Bulletin of the World Health Organization, 55 (6): 659-661 (1977)

A comparison of the serum agar grouping and slide agglutination methods for the identification of *Neisseria meningitidis* strains

P. KUZEMENSKÁ,[1] V. BURIAN,[1] C. E. FRASCH,[2] & E. ŠVANDOVÁ [1]

The serum agar grouping (SAG) and slide agglutination (SA) tests were compared for their ability to identify strains of N. meningitidis. *There was no significant difference between the two methods in respect of their relative sensitivity and detection ability. The most suitable concentration of antimeningococcal serum for SAG was found to be 1:10; however, the most suitable concentration will need to be established for each serum used. The ability of SAG to identify large numbers of* N. meningitidis *strains was confirmed.*

The serological identification of *Neisseria meningitidis* strains is normally carried out by slide agglutination. Recently, however, the serum agar method of Petrie (*1*) has been reintroduced. The merit of this method is its simplicity and its capacity to identify easily many strains of *N. meningitidis*; this would be useful during an outbreak of meningococcal cerebrospinal meningitis.

In this communication, we compare slide agglutination (SA) with serum agar grouping (SAG) and determine the optimum conditions for SAG.

MATERIALS AND METHODS

Strains

The strains of *N. meningitidis* used were:

1. Reference neotype strains: A (M1027), B (M2092), C (M1628), D (M158), X (18/68), Y (19/68), Z (20/68), 29E, W135.

2. Group B neotype strains: S3032, M986, M978, M136, M982, M992, B16B6, M1011, M981, M990, M1080.

3. Field strains isolated from carriers or patients with respiratory infections in Czechoslovakia.

Sera

For SA, rabbit sera prepared by Professor N. A. Vedros, University of California, Berkeley, USA and obtained from WHO were used. Antimeningococcal sera A, B, C, D, X, Y, Z, 29E, and W135 were used both undiluted and diluted 1:10 and 1:20.

For SAG, horse sera provided by WHO were used. Antimeningococcal sera A, B, and C were tested at concentrations of 1:5, 1:10, 1:20, and 1:40; sera Y, 29E, and W135 were tested at a concentration of 1:10.

Slide agglutination

The strains, after culture for 18–20 h on Mueller-Hinton medium in a CO_2 atmosphere at 37°C, were suspended in a drop of distilled water on a slide. Drops of antimeningococcal serum were placed on another slide. One drop of the suspension was added to each drop of serum by loop. After mixing, the slide was agitated by hand and the results were read within 3 min. Agglutination in saline solution was also evaluated. Scores of +++, ++, +, or — were given according to the degree of agglutination.

The strains were classified into the following groups:

(*a*) *Typable*

Monoagglutinable—agglutinating with one antimeningococcal serum.

[1] Centre of Epidemiology and Microbiology, Institute of Hygiene and Epidemiology, Šrobárova 48, 100 42 Prague 10; Czechoslovakia.

[2] US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD 20014, USA.

(*b*) *Nontypable*

Polyagglutinable—agglutinating with two or more antimeningococcal sera.

Autoagglutinable—agglutinating with saline solution.

Nonagglutinable—not agglutinating with antimeningococcal sera or saline solution.

Serum agar grouping

After the strains had been cultured on Mueller–Hinton medium as described above, they were inoculated on to TSB medium containing horse antimeningococcal serum at concentrations of 1 : 5, 1 : 10, 1 : 20, and 1 : 40. The diameter of the inoculum was 6–8 mm and it was possible to accommodate 8–12 inocula on one plate. Inoculated plates were incubated at 37°C in a CO_2 atmosphere, and after 18–20 h the results were read. The results were scored +++, ++, +, or — according to the strength and diameter of the precipitin halo around the growth.

RESULTS

Comparison of SA and SAG

The results are shown in Table 1. The two methods were in agreement for 73 (94.8%) of the 77 strains tested: 44 were positive and 29 negative. Of the four strains that gave different results in the two tests, one was positive with SAG and negative with SA and three were negative with SAG and positive with SA. The 95% confidence interval was 87.2–98.6%; the estimated proportion of disagreement was only 5.2%. There was no statistical difference between the two tests in respect of their relative sensitivity and detection capabilities.

Table 1. Comparison of the results of the serum agar grouping (SAG) and slide agglutination (SA) tests

SA	SAG		
	Positive	Negative	Total
Positive	44	3	47
Negative	1	29	30
Total	45	32	77

Concentration of horse sera in TSB medium for SAG

Sera A, B, and C were tested at concentrations of 1 : 5, 1 : 10, 1 : 20, and 1 : 40. According to the SAG results, the most suitable concentration in TSB medium appeared to be 1 : 10, since at this concentration the majority of SAG results were +++ or ++.

Sera Y, 29E, and W135 were tested only at a concentration of 1 : 10. The results were + or ++ and we can suppose that a concentration of 1 : 5 would be more suitable. However, it was impossible to verify this owing to the small quantity of serum available.

ACKNOWLEDGEMENTS

The technical cooperation of Mrs M. Myšková, Medical Faculty of Hygiene, Charles University, Prague, is gratefully acknowledged.

RÉSUMÉ

COMPARAISON DES MÉTHODES DE GROUPAGE SUR AGAR SÉRIQUE ET D'AGGLUTINATION SUR LAME POUR L'IDENTIFICATION DES SOUCHES DE *NEISSERIA MENINGITIDIS*

Bien que l'agglutination sur lame (SA) soit la méthode habituellement utilisée pour l'identification des souches de *N. meningitidis*, la méthode de groupage sur agar sérique (SAG) a été récemment réintroduite à cet effet. La présente communication relate une expérience visant à comparer les deux méthodes. Dans l'épreuve SAG, les souches ont été inoculées sur un milieu TSB contenant du sérum antiméningococcique de cheval à différentes concentrations. Les boîtes de Pétri inoculées ont été incubées pendant 18–20 heures à 37° dans une atmosphère CO_2, et les résultats ont été notés +++, ++, + ou − selon l'intensité et le diamètre du halo de précipitines autour de la colonie.

On n'a pas constaté de différence statistiquement significative entre les deux types d'épreuve quant à leur pouvoir de détection ou leur sensibilité relative. Sur un total de 77 souches testées, les deux méthodes ont donné des résultats similaires pour 73 souches, avec 44 résultats

positifs et 29 négatifs. La concentration optimale de sérum antiméningococcique s'est révélée être 1 : 10 pour l'épreuve SAG; elle devra néanmoins être déterminée pour chaque type de sérum utilisé.

La méthode SAG a l'avantage de permettre d'identifier aisément un grand nombre de souches de *N. meningitidis*, ce qui peut être très utile en cas d'épidémie de méningite cérébro-spinale méningococcique.

REFERENCES

1. PETRIE, G. F. A specific precipitin reaction associated with the growth on agar plates of *Meningococcus, Pneumococcus* and *B. dysenteriae* (Shiga). *British journal of experimental pathology*, **13**: 380-394 (1932).