

## BRIEF COMMUNICATION

### BACTERIAL ANTIGEN DETECTION IN CEREBROSPINAL FLUID BY THE LATEX AGGLUTINATION TEST

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#### SUMMARY

Eighty purulent cerebrospinal fluid (CSF) samples from patients with clinical evidence of meningitis were studied using the Directigen latex agglutination (LA) kit to determine the presence of bacterial antigen in CSF. The results showed a better diagnostic performance of the LA test than bacterioscopy by Gram stain, culture and counterimmunoelectrophoresis (CIE), as far as *Neisseria meningitidis* groups B and C, and *Haemophilus influenzae* type b are concerned, and a better performance than bacterioscopy and culture considering *Streptococcus pneumoniae*. Comparison of the results with those of culture showed that the LA test had the highest sensitivity for the *Neisseria meningitidis* group C. Comparing the results with those of CIE, the highest levels of sensitivity were detected for *N. meningitidis* groups B and C. Regarding specificity, fair values were obtained for all organisms tested. The degree of K agreement when the LA test was compared with CIE exhibited better K indices of agreement for *N. meningitidis* groups B and C.

**KEYWORDS:** Bacterial meningitis; Latex agglutination; Bacterial antigen detection in CSF.

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#### INTRODUCTION

Bacterial meningitis is mainly caused by bacteria which liberate capsular polysaccharide into the body fluids during infection.

Concerning the most frequent meningitis agents such as *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, rapid methods to detect soluble antigens in cerebrospinal fluid (CSF) have been reported<sup>5, 10, 13</sup>.

Although antigen detection methods do not replace traditional bacteriologic techniques, they are usually more sensitive and specific than the inter-

pretation of a Gram-stained smear. Gram-positive bacteria can appear Gram-negative because of the action of antibacterial agents on the cell wall, leading to misinterpretation of the Gram stain. Very frequently, culture results are adversely affected by prior antimicrobial therapy<sup>11</sup>. Furthermore, results of antigen detection tests are available at least 18h before culture results. The transport conditions of the biological material to be processed may also affect the results of a culture which depends on viable organisms<sup>3</sup>.

Among the less time-consuming methods, the latex agglutination (LA) test has been the most widely used.

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In the present study, a commercial LA kit was evaluated and the results were compared with those of bacterioscopy by Gram stain, culture, and CIE.

### MATERIAL AND METHODS

A total of 80 purulent CSF samples were obtained from patients with clinical evidence of meningitis and analyzed by bacterioscopy using Gram stain, culture, and CIE, as previously described <sup>1</sup>.

LA system tests were performed using the Directigen latex agglutination antigen detection kit, kindly provided by Becton Dickinson Microbiology Systems (Cockeysville, Maryland 21030 USA) following manufacturer recommendations.

The sensitivity and specificity of the LA system were evaluated according to a methodology already described <sup>6, 7, 8</sup>, and compared to culture, the standard diagnostic method, and to CIE, another immunologic diagnostic method.

The presence of antibacterial agents in the CSF samples was detected by a previously described method <sup>9</sup>.

### RESULTS

The results of the Directigen LA antigen detection kit (Table 1) showed a better performance than bacterioscopy by Gram stain, culture and CIE, as far as *N. meningitidis* groups B and C, and *H. influenzae* type b are concerned. The LA test was not applied to some of the CSF samples because of lack of enough CSF after the use of other methods.

Table 1 shows a high percentage of antibacterial agents present in the CSF samples, with a median percentage of 73.4%.

Table 2 shows that the sensitivity of the LA test was highest compared with culture for *N. meningitidis* group C. When the LA test was compared with CIE, the highest values were observed for *N. meningitidis* groups B and C. Regarding *S. pneumoniae*, sensitivity and specificity were not evaluated because no CSF sample was positive for this microorganism, and CIE was not performed for this bacterial agent. Specificity for all other organisms tested showed fair results.

The degree of K agreement when the LA test was compared with CIE was better for *N. meningitidis* groups B and C.

### DISCUSSION

Bacterial meningitis can be caused mainly by *N. meningitidis*, *H. influenzae*, and *S. pneumoniae*. These types of meningitis are frequently fatal when not adequately treated. Moreover, previous antibiotic therapy may impair laboratory diagnosis of meningitis by bacteriologic methods, with a possible decrease in the rate of positive CSF bacterioscopy by Gram stain and culture. On the other hand, bacterial search by immunologic methods may still lead to a diagnosis of these meningitis cases. Nevertheless, in the early phase of meningitis, a sufficient amount of bacterial antigen may not yet be present in CSF to be detected by immunologic methods.

In this study, the results of the Directigen LA test

TABLE 1  
Performance of Gram stain, CIE, culture and the Directigen latex agglutination kit for antigen detection in CSF samples in the presence of antibacterial agents

Organisms	No. of positive CSF samples/No. tested (%)				Antibacterial agents detected in CSF (%)
	Gram stain	Culture	CIE	Latex	
<i>N. meningitidis</i>					
group B	5/51 (5.8)	5/51 (9.8)	4/51 (7.8)	10/46 (21.7)	(72.3)
group C	2/51 (3.9)	1/51 (2.0)	8/51 (15.7)	11/35 (31.4)	(78.0)
<i>H. influenzae</i> type b	3/45 (6.7)	7/45 (15.6)	7/45 (15.6)	8/43 (18.6)	(67.0)
<i>S. pneumoniae</i>	2/38 (5.3)	0/38 (0.0)	-	4/36 (11.1)	(76.3)
Median (%)	(5.4)	(6.9)	(13.0)	(20.7)	(73.4)

TABLE 2

Sensitivity and specificity of the Directigen latex agglutination kit compared with culture and CIE of CSF samples

Methods	Organisms	Sensitivity	Latex Specificity	Kappa indices (K)	K concept	Zo <sup>1</sup>
Culture	<i>N. meningitidis</i> group B	0.400 (n=0.118-0.770)	0.855 (n=0.738-0.924)	0.175	Poor	0.388
	<i>N. meningitidis</i> group C	1.000 (n=0.202-1.000)	0.821 (n=0.702-0.900)	0.139	Poor	0.281
	<i>H. influenzae</i> type b	0.571 (n=0.159-0.750)	0.891 (n=0.753-0.957)	0.320	Weak	0.702
CIE	<i>N. meningitidis</i> group B	1.000 (n=0.510-1.000)	0.857 (n=0.722-0.033)	0.511	Moderate	1.136
	<i>N. meningitidis</i> group C	1.000 (n=0.566-1.000)	0.813 (n=0.647-0.911)	0.539	Moderate	1.376
	<i>H. influenzae</i> type b	0.571 (n=0.159-0.750)	0.889 (n=0.747-0.956)	0.317	Weak	0.695

<sup>1</sup>Zo = observed Z (Z critics = 1.96 for the 0.05 level). See references 6 and 8

proved to be better in diagnostic terms than bacterioscopy, culture, or CIE for all organisms tested.

MORENO-CARVALHO et al. <sup>10</sup>, studying LA reagents for *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* groups A and C but not group B, similarly found a better performance of LA over culture, and also over bacterioscopy.

The LA test was also reported to be comparable to culture for *H. influenzae* type b, and *N. meningitidis* group B by TILTON et al. <sup>14</sup>, but better than culture for *S. pneumoniae*.

DIRKS-GO & ZANEN <sup>4</sup> and WHITTLE et al <sup>16</sup> observed that LA and CIE were very similar in sensitivity and specificity. However, many other authors <sup>2, 5, 12, 14, 15</sup> have reported a slightly more sensitive performance of LA over CIE.

Our better results were probably due to an improvement of the LA kits over the years since 1972.

In our experience, we have observed a large number of CSF samples in which antibacterial agents were detected (73.4%), a somewhat higher level than reported by others (30 to 55%) <sup>9</sup>. This might explain the low positivity observed with the diagnostic methods used.

This same observation was made by MORENO-CARVALHO et al. <sup>10</sup>, concerning a high percentage of negative cultures and bacterioscopy, although these investigators did not test the presence of antibiotics in CSF.

As far as immunologic methods are concerned, the LA test had a higher sensitivity than CIE for *N. meningitidis* groups B and C, but not for *H. influenzae* type b. However, the advantages of the LA test over CIE are speed, simplicity and performance without special equipment, and, similarly to CIE, it can be applied to other body fluids, such as serum, pleural fluid and urine.

## RESUMO

### Detecção de antígenos bacterianos no líquido cefalorraquidiano através do teste de aglutinação de latex.

Oitenta amostras purulentas de líquido cefalorraquidiano (LCR) de pacientes com evidência clínica de meningite foram estudadas empregando-se Kit Directigen de aglutinação de latex (AL) para demonstrar antígeno bacteriano no LCR. Os resultados mostraram que o teste de AL apresentou melhor desempenho diagnóstico do que bacterioscopia através da coloração de Gram, cultura e imunoelctroforese cruzada (IEC) em relação à *Neisseria meningitidis* grupos B e C, e ao *Haemophilus influenzae* tipo b, e melhor do que coloração de Gram e cultura quando *Streptococcus pneumoniae* foi avaliado.

A comparação dos resultados com os de cultura mostrou o maior nível de sensibilidade considerando-se *N. meningitidis* grupo C. Quanto à especificidade, os valores foram satisfatórios para todos os microrganismos testados. O grau de concordância K em relação à IEC exibiu melhores índices K de concordância para *N. meningitidis* grupos B e C.

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