

MAINTENANCE OF CISTERN WATER QUALITY
AND QUANTITY IN THE VIRGIN ISLANDS

R.H. Ruskin and P.S. Callender
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Arthur A. Richards
President

Darshan S. Padda
Vice-President
Research & Land-Grant Programs

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CARIBBEAN RESEARCH INSTITUTE
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ABSTRACT

Almost two-thirds of the residents of the Virgin Islands are not served by a potable water distribution system, with almost 80 percent of them relying on rainfall harvesting techniques with cistern storage. Since the majority of these cisterns serve less than 25 people, they are exempt from the mandates of the Safe Drinking Water Act; yet these cisterns could serve as foci and reservoirs of enteric disease.

Cisterns are readily subject to contamination because they are generally not sealed and are exposed to the environment. In this study of 20 private residential cisterns it was found that if only overall averages were considered, then not one of them would have met the Safe Drinking Water standard of ≤ 1 total coliform/100mL and only 4 of the 20 would have met this limit 50 percent or more of the time.

We determined that the source of the contamination is almost solely environmentally associated, as a result it is felt that a fecal coliform standard would be a better indicator of water quality. What cannot be determined by a coliform test, is the occurrence of Pseudomonas aeruginosa, an opportunistic pathogen known to cause both ear infections and diarrheal disease, and which is frequently found in cistern water.

The best way of controlling these microbial contaminants is via chlorination using common household bleach, which is a very effective chlorinating agent. In addition proper maintenance is necessary such as trimming overhanging trees from roofs, install screens over all opening leading into the cistern, and general cistern maintenance practices.

While the Virgin Islands are blessed with an annual rainfall of between 45-55 inches per year, the average Virgin Islander is very conscientious about water use, and uses between 16 to 54 gallons per day, with less water being used by those on either small cisterns, or where the number of residents is large.

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Table of Contents

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
INTRODUCTION	1
MATERIAL AND METHODS	2
Site Selection and Preparation	2
Sample Collection and Procedure	4
Microbiological Analysis	5
<u>Pseudomonas aeruginosa</u> Analysis	8
Experimental Design	10
Quality Control	14
RESULTS	14
DISCUSSION	31
APPENDIX A	36
APPENDIX B	44
BIBLIOGRAPHY	47

MAINTENANCE OF CISTERN WATER QUALITY AND QUANTITY
IN THE VIRGIN ISLANDS

The U.S. Virgin Islands - comprised of the three major islands of St. Thomas, St. John, and St. Croix, and several small cays and area islets - lie situated at the northwestern most edge of the West Indies, and due east of Puerto Rico. Almost two-thirds of the residents of these islands are not served by a potable water distribution system, with almost 80% of them relying on rainfall harvesting techniques with a cistern storage (36).

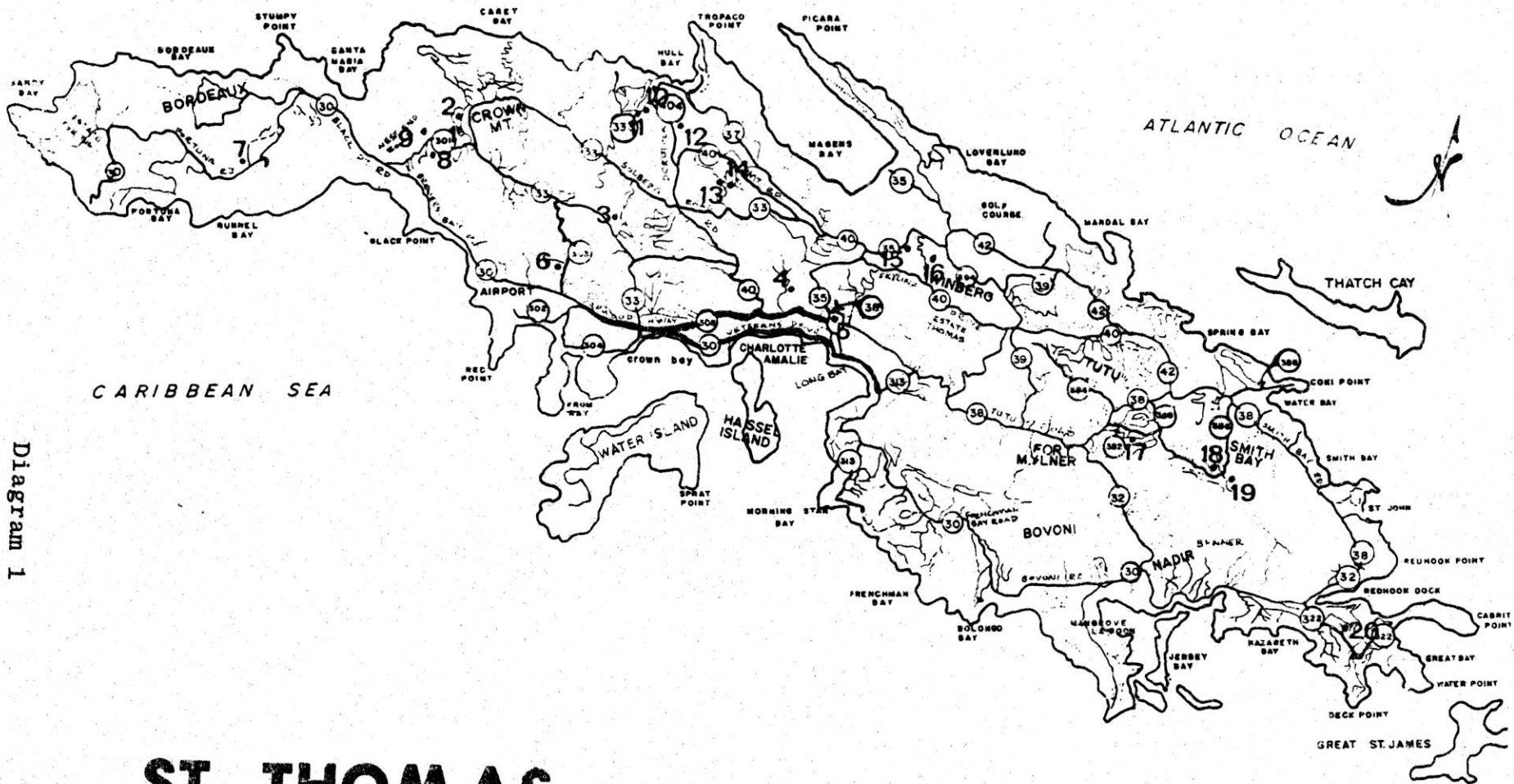
Cisterns are tanks used for the storage of water, and maybe either above ground or below ground; they may be incorporated into the structure of the house, or may be separated from the house. At the present time there are no guidelines covering the treatment or storage of cistern water. There are no water quality standards for cisterns serving less than 25 people since such cisterns are exempt from the mandates of the Safe Drinking Water Act (5). Yet, these cisterns could serve as foci and reservoirs of enteric disease (36). Cisterns are readily subject to contamination because they are generally not sealed and are exposed to the environment (36). Leaves, dirt, insects, frogs, animal droppings, etc., all can find their way into cisterns, and all contribute to the contamination problem (36).

This research was conducted to answer basic questions about cistern water quality, and how it is affected by the hydrology, the demand for water, and the environment; it was also conducted to provide the necessary hard data whereby territorial officials can establish cistern water quality standards which will adequately protect the public's health, and to recommend practices which will maintain the quality of that water.

MATERIALS AND METHODS

Site selection and preparation. Twenty individuals volunteered their time and cisterns for this study. The twenty cisterns were spread across the island of St. Thomas (Diag. 1) in the U.S. Virgin Islands. Each participant was given a questionnaire to fill out pertaining to the environment surrounding the cistern, the frequency with which water had to be bought, the type of roof that serves as a water catchment, the size and maintenance of the cistern, water treatment practices, if any, and accessibility to the roof area, etc.

An on site inspection was then made of each participating home, and the roof catchment area was measured. During the inspection a site was selected and a manual rain gauge (Qualimetrics, Inc., North Highlands, CA) was installed to measure rainfall patterns. A point in the plumbing was also selected, and later a qualified plumber came back and installed a water meter (Precision Meter Inc.) so individual home water usage patterns could be measured.



ST. THOMAS
 UNITED STATES VIRGIN ISLANDS

Diagram 1

MAP OF ST. THOMAS ROUTE SYSTEM		
DEPARTMENT OF PUBLIC WORKS, DIVISION OF ROADS & HIGHWAYS		
DATE 12-20-53.	SCALE: 1" = 0.5 MILES	PREPARED BY CLAYTON S. MITCHELL
COMMISSIONER OF P.W.D. CECIL A. GEORGE	DIR. OF ROADS & HIGHWAYS ALOY W. NIELSEN	

Sample Collection and Procedure. The twenty sample sites were divided into four groups of five according to geographic location; this was done to facilitate the collection of the samples and shorten the lag time between sample collection and sample analysis. A group of five samples were picked up Monday and another group of five were picked up on Tuesday, with the other two groups being picked up on the following Monday and Tuesday. This procedure resulted in a round robin sampling cycle with a duration of two weeks per cycle. Limiting the sampling to Monday and Tuesday allowed for the completion of all analysis, counting, and verification procedures by Saturday of that week. The actual study period ran from September 1, 1986 to May 23, 1987.

Samples were collected in 1 liter amber glass bottles with ground glass stoppers. While chlorine residuals were not expected to be found because most private residents do not regularly chlorinate their cisterns, sodium thiosulfate was added in the event that one may have done so. 0.8 mL of a 10% solution of sodium thiosulfate was added to each sample bottle before the mouth of the bottle was covered by aluminum foil and autoclaved for 15 minutes at 15 psi (3,5). Owners were queried periodically whether they had chlorinated their cisterns since the last visit. Of the study group only four had bothered to chlorinate during the study period and this averaged less than twice for each of the four individuals. The ground glass stoppers were

individually wrapped in aluminum foil and autoclaved with the bottles. After cooling sample labels were affixed to the bottles which were then filled in at the time the samples were collected by the Water Resources Research Center (WRRC) personnel.

Samples were collected by both WRRC personnel and in four instances by the homeowners themselves. These four homeowners were given a lengthy set of typed sampling procedures to follow, and had these procedures demonstrated to them; periodically these individuals were queried about their sampling procedures just to insure that they were followed.

In collecting the samples the faucet was turned on and allowed to flow to waste for at least 2 minutes before the sample was collected (3,5). Initially the samples were packed on ice and returned to the lab (3,5); after the first week, however this became impractical because of the lack of coolers, the expense of ice, and the short transit times between sample collection and sample analysis which typically ran less than 2 1/2 hours. While it has been reported in the literature that die off does occur even after only short intervals (29,30) the die off would be insignificant (39) for the times involved and also tends to simulate actual cistern water temperatures.

Microbiological analysis. Total coliform, fecal coliform, fecal streptococcus, Pseudomonas aeruginosa, and heterotrophic plate count analyses were performed. Under the

Safe Drinking Water Act only total coliform analysis is usually done, however since contamination was suspected, the other usual tests for fecal coliform, fecal streptococcus, and heterotrophic plate count analysis was also performed. P. aeruginosa analysis was performed as it is emerging as a major health concern.

All analysis with the exception of the heterotrophic plate count was done via the membrane filter (MF) technique. The heterotrophic plate count was done using the spread plate technique (3).

Total coliform, fecal coliform, and fecal streptococcus were isolated on m-Endo Agar (Difco Laboratories, Detroit, MI) (3, 5), m-FC Agar (Difco) (3,4,5), and KF Streptococcus Agar (Difco (3,4,5), respectively. The heterotrophic plate count (3) was performed using Standard Plate Count Agar (Difco). All media were prepared in accordance with the manufacture's instructions. Analysis for P.aeruginosa was done using a new medium developed in this laboratory designated m-CX. This medium was developed in response to noted problems with the recommended m-PA Agars listed in Standard Methods for the examination of Water and Waste Water (3) (see Appendix A on the development of m-CX).

Sample volumes of 100,50,25, and 10 mL were filtered. Total coliform, fecal streptococcus, and P. aeruginosa samples were filtered through 0.45m filters (GN-6, 66068; Gelman Sciences Inc., Ann Arbor, MI.) (3,4,5). Fecal coliform samples were filtered through 0.7m filters

(Millipore Corporation, Bedford, Mass.) (3). 0.1 and 1 mL volumes of 3 dilutions ranging from 1 to 10^{-6} were run in duplicate for the heterotrophic plate counts.

Total coliforms, fecal streptococcus and the heterotrophic plate counts were incubated at $35 \pm 0.5^{\circ}\text{C}$ (3,5). The fecal coliform were incubated at $44.5 \pm 0.2^{\circ}\text{C}$ (3,5). The P. aeruginosa plates were first preincubated at $30 \pm^{\circ}\text{C}$ for 3-4 hours and then incubated at $41.5 \pm^{\circ}\text{C}$ (3,7,8,11,20,23,34).

Total coliforms and fecal coliforms were incubated for 24 hours; fecal streptococcus, heterotrophic plate counts, and P. aeruginosa were incubated for 48 hours.

Verification of isolates. Up to five typical and, if present, five atypical colonies were picked from each medium for each sample, and subjected to verification (5, 20). Colonies were counted with the aid of a variable magnification dissecting scope with a minimum magnification of 10x (Parco EMZ745 10L with annular fluorescent illumination; Parco Scientific Co., Bienna, OH) (3,5,20). The heterotrophic plate counts were counted with the aid of a Quebec Colony Counter (3,5,20).

Typical green sheen, and atypical red colonies were picked from the m-Endo Agar plates and verified in Lauryl Tryptose Broth (Difco) and Brilliant Green Bile 2% (Difco) (3,5,20,31,40).

Typical blue and atypical grey or blue-gray colonies from the m-FC Agar plates were verified in Lauryl Tryptose Broth and EC Medium (Difco) (3,4,5,20,33).

Typical red-pink to red colonies from the KF Streptococcus Agar plates were verified in Brain Heart Infusion Broth (Difco) at 35 and $44.5 \pm 0.2^{\circ}$ C and in Brain Heart Infusion with 40% Bile (Difco) at $35 \pm 0.5^{\circ}$ C, and a Catalase test (3,5).

Typical fluorescent yellow to yellow green or blue green, mucoid colonies, and atypical small, clear, flat, non-mucoid colonies were picked from the m-CX Agar plates and verified on Skim Milk Agar (Brown and Foster) (3,5,7,8,10,20,28), Pseudomonas Isolation Agar (Difco) and King's B medium (same as Difco's Pseudomonas F Agar) on a routine basis (Diag 2). Other tests were employed in the development of this medium.

Pseudomonas aeruginosa analysis. Because P. aeruginosa is a well known opportunistic pathogen - especially to the very old, the very young, and hospitalized patients (most notably burn patients) - many states have begun to regulate P. aeruginosa contamination in water supplies. Since 1972, the medium of choice for the isolation P. aeruginosa has been the m-PA agars based on the formula by Levin and Cabelli (28) and thus as modified by Dutka and Kwan (11). This last formula is the one recommended in Standard Method for the Examination of Water and Wastewater, 16th ed. These two formulas have since been superceded by three other

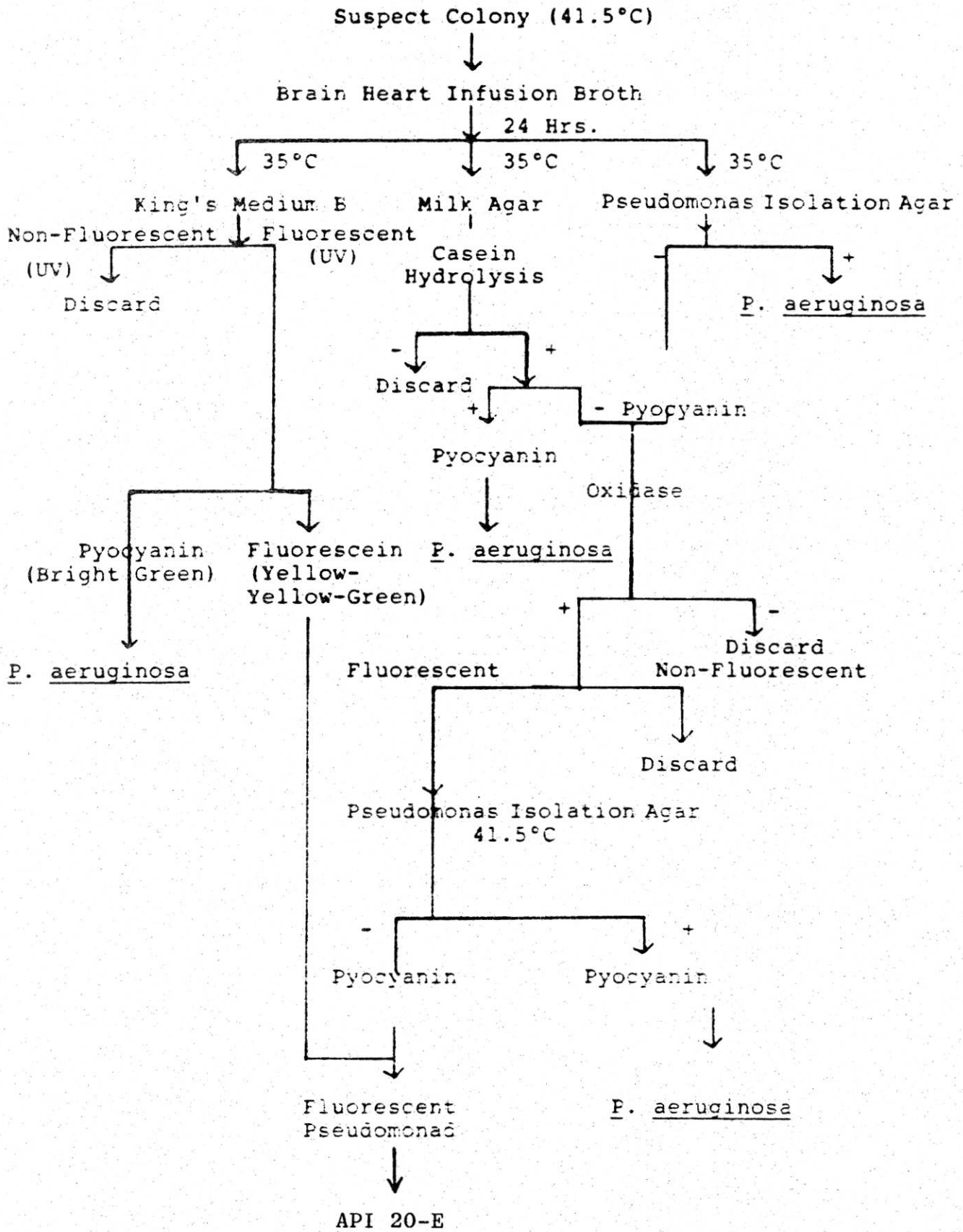


Diagram 2

formulas (7,8) which have yet to be included in Standard Methods. All the m-PA formulas were tested and all suffer from one or more major drawbacks; as a result, we developed a medium in this laboratory based upon the highly successful "A" formula of King, Ward and Raney (25) in an effort to get around these drawbacks. We have designated this formula m-CX and in both informal and more formal testing found it to be as good or better than any of the m-PA agars tested, and better than either of the m-PA formulas listed in Standard Methods, thus used it exclusively throughout. A more complete and detailed discussion of the development of this medium is included as Appendix A of this report.

Experimental Design. As stated earlier, the twenty cisterns were sampled in a round robin system of groups of five. Each sample was analyzed by membrane filtration for total coliform, fecal coliform, fecal streptococcus, P. aeruginosa, and heterotrophic plate count. In addition, conductivity, pH, turbidity, and where applicable, chlorine residual were measured. These analyses constituted the mainline of the research, and were done for each cistern thereby generating a profile of the water quality for a "typical" residential cistern. As the research progressed, it became quite apparent that the "typical" cistern would fall far short of the provisions of the Safe Drinking Water Act. In spite of the apparent contamination, few homeowners complained of any illnesses which they attributed directly to the quality of their cistern water. There was one

reported case however, where the person complained of a prolonged case of diarrhea. The routine analysis of their cistern water revealed high numbers of both total coliform and fecal streptococcus, moderately high numbers of both fecal coliforms and P. aeruginosa, and a heterotrophic plate count greater than 10^5 CFU/mL, thereby establishing a possible direct link between cistern water quality and enteric disease. This link was further strengthened by the results obtained in a series of side experiments to the main line of the research. Many of these side experiments were designed to better understand the dynamics which influence water quality, and maybe suggest a control mechanism.

One such side experiment was done to see what effect the addition of household bleach would have on the bacterial populations in the cisterns. The bleach was added using a chlorination table which would yield a Free Residual Chlorine reading of 1.5-2.0 mg/L (Tables 1 and 2).

Another side experiment looked at the effects leaves had on the addition of microbial contamination to the cistern.

Another experiment looked at how long it would take after chlorination for a cistern to return to pre-chlorination levels.

Table 1

Free Residual Chlorine Using Chlorine Bleach

1.5 Fluid Oz. (44.36 mL) per Load Ton (241 Gal.)-- 1.5-20 mg/L

Load Ton	Gal.	Dose Fluid Oz.	Approx. mL
1	214	1.5 Oz.	45
2	428	3.0	89
3	642	4.5	133
4	856	6.0	178
5	1070	7.5	222
6	1284	9.0	266
7	1498	10.5	311
8	1712	12.0	355
9	1926	13.5	400
10	2140	15.0	446
11	2354	16.5	488
12	2568	18.0	533
13	2782	19.5	577
14	2996	21.0	621
15	3210	22.5	665
16	3424	24.0	710
17	3638	25.5	755
18	3852	27.0	799
19	4066	28.5	843
20	4280	30.0	887

Table 2

Free Residual Chlorine

Using 70% HTC Swimming Pool Chlorine

CAPACITY IN GALLONS	DOSE IN OZ. TO PROVIDE 3-5 mg/L FREE RESIDUAL CHLORINE	CAPACITY IN GALLONS	DOSE IN OZ. TO PROVIDE 3-5 mg/L FREE RESIDUAL CHLORINE
1,000	.95	29,000	27.55
2,000	1.90	30,000	28.50
3,000	2.85	31,000	29.45
4,000	3.80	32,000	30.40
5,000	4.75	33,000	31.35
6,000	5.70	34,000	32.30
7,000	6.65	35,000	33.25
8,000	7.60	36,000	34.20
9,000	8.55	37,000	35.15
10,000	9.50	38,000	36.10
11,000	10.45	39,000	37.05
12,000	11.40	40,000	38.00
13,000	12.35	41,000	38.95
14,000	13.30	42,000	39.90
15,000	14.25	43,000	40.85
16,000	15.20	44,000	41.80
17,000	16.15	45,000	42.75
18,000	17.10	46,000	43.70
19,000	18.05	47,000	44.65
20,000	19.00	48,000	45.60
21,000	19.95	49,000	46.55
22,000	20.90	50,000	47.50
23,000	21.85		
24,000	22.80		
25,000	23.75		
26,000	24.70		
27,000	25.65		
28,000	26.60		

Another studied the effects of turbidity on the effectiveness of chlorination on bacterial populations both before and after sedimentation took place. This turbidity was induced by a simulated rainfall on a gutter full of leaves and collecting the runoff in a sterile bottle for analysis.

One experiment was done to see if reported die off rates for some standard indicator organisms would hold true for cisterns. This would yield information about cistern self-purification chances via bacterial die off (17).

Finally an experiment was done that looked at the types of bacteria found in both the biofilm layer which forms between the water-wall interface, and the sediment layer.

Quality Control. Accepted quality assurance practices (3, 5) were observed throughout this study.

RESULTS

Table 3 presents the overall results of the twenty cisterns studied, and gives the lowest, highest and mean values obtained for each parameter. The mean value was calculated from a total of between 12 and 15 samples.

Table 4 presents all the data for three of the twenty cisterns and is intended to show typical results of a cistern of very good quality (Sample Site # 19); a cistern which is moderately contaminated (Sample Site # 10); and a

Table 3

	Total Coliform			Fecal Coliform			Fecal Streptococcus			P.aeruginosa			Standard Plate Count		
	X _L	\bar{X}	X _H	X _L	\bar{X}	X _H	X _L	\bar{X}	X _H	X _L	\bar{X}	X _H	X _L	\bar{X}	X _H
1	0	125	1300	0	15	153	0	258	1410	0	25	236	4.0×10^1	1.2×10^5	1.7×10^6
2	0	154	2160	0	42	606	0	40	142	0	16	130	1.2×10^2	6.4×10^3	6.9×10^4
3	19	3713	23400	0	80	570	0	214	1734	0	18	65	3.5×10^3	2.3×10^7	3.2×10^8
4	280	2118	9356	0	18	100	0	168	855	0	61	316	5.7×10^2	7.0×10^4	5.6×10^5
5	0	96	1150	0	25	312	0	54	333	0	16	140	1.9×10^2	1.3×10^5	1.6×10^6
6	0	322	3620	0	123	1430	0	117	1330	0	4	24	2.0×10^2	2.0×10^4	1.8×10^5
7	0	222	1240	0	90	760	0	53	310	0	108	600	2.5×10^2	3.8×10^5	3.2×10^6
8	0	193	980	0	31	360	0	176	1280	0	50	598	5.0×10^2	1.6×10^5	1.0×10^6
9	0	11	94	0	1	5	0	16	67	0	195	2210	3.0×10^3	4.9×10^6	4.0×10^7
10	0	3	10	0	0	2	0	152	1120	0	52	326	1.0×10^2	4.3×10^4	2.7×10^5
11	6	952	8450	0	22	162	5	171	936	0	27	266	3.0×10^2	2.3×10^5	1.6×10^6
12	12	356	1360	0	23	81	4	197	541	0	70	522	2.2×10^2	2.0×10^4	9.3×10^4
13	0	148	430	2	45	272	0	54	200	0	13	103	5.0×10^4	9.2×10^4	5.0×10^5
14	0	92	309	0	7	47	3	44	156	0	5	40	2.0×10^2	5.3×10^4	4.6×10^5
15	0	34	195	0	7	41	0	13	99	0	9	39	5.0×10^2	4.0×10^5	2.4×10^6
16	0	2268	8528	0	31	84	1	175	355	0	23	120	1.6×10^2	8.8×10^3	2.2×10^4
17	0	26	300	0	3	48	0	24	114	0	28	271	2.0×10^1	1.5×10^3	9.8×10^3
18	0	618	6240	0	24	114	0	172	500	0	268	2220	0.0×10^0	9.0×10^4	1.2×10^6
19	0	15	94	0	2	15	0	48	243	0	2	11	3.0×10^1	2.7×10^2	5.2×10^2
20	0	1302	6652	0	96	432	0	223	930	0	90	850	1.8×10^2	3.8×10^4	3.2×10^5

Table 4

16

Sample Site#	Total Coliform	Fecal Coliform	Fecal Streptococcus	<u>Pseudomonas aeruginosa</u>	Heterotrophic Plate Count
19	0	0	3	0	2.2×10^2
	28	1	4	0	3.7×10^2
	0	0	24	0	1.6×10^2
	5	7	48	0	2.2×10^2
	94	15	243	1	5.2×10^2
	11	0	0	0	1.3×10^2
	4	0	95	1	7.0×10^1
	7	5	21	0	5.0×10^2
	0	0	0	0	9.0×10^1
	2	1	5	8	3.0×10^1
	23	0	21	0	1.3×10^2
	0	0	11	0	6.0×10^1
	0	0	1	0	4.5×10^1
	32	0	198	11	1.2×10^3
10	0	0	N.D.	120	2.0×10^5
	10	2	26	0	4.0×10^3
	N.D.	N.D.	14	N.D.	2.0×10^2
	0	0	22	32	6.0×10^3
	0	0	78	19	2.6×10^3
	2	0	29	6	1.0×10^2
	1	0	0	2	1.0×10^4
	0	0	97	20	4.5×10^2
	0	0	13	4	5.0×10^2
	2	0	159	326	2.7×10^5
	0	0	35	3	6.0×10^3
	0	0	335	4	9.5×10^4
	3	0	53	51	2.5×10^3

Table 4 continued

Sample Site #	Total Coliform	Fecal Coliform	Fecal Streptococcus	<u>Pseudomonas aeruginosa</u>	Heterotrophic Plate Count
	5	0	1120	86	2.4×10^2
3	6150	570	65	20	5.7×10^5
	20	N.D.	N.D.	N.D.	3.5×10^3
	N.D.	260	N.D.	N.D.	8.0×10^5
	2500	10	36	0	4.9×10^5
	3220	22	0	N.D.	1.2×10^5
	23400	2	1736	35	1.0×10^5
	3750	0	125	8	4.9×10^4
	220	131	186	0	N.D.
	N.D.	N.D.	N.D.	N.D.	3.2×10^8
	35	0	0	2	4.0×10^5
	4420	33	231	6	2.2×10^4
	772	8	59	0	1.2×10^5
	46	4	7	0	3.1×10^4
	TNTC	2	126	6	1.2×10^5

N.D. = No Data

TNTC = Too Numerous to Count

cistern of very poor water quality (Sample Site # 3). These same sample sites also illustrate that there is no correlation between either of the coliform tests or the fecal streptococcus test with the presence or absence of P. aeruginosa.

If the total coliform standard of ≤ 1 total coliform per 100 mL were used for determining residential cistern water quality, and if only overall averages were considered, then not one of the twenty cisterns studied would meet this limit (Table 5). However, four of the twenty would have met this limit 50% or more of the time, with the best of the four being 60% of the time. Table 5 also compares the number of cisterns which would have met a fecal coliform standard of ≤ 1 fecal coliform per 100 mL. Thus, if a fecal coliform standard were in use, eleven of these twenty cisterns would have met this limit 50% or more of the time, with the best of the eleven being 93% of the time.

Table 6 compares those instances where the coliform standard was violated and the occurrence of P. aeruginosa is given for each cistern. Coliform violations were calculated using equation 1.

$$\sum \frac{\text{Number of Samples with Total Coliform} > 1}{\text{Total Number of Samples for each cistern}} \times 100 = \% \text{ violation for each cistern} \quad [1]$$

P.aeruginosa occurrence in each cistern was calculated in a similar way using equation number 2.

Table 5

Percentage of Time
Cistern Would Meet
Total Coliform Standard-vs-Fecal Coliform Std.
of 1/100 mL

Sample Site	<u>Total Coliform Standard</u>	<u>Fecal Coliform Standard</u>
1	37%	47%
2	40	67
3	0	20
4	0	33
5	47	67
6	50	79
7	8	50
8	20	53
9	40	73
10	50	86
11	0	13
12	0	29
13	7	0
14	20	60
15	60	67
16	9	27
17	57	93
18	13	33
19	36	79
20	7	14
Average	25%	50%

Table 6

Coliform Violations
-vs-
Pseudomonas aeruginosa
in Sample

Sample Site	<u>Total Coliform Violations/%</u>	<u>P. aeruginosa Occurrence/%</u>
1	62%	38%*
2	67	64 *
3	100	58
4	100	92
5	50	50 *
6	42	58 *
7	91	82 *
8	77	62 *
9	57	71 *
10	46	92 *
11	100	73
12	100	86
13	93	50
14	79	64 *
15	40	80 *
16	91	55 *
17	43	64 *
18	87	87 *
19	64	29
20	93	86 *
	----- 74 %	----- 67%

* = P. aeruginosa found in the absence of Total Coliform occurred in 70% of the cisterns.

$$\sum \frac{\text{Number of Samples in which } P.aeruginosa \text{ was found}}{\text{Total number of samples for each cistern}} \times 100 = \% \text{ occurrence of } P.aeruginosa \quad [2]$$

The percentage of cisterns in which P. aeruginosa was found in the absence of total coliforms was calculated by equation number 3.

$$\sum \frac{\text{Number of Cisterns in which } P.aeruginosa \text{ is found in absence of Total Coliforms}}{\text{Number of Cisterns in which } P.aeruginosa \text{ was found}} \times 100 = \% \text{ occurrence of } P.aeruginosa \text{ in the absence of Total Coliforms} \quad [3]$$

Table 7 compares the average total coliform data with the average fecal coliform data and the average fecal streptococcus data. Further, the fecal coliform-fecal streptococcus ratio was calculated. This ratio was not done to pinpoint the exact source of the contamination (3), but rather to establish whether the source of the contamination was from man enhanced sources, or from sources other than man (3,36). A ratio greater than 4.1 is considered indicative of pollution derived from wastes composed of human excrement, whereas ratios less than 0.7 suggest pollution due to nonhuman sources. Ratios between 0.7 and 4.4 usually indicate wastes of mixed human and animal sources (3).

The results that while cistern water may be contaminated (the total coliform number), the fecal coliform/fecal streptococcus ratio suggests that the sources of that contamination is probably of nonhuman origin.

Table 7

Sample Site	Ave. Total Coliform	Ave. Fecal Coliform	Ave. Fecal Streptococcus	F.C./F.S. Ratio
1	125	15	258	0.06
2	154	42	40	1.05
3	3713	80	214	0.37
4	2118	18	168	0.11
5	96	25	54	0.46
6	322	123	117	1.05
7	222	90	53	1.70
8	193	31	176	0.18
9	11	1	16	0.06
10	3	0	152	0.00
11	952	22	171	0.13
12	356	23	197	0.12
13	148	45	54	0.83
14	92	7	44	0.16
15	34	7	13	0.54
16	2268	31	175	0.18
17	26	3	23	0.13
18	618	24	172	0.14
19	15	2	48	0.04
20	1302	96	223	0.43

Average Total Coliform

638

Average Fecal Coliform

32

Average Fecal Strepto.

118

One explanation for these high total coliform counts outside of excreta from animals, dust and insects, pertains to the impact that leaves from trees have on cistern water quality. This source is particularly important in forested areas of the island since leaves contain extremely high numbers of total coliform, fecal coliform, and fecal streptococci (16). This was determined by collecting tree leaves in a clean 1 meter long length of gutter, trickling 2 liters of sterile deionized water over the leaves and collecting the runoff in sterile 1L amber bottles. Once back at the laboratory, the water was analyzed for all of our parameters. The results were too numerous to count (TNTC) for all the analyses, with heterotrophic plate counts in the millions.

Thus, a home in which the gutters are full of leaves acts like a giant tea bag: When it rains, the water washes over the leaves which act just like tea leaves, only the "flavor" of the "tea" is bacterial, which then enters into the cistern.

One experiment that was done dealt with the ability of cistern water to self-purify. In natural waters this is a well known phenomenon especially for surface waters and, to a lesser extent, ground water. The mechanisms involved in self purification include: sedimentation, nutrient limitation, competitive microbial flora, predators, aeration, sunlight exposure, water temperatures, water pH,

and travel time downstream or holding time if the water is impounded (17).

In one study of coliform reduction via natural self purification, several different rivers were analyzed. The T90 (the time needed for a 90% reduction of the coliform population to occur) varied from 2.1 to 115 hours (17).

In a separate study only using pure cultures and membrane diffusion chambers, McFeters established the half life (the time needed for half the population to die) of several different bacterial species and several different strains of each. For coliform the half life was determined to be between 17.0 and 17.5 hours.

In cistern water we found that self-purification does not occur, or if it does, it proceeds at a very slow rate. The procedure we followed was to sterilize 2 liters of deionized water which was trickled over a gutter full of leaves 1 meter long, with the run off collected in sterile 1L amber bottles which we used as "mini-cisterns". From these "mini-cisterns", samples were regularly taken and analyzed for all our standard parameters used in this study; after one week we observed essentially no change in any of our numbers and the sample was discarded. There are two possible reasons that come to mind which might explain the lack of observable die-off. One, the interior of a cistern is very dark, thus light inactivation does not occur; and two, there are always nutrients within the cistern which can be utilized. These nutrients can be from the organic matter

which gets washed into the cistern with each rain, as well as from dead bacterial remains.

Turbidity was quite apparent in the "rainwater" after it flowed over leaves and into the "cistern". To see what effect it had on both water quality and chlorination, an experiment was conducted which had four parts. In Part I, the water was analyzed as collected. In Part 2, as collected, but after chlorination. In Part 3 and 4 the sample was centrifuged at 7,000 rpm for 20 minutes, this resulted in a sediment and a water layer, each of which was analyzed before and after chlorination. The results show colonies too numerous to count (TNTC) even at a dilution of 10⁻¹ for all tests in Parts 1 and 2; TNTC for all tests involving the sediment alone in Part 3, but countable results for the water fraction; and finally a few bacteria in the sediment layer of Part 4, but none even in 100 mL of the water layer. This is significant because it strongly suggests that the contaminants are debris associated, and not aqueous associated suspensions.

While the occurrence of P. aeruginosa is a major concern, other opportunistic pathogens do occur in cisterns both in the biofilm layer which occurs along the wall-water interface, and in the sediment layer at the bottom of the cistern. We had a unique chance to study both of these niches when a cistern, which had been cleaned just the year before, was drained for cleaning and repair. The procedure was to use sterile cotton swabs; swab, with rolling motion,

the water-wall interface or the sediment; and put the swabbed sample into various transport media which were then streaked out on several different types of selective media once back at the laboratory. Positive suspect colonies from the selective media were then identified via the API-20E system.

Common to both niches were Escherichia coli, several different strains of Enterobacter, and Streptococcus. Where they differed was in the isolation of specific types of bacteria. P.aeruginosa, Providencia rettgeri, and Morganella morganii (both formerly of the genus Proteus), were isolated only from the water-wall interface swabs. All three are opportunistic pathogens and all have been implicated in diarrhea episodes (15).

From the bottom swabs, however, we only isolated Acinetobacter calcoaceticus, which while an opportunistic pathogen is rarely associated with disease in man (15).

Table 8 shows the effectiveness of using household bleach as a chlorinating agent. Diagram 3 presents the combined results of two experiments. In one, chlorine residual readings were taken each day, when either the free or total residual chlorine level fell below 0.8 mg/L, more bleach was added. In the second, the cistern was heavily dosed one time, chlorine residual readings were taken on a day to day basis until depleted while watching the bacterial levels rise over time.

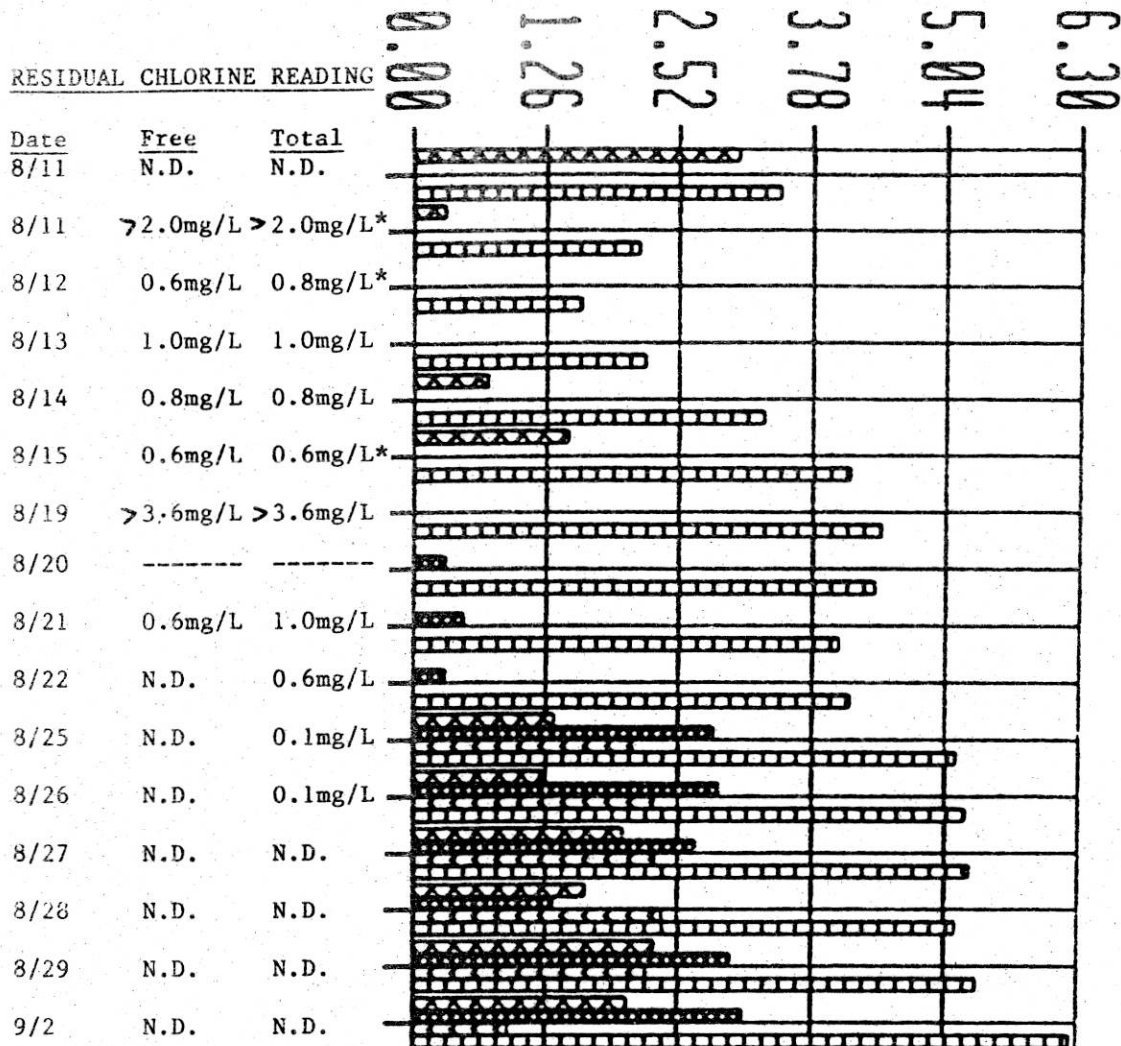
Table 8

Site Sample	Total Coliform	Fecal Coliform	Fecal Streptococcus	<u>P. aeruginosa</u>	Standard Plate Count
#1					
2/11/86					
Pre	1216	---	----	580	3.0×10^3
Post	2	---	----	12	1.4×10^2
% Reduction	99.8%			97.9%	95.3%
10/6/86					
Pre	46	6	----	1	----
Post	0	0	----	0	----
% Reduction	100%	100%		100%	
10/20/86					
Pre	92	17	124	59	2.6×10^5
Post	0	0	0	0	7.9×10^3
% Reduction	100%	100%	100%	100%	97.0%
1/12/87					
Pre	----	0	53	----	----
Post	----	0	15	----	----
% Reduction	----	0	71.7%	----	----
#18					
10/21/86					
Pre	14	0	500	9	2.8×10^4
Post	0	0	0	0	0.0×10^0
% Reduction	100%	0	100%	100%	100%

LOG OF POP.

CHLORINATION STUDY

AUG. 11 - SEPT. 2



*Cistern Dosed

N.D.=Not Detected

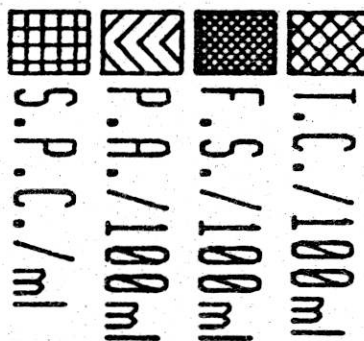


Diagram 3

One unknown concern about chlorination pertains to the development of chlorine resistant strains (35). If chlorination were done too frequently this could result in the occurrence of a chlorine resistant population (31,35).

Indeed, our results tend to suggest one of two things: 1) Such a chlorine resistant strain will develop over a short period of time, or 2) the cistern is developing a heavy spore forming population.

The results of the hydrological study are presented in Table 9. In summary it can be concluded that the average person will use between 15 to 50 gallons of water per day. All participants in this study indicated that they live in areas which they describe as being either wet or moderately wet, though as can be seen from the data in Table 9, the amount of recorded rainfall varied widely. It should be noted that the "official" annual rainfall is between 45 and 55 inches per year. There were two known sites - 8 and 12 - which have kept rainfall records for three or more years. Based on their records the mean rainfall in St. Thomas was 44.91 inches for 1986, and 57.06 inches for 1987; during the study period, the mean rainfall was 40.25 inches.

While it would be very nice to equate water quality with rainfall events, time did not permit an intensive study along these lines; however, there is some evidence that

Table 9

SITE NUMBER	ROOF AREA SQ. FT.	CISTERN CAP. GALLONS	NUMBER OF RESIDENTS	DATE START	DATE END	AVE. WATER USE/DAY	AVE. PER CAPITA USE/DAY	TOTAL RAINFALL
1	518.0	1101.8	1	11/03/86	5/11/87	016.7	16.72	32.85
2	2171.3	16830.0	3	10/03/86	5/11/87	130.3	43.42	32.85
3	2730.0	31910.0	7	10/06/86	5/28/87	181.8	25.97	19.78
4	843.0	23122.2	2	11/18/86	4/16/87	N/D	N/D	13.21
5	374.8	30993.1	2	11/18/86	6/02/87	N/D	N/D	16.08
6	2223.0	23188.0	12	10/23/86	6/02/87	290.8	24.23	24.31
7	1588.8	20781.2	3	10/17/86	6/02/87	118.5	39.50	29.22
8	2551.7	25716.0	6	10/18/86	6/02/87	N/D	N/D	39.08
9	2389.6	17305.7	2	10/18/86	6/05/87	041.4	20.72	30.74
10	1836.0	32871.7	5	10/31/86	6/05/87	172.9	34.58	19.63
11	2742.2	27203.7	4	10/19/86	6/05/87	192.5	47.28	35.91
12	6057.6	31882.7	10	10/19/86	4/16/87	223.7	22.4	40.53
13	2286.0	20259.4	6	10/19/86	2/03/87	100.6	16.77	22.96
14	1454.4	N/D	4	10/20/86	5/04/87	125.3	31.33	13.32
15	1327.2	19896.3	2	11/01/86	6/02/87	059.2	29.60	N/D
16	N/D	N/D	6	10/31/86	3/17/87	N/D	N/D	07.88
17	2078.4	N/D	5	10/31/86	12/2/86	076.3	15.26	12.73
18	961.4	21344.0	3	10/06/86	3/17/87	161.0	53.67	18.64
19	2729.5	N/D	3	11/01/86	6/05/87	101.8	53.13	10.66
20	2286.0	18900.0	3	N/D	6/05/87	N/D	N/D	N/D

suggests that there may be some such correlation. It was noted that there were higher bacterial counts right after a noted rainfall, however since it rained frequently during the study period, this could be a coincidence. Logic dictates though that if rainfall does occur, then any deposits on the roof will be washed into the cistern. We showed that leaves do significantly contribute to cistern water contamination, as does dust and dirt, thus it stands to reason that there should be a correlation between cistern water quality and noted rainfall events. We hope to be able to do an experiment along these lines in the near future.

DISCUSSION

Because cisterns are open and exposed to the environment they are readily subject to contamination from the environment. If the Safe Drinking Water Act limit of 1 total coliform per 100 mL of sample were used as a guideline for determining the health associated risks of cistern water, then only four of the twenty cisterns studied would have met this limit 50% or more of the time; none of the twenty would have met this limit if individual averages were considered.

Outside the usual fecal sources, such as animals, dust and insects, a major source of contamination is tree leaves. These leaves contain not only high numbers of total coliform, but fecal coliform, and fecal streptococci as well. Indeed, when fecal coliform/fecal streptococcus

ratios are considered, the major source/s of this contamination is from nonhuman sources.

Chlorination has been in use since 1908 because it is effective in controlling bacterial population (6,32) - especially enteric pathogens. Chlorox bleach which contains 5% by volume free chlorine is a very effective chlorination agent, giving typically 95-100% bacterial reductions across the board, yet few residents (1 out of 18 - two were controls) chlorinated their cistern at all, and they only did it quarterly.

While very effective, we found that chlorination only lasted for 3-5 days before regrowth began to occur.

The actual duration of a given chlorine residual in a cistern will depend on many factors, yet the role of turbidity cannot be overstated (21,27). Even though an individual might chlorinate his cistern adequately if significant turbidity is present adequate disinfection might not occur for two basic reasons: some of the residual will be used up by the organic compounds in the turbidity; and two, what residual that is left may not penetrate to the center of the turbidity particle where some of the microorganisms will be found (3,4); thus these organisms will survive. Indeed we found that vast numbers of bacteria

were found in the sediment layer at the bottom of the cistern and not in the water column above it. In vitro studies showed that in spite of heavy chlorination, a huge population of indicator bacteria could still be recovered six hours after the chlorination. This is significant because if this layer were disturbed, as say by a large rainfall, it could serve - in addition to any input due to the rain - to resuspend these bacteria in the water; in short the sediment layer could act as a reservoir for future contamination.

Occurrence of P. aeruginosa was found in all the cisterns of our study. While classed as a secondary or opportunistic pathogen it is of particular concern because in immunologically compromised individuals such as the very old, the very young, and burn victims, it is a frequent cause of death. Also, it is a common cause of ear infection and some waterborne diarrheal disease episodes (15,23). Thus, a cistern not properly maintained could prove detrimental to the health of those same individuals if they were to either bathe in or drink water contaminated with P. aeruginosa.

While the occurrence of P. aeruginosa is a major concern, other opportunistic pathogens do occur in cisterns as well. These opportunistic pathogens will be found in the water, in the biofilm and in the sediment layers.

The importance of cistern water to residents of the Virgin Islands and the West Indies as a whole, for cooking, drinking and bathing cannot be overstated. That cistern water can become contaminated is not new. Frequently we take our cistern water for granted, and unless it makes us sick, there is nothing to worry about. This is evident by the almost total lack of chlorination by homeowners of their cisterns. While residents and researchers alike have not been surprised to learn that cistern water is contaminated, the extent to which it is, does come as a surprise. This report delineates the extent of that contamination, and how to treat that contaminated water; but, it must also do two other things if the value of this research is to be fully realized. One thing it must do is shake residential complacency about the health risks associated with its use - that the importance of cistern water quality is exceedingly important, and doubly important where susceptible people such as the very old and the very young may be involved - and thus it must present a way in which contamination of this vital water resource is minimized. To this end we are recommending both a common sense approach, but with added urgency, as well as novel approaches to this problem. These recommendations are as follows:

- 1) All openings leading into or out of the cistern should be screened.
- 2) All trees should be trimmed back so as not to overhang the roof, and provide a free space of 10 to 20 feet.
- 3) Chlorination with household bleach at least quarterly, preferably on a monthly basis, is urged.
- 4) A cistern cleaning at least once every five (5) years - more often in areas where blowing leaves or dust are a problem.

Optionally:

- 5) The collection box or drain pipe be fitted with a first flush device (1) (see Appendix B) which would dump the first few gallons to waste before entering the cistern; and/or the use of a non-charcoal filter to filter the water before it enters the cistern (1) (See Appendix B).
- 6) Have the water analyzed on at least a quarterly basis for fecal coliform (not total coliform since trees are a major source of them) which would be a better indicator of fecal pollution, and for P. aeruginosa since it, more than anything else, is likely to produce disease.

In summary, IF PROPERLY MAINTAINED, cistern water is a SAFE source of water to drink, cook and bathe with - but the maintenance of that water is the homeowners' responsibility.

APPENDIX A

Development of m-CX. As more and more states begin to regulate P. aeruginosa contamination in water, the necessity to formulate a standard method becomes apparent. P. aeruginosa has been examined from time to time as an alternative to the total coliform standard primarily because P. aeruginosa represents a significant health hazard.

especially to hospitalized patients, and may be found in the absence of coliform bacteria. The major drawback to using it as the sole indicator of water quality is that it is ubiquitous in nature and tends to persist in water for extended periods of time. It also fails to meet many of the other criteria of an ideal indicator organism. In addition, P. aeruginosa is hard to enumerate in water, and most of the selective media require large inocula and do not yield a quantitative recovery of the organism (10,28). The coliform standard, however imperfect it may be, comes closest to fulfilling these criteria, and thus why it is used as the indicator of water quality (6,15,32).

There are two accepted techniques used in environmental microbiology to enumerate the organisms. These are the most probable number multiple tube technique (MPN), and the Membrane Filter technique (MF) (3).

The first attempt to quantity P. aeruginosa in water was a MPN method developed by Drake (10). All MPN methods tend to suffer from the same common ailments: the MPN method yields only statistical information; it does not provide a direct count; it is less precise; and it provides a greater percentage of false negative results. In addition, the MPN is more costly, cumbersome, and slower than the MF technique (5).

Recognizing this, Levin and Cabelli in 1972 developed the first MF medium, m-PA dedicated to the recovery of P. aeruginosa from water (28). This medium was designed to satisfy the following criteria (i) accuracy (ii) selectivity (iii) specificity (iv) precision and (v) comparability (28).

As researchers began to evaluate m-PA, it became quite apparent that while it gave better results than the MPN method, the recovery values obtained using m-PA were poor; as a result, the medium has since been modified several times. The first modification came in 1977 by Dutka and Kwan (m-PA-B), (11) then again in 1978 by Broadsky and Ciebin (m-PA-C) (7) and most recently in 1986 by deVicente et. al. (m-PA-D and E) (8). Each modification gave better overall recovery than the previous formula.

While recovery values were chief among the problems associated with the m-PA agars, there were others. Multiple and confusing ranges of colony types and morphology have been noted (7). The definition of a "typical" colony ranges from darkish brown to greenish black centers surrounded by an opaque to translucent white periphery (28), to flat, dry, greenish grey with dark center or black with or without a greenish rim, and dark-brown without a rim and more round with irregular edges (8). To further compound the problem, we found that there are other bacteria which grow on the m-PA agars at 41.5 °C, and which look identical to P.aeruginosa, such as Chromobacterium violaceum.

We have developed a medium to meet all the criteria set out by Levine and Cabelli, but which does not have the problems of their medium. This medium, designated m-CX, is a highly modified Kings A formula (25) (Ruskin, R.H., P.S. Callender, and H.H. Smith. Abstra. Annu. Meet. Am Soc. Microbiol. 1987. Q64, p292). The medium is prepared as follows by adding the following ingredients in g/L.

m-CX Agar

40.0 g	Bacto Peptone
20.0 g	Potassium Sulfate
10.0 g	Xylose
2.8 g	Magnesium Chloride
0.6 g	Cetrimide
13.6 g	Bacto Agar

- 1) Suspend in 1 liter of deionized water
- 2) Adjust the pH to 7.1 ± 0.1
- 3) Add 15 mL of Glycerol
- 4) Heat to boiling in a water bath
- 5) Autoclave at 15 lbs. for 15 minutes
- 6) Cool to 50-60 °C and add the following dry antibiotics:
0.0085g Kanamycin
0.0370g Nalidixic Acid
- 7) Adjust the final pH if necessary to 7.1 ± 0.1 and dispense into 50 x 9 mm petri dishes.

This medium is designed to recover only P. aeruginosa and nothing else. Cefrimide was added to inhibit bacteria other than P.aeruginosa (19, 23). Xylose was added to enhance recovery of those strains of P.aeruginosa which can metabolize it (8, 23). Nalidixic acid was added to inhibit other bacteria. Kanamycin was added as a selective ingredient to repress both P. fluorescens and P. putida (2, 23). Additionally, incubation at 41.5 ± 0.5 °C for a total of 48 hours, after a short preincubation period of 3 at 35.0 ± 0.5 °C, should allow for the growth of only those pseudomonas capable of growing at this elevated temperature (3, 7, 8, 11, 20, 23, 28).

Typical P. aeruginosa colonies on m-CX are easily distinguished from atypicals. Typical colonies are large and mucoid in appearance, creamy greenish-yellow to bluish green in color which may or may not have a tan or brown

center, are usually fluorescent under UV light, and will frequently, but not always, stain the filter and/or the medium with a fluorescent blue green "hot spot". This "hot spot" can usually be observed from the bottom of the plate, and once the colony has been picked. Atypical colonies on the other hand, are usually small, flat, and clear.

Table 10 presents the results of the verification of 1003 colonies - 639 typical colonies and 364 atypical colonies. Of the 639 typical colonies, 569 colonies or 89% were pyocyanic P. aeruginosa; 26 colonies or 4% were fluorescent, non-pyocyanic colonies which may or may not be apyocyanic strains of P. aeruginosa; and only 45 colonies or 7% were non-flourescent, non-pyocyanic false positives. Thus, of the typical colonies, between 89% to 93% are PA, depending on the identification of the apyocyanic fluorescent pseudomonads.

Of the 364 atypical colonies, 73 colonies (20%) were pyocyanic P. aeruginosa; 139 colonies (38%) were fluorescent non-pyocyanic pseudomonads; and 153 colonies (42%) were true atypicals.

There are two possible ways to explain the high percentage of false negatives obtained:

- 1) The data included both early as well as later isolates and misidentification of some typicals occurred; thus some of the atypicals should have been reported as typicals.

Table 10

VERIFICATION BREAKDOWN OF 1003 COLONIES FROM m-CX AGAR

	BIOCHEM. REACTION	TYPICAL CFU - 639			ATYPICAL CFU - 364		
		KINGS B MEDIUM	PS ISOLATION AGAR	MILK AGAR	KINGS B MEDIUM	PS ISOLATION AGAR	MILK AGAR
A	+	93%	89%	90%	43%	20%	35%
	-	7%	10%	7%	53%	75%	60%
B	PYOCYANIC	569			73		
	FLUORESCENT NON-PYOCYANIC	26			139		
	NON-FLUORESCENT NON-PYOCYANIC	45			153		
C	% VERIFIED	89 - 93%			20 - 58%		
	*	*Depends on the ID of fluorescent non-pyocyanic species			*Depends on the ID of fluorescent non-pyocyanic species		

- 2) Stressed P. aeruginosa colonies were recovered, but due to their previous stressed condition failed to exhibit typical size, shape and appearance and were thus misidentified as atypical. This stress could have resulted from environmental factors in the water from which they were recovered, or due to colony numbers above the upper counting limit which resulted in small growth size, etc. and a misidentification.

One thing is sure: of the 1003 colonies, 642 or 64% were pyocyanic P. aeruginosa; another 16% were fluorescent non-pyocyanic pseudomonas which may or may not be apyocyanic strains of P. aeruginosa. Combined, 80% of all colonies are some type of fluorescent pseudomonads capable of growth at 41.5°C in the presence of Centrimide, Kanamycin and Nalidixic Acid. If we remove the 153 colonies which were correctly identified as true atypicals of the remaining 850 colonies, 807 colonies or 95% were either fluorescent and/or pyocyanic. Of that 95%, 76% were pyocyanic P. aeruginosa - thus only 5% were false positives.

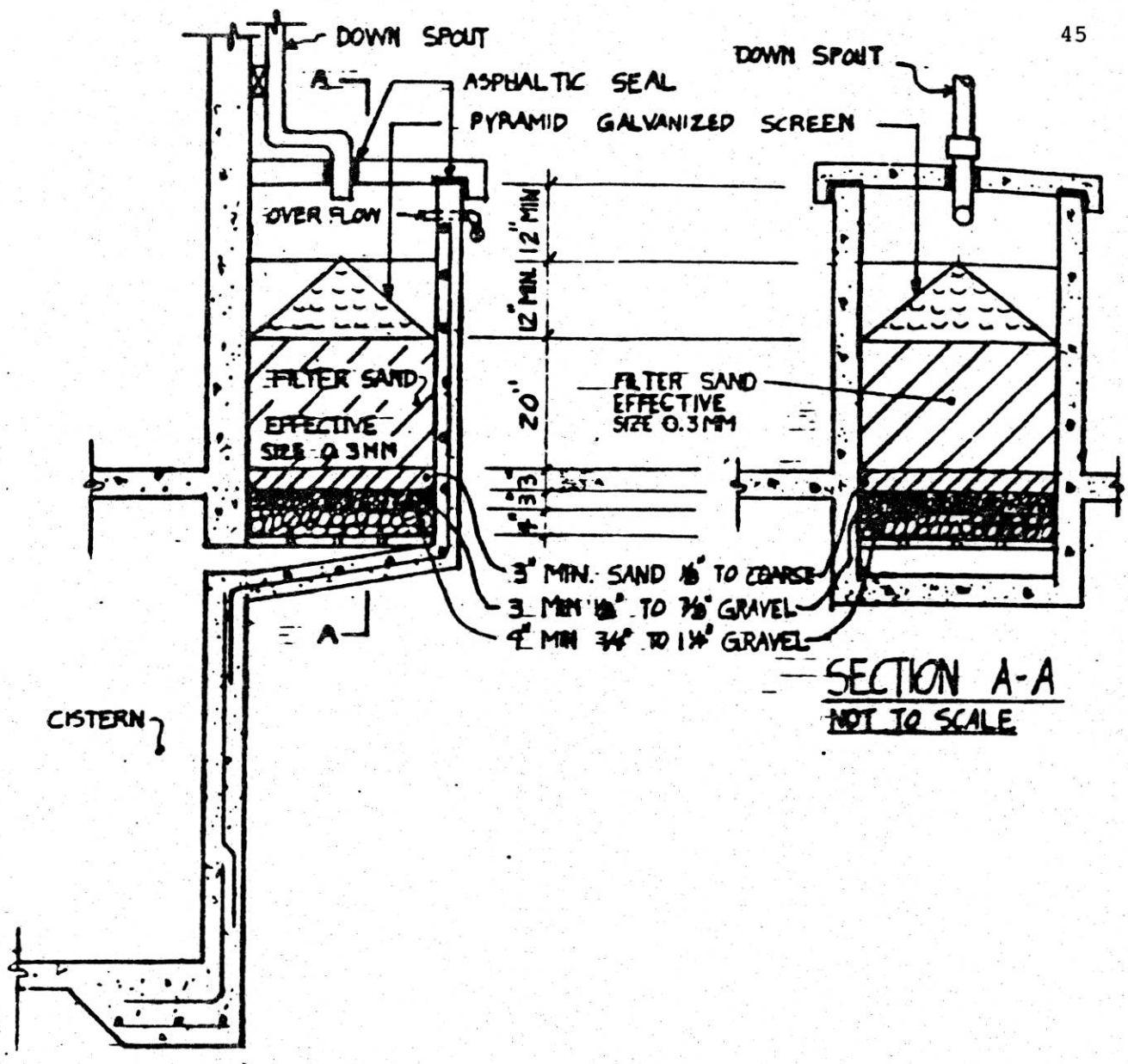
Recently, several researchers have found unidentified fluorescent pseudomonas other than P. aeruginosa which are capable of growth at 41°C. (2,22). However, of these strains, less than 1% were resistant to Kanamycin (2) which

is one of the distinguishing characteristics of P. aeruginosa, and separates it from both P. fluorescens and P. putida (2,23). Since m-CX incorporates Kanamycin, the apyocyanic fluorescent strains should be P. aeruginosa; the only other explanation would be that m-CX recovers the "less than 1%" unidentifiable fluorescent strain.

One major problem noted with the methodologies published on the in m- PA agars as a whole pertains to the question of apyocyanic strains of P. aeruginosa. In all of the flow charts (7, 8, 11, 28), confirmation is based on the formation of pyocyanin, yet in King, Ward and Raney's original paper in which 107 strains were studied, 46% failed to produce pyocyanin (25). Other tests used in the confirmation of P. aeruginosa such as grape like odor (28) are unreliable for identification of apyocyanic strains (23). Any procedure that isolates only pyocyanic strains or limits the confirmation to pyocyanic strains of P. aeruginosa is recovering only a portion of the true population present. Thus, the use of casin hydrolysis (3, 5, 7, 8, 11, 20, 23, 28) alone cannot be used to verify P. aeruginosa. Of the 1003 strains examined, 58 (6%) failed to hydrolyze casin but did produce pyocyanin, fluorescein, or both.

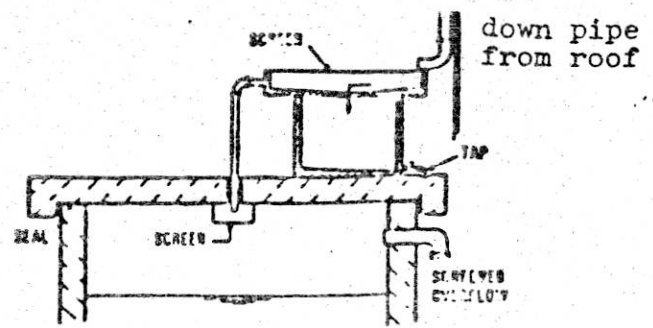
In all cases, results obtained with m-CX were either as good or better than the results obtained with any of the m-PA formulas tested when run in parallel as duplicates.

APPENDIX B

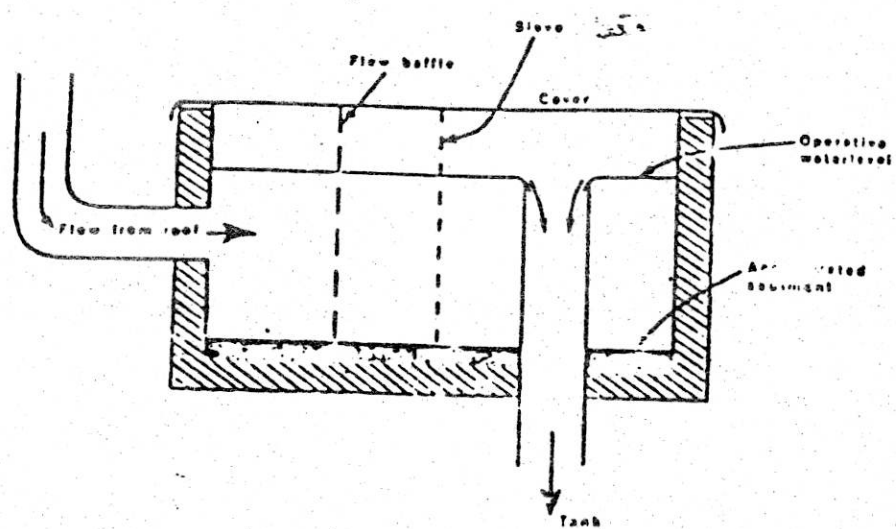


SECTION A-A
NOT TO SCALE

FILTER BOX DETAIL
NOT TO SCALE



Arrangement for diverting the "first foul flush" (Hofkes 1981).



Baffle tank to allow grit and dirt to settle (UNEP 1979).

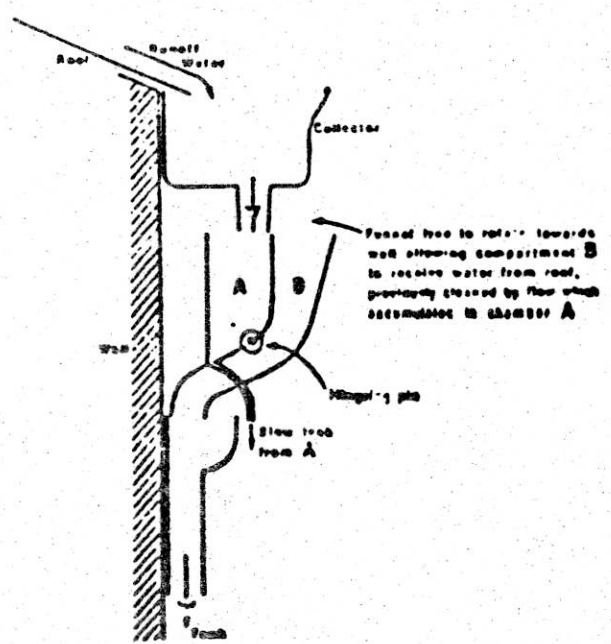


Figure 7. Swing funnel for separation of first rainwater from the later cleaner runoff (UNEP)

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