The membrane filtration method for the examination of coliform organisms in drinking water. A comparison with the tube dilution method

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The authors are indepted to the present head of the laboratory, dr. Einar H. Aandahl, for permission to publish the results.

Background.

The basic research on membrane filters was begun nearly fifty years ago in Germany (15). The principle of membrane filtration was employed also by other countries during the pre-war period, thus by the USSR in the early 1930es. The method did, however, not come into general use until the second world war, when the technique was taken up in Germany for water examination etc. because of the destruction of many of the permanent laboratory institutions.

After the war the method gained an even more widespread use. It was adopted by England, United States and other countries and proved a valuable accessory to the conventional techniques. It became, however, also gradually established as an independent method, especially well suited for small laboratories and under field conditions.

It is not until recent years that the membrane filtration technique has been adopted in Norway. A few public health laboratories have started to use it on a limited scale, thus among others Statens Institutt for Folkehelse, Oslo (16). Norsk institutt for vannforskning, Oslo, is, however, perhaps the institution which has the most extensive experience with the method, and the work in the present study is based upon the detailed technique elaborated by the latter (9).

The situation today it that the membrane filtration technique may be said to be a fully recognized method for bacteriological examination of water. As all laboratory methods it has, however, its limitations and advantages both as judged independently and in comparison with other methods (1, 2, 3, 4, 5, 6, 7, 10, 13, 14).

Purpose.

There is undoubtedly a clear justification for taking up for practical trial the membrane filtration method under diffe-

rent conditions in our country (3, 6, 7). The present decentralization of the public health laboratory services in Norway, and the need for convenient field methods for water examination are among the most important reasons for this.

The present study has been concentrated on the use of the membrane filtration technique for the examination of coliform organisms and has consisted of a comparison with the conventional method, i.e. the tube dilution method, in a material of samples where the two methods have been used in parallel.

The purpose has been under the prevailing conditions to find out to which degree the results as obtained by the membrane filtration technique are correlated to those obtained by the tube dilution method. The main question has been whether or not results by the respective methods follow each other in terms of hygienical classification of water samples. A strict requirement of direct numerical identity of results would obviously be unrealistic in two methods of such widely different nature.

The purpose has finally been to assess practical points concerning the use of personnel, time, equipment, and media in the membrane filtration procedure as compared to the conventional technique.

Methods.

Membrane filtration technique.

The membrane filtration systems used at present are mostly based on simple pressure filtration, often by aid of a suction flask and a water suction pump. The filter itself is prepared from cellulose esters and forms a continuous surface with microscopical pores. The pore size is practically constant, and the filter operates in a manner analogous to a sieve, so that particles, in casu bacteria, are mechanically retained at the surface, contrary to ordinary fiber filters where the particles are mostly retained by absorption within the filter itself.

A filtration equipment manufactured by Membranfiltergesellschaft, Göttingen, Germany, has been used (Model: Bakterien-Nachweisgerät Coli 5). The filter holder can be opened and closed in a simple operation in connection with the insertion of a new filter. On the top there is a recipient cylinder or cone. The whole device is mounted on a suction flask so that the pump, e. g. an ordinary water suction pump, may be coupled. The equipment is made of metal so that sterilization is easy (e. g. by flaming).

The filter type used is also manufactured by Membranfiltergesellschaft and has the type designation MF50 (earlier Co5). The medium pore diameter is 0.6 μ.

As medium has been used Bacto Endo Broth MF (Difco), freshly prepared before each series of examinations (8). About 2.5 ml medium has been pipetted onto a filter paper disk (Whatman no. 16, 50 mm diameter) serving as a vehicle for the medium during incubation. The membrane filter has been placed upon the moist filter paper immediately after filtration of the specimen. Metal ointment boxes have been used instead of Petri dishes in order to secure humidity throughout the incubation period (9).

The *detailed practical procedure* has then consisted of the following: Before use boiling of the membrane filter for 20 minutes in water. Transfer of the filter to the filter holder in the previously sterilized filtration set using a special pincet. Filtering 100 ml of water (three fill-

ings of the recipient cylinder containing 100/3 ml). Transfer of the filter to the medium taking care to avoid air bubbles between the filter and the filter paper disk moistened by the medium (cfr. above). Incubation in an ordinary air incubator at 37° C for 18—20 hours.

The *reading of results* has been done by counting colonies using a lens. All red colonies have been registered irrespectively of whether they have shown metallic sheen or not (12). During the first part of the study a separate recording of the metallic sheen colonies has been performed. The judging of this characteristic has, however, proved difficult due to subjectivity on the part of the examiner and to other uncontrollable factors. The total number of red colonies has been taken to express directly the number of coliform organisms per 100 ml of water. This is the only important modification made in the procedure otherwise adopted from Norsk institutt for vannforskning. The latter institute records only colonies with metallic sheen as coliforms (9). As each water sample consist of a duplicate (two bottles), the final number is in each case the arithmetic mean of two registered values.

Tube dilution technique.

This technique has been carried out according to the procedure currently employed by Statens Institutt for Folkehelse, a procedure originally established by S. D. Henriksen. The concentration of coliform organisms is determined firstly by a presumptive test, secondly by a completed test. The medium is lactose broth to which is added the volume of water to be examined (10 ml, 1 ml, 0.1 ml) in series of five parallels. The positive criterium of coliform organisms has been the formation of gas after 24 and 48 hours' incubation at 37° C. The completed test consists of transfer to a solid medium (lactose-bromothymolblue-agar), differentiation on the basis of acid formation and colonial morphology, inoculation of representatives of the various characteristic colony types into lactose broth and once again the registration of eventual formation of gas after 48 hours at 37° C. Cultures showing gas formation are considered verified as containing coliform organisms.

By counting the number of tubes in the various series of parallels fulfilling the positive criterium (gas formation), and by using statistical tables (McGrady), a so-called most probably number index (MPN-index) is obtained. The final result for each sample is the arithmetic mean of the numbers from the duplicates (the two sampling bottles) (cfr. above).

Statistical methods.

Special statistical evaluation has been limited to those samples in which one or both laboratory methods have indicated bacterial pollution (coliform organisms). The samples in which both methods have indicated no pollution have been kept apart. The aim has been to demonstrate the degree of covariation between the series of results by the respective laboratory methods.

As statistical expression of the main trend has been used the linear correlation coefficient:

$$r = \frac{\Sigma\ (X—M_x)\ (Y—M_y)}{N \cdot S_x S_y}$$

X: numerical result by the tube dilution method (MPN),

Y: numerical result by the membrane filtration method,

M_x: arithmetic mean of results by tube dilution method,

M_y: arithmetic mean of results by membrane filtration method,

S_x: standard deviation of results by tube dilution method,

S_y: standard deviation of results by membrane filtration method,

N: numbers of pairs of results by the two methods.

In order to obtain a more detailed expression of the covariation a system of classes has been established, such as seen below:

Degree of bacterial pollution, classes:	*Numerical interval*	
	Tube dilution method	*Membrane filtration method*
I	0	0
II	1—5	1—5
III	6—30	6—20
IV	31—125	21—75
V	126—275	76—150
VI	276—500	151—275
VII	> 501	> 276

Material.

The material consists of samples of drinking water submitted to Statens mikrobiologiske laboratorium, Lillehammer, during 1966 and 1967. It includes 456 samples in all, each sample being a duplicate consisting of two bottles of water taken at the same time and place and otherwise in an identical manner as far as this has been possible under practical conditions.

The material is reviewed in Table 1. The geographical distribution of the samples is illustrated in Table 2.

Table 1.
DISTRIBUTION OF MATERIAL ACCORDING TO POLLUTION

Year	*Total No. of samples*	*Samples showing bacterial pollution (coliform organisms) by one or both methods*	*Samples showing no pollution by either method*	*Samples totally excluded from statistical examination*)*
1966:	310**)	149	137	24
1967:	146	39	101	6
Sum:	456	188	238	30

*) Reasons for exclusion: poor correspondance between duplicates (one or both methods); result above numerical limit of method (in tube dilution method 1800, in membrane filtration method too dense growth for counting); swarming growth on membrane filters.

**) Included also 22 samples taken in particularly cautious manner from the river Lågen near Lillehammer; used as early series to gain practical experience with techniques.

Table 2.

GEOGRAPHICAL DISTRIBUTION OF MATERIAL

District	*Approximate percentage*
Lillehammer area (Lillehammer, Gausdal, Ringsaker)	60
Vest-Oppland (Gjøvik, Toten, Valdres)	19
Gudbrandsdalen	17
Hedmark (— Ringsaker)	4

(Districts are often poorly defined geographically; therefore only approximate values have been given.)

Results.

The general statistical parameters derived from the material of samples showing bacterial pollution (coliform organisms) by one or both methods are the following:

		Tube dilution method	*Membrane filtration method*
Arithmetic mean	M	69.4	48.2
Standard deviation	S	200.7	108.2
The relationship	S/M	2.89	2.24
Number of samples	N	188	188

Correlation coefficient r + 0.71

The value of r expresses a considerable degree of positive covariation between the two methods if a linear relationship is assumed.

For samples with a low degree of pollution (values between 1 and 20 by the tube dilution method) and samples with a high degree of pollution (above 60 by the same method) the covariation is higher than for the material taken as a whole. Samples with a medium degree of pollution (between 20 and 60 by the tube dilution method) demonstrate on the other hand no clear pattern of covariation between the two methods.

The relation between the standard deviation S and the arithmetic mean M by the two methods, demonstrates more skew distribution of the numerical results obtained by the tube dilution method than by the membrane filtration method. This and other indications derived from the present material have led to the division into numerically non-corresponding classes of pollution as seen above.

Table 3 shows the covariation as expressed through the pollution class system.

Table 3.
CORRELATION OF RESULTS AS EXPRESSED BY POLLUTION CLASSES

Degree of pollution, class)*		*Tube dilution method* I	II	III	IV	V	VI	VII	*Sum* I—VII
	I	238	13	1					252
	II	41	30	9	1				81
Membrane	III	3	5	15	4				27
filtration	IV	4	1	9	15	5		1	35
method	V			1	3	6	2	1	13
	VI			2		3	2	1	8
	VII			2	1	2	3	2	10
Sum	I—VII	286	49	39	24	16	7	5	426

*) Cfr. above.

It is seen that as many as 48 samples showing no pollution by the tube dilution method yield low positive values by the membrane filtration method. The reverse is true only in 14 samples. Assuming that these membrane filtration results are due to real coliform organisms, this finding suggests that the latter method is a more sensitive instrument of detecting low-degree pollution than the tube dilution method.

The tendency of covariation between the two methods is clearly demonstrated if comparisons are made according to the Table 4.

Table 4.
RELATION ACCORDING TO CLASS DIVISION BETWEEN THE TWO METHODS

Tube dilution method, class)*		*Membrane filtration method, class*)*
III	33 out of 39 in	II— IV
IV	22 » » 24 »	III— V
V	14 » » 16 »	IV— VI
VI	7 » » 7 »	V—VII

Membrane filtration method, class)*		*Tube dilution method, class*)*
III	24 out of 27 in	II— IV
IV	29 » » 35 »	III— V
V	11 » » 13 »	IV— VI
VI	6 » » 8 »	V—VII

*) Cfr. above.

An attempt has been made to compare economy and time in the practical performance of the two methods. Calculations have given the following results (Table 5):

Table 5.

ECONOMY AND WORKING TIME

Per sample (duplicate)	*Membrane filtration method*	*Tube dilution method*
Working time		
a) preparation of media	¼ hr.	1—2 hrs.
b) inoculation and reading	¼ »	2 »
Total	½ hr.	3—4 hrs.
Costs of media etc.	2—3 N. kr.*)	1—2 N. kr.*)

*) 1967-prices.

Discussion and conclusions.

When discussing the results of the present examination it should be kept in mind that the membrane filtration method as such is already officially recognized by several countries for various bacterialogical examinations of water, i. e. also for the analysis for coliform organisms.

Considering in addition to this the need in many countries, also Norway, for methods which are easier to perform under conditions outside the advanced microbiological laboratory, there seems to be a clear justification for studies of this kind (6).

It should finally be remembered that the ultimate purpose of this investigation is a practical one, i. e. to examine if the two methods follow each other to such extent that the hygienical conclusion would have been the same irrespectively of whether one or the other method had been used.

Judging from the general correlation between the techniques as expressed by the linear correlation coefficient it is clear that they show a high degree of over-all parallelism of results. If the results showing no pollution by both methods was to be included in the calculation, the coefficient would approach the value + 1.

A more articulate picture is presented by the class division porcedure (Table 3 and 4). If a deviation including one class above and below is accepted in comparing the respective methods, there is a reasonable agreement at all levels of pollution. The most obvious discordance is apparently present on very low levels of pollution. It seems, however, that the membrane filtration technique fails to detect what may be assumed to be coliform organisms only in a minority of samples (14 out of 188).

This purely statistical comparison seems then to justify the conclusion that the

membrane filtration technique, even in the comparatively simple modification used in this investigation, is a valuable supplement to the methodology in this field. Assuming thus that the technique satisfies fundamental requirements to reliability, the practical advantages including the work economy and the rapidness in yielding a result, represent extra elements in its favour.

The present study demonstrates on the other hand that the membrane filtration technique has its own inherent problems. It has not been the purpose of the present study to evaluate such problems, and it is desirable that more work is performed to solve questions perhaps mainly of bacteriological nature, pertaining to the choice of media and incubation temperature, the counting and distinction between different colony types, etc. Attention is, however, drawn to the tendency of lower numerical recordings by the membrane filtration method than by the tube dilution method in high degrees of pollution. It is thought that the main reason for this is overcrowding of the filters, a phenomenon which has been pointed out by other workers (6).

It should thus be emphasized that the technique must still be considered a method only to be used by or under close supervision by experienced microbiologists. This is in accordance with the opinion expressed by others (6).

Summary.

An examination has been done concerning the correlation of results obtained by different methods for the examination of coliforms in drinking water. The techniques used are the membrane filtration method and the tube dilution method (MPN-index). The comparison has been made by aid of statistical methods with the intention of showing the degree of covariation with special reference to practical hygienical conclusions. A high degree of covariation has been found, and in view of the work economy and rapidness it is concluded that the membrane filtration method represents a valuable supplement to the tube dilution method.

Acknowledgements.

The authors are indebted to mrs. Maja Fridan Løkken and stud. med. (now dr.) Erling B. Myhre for valuable technical assistance.

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