Document 1. PILOT PROTOCOL Library Date May 30, 1986

ETHICAL REVIEW COMMITTEE, ICDDR,B

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DHAKA-12

17/6/86

Principal Investigator DR. ANWARUL HUQ Trainee Investigator (if any) _____

Application No. 86-020P Supporting Agency (if Non-ICDDR,B) _____

Title of Study Evaluation of finger prick blood and urine for Salmonella Typhi isolation from suspected patient.

Project status:
(X) 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).

Source of Population:
(a) Ill subjects (Yes) No
(b) Non-ill subjects Yes No
(c) Minors or persons under guardianship Yes No

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)

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)

Are subjects clearly informed about: NA
(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: NA
(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 NA

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.

Principal Investigator

Trainee

JUN 19 1986

REF
QW 138.5.C2.NB.
H795e
1986

86-020P
17/6/86

SECTION I - RESEARCH PROTOCOL (PILOT)

1. Title — Evaluation of Finger Prick Blood and Urine for Salmonella typhi Isolation from Suspected Patient

2. Principal Investigators — Dr. Anwarul Huq
Dr. David A. Sack

Co-Investigator — Mr. Khorshed Alam

3. Starting Date — June 15, 1986

4. Completion Date — December 31, 1986

5. Total Direct Cost — US $2541.40

6. Scientific Program Head

This protocol has been approved by the Disease Transmission Working Group

Signature David A Sack

Date 29 May 1986

7. Abstract :

Finger prick blood of 200 ul and urine samples will be processed by using enrichment methods to evaluate their efficiency in Salmonella typhi isolation. Selinite F broth will be used as the enrichment both and MacConkey and bismuthsulfite will be used as plating media. Suspected colonies growing on either agar will be serologically confirmed as Salmonella typhi. Results from the finger prick blood and urine samples will be compared to the results from the venapuncture blood sample routinely done in the hospital.

8. Reviews :

a. Research Involving Human Subject _____

b. Research Review Committee _____

c. Director _____

SECTION II - RESEARCH PLAN

I. INTRODUCTION

1. Objective : The Immediate goal is to evaluate the use of finger prick blood and urine for the isolation of *Salmonella typhi* by enrichment method.

2. Background

For routine bacteriological isolation of *Salmonella typhi* blood has been the choice of sample. Although, the better recovery of *Salmonella typhi* from bone marrow has been documented (Gilman et al 1975, Robertson et al 1968), blood culture is preferable because of the painful and tedious technique involved in bone marrow collection. Moreover, in the field situation and in the Bangladesh socio-cultural situation even venous blood is sometime difficult to collect. Considering the need to be able to diagnose typhoid fever in the field we propose a study involving finger prick blood for the culture and isolation of *Salmonella typhi* from patients.

Salmonella typhi infected patients may excrete faeces containing up to 10^9 organism per gram of stool but in blood the number is much lower which may reach at most 10^3 per ml, and more commonly 10^2 organisms per ml (or less) in blood. It is therefore necessary to have sufficient volume of blood to detect these organisms. Usually 5 to 7 ml of blood is desirable for any blood culture and with new Dupont isolator, 1.5 ml of blood is required from infants up to 5 years of age.

In our study we plan to collect only 200 ul of blood from finger prick as sample.

Finger prick blood is not generally used for culture because of almost certain contamination with skin organisms. We expect the specimen to be contaminated; however, using enrichment and selective media, and focusing only on Salmonella typhi as our outcome event, contamination will not be a problem.

Urine has been examined as a source for isolating Salmonella from Salmonella infected patient and found to be unsatisfactory. Gilman et al found that only 7% of the urine specimen from the S.typhi patients grew Salmonella colonies on Macconkey's plate when direct streaking was done by them. We propose in our study to re-examine the suitability of urine using enrichment methods similar to those used for blood culture. The enrichment methods should allow detection of organisms at or low aconcentrations as 5 organisms per 100 ml as compared to a sensitivity of 5 organisms per ml in Gilman's study (100 fold increase in sensitivity).

3. Rationale :

Finger prick blood is not considered to be a suitable sample for culture because the volume is low and the sample is usually contaminated. We feel however that culturing both the finger stick blood and urine samples, using enrichment broth and selective agar might detect a substantial proportion of typhoid patients. Such methods might be quite useful in the field.

B. SPECIFIC AIMS

To develop a simple procedure for diagnosing patients with typhoid in the field.

C. MATERIALS AND METHODS :

Two hundred patients with symptoms suggestive of typhoid will be studied in Dhaka Hospital. Patients having the routine test examinations done in our hospital will have the additional tests performed by our group for the study purpose. Patients who received antibiotics during the previous 7 days will be excluded.

Blood : Two hundred microlitre of blood will be collected from the patient on arrival into the hospital after cleaning the finger tip thoroughly with water. Upon collection blood will be transferred into 5 ml of selenite F broth and after 12 hours of enrichment at 37C, broth will be streaked onto MacConkey and bismuth sulfite plates. After overnight incubation at 37C all Salmonella suspected colonies will be serologically tested by slide agglutination with specific polyvalent antisera for Salmonella typhi.

Urine : Twenty millilitre of urine will be collected from the same group of 200 suspected patients and will be transferred into 10 ml tripple strength selenite F broth and incubated at 37C for 12 hours. After incubation, a MacConkey and bismuth sulfite agar agar plate will be streaked for culture and incubated again at 37C overnight. All suspected colonies will be serologically tested by slide agglutination with specific polyvalent antisera for Salmonella typhi.

It is expected that these patients will have all the routine tests done for the identification of Salmonella in our hospital. In case of infant patients Dupont isolator 1.5 will be performed as usual. Results from the routine tests and from our study will be analyzed for evaluation purpose as shown on Table I.

Based on age and sex of patients studied a similar table will be constructed for age group <10 and 10+ and for male and females. Finally, using quantitative data from the DuPont isolation specimens correlation will be calculated for the relation of bacteremia to positivity in the capillary specimen.

D. SIGNIFICANCE

If the results are encouraging, this will be an acceptable means in the field situation where collection of venous blood is generally not possible at present. Also it may be very useful for the infant patient since it sometimes is impossible to draw even 1.5 ml of blood that is required for Dupont isolator.

E. FACILITIES REQUIRED

No extra facilities required other than what we have available at the ICDDR,B.

F. COLLABORATIVE ARRANGEMENT

Nil

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% time	Annual Salary	Project Take	Requirement Dollar
Dr. Anwarul Huq	Manager, MIC	5%			$ 150.00
Mr. Khorshed Alam	Supervisor	5%			$ 100.00

2. SUPPLIES AND MATERIALS

200 culture for blood	200 x 5.00	=	$ 1000.00
200 culture for urine	200 x 3.00	=	$ 600.00
Antisera for Salmonella typhi 3 bottles	30 x 3		$ 90.00

B. BUDGET SUMMARY

C A T E G O R Y	US DOLLAR
1. Personnel	250.00
2. Supplies	1690.00
3 - 12 None	-
	1940.00
Overhead	601.40
US $	2541.40

OVERVIEW OF THE SALMONELLA POSITIVE CASE BY VARIOUS METHOD

Patient Number_____ Date _____

Name _____

Age_____ Sex_____

Routine Tests	Capillary Blood		Urine		Either Capillary blood or urine	
	Positive	Negative	Positive	Negative	Positive	Negative
DuPont Isolator 1.5 Positive Negative						
BiPhasic Soy Broth Positive Negative						

EFERENCES

1. Gilman, R.H., M. Terminel, M.M. Levine, Pablo Hernandez-Mendoza and R.B. Hornick. 1975. Lancet 1211-13.

2. Robertson, R.P., M.F.A. Waham, R.O. Roasch. New Engl.J.of Med. 1968. 278, 171.