

Rapid diagnosis of bacterial meningitis by an enzyme immunoassay of cerebrospinal fluid

M. A. M. SALIH¹, H. S. AHMED², Y. HOFVANDER³, D. DANIELSSON⁴
AND P. OLCÉN^{4*}

Departments of ¹Pediatrics and Child Health and ²Microbiology, Faculty of Medicine, University of Khartoum, Sudan; ³International Child Health Unit, Department of Pediatrics, University Hospital, Uppsala, Sweden; ⁴Department of Clinical Microbiology and Immunology, Örebro Medical Center Hospital, Örebro, Sweden

(Accepted 5 June 1989)

SUMMARY

A total of 250 cerebrospinal fluid (CSF) specimens were analyzed using a rapid enzyme immunoassay (Pharmacia Meningitis EIA-Test) (EIA) for the detection of antigens of *Haemophilus influenzae* type b, *Neisseria meningitidis* (serogroups A,B,C) and *Streptococcus pneumoniae* (25 selected types). The test is performed in less than 1 h and read by the naked eye. EIA and coagglutination (CoA) were compared with a constructed reference that comprised samples which were either positive by culture and/or on direct microscopy (DM), or in which there were positive results with both EIA and CoA for the bacteria covered by the assays. Using this reference for CSF samples assayed in a period between two meningococcal meningitis epidemics, the sensitivity was 0·86 for EIA and 0·69 for CoA, the specificity 0·95 (EIA) and 0·97 (CoA), the predictive value for a positive result 0·81 (EIA) and 0·87 (CoA) and, the predictive value for a negative result 0·96 (EIA) and 0·93 (CoA). Antibiotics had been given to 54% of the patients before admission. All of the 56 samples that were positive in any of the tests taken during an epidemic of group A meningococcal disease were detected by EIA; CoA was negative in 45% and culture/DM was negative in 32%. Sequential dilutions of two CSF samples from which *H. influenzae* type b had been isolated, showed the EIA to be 16-32 times more sensitive than CoA. With both technical feasibility and good sensitivity and specificity, the EIA seems to be useful and reliable for the rapid diagnosis of bacterial meningitis, especially in situations where pre-treatment with antibiotics are likely.

INTRODUCTION

Childhood bacterial meningitis is still a cause of considerable morbidity and mortality for both developing countries and the developed world (1-5). An area with devastating recurrent periods of epidemic meningococcal meningitis extends

* Correspondence: Per Olcén, Department of Clinical Microbiology and Immunology, Örebro Medical Center Hospital, S-701 85 Örebro, Sweden.

from West Africa, over the continent, to Sudan in the east. The name of the meningitis belt has been coined to it (6). Greater Khartoum in Sudan is within this belt and has recently experienced two major epidemics, in 1980 (7) and 1988, during the last of which part of the present study took place. In inter-epidemic periods bacterial meningitis still occurs sporadically. The aetiological spectrum during these periods have not been previously investigated in Sudan.

A correct and rapid aetiological diagnosis in patients with suspected bacterial meningitis is a prerequisite for optimal treatment and significantly reduces mortality and the risks of permanent sequelae. It is also important from an epidemiological point of view by indicating the possible need for prevention by specific immunization of the population (8) and for the administration of chemoprophylaxis to close contacts (9).

Microscopy of Gram stained smears and culture of cerebrospinal fluid (CSF) are the standard methods for the laboratory diagnosis of bacterial meningitis. Microscopy lacks, however, specificity and culture may be time consuming and even negative. Serological tests including direct immunofluorescence (IFL), counter-immunoelectrophoresis (CIE), latex agglutination and co-agglutination (CoA) have been used for the rapid identification of bacterial antigens in CSF for the three most common bacteria causing meningitis i.e. *Haemophilus influenzae* (type b), *Neisseria meningitidis* (serogroups A, B and C) and *Streptococcus pneumoniae* (10–16). However, the need for a fairly sophisticated laboratory for IFL and CIE, and the low sensitivity of the agglutination tests have limited their use. Radioimmunoassays (17) and enzyme immunoassays (EIA) (18–21) have been used for detection of low antigen concentrations but are still largely research methods.

An EIA test without the need of sophisticated equipment was recently developed (Pharmacia Diagnostics Corp., Uppsala, Sweden) for the rapid detection of bacterial antigens in CSF. An evaluation of the system in a developing country, where the incidence of bacterial meningitis is high, was considered pertinent and was the aim of the present study. We have therefore critically assessed the EIA when used in the Sudan both during an inter-epidemic period (1985–6) and during an epidemic of meningococcal meningitis (1988).

MATERIALS AND METHODS

Patients

Children over 1 month of age, admitted to the Children's Emergency Hospital in Khartoum with a clinical diagnosis of meningitis/meningoencephalitis, form the study population. The Inter-epidemic Group of patients, in all 145, consisted of children admitted between April 1985 and October 1986, Saturday through Wednesday. The Epidemic Group of patients, in all 147, consisted of children admitted to the same hospital between 7 a.m. and 1 p.m. on Saturdays and Mondays with suspected meningitis during an epidemic of meningococcal disease (serogroup A) during February–July 1988. These times and days were determined both by work load and resources available to carry out the study.

Handling of CSF specimens

Each of the 292 CSF samples were examined for white blood cell count (WBC), direct microscopy (DM) of a Gram-stained smear, and culture on McConkey, blood and chocolate agar media at 36 °C in a humid atmosphere with 4–5% CO₂. Bacteria isolated by culture were identified with standard procedures. Isolated *N. meningitidis* and *H. influenzae* strains were subjected to serologic grouping and typing as described (12). Turbid CSF samples, control samples from 78 patients with cerebral malaria and/or febrile convulsions, and any sample with a WBC of more than 10 × 10⁶ cells/l were tested within 4 h with CoA at the hospital laboratory in Khartoum. CSF samples that were culture positive, but not tested before, were assayed when diagnosed. EIA was performed on each sample either directly (Epidemic Group) or after storage and transport in liquid nitrogen to Sweden (Inter-epidemic Group). The presence of antimicrobial activity in CSF was tested by placing 50 µl of CSF on a PDM agar plate seeded with a multisensitive *S. epidermidis* as indicator strain. Any CSF sample showing inhibition of growth within the area of application was interpreted as containing antibiotics.

Enzyme immunoassay (EIA)

The EIA tests were performed with kits manufactured by Pharmacia Diagnostics, Uppsala, Sweden (Meningitis EIA-Test lot nr 06/87 and 09/87) and the instructions for transport, storage and use were followed. The test kit consists of a set of three plastic tubes coated with rabbit antisera against *N. meningitidis* (groups A, B and C), *H. influenzae* type b and 25 selected types of *S. pneumoniae*, respectively. The test is performed as follows: 100 µl of corresponding antiserum conjugated to horseradish peroxidase and 100 µl of the CSF sample are mixed in each of the three tubes and incubated for 30 min at room temperature (Rt). After washings (washing solution included in the test kit), 200 µl of substrate (O-phenyldiamine dihydrochloride) is added and incubated for 10 min at Rt. The reaction is stopped by 200 µl of 2N HCl and the development of yellow-orange colour observed by the naked eye is considered a positive reaction. Positive and negative controls are included.

Coagglutination (CoA)

The CoA tests were performed with Phadebact CSF Test kits (Pharmacia Diagnostics, Uppsala) with reagents covering *N. meningitidis* groups A, B, C, Y and W-135, *H. influenzae* type b and the 83 types of *S. pneumoniae*. The instructions of the manufacturer were followed.

Definition of bacterial meningitis

Bacterial meningitis was defined as follows: clinical and laboratory evidence of meningitis with positive culture and/or direct microscopy of CSF, or with positive results in both the serological CSF tests, EIA and CoA.

Laboratory experimental tests

Bacterial strains. The following bacterial strains were used to test the specificity of the EIA and CoA tests: *N. meningitidis* reference strains kindly provided by Dr

Table 1. *Diagnosis of bacterial meningitis by examination of CSF with enzyme immunoassay (EIA), co-agglutination (CoA), culture and direct microscopy (DM)*

Positive result for:	EIA		CoA		Culture		DM	
	P/T	(%)	P/T	(%)	P/T	(%)	P/T	(%)
<i>H. influenzae</i>	20/23	(87)	19/25	(76)	11/25	(44)	4/25	(15)
<i>S. pneumoniae</i>	4/7	(57)	0/7		6/7	(86)	3/7	(43)
<i>N. meningitidis</i>	7/8	(88)	6/8	(75)	4/8	(50)	4/8	(50)
<i>N. meningitidis</i> (epidemic group)	78/79	(99)	31/56	(55)	83/117	(71)	68/117	(58)

P = number positive, T = number tested.

Carl Frash (groups A, B, C, Y and W-135) and clinical strains covering the same groups including two serological variants of group Y; *H. influenzae* reference strains from the Colindale collection (types a,b,c,d,e,f) and 5 clinical type b strains; 24 clinical strains of *S. pneumoniae*; 6 of *Listeria monocytogenes*; 3 of *Escherichia coli* K1; 3 of *Streptococcus agalactiae* and 4 of *Staphylococcus aureus*. All the clinical strains were isolated from CSF samples at our laboratory in Örebro, Sweden. All strains were stored at -70°C .

Direct EIA and CoA of bacterial suspensions

The reference and clinical CSF strains were cultured on conventional solid culture media for 18–24 h and then suspended in sterile phosphate buffered saline pH 7.4 (PBS) to a concentration of 10^8 bacteria/ml according to the McFarland scale. Each suspension was assayed as for the CSF specimens (see above) with EIA and CoA. Where the results differed with the two methods a suspension containing 10^9 bacteria/ml was substituted in the tests.

RESULTS

Assays of CSF samples from children during the inter-epidemic period

CSF specimens from 145 children were examined. Bacterial meningitis was confirmed in 35 cases and suggested in another 9 children with a compatible clinical picture and a positive reaction in one of the serological tests. Fifty-two children had clinical and laboratory features consistent with aseptic meningitis, and 49 had cerebral malaria and/or febrile convulsions, all of whom had negative CSF test results with the four diagnostic methods used.

Table 1 shows the results of the diagnostic tests for *H. influenzae*, *S. pneumoniae* and *N. meningitidis*. The highest positive yield was obtained with EIA by which 31/38 (82%) were reactive, followed by CoA (25/40 = 63%), culture (21/40 = 53%), and microscopy of Gram-stained smears (11/40 = 28%). The highest yields with the EIA and CoA were seen with the reagents for *H. influenzae* and *N. meningitidis*. The CoA gave no reaction with the reagent for pneumococci. In four cases the bacterial meningitis was caused by bacteria belonging to the Enterobacteriaceae group (salmonella and *E. cloacae*). The serological tests were all negative for these CSF specimens. Ten *H. influenzae* strains were available for serotyping and all of them were type b.

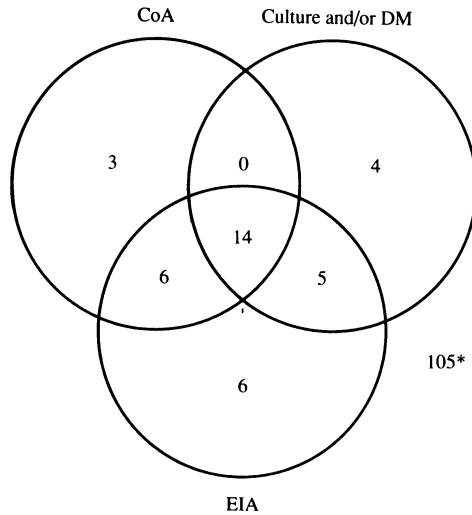


Fig. 1. Venn-diagram showing the results of the CSF analyses of 143 acute samples using culture and direct microscopy (DM), EIA-test and co-agglutination (CoA). Figures within a circle represent positive outcomes with the corresponding test for the bacteria covered by the serological tests, e.g. *H. influenzae*, *S. pneumoniae* and *N. meningitidis*. * Samples negative in all tests.

The overlapping results with the diagnostic tests are shown in Fig. 1. EIA was the only positive test in 6 CSF samples and CoA in 3. All the EIA reactions were clear with all the different test reagents.

Assays of CSF samples from children during the N. meningitidis group A epidemic

CSF specimens from 147 acutely sick children taken during the epidemic were examined. The diagnostic tests were all negative in 29 children who on follow-up proved to have had febrile convulsions. The diagnostic outcome for the remaining 118 samples is presented in Table 1. Of the samples tested, 99% were positive with EIA, 55% with CoA, 71% with culture and 58% by DM. The overlapping results for 56 samples with a positive result in any of the tests and examined with all methods are presented in Fig. 2. It can be seen that all of them were positive with EIA while CoA was negative in 45% and culture/DM was negative in 32%. In 11 samples, the only positive diagnostic test was with the EIA for *N. meningitidis*.

One hundred strains isolated from CSF throughout the epidemic from patients in the study, as well as from other patients, were serogrouped. All of them belonged to serogroup A and were resistant to sulphonamide.

Antibiotic influence on diagnostic outcome

Assay for antimicrobial activity in CSF taken on admission from patients in the Inter-epidemic Group suggested pre-admission administration of antibiotics in 22 of the 41 (54%) cases examined with proven or suggested bacterial meningitis. In 10 of these patients the CSF samples were culture negative (45%); 9 however were positive in one or both of the serological tests. No antimicrobial activity was

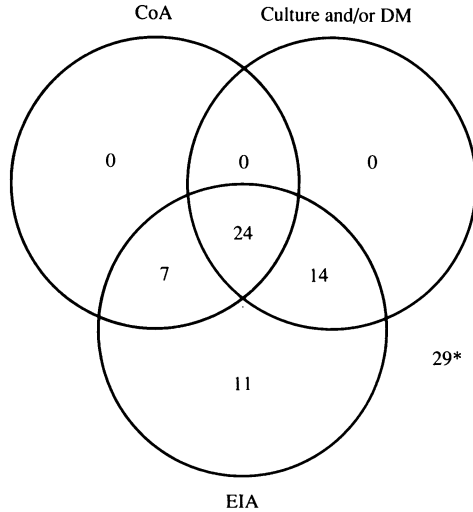


Fig. 2. Venn-diagram showing the results of the CSF analyses of 85 acute samples, taken during an epidemic of *N. meningitidis* group A meningitis, using culture and direct microscopy (DM), EIA-test and co-agglutination (CoA). * Samples negative in all tests.

demonstrated in 19 of the 41 samples. Six (32%) were culture negative but positive in one or both of the serological tests.

In the Epidemic Group, 9 of 115 patients (8%) with proven or suggested meningococcal meningitis had a reliable history of ongoing antibiotic treatment on admission. All of them were positive in EIA and 7 were positive in CoA.

Sensitivity, specificity and predictive values of positive and negative test results with EIA and CoA

For calculation of the significance of the serological diagnostic tests the results with each were compared with a constructed positive reference from the Inter-epidemic Group. The positive reference comprised specimens positive by culture or DM or with positive results in both the EIA and CoA tests for the bacteria covered by the assays. Using this reference the sensitivities were 0.86 for EIA and 0.69 for CoA. The specificity was 0.95 (EIA) and 0.97 (CoA), the predictive value for a positive result 0.81 (EIA) and 0.87 (CoA) and, the predictive value for a negative result 0.96 (EIA) and 0.93 (CoA).

Laboratory tests of specificity and sensitivity of EIA and CoA

The assay results of 62 different suspensions of reference strains and clinical isolates are presented in Table 2. With 10^8 bacteria/ml, a correct result was arrived at in all cases with the EIA whereas CoA missed one clinical isolate of *N. meningitidis* group B, one reference strain of *N. meningitidis* W-135 and 7 out of 24 clinical strains of *S. pneumoniae*. The three *E. coli* K1 strains were all negative in both the tests. No false positive results were noted. With a suspension of 10^9 bacteria per ml four of the *S. pneumoniae* strains became positive with CoA.

Table 2. Analysis of 62 cultured strains with EIA-test and CoA method

Strains*	No.	Correct results with		Remarks
		EIA	CoA	
<i>N. meningitidis</i> clinical (A,B,C, two Y, W-135)	6	6	5	<i>N. meningitidis</i> group B negative in CoA
<i>N. meningitidis</i> ref. strains (A,B,C,Y,W- 135)	5	5	4	<i>N. meningitidis</i> group W-135 negative in CoA
<i>H. influenzae</i> clinical (b)	5	5	5	All positive
<i>H. influenzae</i> ref. strains (a-f)	6	6	6	Only type b is positive
<i>S. pneumoniae</i> clinical	24	24	17	7 negative in CoA
<i>L. monocytogenes</i> clinical	6	6	6	All negative
<i>E. coli</i> K1 clinical	3	3	3	All negative
<i>Str. agalactiae</i> clinical	3	3	3	All negative
<i>S. aureus</i> clinical	4	4	4	All negative
Total	62	62	53	

* Suspension of 10^8 bacteria/ml.

Two CSF samples from which *H. influenzae* type b was isolated and which were positive in both EIA and CoA were diluted twofold in PBS with 10% human serum. The EIA was positive in dilutions 1/32 and 1/16384 whereas the corresponding dilutions for CoA were 1/2 and 1/512 respectively. The concentration of bacterial antigen needed for a positive EIA was therefore about 16-32 times less than that needed for CoA.

DISCUSSION

The diagnostic yield of the EIA for the three most common causative agents of bacterial meningitis was remarkably high in this study in comparison to the two standard methods for analysing CSF specimens, i.e. DM and culture. CoA test was also more sensitive than the latter techniques detecting 63% of the positive specimens in the Inter-epidemic Group compared to 27% and 57% respectively detected by DM and routine cultures, which is contrary to the observations of others (14-16). An explanation may be the high frequency of pre-hospital antibiotic therapy. Supporting this view are the results of the tests on the group of patients with meningococcal meningitis during the epidemic period. The drop in the percentage of pretreatment with antibiotics in this group to 8% was paralleled by an increase in the yields of DM and culture to 58% and 71% respectively.

Table 3. *Diagnostic yield of EIA (A) and CoA (B) compared to the reference* for bacteria covered by the assay in the Epidemic and Inter-epidemic groups*

(A)	Reference			(B)	Reference		
	+	-	Total		CoA	+	-
EIA				CoA			
+	70	17	87	+	51	3	54
-	4	137	141	-	23	151	174
Total	74	154	228	Total	74	154	228

* Positive reference = specimens positive by culture and/or DM, or with positive results with both EIA and CoA.

A low culture yield has repeatedly been observed in studies done on pyogenic meningitis in Khartoum. Only 16% of 125 turbid CSF specimens were positive in one study (22) and Sippel and his associates (7) found only 30% of 80 "hazy to purulent" CSF samples to be culture positive during an epidemic of meningococcal meningitis in 1980. In the latter study 15 CSF samples from 43 patients (35%) that were negative by culture, counter-immunoelectrophoresis and CoA showed evidence of pre-treatment with antibiotics (7).

The EIA was found to be more sensitive than the CoA test in detecting bacterial antigens in CSF samples of both the Inter-epidemic and the Epidemic Groups. With a defined positive reference (Tables 3A & B) EIA showed a sensitivity of 0.95 as compared to 0.69 for CoA. These findings were in agreement with the results using laboratory strains, and of diluted CSF samples that showed the EIA to be about 16-32 times more sensitive than CoA. The lower predictive value for positivity of the EIA-test (80%) in comparison to CoA (94%) is explained by the relatively high proportion of specimens which gave positive results only with the EIA-test ($n = 17$). Six of these samples were from the Inter-epidemic Group and were strongly positive for *H. influenzae*, *N. meningitidis* or *S. pneumoniae*. The remaining 11 samples from the Epidemic Group were all positive in the EIA for *N. meningitidis*. This fact together with the epidemic situation are in support of genuine results with the EIA rather than false positives and might imply that the true predictive value for positivity and specificity are higher than those calculated. The sensitivity for both the serologic diagnostic techniques varied with the bacteriological species. Whereas diagnostic results in the Inter-epidemic Group were about the same for *H. influenzae* and *N. meningitidis*, it was reduced with *S. pneumoniae* since only 4 of 7 (57%) specimens were positive in EIA-test and none was positive in CoA. This could not be explained by pre-hospital treatment with antibiotics, leading to decreased *S. pneumoniae* concentration in CSF below detectable levels, since only one of the seven patients with pneumococcal meningitis had detectable antimicrobial activity in the CSF or plasma samples taken on admission to hospital. On the other hand, difficulty in detecting *S. pneumoniae* with CoA in comparison to culture has not been encountered in previous studies either in Europe or in USA, East or West Africa (14, 15, 23, 24). It is possible that the types of *S. pneumoniae* which are prevalent in Sudan are poorly represented in the COA reagent, but no study has been conducted to

support or disprove this hypothesis. The possibility of unnoticed inactivation of the COA reagent cannot be ruled out.

The EIA showed the highest sensitivity, compared to the other techniques in the Epidemic Group for detecting *N. meningitidis* (sensitivity = 1.00). CoA had a sensitivity of 0.55 which is comparable to that observed by others (7, 15). *N. meningitidis* group A is the predominant strain in Sub-Saharan Africa and its antigen has been easier to detect with serological tests than group B that is prevalent in Europe (25, 26). This fact may contribute to the high sensitivity with the EIA in the present evaluation. Further studies in areas where the meningococcus of serogroup B is the most prevalent serogroup (West Europe, North America) need to be undertaken. The present study shows that the EIA has a sensitivity comparable to results obtained with IFL techniques (15). The EIA used in this study has the advantage to IFL techniques of not needing sophisticated equipment but can be used by any diagnostic laboratory. It has therefore a great potential as a valuable clinical and diagnostic adjunct to Gram stain for a rapid presumptive diagnosis.

From our experience it is a convenient test for routine use in conjunction with cultures that are needed for confirmation of the results, for serogrouping/typing and for antibiotic sensitivity testing of isolated organisms.

ACKNOWLEDGEMENTS

We wish to acknowledge with thanks Professor Mohamed Ibrahim A. Omer of the Department of Pediatrics and Child Health, University of Khartoum and Assistant Professor Jan Kjellander, Department of Clinical Microbiology, Örebro Medical Center Hospital, Örebro for their advices and for placing laboratory facilities to our disposal. Thanks are also due to Dr Ahmed Ibrahim El Hag, Dr Khalid A Osman, Dr Imad Yassin and Dr Zeinab Musa from the University Pediatric Wards (C2) Khartoum Teaching Hospital, Dr Helena Palmgren at the Department of Infectious Diseases in Umeå, Sweden and Mrs Theodora Fredriksson of the International Child Health Unit, Department of Pediatrics, University Hospital, Uppsala for the help they offered at various times throughout the periods of the study. We wish to thank Pharmacia Diagnostics, Uppsala, Sweden for supplying the diagnostic kits. The study was supported financially by grants from the Maud and Birger Gustavsson's fund, and from the Örebro County Council Research Committee.

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