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Paper

–Low Cost PVC Membrane Filtration Devices –

An Alternative to Commercial MF Units for *E.coli* and Other Coliform Testing in Developing Countries

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Abstract

The membrane filtration technique is a standard method for use by laboratories for detecting the presence of *E.coli* and other coliform bacteria in drinking, waste and surface water [APHA, 1998]. For this technique a filtration unit is required, unfortunately these units are in most cases unaffordable in developing countries. The aim of this work was to design an affordable membrane filtration (MF) unit which can easily be built on site, is functional and does not lack in terms of quality of results when compared to already approved devices. Two units were designed and built mainly out of water distribution pipes and associated components made of rigid polyvinyl chloride (PVC), one for laboratory and one for field use. Experiments comparing both PVC units with approved devices for different water samples showed that both PVC units operated using prescribed testing and sanitization procedures were able to give the same results and were easy to use. Both units are proposed as reliable alternatives for smaller laboratories and for operating in the field where electricity is not available for operating an electric suction apparatus. These devices have the potential to greatly expand affordable testing of water quality in remote communities and therefore using them could help ameliorating the health conditions in urban and rural areas of a developing country.

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Nomenclature

Abbreviations

MF	Membrane filtration
PVC	Polyvinyl chloride
TC	Total coliform bacteria
SSFH	Stainless steel filter holder
PSFH	Polyphenylsulfone filter holder
RO	Reverse-osmosis
SW	Surface water
RW	Rain water
WW	Well water
CFU	Colony Forming Units

1 Introduction

Water borne diarrheal diseases are a leading cause of death for children, not only in Cambodia but across the developing world. Cholera, shigellosis, rotavirus, typhoid, dysentery and other diarrheal diseases kill nearly 2 million children each year, accounting for 16 percent of childhood deaths. Diarrheal diseases can be either water- or food-borne, however contaminated water causes 90% of all diarrheal diseases [Bryce J. et al., 2005]. Trough faecal contamination pathogenic microorganisms or parasites enter a water source and can from there close the faecal-oral transmission route to cause disease if the water is not treated properly before drinking or food processing. The most common tests for faecal contamination of water rely on the detection of *E.coli* and other coliform bacteria as indicator organisms due to their numerous presence in sewage and the ease of their detection and quantification. For quantitative analysis of members of the coliform group in water the MF procedure with incubation on chromogenic agar medium is today considered as a standard method used by laboratories [APHA, 1998]. The MF technique is a preferred method for water testing because it permits quantitative analysis of larger samples of water in the laboratory and in the field, it can be used to test for low presence of indicator organisms for example in drinking water where one colony forming unit/100ml is a significant result. In the MF technique water samples are suctioned through a membrane filter that collects all bacteria present in the water sample on its surface. Storage, detection and quantification steps are then applied to determine the concentration of indicator organisms. Existing filtration units for laboratory and field use of different shape and material are produced by companies as Sartorius, Nalgene and Pall. They are primarily intended for sale to universities, Environmental Protection Agencies and industry in Europe and USA. There are a

number of barriers to their use in developing countries as the price, supply chain hindrances and customs difficulties. To reduce the amount of materials which need to be imported to perform the MF technique would clearly reduce the costs and would give opportunity to perform testing for faecal indicators in places where poverty and poor sanitation often led to faecal contamination of drinking water sources. The primary objective of this study was to replace commercially available laboratory and field MF units with units that can be constructed on site with easily accessible and affordable materials, in Cambodia and possibly in other developing countries. Over a long period of time these units have to prove that they are functional and do not show any reduction in the quality of results, if this can be achieved they may be used by different institutions of the water and sanitation development sector as alternative devices until commercial MF units can be afforded or more approved units have been developed on site.

2 Materials and methods

2.1 Standard MF units

Standard Methods for Examination of Water and Wastewater 20th edition defines the design of components for membrane filtration. It states that the - “filter holding assembly is constructed of glass, autoclavable plastic, porcelain, stainless steel or partly disposable plastic” and consists of a “seamless funnel fastened to a base by a locking device or by magnetic force. The design permits the membrane filter to be held securely on the porous plate of the receptacle without mechanical damage and allows all fluid to pass through the membrane during filtration”. Further, Standard Method goes on to state that to avoid contamination of the water sample by the filtration unit, special cleaning, sanitization and sterilization methods need to be followed. For most laboratory systems this implies autoclaving. Stainless steel

systems can alternatively be sterilized over a flame before use. “Field units may be sanitized by dipping or spraying with alcohol and then igniting or immersing in boiling water for 2 min instead”. Sterile disposable units may be used too. “The filtration unit is connected to a vacuum line, an electric vacuum pump, a filter pump operating on water pressure, a hand aspirator or other means of securing a pressure differential of 138 to 207 kPa” that will suck the water sample trough the membrane in a relatively short amount of time.

2.2 PVC MF units

Based on the standard methods two alternative PVC MF units were designed. The first unit is intended for laboratory use and was given the name “PVClab unit”, the second is intended for field use named “PVCfield unit”. All materials used to manufacture the units are available at local markets in and around of Phnom Penh, Cambodia. The units are primarily made of rigid PVC water distribution pipes which are easily available and with all associated components affordable not only in Cambodia but presumably in many countries which fall under the Human Development Index [Ceresana Research, 2008]. Relevant properties of used PVC water distribution pipe material are: softening point >75℃, non-autoclavable, insoluble in water, combustible but self-extinguishing. These properties account for most PVC drinking water distribution pipes [NAPC, 2002] but should be verified with the local supplier. Table 1 shows first material costs for the PVC MF devices, it is assumed that the units are then constructed on site so that no further costs arise. Second it shows prices for commercially available devices, the prices are taken from a HACH Microbiological Products catalogue[1] except for devices from Sartorius where price information was given on demand in March 2011. The price difference between the PVC- and the commercial devices is outstanding.

[1] http://www.hach.com/fmmimghach?/CODE%3A139-146-MICROBIOLOGY16962|1

Table 1: PVC and commercial MF devices and prices

Device	US$
PVC:	
PVC filter holder	1.50
PVClab manifold	12.00
PVCfield base part	3.50
Commercial:	
Stainless steel filter holder, 100mL (Sartorius)	481.00 *
Polyphenylsulfone Filter Holder, 300 mL (Pall)	205.00 *
Stainless steel manifold, three branches (Sartorius)	1050.00 *
Stainless steel manifold, three branches (Nalgene)	910.00 *
Microfunnel™ System for field use (50 disposable funnels and a single place manifold) (Pall)	783.00 *

* Transportation cost and custom tax not included

2.3 PVC filter holder

The two membrane filtration units use the same filter holder, see Figure 1. It is made of one piece of PVC pipe as a base (l=120 mm; ID=40 mm), stainless steel chicken wire (pore size=0.5 mm) to support the membrane filter, one rubber O-ring used in common machine design (D=46 mm) and one PVC pipe fitting (40 x 35 mm reducing couple) used as a funnel (V=50 mL). Detailed information about how to build the filter holder can be taken from the construction manual in the appendix.

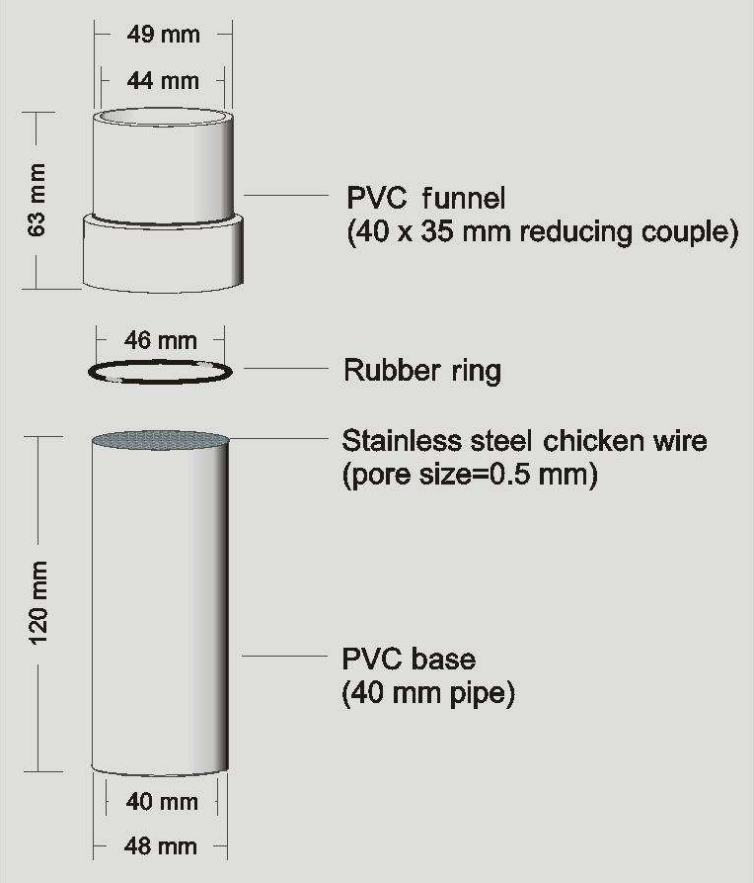

Figure 1: PVC filter holder

2.4 PVClab unit

The PVClab unit is intended for regular laboratory use and utilises an electric suction apparatus that is additional to the system used in the field. In the same way as with commercially available systems multiple filter holders can be connected to a multiple branch manifold which is connected to an electrical suction apparatus. The three

branch manifold of the PVClab Unit is made of a 150 mm half cut PVC Pipe with three equispaced holes (D=20 mm) drilled into the top. Additional PVC fittings, valves, screws and tubing devices are joined together as shown in Figure 2. Detailed information about how to build the three branch manifold can be taken from the construction manual in the Appendix.

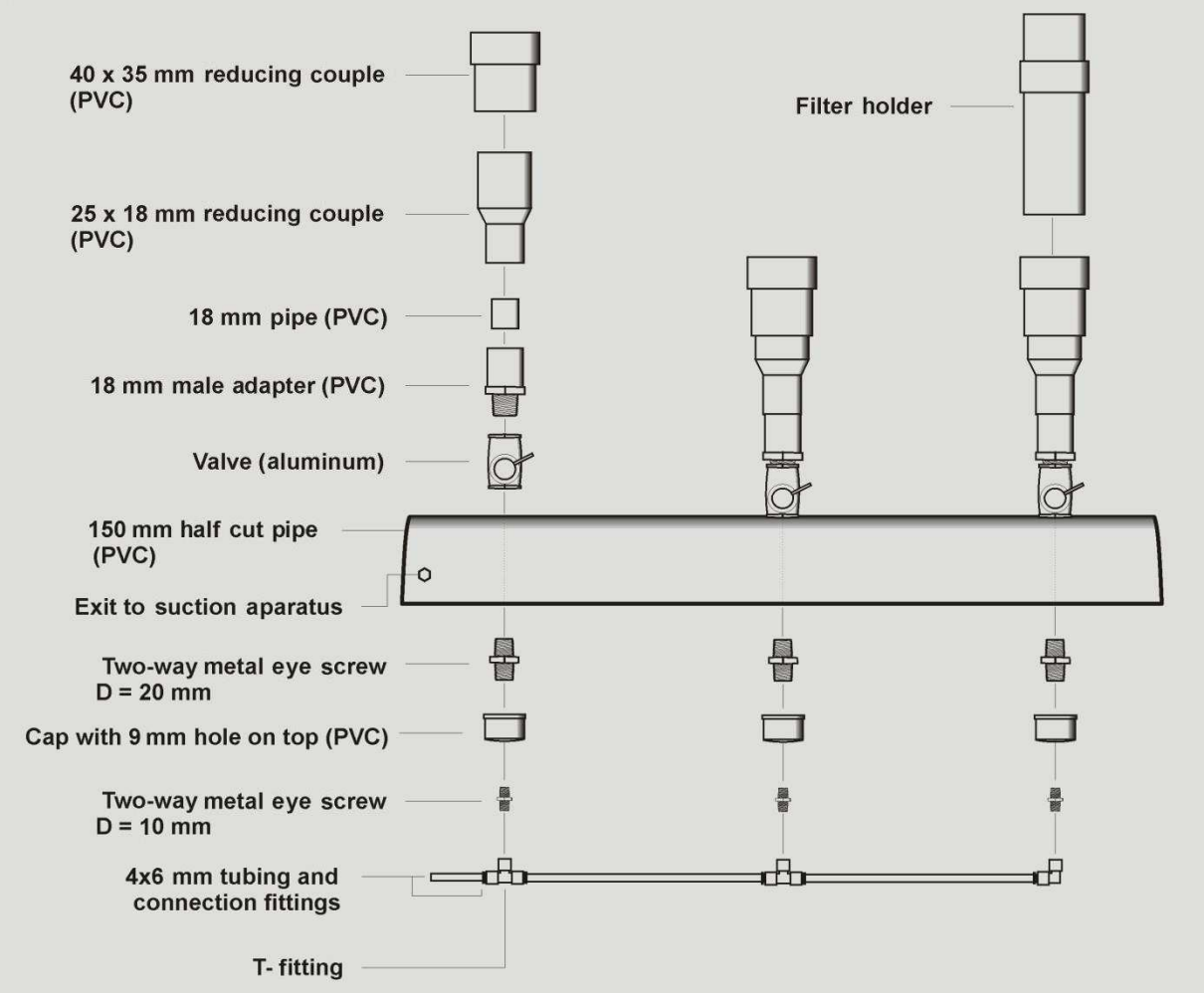

Figure 2: PVClab unit

2.5 PVCfield unit

The PVCfield unit is intended for field use or as a backup system in laboratories during electrical power outages. The primary difference between this unit and the PVClab unit, is the ability to perform MF without electricity, and to combine this with chromogenic media that can be incubated at room temperature, and therefore allow

reliable examination of water quality where electricity is not available. The PVCfield unit uses a syringe (≥100 mL) to create a pressure difference which sucks the water sample through the membrane filter. The unit is made of a 150x150 mm flat PVC base and a reducing couple fixed permanently on top of the base. Additional valves, screws and tubing devices are joined together as shown in Figure 3. Detailed information about how to build the field membrane filtration unit can be taken from the construction manual in the Appendix.

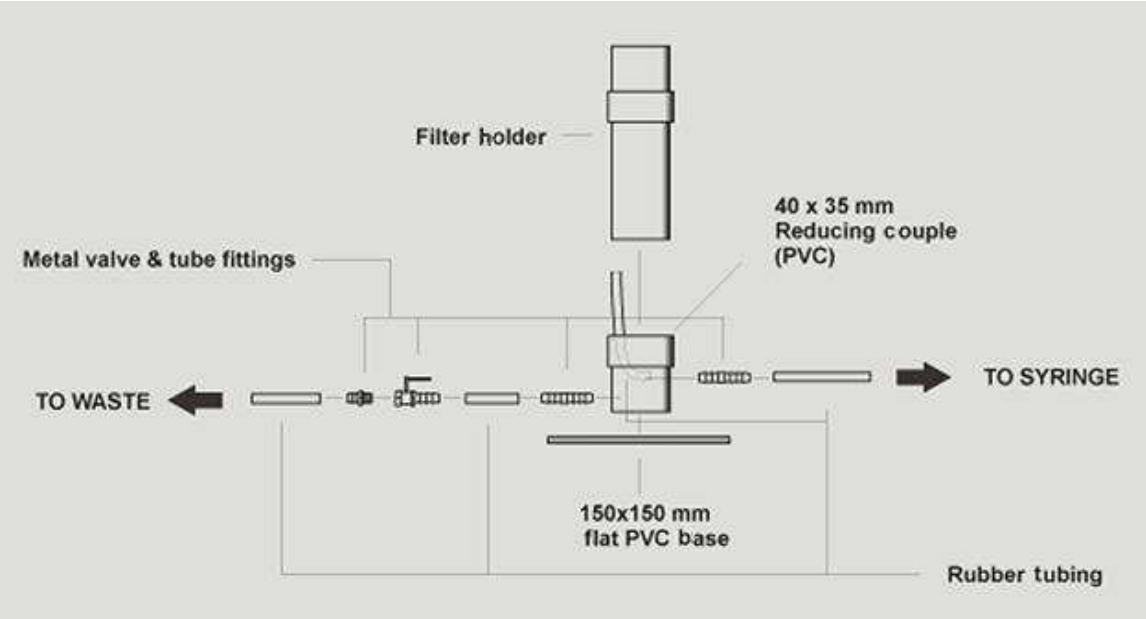

Figure 3: PVCfield unit

2.6 Sanitization and storage

Due to the relatively low softening point and non-fire resistance, PVC filter holders cannot be sterilized, instead the systems need to be sanitized. The following sanitization procedure was developed:

1. After usage the PVC filter holders are sprayed or wiped with alcohol, quickly brushed under tap water and then immersed in 75 ℃ hot water for 20 min.
2. The filter holding devices are then placed into a filter holder storage container covered with aluminium (see Figure 4) where they are cooled down to room temperature before reuse and stored up to 20 days. All components are placed in

a way that allows them to dry quickly as any wet areas may lead to microbiological growth, therefore the black rubber rings are placed vertically into the PVC funnel. The storage container is a simple basket with a perforated PVC plate attached to the inside of the basket.

More detailed information about sanitization and storage can be taken from the testing manual in the Appendix.

Figure 4: Filter holder storage container

2.7 Chromogenic media

A standard method for the detection of *E.coli* and other coliform bacteria in drinking water samples is the MF lactose TTC Tergitol-7 procedure (ISO 9308-1: 2000). In this procedure total coliforms (TC) are detected and enumerated on a membrane filter after sample filtration and incubation on chromogenic media due to a difference in colony colour. When the TC result is positive accompanying tests are required for the confirmation of *E.coli*. These tests are time consuming and more difficult to perform in developing countries with a lack of laboratory equipment, chemical supplies and trained laboratory employees. Nonetheless it is important to distinguish

between *E.coli* and other coliforms as some coliform bacteria can be naturally present in high numbers and are not always a good indicator for a faecal contamination [Stevens et al., 2003]. A method to avoid the difficulties of the MF lactose TTC Tergitol-7 procedure is use of dehydrated selective chromogenic media for simultaneous detection and enumeration of TC and *E. coli* in water samples. Examples of media which were developed as alternatives to the standard ISO procedure are:

- MERCKS' *ChromoCult® Coliform Agar* ("USEPA approved in water testing, AOAC approved for use in processed food testing").
- Micrology Laboratories LLCs' *Coliscan MF* for optional incubation at room temperature ("USEPA Approved for the determination of E. coli and total coliforms for use in National Primary Drinking Water regulations monitoring").
- BIORADS' *RAPID'E.coli2* ("Validation for water testing is pending, NordVal and AFAQ AFNOR approved for use in processed food testing").

The ease of use, the quick detection and the relatively low price make them a useful alternative for drinking water testing in developing countries. In this study dehydrated Rapid E.coli 2 media from BIORAD and Coliscan MF media for optional incubation at room temperature from Micrology Laboratories LLC for simultaneous detection and enumeration of TC and *E. Coli* were used.

2.8 Experimental setup

2.8.1 PVClab unit comparison study

The first comparison study is a 4 month comparison between the PVClab unit, a stainless steel filter holder (SSFH) from Sartorius and a polyphenyl-sulfone filter holder (PSFH) from Pall, both mounted on the same steel manifold (no-name, fabricated in Phnom Penh). Two PVC filter holders are tested on the PVClab unit,

both are named differently (#1 and #2) and are the same over the whole period of testing. Depending on the filter holder material, three different cleaning and storage procedures are applied before testing:

- SSFH is sterilized over the flame before usage and left cooling down to room temperature before testing.
- PSFH (cleaned and dried) is wrapped in aluminium foil, sterilized by autoclaving and stored until usage.
- PVC filter holders are sanitized as described in section 2.6.

Sample water is well water from a shallow well, it is taken just before testing and homogenized in a 500 mL jar by continuous stirring. One water sample is analyzed in a timeframe of max. 3 h on all 4 filter holders, starting with the SSFH followed by the PSFH and both PVC Filter holders. A control experiment is performed on each filter holder before any sample testing or rinsing, therefore 100 mL of pure Reverse-osmosis (RO) water are suctioned through a membrane filter (D = 47 mm, pore size 0.45 µm) which is then treated same as a samples' membrane filter which is explained later. The aim is to see if any filter holder device may contaminate the water sample trough improper sterilization, sanitization or storage procedures. Then the well water sample is suctioned through a membrane filter. Sample volumes suctioned through the filter vary between 100 µL and 10 mL for different samples depending on how contaminated the well is on the day of sampling and testing (based on estimation, e.g. rain or no rain). For each sample volume less than 10 mL, 10-20 mL of RO water are added to the funnel first to ensure a homogeneous distribution of bacteria on the membrane filter for later counting of colonies. Membrane filtration analysis for each sample are carried out in duplicate on all four membrane filter holders, bacterial colony counts are later reported as the mean of these replicates. In between duplicates, membrane filter holders are rinsed with 50

mL of RO water as previous testing did not show any cross contamination for all 4 filter holders. After filtration each membrane is placed on BIORADS' *RAPID'E.coli2* media in a glass petri dish and incubated at 37 ℃ for 21 h +/- 3 h. Violet colonies are counted as *E.coli* (GAL+/GLUC+) and blue-green colonies as other coliforms (GAL+/GLUC-). The number of Totoal Coliforms (TC) is calculated as the sum of violet and blue-green colonies.

2.8.2 PVCfield unit comparison study

In the second comparison study the PVCfield unit is compared to the SSFH unit and the PVClab unit. The PVCfield unit uses a PVC filter holder that is connected to a base part which is connected to a 100 mL syringe (see Figure 3). Both laboratory units are set up the same way as in the first comparison study, sterilization and sanitization procedures for all filter holders as mentioned before. Sample water is either surface water (SW), rain water (RW) or well water (WW). To estimate the right sample volume leading to a countable number of colonies on the membrane (<300) the samples are taken and pretested one day prior the comparison study and then stored at 4 ℃ over night. On the day of testing th e sample is homogenized in a 500 mL jar by continuous stirring. One water sample is then analyzed in a timeframe of max. 2 h on all three units starting with the SSFH unit followed by the PVClab unit and the PVCfield unit. Sample volumes that are suctioned trough the membrane filter vary for different samples depending on the obtained colony counts from the testing performed on the previous day. For each sample volume less than 10 mL, 10-20 mL of RO water are added to the funnel first. Membrane filtration analysis for each sample are carried out in triplicate on all three membrane filter holders, bacterial colony counts are later reported as the mean of these replicates. In between

triplicates, membrane filter holders are rinsed with 50 mL of RO water. Incubation and counting procedures are the same as in the first comparison study.

2.8.3 Media comparison

In the third comparison study the PVCfield unit is compared to the SSFH unit, additionally two different chromogenic media are compared which are first BIORADS' *RAPID'E.coli2* media and second Micrology Laboratories LLCs' *Coliscan MF* media. A difference between both media is that *Coliscan MF* was developed for optional incubation at room temperature and does therefore not require an incubator. Set up of the MF devices aswell as sterilization and sanitization procedures are the same as mentioned before. Sample water is surface water from the Mekong river and is pretested as in the second comparison study. One water sample is analyzed in a timeframe of max. 1h on both units starting with the SSFH unit followed by the PVCfield unit. Sample volumes vary depending on the obtained colony counts of the pretesting. For each sample volume less than 10 mL 10-20 mL of RO water are added to the funnel first to ensure a homogeneous distribution of bacteria on the membrane filter. For one sample a duplicate measurement is performed on the SSFH first with both membrane filters incubated at 37 ℃ on *RAPID'E.coli2* media, the holder is then sterilized over the flame for the next duplicate measurement, this time both membrane filters are incubated at room temperature on *Coliscan MF* media. Same is done for the PVCfield unit with the difference that the filter holder is exchanged with a new one after the first duplicate measurement. In between duplicates membrane filter holders are rinsed with 50 mL of RO water. After incubation *E.coli* and coliform colonies are counted. The counting procedure for membrane filters incubated at 37 ℃ on *RAPID'E.coli2* media is the same as in the prior comparison studies. Membrane filters incubated on *Coliscan MF* media at room

temperature (28 +/- 4 ℃) are incubated for 48 h as recommended by the supplier. Blue-purple colonies are then counted as *E.Coli* (GAL+/GLUC+) and pink colonies as other coliforms (GAL+/GLUC-). The number of TC is calculated as the sum of blue-purple and pink colonies.

3 Results and discussion

3.1 Control samples

Control samples with 100 ml RO water had the goal to reveal any contamination of water samples trough improper sterilization, sanitization or storage procedures of the filter holders. Results show that 5% of all plates were contaminated with a maximum of 3 external (coliform) colonies, *E.coli* has never occurred for any control sample. The contaminations occurred on all different systems (Sartorius, Pall, PVC) and cannot be referred to the PVC filter holders in particular, rather are they for all three systems a result of contaminated RO water, contamination was found to occur in the RO water storage tank occasionally. Therefore the constructed PVC Filter holders did not cross-contaminate drinking water samples with the applied sanitization and storage procedure.

3.2 Colony counting

All membranes incubated on *RAPID'E.coli2* media showed equally formed, distinguishable and therefore countable colonies up to a number of ~300 colonies per plate during the entire time of study. No difference for any MF unit was observed. Little difficulties occurred with membranes incubated on *Coliscan MF* media, some colonies were difficult to count and some not countable due to non clear colony formation, this was especially true for colony counts > 100. This result can be associated with the media itself and not with the MF unit, it is discussed in more detail in section 3.4.

3.3 PVClab unit

A person experienced in construction of mechanical systems is of advantage when constructing the PVClab unit. Essential was the correct construction of the PVC filter holder, the PVC funnel needs to sit firmly on its base not allowing any leakage. Only when this has been achieved the filter holder is ready for sample testing. Deformations and other mechanical or thermal wears of the filter holders were not spotted during the entire time of testing when following the developed method of testing, sanitization and storage. However, deformation was observed in the process of sanitization when the temperature of the water exceeded 90 ℃, therefore it is important to keep the temperature at 75 ℃. The PVC 3-branch manifold shown by Figure 2 was the most robust from several alternatives that were built. Over the whole period of testing all parts of the manifold stayed intact, no mechanical or other types of wears were observed. No disadvantages can be reported in terms of handling, once built the whole PVClab unit felt robust and easy to work with. Regarding time consumption and space the PVC system can be put on a par with the PSFH unit but is at a disadvantage compared to the SSFH unit, more time and space are required for sanitization and storage of the PVC filter holders. Shown by Figures 5 and 6 is the result of the 4 month comparison study between the PVClab unit, the SSFH unit and a PSFH unit. *E.coli* and TC colony counts for 22 well water samples were compared, the goal was to see if the PVClab unit can give the same results over a period of 4 month. Results revealed very similar *E.coli* and TC colony counts for all different systems after incubation of the membrane filters on *RAPID'E.coli2* media, differences in colony counts are casual and cannot be referred to any system in particular. Also obvious is that the performance of both PVC filter holders was stable over a period of 4 month, no degradation in quality of results can be observed. Same results were observed in the second comparisson study shown by Figures 7

and 8, when excluding the PVCfield unit the Figures show another successful comparisson between the SSFH unit and the PVClab unit. Over the entire time of testing the PVClab unit with its testing and sanitization procedure showed that later colony counts are the same as for approved membrane filtration units, it is therefore seen a reliable alternative to approved membrane filtration units.

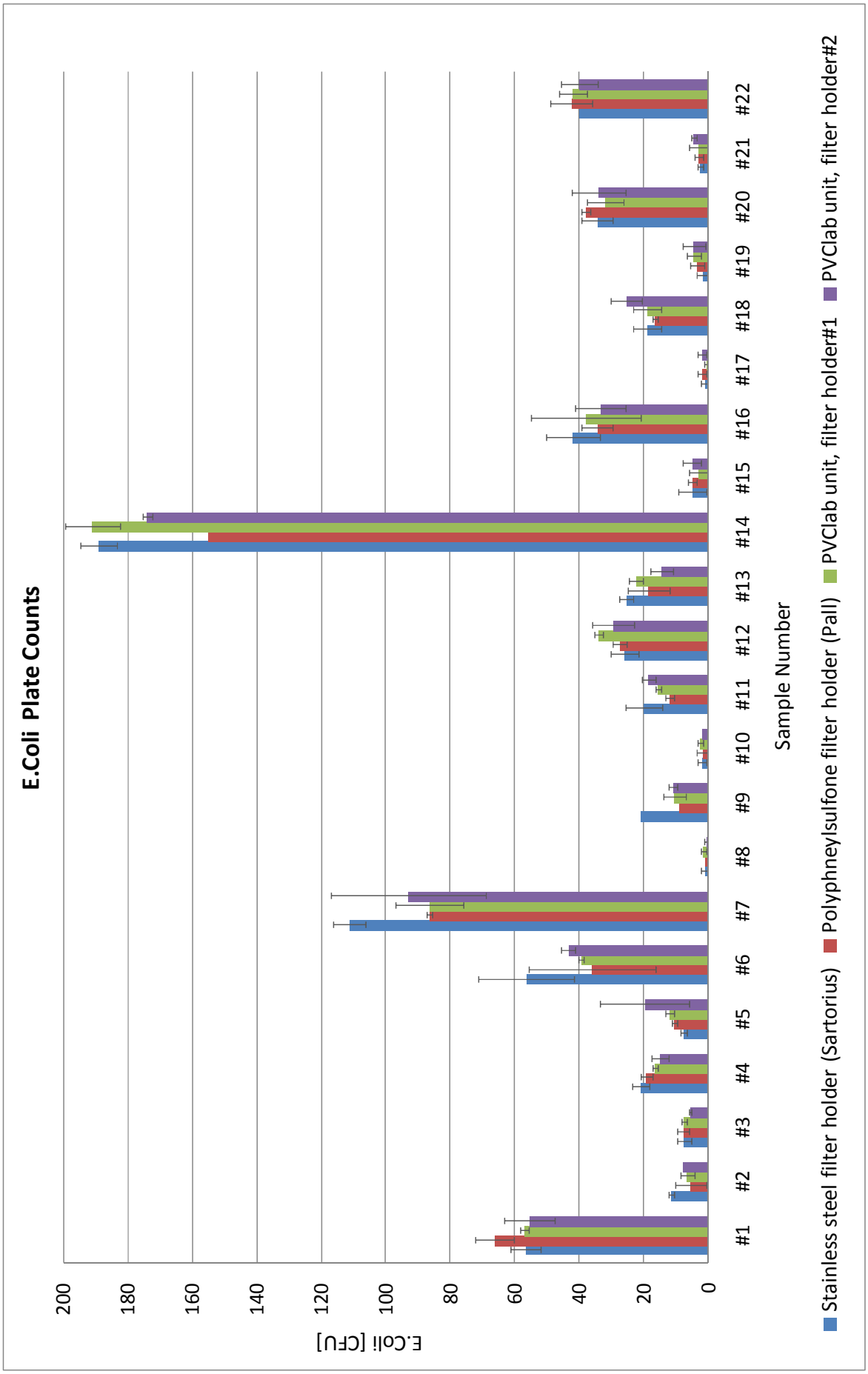

Figure 5: PVClab unit comparison study, *E.coli* plate counts.

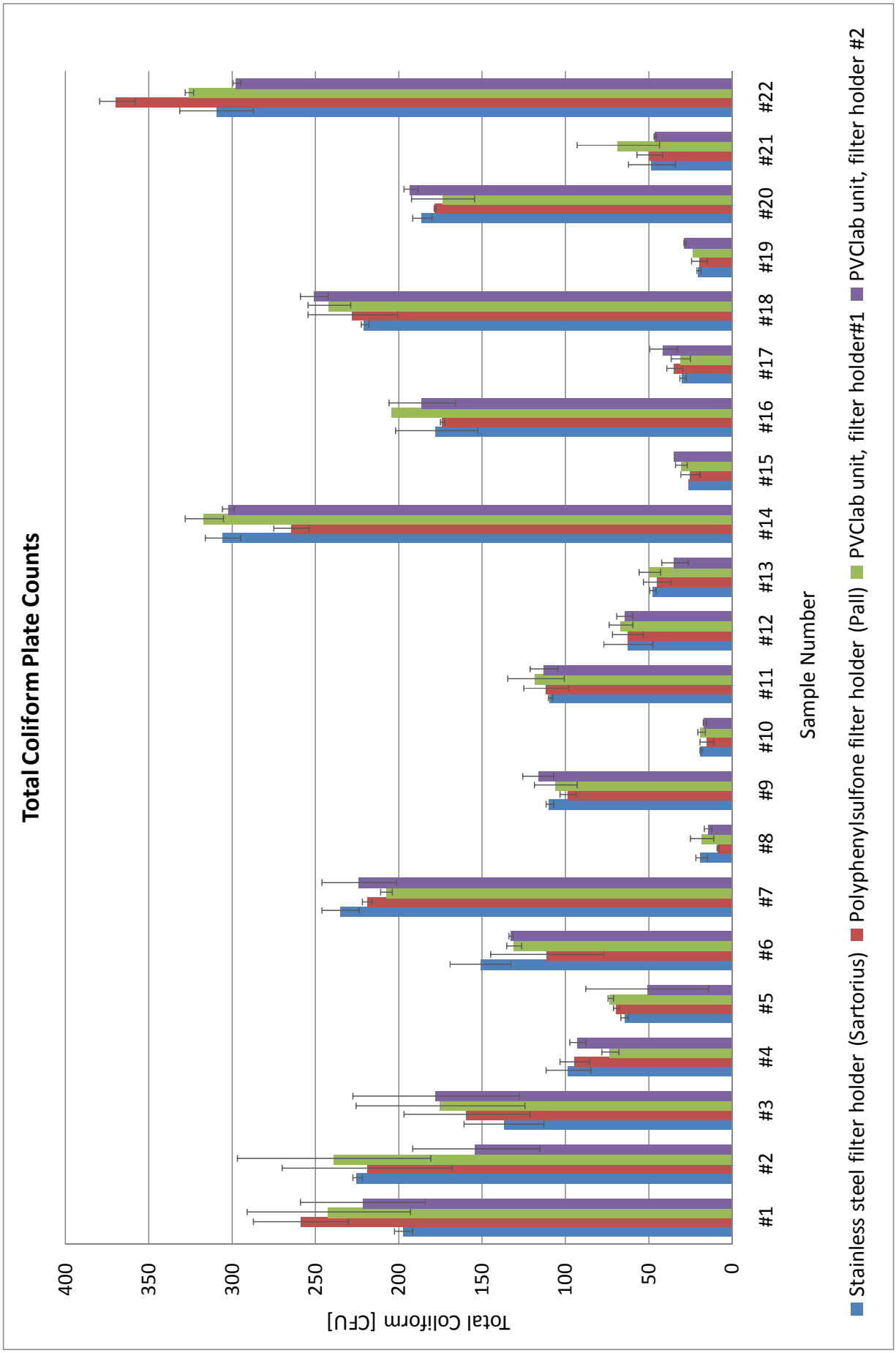

Figure 6: PVClab unit comparison study, TC plate counts.

3.4 PVCfield unit

Essential for this unit is again the construction of the PVC filter holder which is the same as for the PVClab unit. No mechanical or thermal wears were observed for any filter holder during the time of testing. The base part of the unit is a relatively simple construction as obvious from Figure 3, it was airtight and stayed intact with no mechanical wears observed. The 100 mL syringe used provided a pressure difference which left a membrane filter that was dry enough to be placed on nutrient media after filtration. Different laboratory staff people tested the unit and reported it to be easy to work with and of little effort. Figures 7 and 8 show the result of the comparison study between the PVCfield-, the SSFH- and the PVClab unit. Figures compare *E.coli* and TC colony counts for 19 water samples; water samples were either SW, RW or WW. The PVC field unit showed very similar *E.coli* and TC colony counts compared to the SSFH- and the PVClab unit for all water samples, minor differences in colony counts were casual and cannot be referred to any system in particular. Same results were observed in the third comparisson study shown by Figures 9 and 10, when excluding the data obtained on *Coliscan MF* media the Figures show another successful comparisson between the SSFH unit and the PVCfield unit for 6 RW samples. The PVCfield unit with its testing and sanitazion procedure showed for all samples the same results as an approved MF unit. The PVCfield unit is therefore seen as a reliable alternative for *E.coli* and other coliform testing in the field or in the laboratory as a backup system that does not require electrical power.

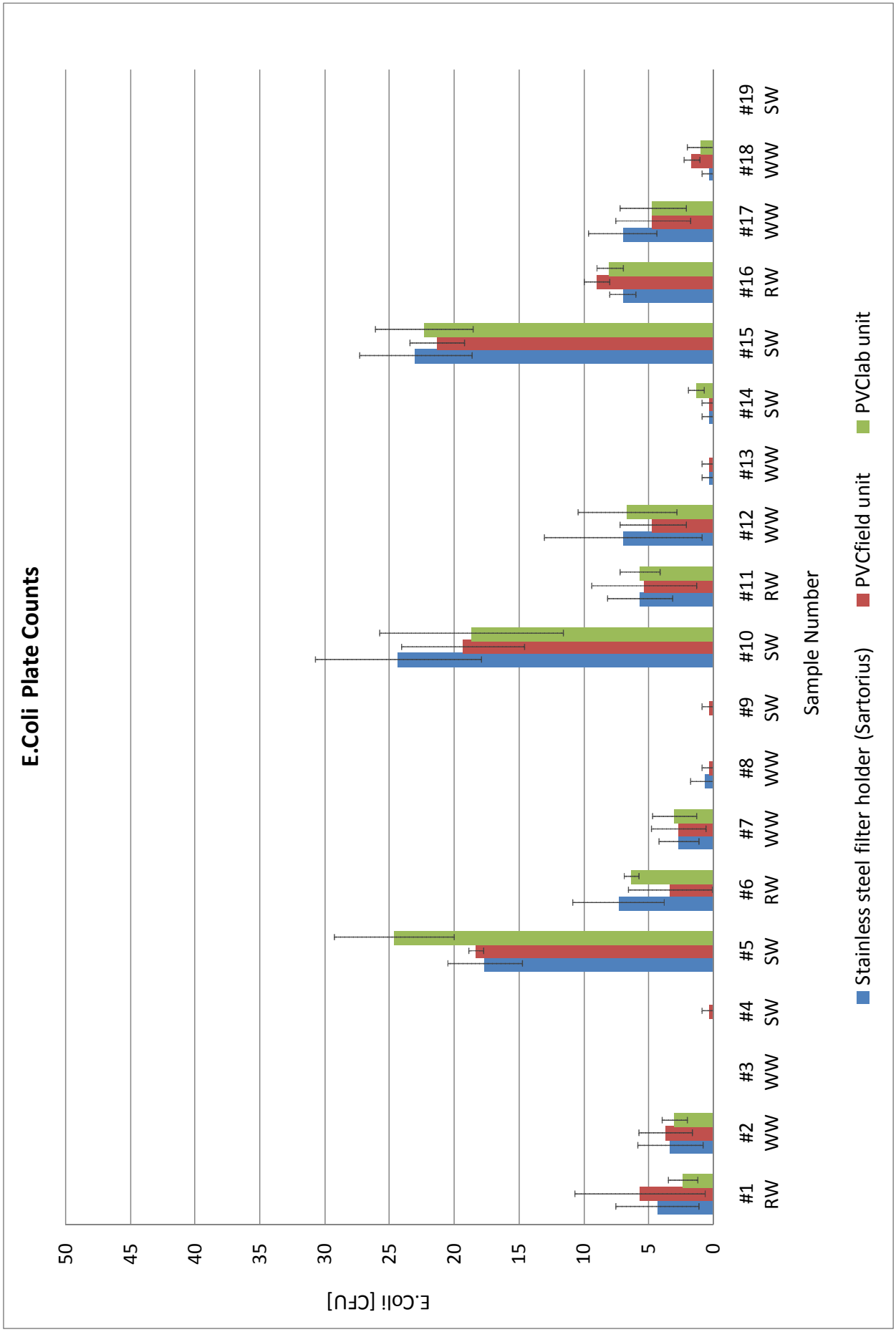

Figure 7: PVCfield unit comparison study, *E.coli* plate counts.

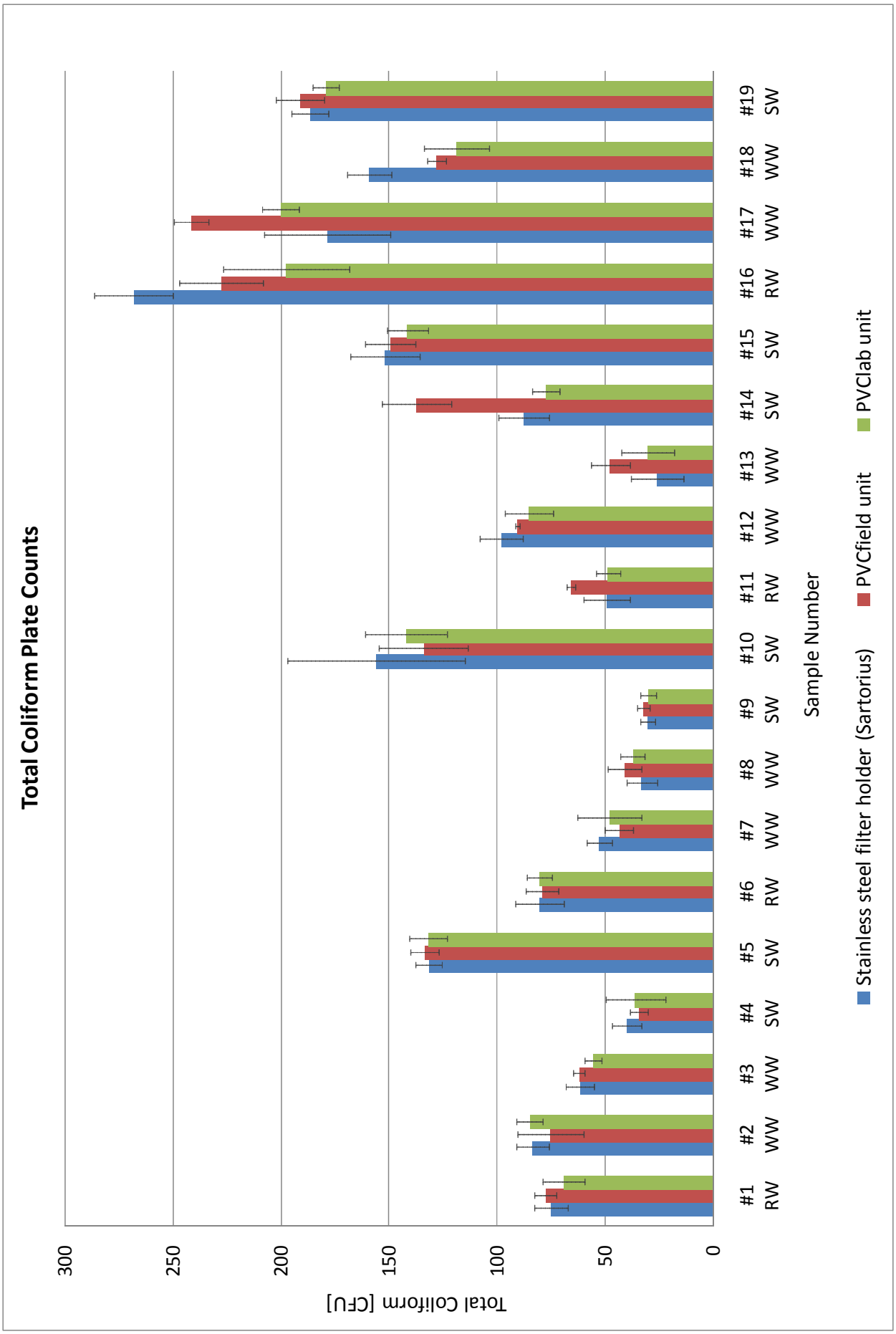

Figure 8: PVCfield unit comparison study, TC plate counts.

Nongovernmental organizations in Cambodia state that nutrient media which can be incubated at room temperature is needed for water testing in rural areas. Figures 9 and 10 show the result of a media comparison study. Two different media were compared on the SSFH- and on the PVCfield unit, *RAPID'E.coli2* media and *Coliscan MF* media for optional incubation at room temperature. Results show that similar *E.coli* colony counts were obtained on both nutrient media for both units. TC counts instead did not show the same similarity for samples with a TC number > 100. Colony counting of other coliforms than *E.coli* on *Coliscan MF* media was in many cases not possible as colonies could not be separated from each other visually. In Figure 8 this is obvious from columns with no error bars or entire columns that are missing (in these cases both plates of the duplicate measurement could not be evaluated). These difficulties are associated with the media itself and not with the PVCfield unit. The obtained results indicate that the PVCfield unit in combination with *Coliscan MF* media may be an opportunity to do testing for fecal indicators in the field, counting problems seem to occur when TC numbers are > 100. An additional comparison study may be necessary to underline this statement.

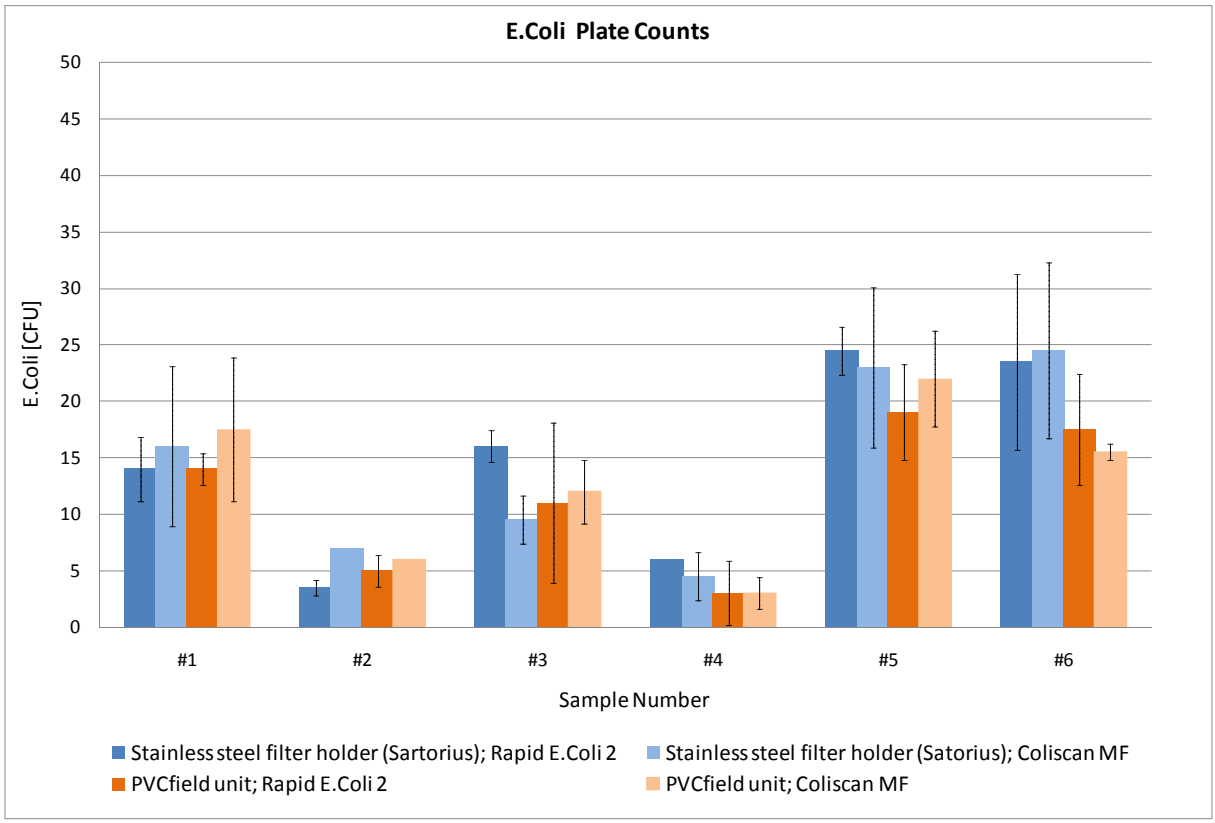

Figure 9: Media comparison study, *E.coli* plate counts.

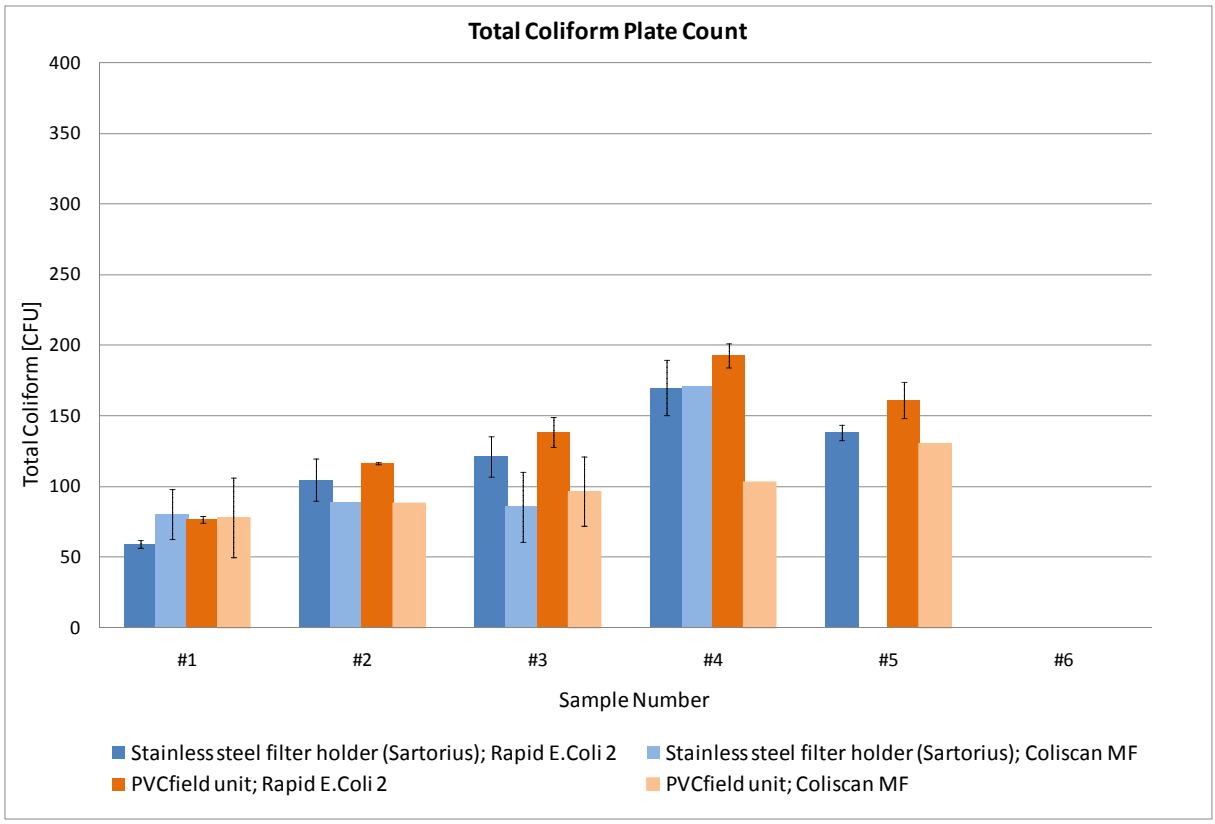

Figure 10: Media comparison study, TC plate counts.

4 Conclusion

Both PVC MF units are relatively easy to build with easily accessible and affordable materials. Experiments comparing both PVC units with approved devices for different water samples showed that both PVC units operated using prescribed testing and sanitization procedures were able to give the same results and were easy to use. Both units are proposed as reliable alternatives for smaller laboratories and for operating in the field where electricity is not available for operating an electric suction apparatus. These devices have the potential to greatly expand affordable testing of water quality in remote communities and therefore using them could help ameliorating the health conditions in urban and rural areas of a developing country. Sources of faecal water contamination could thus be identified and in many cases resolved by relatively simple means and with little management effort.

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