Selectagerm: A new approach to clinical bacteriology

P. W. GREAVES

From the Public Health Laboratory, City and Sherwood Hospitals, Hucknall Road, Nottingham

SYNOPSIS A method of processing clinical specimens received at the laboratory on cotton wool swabs is described. The method uses a range of selective culture media to assist in the isolation of potential pathogens from mixed cultures. Because the culture media are distributed in 2 millilitre volumes in the small squares of divided plastic petri dishes it is economically possible to use the same series for every specimen. This facilitates the rapid processing of large numbers of swabs and allows the isolation of pathogens from sites where they might otherwise go unnoticed.

Many laboratories use selective culture media as well as non-inhibitory media when seeking specific pathogens from sites with a normal bacterial flora or when a mixed infection is suspected. Material from the initial inoculum on a non-selective plate must be serially spread over a large area of medium so that well isolated individual colonies of all of the bacteria present may be detected. However, with a suitable selective medium, meticulous 'plating out' of the inoculum is usually unnecessary; only a small portion of the surface need be used and several different specimens may be examined on one plate.

This paper describes a method of using 10 selective culture media which among them will isolate all of the aerobic bacteria commonly encountered in clinical bacteriology. The media are distributed in 2 ml volumes in each of the 25 squares of 4 in. divided petri dishes (Dyos Plastics Ltd.) and inoculated with a standard loopful (0.01 ml) of a suspension of bacteria derived from clinical specimens received at the laboratory on cotton wool swabs. Because of the small volume of selective medium involved the same basic set can be economically employed for a wide variety of specimens. This makes possible the isolation of certain pathogens from sites where they might otherwise go unnoticed.

Materials and Methods

The following culture media were employed.

NO. 1 FOR *Staphylococcus aureus*

Oxoid Dnase agar (CM 321) with 0.025% methyl green (CI 42585), polymyxin (500 units/ml), nalidixic acid (10 µg/ml), and amphotericin B (2.5 µg/ml).

NO. 2 FOR STREPTOCOCCI AND PNEUMOCOCCI
Oxoid BAB no. 2 (CM 271) with 7% defibrinated horse blood, polymyxin (50 units/ml), nalidixic acid (10 µg/ml), and 1:500 000 crystal violet.

NO. 3 FOR PATHOGENIC *Neisseriae*

Oxoid DST agar (CM 261) with 7% lysed horse blood, polymyxin (15 units/ml), vancomycin (5 µg/ml), trimethoprim (5 µg/ml), and amphotericin B (2.5 µg/ml).

NO. 4 FOR CORYNEBACTERIA

Oxoid Hoyle's base (CM 83) with 7% lysed horse blood, 0.035% potassium tellurite, and amphotericin B (2.5 µg/ml).

NO. 5 FOR *Haemophilus influenzae*

Chocolate agar containing Oxoid BAB no. 2 (CM 271), 7% defibrinated horse blood, bacitracin (1.5 units/ml), and amphotericin B (2.5 µg/ml).

NO. 6 FOR *Candida albicans*

Oxoid malt extract agar (CM 59) with 0.014% potassium tellurite and chloramphenicol (40 µg/ml).

NO. 7 FOR *Pseudomonas aeruginosa*

Oxoid BAB no. 2 with 0.025% cetrимide and nalidixic acid (10 µg/ml).

NO. 8 FOR THE *Proteus* GROUP

Phenylalanine agar (Shaw and Clarke, 1955) with polymyxin (500 units/ml), bacitracin (5 units/ml), and amphotericin B (2.5 µg/ml).

NO. 9 FOR *Klebsiella aerogenes*

Oxoid Christensen's urea agar (CM 53) with carbenicillin (75 µg/ml) and amphotericin B (2.5 µg/ml).

NO. 10 FOR *Esch. coli* AND OTHER COLIFORM ORGANISMS

Oxid MacConkey agar (CM 7) with 0.5% sucrose, bacitracin (5 units/ml), and amphotericin B (2.5 µg/ml).

Each of these culture media was tested for the following properties.

For its ability to support the growth of different strains of the appropriate test organism from a small inoculum. Except for three strains of *C. diphtheriae* from the National Collection of Type Cultures each of the organisms used was freshly isolated from routine clinical material on a non-inhibitory medium. As far as possible only epidemiologically unrelated strains were employed. Viable counts were determined on blood agar and on a selective medium by the method of Miles and Misra (1938).

For its ability to inhibit different numbers of 'contaminating' organisms in a series of artificially prepared mixtures. Twenty-five mixtures, each containing *Staphylococcus aureus*, *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Corynebacterium diphtheriae*, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella aerogenes*, and *Escherichia coli*, but in different proportions, were inoculated on batches of media which had been stored at 4°C for varying periods up to 10 days.

For its performance in routine clinical bacteriology in comparison with the methods currently employed in this laboratory and in a second independent laboratory. Material received on cotton wool swabs was processed by first inoculating the plates of culture media normally employed and then breaking the swab into a bijoux bottle containing 1 ml nutrient broth. After standing at room temperature for five to 10 minutes to allow the swabs to become thoroughly moistened the bijoux bottles were shaken vigorously in the horizontal plane and a standard loopful of the resulting suspension was inoculated onto individual squares of each selective culture medium.

Results

MEDIUM NO. 1 FOR *Staphylococcus aureus*

This medium was designed to isolate *Staphylococcus aureus* and to inhibit almost all other organisms, including *Staphylococcus epidermidis*. It is a combination of the selective medium of Finegold and Sweeney (1961) and the modification by Smith, Hancock, and Rhoden (1969) of the Dnase medium described by Jeffries, Holtman, and Guse (1957).

Most coagulase-positive staphylococci grew well and showed a positive Dnase reaction after over-

night incubation, but about 10% grew poorly and isolated colonies of these were Dnase negative. These could be rapidly differentiated from the few strains of coagulase-negative staphylococci which grew on this medium by placing a large inoculum on a thin plate of conventional Dnase agar and testing for Dnase production after incubation at 37°C for four hours (Jeffries *et al.*, 1957).

Most strains of streptococci grew on the medium, and some were Dnase positive. No other organisms tested grew on this medium and there was no significant difference in the recovery of the test organisms from artificial mixtures after the plates had been stored at 4°C for 10 days.

MEDIUM NO. 2 FOR STREPTOCOCCI

This is crystal violet blood agar (Garrod, 1942) incorporating nalidixic acid in order to inhibit 'coliform' organisms (Beerens and Tahon-Castel, 1966) and polymyxin to inhibit *Pseudomonas aeruginosa* (Kidson, 1967).

All of the strains of streptococci examined grew equally well on the selective medium and on blood agar, giving typical haemolytic reactions. None of the other bacterial species tested were capable of growth even when present in large numbers and there was no deterioration in performance after storage at 4°C for 10 days.

MEDIUM NO. 3 FOR PATHOGENIC NEISSERIAE

This medium (Lancet, 1970; Phillips, Humphrey, Middleton, and Nicol, 1972) has been used in this laboratory for the isolation of *Neisseria gonorrhoeae* for the past two years.

Most strains of gonococci and all of the strains of meningococci used in this study grew well and none of the other bacteria tested grew on the medium even when present in large numbers. However, three strains of gonococci failed to grow and problems may arise if such strains are locally numerous. There was no difference in performance of the medium after storage at 4°C for 10 days.

MEDIUM NO. 4 FOR CORYNEBACTERIA

This is the traditional Hoyle's medium (Hoyle, 1941) which has been used for many years for the isolation of *Corynebacterium diphtheriae*. Some strains of staphylococci and streptococci can grow on this medium when present in large numbers, but the colonial forms are easily distinguished from those of the corynebacteria. Each of the stock strains of the diphtheria bacillus grew well and viable counts were equivalent to those on blood agar. The medium can be stored for more than 10 days at 4°C without deterioration.

MEDIUM NO. 5 FOR *Haemophilus influenzae*

This is described by Jarvis and Ewins (1968) and during the preliminary trials was the least satisfactory of the media since it failed to inhibit enterobacteria, hence isolation of *Haemophilus influenzae* from the artificial mixtures was difficult although there was seldom any trouble in isolating this organism from clinical material.

The inhibitory effect of the bacitracin was reduced on prolonged storage and some strains of staphylococci were then able to grow.

MEDIUM NO. 6 FOR *Candida albicans*

This has been in regular use in this laboratory for many years and is highly selective for organisms of the *Candida* group. Colonies are small after overnight incubation and there is seldom any evidence of tellurite reduction at this stage; best results are obtained after incubation for 48 hours. This medium also stores well.

MEDIUM NO. 7 FOR *Pseudomonas aeruginosa*

This was described by Lilly and Lowbury (1972). All of the strains of *Pseudomonas aeruginosa* examined grew well and gave a positive oxidase reaction after overnight incubation. The results of the viable counts were equivalent to those on blood agar and most strains showed classical pigmentation, although in some cases this was more easily seen after 48 hours. None of the other species of bacteria examined grew on this medium and there was no significant difference in performance after storage at 4°C for 10 days.

MEDIUM NO. 8 FOR THE *Proteus* GROUP

This medium was devised for the immediate group identification of *Proteus* strains. All of the strains of *Proteus* examined grew freely on the medium and gave a positive reaction for phenylalanine deaminase after overnight incubation. As well as the usual test strains, several hundred strains of other 'coliform' bacilli isolated from the urinary tract were examined. None grew on the freshly prepared medium, but the plates were less inhibitory after storage at 4°C for more than seven days. No strains of *Serratia marcescens* were available for testing; these would be expected to grow on the medium but would give a negative reaction in the test for phenylalanine deaminase.

MEDIUM NO. 9 FOR *Klebsiella aerogenes*

This was designed for the immediate recognition of *Klebsiella aerogenes* and its differentiation from mucoid strains of *Escherichia coli*. The majority of strains of *Klebsiellae* grew well on the medium and proved capable of hydrolysing urea, but a few

strains proved sensitive to the concentration of carbenicillin employed. Occasional species of *Enterobacter* and strains of carbenicillin-resistant *Pseudomonas aeruginosa* also grew but did not hydrolyse urea. There was no significant difference in performance of the medium after storage at 4°C for 10 days.

MEDIUM NO. 10 FOR *Escherichia coli* AND OTHER COLIFORM ORGANISMS

Pseudomonas aeruginosa, *Klebsiella aerogenes*, and all species of *Proteus* grow on this medium, as well as *Escherichia coli* and other coliform organisms. It is thus difficult to separate *Escherichia coli* from mixtures of enterobacterial species. The medium is included in order to detect 'coliforms' other than those for which special culture media have been devised. The majority of Gram-positive organisms are inhibited for at least 10 days after preparation.

The results of examination of the artificial mixtures showed that any organism except *Haemophilus influenzae* and *Escherichia coli* could be isolated on the appropriate selective medium if it was present in numbers greater than 500 cells per cm³, irrespective of the numbers of other organisms present. Small numbers of *Escherichia coli* and *Haemophilus influenzae* escaped recognition in the presence of greater numbers of other coliform bacilli. Table I shows the spectrum of growth of the test organisms on these media.

ISOLATIONS FROM CLINICAL SPECIMENS

At this stage, the Selectagerm system seemed sufficiently promising to warrant direct comparison with the methods used in the routine diagnostic laboratory. All swabs received over a period of five days were therefore examined by both methods.

Respiratory specimens, including ear, nose, and throat swabs were examined routinely on blood agar medium and crystal-violet-nalidixic acid blood agar (CVNA) for haemolytic streptococci. Hoyle's medium, *Candida* medium, and chocolate agar were included where relevant. There were 61 of these.

Vaginal swabs were examined routinely on cysteine-lactose-electrolyte-deficient agar (CLED) and *Candida* medium. Reduced neomycin blood agar for anaerobic culture and chocolate and crystal-violet-nalidixic acid blood agar media were included where appropriate. There were 91 of these.

Other swabs, including swabs from postoperative wounds and a wide variety of other septic conditions, were examined on blood agar or electrolyte-deficient agar medium according to the site of the infection. Cultures for anaerobes and other specific organisms such as haemolytic streptococci were included where appropriate. There were 85 of these.

	Medium Number									
	1	2	3	4	5	6	7	8	9	10
<i>Staph. aureus</i>	+++ ¹	-	-	d ²	-	-	-	-	-	-
" <i>epidermidis</i>	d	-	-	-	-	-	-	-	-	-
<i>Strep. pyogenes</i>	+++	+++	-	-	-	-	-	-	-	-
" <i>agalactiae</i>	+++	+++	-	-	-	-	-	-	-	-
" <i>faecalis</i>	+++	+++	-	d	-	-	-	-	-	-
" <i>viridans</i>	+++	+++	-	d	-	-	-	-	-	-
" <i>pneumoniae</i>	+	+++	-	-	-	-	-	-	-	-
<i>H. influenzae</i>	-	-	-	-	+++	-	-	-	-	-
<i>P. septica</i>	-	-	-	-	-	-	-	-	-	-
<i>N. gonorrhoeae</i>	-	-	+++	-	-	-	-	-	-	-
<i>N. meningitidis</i>	-	-	+++	-	-	-	-	-	-	-
<i>N. catarrhalis</i>	-	-	-	-	-	-	-	-	-	-
<i>C. diphtheriae</i>	-	-	-	+++	-	-	-	-	-	-
<i>Ps. aeruginosa</i>	-	-	-	-	+++	-	+++	-	d	+++
<i>Ac. anitratus</i>	-	-	-	-	+++	-	-	-	-	+++
<i>Pr. mirabilis</i>	-	-	-	-	+++	-	-	+++	-	+++
<i>Ct. freundii</i>	-	-	-	-	+++	-	-	-	-	+++
<i>K. aerogenes</i>	-	-	-	-	+++	-	-	-	+++	+++
<i>E. coli</i>	-	-	-	-	+++	-	-	-	-	+++
<i>Sh. sonnei</i>	-	-	-	-	+++	-	-	-	-	+++
<i>Ent. cloacae</i>	-	-	-	-	+++	-	-	-	++	+++
<i>Candida albicans</i>	-	-	-	-	-	+++	-	-	-	-

Table I List of organisms examined and spectrum of growth on the various selective media

¹+++ = growth equivalent to that on blood agar.²d = doubtful, some strains are capable of growth.

Fifty-six of the 237 swabs showed no bacterial growth with either method. In a further 76 specimens the same organisms were isolated by both and when two or more organisms were isolated from the same specimen the relative proportions of each were similar. From the remaining 105 specimens the service laboratory isolated 44 strains not detected by Selectagerm and 88 strains were isolated by Selectagerm alone. With the exception of *Staphylococcus epidermidis*, which Selectagerm is designed to inhibit, none of the strains isolated by the service laboratory alone produced more than 50 colonies, and several less than 10 colonies, on any plate, whereas many of the strains only isolated by Selectagerm were present in profuse or confluent growth on the appropriate

selective medium. The results of this trial are shown in table II.

Since this trial compared the results of the Selectagerm system with those from a laboratory where there was already a wide use of selective culture media, a further comparative trial was undertaken with material that had already been examined in another, independent laboratory. The results, which closely resembled those from the earlier exercise, are shown in table III.

Discussion

The Selectagerm system offers three main advantages over more conventional bacteriological methods.

	Number of Isolates			Proportion of Isolates	
	Both Methods	Service Laboratory Only	Selectagerm Only	Service Laboratory (%)	Selectagerm (%)
<i>Staph. epidermidis</i>	6	19	5	83	37
<i>Staph. aureus</i>	22	8	11	73	81
β -streptococci	7	5	4	75	69
Enterococci	30	4	12	74	91
Other streptococci	59	1	9	87	99
<i>H. influenzae</i>	2	0	22	8	100
<i>Esch. coli</i>	30	4	12	74	91
<i>Klebsiella</i>	2	0	3	40	100
<i>Proteus</i>	8	0	6	78	100
<i>Ps. aeruginosa</i>	7	0	2	78	100
<i>Candida</i>	11	3	2	88	81
Total	184	44	88	72	86
Total excluding <i>Staph. epidermidis</i>	178	25	83	71	91

Table II Numbers of bacterial isolates from 237 swabs examined by the Selectagerm method and by conventional laboratory techniques

	Number of Isolates			Proportion of Isolates	
	Both Methods	Independent Laboratory	Selectagerm	Independent Laboratory (%)	Selectagerm (%)
<i>Staph. epidermidis</i>	—	19	4	83	17
<i>Staph. aureus</i>	42	3	10	82	95
β -Haemolytic streptococci	6	3	1	90	70
Enterococci	16	2	7	72	92
Other streptococci	4	1	2	71	86
<i>H. influenzae</i>	2	—	1	67	100
<i>Esch. coli</i>	15	2	2	89	89
<i>Klebsiella</i>	4	—	—	100	100
<i>Proteus</i>	6	4	1	91	64
<i>Ps. aeruginosa</i>	4	1	—	100	80
<i>Candida</i>	3	—	—	100	100
Total	102	35	28	83	79
Total excluding <i>Staph. epidermidis</i>	102	16	24	83	89

Table III Numbers of bacterial isolates from 135 swabs examined by the Selectagerm technique and in an independent hospital laboratory

Specimens may be examined on a wide variety of selective culture media at a cost of little more than one additional blood agar plate and organisms may be isolated which would otherwise be missed. Many of the strains isolated can be immediately identified, at least to generic level, by virtue of their growth or specific biochemical reactions on the appropriate culture medium, and the relative proportions of the different organisms present in a lesion can be readily determined.

A surprising feature of the trial was the number of occasions when single organisms were isolated in profuse growth by Selectagerm from specimens which yielded little or no growth by conventional methods. An analysis of the results suggests that this is less likely to happen when swabs travel to the laboratory in transport medium, and that agitation in broth effects a better recovery of organisms from dry swabs than pressure on the surface of agar plates. However, since there are bacteria which will not grow on any of the selective media, and some specimens which yield less than 500 organisms per cm³ of broth suspension, it is still necessary to use a non-inhibitory medium as well as the Selectagerm set.

This system is currently in use at our own laboratory together with reduced neomycin blood agar plates for anaerobic culture.

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