Date 21/5/87

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Mahbubur Rahman Trainee Investigator (if any) Mahmuda Yasmin

Application No. 87-012P Supporting Agency (if Non-ICDDR,B) _____

Title of Study Rapid diagnosis of Typhoid Fever by detecting Salmonella typhi antigen using Coagglutination technique

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve:
   (a) Physical risks to the subjects Yes (No)
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects Yes (No)
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes No
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) Yes (No)
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids Yes (No)
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used Yes (No)
   (c) Physical risks Yes (No)
   (d) Sensitive questions Yes (No)
   (e) Benefits to be derived (Yes) No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure (Yes) No
5. Will signed consent form be required:
   (a) From subjects Yes (No)
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects Yes No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ___ Protocol (Required)
   ___ Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(PTO)

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Mahbubur Rahman
Principal Investigator

Mahmuda Yasmin
Trainee

MAY 25 1987

Attachment 1a.

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The /Committee will not consider any application which does not include an abstract summary. The abstract should summarize the purpose of the study, the methods and procedures to be used, by addressing each of the following items. If an item is not applicable, please note accordingly:

1. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and an assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.

5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

   (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course of action.

   (c) If there is a potential risk to the subject or privacy of the individual is involved in any particular procedure include a statement in the consent form stating whether or not compensation and/or treatment will be available.

6. If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

The statement to the subject should include information specified in items 2, 3, 4, 5(c) and 7, as well as indicating the approximate time required for participation in the activity.

87-0124
21/5/87

SECTION - I: RESEARCH PROTOCOL

1.	TITLE	: Rapid diagnosis of Typhoid Fever by detecting Salmonella typhi O-antigen using coagglutination technique.
2.	PRINCIPAL INVESTIGATOR	: Mahbubur Rahman
3.	CO-INVESTIGATORS	: Asma Khanam Anwar Hossain
4.	CONSULTANT	: David A. Sack
5.	TRAINEE INVESTIGATOR	: Mahmuda Yasmin
6.	STARTING DATE	: When fund is available
7.	CLOSING DATE	: Six months after starting date
8.	TOTAL DIRECT COST	: 3600/=
9.	SCIENTIFIC PROGRAM HEAD	: This protocol has been approved by the Laboratory Sciences and Epidemiology Division.

Signature of Scientific Program Head David A Sack

Date 22 April 1987

10. ABSTRACT SUMMARY:

A total of 60 patients consisting of 30 culture proven typhoid patients, aged 6 months to 60 years and 30 non-typhoid febrile controls will be studied. This study will be done in collaboration with typhoid fever study by Asma Khanam which is going on in ICDDR,B Hospital. Salmonella typhi O-antigen will be detected in patient's serum, stool and urine by coagglutination test, collected on hospital day 1, 5 and 14. This study will not require any additional blood or venipuncture. The results will be compared with blood culture and widal test, performed on the same day. Coagglutination, a particle agglutination test in which formaldehyde stabilized, heat treated Staphylococcus aureus cowan-1 strain containing high amount of protein A in the all wall is used as reagent after treating the cells with high titer specific antiserum. Protein A binds FC fragment of IgG keeping Fab fragment free and in the presence of homologous antigen, the sensitized staphylococcal cells produce visible agglutination on slide. Using this technique an attempt will be made for rapid diagnosis of typhoid fever by detecting Salmonella typhi O-antigen as the results will be available within 5h.

11. REVIEW:

a) Research involving human subjects

b) Research Review Committee

c) Director

d) Ethical Review Committee

e) Controller

SECTION - II: RESEARCH PLAN

A. - INTRODUCTION:

1. Objectives:

To detect Salmonella typhi O-antigen from serum, stool and urine of suspected typhoid patients by coagglutination technique and compare the results with blood culture and widal test with an idea to develop a rapid diagnostic test of typhoid fever.

2. Background:

Typhoid fever is a prevalent infectious disease of man with multi-system involvement and marked morbidity and mortality in developing countries where the disease is endemic (1). The disease is caused by ingestion of *S. typhi* by a susceptible host and runs a prolonged course with high rate of complication (2) Typhoid Fever runs a course of about 3-6 weeks in untreated cases (3). It represents about 1.5% of diarrhoeal patients attending to ICDDR,B (14). The major symptoms and signs of typhoid fever is relatively non-specific which makes the diagnosis difficult particularly in early part of illness (4). Typhoid fever is suspected and diagnosed usually during 2nd week of illness when the patient is toxic and has been suffering from continued fever. As a result, incorrect and inappropriate therapy is not uncommon (2). The disease is diagnosed by isolation of *S. typhi* from blood culture which is the only dependable method at present (4). Blood culture is positive in 80% of untreated patients seen in 1st week of illness (5) and isolation rate decreases gradually as

the disease continues. Blood culture takes about 2 to 3 days and often gives false negative result when infecting organism is low in blood and patient has received antibiotic (4). Stool culture is less sensitive and only positive in 33-66% of patients during second through fourth week of illness (5, 6). Urine culture is also less sensitive being positive in 25-33% of typhoid patients. Agglutinin against *S. typhi O*, and vi antigen is formed irregularly during the course of the illness. Many immunodiagnostic tests are available for demonstration of antibody in patient's serum. The commonly used widal test is not a reliable diagnostic test (7). Moreover in endemic areas the agglutinin is present in normal healthy person. In Dade County epidemic, only 24% of patients showed 4 fold or greater rise in antibody titer against *S. typhiO* antigen, the titer actually decreased or remained low in 60% of patients during therapy (8). Agglutinin against against H antigen, irrespective of change of titer has no value in diagnosis. Vi antibody is evoked by acute *Salmonella typhi* infection and also found in 70% of known carrier in one study (5). Thus widal test plays a minor role in diagnosis of typhoid fever, at times it can help, but at other time it may cause confusion (5).

S. typhi has O antigen which is lipopolysaccharide part of cell wall, H flagellar antigen composed of protein flagellin and vi antigen which is capsular polysaccharide. In a number of studies attempts have been made to detect these antigens in clinical specimens for diagnosis of *S. typhi* infection using different immunological methods. ELISA (9), counter immunoelectrophoresis

(10) and coagglutination test (11, 12, 13) has been tried to detect Salmonella antigens in Typhoid patients. ELISA and counter immunoelectrophoresis for *S. typhi* antigens are expensive, time consuming and less sensitive.

Coagglutination, a modified agglutination test has been found highly specific and sensitive for detection of these antigens in typhoid patient's serum and urine in a limited number of patients in three studies (11, 12, 13). In one study (13) this technique was used to detect O-antigen in patient's serum. The sensitivity, specificity and accuracy indices of coagglutination test in this study were over 95%, indicating that it may be a reliable test for typhoid fever. In another study for detection of these antigens in urine by this method, antigens of *S. typhi* were positive in 29.3% of cases compared to blood culture which was positive in 21.8%. The Salmonella agglutinins were positive in 28.8% of cases only (11).

Attempts have not been taken to detect antigens in stool which may be helpful particularly in diarrhoeal patients. None of the above studies have mentioned or observed the effect of antibiotic therapy on antigen detection which may help in diagnosis of partially treated culture negative patients.

At ICDDR,B, about 100 hospitalised diarrhoeal patients per year are diagnosed to have typhoid fever and diagnosis is made by positive blood culture mostly during 2nd and 3rd week of illness (14). At present one study on typhoid fever is going on. The clinical specimens for this study will be taken from the ongoing protocol.

An attempt to detect S. typhi O-antigens in serum, urine and stool will be made in suspected typhoid patients and non-typhoid febrile controls. This study may help to make early diagnosis typhoid fever by detecting antigens in clinical samples (serum, urine and stool) of febrile patients.

3. Rationale:

More than 100 culture proven typhoid patients are admitted into ICDDR,B with diarrhoeal complaints (1981). Diagnosis of this disease is made by isolation of bacteria from blood. Eighty percent of typhoid patients gives positive blood culture in 1st week and positivity decreases with increased duration of illness. In addition blood culture gives false negative result when bacterial count is low in blood. Due to initial non-specific presentation of typhoid fever; the patient is often treated with antibiotic making diagnosis very difficult. Antibody response during typhoid fever does not corelate well with disease process. Thus blood culture and/or antibody estimation do not help very much for early diagnosis of typhoid fever. Antigen detection in serum and urine in limited number patients is found very encouraging in early diagnosis of typhoid fever. Moreover coagglutination technique is very simple, inexpensive, easy to perform and gives result within a short time. Thus the study may help for rapid diagnosis of typhoid fever.

B. SPECIFIC AIMS:

1) To detect Salmonella typhi O-antigen in clinical samples by coagglutination technique and compare it with antibody detecting test and blood culture to establish a rapid diagnostic test of typhoid fever.

C. MATERIALS AND METHODS:

1. Clinical specimens:

Blood: 0.25-0.5ml serum will be taken during widal test for the cases. Control sera will be collected from pathological and biochemistry laboratory and only from non-typhoid febrile patients. Blood culture and widal test will be done by mother protocol on day 1, 5 and 14.

Urine: Urine will be collected on day 1, 5 and 14. Urine will be centrifuged and supernatant will be collected and kept in the refrigerator until tested for antigen. Urine will also be concentrated for detecting antigen.

Stool: Stool or rectal swab will be collected on day 1 and 5. One rectal swab or 0.25ml of stool will be incubated to 2ml of selenite F broth for 4h. Finally the broth will be tested for Salmonella typhi O-antigen. Stool or rectal swab will be cultured on days 1, 5, 14 as a part of ongoing typhoid study.

2. Antisera:

It will be raised in rabbit by intravenous injection of non-motile, non-capsulated strain of S. typhi treated with alcohol and acetone.

3. Preparation of coagglutination reagent:

Method of Kronvall modified by Edwards and Larson will be used to prepare the reagent (15, 16).

(i) Preparation of stabilized protein A rich staphylococcus cell:

Staphylococcus aureus cowan 1 strain will be grown in trypticase soy broth (BBL) overnight at 37°C in a shaker. The cells will be washed 3 times with phosphate buffered saline (PBS-0.03M phosphate, 0.12M Nacl, pH-7.2) and suspended in 0.5% formaldehyde in PBS. The suspension is incubated at room temperature for 3h. The treated bacteria will be washed three times with PBS and reconstituted to a final concentration of 10% (v/v) in PBS. This suspension will be heated at 80°C for one hour in a waterbath and cooled quickly. The heat treated cells will be washed two times with PBS and reconstituted 10% (v/v) in PBS to store at 4°C until used.

ii) Preparation of antibody coated staphylococcus

One ml of stabilized Staphylococcus suspension will be added to 0.1ml of specific antiserum. After thorough mixing, the suspension will be incubated for 3h at room temperature with gentle hand shaking at approximately 0.5h intervals. Suspension will be centrifuged at 2500 rpm for 20 minutes and supernatant will be discarded. The pellet will be washed once with PBS and reconstituted to 2% (v/v) suspension in PBS with 0.1% sodium azide for use in the study. The coagglutination reagent will be stored at 4°C until used.

iii) Control reagent:

A 2% (v/v) suspension of formaldehyde stabilized, heat treated Staphylococcal cells in PBS with 0.1% sodium azide, sensitized with preimmunized normal rabbit serum will be used as negative control reagent.

iv) Procedure for coagglutination test:

Two rectangles will be drawn on a glass slide. One drop of test specimen will be added on to each of rectangles. One drop of coagglutination reagent will be added to one rectangle and one drop control reagent to other. After mixing thoroughly with a loop, the results are read at the end of two minutes against a dark background for agglutination. In doubtful cases positive control will be used. Absorption of test specimen with 10% (v/v) non-sensitized Staphylococcal cells and/or heating at 80 °C for 5 minutes will be used to remove non-specific reaction, if any.

4. Widal Test:

This test will be done by mother protocol. Seroconversion will be considered if there is four fold or more increase in antibody titer.

D. SIGNIFICANCE:

If *S. typhi* O-antigen detection in clinical specimens by this method is found sensitive and specific, coagglutination test can be used as early screening test of typhoid fever. The test results will be available within 5h and this may dictate appropriate antibiotic therapy. False negative typhoid cases by

culture may be diagnosed by this antigen detection technique. Antisera raised in the rabbit in our laboratory may be used for other purpose.

F. FACILITIES REQUIRED:

1. Office Space: Not needed
2. Laboratory Space: ICDDR,B Lab space will be used
3. Hospital Resources: From mother protocol - Typhoid protocol of Asma Khanam - No extra help is needed.
4. Animal Resources: Rabbits are required to raise antibody
5. Logistic Support: Data processing, preparation of manuscript, etc.

REFERENCES

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SECTION - I: BUDGET

A. DETAILED BUDGET:

1. Personnel Services:

Name	Position	% Effort	Taka	Dollar
Mahbubur Rahman	Principal Investigator	10%	---	---
Asma Khanam	Co-Investigator	10%	---	---
Anwar Hossain	Co-Investigator	10%	---	---
Mahmuda Yasmin	Trainee Investigator	70%	---	360
	Research Officer	100%	---	1900

2. Supplies and Materials:

a.	Clinical supplies and Lab materials		500
b.	Animal Resources - Rabbit		140
c.	Test Tubes - Slides etc.		200
3.	Patient Hospitalization	---	---
4.	Outpatient care	---	---
5.	Travel of persons	---	---
6.	Transportation of things	---	---
7.	Rent, Communication and utilities	---	---
8.	Xerox	---	100
9.	Publication and printing	---	200
10.	Other contractual services	---	---
11.	Construction, Renovation, Alteration	---	---
12.	Others	---	200
	Sub-Total		US $ 3600
	Indirect cost (31%)		
	Grand Total		

ABSTRACT SUMMARY

1. Serum, Urine and stool samples will be collected from 30 culture positive typhoid patients and 30 nontyphoid patients.

2. No potential risk.

3. Not needed.

4. All data will be handled confidentially and in coded format.

5. Not needed.

6. Not needed.

7. Early diagnosis of the disease will be helpful for proper treatment of the patients.

8. No