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ICDDRB: Centre for Health & Population Research RRC APPLICATION FORM

DMONO

RESEARCH PROTOCOL

Protocol No.: 2001—003

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RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: Evaluation of lactoferrin (Latex agglutination-Leuko-Test® and a new dip stick-Leuko-Stick®-Test) and modified guaiac-test (Colo-Rectal®-Test) as screening tests in the diagnosis and differentiation of inflammatory and non-inflammatory diarrhoea in patients with acute infectious diarrhoea.

Theme: *(Check all that apply)*

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☐ Environmental Health
- ✓ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: **Lactoferrin test, Screening test, Inflammatory diarrhoea**

Principal Investigator: **Dr. H. Ashraf** **Division:** **CSD** **Phone:** 2313

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CSD

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Co-Principal Investigator(s): **Dr. Johannes Beltinger**

Co-Investigator(s): **Dr. Nur Haque Alam**
Dr. P.K. Bardhan
Dr. S.A. Sarker
Dr. A.S.G. Faruque

Student Investigator/Intern: **Nil**

Collaborating Institute(s): **University of Basel, Switzerland (Prof. Klaus Gyr)**

Population: Inclusion of special groups *(Check all that apply)*:

Gender
- ✓ Male
- ✓ Females

Age
- ✓ 0 – 5 years
- ✓ 5 – 9 years
- ✓ 10 – 19 years
- ✓ 20 +
- ✓ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify _____)
- ☐ Animal

Project / study Site *(Check all the apply)*:

- ✓ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh _____
- ☐ Outside Bangladesh
  name of country: _____
- ☐ Multi centre trial
  (Name other countries involved)
  _____

Revised on: July 2001

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Type of Study *(Check all that apply)*:

- ☐ Case Control study
- ☐ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- ✓ Others: **Diagnostic tests**

Targeted Population *(Check all that apply)*:

- ✓ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process *(Check all that apply)*:

- ☐ Written
- ☐ Oral
- ✓ None
- ☐ Bengali language
- ☐ English language

Proposed Sample size:

Total sample size: 350 ☐

Sub-group _____ ☐ _____ ☐

_____ ☐ _____ ☐

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☐ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☐ Existing data available via public archives/source
- ✓ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes/No

☐ ✓ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ✓ Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ✓ a. place the subject at risk of criminal or civil liability?

☐ ✓ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ☐ greater than minimal risk
- ☐ no risk
- ☐ no more than minimal risk
- ✓ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

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s/No

☐ Is the proposal funded?

If yes, sponsor Name: University of Basel, Switzerland (Prof. Klaus Gyr)

s/No

☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1) _____

(2) _____

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

tes of Proposed Period of Support	Cost Required for the Budget Period ($)
(Day, Month, Year - DD/MM/YY)	*a. 1st Year*	*2nd Year*	*3rd Year*	*Other years*
Beginning date: As soon as possible	US$12,000	x	x	x
End date: 1 year from the starting date	*b. Direct Cost :* US$12,000	*Total Cost :* US$12,000		

pproval of the Project by the Division Director of the Applicant

e above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewer: ie protocol has been revised according to the reviewer's comments and is approved.

me of the Division Director	Signature	04/02/2001 Date of Approval

rtification by the Principal Investigator

ertify that the statements herein are true, complete d accurate to the best of my knowledge. I am aware it any false, fictitious, or fraudulent statements or ims may subject me to criminal, civil, or administra- e penalties. I agree to accept responsibility for the ientific conduct of the project and to provide the re- ired progress reports if a grant is awarded as a result this application.

Signature of PI

Date: 31.01.2001

Name of Contact Person (if applicable)

Table of Contents

☐ Check here if appendix is included

POJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigator

Project Name: Evaluation of lactoferrin (Latex agglutination-Leuko-Test® and a new dip stick-Leuko-Stick®-Test) and modified guaiac (Colo-Rectal®-Test) as screening tests in the diagnosis and differentiation of inflammatory and non-inflammatory diarrhoea in patients with acute infectious diarrhoea

Total Budget: US $ 12,000 Starting Date: As soon as possible Ending Date: 1 year from starting date

In developing countries like Bangladesh infectious diarrhoea is one of the most important causes of death, especially in young children. Early differentiation between inflammatory and non-inflammatory diarrhoea is important because of the broad spectrum of underlying causes of acute diarrhoea. Most cases of acute diarrhoea are self-limiting and need only symptomatic therapy and most importantly adequate fluid substitution and nutritional support. *Shigella* and *Salmonella* species, *Campylobacter, Clostridium difficile, EIEC, EHEC* and invasive parasites like *Entamoeba histolytica* however, can cause severe intestinal inflammation and need focused diagnosis and sometimes-specific antibiotic treatment. The unselected and widespread use of antibiotics in the treatment of acute diarrhoeal illnesses especially in developing countries are an unnecessary use of hundred of millions of dollars in developing countries and in addition to the likelihood of inappropriate therapy are contributing to a dramatic increase of resistant strains which limit treatment options severely. This not only affects the costs of treatment but will possibly lead to an increase in mortality rates by creating multiple resistant strains. Strain resistance will not only affect the population in developing countries but also travelers who are often prone to acute diarrheal illnesses. The clinical differentiation between inflammatory and non-inflammatory diarrhoea is in most cases impossible because of the lack of clear evidence of macroscopic visible blood or pus in the stool or even fever. The microscopic examination of stool and staining for stool leukocytes with methylene blue has been widely recommended to be a rapid, simple and reliable screening test to distinguish inflammatory from non-inflammatory diarrhoeas. Stool microscopy requires an experienced technician and basic laboratory equipment. In addition, it should be performed on fresh stool samples because storage destroys the morphology of the leukocytes. In 1983, a modified guaiac test to detect occult blood in faeces was shown to yield a good correlation with the feacal leukocyte examination in patients suffering from acute inflammatory diarrhoea. These data were confirmed by Beltinger in 1997 and Bardhan in 2000 in a prospective study in Bangladesh in a large number of patients. According to the study, occult blood in the stool has the same sensitivity and positive predictive value as stool microscopy. Guerrant and coworkers used a marker for leukocytes, feacal lactoferrin in stool specimens as reference for an inflammatory process. Lactoferrin is a 77-kDa iron-binding glycoprotein that facilitates the production of hydroxyl radicals and chelates iron, preventing its accessibility to microorganisms and thus inhibiting their growth. It is present in high amounts especially in the granula of polymorphonuclear leukocytes. Invasive enteropathogens, which interact with intestinal epithelial cells or even cross the epithelial barrier, attract inflammatory cells in the submucosa such as polymorphonuclear leukocytes. Based on an antilactoferrin latex agglutination test (Leuko-Test, Techlab, Blacksburg, VA) the semiquantitative measurement of the titer gave evidence for an inflammatory process and therefore a possibility to distinguish between inflammatory and non-inflammatory diarrhoea. A new developed test to look for lactoferrin; a lactoferrin dip stick (Leuko-Stick, Techlab, Blacksburg, VA) test uses an immunochromatographic method, which has been widely used in pregnancy tests to simplify the diagnostic procedure. In a meta analysis to evaluate the diagnostic value of different feacal screening tests, which included 25 large studies, the feacal lactoferrin test had the highest diagnostic performance for the discrimination of inflammatory from non-inflammatory diarrhoeas. The aim of this study is to

evaluate the lactoferrin test (Latex agglutination-Leuko-Test and a new dip stick-Leuko-Stick Test) alone and in combination with the occult blood (Colo-Rectal Test^{R}) test in the diagnosis and differentiation of inflammatory vs. non-inflammatory diarrhoea in patients with acute diarrhoea and to compare these results with the number of leukocytes in the stool, the stool microscopy and the results of stool culture. This study will be using the surveillance system of the ICDDR,B where stool specimens are randomly collected and stool culture performed. The study will try to set up an easy, inexpensive screening test and algorithm, which will be of use under field conditions where no laboratory facilities are available. It is also intended to reduce the costs for unnecessary stool culture and the unreflected use of antibiotics.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Dr. Hasan Ashraf	Medicine/Gastroenterology	PI
2. Dr. Johannes Beltinger	Medicine/Gastroenterology	Co-PI
3. Dr. Nur Haque Alam	Medicine/Gastroenterology	Co-Investigator
4. Dr. P.K. Bardhan	Medicine/Gastroenterology	Co-Investigator
5. Dr. S.A. Sarker	Medicine/Gastroenterology	Co-Investigator
6. Dr. A.S.G. Faruque	Epidemiologist	Co-Investigator
7. Prof. Klaus Gyr	Medicine/Gastroenterology	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

1. Lactoferrin test (Latex agglutination test and a new dip stick test) alone and in combination with occult blood test is an useful screening test in the diagnosis and differentiation of inflammatory vs. non-inflammatory diarrhoea in patients with acute infectious diarrhoea. A positive lactoferrin test result actually indicates an increased level of faecal leucocytes suggestive of inflammatory diarrhoea and a negative result indicates non-inflammatory diarrhoea.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

To evaluate the lactoferrin test (Latex agglutination test and a new dip stick test) alone and in combination with the occult blood test in the diagnosis and differentiation of inflammatory vs. non-inflammatory diarrhoea in patients with acute diarrhoea and to compare these results with stool microscopy and stool culture reports. The proposed test is a substitute of the faecal microscopy and not a gold standard.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).

In developing countries infectious diarrhoea is one of the most important causes of death, especially in young children. Attack rates attributable to diarrhoeal disease in young children are estimated 6-12 episodes/child per year and overall death rate of 3.2-3.5 million/year (Bern *et al.*, 1992; Savarino and Bourgeois, 1993). Primary prevention by improving hygiene or by administrating an effective vaccine is not likely to be feasible in the near future in these areas. Despite great improvement in the management of acute diarrhoea and a remarkable reduction in death rates the number of fatalities still remains unacceptably high (Claeson and Merson, 1990; WHO, 1999). Therefore, the early initiation of effective antimicrobial therapy for patients suffering from severe inflammatory diarrhoea will remain a major strategy to reduce mortality rates (Ronsmans *et al.*, 1988; Richards *et al.*, 1993).

Early differentiation between inflammatory and non-inflammatory diarrhoea is important because of the broad spectrum of underlying causes of acute diarrhoea. Most cases of acute diarrhoea are self-limiting and need only symptomatic therapy and most importantly adequate fluid substitution and nutritional support. *Shigella* and *Salmonella* species, *Campylobacter, Clostridium difficile, EIEC, EHEC* and invasive parasites like *Entamoeba histolytica* however, can cause severe intestinal inflammation and need focused diagnosis and sometimes-specific antibiotic treatment.

The unselected and widespread use of antibiotics in the treatment of acute diarrhoeal illnesses especially in developing countries are an unnecessary use of hundred of millions of dollars in developing countries and in addition to the likelihood of inappropriate therapy are contributing (Harris and Black, 1991;DuPont, 1993) to a

dramatic increase of resistant strains which limit treatment options severely (Ronsmans *et al.*, 1996). This not only affects the costs of treatment but will possibly lead to an increase in mortality rates by creating multiple resistant strains. Strain resistance will not only affect the population in developing countries, but also travellers who are often prone to acute diarrhoeal illnesses (Caeiro and DuPont, 1998;WHO, 1998).

The clinical differentiation between inflammatory and non-inflammatory diarrhoea is in most cases impossible because of the lack of clear evidence of macroscopic visible blood or pus in the stool or even fever.

The microscopic examination of stool and staining for stool leukocytes with methylen blue has been widely recommended to be a rapid, simple and reliable screening test to distinguish inflammatory from non-inflammatory diarrhoeas (Willmore and Shearman, 1918; Wolf, 1969; Harris *et al.*, 1972; Satterwhite and DuPont, 1976; Pickering *et al.*, 1977; Maki *et al.*, 1979; Alvarado, 1983) Stool microscopy, however requires an experienced technician and basic laboratory equipment. In developing countries, where rates of diarrhoeal illness are high, most people do not have access to such services or even to physicians. In addition stool microscopy should be performed on fresh stool samples because storage destroys the morphology of the leukocytes.

In 1983, a modified guaiac (Colo-Rectal®-Test) to detect occult blood in faeces was shown to yield a good correlation with the feacal leukocyte examination in-patients suffering from acute inflammatory diarrhoea (Vogtlin *et. al.*, 1983). These data were confirmed by Beltinger *et al.* (Beltinger *et al.*, 1997) and Bardhan *et. al.* (Bardhan *et al.*, 2000) in a prospective study in Bangladesh in a large number of patients. According to this study, occult blood in the stool has the same sensitivity and positive predictive value as stool microscopy.

In this study the predictive value of different cut-off points of the stool microscopy was shown. The best predictive value to give evidence for a mediator of inflammatory diarrhoea was a number of > 20 leukocytes per high power field in the stool microscopy followed by the combination of >10 erythrocytes and >1 leukocytes per high power field. These data were confirmed in other studies, one on pediatric patients (Patwari *et. al.*, 1993) and in-patients with shigellosis. Clinical information alone however was insufficient to be used as a predictor to distinguish between inflammatory and non-inflammatory diarrhoea (Bardhan *et. al.*, 2000).

Guerrant and coworkers used a marker for leukocytes, feacal lactoferrin in stool specimens as reference for an inflammatory process. Lactoferrin is a 77-kDa iron-binding glycoprotein that facilitates the production of hydroxyl radicals and chelates iron, preventing its accessibility to microorganisms and thus inhibiting their growth. It is present in various concentrations in several body fluids including colostrum, mature milk, vaginal mucus, blood, sputum and saliva (Martins *et al.*, 1995) and is found in high amounts especially in the granula of polymorphonuclear leukocytes. Invasive enteropathogens which interact with intestinal epithelial cells or even cross the epithelial barrier attract inflammatory cells in the submucosa such as polymorphonuclear leukocytes. Based on an antilactoferrin latex agglutination test (Leuko-Test® test, Techlab, Blacksburg, VA) the semiquantitative measurement of the titer gave evidence for an inflammatory process and therefore a possibility to distinguish between inflammatory and non-inflammatory diarrhoea (Miller *et al.*, 1994). A new developed test to look for lactoferrin; a lactoferrin dipstick test (Leuko-Stick®) uses an immunochromatographic method, which has been widely used in pregnancy tests to simplify the diagnostic procedure.

In a meta analysis to evaluate the diagnostic value of different feacal screening tests which included 25 large studies (Huicho *et al.*, 1996), the fecal lactoferrin test had the highest diagnostic performance for the discrimination of inflammatory from non-inflammatory diarrhoeas. One limitation of this test remains the possible interaction of the anti-lactoferrin antibodies with human milk lactoferrin especially in breast-fed infants. In a prospective study in Mexico where the lactoferrin test was used to study infants below 2 years, the lactoferrin test was shown to have the same sensitivity in breast vs non breast fed children but a lower specificity (Huicho *et al.*, 1997). In Bangladesh, however, these group of children are the one most affected by acute diarrhoeal illnesses (Baqui *et al.*, 1992).

This study will be using the surveillance system of the ICDDR,B where stool specimens are randomly collected and stool culture performed.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Study population

The study will be conducted at the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Patients enrolled in the study will belong to 2% systematic sample of all patients registering at the out patient department of the hospital (Dhaka Hospital Surveillance System) (Stoll *et. al.*, 1982).

History and physical examination will be performed by a physician, noting particularly clinical features such as the presence of visible blood and/or mucus in the stool, fever (rectal temperature >38°C), abdominal pain or tenderness, tenesmus and the degree of dehydration. Patient enrolment will continue for one calendar year and all patients belonging to the surveillance protocol during this period will be included.

Patients are categorized as having one of the following clinical types of diarrhoea: acute watery diarrhoea (AWD), invasive diarrhoea (ID), or persistent diarrhoea (PD). Stool samples for microscopical examination, and rectal swabs for culture are obtained from every patient in the surveillance group. There are no restrictions as to age.

Laboratory Methods

Stool microscopic examination will be performed within one hour of collection of stool by an experienced technician. The average numbers of leukocytes as well as red blood cells per high power field (hpf) will be determined in a wet mount preparation. Samples are microscopically examined for red blood cells (recorded as 0, 1-10, 11-20, 21-50, >50) per high power field (hpf), white blood cells (1-10, 11-20, 21-50, >50) per hpf, stool fat (no, few, moderate, many) and parasites (especially motile trophozoites of *E. histolytica*), by a direct smear without concentration techniques.

The rectal swabs will be taken and plated directly on different culture media (Salmonella-Shigella (SS), Taurocholate-tellurite-gelatine and Mac Conkey's agar) and examined for *Salmonella, non-typhoid Salmonella, Shigella, Vibrio cholera, Aeromonas* and *Plesiomonas* by standard methods. Rectal swaps will also be plated on Campy-BAP agar to isolate *Campylobacters*, and a pool of 5 colones of *E. coli* from MacConkey agar for *diarrhogenic E. coli* by DNA probing. Patients will also be tested for Rotavirus by ELISA.

In addition, the following tests for the detection of faecal occult blood and stool leucocytes will be performed in the clinical pathology laboratory.

1. Modified guaiac-test (Colo-Rectal®-Test, Roche AG, Switzerland). This test is based on the peroxidase activity of haemoglobin, which causes a colour change in the presence of H_2O_2 and guaiac. The detection limit is 1-2ml blood per 100g stool, according to the manufacturer. The result will be considered positive when the test fields turns blue after adding the reagent or negative when there is no colour change.

2. A lactoferrin test latex agglutination test (Leuko-Test®, TechLab®, VA, USA) will be performed according to the instructions by the manufacturer:50µl of stool will be mixed with a buffered solution containing 0.1% sodium azide. Latex beads coated with antibodies against lactoferrin will be reacted with the stool sample on an agglutination card. A positive control and a negative control will monitor the quality of the test.

3. At the same time the Leuko-Stick® Test (TechLab®, VA, USA) a new rapid immunochromatographic test will be performed. The same dilution as with the LeucoTest® will be used and four drops of the diluted sample are transferred to a well on the test stripe. After five minutes incubation a positive result will show a red line in the test well and in a control well.

Sample size

If we assume a number of approximately 100,000 patients per year in the Dhaka Hospital of ICDDR,B, there would be 2000 patients included in the 2% surveillance study. Assuming 30% positive stool culture we could include 600 patients with a positive result in stool culture (including inflammatory and non-inflammatory cases). With a power of 80 we would need 350 patients to prove an increase of the sensitivity and specificity of the occult blood test / stool microscopy to differentiate between inflammatory and non-inflammatory diarrhoea by 15%.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

The present study will be conducted in the Clinical Research and Service Centre (CRSC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). This centre treats more than 100,000 diarrhoeal patients each year, majority of whom are children under 5 years. Patients enrolled in the study will belong to 2% systematic sample of all patients registering at the out patient department of the hospital (Dhaka Hospital Surveillance System) (Stoll *et. al.*, 1982).

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

Data management and analyses plan:

For the purpose of comparison, the isolation of pathogens from the faecal samples will serve as the gold standard. Statistical significance for proportions will be tested by the chi-square test and Fisher exact test. Sensitivity (proportion of invasive diarrhoea patients correctly identified), specificity (proportion of non-invasive diarrhoea patients correctly identified), positive predictive value (proportion of patients with positive test result who will have invasive diarrhoea), negative predictive value (proportion of patients with negative test result who will have non-invasive diarrhoea), accuracy (proportion of total patients correctly identified), and likelihood ratio (ratio of the probability of a positive test result in invasive diarrhoea with the corresponding probability in non-invasive diarrhoea) will be calculated according to the standard methods. Optimal predictive parameters will be defined by constructing a Receiver Operating Characteristic (ROC) curve. P-value will be set at <0.05. Subgroup analysis of data for the breastfed and non-breastfed children will be done separately.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Not required.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

1. Alvarado, T. (1983). Faecal leucocytes in patients with infectious diarrhoea. *Trans R Soc Trop Med Hyg* 77(3):316-20.

2. Baqui, A.H., R.E. Black, R.B. Sack, M.D. Yunus, A.K. Siddique and H.R. Chowdhury (1992). Epidemiological and clinical characteristics of acute and persistent diarrhoea in rural Bangladeshi children. *Acta Paediatrica* **381 (Suppl)**:15-21.

3. Bardhan, P.K., J. Beltinger, R. W. Beltinger, A. Hossain, D. Mahalanabis and K. Gyr (2000). Screening of patients with acute infectious diarrhoea: evaluation of clinical features, faecal microscopy, and faecal occult blood testing. *Scand J Gastroenterol* **35**(1):54-60.

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5. Bern, C., J. Martiness, I. De Zoysa and R.I. Glass (1992). The magnitude of the global problem of diarrhoeal disease: a ten-year update. *Bulletin of the World Health Organisation* **70**:705-714.

6. Caeiro, J.P. and H.L. DuPont (1998). Management of travellers' diarrhoea. *Drugs* **56**(1):73-81.

7. Claeson, M. and M.H. Merson (1990). Global progress in the control of diarrhoeal diseases. *Pediatric Infectious Disease Journal* 9:345-355.

8. DuPont, H.L. (1993). Diarrhoeal disease: Current concepts and future challenges. Antimicrobial therapy and prophylaxis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**(Suppl 3): 31-34.

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12. Huicho, L., Campos, J. Rivera and RL. Guerrant (1997). Fecal screening tests in the approach to acute infectious diarrhea: a scientific overview. *Pediatric Infectious Disease Journal* **15**(6): 486-94).

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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The results of the study will be disseminated through publishing in the international journals and presenting in the national and international conferences.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

This is a collaborative study between the University of Basel, Switzerland and Clinical Sciences Division, ICDDR,B. The collaboration is organized by Prof. Klaus Gyr, Department of Internal Medicine, University of Basel, Switzerland.

Detailed Budget for New Proposal

Project Title: Evaluation of lactoferrin (Latex agglutination- Leuko-Test® and a new dip stick-Leuko-Stick™ test and modified guaiac (Colorectal®-test) as screening test in the diagnosis and differentiation of inflammatory and non-inflammatory diarrhoea in patients with acute infectious diarrhoea.

Name of PI: Dr. Hasan Ashraf

Protocol Number: 2001-003 Name of Division : Clinical Sciences Division

Funding Source : Amount Funded (Total): US $ 12,000

Starting Date: As soon as possible Closing Date: 1 year from the starting date

SL.No.	Account Description	Salary Support		US $ Amount Requested		
	Personnel	Position	Effort %	1st Year		Total
1.	Dr. Hasan Ashraf	PI	10%	1,355		1,355
2.	Dr. Nur Haque Alam	Co-Inv	5%	862		862
3.	Research Officer		100	3,683		3,683
4.	Lab. Attendant-4		100x4	3,600		3,600
	Sub-Total			9,500		9,500
	Supplies and Materials (Description of items)					
	Office supplies			500		500
	Non-stock			500		500
	Sub Totals			1000		1000
	Other Contractual Services					
	Printing and Publication			500		500
	Fax, DHL & other utilities			500		500
	Sub Total			1000		1000

Interdepartmental Services				1st Year		Total
	Transport			500		500
	Sub Total			500		500
	TOTAL COST			12,000		12,000

16/7/01

Md. Bozlur Rahman
Manager, Budget & Control
ICDDR,B: Centre for
Health & Population Research
Mohakhali, Dhaka-1212
Bangladesh

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Salaries of personnel are requested according to the percentage of work of each person involved in the study. The study materials like diagnostic kits will be provided free of cost by the respective companies. The stool microscopy and culture results will be collected from the Surveillance study.

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included ☑
2. Approval of the Division Director on Face Sheet ☑
3. Certification and Signature of PI on Face Sheet, #9 and #10 ☑
4. Table on Contents ☑
5. Project Summary ☑
6. Literature Cited ☑
7. Biography of Investigators ☑
8. Ethical Assurance ☑
9. Consent Forms ☐
10. Detailed Budget ☑