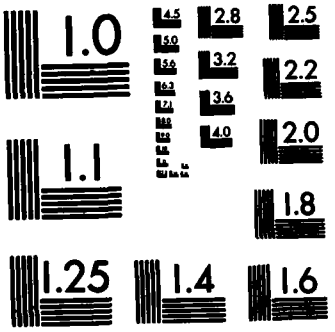
AD-A123 159 A SIMPLE KIT SYSTEM FOR RAPID DIAGNOSIS OF CEREBROSPINAL MENINGITIS IN DEVELOPING AREAS(U) NAVAL HEALTH RESEARCH CENTER SAN DIEGO CA W R SANBORN ET AL. 1/1

UNCLASSIFIED 01 OCT 82 NAVHLTHRSCHC-82-21 F/G 6/5 NL



A SIMPLE KIT SYSTEM FOR RAPID DIAGNOSIS OF CEREBROSPINAL MENINGITIS IN DEVELOPING AREAS

W. R. SANBORN
I. M. TOURE

REPORT NO. 82-21

NAVAL HEALTH RESEARCH CENTER
P. O. BOX 85122
SAN DIEGO, CALIFORNIA 92138

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
BETHESDA, MARYLAND

A Simple Kit System for Rapid Diagnosis of Cerebrospinal Meningitis in Developing Areas

by

Warren R. Sanborn[1,3]

and

Isak M. Toure[2]

Report No. 82-21 supported by Naval Medical Research and Development Command, Bethesda, Maryland, Department of the Navy, under research Work Unit M0095-PN.002-5068. The views presented in this paper are those of the author(s). No endorsement by the Department of the Navy has been given or should be inferred.

Footnotes:

1. Biological Sciences Division, Naval Health Research Center, P.O. Box 85122, San Diego, CA 92138, U.S.A.
2. Section Biologie, Centre Muraz, B.P. 153, Bobo-Dioulasso, Upper Volta, West Africa
3. Present address: Portable Rapid Diagnostic Technology, Inc., P.O. Box 667, Solana Beach, CA 92075

SUMMARY

Acute infectious diseases often occur in areas where conventional diagnostic laboratory services do not exist. Nevertheless, early and specific etiologic diagnosis is often the key to successful therapy and control of serious infections. A simplified laboratory kit was developed through laboratory and field studies for the rapid diagnosis of cerebrospinal meningitis. This study was designed to assess the effectiveness of this kit, employing coagglutination tests, when operated by rural medical attendants. Field studies conducted in West Africa revealed that medical attendants could, with a minimum of technical familiarization, rapidly and accurately diagnose bacterial meningitis cases. This kit, employing inert particle aggregation tests (coagglutination or latex agglutination) appears to be appropriate technology that can effectively support primary health care delivery in developing countries.

INTRODUCTION

Acute bacterial infections comprise a major portion of the health problems faced by peoples of developing countries. In sub-Sahara Africa a continuing concern is cerebrospinal meningitis (CSM), a seasonal disease with most cases occurring from January through April. Specific diagnosis early in the course of this disease can provide significant aid in the choice of appropriate anti-microbial therapy and can indicate need for specific vaccination for prevention. Conventional diagnostic laboratories are not available to the majority of peoples in this region, since 75-80% live in rural areas where transportation and communication are major difficulties.

The coagglutination test (COAG) is a sero-diagnostic technique of relatively recent development and is adaptable to rapid diagnostic test systems. Killed and stabilized cells of Staphylococcus aureus are employed as inert particles to carry antisera that can specifically identify antigens of various pathogenic bacteria (3,6,8,10). For bacterial meningitis the antisera are usually directed against Neisseria meningitidis, Haemophilus influenzae, or Streptococcus pneumoniae. COAG reagents prepared with these antisera will specifically detect and identify antigens of homologous bacteria in either clinical specimens or cultures (4,11,15,16). Strength and quality of antiserum used for reagent preparation appear to be the major factors in variation of sensitivity (4).

COAG tests appear to offer the potential for rapid diagnosis of infectious diseases at the primary health care delivery level since they are simple to perform. These tests might thus provide practical and economical alternatives to conventional laboratory methods that in many areas are not available. This report describes preliminary field evaluations of portable COAG kits for the rapid, bed-side diagnosis of meningitis.

MATERIALS AND METHODS

Studies were conducted during the 1981 CSM season in the town of Koutiala in Mali and the village of Penny in Upper Volta. They were chosen for their relative proximity to the laboratory at Centre Muraz in Bobo-Dioulasso, Upper Volta, and for their differing characteristics. Koutiala has a small hospital, running water, electricity, the services of a physician, but no diagnostic microbiology capacity. Penny has no hospital, no dispensary, no running water, no electricity, and no physician. Both Koutiala and Penny experienced outbreaks of CSM that were thought, from previous experience, to be due to N. meningitidis group C. Cerebrospinal fluid (CSF) specimens were evaluated at the patients' locations and at Centre Muraz.

The diagnostic materials were packaged in a plastic box measuring 13 x 8 x 5 cm. COAG tests were used as the diagnostic tool. The box also contained plastic ampules for reagents, microscope slides, bacteriological loops, a loop decontamination system, and an alcohol burner. Contents were packed in protective insulating plastic foam (Figure 1).

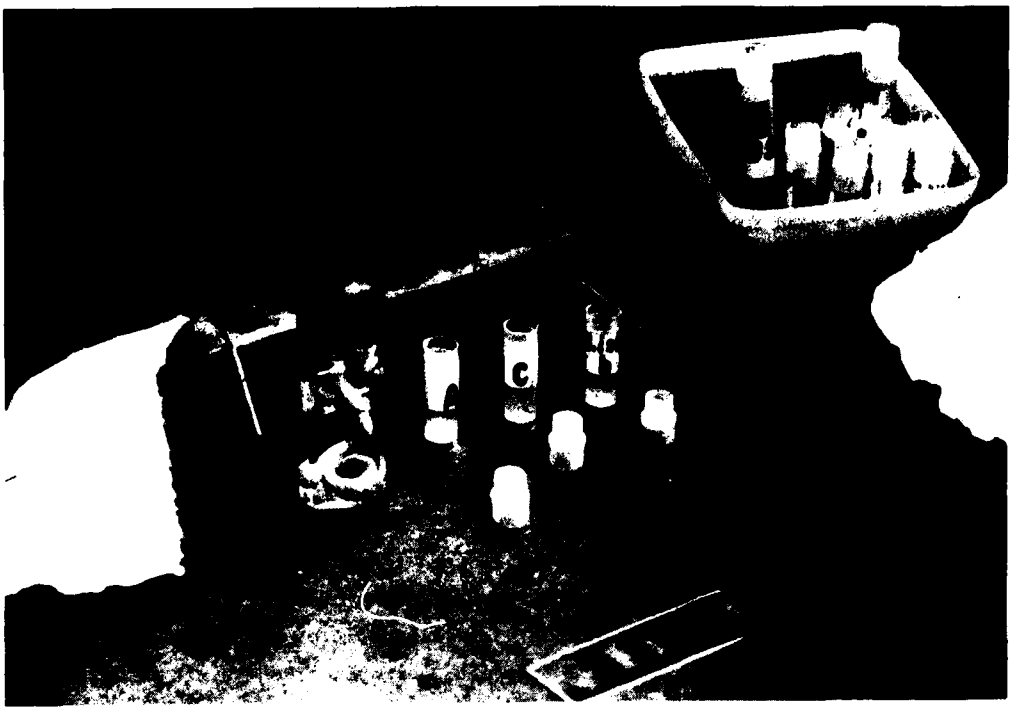


Figure 1: Simple, reusable kit for performing coagglutination, latex agglutination, or other inert particle aggregation diagnostic tests.

The COAG diagnostic tests were performed by local attendants who were trained for about 6 hours over a 2-day period. The instructor (I.M.T.) was familiar with local languages and customs. Training included proper use of bacteriological loops for specimens and reagents, mixing of reagents on slides, and reading COAG tests with positive and negative controls. Specimens and reagents were mixed on glass microscope slides that were rocked back and forth for 1 minute. Then the mixture was observed for clumping.

COAG reagents were prepared as previously described (8), although the harvested staphylococcal cells were heated at 100°C for 20 minutes prior to the washing and fixation steps. The sensitizing antisera employed were N. meningitidis group A and C (Neisseria Repository, University of California, Berkeley), H. influenzae type b (Hyland Division of Travenol Laboratories), and S. pneumoniae Omniserum (Statenserum Institut, Copenhagen). COAG reagents were refrigerated until taken to the field, where they were then continually exposed to the local ambient temperature but were protected from direct sunlight. The temperature reached 30°C in Koutiala during the 3 week test period, and ranged from 30 to 40°C during the 8 week test period in Penny.

CSF specimens were 6 to 72 hours old when re-tested at Centre Muraz. The same lots of COAG reagents were used at the laboratory as were used in the field.

RESULTS

Thirty CSF specimens were evaluated from Koutiala (Table 1). In 28 of these, the results from both examinations agreed. Thus, the agreement between CSF results obtained by the attendant in Koutiala and the Centre Muraz laboratory was 93%. One case identified as H. influenzae meningitis by the laboratory was found to be negative by the attendant. No cases of meningococcal meningitis were identified.

TABLE 1

Comparison of Coagglutination Test Results for Meningitis Diagnosis Performed by African Medical Attendants with Examinations Performed by an Established Microbiology Laboratory

Etiologic Agent	Koutiala		Penny	
	Attendant	Control	Attendant	Control
N. meningitidis Gr. A	0	0	1	3
N. meningitidis Gr. C	0	0	0	1
N. meningitidis Gr. A. A+C*	0	0	4	1
S. pneumoniae	21	21	8	8
H. influenzae type b	6	7	2	3
Negative	3	2	5	5
Totals	30	30	20	20

* Cross reaction due to excess antigen. Following dilution in the control laboratory, these yielded specific reactions.

Twenty CSF specimens were evaluated from Penny (Table 1). The results of both were the same for 16 CSF specimens, making the agreement between the attendant's results and those of the laboratory to be 80%. An excess of antigen in CSF from 4 patients caused a non-specific diagnosis by the attendant. These were also noted at Centre Muraz, but following 1:10 dilution, these yielded specific reactions.

A bacterial etiology for infection was identified in 43 of the 50 CSF samples. S. pneumoniae was the most common agent, H. influenzae was next, and N. meningitidis comprised only 9%.

The COAG reagents showed no loss of reactivity when exposed in the field to elevated ambient temperatures for as long as 8 weeks.

DISCUSSION

"Health to all peoples of the world by the year 2000" is a goal that has been set by the World Health Organization. If this goal is to be achieved, new and novel approaches to health care delivery must be employed. This study of portable, rapid diagnostic kits for meningitis that incorporate simple, accurate COAG tests demonstrates that modern laboratory technology can be adapted to primary health care delivery in rural areas of developing countries.

The COAG reagents were stable under the harsh temperature and environmental conditions found in sub-Sahara Africa, and unpublished studies have shown that COAG reagents also withstand freezing and thawing. Their stability contrasts with the relative delicacy of other inert particle aggregation test reagents, such as latex agglutination reagents. The simplicity of the COAG tests permitted

satisfactory instruction of local primary health care workers, i.e. medical attendants, within 6 contact hours, and they then performed independently with a high degree of accuracy. COAG reagents can be prepared locally using minimum equipment and locally procured materials, thus affecting significant economy over commercial reagent purchase (10).

Simple rapid diagnostic test kits in this study provided bed-side diagnostic information that was immediately useful for patient care. The traditional non-specific diagnosis of "meningitis", based solely on clinical observations, was transformed into a specific etiologic diagnosis. These CSM cases were initially assumed to be due to N. meningitidis group C because of the previous year's findings in Bamako, Mali, and other earlier studies (9). The rapid diagnostic tests revealed that 91% of patients were infected with S. pneumoniae and H. influenzae. This specific etiologic information directed appropriate patient therapy and would have prevented the inappropriate administration of specific meningococcal vaccines. Had the vaccines been given, there would have been apparent failures possibly disillusioning medical authorities and local inhabitants. Rapid, on-site, specific diagnostic capability is a valuable asset for both patient care and public health.

COAG tests have been reported for a variety of diseases including typhoid fever (13), Salmonella gastroenteritis (14), cholera (1,15), bacterial pneumonias (7), dengue fever (antibody) (2), hepatitis B (12), and leishmaniasis (5), to name a few examples. Thus, it appears that its potential applications are broadening. When this simple test, whose reagents can be prepared locally, is employed in a simple portable kit, it appears that this concept may be particularly appropirate for rural areas of Africa where medical facilities are limited and the population is large. Thus, this technology may offer useful diagnostic support for improvement of treatment and control measures for many tropical infections.

REFERENCES

1. Andrade, J.R.C. and Macedo Sores, Y.C.M. Coagglutination method for identification of Vibrio cholerae. Revista de microbiologica 10:111-120(1979).

2. Cheong, C.Y. et al. Staphylococcal agglutination - inhibition reaction: A rapid and simple test for dengue antibodies. Singapore Medical Journal 16:194-195(1975).

3. Christensen, P. et al. New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. Infection and Immunity 7:881-885 (1973).

4. Dirks-Go, S.I.S. and Zanen, H.C. Latex agglutination, counterimmunoelectrophoresis, and protein A coagglutination in diagnosis of bacterial meningitis. Journal of Clinical Pathology 31:1167-1171(1978).

5. Dishon, T. et al. Coagglutination and indirect hemagglutination in the detection of an excreted immunologically active substance from Leishmania. Israel Journal of Medical Sciences, 17:245-248(1981).

6. Edwards, E.A. and Hilderbrand, R.L. Method for identifying Salmonella and Shigella directly from the primary isolation plate by coagglutination of protein A - containing staphylococci sensitized with specific antibody. Journal of Clinical Microbiology 3:339-343(1976).

7. Edwards, E.A. et al. Rapid detection of pneumococcal antigens in sputum using a coagglutination test. Military Medicine 145(4), 256-258(1980).

8. Edwards, E.A. and Larson, G.L. New method of grouping Beta-hemolytic streptococci directly on sheet blood agar plates by coagglutination of specifically sensitized protein A-containing staphylococci. Applied Microbiology 28:972-976(1974).

9. Lapeyssonnie, L. La meningite cerebrospinale en Afrique. Bulletin of the World Health Organization (suppl.) 3-114(1963).

10. Lesmana, M. et al. A coagglutination method for presumptive identification of Salmonella typhi. South East Asian Journal of Tropical Medicine Public Health. 11:301-306(1980).

11. Olcen, P. et al. The use of protein A-containing staphylococci sensitized with anti-meningococcal antibodies for grouping Neisseria meningitidis and demonstration of meningococcal antigen in cerebrospinal fluid. Acta Pathology Microbiology Scandinavia, Section B. 83:387-396(1975).

12. Ragagopalan, M.S. and John, T.J. Sensitivity of passive bacterial agglutination for detection of hepatitis B surface antigen. Journal of Clinical Microbiology 16:549-551(1982).

13. Rockhill, R. et al. Detection of Salmonella typhi D, Vi, and antigens, by slide coagglutination, in urine from patients with typhoid fever. Journal of Clinical Microbiology 11:213-216(1980).

14. Sanborn, W.R. et al. Enrichment culture coagglutination test for rapid, low-cost diagnosis of salmonellosis. Journal of Clinical Microbiology 12:151-155(1980).

15. Sanborn, W.R. and Dutta, S.R. Cholera diagnosis by a coagglutination test. Abstract of Annual Meeting, American Society for Microbiology, C15:312(1979).

16. Thirumoorthi, M.C. and Dajani, A.S. Comparison of staphylococcal coagglutination, latex agglutination, and counterimmunoelectrophoresis for bacterial antigen detection. Journal of Clinical Microbiology 9:28-32(1979).

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 82-21	2. GOVT ACCESSION NO. A123159	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* A Simple Kit System for Rapid Diagnosis of Cerebrospinal Meningitis in Developing Areas		5. TYPE OF REPORT & PERIOD COVERED FINAL 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) W.R. Sanborn Isak M. Toure		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Health Research Center San Diego, CA 92152		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS M0095-PN.002-5068
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research & Development Command Bethesda, MD 20814		12. REPORT DATE 1 Oct. 1982 13. NUMBER OF PAGES 7
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)* Bureau of Medicine & Surgery Department of the Navy Washington, DC 20372		15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Meningitis
Rapid Diagnosis
Coagglutination
Field Kit

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

A simplified portable diagnostic kit employing coagglutination reagents has been developed for the rapid, bed-side diagnosis of cerebrospinal meningitis. Field trials employing this kit were conducted in rural sub-Sahara Africa to diagnose the etiologic agents of meningitis outbreaks. West African village medical attendants successfully used the kit to rapidly and specifically diagnose meningitis cases. Other acute infections can also be rapidly diagnosed in a similar manner. This kit offers appropriate technology for support of primary health care delivery in rural regions of developing countries.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*


FILM
2-83