الكشف عن السالمونيلا والكشافات البيولوجية في المياه المعالجة باستخدام طريقة الترشيح خلال مهد من الشاش المعقم

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أجريت هذه الدراسة لتقييم طريقة الترشيح خلال مهد من الشاش المعقم كطريقة بسيطة واقتصادية . كانت قدرة الحجز الكلية ١٠ خلية لكل مهد.

ظهرت كفاءة هذه الطريقة بصورة واضحة وكمية عند تركيز عينات المياه المعالجة جزئيا أو كليا.

أوضحت النتائج أيضا امكانية عزل ميكروبات السالمونيلا والبكتيريا العنقودية الملونة بالاضافة الى كشافات التلوث من عينات المياه الخام وكذلك من عينات اخذت من مختلف خطوات المعالجة.

لوحظ أن البكتريا الصامدة للأحماض والبكتيريا العنقودية وكذلك الخميرة أكثر مقاومة لعمليات الكلورة والمعالجة من مجموعة بكتيريا القولون.

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A SWAB TECHNIQUE FOR DETECTION OF SALMONELLAE AND OTHER BIOINDICATORS FROM TREATED WATER

(With 4 Tables & 1 Fig.)

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SUMMARY

This study was carried out to evaluate a simple, inexpensive, continious flow swab sampler (CSS) technique. The retaining capacity of the filtration pad (400x30 cm, surgical cotton gauze) is limited at a maximum count of 10^6 organisms/swab. This technique is efficient as a quantitative, or at least rough quantitative, when it is used for clean water sampling. Samonella enteritidis, Salm. typhimurium and coloured Staphylococci organisms in addition to other bioindicators were isolated from Nile water at the intake and along the treatment plant. Acid-fast, staphylococci and yeasts commonly resist chlorination effects than the coliform group.

INTRODUCTION

MOORE, 1948 presented the cotton gauze swab technique for the isolation of Salmonellae from polluted waters. Since that time and later on, this technique is known as Moore swab or swab technique. This technique has been used in numerous occasions during epidemic investigations and environmental surveys for isolating Salmonellae (WELLS, et al. 1971) Vibrio cholera from sewers and saptic tanks (ISAACSON, et al. 1974; ISAACSON, 1975 and BARRET, et al. 1980) and enteroviruses (GRAVELLE & CHIN, 1961 and LIU, et al. 1971).

SINCE, 1948, when Moore presented his technique, as a hanging pads in the water current, many modifications were introduced to meet different needs. One of the most important needs is the adoption of that technique to the stagnant or nonflowing water sources in addition to the determination of the detected microorganisms on quantitative basis. These concepts were successfully gained in the field of virology (COIN, et al. 1964; LIU, et al. 1971 and APHA, 1985).

This investigation was performed to determine the efficiency of a swab sampler in concentrating Salmonellae and other microbial agents from water of different qualities. The effect of the filtered volume during the filtration processes on the retained organisms in addition to their accumulation within the swab strip during processing were also considered.

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MATERIAL and METHODS

I. Sampler device and preparation:

Swabs were made by cutting pieces of cotton gauze, 4 meters length by 30 cm width. Swabs were wrapped in heavy paper and sterilized by autoclaving.

Filter holders made of plastic bottles in which chemical reagent glass bottles are delivered. These bottles are made of autoclavable plastic, screw capped, 1.5 litres capacity. Holders were made by punching the bottom of each bottle, by means of a hot 4 mm. diameter punch to 12 holes. The sterile gauze swab was packed into the sterile holder under aseptic condition so as to avoid forming channels completely through the packing for water to flow through. The gauze was enough to form a firm but still compressible (about two - thirds original length). The sterile filters were then sealed until use.

II. Processing:

1- Sample Concentration.

The upper side of the filter (Screw capped) was opened and the designed volume of water sample was poured into the holder and allowing it to flow through the gauze to the punches until stop dropping.

2- Elution.

Filter contents (gauze and associated sample concentrate) were aseptically transferred into a sterile wide mouth bottle (2 liters capacity) containing 100 ml. eluent (3% sterile beef extract) and shaked well. Cotton gauze was thoroughly squeezed twice after rewetting. The extracted eluent was aseptically aspirated into other sterile graded container. Other 100 ml. eluent were added to the cotton gauze and squeezed twice again. Gathering the collected eluents to have a final volume fixed to 300 ml. The collected eluent (300 ml) was mixed well and prepared for bacteriological examination, at the suitable concentration for each parameter by using the M-F technique (APHA, 1985). Total viable bacterial counts were estimated by the poured plate technique (APHA, 1985).

5- Tested parameters.

The prepared samples were subjected to the following tests:

a. Total viable bacterial counts (at 22°C & 37°C).
b. Total coliform count.
c. Acid-fast bacilli count.
d. Staphylococcus count.
e. Yeast count.
f. Salmonellae recovery (APHA, 1985).

Methods of determination were described in details by EL-HAWAARY and KHALAFALLA (1986).

RESULTS

Experiments were carried out to determine whether microorganisms adsorption onto the filtration swab could increase as the sample volume increased. Primary sedimented sewage sample (3.8 litres) was diluted to 10^{-1} by deionized sterile tap water and thoroughly mixed. Five different volumes were designed to represent the five samples volumes as 1, 2, 5, 10 or 20 litres per swab. Results of two experiments as average values are presented in table 1 and figure

1. Data in table 1 indicate that, an increase in sample volume from 1 to 20 litres did not necessarily result a parallel increase in the detected counts. The detected numbers in all tested cases are ranged from 10^3 to 10^5 while the actual original numbers filtered through the swabs were ranged between 10^6 to 10^{10}. Moreover, figure 1 indicates the detection percentage of some parameters and their original filtered numbers. Table 1 and Fig. 1. It can be easily noticed that while the original numbers increased, by increasing sample volume, the rate of recovery is decreased. This is mainly due to the retaining capacity of the filtration pads which is limited by a maximum numbers of organisms to be retained i.e. 10^5 to 10^6 or the leaching effect of the large filtered volumes. To evaluate the effect of filtered volume on the retain of a fixed number of organisms each of 1, 5, 10, 25 or 50 litres of deionized sterile tap water was inoculated by 100 ml. of primary settled sewage and thoroughly mixed before filtration. The data in table 2 indicate that, whether sample volume is 1 litre or more, up to 50 litres, the recovered counts were in the range of the same log or nearly so (10^2 - 10^5) for all tested parameters. Table 2. This could show that the retaining capacity of the filtration pad is the limiting factor for the recovery rate. Thus when the numbers of filtered organisms were higher, the rate of recovered ones became lower.

The value of using this swab sampler for tracing microorganisms especially Salmonellae and coloured staphylococci during water treatment was demonstrated in El-Maadi water works. Efficiency of treatment in removing microorganisms from raw Nile water during each process along the treatment plant was illustrated in table 3.

The data indicate the presence of yeasts, acid-fast and staphylococci in all tested samples collected from raw Nile water at the intake and along the treatment processes and also from finished post-chlorinated water. On the other hand, total coliform organisms failed to withstand pre and post-chlorination, in most of the tested samples (13 samples out of 21). Coloured staphylococci could not be detected from two samples of post-chlorinated water (2 samples out of 7).

By using the swab sampler, it was possible to detect two Salmonellae serotypes, by filtration of 20 litres for each sample taken from the intake of the treatment plant. The isolated serotypes are Salm.enteritidis and Salm.typhimurium, each isolated from 2 different samples, (Table 4). The same two Salmonellae serotypes were also detected, by filtration of 20 litres sample by the swab sampler, in 4 cases along the treatment steps, i.e. one case after prechlorination and clarification (Salm.typhimurium), two cases after filtration (Salm.enteritidis & Salm.typhimurium) and one case (Salm.enteritidis) from finished post-chlorination water (Table 4).

The incidence of pathogenic organisms i.e. Salmonellae and pathogenic staphylococci compared to the presence or absence of total coliform organisms, in the same concentrate, are indicated in table 4. It was obvious that there was no discripancies between the existance of total coliforms on one hand and both of the two pathogens on the other, in raw Nile water (Intake). Along the treatment processes i.e. after clarification, filtration and post-chlorination, there were many discripancies between Salmonellae and coliforms and between coliforms and coloured staphylococci (Table 4).

DISCUSSION

In this investigation, a continuous flow swab sampler (CSS) was designed and evaluated for its efficiency in concentrating low multiplicities of different organisms from treated water.

The merits of this device for field use are the simplicity and low costing during current use, due to lack of need for sophisticated equipment. Moreover, it is efficient in sampling large volume from stagnant or nonflowing water sources, providing a sample taken at a specific time.

Although the rate of detection by the (CSS) technique is very low (0.001-0.72%), it is very efficient and sensitive towards any of the tested bacterial parameters. This shortage in recovery efficiency, compared with membrane filtration (SPIRA & AHMED, 1981) is due to the retaining capacity of the filtration pads. From the available results, it can be safely said that CSS technique is very efficient in concentrating bacteria from samples bearing low numbers of organisms as in clean natural sources and partially or completely treated water. To ensure quantitative recovery, total counts of each parameter, in concentrated sample after filtration, should not exceed 10^6 organisms. Within this range of counts, CSS technique seems to be a good quantitative technique.

Comparison between results obtained by the CSS technique and those obtained by ordinary methods recomended by APHA (1985), during surviellance of El-Maadi water works (EL-DIB, et al. 1986), indicated that range of counts, representing different detected parameters, in the same time of sampling, were mostly in the same log of count. Furthermore, the ordinary recommended methods failed to detect coliform or/and faecal streptococci in most of the clarified and in all post-chlorinated finished waters. Meanwhile, it was possible to detect yeasts, acid-fast and staphylococci organisms along the treatment processes by using the CSS technique. Moreover, two serotypes of Salmonellae group (Salm.enteritidis and Salm.typhimurium) in addition to coloured staphylococci were also detected along the treatment plant, and post-chlorinated finished water, in the presence or absence of coliform bacteria (Table 4).

Bacterial outbreaks characterized by diarrhea, headach and fever were attributed to drinking water. Among the etiologic agents, different species of salmonella and shigella and campylobacter were detected in the presence of low or mostly in the absence of any coliform organism (LIPPY & ERP, 1976 and CRAUN, 1977 & 1981). During the present investigation, it was noted that while coliform organisms failed to withstand pre and post-chlorination, in most cases, yeasts, staphylococci and acid-fast organisms are clearly persist chlorination and treatment facilities. Therefore, their is no discrepancies between the existence of any of the two detectd pathogens and each of yeasts, staphylococci or acid fast as a bioindicator. So, any of these groups of organisms can be safely used as a good indicator for potential health hazard of drinking water.

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Table (1)

Effect of Sample Volume on the Recovered Counts by the Swab Sampler Technique

Parameter		Original Counts/liter	Detected Counts After Filtration of: 1 Liter	2 Liters	5 Liters	10 Liters	20 Liters
Total Counts at:	22°C	1.1×10^{8}	4.2×10^{5} (0.400)*	2.3×10^{5} (0.100)	2.9×10^{5} (0.050)	9.0×10^{5} (0.090)	7.5×10^{5} (0.030)
	37°C	1.0×10^{8}	7.2×10^{5} (0.720)	3.6×10^{5} (0.180)	3.0×10^{5} (0.060)	4.8×10^{5} (0.050)	7.4×10^{5} (0.040)
Total Coliforms		2.5×10^{7}	4.2×10^{4} (0.170)	4.8×10^{8} (0.100)	6.6×10^{4} (0.060)	9.6×10^{4} (0.040)	6.0×10^{4} (0.010)
Faecal Streptococci		7.5×10^{6}	3.9×10^{3} (0.050)	7.5×10^{3} (0.050)	4.5×10^{3} (0.010)	6.0×10^{3} (0.010)	3.0×10^{3} (0.010)
Acid-Fast		2.7×10^{8}	6.0×10^{4} (0.022)	1.4×10^{5} (0.026)	1.1×10^{5} (0.008)	2.0×10^{5} (0.007)	1.6×10^{5} (0.003)
Staphylococci:	Total	7.8×10^{8}	1.2×10^{5} (0.020)	7.2×10^{4} (0.002)	1.4×10^{5} (0.004)	1.1×10^{5} (0.002)	1.3×10^{5} (0.001)
	Coloured	4.5×10^{8}	6.6×10^{4} (0.014)	2.8×10^{4} (0.002)	3.9×10^{4} (0.002)	4.2×10^{4} (0.001)	1.9×10^{3} (0.001)
Yeasts		1.8×10^{8}	2.0×10^{5} (0.110)	3.6×10^{5} (0.100)	3.3×10^{5} (0.040)	5.1×10^{5} (0.030)	2.8×10^{5} (0.01)

* Percentage of recovery.

Table (2)

Effect of Filtration Volume on the Recovery of Fixed Bacterial Load by Swab Sampler Technique

Dilution Rate	Filtered volume	T.V.C./1ml at: 22°C	37°C	Total* coliform	Faecal streptococci	Acid-fast	Staphylococci total	coloured	Yeasts
0	Original	5.2×10^{8}	5.8×10^{8}	1.0×10^{7}	1.3×10^{7}	2.8×10^{8}	8.4×10^{8}	3.6×10^{8}	6.0×10^{8}
1 : 10	1 L	4.8×10^{3}	8.9×10^{3}	1.9×10^{4}	2.3×10^{3}	6.0×10^{4}	7.2×10^{4}	2.3×10^{4}	9.0×10^{3}
1 : 50	5 L	2.1×10^{4}	1.5×10^{4}	7.9×10^{3}	3.0×10^{3}	4.2×10^{4}	2.6×10^{4}	8.4×10^{3}	9.0×10^{3}
1 : 100	10 L	2.0×10^{4}	1.7×10^{4}	2.8×10^{4}	1.5×10^{3}	5.7×10^{4}	1.4×10^{5}	7.2×10^{3}	1.2×10^{4}
1 : 200	20 L	1.6×10^{4}	1.9×10^{4}	5.7×10^{4}	7.5×10^{2}	6.0×10^{4}	1.4×10^{4}	7.2×10^{3}	1.2×10^{4}
1 : 500	50 L	2.3×10^{4}	1.8×10^{4}	8.4×10^{3}	7.5×10^{2}	4.5×10^{4}	3.6×10^{4}	1.4×10^{4}	7.5×10^{3}

* All parameters were calculated/100 ml except total viable counts (T.V.C.)/1 ml.

Table (3)

Counts of Bioindicator Parameters During Water Treatment in El-Maadi Water Works Using the Swab Sampler Technique

Sample Source		Counts of tested parameters / 100 ml elluent				Total Staphylococci	Coloured Staphylococci	Yeasts
		T.C. (22°C)	T.C. (37°C)	T. Coliform	Acid-fast			
Raw Nile Water	Max.	$2.4\times10^{9}_{7}$	$4.8\times10^{8}_{7}$	1.8×10^{4}	$4.0\times10^{2}_{1}$	3.0×10^{3}	$2.6\times10^{2}_{1}$	$8.9\times10^{2}_{1}$
	Min.	2.4×10^{8}	2.4×10^{8}	6.0×10^{2}	8.0×10^{1}	6.5×0^{2}	3.0×10^{1}	9.0×10^{1}
	Aver.	6.7×10^{8}	2.3×10^{8}	7.0×10^{3}	1.7×10^{2}	1.3×10^{3}	1.3×10^{2}	3.9×10^{2}
Primary chlorinated clarified water	Max.	1.2×10^{4}	1.0×10^{4}	1.2×10^{3}	$1.2\times10^{2}_{1}$	2.3×10^{3}	$1.0\times10^{3}_{1}$	$6.4\times10^{2}_{1}$
	Min.	2.0×10^{2}	2.5×10^{2}	0.0	4.0×10^{1}	1.0×10^{2}	3.2×10^{1}	6.0×10^{1}
	Aver.	2.5×10^{3}	2.2×10^{3}	2.6×10^{2}	8.0×10^{1}	8.8×10^{2}	5.5×10^{1}	3.3×10^{2}
	Reduc-tion.(%)	99.99	99.99	96.3	53.0	32.3	57.7	15.4
Filtered Water	Max.	8.0×10^{3}	7.6×10^{2}	3.0×10^{1}	$1.4\times10^{2}_{1}$	$7.4\times10^{3}_{1}$	1.0×10^{3}	7.2×10^{2}
	Min.	1.6×10^{2}	1.3×10^{2}	0.0	3.0×10^{1}	8.0×10^{1}	4.0	1.6×10^{2}
	Aver.	1.6×10^{3}	3.6×10^{2}	7.1	6.6×10^{1}	1.4×10^{3}	2.3×10^{2}	3.8×10^{2}
	Reduc-tion.(%)	36.0	83.0	97.3	17.5	59.1*	318.2*	15.2*
Finished postchlorinated Water	Max.	9.0×10^{2}	6.2×10^{2}	6.3	$4.0\times10^{1}_{1}$	4.0×10^{2}	4.0×10^{1}	$3.9\times10^{2}_{1}$
	Min.	6.0×10^{2}	1.2×10^{2}	0.0	$1.0\times10^{1}_{1}$	8.0	0.0	2.0×10^{1}
	Aver.	2.9×10^{2}	2.8×10^{2}	0.9	2.1×10^{1}	1.6×10^{2}	1.5×10^{1}	1.8×10^{2}
	Reduc-tion.(%)	81.9	22.2	87.3	68.2	88.6	93.5	52.6
Final treatment efficiency (%)		99.99	99.99	99.98	87.6	87.7	88.5	53.8

* These percentages represent an exeptional increase in detected counts.

Table (4)

Discrepancies Between (A) Coliforms and Salmonellae and (B) Coliforms and Coloured Staphylococci Bearing Samples by Using the Swab Sampler Technique

(A) Salmonellae:

Detected Parameter		Raw Nile Water (Intake)		Sample Source: Pre chlorinated Clarified water		Filtered Water		Finished post-chlorinated water		Total
Coliform		+	-	+	-	+	-	+	-	
	+	4 (a,b)	0	0	1 (a)	1 (a)	1 (b)	1 (a)	0	8
	-	3	0	4	2	2	3	0	6	20
Total		7	0	4	3	3	4	1	6	28

(B) Coloured Staphylococci

Coliform		+	-	+	-	+	-	+	-	
Coloured Staphylococci	+	7	0	4	3	3	4	1	4	26
	-	0	0	0	0	0	0	1	1	2
Total		7	0	4	3	3	4	2	5	28

a: Salm. enteritidis
b: Salm. typhimurium

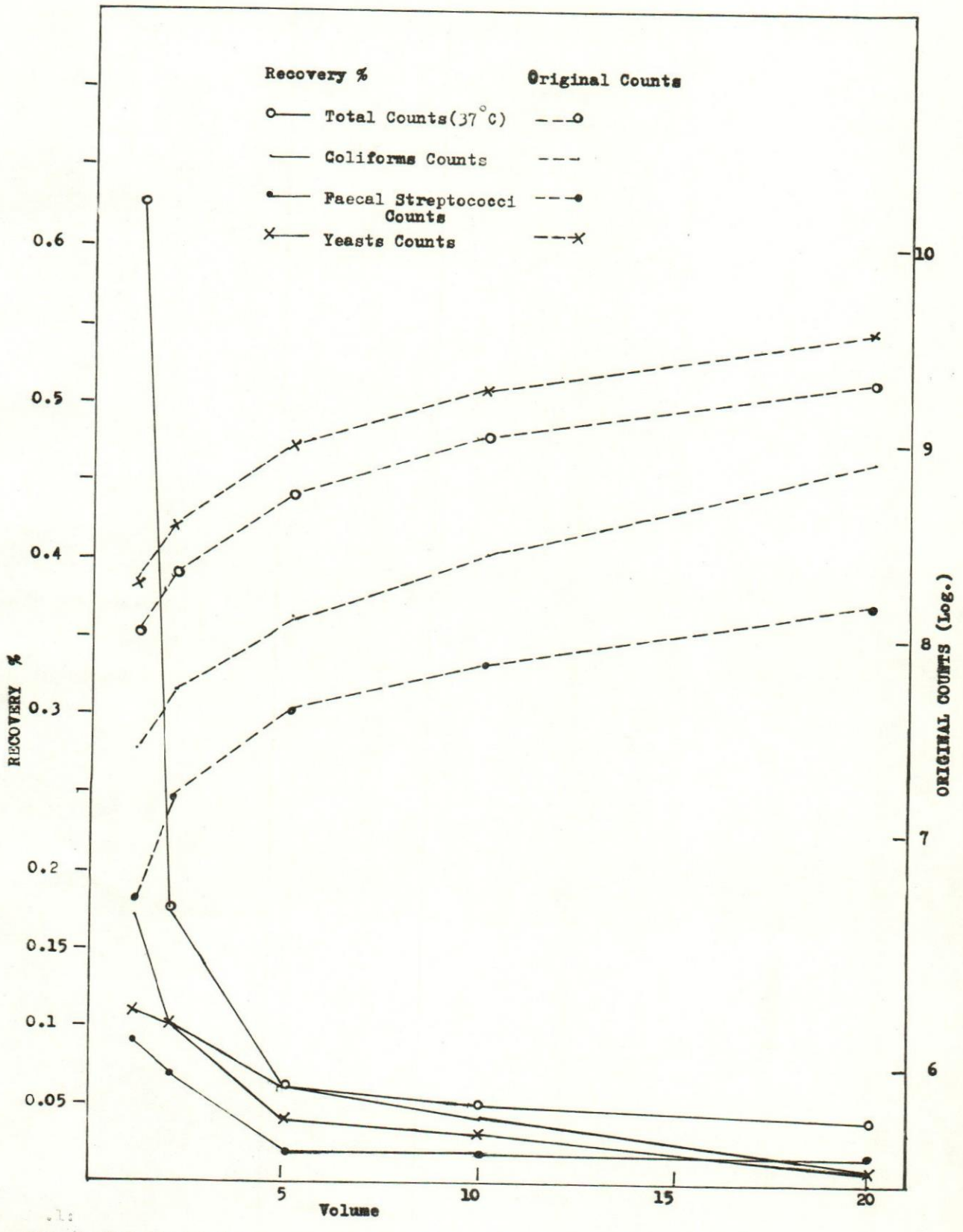

Fig. 1: Relation between recovery percentage and original filtered counts