Attachment 1.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 25-3-87 27/3

Principal Investigator Dr. B. A. Kabir

Application No. 87008P

Title of Study Bacteroides fragilis diarrhea

Trainee Investigator (if any) MRS. TASMINA RAHMAN

Supporting Agency (if Non-ICDDR,B)

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.

Principal Investigator

Trainee

APR - 1 1987

87 cc 8P 29/3

SECTION I - RESEARCH PROTOCOL (Pilot)

1. Title : Preliminary Investigation of the Anaerobic Bacterium Bacteroides fragilis as an etiologic agent of diarrheal disease in Bangladesh

   Short title (Bacteroides fragilis diarrhea)

2. Principal Investigator : Dr. Bradford A. Kay

   Co-investigators : Dr. David A. Sack
   Mrs. Khaleda Haider

   Research Trainee : Mrs. Tasmina Rahman

3. Starting Date : April, 1987

4. Completion Date : September, 1987

5. Total Direct Cost : US $ 4993.52

6. Scientific Program Head :

   This protocol has been approved by the Laboratory Sciences and Epidemiology Division

   Signature of Scientific Program Head : David A. Sack

   Date : 27.3.87

7. Abstract Summary :

Bacteroides fragilis, an anaerobic bacillus and common inhabitant of the intestines of man and animals, has recently been described as a potential cause of human diarrhea. The enteric disease syndrome in man is similar to that recently identified in cattle and sheep. One study has been made in the United States to investigate the role of B. fragiles in human diarrheal disease. In that study enterotoxigenic B. fragilis were reported to be associated with watery diarrhea of one week or more duration and associated with intestinal cramping, often accompanied by fever, vomiting and blood in the stools. This proposal is for a

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective :

The objective of this research is to determine if toxinogenic Bacteroides fragilis is a cause of diarrheal disease in the hospitalized patient population at ICDDR,B.

2. Background

Bacteroides fragilis, a Gram-negative, non-spore-forming, obligately anaerobic bacterium is a common inhabitant of the intestinal tract of warm blooded animals and man. The bacterium has long been recognized as an occasional cause of extra-intestinal anaerobic infections in man (8,9,16) and has recently been recognized as a significant pathogen of animals.

In 1984 Myers et al.(13) described an enterotoxin-like activity of B. fragilis. These toxigenic strains were isolated from lambs with diarrheal disease. These investigators noted that enterotoxic activity was expressed in the calf and lamb ileal loop assay methods (12) but not in the infant mouse method used for detecting Escherichia coli heat stable toxin (ST) (4). The same authors later reported the isolation of toxigenic B. fragilis cultures from diarrheic calves as well as lambs (2,14).

Recent evidence has implicated toxinogenic B. fragilis isolates in human diarrheal illness (15). Enterotoxigenic strains of this organism were isolated from 8 of 44 diarrheic individuals ranging from five months to 69 years of age. Isolates of B.

fragilis made from these patients produced a heat-labile classical enterotoxin. Enterotoxigenic strains were able to kill adult rabbits (with ligated caeca) when 10^9 colony forming units were injected into the ileum. Rabbit disease was characterized by mucoid, bloody diarrhea, and severe necrotizing colitis. The bacterium was noted to colonize the caudal small intestine and colon of the rabbit. Non-enterotoxigenic strains of B. fragilis were unable to establish enteric disease in rabbits.

Diarrheal diseases remain the most common and single greatest health problem in developing countries. However even under ideal laboratory conditions the identification of an etiologic agent is made in only 70-80% of cases. The growing awareness of the profound impact of diarrheal diseases on people in the developing world has warranted intensive research on known agents and the identification of new agents which may cause diarrheal diseases. There is strong suggestive evidence that bacteria in addition to those currently recognized, cause diarrhea. In studies carried out in Kenya (18) and Morocco (19) to evaluate the effect of an antibiotic as a prophylactic for travellers diarrhea, no etiologic agent could be identified in nearly 40% of cases. Thoren et al. (23), studying pediatric diarrhea in Addis Ababa, Ethiopia, could establish microbial etiology in only 70% of patients. Similarly Dupont working with American students newly arrived in Mexico, reported that 22% of diarrheal cases presented with unknown etiology. Black and colleagues (1) reported that in the 9-11 month old age group, a rate of more than 4 episodes of diarrhea per year was common,

and that the etiology of much of these episodes of infectious diarrheal disease remained to be known. Sutoto (21) studied the incidence of diarrhea in North Jakarta. He found that 45% of cases were caused by enteropathogenic bacteria, and rotavirus, but in over 50% of episodes the causative agent could not be determined.

Merson (11) identified the recognition of new etiologic agents and the elucidation of their roles in acute and chronic diarrhea as important areas for future research. Similarly, the Control of Diarrheal Disease program (CDD) of the World Health Organization has set as one of their goals the identification of new viral and bacterial agents as a cause of diarrhea. This protocol may help up to evaluate the potential role of toxigenic B. fragilis in diarrhea.

3. Rationale :

The reasons for the lack of identification of etiology of a third or more of diarrheal specimens are undoubtedly numerous and multifactorial. However, a potential major contributer to this group is undoubtedly the existence unrecognized pathogens. During the past fifteen years many newly described etiologic agents of diarrheal disease have been identified. Among them, enterotoxigenic Escherichia coli is now known to be one of the most frequent causes of diarrheal disease. Recognizing the ability of this normal gut inhabitant to acquire a variety of virulence-associated genes and become etiologic, has permanently altered the way in which so-called "normal flora" is viewed.

Perhaps in a similar fashion, B. fragilis has been considered normal flora in the human intestinal tract. As such it has not been suspected of playing a role in diarrheal illness. However, if mechanisms exist similar to those seen with E. coli, wherein "non pathogenic" strains can become toxinogenic and thus etiologic, this organism may be an unrecognized but significant cause of diarrhea. There is therefore clear evidence for research to be undertaken to rapidly assess the significance of the report by Meyers and colleagues(15).

The recent development of the ICDDR,B anaerobe laboratory and the immediate availability of trained technical staff facilitates the immediate beginning of this investigation.

B. SPECIFIC AIMS

1. to isolate and identify Bacteroides fragilis from patients with diarrheal disease who have had no other pathogen identified.

2. to determine, by various in-vivo and in-vitro tests, the toxigenicity of the B. fragilis isolates made in this study.

3. to characterize the clinical presentation of patients from which toxinogenic B. fragilis have been isolated.

4. to biochemically characterize the bacterial isolates and, chracterize the toxin produced by isolates of B. fragilis.

5. to examine the plasmid profiles of toxinogenic and non-toxinogenic B. fragilis isolates to determine if potential genotypic and/or phenotypic markers are associated with toxin production.

C. METHODS OF PROCEDURE

Materials:

1. Collection of samples:

a. Anaerobic transport medium (modified Cary and Blair transport medium) (PRAS).

b. Sterile cottion swabs.

c. Empty test tubes.

d. Empty sterile containers to collect stool.

e. Candle jar

2. Inocculation and Incubation of the sample.

a. PINN medium (2)

TBA Tryptose blood agar (TBA) with YE with yeast extract	- 34 g
Defibrinated bovine blood	- 50 ml
Polymixin B	- 5 IU
Irgason	- 2 ug
Novobiocin	- 30 ug
Nalidixic acid	- 32 ug

b. Brucella agar with 5% sheep blood, Vitamin K, and hemin (BA)

c. Bacteroides Bile esculine agar (BBL)

BBE (10)

Tryptose soy agar (BBL)	.....	40.0 g
Oxgall	....	20.0 g
Esculine	....	1.0 g
Ferric ammonium citrate	... .	0.5 g
Hemin solution (5mg/ml)	....	2.0 ml
Gentamicin Solution (40 mg/ml)	... ...	2.5 ml
D/W	....	1000 ml

d. Glove box with incubator

e. Mixed gas (Biological gas) (22)

Co_2 - 10%

H_2 - 10%

N_2 - 80%

f. Nitrogen gas (Normal)

g. Candle jar -

3. Identification

a. BA

b. Chocolate agar

c. Tryptose blood agar (TBA) (Difco Laboratories, Detroit Mich)

d. Kanamycin - bile - esculine agar (KBE)(3)

e. Catalase - (3% H_2O_2), Brain-heart infusion agar with 0.5% yeast extract (BHI - YE; Difco Laboratories), Hemin Solution (0.1 ml), 0.01 N NaOH.

f. Indole reagent, 2% tryptone (Difco), Xylene, Ehrlich reagent)

g. Biochemical tests - (6,8)

i. Rhamnose

ii. Trehalose

iii. Mannitol

h. API

i. Antibiotic discs (22)

Vancomycin - 5 ug

Colistin - 10 ug

Kanamycin -100 ug

4. Patient selection

Patients will be selected for inclusion in this study based on their clinical and laboratory findings. All inpatients (hospitalized) with a history of acute or chronic diarrhea from which no etiologic agent has been isolated will be eligible for study. As the clinical presentation of B. fragilis diarrhea in man is poorly understood, a broad range of patients with varying characteristics will be selected. Patients from all age groups (infants, adolescent, young adult, adult and older adults) will be examined. Bloody and non-bloody diarrhea without recognized etiology will be the single most important criteria. Also there is evidence that prolonged diarrhea (over two weeks duration) may be associated with B. fragilis. Therefore, patients with both chronic and prolonged diarrhea (more than two weeks) will be examined.

Patients enrolled in the 4% surveillence population which have diarrhea and no pathogen isolated will be particularly of interest, as a large number of tests will already have been done to determine etiology.

Candidates for inclusion into the study will be sampled by rectal swab for anaerobes at the same time the rectal swabs are taken for other organisms thus reducing the discomfort to the patient. These specimens will be plated directly on to the isolation media. Bacteroides fragilis will only be identified and characterized when all tests for other pathogens are completed and negative results have been obtained. This delay should not pose a problem, as Bacteroides can take two to three days to form mature colonies. The identification of other pathogens (routine enterics) can be done at this time.

Methods:

1. Collection of samples.

   a. Stools or rectal swabs will be collected in anaerobic transport medium with sterile cotton swabs. Swabs will be kept in sterile test tubes. Both medium and swab tubes should be kept in the glove box for 24 hours to pre-reduce the media. (22).

   b. For stool collection, sterile containers should be kept in the glove box prior to sample collection and should be transported in candle jars after sample collection.

Transportation:

Samples should be sent to the Lab in an insulated container when collected other than at the ICDDR,B hospital.

2. Set-up:

a. Stool or R/S should be set-up in the anaerobic chamber on pre-reduced Brucella agar, PINN, and BBE, for anaerobic incubation. Streaking for isolation should be done in 4 quardrauts for semi-quantitation (Growth in 1st area equals 1+, 2nd area 2+ etc.)

b. A slide should be prepared for Gram stain reading to correlate with the culture result.

3. Incubation

Plates are incubated anaerobically for 48 hours at 37°C.

4. Isolation:

a. After 48 hours of incubation isolation of colony types resembling Bacteroides fragilis should be done.

b. 1/4th of each colony type should be sub-cultured to each of chocolate and Brucella or TBA plate.

The Chocolate agar plate should be incubated in a candle jar for 48 hrs. at 37°C and the Brucella or TBA plate should be incubated anaerobically for 48 hrs. at 37°C.

c. A Gram stain of each colony type should be done at the time of subculture. The organism that grows on Brucella agar and not on chocolate agar should be considered an anaerobe and further identified.

5. Identification:

Only anaerobic Gram negative rods should be considered for further identification. All other organisms will be excluded in this study for further identification.

a. Catalase test: 3% H_2O_2 should be used for catalase test. Catalase positive bacteria will be considered for further test. All other organism should be interpreted as anawrobic Gram negative rod.

b. Spot Indole test:

Indole negative organisms should be tested further Indole positive organism will be interpreted as anaerobic Gram negative bacteria.

c. Biochemicals:

Biochemical test will be performed by API method.

d. Growth in 20% bile broth can be observed for B. fragilis.

6. For further confermation following tests can be done.

a. Antibiotic disk susceptibility test.

Vancomycin, colistin and kanamycin disc should be used.

B. fragilis is resistant to all of these three.

b. Kanamycin-bile-esculine agar.

B. fragilis and some other species of Bacteroides turn this medium black.

To presumtively identify isolates to species, catalase, indole, Rhamnose, trehalose and mannitol fermentation can be performed

(5,6). All should be incubated for 48 to 72 hrs, and those with poor growth were incubated for a total of 7 days.

Catalase:

Agar stabs of BHI-YE should be melted on the day they are to be incubated.

Autoclaved stock hemin solution (0.1 ml) containing 350 ug of hemin per ml of 0.01N NaOH should be added to 7 ml of agar, and the medium is to be solidified on a slant.

After incubation, growth on the slants are to be exposed to air for 30 min, a loopful of bacterial growth should be transfered to a glass slide, and 3% H_2O_2 should be added.

Evolution of bubbles should be considered a positive test for catalase.

Indole :

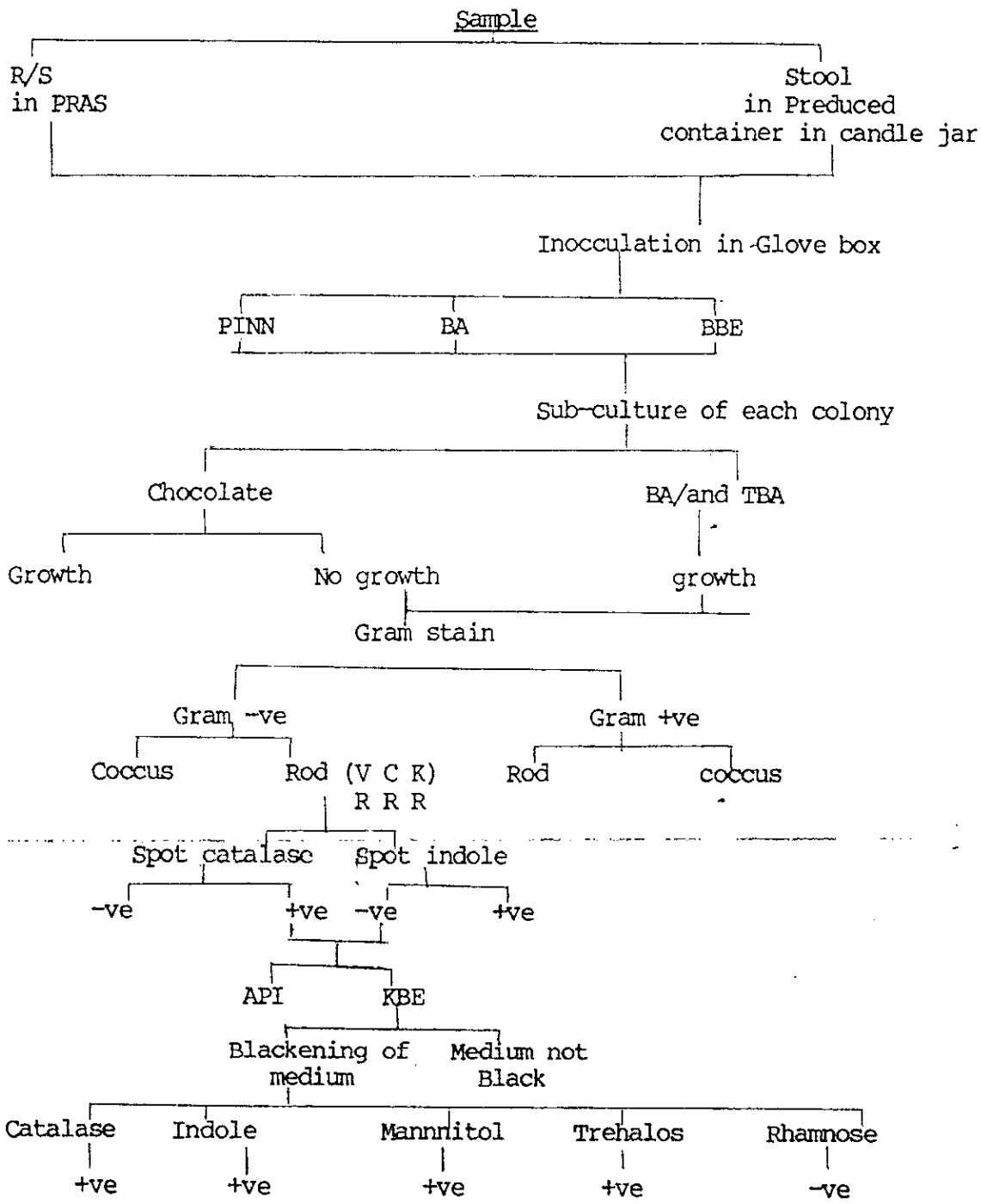
Isolates should be grown in 2% tryptone (Difco. Laboratories semisolid agar, pH 7.2. Indole are to be extracted with Xylene to form a layer on top. Ehrlich reagent (0.5 ml) should be added slowy down the side of the tube. If Indole is present, a red ring should form between the Xylose and the reagent.

Fermantation:

For formantration reactions, 0.6% (wt/vol) carbohydrates should be added to thioglycolate medium without glucose or indicator (pH 7.2; Difco Laboratories).

After good growth of the isolates, 1% bromothymol blue is to be added.

FLOW DIAGRAM FOR STOOL PROCESSING



Enterotoxin studies :

To determine whether B. fragilis produces a classical enterotoxin, ETBF isolates will be cultured in brain heart infusion broth. After anaerobic growth for 48 hr, the cells will be centrifuged at 12,000 g for 2 min. The supernatant will then be passed through a 0.22 u membrane filter and a portian will be plated on Brucella agar to ensure sterility.

The filtrate will then be evaluated by the rabbit and lamb ileal loop tests (13), Y1 adrenal cell (17) and CHO cell assays (7) enterotoxin for production.

Enteropathogenicity of ETBF :

B. fragilis will be grown anaerobically for 24 hours in brain-heart infusion broth (BHI) (Difco). One ml of cell suspension (approx. 5 x 10^9 colony forming units) will be injected directly into the ileum of a 1.8 to 2.2 kg rabbit in which the ceacum (but not the small intestine) has been ligated, as previously described (20). Control rabbits will be injected with 1 ml of sterile BHI broth.

All rabbits will be observed for clinical signs of enteric disease for 6 days post-challenge (P.C).

D. SIGNIFICANCE

The results of this investigation may identify a previously unrecognized agent of diarrheal disease in Bangladesh. Accordingly, such a finding would identify future areas of research in the areas of identification, treatment, prevention and control of B. fragilis induced diarrhea.

E. FACILITIES REQUIRED

No additional facilities will be required other than those which now exist in the Department of Laboratory Services. The present anaerobic laboratory will need some slight maintenance, but will not need extensive renovations or changes.

F. COLLABORATIVE ARRANGEMENTS

Extensive collaboration with scientists outside of ICDDR,B will not be necessary. However, Dr. R. Bradly Sack of the Johns Hopkins University has offered to assist with advise and if need be stock cultures of toxigenic B. fragilis isolates.

SPECIAL PATHOGENS STUDY

Information Form

1. Patient location:
2. Hospital number:
3. Laboratory number (Microbiology):
4. Patient Name:
5. Age :
6. Sex :
7. Patient address :
8. History :

   Nutritional Status :

   Hydration status on admission :

   Diarrhea :

   Duration (in days) :

   Type (Bloody, mucoid, watery) :

   Fever :

   Vomiting :

   Antibiotics given (yes/no) :

   Which :

   Duration :

9. Clinical diagnosis :
10. Lab. tests ordered : (write in what has been ordered)

    Microbiology :

    Clinical Pathology :

    Hematology :

    Chemistry :

ABSTRACT SUMMARY

The protocol entitled "Preliminary Investigation of the Anaerobic Bacterium Bacteroides fragilis as an etiologic agent of Diarrheal Disease in Bangladesh" will address the issue of etiology of diarrheal disease. The protocol will add a culture for Bacteroides fragilis to the existing laboratory work being done for all hospitalized or surveillance patients selected for inclusion in the study. No patients will be hospitalized specifically for this protocol. Patients will be enrolled in the study only when routine culture examinations are negative for recognized pathogens. No physical risks are involved to the patient. No blood or tissues are needed for study. Patients should directly benefit from this study as treatment for prolonged diarrhea by B. fragilis will be possible. Subjects will be informed about the project and will be asked to volluntarily participate. All patient information will be held in confidence and patients will not need to be identified by name. The results of this protocol may significantly add to the knowledge of previously unrecognized etiologic agents of diarrheal disease in Bangladesh. Finally, the study may afford an opportunity for the early diagnosis and treatment of future cases of diarrhea caused by Bacteroides fragilis.

LITERATURE CITED

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ICDDR,B
BUDGET PROPOSAL
(In US $)

PARTICULARS

Program name: LSED ..... Protocol title: Preliminary Investigatic of the Anaerobic Bacterium Bacteroides fragilis as an etiologic agent of diarrhoeal disease in Bangladesh.

P. I's name: Dr. Bradford A. Kay

Protocol no: ..... Starting date: April, 1987

Budget code: ..... Completion date: September, 1987

A/C No.	EXPENSE CATEGORY Description	Refer Page	Column A 1st year Jan.-Dec.	Column B 2nd year Jan.-Dec.	Column C 3rd year Jan.-Dec.	Column D Total Project Cost
3100	Local Salaries	2				1200.00
3200	Intl. Salaries	8				-
3300	Consultants	14				-
3500	Travel Local	15				-
3600	Travel Intl.	16				-
3700	Supplies & Mat.	18				1450.00
3800	Other Costs	19				100.00
4800	Inter Deptl. Ser.	20				2243.52
	Total Direct cost					4993.52
0000	Indirect cost = 31% of total direct cost					
	TOTAL OPERATING COST					
0300	Capital expenditure Refer page no. 21					-
	TOTAL PROJECT COST					

P.I.'s signature

Reviewed by Budget & Finance

PERSONNEL REQUIREMENT-(LOCAL STAFF) 1st/2nd/3rd year

	No.of positions	No. of man months	$ Amount
A. Direct Project/Protocol/Branch Staff at starting date Sourced from Page 3	one	6	1200.00
Add:			
B. New recruitments Sourced from Page 4			
C. Staff allocated from other area Sourced from Page 5			
(i) Sub-Total			
Less:			
. Separations Sourced from Page 6			
. Staff allocated to other area Sourced from Page 7			
(ii) Sub-Total			
(i)-(ii) TOTAL			* 1200.00

*Agrees with Page 1 A/C No.3100

getB6.1-3
z 13

LOCAL STAFF (EXISTING) FOR 1ST/2ND/3RD YEAR

(From.....To 31-12-19....)

ob designation	Level	A No. of positions at starting date	B No. of months at 31.12.19__	C Man months at 31.12.19__ (A x B)	D ** Rate per month	E $ Amount (C x D)
search Officer	GS-V	One	6	6	200	1200.00
TOTAL	*	XXXXXXXXXXX	*	XXXXXX		*1200.00

* AGREES WITH PAGE 2 ROW A

ease contact Budget
fice (Ext. 280) for rates.

idget86.1-3
:iz-13.

SUPPLIES AND MATERIALS-1ST/2ND/3RD YEAR

A/C Code	Item Description	$ Amount
3701	Drugs (used for medication in the hospitals and field stations)	100.00
3702	Glassware (bottle, beaker,cylinder, petridish, aluminium seal, slides, stopper, tube etc.)	100.00
3703	Hospital supplies (bandage, gauze, blade, bowl, catheter, cotton, needle, syringe, soluuion, leukoplast, towel etc.)	100.00
3704	Stationery and office supplies (battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)	
3705	Chemicals and media (acid, reagent, dextrose, sodium, bactoagar etc.)	
3706	Materials for uniform (cloth, button etc. required for making uniforms)	
3707	Fuel, oil and lubricants (diesel, mobil, petrol, kerosene etc.)	
3708	Laboratory supplies (aluminium foil, bag, blade, brush, cap, container, film X-Ray etc.) biological gas, etc.	500.00
3709	Housekeeping supplies (aerosol, battery, wiping cloth, duster, lock and key etc.)	
3710	Janitorial supplies (bleaching powder, brush, detol, detergent, insecticide, soap etc.)	
	Page total (balance b/d)	800.00

Contd. to page 18

SUPPLIES AND MATERIALS-1ST/2ND/3RD YEAR

(Contd. from Page No. 17)

A/C code	Item description	$ Amount
	Page total from page 17 (balance c/d)	
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)	500.00
	Sub-Total	
3713	Freight and other charges add 30% for import	150.00
	TOTAL	1450.00

AGREES WITH
PAGE 1
A/C 3700
COLUMN D

Note For rates please contact Supply Ext.260 (add 10% to rates for inflation)

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OTHER COST-1ST/2ND/3RD YEAR

A/C code	Accounts Description	$ Amount
3800	**Repairs and maintenance** (maintenance and repairs of vehicles, equipments, furniture and building)	100.00
3900	**Rent, communication and utilities** (postage, telephone, telegram, electricity etc.)	
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.)	
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, subsistance paid to the staff)	
	TOTAL	100.00

AGREES WITH
PAGE 1
A/C No.3800
COLUMN D

**INTERDEPARTMENTAL SERVICES-1ST/2ND/3RD YEAR

A/C code	Service Area	$ Amount
4801	Computer	50.00
4802	Transport Dhaka	100.00
4803	Transport Matlab	
4804	Water transport-Matlab	
4805	Transport Teknaf	
4806	Xerox	50.00
4807	Pathology	100.00
4808	Microbiology tests	500.00
4809	Biochemistry	100.00
4810	X-Ray	
4811	I.V. Fluid	50.00
4812	Media	250.00
4813	Patient hospitalisation study	
4814	Animal research 50 rabbits, 6 sheeps	993.52
4815	Medical illustration	
4817	Telex	50.00
4818	Out patient care	
4819	Maintenance charges	
4820	Vehicle maintenance charges	
4821	Library service charges	
4830	Transport subsidy	
	TOTAL	* 2243.52

** Please contact Cost Office on Ext. 281. for rates.

*AGREES WITH PAGE 1 A/C 4800

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BACTEROIDES DIARRHOEA STUDY

CONSENT FORM

Diarrhea is a common and serious disease in Bangladesh. We have recent information about a new pathogen which causes watery diarrhea. As you are/your child is admitted in ICDDR,B hospital you will receive the best possible care during your stay. A series of routine examinations will be done for the known diarrhea causing pathogens. We wish to take an additional rectal swab/and stool sample to examine for a new pathogen. For this study you/your child will not receive any physical injury. Your doctors will be informed about your laboratory tests, so that they can treat you with the best medicine. We would like you to participate in this study so that we may treat you and future patients better.

If you do not want to participate in the study, you will still be well treated like other patients in this hospital. If you have any question, please ask them now.

If you wish to participate in the study voluntarily, then please sign your name or give a left thumb impression below.

_____ _____

Signature of Investigator

Signature/left thumb impression of patients guardian

Date _____

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