

# Use of antiserum agar plates for serogrouping of meningococci

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**SUMMARY** A convenient and reliable method for serogrouping meningococci, based on immunospecific precipitation haloes in antiserum-agar plates, is described. It gave concordant results with conventional slide-agglutination in 900 strains of groups A, B or C tested. The antiserum-agar can also be used as a primary isolation medium for detecting nasopharyngeal carriers of a certain serogroup if antibiotics are added.

Serogrouping of meningococci in plates containing nutrient agar and specific hyperimmune serum was reported by Petrie (1932), who described the distinctive haloes that formed around individual colonies when the serum antibody in the agar reacted with the liberated polysaccharide antigen. In spite of occasional use (Pittman *et al.*, 1938; Ouchterlony, 1949; Slaterus, 1961) this simple identification technique has not become a routine method in clinical laboratories. Diagnostic laboratories have used slide-agglutination with commercial antisera; this is difficult to standardise, and time-consuming when large numbers of strains are tested.

The antiserum agar technique has been used recently with success for typing *Haemophilus influenzae* type b and cross-reacting organisms (Bradshaw *et al.*, 1971; Schneerson *et al.*, 1972). The addition of bacitracin to the agar resulted in a medium suitable for a direct study of nasopharyngeal swabs (Michaels and Stonebraker, 1975). This antiserum-antibiotic medium was selective for *Haemophilus* and showed precipitation haloes around *H. influenzae* type b colonies.

During the present meningitis epidemic in Finland caused by group A meningococci (Mäkelä *et al.*, 1975; Peltola *et al.*, 1976), we were faced with the task of serogrouping thousands of meningococcal strains and needed a rapid, reliable grouping method. A modification of the antiserum-agar method proved applicable, and we report our experience, comparing it with conventional slide-agglutination. On the basis of this experience we now use plates with

antiserum to either group A, B or C meningococci for serogrouping strains isolated by conventional culture methods. If a precipitation halo is not seen, the strain is then tested by slide agglutination with antisera to groups X, Y, and Z (and others, if needed). When looking for nasopharyngeal carriage of meningococci we culture swabs directly on antiserum-agar plates with added antibacterial agents (antiserum-antibiotic plates) Van Peenen *et al.*, 1965; Thayer and Martin, 1966).

## Material and methods

### ANTISERUM-AGAR PLATES

The TSB base consisted of 30.0 g of trypticase soy broth (BBL) and 15.0 g of Bacto agar (Difco) in 1000 ml of distilled water. The base was autoclaved at 121°C for 15 minutes. After cooling to 50°C the antiserum (heated to 45°C) was added in the recommended dilution (Table 1) and mixed well. The plates were poured rather thin, about 15 ml of medium per plate of 9 cm diameter.

Table 1 *Meningococcal grouping sera for antiserum-agar plates*

Animal	Immunising strain	Approximate anti-polysaccharide antibody (mg/ml)	Recommended dilution
Horse 49	Group A, strain A-1*	3.2	1:15
Horse 46	Group B, strain B-11*	1.2	1:12
Burro 211	Group C, strain C-11*	4.5	1:30

\*Gotschlich *et al.* (1969)

## ANTISERUM-ANTIBIOTIC PLATES

These were made similarly but vancomycin (only 1 U/ml), trimethoprim (3.8 µg/ml), colistin (7.5 µg/ml), and nystatin (12.5 U/ml) were added to the antiserum-agar at 50°C.

All plates were stored at 4°C in tight plastic bags to avoid drying. Thus stored, precipitating activity remained for at least three months. The antimicrobial activity decreased gradually and therefore the antiserum-antibiotic plates were not used for more than two weeks.

## ANTISERA FOR THE PLATES

Table 1 lists the animals and bacterial strains used for immunisation and the approximate antibody content of the antisera. The animals were immunised according to Alexander *et al.* (1946) with the following modifications: Approximately 12-15 colonies taken from a 24-hour growth on TBS agar were transferred to 500 ml of Brain Heart Infusion broth and vigorously shaken at 37°C for 5.5 hours. The growth was centrifuged at 10 000 × *g*, at 4°C for 20 minutes, the supernatant was decanted, and the pellet suspended in 20 ml of 0.5% formalin in phosphate-buffered saline, pH 7.4. This bacterial suspension was stored at 4°C and discarded after one week. Serum was collected at days 4, 5, 6, and 8 after the last injection. In most cases, good antisera were produced after the second injection series and the animals were injected three to four times annually. The serum was collected under sterile conditions, centrifuged at 10 000 × *g*, at 10°C for 20 minutes, and stored at -20°C.

Standard media and methods were used for the isolation and biochemical identification of *Neisseriae* (Lennette *et al.*, 1974). Slide-agglutination of meningococci was performed with commercial antisera (Wellcome for group B, Difco for groups A, C, X, Y, and Z).

## THE MATERIAL

Nasopharyngeal samples of healthy persons were collected for screening for carriage of meningococci. Immediately after sampling the charcoal-coated swabs were placed in Stuart's transport medium (Gästrin *et al.*, 1968) and cultured within 6 hours. In addition, we used bacterial strains isolated from clinical specimens in several diagnostic laboratories and sent to the Department of Bacteriology and Immunology, University of Helsinki.

## Results

## SEROGROUPING OF MENINGOCOCCI

Meningococci isolated and identified by routine methods were streaked radially on each of the three

antiserum-agar plates (without antibiotics). Eight to 10 strains were tested on the same plate. The plates were incubated at 36°C in candle jars.

Precipitation haloes were scarcely visible after 18 hours. After 42 hours the haloes were usually quite distinct, reaching about 2-3 mm from the edge of the growth, and remained so for a further 18 hours before beginning to spread and thin out. Therefore the reading of the precipitation reactions was always done at 42 and 60 hours but not later. Keeping the plates at 4°C for 18 hours after the primary incubation did not increase the precipitation. The medium had to be clear and in a fairly thin layer for best visibility of the precipitin reactions. At first we tested agarose, as recommended by others (Bradshaw *et al.*, 1971; Schneerson *et al.*, 1972; Michaels and Stonebraker, 1975), but since simultaneous culturing of about 50 strains of different serogroups on plates prepared with either Bacto agar or agarose showed no differences in the precipitation reactions, we later used only the much cheaper Bacto agar.

The precipitin reactions were always specific, as demonstrated by comparison with simultaneous slide agglutination results for several hundred strains (Table 2).

## SCREENING FOR SPECIFIC SEROGROUPS OF MENINGOCOCCI

When nasopharyngeal swabs were cultured on both conventional Thayer-Martin agar and the three antiserum-antibiotic plates the results were as shown in Table 3. The selectivity of all plates was very satisfactory; besides *Neisseriae* only occasional *Pseudomonas*, *Proteus*, and *Candida* strains were seen. The antimicrobial agents in the medium did not affect the precipitation reaction (Figure). The advantage of the antiserum-antibiotic plate method was the detection of more than one serogroup in a sample: more than one strain was found at one time in approximately 12% of the carriers.

In large screening programmes, we cultured four samples on the same plate, but if growth happened to be heavy it was sometimes difficult to notice the precipitation of a scarce minority population in a sample. Results were improved by spreading the inoculum with a loop, and we would recommend inoculating only one or two samples on one plate.

## Discussion

The antiserum plates were convenient, specific, and suitable for grouping many meningococcal strains at the same time. The simultaneous use of different antiserum plates containing antibiotics permitted large-scale study of meningococcal carrier rates and

**Table 2** Numbers of *Neisseria* strains giving precipitation haloes in different antiserum-agar plates (containing antiserum to meningococcal groups A, B or C). Reactions recorded after 48 h incubation

	No. of strains tested	No. of strains producing haloes	No. of strains producing haloes		
			Anti-group A plates	Anti-group B plates	Anti-group C plates
<b>Meningococci* of:</b>					
Group A	400	400	0	0	0
Group B	400	0	400	0	0
Group C	100	0	0	0	100
Group X	10	0	0	0	0
Group Y	90	0	0	0	0
Group Z	60	0	0	0	0
Nongroupable	300	0	0	0	0
<i>N. gonorrhoeae</i> †	30	0	0	0	0
<i>N. lactamica</i> ‡	10	0	0	0	0

\*The meningococcal strains were serogrouped by slide-agglutination. Groups A, B, C, and Y include nasopharyngeal strains from symptomless carriers and strains from cerebrospinal fluid or blood of patients with meningococcal disease. Groups X, Z, and the nongroupable strains were all from carriers.

†From urethral or cervical samples of patients with gonorrhoea.

‡From the nasopharynx of healthy children.

**Table 3** Nasopharyngeal carriage of meningococci detected by conventional Thayer-Martin agar or by antiserum-antibiotic plates containing antiserum to meningococci groups A, B, or C

Samples*	No.	Carriers detected by		No. of strains of serogroup								Total‡ No. of strains
		Thayer-Martin agar		Antiserum-antibiotic agar	A	B	C	X	Y	Z	Non-groupable	
		No.	(%)									
1 week	150	46	(31)	48†	7	17	1	0	2	3	23	53
5 weeks	140	66	(47)	66	20	13	4	0	1	3	29	70
9 weeks	128	67	(52)	67	19	15	2	0	4	2	38	80

\*Swabs taken from recruits in one garrison 1, 5, and 9 weeks after entering service.

†In two cases meningococci were not found on the Thayer-Martin agar.

‡Includes strains from carriers with more than one serogroup.

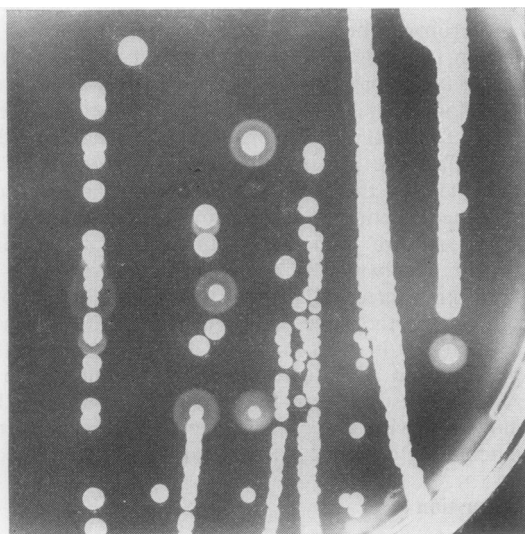


Figure Mixture of groups A and B meningococci streaked on an antiserum-antibiotic plate containing meningococcal group B antiserum. Colonies of group B meningococci are surrounded by precipitation haloes. Plate was grown for 42 h at 36°C in a candle jar.

made it possible to find more than one groupable strain in individual nasopharyngeal samples. The selective antiserum-antibiotic plates are especially convenient in an epidemic or in local outbreaks where carriers of a certain serogroup are looked for. A drawback of the method is the large amount of specific antiserum required.

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