Title of Study: Secretory IgA antibody in saliva of children with shigellosis

Principal Investigator: CONSTANCE SCHULTZ
Trainee Investigator (if any):
Publication No.: 88-013
Supporting Agency (if Non-ICDR,B): USAID

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).

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] NA
   (c) Physical risks [Yes] [No] NA
   (d) Sensitive questions [Yes] [No] NA
   (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]

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:
   NA 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 Ctte for review.

The Principal Investigator agrees 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
SECTION I - RESEARCH PROTOCOL

1. TITLE : Secretory IgA antibody in saliva of children with shigellosis

2. PRINCIPAL INVESTIGATOR : Ms. Constance Schultz

COINVESTIGATOR : Dr. Firdausi Qadri
Dr. Faruque Ahmed

3. STARTING DATE : May 1988

4. COMPLETION DATE : May 1989

5. TOTAL DIRECT COST : US$ 13,400
(Funding source: USAID)

6. ASSOCIATE DIRECTOR : Dr. Ivan Ciznár

This protocol has been approved by the Laboratory Sciences Division.

Signature of Associate Director: [Signature]

Date: April 12, 1988

7. ABSTRACT SUMMARY

This study is meant to investigate the secretory IgA antibody response during shigellosis in children. Saliva samples from patients will be collected on day of diagnosis, day 7 and day 14 of illness. Saliva from children of a local school and from children who just arrived in Bangladesh from non-endemic areas, both without any history of recent diarrhoea, will be used as control.

The antibody response will be analyzed by ELISA and Western-blot, using outer membrane proteins (OMPs) and lipopolysaccharide (LPS), prepared from Shigella dysenteriae.
type 1 and *Shigella flexneri* strains. *Shigella* toxin extracted from a *Shigella dysenteriae* type 1 strain will also be used in ELISA and Western-blot analysis. Unstimulated saliva samples will be used, after heating at 56°C and centrifugation. It is expected that these analyses will enable us to observe a rise in the IgA antibody titer in saliva during *Shigella* infection.

8. REVIEW

a) Ethical Review Committee

b) Research Review Committee

c) Director
SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective
To determine secretory IgA antibody response to Shigella infection in saliva from children.

2. Background
Vaccine development against shigellosis is one of the highest priorities in the WHO, Diarrhoeal Disease Control program (16). Since ORS alone is not sufficient for the treatment of shigellosis and resistance to antibiotics is expanding rapidly, immunoprophylaxis is one area of research attracting attention and gaining importance.

Development of an effective vaccine against shigellosis will substantially benefit from the knowledge of the immune response of the human body to pathogens. In addition, a knowledge of the immunogenic components of the pathogen is required. In intestinal infections, the local mucosal immune response is of great importance since it is the first step in defense against a pathogen. However, it is difficult to study this immune response without the use of invasive methods. As an alternative to this, saliva may be used to study the local humoral intestinal immunity.
Antigenic stimulation of lymphocytes residing in the Peyer's patches in the small intestine leads to proliferation and migration of the cells into the regional lymph nodes, the lymph and the circulation. The antigenically stimulated mature lymphocytes return to the small intestinal mucosa or settle in the various exocrine glands and differentiate into IgA secreting plasma cells. It is because of this mechanism of migration and homing of lymphocytes that the IgA excreted by the salivary glands is of similar specificity to the IgA secreted by the plasma cells which have returned to the intestinal mucosa (1,11). Various studies showed evidence that transport of circulating monomeric IgA into the secreting glands is minimal (11). It is for this reason that by measuring the salivary IgA levels in patients, one can evaluate the local humoral immune response in the small intestine.

Mellander et al. (9,10) have shown that infants can respond to exposure to E. coli antigens with production of salivary secretory IgA antibodies. Mestecky et al. (12) have immunized volunteers orally with capsulated and killed Streptococcus mutans which induced a significant rise in the secretory IgA titer in the saliva.
In Bangladesh, Islam et al. have detected salivary IgA antibodies against *Giardia lamblia* in a cohort study (4). There are no reports, however, regarding the presence of salivary IgA antibodies in patients with shigellosis (2). Several animal studies, focused on the local immune response after stimulation by *Shigella* spp., have shown an increase in local antibody production in the gut (5,8).

In shigellosis, various components of the bacteria may be responsible for generating an immune response. Of these, the outer membrane proteins (OMPs), lipopolysaccharides (LPS) and the *Shigella*-toxin appear to be important and are being studied so as to understand their immunogenic properties (8,13). Since secretory IgA in saliva represents the intestinal secretory immune response, it should be possible to investigate the immunogenicity of OMP, LPS and *Shigella*-toxin by measuring the salivary IgA levels against each of these bacterial components.

3. **Rationale**

The mucosal immune system provides the body with a major barrier against nutritional, as well as microbiological antigens. The importance of the mucosal immune system can be seen in patients with deficiency in secretory IgA. These patients suffer from recurrent respiratory and intestinal infections.
(3). Understanding the various factors involved in mucosal immunity is of great importance for development of an effective vaccine. This study aims to give insight into the production of salivary IgA antibodies during shigellosis and to assess whether salivary IgA can be used as an indicator of the intestinal immune response.

In developing a method to estimate the amount of IgA antibodies in saliva during shigellosis, one may find a non-invasive procedure for the measurement of antibody response. Thus, this study might also be of relevance in its clinical and epidemiological application.

B. SPECIFIC AIMS

a) To set up enzyme-linked assays for measuring IgA antibodies in saliva against OMPs, LPS and toxin obtained from *S. dysenteriae* type 1 and *S. flexneri* antigens.

b) To observe a rise in the secretory IgA antibody titer in saliva during the course of a *Shigella* infection.

C. MATERIALS AND METHODS

a) Patients saliva

Unstimulated saliva from 10 children with *S. dysenteriae* type 1 infection and 10 children with
*S. flexneri* infection (bacteriologically confirmed), aged 2-12 years, will be collected on the day of diagnosis (2nd day of hospitalization) and on day 7 and day 14 of illness. Maximal duration of diarrhoea before attending the hospital will be 4 days. In case of breastfeeding, saliva will be collected 2 hours after feeding to prevent contamination with secretory IgA in breast milk. In order to obtain a statistically satisfactory number of children, it will be necessary to collect samples from children attending both the Dhaka and the Matlab treatment centres.

b) **Control saliva**

Saliva from 10 children in the same age group as the test group will be used. Only children without a history of recent *shigella* infection will be included. These children will be found in a local social welfare school (Samaj Unnayan School, Siddeswari, Dhaka).

Further saliva of 10 children who have recently arrived in Bangladesh from a non-endemic area and without any history of shigellosis will be used as control (American International School, Dhaka).

c) **Collection and treatment of saliva**

Unstimulated saliva samples will be collected either by suction with a dropper, or by spitting into a beaker. The samples will be heated at 56°C for 15 min to
prevent enzymatic degradation (4), centrifuged at 5,000 X g and stored in aliquots at -70°C.

d) **Stool samples**

Stool samples from the patients group will be examined for *Salmonella*, *Shigella*, *V. cholera* and *E. coli* ST/LT on admission as is normal procedure in the ICDDR,B treatment centres. Stool samples will also be collected on day 7 and/or day 14 in case of persistent or recurrent diarrhoea only.

e) **Analysis of antibody in saliva**

i) **Antigen**

OMP (13) and LPS (15) for ELISA and Western-blotting will be prepared using strains *S. dysenteriae* type 1 No. 26406 and *S. flexneri* 2a No. 611. In case of different *Shigella flexneri* serotypes, homologous strains will be used. These strains are at present stored at -70°C. These bacteria will be subcultured and grown at 37°C with shaking in Trypticase Soy Broth containing 0.6% Yeast Extract. For preparation of *Shigella* toxin for ELISA and Western-blotting, *S. dysenteriae* type 1 strain No. 26406 will be used, using methods developed in the Immunology laboratory.
ii) **ELISA**

Microtitre plates (polystyrene) will be coated with OMP, LPS and *Shigella* toxin. After washing, serially diluted saliva samples will be added. Anti-human IgA conjugated to horseradish peroxidase will be used to determine the IgA content of the saliva. For visualization, 4-Chloro-1-Naphtol will be used as substrate and colour development will be measured at 540nm (6,13).

iii) **Western-blotting**

OMPs, LPS and *Shigella*-toxin, separated by 13.5% SDS-polyaorylamide gel electrophoresis (7), will be blotted onto nitrocellulose sheets in the transblot apparatus of Bio-Rad (14). Saliva samples will be used to probe specific antigens. Rabbit anti-human IgA linked to horseradish peroxidase will be used to detect recognition using 4-Chloro-1-Naphtol as substrate.

9) **Statistical analysis**

In order to measure the significance value of the obtained data the Student’s t-test or other appropriate statistical evaluations will be carried out.
REFERENCES


2. Mezz, Medline (1966 to April 1988); bibliography on antibody (IgA, IgG, or IgM) in saliva.


ABSTRACT SUMMARY

This study is meant to investigate the secretory IgA antibody response during shigellosis in children. By measuring the salivary IgA levels in patients, one can evaluate the local immune response in the small intestine.

Saliva will be analyzed using ELISA and Western-blot.

It is expected that knowledge of the local immune system can give a valuable contribution to the development of an effective vaccine.

1) Since the majority of infections occurs in children, it is of more relevance to study the immune response in this high-risk group.
2) There are no risks involved.
3) N.A.
4) Patients will be identified by a study number, data will be handled with proper care.
5) All guardians will be asked to sign a consent form.
6) N.A.
7) No personal benefit for the patient; society can benefit if this study gives a relevant contribution to the development of a Shigella vaccine, as well as to the development of non-invasive methods for establishing a rapid diagnosis.
8) Saliva will be collected.
CONSENT FORM

At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), research is carried out for a better understanding how to prevent people from being attacked by blood dysentery. We are studying the defense mechanism of the body against the bacteria which cause blood dysentery. We sincerely ask for your cooperation in this study, hoping that it will help us to gain a better insight into the processes involved.

If you kindly agree to participate in this study, you may expect the following:

a) We will collect some saliva from your child on the day of admission and day 7 and day 14 of illness. Your child will be asked to spit into a beaker or we will use a glass dropper.

b) On the same days we will collect a stool sample from your child, using a rectal swab, in case there has been recurrent diarrhoea.

c) We will visit your house to collect the samples in case you have already left the hospital before the completion of the study.

d) Your medical records will be kept confidential.

e) You do not have to participate in the study. Your decision to join or not to join the study will not in any way affect your medical treatment while you are in the hospital. Once you enter the study, you are free to leave the study without any risk of losing your medical care.

f) We will be ready to answer any of your questions concerning the study.

If you agree to participate in this study, please sign your name here.

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Signature (left thumb impression) of patient or guardian

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Signature of investigator

Date


"প্রযুক্তি গতি"

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আনুষ্ঠানিক উদ্যানের গবেষণা কেন্দ্র, বাংলাদেশ এর রাজশাহী অঞ্চলের অভ্যন্তর থেকে বিভাগে রক্ষা গবেষণা করার জন্য এই প্রক্রিয়া চলমান হয় । আরো রক্ষা গবেষণার কারণ বে নাটকের সার্বিক সার্ব্বনাশী ধর্মের একটি বিষয় হয় তাহে এই গবেষণা করে আপনার সত্ত্বায় ব্যক্তি করিতে পারে, এই গবেষণার পর গতি হয় যে বাইরে গতি হয় নাই যাহা গতি হয় ।

এখন আপনি এই কর্মপ্রবেশ অংশ গ্রহণে ইচ্ছুক হন তবে নিম্নলিখিত বিষয়ের তথ্যগুলোর আপনাকে সর্বনিম্ন দিকে হবে ।

1। আপনার আগবর্তী নিশ্চিত হয় যে কিছু লামা (গ্রুপ) নিয়ে এই দিনের বিষয় হবে এবং অস্ত্রাদির প্রথম এবং দ্বিতীয় দিনে আপনার বিশ্বাসে একটি গার্ড চুলি চালু করে আমার একটি বাণী করছে যার অনুসারে হবে নাই হবে অভ্যন্তর চালু করে নাই হবে ।

2। তোমাদের এই নিবন্ধের মাধ্যমে আপনার নিশ্চিত হয় যে পোলিশনার সার্ব্বিরের করে সংগঠন করা হবে বলি আমার চালান চালান হবে ।

3। বলি আপনি কর্মপ্রবাহ এথাল্যান্ডে বাহি চলে যাবে আপনা আপনার বাহি চলে যাবে (পোলিশনার) সংগঠন করবে ।

4। আপনার কর্ম চালনার বল্ল গর পোলিশন রাখা হবে ।

5। আপনার এই কর্মপ্রবেশ করোনার নামে, আপনার নিজস্ব করা যা আপনি এই কর্মপ্রবেশ একবার অপ্রমাণ করবে, তদ্বিপদে যে কোন সদরই আপনি এই কর্মপ্রবেশের প্রতি উপস্থাপন করলে এটি আপনার হাতগুলোর সুবিধা পেতে কোন পৃথপিছু দৃষ্টি হবে না ।

6। আপনা এই কর্মপ্রবেশ করতে যে কোন ধরণের সমর্থন দিয়ে তাই আপনি ।

আপনি এই গবেষণা কর্মপ্রবেশ রাখো হেন একবারের এই সর্ব্বচেষ্টা পরে নই অথচ বাচ যাদের জন্য গুরুত্বপূর্ণ হয় নিয় ।

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গুরুর/রোগীর নাম হাতের রস্কাঙ্গুলির হাঁট নিয় ।

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পরিচয়ের গৃহীত চারিদিঘি ।
CONSENT FORM

At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), research is carried out for a better understanding how to prevent people from being attacked by blood dysentery. We are studying the defense mechanism of the body against the bacteria which cause blood dysentery. We sincerely ask for your cooperation in this study, hoping that it will help us to gain a better insight into the processes involved.

If you kindly agree to participate in this study, you may expect the following:

a) We will collect some saliva from your child on the day of admission and day 7 and day 14 of illness. Your child will be asked to spit into a beaker or we will use a glass dropper.

b) On the same days we will collect a stool sample from your child, using a rectal swab, in case there has been recurrent diarrhoea.

c) In case you have left the hospital before the completion of the study, we would like you to visit the hospital on day 7 and/or day 14 of illness in order to collect the samples.

d) Your medical records will be kept confidential.

e) You do not have to participate in the study. Your decision to join or not to join the study will not in any way affect your medical treatment while you are in the hospital. Once you enter the study, you are free to leave the study without any risk of loosing your medical care.

f) We will be ready to answer any of your questions concerning the study.

If you agree to participate in this study, please sign your name here.

________________________________________
Signature (left thumb impression)
of patient or guardian

________________________________________
Signature of investigator

________________________
Date
"সন্দি পদের নামে"  

(2CDR,B)  
আসামীরিক ইউরোপ গবেষণা কেন্দ্র, বাংলাদেশ এ রূপ আমাদের আকর্ষণ থেকে বিভাগে রকা শুরু চায় যে গবেষণা চালাতে। আমরা রকা আমাদের কাজ হয়। ব্যাকটেরিয়া তাদের পরিপ্রেক্ষিতের বা পরিস্থিতি ব্যবস্থা তা বুঝতে চেষ্টা করি। এই গবেষণা করে আমরা এর ধারণার তালিকায় সমস্যাকরণ করি, যা আমাদের এই গবেষণার বিষয়কে পরিচালনায় বুঝতে পারিস্থিতিক।

যদি আপনি এই কার্যক্রম অংশ গ্রহণ ইচ্ছুক হন তবে নিবন্ধিত বিষয়গুলোতে আপনাকে সন্দেহ নিয়ে চাহে না-

1। আমরা আপনার সিদ্ধান্তের রূপ থেকে কিন্তু প্লান (শ্রুতি) নিয়ে যে নিয়ে একটি চর্চা হয় এবং অনুশীলন সম্পন্ন এবং চর্চা নিয়ে আপনার সিদ্ধান্তের একটি প্লান থেকে হবে অথবা একটি চর্চা সম্পন্ন করা হবে।

2। সেই ক্ষেত্রে বিশেষায়তন আপনার সিদ্ধান্ত হল প্লেইথার রেকর্ড গোষ্ঠী নিয়ন্ত্রণ করে সাধারণ হবে যদি ব্যাংকরিতা চাপ থাকে।

3। প্রয়োজনের বোধ থেকে আপনার বাড়ি থেকে আরা অনুশীলন ৭ থেকে ১৪সালের মধ্যে সমূহ সম্পন্ন করে যদি আপনি কার্যক্রম অনলাইনের মাধ্যমে হয় তাহলে বাড়ি চালু হবে।

4। আপনার সমস্ত আপনার বাড়ি পর লোগন বাধা হবে।

5। আপনার এই কার্যক্রম মোটামুটি যে আমাদের ভাব, আপনার বাধা করা হবে বা না করে কোন কোন কোন করার বাণিজ্যের বুদ্ধিসম্পদের বাণিজ্য বর্তমান হবে না, যদি আপনি এই কার্যক্রম একবার পরিপ্রেক্ষিত করেন, হয় তাহলে কোন মতো আপনি এই কার্যক্রম থেকে অবহেলা হয়ে পারেন এবং আপনার হালাতদের মূল্যায়ন থেকে কোন অপরিসীম দক্ষতা হবে না।

6। আমরা এই কার্যক্রম সমূহের বে কোন ধরণের প্লেইথার উচ্চতার নিয়ে চেষ্টা করি।

আপনি এই গবেষণা কার্যক্রম রাজি হলে আমাদের এই সন্দেহ পরে নাই যদিও বাড়ি হয়ের সম্প্রতি।

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প্রকাশক ও লেখকের শুভ আশা ও বৃদ্ধিসম্পদির শেষ নিবন্ধ

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প্রকাশকের মুখ্য  চারিদিন
To the parents of 

At the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) in Dhaka research is carried out on all aspects of diarrhoeal diseases. Studies are carried out on the causes and treatment of diarrhoea, socio-economic aspects involved and also vaccine development.

We are planning to study the immune response of the body against the bacteria *Shigella* which cause bacillary dysentery. For this purpose, we will use saliva samples from children with shigellosis. We also need "control" saliva samples from healthy children without any history of bacillary dysentery and from non-endemic parts of the world.

We, therefore, would like to ask for your cooperation by giving us permission to collect some saliva (1-2 ml) from your child. The saliva will be collected at the American International School. We will call the child down from class for five minutes and he/she will just have to spit into a beaker. Besides this, we would like you to fill in the attached questionnaire.

All data will be handled with utmost discretion.

In case you have any further inquiries, please contact us at ICDDR,B (phone 600171-8, extension 243/286, Constance Schultz).

Thanking you for your cooperation.

Constance Schultz
Research Fellow

Dr. Firdausi Qadri
Scientist

Dr. Ivan Ciznar
Associate Director
Laboratory Sciences Division
QUESTIONNAIRE

1. Are you willing to help us in this study by permitting your child to participate?
   YES   NO

   If "yes", please answer the following questions:

2. Did your child have diarrhoea after arrival in Bangladesh?
   YES   NO

3. In case of diarrhoea, was a stool sample examined?
   YES   NO

   Diagnosis

4. Were there any family members who contracted diarrhoea after arrival in Bangladesh?
   YES   NO

5. In case of diarrhoea, were stool samples examined?
   YES   NO

   Diagnosis

6. Where did your child live in the previous years, before coming to Bangladesh?
DATA FORM

NAME : 

AGE : 

I.C. No. : 

STUDY NO. : PS 

-----------------------------------------------

ON ADMISSION : ______________________________ (DATE) 
- DURATION OF DIARRHOEA : _______ DAYS (MAX. 4 DAYS) 
- ASPECT OF STOOL : ________________________________ 
- GRADE OF DEHYDRATION : MILD [ ] MODERATE [ ] SEVERE [ ] 
- DIAGNOSIS : S. dysenteriae 1 [ ] 
- S. flexneri [ ] 
- TREATMENT : 

- SALIVA SAMPLE COLLECTED : YES [ ] NO [ ] 

-----------------------------------------------

DAY 7 : ______________________________ (DATE) 
- SALIVA SAMPLE COLLECTED : YES [ ] NO [ ] 
- RECTAL SWAB COLLECTED : YES [ ] NO [ ] 
- RESULT : 

-----------------------------------------------

DAY 14 : ______________________________ (DATE) 
- SALIVA SAMPLE COLLECTED : YES [ ] NO [ ] 
- RECTAL SWAB COLLECTED : YES [ ] NO [ ] 
- RESULT : 

-----------------------------------------------
<table>
<thead>
<tr>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan-June</td>
<td>Whole Ye</td>
<td>Proposed</td>
</tr>
<tr>
<td>Draft amount:</td>
<td>USD</td>
<td></td>
</tr>
<tr>
<td>Completion Date: May 1999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starting Date: May 1998</td>
<td></td>
<td></td>
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<tr>
<td>Name of P.I. / Branch Head / Division Head:</td>
<td>Conference Schultze / Dr. Jean Clarke</td>
<td></td>
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<tr>
<td>Department / Secretary for Science:</td>
<td>Laboratory Sciences Division</td>
<td></td>
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<td>Proposal Name:</td>
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Page 1 of 22
<table>
<thead>
<tr>
<th>Description</th>
<th>No. of Positions</th>
<th>No. of Man Months</th>
<th>$ Amount</th>
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<tbody>
<tr>
<td>1. Direct Project/Protocol/Branch Staff at 1.1.1988</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sourced from Page 3</td>
<td></td>
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<td>2. Add:</td>
<td></td>
<td></td>
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<tr>
<td>New Recruitments</td>
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<td></td>
<td></td>
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<tr>
<td>Sourced from Page 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manpower allocated from other area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sourced from Page 5</td>
<td></td>
<td></td>
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<td>(i) Sub-Total</td>
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<tr>
<td>3. ESA:</td>
<td></td>
<td></td>
<td></td>
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<td>Separations</td>
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</tr>
<tr>
<td>Sourced from Page 6</td>
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<td></td>
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</tr>
<tr>
<td>Manpower allocated to other area</td>
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</tr>
<tr>
<td>Sourced from Page 7</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(ii) Sub-Total</td>
<td></td>
<td></td>
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<tr>
<td>(i)-(ii) TOTAL</td>
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*AGREES WITH PAGE 1 A/C NO. 3100 COLUMN C

get87:1-3
NEW RECRUITMENT—(LOCAL STAFF)—1988

<table>
<thead>
<tr>
<th>Job Title</th>
<th>Start</th>
<th>No.</th>
<th>No. of</th>
<th>**Rate</th>
<th>Level</th>
<th>Date</th>
<th>of</th>
<th>months</th>
<th>of man</th>
<th>per</th>
<th>$ Amount</th>
<th>31,1988</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research Officer</td>
<td>GS-5</td>
<td>May '88</td>
<td>1</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td>352</td>
<td></td>
<td></td>
<td>4,224</td>
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</tr>
</tbody>
</table>

TOTAL: $:XXXXXXXX: 12:XXXXXXXX: *4,224

** See Annexure-A for rates

* AGREES WITH PAGE 2 ROW B
### MANPOWER ALLOCATED FROM OTHER AREA (LOCAL STAFF) - 1988

<table>
<thead>
<tr>
<th>Name &amp; Job Title</th>
<th>Level</th>
<th>Code of other area</th>
<th>No. of posit.</th>
<th>No. of months</th>
<th><strong>Rate per month</strong></th>
<th>$ Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Health Worker</td>
<td>GS-3</td>
<td>23 01 20</td>
<td>1</td>
<td>2</td>
<td>219</td>
<td>438</td>
</tr>
<tr>
<td>Male Health Worker</td>
<td>GS-3</td>
<td>23 01 20</td>
<td>1</td>
<td>2</td>
<td>219</td>
<td>438</td>
</tr>
</tbody>
</table>

**TOTAL**

* 2
* 4
* 876
* 438

**See Annexure-C for average rate/month**

* AGREES WITH PAGE 2 ROW C**
## SUPPLIES AND MATERIALS-1988

<table>
<thead>
<tr>
<th>A/C Code</th>
<th>Item Description</th>
<th>$ Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>3701</td>
<td>Drugs (used for medication in the hospitals and field stations)</td>
<td></td>
</tr>
<tr>
<td>3702</td>
<td>Glassware (Bottle, beaker, cylinder, petridish, aluminium seal, slides, stopper, tube etc.)</td>
<td>300</td>
</tr>
<tr>
<td>3703</td>
<td>Hospital supplies (bandage, gauze, blade, bowl, catheter, cotton, needle, syringe, solution, leukoplast, towel etc.)</td>
<td></td>
</tr>
<tr>
<td>3704</td>
<td>Stationery and office supplies (Battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)</td>
<td>200</td>
</tr>
<tr>
<td>3705</td>
<td>Chemicals and media (Acid, reagent, dextrose, sodium, bactoagar etc.)</td>
<td>1,250</td>
</tr>
<tr>
<td>3706</td>
<td>Materials for uniform (Cloth, button etc. required for making uniforms)</td>
<td></td>
</tr>
<tr>
<td>3707</td>
<td>Fuel, oil and lubricants (Diesel, mobil, petrol, kerosene etc.)</td>
<td></td>
</tr>
<tr>
<td>3708</td>
<td>Laboratory supplies (Aluminium foil, bag, blade, brush, cap, container, film X-Ray etc.)</td>
<td>50</td>
</tr>
<tr>
<td>3709</td>
<td>Housekeeping supplies (Aerosol, battery, wiping cloth, duster, lock and key etc.)</td>
<td>50</td>
</tr>
<tr>
<td>3710</td>
<td>Janitorial supplies (Bleaching powder, brush, detol, detergent, insecticide, soap etc.)</td>
<td></td>
</tr>
</tbody>
</table>

Page total (balance c/f) 1,850

(Contd. to page No. 18)
**SUPPLIES AND MATERIALS-1988**

(Contd. from Page No. 17)

<table>
<thead>
<tr>
<th>A/C Code</th>
<th>Item Description</th>
<th>$ Amount</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>Page total from page No. 17 (balance b/f):</strong> 1,850</td>
<td></td>
</tr>
<tr>
<td>3711</td>
<td><strong>Tools and spares</strong> (Automobile spares, tyres, tubes, battery, stores required for maintenance services etc.)</td>
<td></td>
</tr>
<tr>
<td>3712</td>
<td><strong>Non-stock supplies</strong> (Materials not normally kept in stock and purchased only against specific requisitions)</td>
<td>2,000</td>
</tr>
<tr>
<td></td>
<td><strong>Sub-Total</strong></td>
<td>3,850</td>
</tr>
<tr>
<td>3713</td>
<td><strong>Freight and other charges</strong> &lt;br&gt;Add 30% to above sub-total for imports</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td><strong>TOTAL</strong></td>
<td>$4,450</td>
</tr>
</tbody>
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*AGREES WITH PAGE 1 <br>A/C 3700 <br>COLUMN C*

Note: For rates please contact Supply Ext. 260. <br>Add 10% for inflation

Budget 87.18
<table>
<thead>
<tr>
<th>A/C Code</th>
<th>Accounts Description</th>
<th>$ Amount</th>
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<tbody>
<tr>
<td>3800</td>
<td><strong>Repair and maintenance</strong>&lt;br&gt;(Maintenance and repairs of vehicles, equipments, furniture and building)</td>
<td></td>
</tr>
<tr>
<td>3900</td>
<td><strong>Rent, communication and utilities</strong>&lt;br&gt;(Postage, telephone, telegram, electricity etc.)</td>
<td>100</td>
</tr>
<tr>
<td>4100</td>
<td><strong>Bank charges</strong></td>
<td></td>
</tr>
<tr>
<td>4200</td>
<td><strong>Legal and professional expenses</strong>&lt;br&gt;(Professional membership fee, legal fee, audit fee etc.)</td>
<td></td>
</tr>
<tr>
<td>4300</td>
<td><strong>Printing and publication</strong>&lt;br&gt;(Printing of forms, books, journals, reprints etc.)</td>
<td>100</td>
</tr>
<tr>
<td>4400</td>
<td><strong>Hospitality &amp; donation</strong>&lt;br&gt;(Guest house accommodation, donations, hospital food, lunch, refreshment etc.)</td>
<td>100</td>
</tr>
<tr>
<td>4500</td>
<td><strong>Service charges</strong>&lt;br&gt;(Porter, labour, washing, laundry and other misc. exp.)</td>
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</tr>
<tr>
<td>4600</td>
<td><strong>Staff development and training</strong>&lt;br&gt;(Training course fee, training materials, stipend, scholarship, subsistence paid to the staff)</td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL** * 300

*AGREES WITH*

PAGE 1
A/C No. 4000
COLUMN C

Budget 87.19
**INTERDEPARTMENTAL SERVICES-1988**

<table>
<thead>
<tr>
<th>A/C Code</th>
<th>Service Area</th>
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<tbody>
<tr>
<td>4801</td>
<td>Computer</td>
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</tr>
<tr>
<td>4802</td>
<td>Transport Dhaka</td>
<td>500</td>
</tr>
<tr>
<td>4803</td>
<td>Transport Matlab</td>
<td>500</td>
</tr>
<tr>
<td>4804</td>
<td>Water transport-Matlab</td>
<td>500</td>
</tr>
<tr>
<td>4805</td>
<td>Transport Teknaf</td>
<td></td>
</tr>
<tr>
<td>4808</td>
<td>Xerox and mimeograph</td>
<td>100</td>
</tr>
<tr>
<td>4807</td>
<td>Pathology</td>
<td></td>
</tr>
<tr>
<td>4808</td>
<td>Microbiology tests</td>
<td></td>
</tr>
<tr>
<td>4809</td>
<td>Biochemistry</td>
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</tr>
<tr>
<td>4810</td>
<td>X-Ray</td>
<td></td>
</tr>
<tr>
<td>4811</td>
<td>I.V. fluid</td>
<td></td>
</tr>
<tr>
<td>4812</td>
<td>Media</td>
<td></td>
</tr>
<tr>
<td>4813</td>
<td>Patient hospitalisation study</td>
<td></td>
</tr>
<tr>
<td>4814</td>
<td>Animal research</td>
<td></td>
</tr>
<tr>
<td>4815</td>
<td>Medical illustration</td>
<td>400</td>
</tr>
<tr>
<td>4817</td>
<td>Telex</td>
<td></td>
</tr>
<tr>
<td>4818</td>
<td>Out patient care</td>
<td></td>
</tr>
<tr>
<td>4819</td>
<td>Maintenance charges</td>
<td></td>
</tr>
<tr>
<td>4820</td>
<td>Vehicle maintenance charges</td>
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</tr>
<tr>
<td>4821</td>
<td>Library service charges</td>
<td>50</td>
</tr>
<tr>
<td>4822</td>
<td>Staff Clinic Charges - Dhaka</td>
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</tr>
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(Contd. to page No. 21)