Technical method

Application of the Stomacher for rapid homogenization of sputum and the serial streak dilution method for quantitative culture

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Techniques for sputum homogenization and quantitative cultural methods have enabled clinical microbiologists to make a more objective assessment of the bacterial flora found in lower respiratory tract infection. This paper describes the application of the Colworth Stomacher for rapid and effective homogenization of sputum and the use of a calibrated loop for quantitative culture of the homogenate.

The Stomacher (A. J. Seward, London SE1 9U6) has been designed for the blending of powders, liquids, samples for microbiological analysis, emulsification, and extraction. A suitable plastic bag containing the sample is placed in the stomaching area and sealed near the top by the closed Stomacher door. Two paddles alternately squeeze the bag and its contents against the inner aspect of the door (Sharpe and Jackson, 1972).

Method

On arrival at the laboratory the sputum samples were examined macroscopically for the presence of pus. All subsequent manoeuvres took place in the safety cabinet (fig 1). An equal volume of quarter-strength Ringer's solution was added to the sample to give a 1:2 dilution. This was poured from the sample container into a clean non-sterile polyethylene bag (Stomacher 80 bag measuring 18 × 10 cm) which had been inscribed with the laboratory specimen number using a marker pen, and processed in the machine.

A large bag (Stomacher 400 bag measuring 31 × 18 cm) was used for volumes greater than 5 ml. Homogenization of the sputum sample was usually complete within 30 seconds although unusually tenacious specimens sometimes required a minute or more. Slides were prepared for Gram and Ziehl-Neelsen staining. A calibrated 0·01 ml nichrome wire loop was dipped into the homogenate and spread evenly on to one-half of a blood agar and chocolate agar plate. After flaming, a diagonal streak was made with the flat of the loop, which was bent to a convenient angle, through the well inoculum into one of the lower quadrants of the plate, and the material so picked up was evenly spread. The loop was again flamed and the manoeuvre repeated into the remaining quadrant of the plate. After overnight aerobic incubation at 37°C the plates were inspected for pathogenic bacteria, and a quantitative estimate was made as shown in fig 2 (Lindsey, 1959). Tenfold dilutions were prepared from the remainder of the homogenate in quarter-strength Ringer's solution and total colony counts were performed by the drop count method of Miles and Misra (1938).

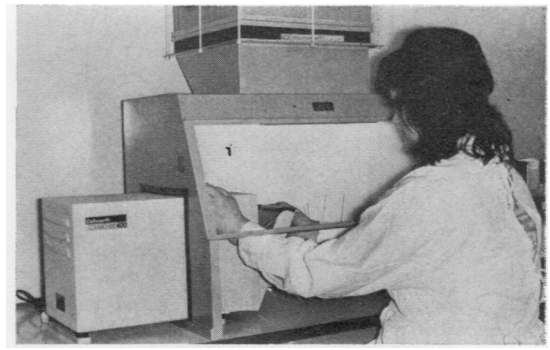

Fig 1 *The safety cabinet modified to accommodate the Stomacher.*

The remainder of the homogenate was tipped back into its original sample pot after which the open end of the bag was sealed in the flame of a Bunsen burner, placed in an aluminium pot containing disinfectant stored within the cabinet, and finally autoclaved.

Results

Four hundred and sixty-two samples of homogenized sputa were examined and colony counts obtained by the serial dilution streaking technique and the dilutions method were compared in parallel. The coefficient of correlation was 0·81.

Quantitative microbiological examination of sputa from patients with pneumonia studied by Monroe

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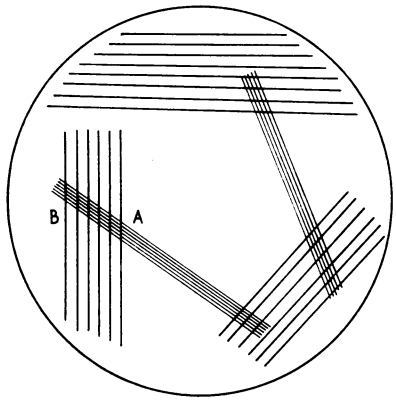

Fig 2 *Quantitative estimation of homogenized sputum (by serial-dilution) using the 0·01 ml calibrated loop. Starting with 1:2 dilution in the well inoculum it may be calculated that in streak A 5 bacterial colonies indicates 10^7 micro-organisms per ml in the original sample, 10 colonies, 2×10^7, etc. Streak B, 1 colony = 2×10^8, 10 colonies, 2×10^9, etc. In infected sputa micro-organisms present in streak A would normally be isolated in almost pure culture.*

et al (1969) suggested that pathogenic bacteria responsible for the infection were generally present in numbers of 10^7 per ml or more, and this work was confirmed by Pirtle *et al* (1969). Accepting 10^7 organisms per ml as a level of significant numbers, the serial dilution technique achieved a differentiation between significant and non-significant levels comparable to the dilution and colony plate count method (table).

No. of Samples	*Method*	
	Dilution	*Serial Dilution (Loop)*
316	Significant	Significant
17	Not significant	Significant
42	Significant	Not significant
87	Not significant	Not significant

Table *Comparison of results obtained in 462 sputa examined by dilution and loop methods*

The test null hypothesis shows that the proportion of samples accepted as significant or not significant are the same and that the loop method does not significantly differ from the other:

X^2, with Yates' correction = 3·32, df = 1; not significant at 95% level.

Comment

Homogenization of sputum by the Stomacher achieved a satisfactory homogenate in one or two minutes whereas liquefaction by protein digestion agents, such as N-acetyl-l-cysteine or Pancreatin-trypsin, may require as long as 30 minutes or more. It had the added advantage that samples arriving late in the day could be processed with minimal inconvenience for the laboratory staff. Homogenization was equally effective when two or three sample bags were placed together in the Stomacher but we do not favour this practice because of the possibility of confusion. There is a very small risk of bag failure but placing the sample bag inside another bag of larger size provides complete protection. Figure 1 illustrates how readily the machine was accommodated within the safety cabinet. An airtight rubber seal surrounding the casing eliminated significant loss in airflow efficiency.

Sputa samples which require to be cultured for tubercle bacilli could, if necessary, be left overnight, whereas samples which have undergone mucolytic digestion need to be processed immediately. Another advantage of Stomacher homogenization is that bacteriology and enzyme and antibiotic assay can be done on the same homogenate, which is not possible with material treated by mucolytic digestion.

The disadvantage of the method is the need to pour the specimen from the sample container into the bag for homogenization and back again after processing. Sound bacteriological bench technique carried out within the safety cabinet should reduce contamination and infection risks to a minimum.

Serial dilution streaking, as described by Lindsey (1959), using a standard volume loop, is extremely simple and reproducible and permits a quantitative

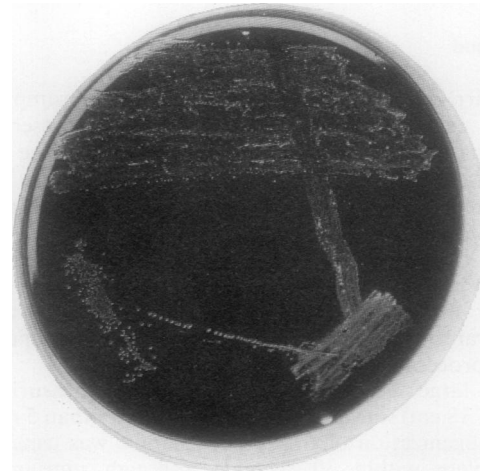

Fig 3 *Appearance of chocolate agar plate inoculated by the streak dilution technique after overnight incubation at 37°C. A pure growth of* Haemophilus influenzae *is present along streaks A and B.*

estimate of the numbers of micro-organisms present in the original sample. Pathogenic organisms isolated from patients producing mucopurulent sputa were frequently present in virtually pure culture along the 10^7 per ml dilution streak (fig 3). However, 'coliforms' cultured from sputa produced in patients who were already receiving broad-spectrum antimicrobial therapy were sometimes isolated at 10^8 per ml. Examination of the Gram film and clinical assessment of the patient were necessary before deciding upon their significance. One case of *Escherichia coli* pneumonia was seen in this series, and the colony count in both dilution and loop streak methods was greater than 10^9 per ml.

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Letters to the Editor

Isolation of Vibrio cholerae

I would like to add two suggestions to the lucid instructions by Furniss and Donovan (*J. clin. Path.*, 1974, **27**, 764-766) for the isolation and identification of the cholera vibrio.

One is that if the thiosulphate-citrate-bile-salt-agar (TCBS) medium is preferred to Monsur's, one should also use a non-inhibitory medium, because we find that the dehydrated TCBS from one British supplier is fairly inhibitory even to fresh isolates and becomes almost completely inhibitory about two weeks after the container has been opened. Storage may be better in a cooler climate, but this needs to be defined. A simple non-selective medium can be made from peptone agar or nutrient agar with 0·5% bile salt or 0·1% Teepol adjusted to pH 8·0-8·5.

The second point is the importance of colony morphology. There are very few faecal bacteria having the flat, almost transparent, bluish colonies of the cholera vibrio. This is of course better seen on a transparent medium than on TCBS. A rare strain may have a wrinkled centre on overnight incubation: a rugose variant. However, the rest of the colony will give a clue. With a positive oxidase reaction and the usually strongly positive agglutination in a slide test, there is seldom any difficulty in identifying the vibrio within one day with reasonable confidence.

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SI units, pH and cH^+

We were disappointed in the UK-SI Committee's reply (Baron *et al*, 1975) to the comments by Bold and his colleagues (1975) regretting the lack of guidance as to whether an SI unit should be used to report H^+ concentration. It is precisely because it is an area of scientific controversy that expert guidance would have been appreciated. This particular topic has after all been controversial ever since Sörensen (1909a,b) defined pH formally as—

$$pH = \frac{1}{\log [H^+]}$$

whereas in actual practice

$$pH = pH_s + \frac{(E - E_s)\,F}{2{\cdot}3026\,RT}$$

The debate as to whether the special symbol $p\alpha H$ should be used to denote H^+ activity as opposed to H^+ concentration continued until Peters and van Slyke (1931), in their classic work, pronounced in favour of the commonsense use of pH to cover both situations.

Our own preference for reporting H^+ in acid-base work in nanomole/litre has been expressed elsewhere (Howorth, 1974), but a further reason for resolving the arguments about activity/concentration of ions is the increasing use of electrodes other than the H^+-glass electrode in chemical pathology. For example, we expect to have in routine use within a few months a non-colorimetric serum electrolyte analyser based entirely on specific ion electrodes:

Na^+ electrode	:	for Na^+
K^+	,,	: for K^+
H^+	,,	: for total CO_2
NH_3	,,	: for urea
O_2	,,	: for glucose

Many other specific ion electrodes, for example, for calcium and halides, are routinely used elsewhere, as well as CO_2 and O_2 electrodes in acid-base work.

If the assumptions about activity coefficients implicit in converting electromotive activity to concentration units are unacceptable to the UK-SI Committee and others, we would all have to consider reporting Na^+ results as pNa, K^+ as pK, and so on (Svendsen, 1966). We ask, is it really a useful concept to report urea as