Attachment 1.

Date 25.10.87

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Khaleda Haider

Trainee Investigator (if any) 20

Application No. 87-025

Supporting Agency (if Non-ICDDR,B)

Title of Study Association of extra-cellular hydrolytic enzyme with virulence of Shigellae

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: NA
   (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) NO 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: 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.

Khaleda Haider
Principal Investigator

Trainee

OCT 28 1987

SECTION I - RESEARCH PROTOCOL

1. Title : Association of extracellular hydrolytic enzymes with virulence of Shigellae.

2. Principal Investigator : Khaleda Haider

   Co-Investigators : Dr.Md. Showkat Ali
   Dr. F. Qadri

   Consultants : Dr. Ivan Ciznar

3. Starting date : December 01,1987

4. Completion date : December 30, 1988

5. Total Direct Cost : US$ 25,286.00

6. Scientific Program Head : Dr. Ivan Ciznar

This protocol has been approved by the Laboratory Sciences & Epidemiology Division.

Signature Ivan Ciznar

Date Oct. 25, 1987

7. Abstract Summary:

The present investigation aims to correlate the presence of extracellular enzymes with virulence in Shigellae. Qualitative and quantitative difference will be determined in enzymes present in each pairs of virulent and avirulent strains that will be used in the study. For these purposes 5 pairs of strains each of S. dysenteriae 1, S. flexneri 2a, S. boydii (12-15) and S. sonnei Form I will be studied. In addition rough mutants of S. dysenteriae 1 and S. sonnei will also be screened (5 each).

It is hoped that this study may give us information regarding the qualitative and quantitative differences in extra cellular enzymes produced by different Shigella species. In addition to this differences between isogenic pairs of virulent and avirulent strains will be helpful in understanding the role of enzymes in pathogenecity of the bacteria.

8. Reviews:

a. Ethical Review Committee _____

b. Research Review Commitee _____

c. Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objectives:

To study the extracellular enzymes secreted by *Shigella* species and to see if qualitative and quantitative differences exists between virulent and avirulent pairs of isogenic strains.

2. Background:

A. Extracellular enzymes in some gram negative bacterial pathogens other than Shigella.

Extracellular hydrolytic enzymes have been implicated as important virulence factors in several microbial diseases including those caused by Erwina (1), *Pseudomonas aeruginosa* (2), *Serratia marceseus* (3) and *Vibrio cholerae* (4). Levels of the enzyme, Aminopeptidase, has been used to distinguish between virulent and avirulent *Erwina* species (1). The hydrolytic enzymes have also been used by other investigators to identify member of the family Enterobacteriaceae (5). Mucinase, lecithinase, collagenase, and neuraminidase have been detected in *Vibrio cholerae* (6,7). Neuraminidase is thought to play an important role in the pathogenesis of *V. cholerae* (8). It has been shown that mutants of *V. cholerae*, that were deficient in protease production, showed reduced proteolytic activity and showed a dramatic loss of virulence in the infant mouse, although toxin was still produced by them. A number of investigators have shown that the mucinolytic enzyme in *V. cholerae* is able to produce desquamation of the intestinal mucosa of guinea pigs and increase the permeability of the intestinal wall of mice (9). It has also been shown that

intestinal contents of animals experimentally infected with cholera organisms contain mucinolytic activity (6). Interestingly it was also observed that sera of convalescent cholera patients contains antibodies which neutralizes the action of mucinase (9). All these studies suggest that mucinolytic enzymes may have some role to play in the pathogenecity of V. cholera.

B. Extra cellular enzymes in Shigellae.

Shigellosis is a complex infection and still remains a significant cause of morbidity and mortality in developing countries.

Members of the genus Shigella when ingested by man can cause watery diarrhoea followed by dysentery syndrome (10). The fundamental characteristics of virulence caused by Shigellae is their ability to adhere and to invade epithelial cells and subsequently multiply therein. After invasion, the proliferating Shigella elaborates an enterotoxin, although its role in the initiation of diarrhoea is controversial. The ability to adhere to epithelial surfaces is an important factor for the pathogenesis of such organisms.

A literature survey on Shigella studies have indicated that enzymes may be useful for identification purposes or as markers of virulence.

1. Enzymes as tools for identification : Esterases have been used to differentiate Shigella flexneri 6 from S. flexneri 1-5 (11). Gamma-glutamyltransferase was detected in S. dysenteriae 3-9, S.

flexneri 6, some S. boydii serotypes but absent in most other Shigella species and serotypes that were studied (12). Similarly report on the use of aminopeptidases for identification of Shigella using the chromagenic substrate, chromozym PL has been suggested (13).

2. Enzymes in Virulence : In 1956 Formal and Lowenthal (9) found mucinase to be present in some strains of S. flexneri 2a, X and Y variants, although it was not found in other S. dysenteriae, S. sonnei and S. boydii strains. In the next 20 years following this observation relatively little work was done to study the extracellular enzyme patterns in Shigella species. However, in the last decade interest on this subject has been revived and several studies have been carried out which suggest that enzymes may be used as markers of virulence. Important among these are Glycerol kinase produced by virulent strains of S. flexneri 2a (14). It was shown that a smooth colonial variant of S. flexneri 2a lost the ability to penetrate HeLa cells and also lost Glycerol kinase activity. Transduction of the Glycerol kinase gene of Shigella into the avirulent Shigella strains restored the ability of 50% of the strains to penetrate HeLa cells. On the basis of this observation it was suggested that more than one gene affects Glycerol kinase activity in Shigella, of which only one may be associated with penetration. In another study Belaia et al. (15) have shown that the activity of Cathepsin D, a lysozomal enzyme, in the splenocytes depends on the virulence of the Shigella strain. Virulent Shigella strains induced pathological labilization of the lysosomal membranes whereas avirulent strains

induced only the transient activity of the enzyme in the lysosomes without any essential changes in the membrane permeability . On the basis of these data it has been pointed out that there is a possibility of differentiating virulent and avirulent Shigella strains by determining the enzyme activity of splenocytes in infected animals.

It is now presumed that the process of penetration by Shigella into epithelium is connected with the enzymatic hydrolysis of the cell wall. It has been shown that S. flexneri 4b produces blood group B alpha glycosidase and it was suggested that S. flexneri 4b could proliferate within the ileocolonic environment by enzymatically degrading mucin glycoprotein sugars (16). Further studies on the role of enzymes in the bacterial penetration or adhesion by modifying surface component of the epithelial cells have however not been carried out. The mechanism by which Shigella attaches to the epithelial surface has not been clarified but evidence suggests that a number of factors are responsible for such attachment. LaBrec et al. (17) postulated that epithelial cell penetration is an essential step in shigellosis. Recently Hale and coworkers (18,19) have shown that penetration into tissue culture cells require active metabolic participation from both the bacteria and the host cell. Therefore, it is understandable that the role of extracellular enzymes may be related to the pathogenic potential of Shigella and as such may be important (20).

Since, not enough is known regarding the relationship of enzymatic activities to pathogenicity in Shigella, the present investigation has been undertaken.

3. **Rationale:**

Several attributes have been identified by in vitro and in vivo studies as being necessary for expression of virulence by Shigella. Among these attributes, toxin production and plasmid mediated invasiveness have been shown to be important but biochemical basis of pathogenesis remain obscure. The present study on enzymatic role in virulence will be of great value from biochemical as well as immunological point of view. Results of this research will give us information whether extracellular hydrolytic enzymes has any correlation quantitatively and/or qualitatively with their respective virulence factor or factors. As a corollary to the above, this study may help to develop an additional diagnostic technique using enzyme assays to differentiate between serotypes of Shigella species which may further provide a rapid and sensitive method for the diagnosis of Shigella species over existing methodologies.

B. **SPECIFIC AIMS**

1. To detect presence and levels of hydrolytic enzymes present in the culture filtrate obtained from growth of 5 pairs of isogenic strains each of S. dysenteriae 1, S. flexneri 2a, S. boydii (12-15), and S. sonnei Form I; and rough variants of S. dysenteriae 1 and S. sonnei.

2. To see if differences in enzyme activity can be detected between (a) virulent and naturally occuring avirulent smooth strains, (b) rough variants isolated in the laboratory.

3. The results obtained from the above study may help to understand which hydrolytic enzymes should be used to set up a method based on enzyme assays for easy classification of strains of Shigellae.

C. MATERIALS AND METHODS

1. Bacterial Strains:

List of bacterial strains which will be used in this study are shown in Table - 1.

Table - 1

Strains used in the study

Shigella species	Strain No.	Sereny	Congored binding
S. dysenteriae 1	Z-24623	+ & -	+ & -
	Z-7828	+ & -	+ & -
	Z-26406	+ & -	+ & -
	Z-1670	+ & -	+ & -
	Z-3351	+ & -	+ & -
S. flexneri 2a	Z-10957	+ & -	+ & -
	Z-611	+ & -	+ & -
	Z-613	+ & -	+ & -
	Z-1506	+ & -	+ & -
	A-18	+ & -	+ & -
S. sonnei Form I (smooth)	Z-6570	+	+
	Z-13020	+	+
	Z-6142	+	+
	Z-6362	+	+
	Z-6577	+	+
Form II (rough)	Z-27684	-	-
	Z-19784	-	-
	Z-14007	-	-
	Z-13030	-	-
	Z-13188	-	-
S. boydii (12-15)	Z-10854	+ & -	+ & -
	Z-7879	+ & -	+ & -
	Z-10426	+ & -	+ & -
	Z-31145	+ & -	+ & -
	Z-24873	+ & -	+ & -

All these strains of virulent and avirulent strains will be grown in different growth media i.e. Trypticase Soy broth with 0.3% yeast extract, and Minimal medium, and their enzyme activities

will be measured to find out the optimal growth condition for producing optimum level of activities of each enzyme. Growth curve relating CFU/ml to optical density at 600 nm for each Shigella species in appropriate media will be carried out.

Initial screening of the enzymatic activities of the Shigella strains will be assayed qualitatively using API ZYM kit (ADI system, S.A., France) (21). The 19 enzymes which can be assayed by this kit are Acid phosphatase, Alkaline phosphatase, Esterase, Lipase esterase, Lipase, Leucine arylamidase, Valine arylamidase, Cystine arylamidase, Trypsin, Chymotrypsin, Naphthol - As-BI-phosphohydrolase, Alpha-galactosidase, Beta-gulactosidase, Beta glucuronidase, Alpha-glucosidase, Beta-glucosidase, N-acetyle-beta-glucosaminidase, Alpha-mnannosidase and Alpha-fucosidase. Those enzymes which will be detected by API ZYM kit from virulent Shigella strains will be used for further investigation.

2. **Experimental procedures:**

Strains of Shigellae stored at -70 °C will be cultured on MacConkey's Agar at 37 °C for 18 hrs. Single isolated colonies will be picked up from the plate and will be grown in TSB with 0.3% yeast extract at 37 °C for 6 h. Aliquots of 0.1 ml of the culture will be used as starter culture for inoculate experimental medium and incubated at 37 °C with continuous shaking for 18 h. At least triplicate estimation will be carried out for each strain. Optical density of the Culture media will be determined at 600 nm and the number of bacterial cells present in the culture will be determined. Also 1 ml of the growth media will be diluted in PBS and plated on TSA agar to determine the

total number of cells. Appropriate broth culture will be used for culturing Shigella strains for a fixed time at 37°C.

The culture will then be harvested at 12,000 g for 15 min at 10°C and the supernatant will be filtered through a millipore filter (0.45 mu). The filtrate will be then dialysed against appropriate buffer with constant stiring and atleast with 2 changes at 4°C for 18 h. The dialysed culture supernatant will be used for the assay of enzyme activity. The pellet will be washed with sterile phosphate buffer saline (PBS) and the total number of cells will be determined by diluting the cells in PBS and plating on TSA plates with 0.3% yeast extract.

3. **Enzyme assay**

For the enzyme assays described below two kinds of reagent blanks will be used. One will contain all the solution except substrate while another will contain everything except culture supernatant.

Protease:

Proteolytic activity of the culture filtrate will be measured according to the method of RinderKnecht et al. (22) using Hide powder Azure as substrate. Reaction mixture will contain in a final volume of 1 ml, 20 mg of substrate, 20 mM Tris-HCl (pH 7.8) and culture filtrate. After incubation at 37°C, tubes will be centrifuged in an eppendorf microfuge, and the absorbance of the supernatant fractions will be determined at 595 nm.

Phosphatases:

Acid phosphatase and alkaline phosphatase activity will be assayed according to the method described by Walter and Schutt (23). The reaction mixture will contain 1 mM P-nitrophenyl phosphate, 0.1 ml of culture supernatant and buffer in a final volume of 0.5 ml. Buffer to be used for acid phosphatase and alkaline phosphatase respectively are 50 mM citrate buffer (pH 5.5) and 50 mM glycine-NaOH buffer at pH 10.5. The P-nitrophenol liberated after 1 hr incubation at 37 °C will be estimated colorimetrically at 410 nm.

Glycosidases:

For the standard routine procedure, the reaction mixture will contain 0.1 M tris phosphate (pH 5.6) and P-Nitrophenyl - alpha /beta sugar pyranoside and will be incubated at 37 °C for 1 hr Glycosidase activity will be detected by measuring the liberated P-nitrophenol at alkaline pH at 410 nm (24).

Amino Peptidase:

Amino peptidase activity will be assayed as described by Giammanco et al., (25). The incubation mixture for the measurement of amino peptidase activity will contain 100 mM Tris - PO_4 pH-7-.8, 1 mM amino acid - napthylamides and the culture supernatant in total volume of 1.0 ml. The reaction will be stopped by adding fast blue BB and the absorbance at 400 nm will be measured.

Mucinase:

Culture supernatant will be assayed for mucinase activity by the method of Kusama and Craig (26). Mucine will be prepared in phosphate buffered saline and 50 ml volumes of serial two fold dilution will be

dispensed into the wells of a microtitre plate. Culture supernatant will be added and reaction volumes will be made upto 0.1 ml with PBS. After 1 hr incubation at 37 °C 25 ul of Cetyltrimethyl ammonium bromide (1% wt/vol) will be added, and the resulting turbidiy will be assayed after 5 min. by measuring the absorbance at 492 nm.

Lipase:

Lipase activity will be determined according to the method of Wretlind et al. (27) using P-nitrophenyl caprylate. The assay solution will contain 7 mM substrate, 100 mM Na-phosphate buffer pH 7.4, and culture filtrate in a final volume of 1 ml. Optical density of the solution will be measured at 410 nm.

Phospholipase:

Phospholipase activity will be assayed according to the method of Dawson (28). The assay solution will contain 0.03 M lysolecithin, 0.3 M phosphate buffer (pH 6.4) and culture filtrate in a final volume of 3 ml. Optical density of the solution will be measured at 520 nm.

Neuraminidase:

Neuraminidase activity will be determined by the method of Aminoff (29). The enzyme activities will be assayed by releasing. N-acetyl neuraminic acid from sialomucoid in a 0.1 M accetate buffer (pH 5.5). The dialysed supernatant will be incubated with the mucin solution. The final volume of the reaction mixture will be made up to 0.5 ml with the buffer. The amount of N-acetyl neuraminic acid released and the amount bound in the assay mixtures will be determined by the thiobarbituric acid method of Aminoff.

Enzyme Unit:

Unit of enzyme activity for the enzymes (glycosidases, aminopeptidase, acid and alkaline phosphatases, lipase, phospholipase, neuraminidase and esterase) is defined as that amount which hydrolyses 1 u mol of substrate per min. under the experimental conditions.. One unit of protease and mucinase is defined as the amount which hydrolyses 1 mg of substrate per hour under experimental condition. Enzyme activity will be measured at appropriate wavelengths in the Pye Unicam recording spectrophotometer.

Table - 2: Enzyme classes to be tested and nature of enzymatic reaction.

Enzyme class	Enzyme number (Enzyme commission)	Substrate class	Bond attacked	Product released.
Glycosidases	EC 3.2.21-24, 30-31	P-No2-phenyl glycosides	glycosidic	P-NO2-phenol.
Phosphatases:				
Alkaline Acid	EC 3.1.3.1 EC 3.1.3.2	P-NO2- phenyl-phosphate	phosphoester	-do-
Aminopeptidases	EC 3.2.1.18	Aminoacid-Beta-napthylamides	amide	Beta-napthyl amine
Lipase	EC 3.1.1.3	P-NO2-Phenyl Falty acid	ester	P-NO2 phenol
Esterase	EC 3.1.1.1	Beta-napthol,ester	ester	Beta-napthol
Protease	EC 3.4.21.3	Hide powder	amide	Remazobrilliant blue
Phospholipase	EC 3.1.1.4	Lyso-lecithin	ester	glycerophosphoryl choline.
Neuraminidase	EC 3.2.1.18	Sialomucoid	ester	N-acetyl neuraminic acid
Mucinase	EC 3.2.1.19	Ovomucin	glycosidic	N-acetyl glucosamine

Table 3: Summary of assay conditions for monitoring enzyme activities.

Enzyme	Substrate concentration	pH	Buffer
Glycosidases	1X10-3M	5.6	0.1M Tris phosphate
Aminopeptidase	1X10-3M	7.8	-do-
Phosphatases	1X10-3M	5.5,10.5	50 mM citrate, 50 mM glycine -NaOH buffer
Protease	20 mg/ml	7.8	0.02 M Tri. Hcl
Lipase	7 x 10-3M	7.4	0.3 M P04
Phospholipase	0.03M	6.4	0.3 M PO4
Neuraminidase	10 mg/ml	5.5	0.1M Acetate buffer
Mucinase	10 mg.ml	7.0	0.1N Na-phosphate
Esterase	1 x 10-3M	7.2	0.1M Tris.Phosphate

4. Virulence Tests:

a) Sereny test will be performed according to the method described by Sereny et al. (30).

b) HeLa cell invasion; (31).

Tissue culture flasks will be seeded with approximately 1.5×10^5 HeLa cells and grown to near confluency. Before bacterial inoculation, monolayers will be washed three times with phosphate buffer saline, pH 7.4. A broth cultures of approximately 10^8 cells will be prepared and 100 µl/well will be added. The plate will be inocubated at 37°C for 1.5 - 2 hrs. Media will then be removed and wells will be washed again. Wells will be refilled with MEM media containing gentamicin (1.5 ml/well) and incubate at 37°C for 1.5 - 2 .hrs. Wells will be washed again with PBS three times. HeLa cells will be lysed by treating with Triton X-100 at a concentration which does not affect the bacteria. The cell suspension containing the bacteria will be counted by direct plating. The number of colonies will be counted and expressed as the percentage of the initial inoculum.

D. SIGNIFICANCE

The proposed study on the analysis of different hydrolytic enzymes may help us to understand the mechanism of virulence and the contributions of these factors to the attachment and adherance of Shigella spp. to the mamalian cells.

F. COLLABORATIVE ARRANGEMENT

This work will be undertaken in collaboration with Dhaka University, Bangladesh. Dr. Showkat Ali, Assistant Professor of the department of Biochemistry, Dhaka University will be actively involved in this project.

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ICDDR,B
1988 BUDGET PROPOSAL
(In US $)

PARTICULARS

Division Name: Laboratory Sciences Division

Protocol/Branch name: Bacterial Genetics

Name of P.I./Branch Head/Division Head: Khaleda Haider

Budget Code: ..... Starting Date: December 01, 1987

Protocol No: ..... Completion Date: December 30, 1988

Donor Name: ..... Grant Amount: 25,286

EXPENSE CATEGORY		Column A: Actual Jan.-June 1987	Column B: Estimated Whole Yr. 1987	Column C: Proposed 1988
A/C No.	Description	Refer Page		
3100	Local Salaries	2		9340
3200	Intl. Salaries	8		1001
3300	Consultants	14		
3500	Travel Local	15		
3600	Travel Intl.	16		
3700	Supplies & Mat.	18		9945
4000	Other Costs	19		600
4800	Inter Deptl. Ser.	21		4400
	Total Direct Operating Cost			25,286
0300	Capital Expenditure Refer Page 22			
	TOTAL DIRECT COST			25,286

P.I./Branch Head Associate Director Reviewed by Budget & Finance

Budget Code: _____

PERSONNEL REQUIREMENT-(LOCAL STAFF) 1988

	No.of Positions	No. of Man Months	$ Amount
A. Direct Project/Protocol/Branch Staff at 1.1.1988 Sourced from Page 3	1	10	5500
Add:	1	12	3840
B. New Recruitments Sourced from Page 4			
C. Manpower allocated from other area Sourced from Page 5			
(i) Sub-Total			
Less:			9340
D. Separations Sourced from Page 6			
E. Manpower allocated to other area Sourced from Page 7			
(ii) Sub-Total			9340
(i)-(ii) TOTAL			*

*AGREES WI
PAGE 1
A/C NO.31
COLUMN C

Budget Code: _____

LOCAL STAFF (EXISTING) AT 1.1.1988

Job title	Level	A No. of Positions 30.6.87	B No.of New Recruits July-Dec.87 (+)	C No. of Separations July-Dec.87 (-)	D No. of Positions at 1.1.88 (A+B-C)	E Man Month at 1.1.88 (D x 12)	F ** Rate per Month	G $ Amount (E x F)
ASSISTANT SCIENTIST	NO.B	1	-	-	1	10	550	5500
				TOTAL	* 1	* 10	XXXXXX	* 5500

** See annexure C for average rate per month.

* AGREES WITH PAGE 2 ROW A

Budget Code:_____

NEW RECRUITMENT-(LOCAL STAFF)-1988

	A	B	C	D	E=(CxD)	F	G=(ExF)
Job Title	Level	Start. date	No. of posit.	No. of months at Dec. 31,1988	No. of man months	**Rate per month	$ Amount
SENIOR RESEARCH OFFICER VI			1	12	12	320	.3840
		TOTAL	* 1	XXXXXXX	* 12	XXXXXXXX	* 3840

** See Annexure-A for rates

* AGREES WITH PAGE 2 ROW B

Budget Code: _____

CONSULTANTS 1988
(Local and International)

Name & Job Title	Travel From-To-From	No. of days	** Perdiem Rate	** Perdiem Total	***Honorarium Rate	***Honorarium Total	**** Travel cost	Total $ Amount
Dr. Showkat Ali	Bangladesh	11 months			US$ 91/-			1001
							TOTAL	*1001

** Check with Finance Ext. 214 for perdiem rate.
*** Check with Personnel Tel. 601100 for honorarium rate.
**** Check with Travel Tel. 602836 for ticket cost. Travel cost includes transit perdiem, ticket cost and ground transportation.

*AGREES WITH PAGE 1 A/C 3300 COLUMN C

Budget Code: _____

SUPPLIES AND MATERIALS-1988

A/C Code	Item Description	$ Amount
3701	**Drugs** (used for medication in the hospitals and field stations)	
3702	**Glassware** (Bottle, beaker, cylinder, petridish, aluminium seal, slides, stopper, tube etc.)	1000
3703	**Hospital supplies** (bandage, gauze, blade, bowl, catheter, cotton, needle, syringe, solution, leukoplast, towel etc.)	
3704	**Stationery and office supplies** (Battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)	500
3705	**Chemicals and media** (Acid, reagent, dextrose, sodium, bactoagar etc.)	1000
3706	**Materials for uniform** (Cloth, button etc. required for making uniforms)	50
3707	**Fuel, oil and lubricants** (Diesel, mobil, petrol, kerosene etc.)	
3708	**Laboratory supplies** (Aluminium foil, bag, blade, brush, cap, container, film X-Ray etc.)	50
3709	**Housekeeping supplies** (Aerosol, battery, wiping cloth, duster, lock and key etc.)	50
3710	**Janitorial supplies** (Bleaching powder, brush, detol, detergent, insecticide, soap etc.)	
	Page total (balance c/f)	2,650

(Contd. to page No. 18)

Budget Code: _____

SUPPLIES AND MATERIALS-1988

(Contd. from Page No. 17)

A/C Code	Item Description	$ Amount
	Page total from page No.17 (balance b/f)	2,650
3711	Tools and spares (Automobile spares, tyres, tubes, battery, stores required for maintenance services etc.)	
3712	Non-stock supplies (Materials not normally kept in stock and purchased only against specific requisitions)	5,000
	Sub-Total	7,650
3713	Freight and other charges Add 30% to above sub-total for imports.	2,295
	TOTAL	* 9,945

*AGREES WITH PAGE 1 A/C 3700 COLUMN C

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

Budget Code: _____

OTHER COST-1988

A/C Code	Accounts Description	$ Amount
3800	**Repairs and maintenance** (Maintenance and repairs of vehicles, equipments, furniture and building)	
3900	**Rent, communication and utilities** (Postage, telephone, telegram, electricity etc.)	100
4100	**Bank charges**	
4200	**Legal and professional expenses** (Professional membership fee, legal fee, audit fee etc.)	
4300	**Printing and publication** (Printing of forms, books, journals, reprints etc.)	500
4400	**Hospitality & donation** (Guest house accommodation, donations, hospital food, lunch, refreshment etc.)	
4500	**Service charges** (Porter, labour, washing, laundry and other misc. exp.)	
4600	**Staff development and training** (Training course fee, training materials, stipend,scholarship, subsistence paid to the staff)	
	TOTAL	* 600

*AGREES WITH PAGE 1 A/C No.4000 COLUMN C

Budget Code: _____

**INTERDEPARTMENTAL SERVICES-1988

A/C Code	Service Area	$ Amount
4801	Computer	1, 200
4802	Transport Dhaka	200
4803	Transport Matlab	
4804	Water transport-Matlab	
4805	Transport Teknaf	
4806	Xerox and mimeograph	500
4807	Pathology	
4808	Microbiology tests	
4809	Biochemistry	
4810	X-Ray	
4811	I.V. fluid	
4812	Media	1,000
4813	Patient hospitalisation study	
4814	Animal research	1,000
4815	Medical illustration	500
4817	Telex	
4818	Out patient care	
4819	Maintenance charges	
4820	Vehicle maintenance charges	
4821	Library service charges	
4822	Staff Clinic Charges - Dhaka	
	Page total (balance c/f)	4400

(Contd. to page No. 21)

Budget Code: _____

**INTERDEPARTMENTAL SERVICES-1988

(Contd. from Page No. 20)

A/C Code	Service Area	$ Amount
	Page total from page # 20 (balance b/f)	4400
4823	Staff Clinic Charges - Matlab	
4824	Bacteriology Test	
4830	Transport Subsidy	
	TOTAL	* 4400

*AGREES WITH PAGE 1 A/C 4800 COLUMN C

** See Annexure-B for rates.

ANNEXURE-A

ICDDR,B

SALARY RATES 1988

Pay Level	Salary and benefits per month US$
***International**	
P5	5,430
P4	4,720
P3	4,200
P2	3,750
P1	3,290
National Officers	
NO F	1,420
NO E	1,110
NO D	820
NO C	600
NO B	480
NO A	410
General Services	
GS 6	320
GS 5	220
GS 4	170
GS 3	140
GS 2	110
GS 1	100
CHW	50

*** US $ 15,000 to be added for new recruits and separations.**

BDGANX.A/(Sept.86)

240 170
12
~~4800~~
5,280/