, and finally,
- the number of times they collected water throughout the day

Just keep your data to these points and you'll be fine.
Cheryl

Cheryl L. Byers, MHA, CIP
Assistant Vice President for Research Compliance
Research Integrity Officer
University of South Florida

Appendix B: Relationship Between Inside and Outside Pipe Diameters

Table B.1 – Inside and outside pipe diameters

Inside Diameter (mm)	Outside Diameter (mm)
15	21
20	27
26	34
33	42

Appendix C: Copy of the Survey Given to Pitcher Pump System Users in Tamatave

Section	No.	Question	Options	Response
Demographics	1	Respondent		
	2	Fokontany		
	3	GPS		
	4	Age of Respondent		
	5	Number of Households		
	6	Age Distribution Under 5 years 5 - 17 years 18 - 35 years 36 - 60 years Over 60 years Total		
Water Usage	7	Where do you collect cooking and drinking water?	Pitcher pump JIRAMA connection River Spring Rainwater Other (explain):	
	8	What do you use water from the Pitcher pump for?	Drinking Cooking Hand washing Hygiene/bathing Household maintenance Washing clothes Other (explain):	
	9	What water do you treat?	Drinking Cooking/kitchen Other (explain): Do not treat water	
	10	Why do you not treat the water?	N/A	
	11	What method do you use for treating water?	None Boiling Chlorine disinfection Solar disinfection Other (explain):	
	12	Number of collections from all water sources throughout the day		
	13	Number of collections from Pitcher pump throughout the day		
	14	How many receptacles are used for collection?		
		Interviewer: look at the receptacle and fill in the volume	Bucket 5 L Bucket 10 L Bucket 15 L Jerry can 15 L Jerry can 20 L Jerry can 25 L	

Appendix C - Cont.

Section	No.	Question	Response	
Sanitation	15	Do you have a latrine?	Yes No	
		If not, where does your family defecate?		
	16	Can we look at the latrine?	Yes No	
	17	How many meters is the latrine pit?		
	18	How often does your family see an incidence of diarrhea?		
	19	Interviewer notes		
Pitcher Pump	20	When was the pump installed initially?		
	21	Who built and installed the pump?		
	22	What was the total cost of pump and installation?		
	23	How is the water quality from the pump?		
	24	How deep is the water table here?		
	25	How long is the pipe?		
	26	Is the water from the pump consistent throughout the year?	Yes No	
		If no, why not?	The pump goes dry Some months the water is very dirty Other (explain):	
	27	What was the most recent repair?	Leather check valves Pipe Well screen Lever	
		How much does it cost?		
		When was this part last repaired?		
		How often is this reparation completed?		
	28	Interviewer notes		

Appendix D: Survey Results from Pitcher Pump Users in Tamatave

Table D.1 – Results from the survey questions asked during household visits in Tamatave

Location	# of households served by the pump
Mangarivotra N.1	1
Mangarivotra N.2	8
Mangarivotra N.3	1
Mangarivotra N.4	2
Mangarivotra N.5	4
Mangarivotra N.6	3
Mangarivotra N.7	3
Mangarivotra N.8	2
Mangarivotra N.9	3
Ankirhiry.1	2
Ankirhiry.2	2
Ankirhiry.3	8
Ankirhiry.4	10
Ankirhiry.5	2
Ambalakisoa.1	10
Ambalakisoa.2	3
Ambalakisoa.3	5
Ambalakisoa.4	6
Ambalakisoa.5	9
Ambalakisoa.6	16
Ambalakisoa.7	2
Ambalakisoa.8	6
Ambalakisoa.9	4
Ambalakisoa.10	10
Ambalakisoa.11	11
Ambalakisoa.12	5

Appendix D – Cont.

Table D.1 – Cont.

Location	Cooking/Drinking Water Sources					Pitcher Pump water use							What water do you treat?			
	Pump	JIRAMA	River	Spring	Rainwater	Drinking	Cooking	Hand washing	Hygiene/ bathing	Household maint.	Washing clothes	Other	Drinking	Cooking/ kitchen	Other	Do not Treat
Mangarivotra N.1	X	X				X	X	X	X	X	X		X			
Mangarivotra N.2		X						X	X	X	X					X
Mangarivotra N.3	X					X	X	X	X	X	X		X			
Mangarivotra N.4	X					X	X	X	X	X	X					X
Mangarivotra N.5	X					X	X	X	X	X	X					X
Mangarivotra N.6	X	X					X	X	X	X	X					X
Mangarivotra N.7	X					X	X	X	X	X	X		X			
Mangarivotra N.8	X					X	X	X	X	X	X		X			
Mangarivotra N.9	X	X				X	X	X	X	X	X					X
Ankirhiry.1	X	X					X	X	X	X	X					X
Ankirhiry.2	X	X						X	X	X	X					X
Ankirhiry.3	X					X	X	X	X	X	X					X
Ankirhiry.4	X					X	X	X	X	X	X		X			
Ankirhiry.5	X	X					X	X	X	X	X					X
Ambalakisoa.1	X	X				X	X	X	X	X	X		X			
Ambalakisoa.2	X	X				X	X	X	X	X	X		X	X		
Ambalakisoa.3	X					X	X	X	X	X	X		X			
Ambalakisoa.4	X	X				X	X	X	X	X	X		X			
Ambalakisoa.5	X	X					X	X	X	X	X					X
Ambalakisoa.6	X					X	X	X	X	X	X		X			
Ambalakisoa.7	X	X						X	X	X	X					X
Ambalakisoa.8	X					X	X	X	X	X	X					X
Ambalakisoa.9	X					X	X	X	X	X	X		X			
Ambalakisoa.10	X					X	X	X	X	X	X					X
Ambalakisoa.11	X					X	X	X	X	X	X					X
Ambalakisoa.12	X	X					X	X	X	X	X					X

Appendix D – Cont.

Table D.1 - Cont.

Location	Water Treatment and Usage							
	Why do you not treat the water	Water Treatment Method					# of water collections from all sources	# of water collections from Pitcher Pumps
		None	Boiling	Chlorine	SODIS	Other		
Mangarivotra N.1					X		20	18
Mangarivotra N.2	JIRAMA connection is already clean	X					24	21
Mangarivotra N.3			X				13	13
Mangarivotra N.4	The water from the pump is already clean	X					8	8
Mangarivotra N.5	The water from the pump is already clean	X					20	18
Mangarivotra N.6	JIRAMA connection is already clean	X					4	3
Mangarivotra N.7			X				26	26
Mangarivotra N.8			X				7	7
Mangarivotra N.9	JIRAMA is already clean	X					10	8
Ankirhiry.1	JIRAMA water is already clean	X					30	25
Ankirhiry.2	JIRAMA water is already clean	X					12	10
Ankirhiry.3	Water from pump is clean	X					10	10
Ankirhiry.4			X				8	8
Ankirhiry.5	Using JIRAMA					JIRAMA	4	3
Ambalakisoa.1			X				13	11
Ambalakisoa.2			X				17	15
Ambalakisoa.3			X				25	25
Ambalakisoa.4			X				10	8
Ambalakisoa.5	JIRAMA water already clean	X					17	14
Ambalakisoa.6			X	X			17	17
Ambalakisoa.7	JIRAMA water already clean	X					8	6
Ambalakisoa.8	Water from pump is clean	X					10	10
Ambalakisoa.9			X				8	8
Ambalakisoa.10	Water from the pump is clean and has been cared for	X					30	30
Ambalakisoa.11	Water from pump is already clean	X					15	15
Ambalakisoa.12	Water from JIRAMA is already clean	X					19	16

Appendix D – Cont.

Table D.1 – Cont.

Location	Water Collection						
	How many receptacles for collection	Size of receptacle					
		Bucket 5L	Bucket 10L	Bucket 15L	Jerry 15L	Jerry 20L	Jerry 25L
Mangarivotra N.1	1		X				
Mangarivotra N.2	1			X			
Mangarivotra N.3	1		X				
Mangarivotra N.4	2			X			
Mangarivotra N.5	2			X			
Mangarivotra N.6	1			X			
Mangarivotra N.7	1			X			
Mangarivotra N.8	1			X			
Mangarivotra N.9	1		X				
Ankirhiry.1	2		X				
Ankirhiry.2	1			X			
Ankirhiry.3	2		X				
Ankirhiry.4	1			X			
Ankirhiry.5	1		X				
Ambalakisoa.1	2		X				
Ambalakisoa.2	2			X			
Ambalakisoa.3	1			X			
Ambalakisoa.4	2		X				
Ambalakisoa.5	2		X				
Ambalakisoa.6	2			X			
Ambalakisoa.7	2		X				
Ambalakisoa.8	2		X				
Ambalakisoa.9	1			X			
Ambalakisoa.10	1			X			
Ambalakisoa.11	2		X				
Ambalakisoa.12	2			X			

Appendix D - Cont.

Table D.1 – Cont.

Location	Sanitation		
	Latrine	Depth of pit (m)	Notes
Mangarivotra N.1	Y	4	Concrete floor; clean structure
Mangarivotra N.2	Y	2	concrete structure, foot rests are plastic; plastic drum with holes
Mangarivotra N.3	Y	1	simple pit; no real superstructure; close to pit
Mangarivotra N.4	Y	1.5	Plastic drum with holes in the bottom; downhill from pump
Mangarivotra N.5	Y	3	Downhill of pump; pit; ok superstructure
Mangarivotra N.6	Y	2	Poor condition, but w/a roof; simple pit
Mangarivotra N.7	Y	4	Poor condition, but w/a roof; simple pit
Mangarivotra N.8	N	NA	Defecate in coffee fields
Mangarivotra N.9	Y	Don't know	Pit; no cover; superstructure
Ankirhiry.1	Y	3	
Ankirhiry.2	Y	2	Concrete platform; good structure
Ankirhiry.3	Y	1	Pit; no cover; superstructure
Ankirhiry.4	Y	2	No roof/door; close to pump
Ankirhiry.5	Y	2	Concrete;clean;pit;structure
Ambalakisoa.1	Y	3	pit;full; poor condition
Ambalakisoa.2	Y	2	pit; concrete slab; shower close
Ambalakisoa.3	N		Defecate near canal
Ambalakisoa.4	Y	2	
Ambalakisoa.5	N		Down by the canal
Ambalakisoa.6	Y	2	
Ambalakisoa.7	Y	1.5	Shared latrine w/Ambalakisoa.6
Ambalakisoa.8	Y	2	Pour flush; setic;clean
Ambalakisoa.9	N		Shared with another compound
Ambalakisoa.10	Y	2	Pit;good cover;ok structure
Ambalakisoa.11	N		
Ambalakisoa.12	Y	3	Pit; concrete slab;tin structure

Appendix D – Cont.

Table D.1 – Cont.

Location	Pump Information			
	Date of pump installation	Age of well (yr)	Cost of pump and installation	How is the water quality?
Mangarivotra N.1	2008	4	Don't remember	Clear and clean
Mangarivotra N.2	2009	3	50,000	Clean
Mangarivotra N.3	2009	3	20,000	Clean
Mangarivotra N.4	2002	10	Don't remember	Clean
Mangarivotra N.5	2001	11	Don't remember	Clean
Mangarivotra N.6	1992	20	Don't remember	Clean
Mangarivotra N.7	1982	30	Don't remember	Clean
Mangarivotra N.8	2007	5		Clean
Mangarivotra N.9	2011	1	120,000	Clean
Ankirhiry.1	1992;2012 significant work	20	30,000	Clear and clean
Ankirhiry.2	2007	5	70,000	Clean
Ankirhiry.3	2005	7	50,000	Clean
Ankirhiry.4	2008	4	20,000	Clean
Ankirhiry.5	Over 10 yrs ago	10	Don't remember	Clean
Ambalakisoa.1	1995	17	60,000	Clean
Ambalakisoa.2	2003	9	Don't remember	Clean
Ambalakisoa.3	1997	15	50,000	Clean
Ambalakisoa.4	Over 20 yrs ago	20	40,000	Clean
Ambalakisoa.5	2000	12	50,000	Clean
Ambalakisoa.6	2002	10	80,000	Clean
Ambalakisoa.7	2012	0	200,000	Clean
Ambalakisoa.8	1995	17	40,000	Clean
Ambalakisoa.9	2010	2	160,000	Clean
Ambalakisoa.10	1986	26	50,000	Clean
Ambalakisoa.11	2010	2	100,000	Clean
Ambalakisoa.12	2002	10	200,000	Clean

Appendix D – Cont.

Table D.1 - Cont.

Location	Pump Information			
	How deep to the water table?	Depth of Pipe (m)	Constant water supply?	If no, why not?
Mangarivotra N.1	5	6	Y	
Mangarivotra N.2	5.3	6	Y	
Mangarivotra N.3	6	7	Y	
Mangarivotra N.4	7	8	Y	
Mangarivotra N.5	6	7	Y	
Mangarivotra N.6	2.5	6	Y	
Mangarivotra N.7	6	6	Y	
Mangarivotra N.8	6	7	Y	
Mangarivotra N.9	6	7	Y	
Ankirhiry.1	5.3	5.5	Y	
Ankirhiry.2	8.5	8	Y	
Ankirhiry.3	2	4	Y	
Ankirhiry.4	3	6	Y	
Ankirhiry.5	5.4	6	Y	
Ambalakisoa.1	3	4	Y	
Ambalakisoa.2	4	5	Y	
Ambalakisoa.3	10	10	Y	
Ambalakisoa.4	7	8	Y	
Ambalakisoa.5	7	8	Y	
Ambalakisoa.6	8	10	Y	
Ambalakisoa.7	6	6.5	Y	
Ambalakisoa.8	5	6	Y	
Ambalakisoa.9	8	8.5	Y	
Ambalakisoa.10	7	7	Y	
Ambalakisoa.11	8	8.5	Y	
Ambalakisoa.12	7	8	Y	

Appendix D – Cont.

Table D.1 – Cont.

Location	Pump Repairs							
	Most recent repair							
	Leather check valves	Pipe	Well screen	Lever	Cost	Date	Frequency of repair	Priming necessary?
Mangarivotra N.1	X				2600	12-sep	every 6 mo	Y
Mangarivotra N.2	X				5000	12-nov	1-2 times per month	Y
Mangarivotra N.3	X				5000	12-nov	1-2 times per month	N
Mangarivotra N.4		X			40000	12-dic	Every 3 years	Y
Mangarivotra N.5	X	X			6000;40000	Nov. 12;Oct. 12	Every month; do not know	N
Mangarivotra N.6	X				6000	12-nov	Every 4 mo	N
Mangarivotra N.7	X				1300	12-jun	Once a year	N
Mangarivotra N.8								N
Mangarivotra N.9				X	2500	12-sep	Once until now	N
Ankirhiry.1			X		10000	12-nov	Every 2 years	Y
Ankirhiry.2	X				6000	12-nov	Every 6 wks	Y
Ankirhiry.3	X				5000	12-oct	Every 3 months	N
Ankirhiry.4	X				6000	12-nov	Every month	Y
Ankirhiry.5	X				12000	12-oct	Every 6 month	N
Ambalakisoa.1	X				7500	12-nov	Every 6 mo	N
Ambalakisoa.2	X				12000	12-may	Eery 6-7 mo	N
Ambalakisoa.3	X				5000	12-ago	Every 6 mo	N
Ambalakisoa.4	X				15000	12-oct	Once a year	N
Ambalakisoa.5	X				8000	12-nov	Every 2 mo	N
Ambalakisoa.6	X				40000	12-jun	Every 8 mo	
Ambalakisoa.7					NA	NA		
Ambalakisoa.8	X		X		10000;30000	12-oct	Every 6 mo; every 15 yrs	N
Ambalakisoa.9	X				10000	12-sep	Every 5 mo	N
Ambalakisoa.10	X				9000	12-nov	Every 4 mo	N
Ambalakisoa.11	X				6000	12-dic	Every mo	N
Ambalakisoa.12	X				4000	12-dic	Every mo	N

Appendix D – Cont.

Table D.1 – Cont.

Location	# of households served by the pump
Antanambao Veriery.1	5
Antanambao Veriery.2	2
Andranomadio.1	3
Andranomadio.2	5
Andranomadio.3	5
Andranomadio.4	2
Andranomadio.5	3
Andranomadio.6	2
Andranomadio.7	10
Andranomadio.8	4
Mangarivotra S.1	5
Mangarivotra S.2	15
Mangarivotra S.3	6
Mangarivotra S.4	8
Mangarivotra S.5	3
Mangarivotra S.6	1
Mangarivotra S.7	7
Mangarivotra S.8	1
Mangarivotra S.9	10
Mangarivotra S.10	3
Mangarivotra S.11	12
Mangarivotra S.12	1
Mangarivotra S.13	2
Mangarivotra S.14	1
Tanambao V.1	4
Tanambao V.2	3
Tanambao V.3	2

Appendix D – Cont.

Table D.1 - Cont.

Location	Cooking/Drinking Water Sources					Pitcher Pump water use							What water do you treat?			
	Pump	JIRAMA	River	Spring	Rainwater	Drinking	Cooking	Hand wash	Hygiene /bathing	House Maint.	Washing clothes	Other	Drinking	Cooking /kitchen	Other	Do not Treat
Antanambao Veriery.1	X					X	X	X	X	X	X					X
Antanambao Veriery.2	X					X	X	X	X	X	X		X			
Andranomadio.1	X	X						X	X	X	X					X
Andranomadio.2	X	X						X	X	X	X					X
Andranomadio.3	X	X						X	X	X	X					X
Andranomadio.4	X	X						X	X	X	X					X
Andranomadio.5	X	X						X	X	X	X					X
Andranomadio.6	X	X						X	X	X	X					X
Andranomadio.7	X	X						X	X	X	X					X
Andranomadio.8	X	X						X	X	X	X					X
Mangarivotra S.1	X					X	X	X	X	X	X		X			
Mangarivotra S.2	X					X	X	X	X	X	X		X			
Mangarivotra S.3	X	X						X	X	X	X					X
Mangarivotra S.4	X	X				X	X	X	X	X	X		X			
Mangarivotra S.5	X					X	X	X	X	X	X		X			
Mangarivotra S.6	X	X						X	X	X	X					X
Mangarivotra S.7	X					X	X	X	X	X	X					X
Mangarivotra S.8	X					X	X	X	X	X	X					X
Mangarivotra S.9	X					X	X	X	X	X	X		X			
Mangarivotra S.10	X	X						X	X	X	X					X
Mangarivotra S.11	X	X						X	X	X	X					X
Mangarivotra S.12	X	X				X	X	X	X	X	X		X			
Mangarivotra S.13	X					X	X	X	X	X	X		X			
Mangarivotra S.14	X	X						X	X	X	X					X
Tanambao V.1	X					X	X	X	X	X	X		X			
Tanambao V.2	X	X						X	X	x	X					X
Tanambao V.3	X	X				X	X	X	X	X	X		X			

Appendix D – Cont.

Table D.1 - Cont.

	Why do you not treat the water	Water Treatment Method: None	Boiling	Chlorine	SODIS	Other	# of water collections from all sources	# of water collections from Pitcher Pumps
Antanambao Veriery.1	Water is clean and dependable	X					10	10
Antanambao Veriery.2			X				8	8
Andranomadio.1	JIRAMA water is already clean	X					2	1
Andranomadio.2	JIRAMA water is already clean	X					10	8
Andranomadio.3	JIRAMA water is already clean	X					11	9
Andranomadio.4	JIRAMA water is already clean	X					30	25
Andranomadio.5	JIRAMA water is already clean	X					20	17
Andranomadio.6	JIRAMA water is already clean	X					25	20
Andranomadio.7	JIRAMA water is already clean	X					16	14
Andranomadio.8	JIRAMA water is already clean	X					35	30
Mangarivotra S.1			X				8	8
Mangarivotra S.2			X				13	13
Mangarivotra S.3	JIRAMA water is already clean	X					30	25
Mangarivotra S.4			X				40	30
Mangarivotra S.5			X				10	10
Mangarivotra S.6	JIRAMA water is already clean	X					10	8
Mangarivotra S.7	Water from the pump is already clean	X					26	26
Mangarivotra S.8	Water from the pump is already clean	X					5	5
Mangarivotra S.9			X				4	4
Mangarivotra S.10	JIRAMA water is already clean	X					24	20
Mangarivotra S.11	JIRAMA water is already clean	X					20	18
Mangarivotra S.12			X				15	13
Mangarivotra S.13			X				10	10
Mangarivotra S.14	JIRAMA water is already clean	X					20	18
Tanambao V.1			X				10	10
Tanambao V.2	JIRAMA water is already clean	X					13	10
Tanambao V.3			X				9	7

Appendix D – Cont.

Table D.1 - Cont.

Location	Water Collection						
	How many receptacles for collection	Size of receptacle					
		Bucket 5L	Bucket 10L	Bucket 15L	Jerry 15L	Jerry 20L	Jerry 25L
Antanambao Veriery.1	1			X			
Antanambao Veriery.2	1			X			
Andranomadio.1	1					X	
Andranomadio.2	1			X			
Andranomadio.3	2			X			
Andranomadio.4	2		X				
Andranomadio.5	2			X			
Andranomadio.6	2		X				
Andranomadio.7	2			X			
Andranomadio.8	2			X			
Mangarivotra S.1	2		X				
Mangarivotra S.2	2		X				
Mangarivotra S.3	2			X			
Mangarivotra S.4	2			X			
Mangarivotra S.5	2			X			
Mangarivotra S.6	1		X				
Mangarivotra S.7	2		X				
Mangarivotra S.8	1			X			
Mangarivotra S.9	1			X			
Mangarivotra S.10	2			X			
Mangarivotra S.11	2			X			
Mangarivotra S.12	1		X				
Mangarivotra S.13	1			X			
Mangarivotra S.14	2		X				
Tanambao V.1	1		X				
Tanambao V.2	1			X			
Tanambao V.3	1		X				

Appendix D – Cont.

Table D.1 - Cont.

Location	Sanitation		
	Latrine	Depth of pit (m)	Notes
Antanambao Veriery.1	Y	2	Pour flush; clean
Antanambao Veriery.2	Y	1.5	
Andranomadio.1	Y	3	Pit;no cover; superstructure
Andranomadio.2	Y	2	Pour flush;concrete;+1close
Andranomadio.3	Y	2.5	Pit;hole in water table
Andranomadio.4	Y	2	Pour flush;clean
Andranomadio.5	Y	1	
Andranomadio.6	Y	Don't know	Inside house; septic; pour flush
Andranomadio.7	Y	1.5	Pit; hole in water table
Andranomadio.8	Y	2.5	Pour flush; septic tank
Mangarivotra S.1	Y	3	Pit; no door; downhill ofpump
Mangarivotra S.2	N	N/A	Defecate in Canal
Mangarivotra S.3	Y	1.5	Pit; concrete slab; clean
Mangarivotra S.4	Y	2	Pit;concrete slab;metal structure
Mangarivotra S.5	Y	2	pit; concrete slab
Mangarivotra S.6	Y	3	Pti;concrete slab; structure
Mangarivotra S.7	Y	2	Pit;concrete slab; structure
Mangarivotra S.8	N	N/A	Did not indicate where family defecates
Mangarivotra S.9	Y	3	Poor;barrel;structure
Mangarivotra S.10	Y	2	Pit;concrete slab;metal structure
Mangarivotra S.11	Y	2	Pit;concrete slab;metal structure
Mangarivotra S.12	Y	2	Pour flush;septic;clean
Mangarivotra S.13	Y	1.5	Pit;dirty;metal structure
Mangarivotra S.14	Y	1.5	Pit; no cover; metal strucutre
Tanambao V.1	Y	Don't know	No latrine; use canal
Tanambao V.2	Y	1.5	Poor; no roof; tarp walls; downhill of pump
Tanambao V.3	Y	2	Pit; concrete slab; metal structure; clean

Appendix D – Cont.

Table D.1 – Cont.

Location	Pump Information			
	Date of pump installation	Age of well (yr)	Cost of pump and installation	How is the water quality?
	2012	0	305,000	Clean
Antanambao Veriery.1	2012	0	300,000	Clean
Antanambao Veriery.2	2006	6	Self-built	Dirty
Andranomadio.1	2012	0	90,000	Dirty
Andranomadio.2	2007	5	100,000	Clean
Andranomadio.3	2002	10	50,000	A little dirty
Andranomadio.4	2002	10	40,000	A little dirty
Andranomadio.5	2000	12	Don't remember	Clean
Andranomadio.6	2010	2	100,000	A little dirty
Andranomadio.7	2000	12	50,000	Clean
Andranomadio.8	2012	0	130,000	Clean
Mangarivotra S.1	2007	5	120,000	Clean
Mangarivotra S.2	1994	18	60,000	Clean
Mangarivotra S.3	1988	24	Don't remember	Clean
Mangarivotra S.4	1994	18	50,000	Clean
Mangarivotra S.5	2012	0	120,000	Clean
Mangarivotra S.6	Over 30 yrs ago	30	Don't remember	Clean
Mangarivotra S.7	2012	0	150,000	Clean
Mangarivotra S.8	1992	20	Don't remember	Clean
Mangarivotra S.9	2003	9	80,000	Clean
Mangarivotra S.10	1998	14	100,000	Clean
Mangarivotra S.11	2002	10	60,000	Clean
Mangarivotra S.12	2004	8	160,000	Clean
Mangarivotra S.13	2005	7	140,000	Clean
Mangarivotra S.14	2008	4	190,000	Clean
Tanambao V.1	2012	0	100,000	Clean
Tanambao V.2	1980	32	Don’t remember	Clean
Tanambao V.3	2012	0	305,000	Clean

Appendix D – Cont.

Table D.1 – Cont.

Location	Pump Information			
	How deep to the water table?	Depth of Pipe (m)	Constant water supply?	If no, why not?
Antanambao Veriery.1	12	13	Y	
Antanambao Veriery.2	12	13	y	
Andranomadio.1	5	6	Y	
Andranomadio.2	2	3.5	N	Some months the water is very dirty
Andranomadio.3	3	5	N	Some months the water is very dirty
Andranomadio.4	2	4	Y	
Andranomadio.5	3	4	N	Some months the water is dirty
Andranomadio.6	4	5	Y	Some months the water is dirty
Andranomadio.7	2	3	N	Some months the water is dirty
Andranomadio.8	3	5	N	Some months the water is dirty
Mangarivotra S.1	10	10	Y	
Mangarivotra S.2	9	9	Y	
Mangarivotra S.3	13	13	Y	
Mangarivotra S.4	6	7	Y	
Mangarivotra S.5	4	5	Y	
Mangarivotra S.6	4	5	Y	
Mangarivotra S.7	6	7	Y	
Mangarivotra S.8	5	6	Y	
Mangarivotra S.9	6	7	Y	
Mangarivotra S.10	6	7	Y	
Mangarivotra S.11	5.5	6	N	Some months the water is dirty
Mangarivotra S.12	5	5 or 6	Y	
Mangarivotra S.13	3.5	4	Y	
Mangarivotra S.14	5	6	Y	
Tanambao V.1	8	8.5	Y	
Tanambao V.2	9	10	Y	
Tanambao V.3	6	7	Y	

Appendix D – Cont.

Table D.1 – Cont.

Location	Pump Repairs							
	Most recent repair							
	Leather check valves	Pipe	Well screen	Lever	Cost	Date	Frequency of repair	Priming necessary?
Antanambao Veriery.1								N
Antanambao Veriery.2								Y
Andranomadio.1	X				8000	11-dic	Twice a year	Y
Andranomadio.2								N
Andranomadio.3	X				14000	12-nov	Every 5 mo	Y
Andranomadio.4	X				5000	12-nov	Every month	N
Andranomadio.5	X				20000	12-oct	Every 2 mo	Y
Andranomadio.6	X				21000	12-nov	Every 6 mo	N
Andranomadio.7	X				4500	12-oct	Every 4 mo	N
Andranomadio.8	X				8000	12-jun	Every month	N
Mangarivotra S.1								N
Mangarivotra S.2	X				9000	12-dic	Every 2 mo	N
Mangarivotra S.3	X				5000	12-oct	Every 3 mo	N
Mangarivotra S.4	X				5000	12-oct	Every 4 mo	N
Mangarivotra S.5	X				8000	12-nov	Every month	N
Mangarivotra S.6								Y
Mangarivotra S.7	X				6000	12-jun	Once a year	N
Mangarivotra S.8								N
Mangarivotra S.9	X				3500	12-nov	Every 2 mo	N
Mangarivotra S.10	X	X			4000;20000	Dec 12/Jun 12	Every 2 mo/ every 10 yrs	Y
Mangarivotra S.11	X				3000	12-jun	Once a year	Y
Mangarivotra S.12	X				10000	12-nov	Every 6 mo	N
Mangarivotra S.13	X				10000	12-sep	Every 3 mo	N
Mangarivotra S.14	X			X	8000/10000	Oct 12/June 12	Every 6 mo; once until now	Y
Tanambao V.1	X				10000	12-nov	Every 6 mo	N
Tanambao V.2					NA			N
Tanambao V.3	X				10000	41802	Every 6 mo	Y

Appendix D – Cont.

Table D.2 – Cost of the Pitcher Pump systems after the year 2000

Number	Cost (1000 Ar)	Year of Construction
1	50	2000
2	50	2000
3	80	2002
4	200	2002
5	50	2002
6	40	2002
7	60	2002
8	80	2003
9	160	2004
10	50	2005
11	140	2005
12	70	2007
13	100	2007
14	120	2007
15	20	2008
16	190	2008
17	50	2009
18	20	2009
19	160	2010
20	100	2010
21	100	2010
22	70	2011
23	200	2012
24	305	2012
25	300	2012
26	90	2012
27	100	2012
28	130	2012
29	120	2012
30	150	2012
Summary Statistics		
Calculation	MGA (1000)	US$
Median	100	45.5

Appendix D – Cont.

Table D.3 – Distance between the Pitcher Pump system and on-site sanitation

Location	Horizontal Distance	Pacing
MangarivotraN.1	9.0	9.6
MangarivotraN.2	17.0	15.7
MangarivotraN.3	4.0	8.1
MangarivotraN.4	20.0	20.8
MangarivotraN.5	21.7	22.9
MangarivotraN.6	10.0	10.0
MangarivotraN.7	12.3	12.3
MangarivotraN.8	14.2	14.2
MangarivotraN.9	12.4	12.4
MangarivotraN.10	12.8	7.9
MangarivotraN.11	5.5	5.6
MangarivotraN.12	3.0	9.6
MangarivotraN.13	5.6	9.0
MangarivotraN.14	9.6	12.0
MangarivotraN.15	9.0	12.0
Ankirihiry 1	12.0	11.9
Ankirihiry 2	12.0	5.9
Ankirihiry 3	15.0	11.1
Ankirihiry 4	3.0	2.2
Ankirihiry 5	4.0	4.0
Ambalakisoa.1	17.0	13.8
Ambalakisoa.2	11.0	10.8
Ambalakisoa.4	10.3	10.3
Ambalakisoa.8	0.0	3.2
Ambalakisoa.10	4.0	4.0
Andranomadio.1	7.0	8.5
Andranomadio.2	7.0	7.8
Andranomadio.3	17.0	17.0
Andranomadio.4	25.0	6.7
Andranomadio.5	9.0	13.5
Andranomadio.6	2.0	2.0
Andranomadio.7	4.0	2.7

Appendix D – Cont.

Table D.3 - Cont.

Location	Horizontal Distance	Pacing
Tanambao Veriery.1	19.0	15.0
Tanambao Veriery.2	5.0	3.8
MangarivotraS.1	3.0	4.2
MangarivotraS.3	5.0	7.0
MangarivotraS.4	3.0	4.3
MangarivotraS.5	7.0	8.6
MangarivotraS.6	5.0	6.2
MangarivotraS.7	7.0	6.2
MangarivotraS.9	16.0	16.0
MangarivotraS.10	3.0	7.6
MangarivotraS.11	9.0	10.8
MangarivotraS.12	7.0	7.3
MangarivotraS.13	10.0	8.6
MangarivotraS.14	11.0	9.7
MangarivotraS.15	10.0	10.0
MangarivotraS.16	5.5	5.5
MangarivotraS.17	12.0	12.0
MangarivotraS.18	5.0	5.0

Appendix E: Phase I Thermotolerant Coliform Sampling Results

Table E.1 – Thermotolerant coliform sampling results from Phase I

Sample Location	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Average TTC (cfu/100 ml)
MangarivotraN.1	88	123	49				86.7
MangarivotraN.2	44	37					40.5
MangarivotraN.3	0	0					0
MangarivotraN.4	TNTC	TNTC					200
MangarivotraN.5	4	5					4.5
MangarivotraN.6	165	121	91				125.7
MangarivotraN.7	0	0					0
MangarivotraN.8	0	0	0	14			3.5
MangarivotraN.9	0						0
MangarivotraN.10	23						23
MangarivotraN.11	104	TNTC	TNTC	TNTC			200
MangarivotraN.12	0						0
MangarivotraN.13	17						17
MangarivotraN.14	7						7
MangarivotraN.15	23						23
MangarivotraN. 16	1						1
Ankirihiry 1	9	6					7.5
Ankirihiry 2	TNTC	TNTC					200
Ankirihiry 3	0	2					1
Ankirihiry 4	89	60					74.5
Ankirihiry 5	4	3					3.5
Ambalakisoa.1	13	28					20.5
Ambalakisoa.2	6	2					4
Ambalakisoa.3	1	2					1.5
Ambalakisoa.4	0	0					0
Ambalakisoa.6	0	0	1	1			0.5
Ambalakisoa.7	TNC	TNC					200
Ambalakisoa.8	0	0					0
Ambalakisoa.9	0	0					0
Ambalakisoa.10	0	0					0
Ambalakisoa.11	4	4					4
Andranomadio.1	34	50					42
Andranomadio.2	10	Unclear	0	6			5.3
Andranomadio.3	11	19					15
Andranomadio.4	10	14					12

Appendix E – Cont.

Table E.1 – Cont.

Sample Location	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Average TTC (cfu/100 ml)
Andranomadio.5	10	5					7.5
Andranomadio.6	8	Error	1	1			3.3
Andranomadio.7	2	6					4
Andranomadio.8	1	NA					1
Tanambao Veriery.1	86	85					85.5
Tanambao Veriery.2	4	4					4
MangarivotraS.1	TNTC	TNTC					200
MangarivotraS.2	136	127					131.5
MangarivotraS.3	TNTC	TNTC					200
MangarivotraS.4	TNTC	TNTC					200
MangarivotraS.5	0	0					0
MangarivotraS.6	Unclear	68	136	138	116		114.5
MangarivotraS.7	0	0					0
MangarivotraS.8	12	6					9
MangarivotraS.9	TNTC	TNTC	TNTC	TNTC			200
MangarivotraS.10	1	1					1
MangarivotraS.11	0	0					0
MangarivotraS.12	3	12					7.5
MangarivotraS.13	2	3					2.5
MangarivotraS.14	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	200
MangarivotraS.15	0						0
MangarivotraS.16	48						48
MangarivotraS.17	TNTC	TNTC	TNTC	TNTC	5700	2880	4290
MangarivotraS.18	30	22	16	27			23.75

Appendix F: Phase II Thermotolerant Coliform Sampling Results

Table F.1 – Thermotolerant coliform sampling results from Phase II

Date	Well Depth	Sample 1	Sample 2	Sample 3	Average TTC (cfu/100 ml)	Log10 CFU
1/9/2013	6.5	110	62	67	79.67	1.90
	7.7	308	235	264	269.00	2.43
	8.66	236	213	183	210.67	2.32
	9.35	246	135	NA	190.50	2.28
1/11/2013	6.5	792	798	786	792.00	2.90
	7.7	464	624	600	562.67	2.75
	8.66	236	430	432	366.00	2.56
	9.35	460	348	266	358.00	2.55
4/6/2013	6.5	335	104	NA	219.50	2.34
	7.7	136	236	324	232.00	2.37
	9.35	203	190	90	161.00	2.21

Appendix G: Raw Data from the Investigation into Effect of Alternative Temperatures on Three FIB Test Kits

Table G.1 – Raw data from standard methods - mTec

Dilution (mL)	*E. coli* (cfu)	*E. coli* (cfu/100 ml)
Blank	0	0
1	4	400
1	7	700
1	5	500
5	61	1220
5	69	1380
5	34	680
7	59	843
7	100	1429
7	96	1371
10	130	1300
10	151	1510
10	NA	NA

Table G.2 – Raw data from standard methods - mEndo

Dilution	Total Coliform (cfu)	Total Coliform (cfu/100 ml)
Blank	0	0
0.05	3	6000
0.05	7	14000
0.05	4	8000
0.1	5	5000
0.1	12	12000
0.1	10	10000
0.5	37	7400
0.5	30	6000
0.5	48	9600

Appendix G - Cont.

Table G.3 – Raw data from Coliscan MF at 22.0°C

At 22.0°C					
Dilution	*E. coli* (cfu)	*E. coli* (cfu/100 ml)	General Coliform (cfu)	Total Coliform (cfu)	Total Coliform (cfu/100 ml)
Blank	0	0	0	0	0
0.1	1	1000	33	34	34000
0.1	0	0	12	12	12000
0.1	1	1000	18	19	19000
0.5	6	1200	52	58	11600
0.5	4	800	43	47	9400
0.5	6	1200	60	66	13200
1	12	1200	98	110	11000
1	4	400	58	62	6200
1	10	1000	96	106	10600
5	32	640	TNTC	TNTC	TNTC
5	33	660	TNTC	TNTC	TNTC
5	42	840	TNTC	TNTC	TNTC
7	TNTC	TNTC	TNTC	TNTC	TNTC
7	TNTC	TNTC	TNTC	TNTC	TNTC
7	TNTC	TNTC	TNTC	TNTC	TNTC

Appendix G – Cont.

Table G.4 – Raw data from Coliscan MF at 35.0°C

At 35.0°C					
Dilution	*E. coli* (cfu)	*E. coli* (cfu/100 ml)	General Coliform (cfu)	Total Coliform (cfu)	Total Coliform (cfu/100 ml)
Blank	0	0	0	0	0
0.1	1	1000	23	24	24000
0.1	3	3000	13	16	16000
0.1	4	4000	14	18	18000
0.5	11	2200	60	71	14200
0.5	16	3200	46	62	12400
0.5	11	2200	72	83	16600
1	23	2300	52	75	7500
1	22	2200	53	75	7500
1	11	1100	97	108	10800
5	91	1820	TNTC	TNTC	TNTC
5	70	1400	TNTC	TNTC	TNTC
5	68	1360	TNTC	TNTC	TNTC
7	98	1400	TNTC	TNTC	TNTC
7	105	1500	TNTC	TNTC	TNTC
7	100	1429	TNTC	TNTC	TNTC

Appendix G - Cont.

Table G.5 – Raw data from Coliscan MF at 44.5°C

At 44.5°C					
Dilution	*E. coli* (cfu)	*E. coli* (cfu/100 ml)	General Coliform (cfu)	Total Coliform (cfu)	Total Coliform (cfu/100 ml)
Blank	0	0	0	0	0
0.1	2	2000	0	2	2000
0.1	3	3000	0	3	3000
0.1	0	0	0	0	0
0.5	5	1000	0	5	1000
0.5	3	600	0	3	600
0.5	0	0	0	0	0
1	2	200	0	2	200
1	1	100	0	1	100
1	0	0	0	0	0
5	34	680	0	34	680
5	2	40	0	2	40
5	37	740	0	37	740
7	1	14	2	3	43
7	49	700	4	53	757
7	28	400	6	34	486

Appendix G – Cont.

Table G.6 – Raw data from Colilert at 22.0°C

22.0°C								
	Total Coliforms				*E. coli*			
Dilution	Big	Small	MPN	MPN/100 ml	Big	Small	MPN	MPN/100 ml
Blank	0		0	0	0		0	0
0.5	20	2	28	5500	2	0	2	400
0.5	20	1	26	5240	1	0	1	200
0.5	22	2	31	6180	2	0	2	400
1	35	6	68	6830	4	0	4	410
1	32	4	56	5560	5	0	5	520
1	30	2	47	4710	1	0	1	100

Table G.7 – Raw data from Colilert at 35.0°C

At 35.0°C								
	Total Coliforms				*E. coli*			
Dilution	Big	Small	MPN	MPN/100 ml	Big	Small	MPN	MPN/100 ml
Blank	0	0	0	0	0	0	0	0
0.5	24	4	37.3	7460	6	0	6.3	1260
0.5	28	3	44.1	8820	5	0	5.2	1040
0.5	28	6	48.8	9760	5	0	5.2	1040
1	42	9	107.6	10760	6	0	6.3	630
1	40	10	98.5	9850	12	1	14	1400
1	44	4	105.4	10540	8	0	8.6	860

Table G.8 – Raw data from Colilert at 44.5°C

At 44.5°C								
	Total Coliforms				*E. coli*			
Dilution	Big	Small	MPN	MPN/100 ml	Big	Small	MPN	MPN/100 ml
Blank	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0

Appendix G – Cont.

Table G.9 – Raw data from modified 3-M at 22.0°C

At 22.0°C					
Dilution	*E. coli* (cfu)	*E. coli* (cfu/100 ml)	General Coliform (cfu)	Total Coliform (cfu)	Total Coliform (cfu/100 ml)
Blank	0	0	0	0	0
0.1	2	2000	57	59	59000
0.1	2	2000	58	60	60000
0.1	2	2000	51	53	53000
0.5	0	0	0	0	0
0.5	7	1400	140	147	29400
0.5	6	1200	139	145	29000
7	11	157	TNTC	TNTC	TNTC
7	12	171	TNTC	TNTC	TNTC
7	14	200	TNTC	TNTC	TNTC

Table G.10 – Raw data from modified 3-M at 35.0°C

At 35.0°C					
Dilution	*E. coli* (cfu)	*E. coli* (cfu/100 ml)	General Coliform (cfu)	Total Coliform (cfu)	Total Coliform (cfu/100 ml)
Blank	0	0	0	0	0
0.1	6	6000	55	61	61000
0.1	4	4000	47	51	51000
0.1	1	1000	79	80	80000
0.5	10	2000	145	155	31000
0.5	11	2200	161	172	34400
0.5	17	3400	121	138	27600
7	TNTC	TNTC	TNTC	TNTC	TNTC
7	TNTC	TNTC	TNTC	TNTC	TNTC
7	TNTC	TNTC	TNTC	TNTC	TNTC
10	TNTC	TNTC	TNTC	TNTC	TNTC
10	TNTC	TNTC	TNTC	TNTC	TNTC
10	TNTC	TNTC	TNTC	TNTC	TNTC

Appendix G – Cont.

Table G.11 – Raw data from modified 3-M at 44.5°C

At 44.5°C					
Dilution	*E. coli* (cfu)	*E. coli* (cfu/100 ml)	General Coliform (cfu)	Total (cfu)	Total coliform (cfu/100 ml)
Blank	0	0	0	0	0
0.5	1	200	0	1	200
0.5	0	0	0	0	0
0.5	3	600	0	3	600
7	58	829	0	58	829
7	26	371	0	26	371
7	13	186	0	13	186
10	69	690	0	69	690
10	68	680	0	68	680
10	80	800	0	80	800

Appendix H: Raw Data from Volunteer Experiments Testing the FIB Kits

Table H.1 – Raw data from when volunteers prepared the plates – standard methods

Group	Plate	mTec (cfu)	mEndo (cfu)
Me	1.0	2	63
	2.0	5	51
	3.0	8	--
1	1.0	4	19
	2.0	5	8
	3.0	3	11
2	1.0	4	81
	2.0	5	13
	3.0	0	27
3	1.0	5	46
	2.0	3	50
	3.0	3	36
4	1.0	7	58
	2.0	0	36
	3.0	8	50
5	1.0	0	46
	2.0	3	42
	3.0	4	79

Appendix H – Cont.

Table H.2 – Raw data from when volunteers prepared the plates – Coliscan MF

Group	Plate	*E. coli* (cfu/100 ml)	General Coliforms (cfu/100 ml)
Me	1.0	6	83
	2.0	5	86
	3.0	NA	NA
1	1.0	14	127
	2.0	4	127
	3.0	NA	NA
2	1.0	8	TNTC
	2.0	6	TNTC
	3.0	7	TNTC
3	1.0	4	TNTC
	2.0	5	TNTC
	3.0	0	TNTC
4	1.0	4	TNTC
	2.0	0	TNTC
	3.0	NA	NA

Appendix H – Cont.

Table H.3 – Raw data from when volunteers prepared the plates – Colilert

Group	Plate	Total Coliform (MPN)	E. coli (MPN)
Me	1.0	TNTC	3.1
	2.0	TNTC	2
	3.0	248.1	5.2
Group 1	4.0	TNTC	2
	5.0	TNTC	4.2
	6.0	290.9	0
Group 2	7.0	TNTC	6.3
	8.0	TNTC	2
	9.0	158.5	2
Group 3	10.0	TNTC	2
	11.0	TNTC	3.1
	12.0	461.1	2
Group 4	13.0	TNTC	1
	14.0	TNTC	2
	15.0	517.2	2
Group 5	16.0	TNTC	3.1
	17.0	TNTC	1
	18.0	547.5	3.1

Appendix H – Cont.

Table H.4 – Raw data from when volunteers prepared the plates – modified 3-M

Group	Plate	*E. coli* (cfu/100 ml)	General Coliform (cfu/100 ml)
Me	1.0	6	TNTC
	2.0	18	TNTC
	3.0	10	TNTC
1	1.0	6	TNTC
	2.0	8	TNTC
	3.0	13	TNTC
2	1.0	5	TNTC
	2.0	5	TNTC
	3.0	6	TNTC
3	1.0	6	TNTC
	2.0	1	TNTC
	3.0	NA	TNTC
4	1.0	6	TNTC
	2.0	6	TNTC
	3.0	5	TNTC

Appendix H – Cont.

Table H.5 – Raw data from when volunteers counted the plates – standard methods

Test 1: MF		
Person	*E. coli* (cfu)	Total Coliform (cfu)
1	60	50
2	42	40
3	53	36
4	55	78
5	46	22
6	59	30
7	60	31
Average	53.6	41.0
SD	7.1	18.5
Me	61	37

Test 2: MF		
Person	*E. coli* (cfu)	Total Coliform (cfu)
1	40	41
2	31	43
3	42	35
4	49	48
5	38	23
6	41	36
Average	40.2	37.7
SD	5.8	8.6
Me	34	48

Test 2: MF		
Person	*E. coli* (cfu)	Total Coliform (cfu)
1	77	29
2	69	25
3	66	29
4	72	36
5	46	16
6	76	32
Average	67.7	27.89
SD	11.4	6.9
Me	69	30

Appendix H – Cont.

Table H.6 – Raw data from when volunteers counted the plates – Colilert

Test 1: Colilert		
Person	*E. coli* (MPN)	Total coliform (MPN)
1	4.1	57.3
2	5.2	60.2
3	3.1	148.3
4	3.1	148.3
5	3.1	165
6	5	148.3
7	5.2	130.9
8	3.1	165
9	8.6	165

Test 3: Colilert		
Person	*E. coli* (MPN)	Total coliform (MPN)
1	5.2	45.9
2	5.2	46.5
3	5.2	153.9
4	5.2	153.9
5	5.2	172
6	6.3	153.9
7	4.1	172
8	5.2	179.3

Test 2: Colilert		
Person	*E. coli* (MPN)	Total coliform (MPN)
1	4.1	48.7
2	4.1	59.4
3	5.2	130.9
4	5.2	130.9
5	5.2	137.9
6	5.2	126.6
7	5.2	143
8	3.1	137.9

Appendix H – Cont.

Table H.7 – Raw data from when volunteers counted the plates – Coliscan MF

Test 1: Coliscan MF		
Person	*E. coli* (cfu)	General Coliform (cfu)
1	9	72
2	9	54
3	9	83
4	9	83
5	9	38
6	12	83
7	13	62
8	9	52
Average	9.9	65.9
SD	1.6	17.1

Test 3: Coliscan MF		
Person	*E. coli* (cfu)	General Coliform (cfu)
1	11	77
2	12	76
3	10	62
4	11	82
5	11	86
6	11	82
7	14	34
Average	11.4	71.3
SD	1.3	18.2
Me	11	72

Test 2: Coliscan MF		
Person	*E. coli* (cfu)	General Coliforms (cfu)
1	15	58
2	9	65
3	14	74
4	15	70
5	15	54
6	12	20
7	19	83
Average	14.1	60.6
SD	3.1	20.4
Me	16	46

Appendix H – Cont.

Table H.8– Raw data from when volunteers counted the plates – modified 3-M

Test 1: modified 3-M		
Person	*E. coli* (cfu)	General Coliforms (cfu)
1	10	TNC
2	10	TNC
3	10	182
4	10	190
5	10	TNC
6	10	207
7	10	152
8	10	140
Average	10	174.2
SD	0	27.6

Test 3: modified 3-M		
Person	*E. coli* (cfu)	General Coliforms (cfu)
1	15	209
2	14	--
3	13	--
4	15	135
5	15	179
6	14	--
7	12	123
Average	14.0	161.5
SD	1.2	39.8
Me	17	121

Test 2: modified 3-M		
Person	*E. coli* (cfu)	General Coliforms (cfu)
1	11	TNC
2	9	TNC
3	11	162
4	11	190
5	12	TNC
6	9	158
7	11	206
Average	10.6	179.0
SD	1.13	23.0
Me	11	161

Appendix I: Permission to Use Tables and Figures

Dear Ms Wahlstrom-Ramler

Thank you for your enquiry. On behalf of the World Health Organization, we are pleased to grant you permission to reproduce the WHO item detailed in the form below.

Figure 3: Progress towards the MDG drinking water target, 2010 (page 6)

This permission is subject to the following conditions:

- This is a non-exclusive permission to reproduce the material detailed below.
- Please ensure that the original WHO source is appropriately acknowledged with either (i) the appropriate bibliographical reference (including publication title, author, publisher, volume/edition number, page numbers, Copyright notice year) or (ii) in the case of materials published on the WHO web site, publication title, the URL reference and the date accessed.
- The material will be reproduced as it was published by WHO and no changes should be made to the content or meaning. Publishers may reformat the material in the style of the publication.
- The use of WHO materials should be factual and used in an appropriate context;
- The material should not be reproduced for use in association with product marketing or promotional activities. In no event should the WHO information products be used in promotional materials, product brochures, web sites, annual reports, and other commercial or company-sponsored publications for distribution to, and/or non-educational presentations for, either the general public, or pharmacists, doctors, nurses, etc.
- There should be no suggestion that WHO endorses any specific company or products in the (article, book etc.) or in the manner of distribution of the article, book etc.).
- The WHO logo and emblem shall not be reproduced.

WHO reserves the right to withdraw the permission in the event a condition is not respected

WHO will not charge any fee for the above permission, however we would like you to please provide me with **1 original** hard copy and an electronic file of your final publication for our records, specifically showing where/how WHO material appears and how it is referenced on your product . Please send directly to this address:

Ms Dolores Campanario

Hello,

Appendix I - Cont.

I am writing a Master's Thesis titled, *Drinking Water in the Developing World: Sources of Fecal Contamination in Pitcher Pump Systems and Measurement Alternatives* at the University of South Florida in Tampa, Fl. I would like to get permission to adapt and reproduce the following table in my thesis:
From: Developing Groundwater: A guide for Rural Water Supply, by: MacDonald et al., 2005

Table 6.4 (pg 252): Typical aquifer properties and feasibility of using horizontal separation.

I am specifically using the first and third columns (rock types and range of hydraulic conductivity, respectively) but not including the Fractured rocks row.

Thank you for your time.

Sincerely,

Meghan Wahlstrom

Denise Hastings Denise.Hastings@practicalaction.org.uk Mar 18 (6 days ago)

to me

Hi Meghan

We grant permission for you to adapt the table for your thesis.

Please ensure that you show full citation

Many thanks

Denise