Appendix-2

icddr,b KNOWLEDGE FOR GLOBAL LIFESAVING SOLUTIONS	**RRC APPLICATION FORM**

RESEARCH PROTOCOL NUMBER: 2007-008	**FOR OFFICE USE ONLY**		
	RRC Approval:	☒ Yes /	☐ No	Date:21 Mar 2007
	ERC Approval:	☒ Yes /	☐ No	Date:9 May 2007
	AEEC Approval:	☐ Yes /	☐ No	Date: Not applicable

Protocol Title: A pilot study for capacity building for a multi-centre, randomized trial for treatment of kala azar in Bangladesh

Short title (in 50 characters including space):

Theme: (Check all that apply)

- ☐ Nutrition
- ☒ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine Evaluation
- ☐ HIV/AIDS
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: kala azar; visceral leishmaniasis; diagnosis; drug resistance

Relevance of the Protocol:

Visceral leishmaniasis or kala azar is caused by a protozoan parasite, *Leishmania donovani* which is transmitted by a sandfly species *Phlebotomus argentipes*. Between 1994 and 2005, over 81,000 people have been reported to have kala azar in Bangladesh. However, its is believed that the accurate number of cases will be higher than the reported. The disease is common in 34 districts of Bangladesh, and among them Mymensingh district alone contributed over 50% of total cases of kala-azar. Improvement in case detection of kala azar is necessary in endemic areas for both surveillance purposes and better control. Therefore, the aim of this proposed study ate to evaluate some newer diagnostic tests using blood and urine samples of suspected cases; and to assess response to treatment with sodium stibogluconate to which resistance has been reported in India. The other aim of this pilot study is to build up capacity for a planned Phase-III clinical trial with a newer drug, sitamaquine, in Bangladesh.

Centre's Priority (as per Strategic Plan, to be imported from the attached Excel Sheet):
4.2. Develop and/or evaluate rapid or simple diagnostic tests to improve case detection and surveillance.

Programmes:

- ☐ Child Health Programme
- ☐ Nutrition Programme
- ☒ Programme on Infectious Diseases & Vaccine Science
- ☐ Poverty and Health Programme
- ☐ Health and Family Planning Systems Programme
- ☐ Population Programme
- ☐ Reproductive Health Programme
- ☐ HIV/AIDS Programme

Principal Investigator (Should be a Centre's staff)

Dr. Kazi M. Jamil

Address (including e-mail address):
Associate Scientist, CSD, ICDDR,B
E-mail: jamil@icddrb.org

DIVISION:

- ☒ CSD
- ☐ LSD
- ☐ HSID
- ☐ PHSD

Co-Principal Investigator(s): Internal

Dr. Rashidul Haque
Scientist and Head, Parasitology Lab
Laboratory Sciences Division, ICDDR,B
E-mail: rhaque@icddrb.org

Co-Principal Investigator(s): External:
(Please provide full official address including e-mail address and Gender)

Co-Investigator(s): Internal:
Dr. Stephen P. Luby
Head
Programme in Infectious Disease and Vaccine Sciences (PIDVS)
ICDDR,B
E-mail: sluby@icddrb.org

Co-Investigator(s): External
(Please provide full official address including e-mail address and Gender)

Student Investigator(s): Internal (Centre's staff):

Student Investigator(s): External:
(Please provide full address of educational institution and Gender)

Collaborating Institute(s):

Institution # 1

Country	Bangladesh
Contact person	Prof Md. Abdul Ghani, FCPS (Medicine)
Department (including Division, Centre, Unit)	Dept of Medicine
Institution (with official address)	Community-based Medical College, Bangladesh (CBMC,B) Mymensingh, Bangladesh
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 2

Country	
Contact person	
Department (including Division, Centre, Unit)	
Institution (with official address)	
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Institution # 3

Country	
Contact person	
Department (including Division, Centre, Unit)	
Institution (with official address)	
Directorate (in case of GoB i.e. DGHS)	
Ministry (in case of GoB)	

Note: If more than 3 collaborating institutions are involved in the research protocol, additional block(s) can be inserted to mention its/there particular(s).

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

Gender
- ☒ Male
- ☒ Female

Age
- ☐ 0 – 4 years
- ☒ 5 – 9 years
- ☒ 10 – 19 years
- ☒ 20 – 64 years
- ☐ 65 +

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

NOTE It is the policy of the Centre to include men, women, and children in all research projects involving human subjects unless a clear and compelling rationale and justification (e.g. gender specific or inappropriate with respect to the purpose of the research) is there. Justification should be provided in the `Sample Size' section of the protocol in case inclusiveness of study participants is not proposed in the study.

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: Mymensingh
- ☐ Outside Bangladesh

  Name of Country:
- ☐ Multi Centre Trial

(Name other countries involved):

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:

NOTE: Does the study meet the definition of clinical studies/trials given by the International Committee of Medical Journal Editors (ICMJE)? Yes ☐ No ☒

Please note that the ICMJE defined clinical trial as "*Any research project that prospectively assigns human subjects to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome*".

If YES, after approval of the ERC, the PI should complete and send the relevant form to provide required information about the research protocol to the Committee Coordination Secretariat for registration of the study into websites, preferably at the www.clinicaltrials.gov. It may please be noted that the PI would require to provide subsequent updates of the research protocol for updating protocol information in the website.

Targeted Population (Check all that apply):

- ☒ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal group
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process (Check all that apply):

- ☒ Written
- ☐ Oral
- ☐ None
- ☒ Bengali Language
- ☐ English Language

Proposed Sample Size: 300

Sub-group (Name of subgroup (e.g. Men, Women) and Number

Name	Number	Name	Number
(1) Case	200	(3)	
(2) Control	100	(4)	

Total sample size: 300

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/sources
- ☒ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes	No	
☒	☐	Is the information recorded in such a manner that study participants can be identified from information provided directly or through identifiers linked to the study participants?
☐	☒	Does the research deal with sensitive aspects of the study participants' 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:

Yes	No	
☐	☒	Place the study participants at risk of criminal or civil liability?
☐	☒	Damage the study participants' financial standing, reputation or employability, social rejection, lead to stigma, divorce etc.?

Do you consider this research (Check one):

☐ Greater than minimal risk ☐ No more than minimal risk

☒ Only part of the diagnostic test [is this an appropriate response?]

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, 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".

Yes/ No

☒ ☐ Is the proposal funded?

If yes, sponsor Name: (1) IHP grant from Govt of Bangladesh

(2) GlaxoSmithKline (UK)

Yes/No

☐ ☒ Is the proposal being submitted for funding?

If yes, name of funding agency: (1)

(2)

Do any of the participating investigators and/or member(s) of 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, a written statement of disclosure to be submitted to the Centre's Executive Director.

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning Date : 01/06/2007

End Date : 31/05/2008

Cost Required for the Budget Period ($)

Years	Direct Cost	Indirect Cost	Total Cost
Year-1	86,630	27,722	**114,352**
Year-2			0
Year-3			0
Year-4			0
Year-5			0
Total	86,630	27,722	**114,352**

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept the responsibility for the scientific conduct of the project and to provide the required progress reports including updating protocol information in the SUCHONA (Form # 2) if a grant is awarded as a result of this application.

_____ _____

Signature of PI **Date**

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewers' comments and is approved.

Dr. M. A. Salam

| --- | --- | --- |
| Name of the Division Director | Signature | Date of Approval |

Appendix-3

Table of Contents

☒ Check here if appendix is included

Project Summary

Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Also 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.

Principal Investigator(s): Dr. Kazi M. Jamil
Research Protocol Title: A pilot study for capacity building for a multi-center randomized trial for treatment of kala azar in Bangladesh
Total Budget US$: **114,352** Beginning Date : 01/04/2007 Ending Date: 31/03/2008
Kala azar (KA) or visceral leishmaniasis (VL) is endemic in several districts of Bangladesh with the highest incidence in Mymensingh, Pabna and Tangail districts. ICDDR,B is involved in a project for improving the surveillance of KA in Trishal, Mymensingh since 2005. Improvement of case detection is necessary for both surveillance purposes and better control. The aims of this pilot study are to assess some newer techniques for diagnosis of KA using blood and urine samples of suspected cases; and evaluate response to treatment with sodium stibogluconate to which resistance has been reported in India, considered to be a part of the same zone harboring the disease agent *Leishmania donovani* and transmitted by the same vector *Phlebotomas argentipes* (sand-fly). No data is currently available on response to sodium stibogluconate in kala azar patients in Bangladesh. Although a number of new drugs have been evaluated in the treatment of KA in India and Kenya, no trial has so far been conducted in Bangladesh. A team of researchers from GlaxoSmithKline (UK) had recently visited Bangladesh to evaluate if it would be possible to conduct a Phase-III clinical trial with sitamaquine. They interacted with scientists of ICDDR,B and expressed their interest to help develop ICDDR,B's capacity in order to include Bangladesh as one of the sites for the planned, multi-centre, Phase-III trial of sitamaquine; India and Nepal are two other possible sites for the trial. The aims of the proposed study are to train physicians and laboratory personnel in preparation for the future drug trial(s) on KA as well as to compare different tests for its diagnosis that might improve case detection at the field level and used for research purposes.

Key Personnel (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the P
1. Dr. Kazi M. Jamil	Internal Medicine/Nutrition	PI
2. Dr. Rashidul Haque	Parasitology	Co-PI
3. Dr. Stephen P. Luby	Infectious Disease	Co-Investiga

Description of the Research Project

Hypothesis to be Tested:

Concisely list in order, 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.

The study will not test any hypothesis.

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.

Primary objective: To develop capacity to evaluate newer regimens for treatment of kala azar in Bangladesh.

Secondary objectives:

1. To evaluate novel diagnostic tests for kala azar including rK39 dipstick test in serum; KATEX in urine; and PCR (polymerase chain reaction) in blood.
2. To evaluate response to treatment with 28 injections of SAG using PCR in blood samples.

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.

Visceral leishmaniasis (VL), also known as kala azar (KA), is a vector-borne disease, caused by the parasite *Leishmania donovani* that affects about 300,000 people in India, Bangladesh and Nepal each year [1]. The disease is usually fatal if left untreated. The reported number of cases of KA has increased in Bangladesh from 3,978 cases in 1993 to 8,920 cases in 2004. However, these figures are considered to be gross underestimates, with an estimated incidence of 15,000-30,000 cases per year. In Bangladesh, 20 million people are considered to be at risk for VL. Until recently, sodium antimony gluconate (SAG) was the mainstay of treatment of VL in Bangladesh. Because of the reported cardiotoxicity and need to administer SAG injections intramuscularly for 28 days, the medical community considers development of effective newer drugs for treatment of VL as very important. In recently conducted limited clinical trials, two oral drugs, miltefosine and sitamaquine, have shown good efficacy in the treatment of VL in India [2-4]. Miltefosine, initially developed as an anti-cancer agent, is relatively expensive and is also contraindicated during pregnancy. Sitamaquine has been developed to offer treatment for VL in the endemic countries with limited resources. However, reported renal toxicity in association with this drug, as noted in limited clinical trials, need to be evaluated in larger randomized trials to finally determine the clinical usefulness as well as safety of Sitamaquine..

GlaxoSmithKline (GSK) undertook major initiatives to evaluate the efficacy of sitamaquine in the treatment of VL. Phase-II trials conducted in India and Kenya have produced encouraging results, paving the way for large, multi-centre, Phase-III trial. Bangladesh with its high burden

of VL, concentrated in relatively smaller geographic regions (12 upazillas of Mymensingh, a few upazillas of Rajshahi and Tangail), makes it an ideal site for evaluation of sitamaquine as an anti-leishmanial agent. A team from GSK-UK had recently visited Bangladesh and expressed their keen interest to work in collaboration with ICDDR,B for including Bangladesh as one of the sites of the planned Phase-III trial of sitamaquine expected to be conducted towards the end-2007. After visiting the endemic areas in Mymensingh, the area with highest burden of VL in Bangladesh, the GSK team also felt the need for capacity-building to meet the ethical requirements for conducting the sitamaquine trial. The capacity building would include: (1) training of physicians to perform splenic aspiration for parasitological diagnosis (Gold Standard) of VL, and (2) setting up of a tertiary referral facility for management of complications either due to the invasive diagnostic procedure or the disease itself. The team visited two large hospitals in the area and identified Community-based Medical College Hospital (CBMCH), Mymensingh as a potential tertiary care facility for this purpose.

ICDDR,B has an ongoing surveillance project on kala azar in Trishal, Mymensingh, and plans to evaluate some newer diagnostic tests that hold promise as alternative to current invasive tests, such as bone-marrow or splenic aspirations, with comparable sensitivity and specificity. The capacity building for Phase-III clinical trial of sitamaquine planned for 2008 will provide an excellent opportunity to make evaluation of these tests. We thus propose to evaluate PCR as a means for diagnosis of kala azar and compare it with 'splenic aspiration' followed by parasitological examination as the Gold Standard. We will also evaluate KATEX in urine of the cases enrolled before and after treatment with SAG, to ascertain the sensitivity and specificity of the tests in Bangladeshi KA patients [5-7].

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.

Among all the endemic areas of Bangladesh, KA is most prevalent in the district of Mymensingh (M&PDC Unit DGHS, 2004). In collaboration with the Government of Bangladesh, ICDDR,B has initiated a project to improve case detection and surveillance of KA in the area. The patients for the proposed study will be selected from three upazilla health complexes in Mymensingh per discussion with the government agencies working in these areas. If necessary, we will go for active surveillance to enroll required number of subjects for the study.

Patients

All patients older than 5 years living in the identified study areas attending the selected health complexes with febrile illness will be screened, beginning from May-June 2007. The time of enrollment of subjects will depend of completion of training of physicians and lab technicians involved with the study. In total, 200 consecutive cases meeting the following diagnostic criteria for KA will be enrolled into the study: (i) fever for >2 weeks; (ii) at least one of the the following criteria- splenomegaly, darkening of the skin, and weight loss; and (iii) a positive

rK39 dipstick test. Exclusion criteria will include: (1) children under five years of age, (2) pregnant women, and (3) patients who are suffering simultaneously from any other serious illness which is unrelated to kala azar (for example, tuberculosis, cancer, etc). The steps to be followed during enrollment and the initial study procedures are shown in Scheme-1. Patients (or their parents/guardians in the event of minors) meeting the above criteria, will be explained about the risk of treatment with SAG and the risk involved in splenic aspiration procedures by expert physicians. Informed consent (verbal or written) will then be obtained from the patients (or their parents/guardians in the event of minors) before inclusion into the study. The consent form will be read out to those who cannot read and a thumb-print or verbal consent will be obtained in the presence of two witnesses. If a subject fulfils entry criteria (fever, rK39 positive etc) but subsequent splenic aspirate turns negative for LD, they will be withdrawn from the study and their further investigations will be done, as needed, outside of the study. One hundred healthy individuals living in the same area will be enrolled as controls for evaluating PCR and other diagnostic tests of KA. Our field workers will visit houses in the community and collect blood samples from the healthy volunteers with their informed consent.

Study procedures and assessments

Splenic aspiration:

Splenic aspiration will be performed in 200 consecutive patients who will present with clinical features of KA with a positive rK39 dipstick test, if they give informed consent to participate in the study. As shown in **Scheme-I** below, splenic aspiration will be performed for confirmation of diagnosis on study day 1, and then again on day 29 to assess cure from the disease. Thus, when a subject is found to be positive by rK39 dipstick test, standard pre-procedure tests for splenic aspiration e,g. hemoglobin estimation, bleeding time, clotting time, and platelet counts, will be performed. The study personnel will arrange transportation of the patients from the health complex to the Community-based Medical College Hospital (CBMCH) in Mymensingh where splenic aspiration will be performed by a trained medical officer under the direct supervision of a senior consultant experienced in performing the procedure. Splenic aspiration will be performed only in those who are not severely anemic (Hb $\geq$ 6 mg/dl), and have platelet counts of >50,000/μl of blood. Prothrombin time will also be checked before splenic aspiration and those with a PT more than 4 seconds prolonged will not be eligible for the study. Patients will then be shifted to a post-operative room following their splenic aspiration, and will be observed for any signs of hemorrhage for the next 24 hours. All necessary treatment will be provided in the event of hemorrahage following the procedure, including blood transfusion. The department of surgery at CBMCH is well-equipped and capable of conducting major operations like emergency splenectomy if needed. If necessary, the patients may also be transported to Mymensingh Medical College Hospital, for more specialized treatment and support.

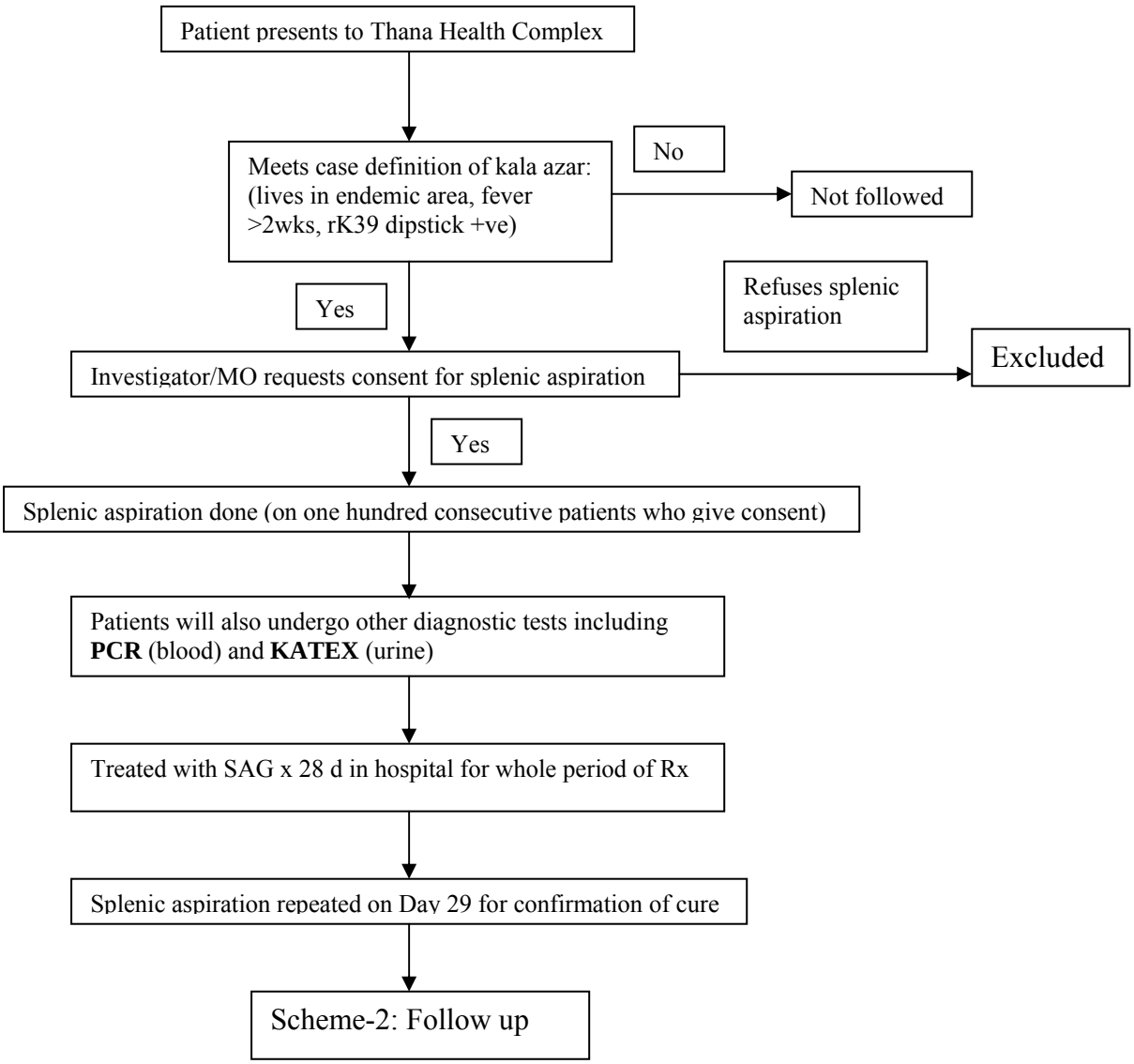
Treatment and follow up

In Bangladesh, intramuscular administration of sodium antimony gluconate (SAG; Albert David Ltd., India), in a dose of 20 mg/kg body weight, with a maximum dose of 850 mg/day, for twenty eight days is the current standard treatment for patients with KA. The pulse and respiratory rates, blood pressures, and temperature will be monitored at 12 hourly intervals during this treatment period. EKG will be performed if clinically indicated. The treatment will be stopped if signs of severe toxicity (cardiotoxicity, renal failure, etc) develop, and the patient will then be treated with standard dose of amphotericin B.

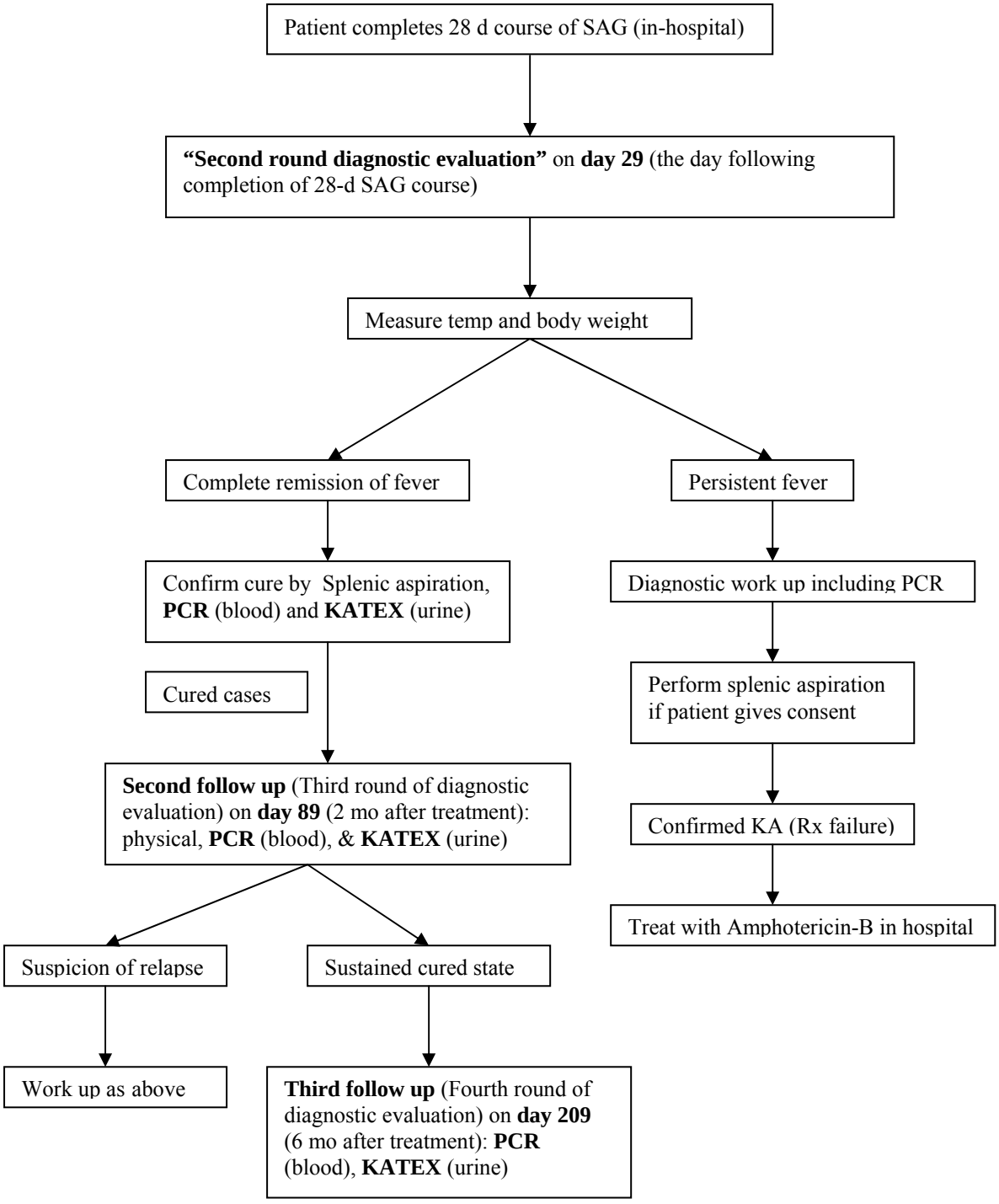
Hospitalization: All patients under treatment will be offered to stay at Community-based Medical College Hospital for the entire period of treatment with SAG. The patients will also be hospitalized for treatment with amphotericin B when there is treatment failure or toxicity with SAG.

Follow up: At the end of treatment, the patients will be evaluated for cure by physical examination and splenic aspiration. The subjects will then be followed for six months as shown in Scheme-2.

SCHEME-1: Enrolment of Subjects and algorithm of initial study procedures



SCHEME-2: Follow up for 6 months after treatment with SAG (including alternative treatment plan for drug resistance/relapse)



Diagnostic tests to be evaluated

rK39 dipstick test

A finger-prick blood specimen will be collected in capillary tubes for rK39 dipstick test [8]. This method is rapid and time saving. One to two drops of blood and then 1-2 drops of buffer will be placed on the dipstick pad, which will be then observed for approximately 5 minutes.

Parasitological diagnosis

Splenic aspirate will be used for parasitological diagnosis. A slide of splenic aspirates will be prepared for Giemsa staining. A portion of the aspirate will also be cultured in NNN medium, and the remaining portion will be used for PCR. Two lab technicians from ICDDR,B will be trained on staining techniques and reading of the slides. Randomly selected slides will also be sent to reference labs elsewhere for Quality Control.

Polymerase chan reaction (PCR)

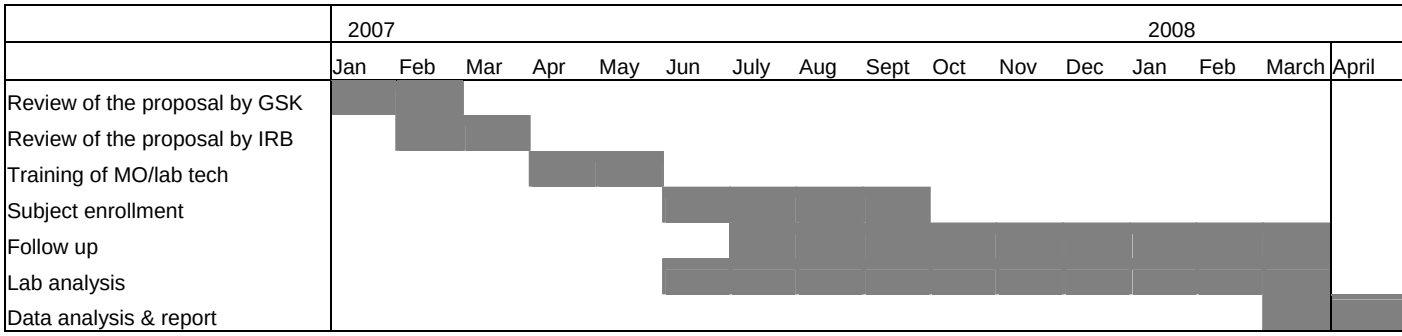
Leishmania donovani in the collected blood samples.will be detected using the methods described by Salotra *et al* [9].

Sample collection and DNA isolation: Collected blood specimen will be transported to the Parasitology Laboratory of ICDDR,B, and will be transferred to 4°C and will be processed generally on the same day. Blood (0.2 to 1 ml) will be treated with RBC lysis buffer (114 mM sodium phosphate [pH 8.0], 1 mM NH_4Cl), and the buffy coat will be isolated. DNA from parasite cultures as well as from clinical samples (skin scrapings, bone marrow, or blood) will be isolated by overnight lysis in NET buffer with proteinase-K (100 µg/ml) and 1% sodium dodecyl sulfate. DNA will be extracted by phenol-chloroform extraction and ethanol precipitation. In a few samples, DNA will be isolated from 0.2 ml of blood using a QIAamp DNA blood minikit (Qiagen) in order to determine if this method provided any advantage over the phenol-chloroform method for DNA extraction.

Oligonucleotide primers: The 792-bp *L. donovani* kinetoplast mini-circle sequence (accession no. Y11401) will be analyzed using PC-Gene software, and appropriate primers will be identified. The two primers will be used 5'-AAATCGGC TCCGAGGCGGGAAAC-3' and 5'-GGTACACTCTATCAGTAGCAC-3', together designated as the LdI primers. These will be synthesized using an Applied Biosystems DNA-RNA synthesizer (model 394). The LdI primers amplify a fragment of approximately 600 bp that is seen on the gels.

PCR amplification: DNA from cultured parasites (1 ng) and from clinical samples (100 ng) will be taken for amplification using the LdI primers described above. The reaction mixture (50 µl) will contain 10 mM Tris-HCl (pH 8.3) 50 mM KCl, 1.5 mM $MgCl_2$, a 200 µM concentration of each deoxynucleoside triphosphate, 50 ng of each primer, and 1.25 U of *Taq* DNA polymerase (Gibco BRL). Each reaction mixture will be overlaid with mineral oil, and amplification will be performed in a thermal cycler (Perkin-Elmer, Warrington, Great Britain) programmed for 40 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and extension at 72°C for 2 min, preceded by an initial denaturation of 2 min at 94°C. Final extension will be for 3 min at 72°C. Products will be analyzed by electrophoresis in 1% agarose gel containing ethidium bromide (0.5 µg/ml) in TAE buffer (0.04 M Tris acetate, 0.001 M EDTA) and photographed under UV illumination.

Duration of the study: One year from the date of start (April 2007 – March 2008)



Sample Size Calculation and Outcome Variable(s)

The sample size of 200 cases and 100 controls is based on practical considerations for meeting the primary objective of capacity development and the study is not formally powered for hypothesis testing. The emphasis for investigating sensitivity and specificity of the new diagnostic tests will be on presenting descriptive statistics and confidence intervals.

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 equipment that will be required for the study. For field studies, describe the field area including its size, population, and means of communications.

The study will be carried out in rural areas of Trishal, Mymensingh. Trishal is one of the most prevalent kala azar areas in Bangladesh. Data provided by the Civil Surgeon of Mymensingh shows that over 1,000 cases of KA were diagnosed in 2005 and 2006 at Trishal Thana Health Complex. However, it is generally believed that the number of cases will be higher if an active surveillance is conducted. ICDDR,B is currently conducting three KA studies in Trishal, and we also have a good rapport with the local communities.

Kala azar patients will be provided treatment at Community Based Medical College Hospital (CBMCH), which is one of the oldest private medical colleges in Bangladesh. This hospital has all the facilities required for management of critically ill patients. Experienced surgeons from the hospital will be involved during spleen aspiration. All patients will be hospitalized during treatment and emergency services will be provided by the hospital. Prof. AIM Mafakharul Islam, Principal of CBMCH has consented to provide necessary support in this regard. All laboratory investigations will be carried out in the Parasitology Laboratory under the Laboratory Sciences Division of ICDDR, B, Dhaka, Bangladesh. The laboratory is well equipped to support this type of study.

Data Safety Monitoring Plan (DSMP)

All clinical investigations (biomedical and behavioural intervention research protocols) should include the Data and Safety Monitoring Plan (DSMP) to provide the overall framework for the research protocol's data and safety monitoring. It is not necessary that the DSMP covers all possible aspects of each elements. When designing an appropriate DSMP, the following should be kept in mind.

a) All investigations require monitoring;

b) The benefits of the investigation should outweigh the risks;
c) The monitoring plan should commensurate with risk; and
d) Monitoring should be with the size and complexity of the investigation.

Safety monitoring is defined as any process during clinical trails that involves the review of accumulated outcome data for groups of patients to determine if any treatment procedure practised should be altered or not.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical software 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.

The sensitivity (proportion of correctly identified true positive results) and specificity (proportion of correctly identified true negative results) will be calculated for each diagnostic test, and 95% confidence intervals for these proportions will be calculated using Wilsons method [10].

The positive and negative predictive values will also be calculated using the method described by Altman et al [10]. The positive predictive value is the proportion of patients with a positive result for the experimental diagnostic test who are correctly diagnosed by parasitological examination. Similarly the negative predictive value is the proportion of patients with a negative diagnostic test result who are correctly diagnosed by parasitological examination. The 95% CI for the positive and the negative predictive values will be determined. These parameters will allow the comparison of each diagnostic test with the 'gold standard' parasitological examination.

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.

Sodium stibolgluconate (SAG) is the currently recommended first-line drug for treatment of kala azar in Bangladesh. It is a toxic drug and requires painful intra-muscular injections for 28 days. The proposed study will help evaluate the response to treatment with the currently recommended doses of SAG and also pave the way for clinical trials with orally effective drugs, such as sitamaquine, which may play a key role in controlling the disease in Bangladesh. The study will follow "Good Clinical Practice" and other national treatment guidelines for treatment of kala azar in Bangladesh. Written informed consent will be obtained from the participating patients or their parents/ guardians in case of the minors. As mentioned in the consent form, the participants can withdraw themselves from the study any time after enrolment. They will receive the same treatment facility at the government health care centres even after withdrawal from the study at their own will.

Use of Animals

Describe in the space provided the type and species of animals that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No use of animal

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. Caryn Bern, Allen W. Hightower, Rajib Chowdhury, Mustakim Ali, Josef Amann, Yukiko Wagatsuma, Rashidul Haque, Katie Kurkjian, Louise E. Vaz, Moarrita Begum, Tangin Akter, Catherine B. Cetre-Sossah, Indu B. Ahluwalia, Ellen Dotson, W. Evan Secor, Robert F. Breiman, and James H. Maguire. Risk factors for kala-azar in Bangladesh. *Emerging Infectious Diseases* 11(5): 655-662, 2005.
2. Monique Wasunna, Juma R. Rashid, Jane Mbui, George Kirigi, Dedan Kinoti, Hudson Lodenyo, J. Mark Felton, Antony J. Sabin, and John Horton. A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. Am J Trop Med Hyg 73(5): 871–876, 2005.
3. Tara K. Jha, Shyam Sundar, Chandreshwar P. Thakur, J. Mark Felton, Antony J. Sabin, and John Horton. A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India. Am J Trop Med Hyg 73(6): 1005–1011, 2005.
4. Shyam Sundar, T. K. Jha, C. P. Thakur, Juergen Engel, Herbert Sindarmann, Christina Fischer, Klaus Junge, Anthony Bryceson, and Jonathan Berman. Oral miltefosine for Indian visceral leishmaniasis. N Engl J Med 347:1739-46, 2002.
5. Mohammad Zahidul Islam, Makoto Itoh, Rusella Mirza, Iftikhar Ahmed, A. R. M. Saifuddin Ekram, Abdul Halim Sarder, S. M. Shamsuzzaman, Yoshihisa Hashiguchi, and Eisaku Kimura. Direct agglutination test with urine samples for the diagnosis of visceral leishmaniasis. Am J Trop Med Hyg 70(1): 78–82, 2004.
6. R. Maurya, R. K. Singh, B. Kumar, P. Salotra, M. Rai, and S. Sundar. Evaluation of PCR for diagnosis of Indian kala-azar and assessment of cure. J Clin Microbiol 43 (7): 3038-3041, 2005.
7. François Chappuis, Suman Rijal, Alonso Soto, Joris Menten, Marleen Boelaert. A meta-analysis of the diagnostic performance of the direct agglutination test and rK39 dipstick for visceral leishmaniasis BMJ 333:723-727, 2006.
8. Bern C, Jha SN, Joshi AB, Thakur GD, Bista MB. Use of the recombinant K39 dipstick test and the direct agglutination test in a setting endemic for visceral leishmaniasis in Nepal. *Am J Trop Med Hyg* 63:153–7, 2000.
9. Salotra P, Sreenivas G,Pogue GP, Lee N, Nakhasi HL, Ramesh V, and Negi NS. Development of a species-specific PCR assay for detection of Leishmania donovani in clinical samples from patients with kala-azar and post-kala-azar dermal leishmaniasis. J Clin Microbiol. 39(3):849-54, 2001.
10. Altman D G, *et al*. Statistics With Confidence. London: BMJ Publishing Group; 2nd edition 2000; 46-47, 105-107.

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 the People's Republic of Bangladesh through a training programme.

We anticipate that this research will result in several international publications. If appropriate, this work will facilitate to launch a VL prevention campaign in Bangladesh.

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.

The participants will be admitted to Community-based Medical College Hospital (CBMCH) in Mymensingh during the entire period of treatment with sodium stibogluconate, or with amphotericin-B. Thus CBMCH will be a collaborating partner of ICDDR,B in the conduct of this study.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

(Note: Biography of the external Investigators may, however, be submitted in the format as convenient to them)

1 Name: Kazi Mohammad Asif Jamil

2 Present Position: Associate Scientist, CSD

3. EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*			
INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
Chittagong Medical College, Chittagong, Bangladesh	M.B.B.S.	1988	Medicine
The University of Tokyo School of Medicine, Tokyo, Japan	Ph.D.	1995	Medical Sciences
The University of California Davis, Davis, USA	Postdoctoral fellowship	2001	Nutrition

4.0 List of ongoing research protocols:

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2005-022	01/07/2005	31/12/2007	20
2006-014	01/04/2006	31/12/2007	20
IHP-related activity	01/07/2005	30/09/2007	50

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3. As Co-Investigator

Protocol Number	Starting date	End date	Percentage of time
2006-27	01/10/2006	31/03/2009	05

5 Publications

Types of publications	Numbers
a. Original scientific papers in peer-review journals	6
b. Peer reviewed articles and book chapters	0
c. Papers in conference proceedings	0
d. Letters, editorials, annotations, and abstracts in peer-reviewed journals	6
e. Working papers	0

6 Recent publications including publications relevant to the present research protocol:

(Kala azar is a new area of research for K.M. Jamil who is currently involved as PI in a project on improving surveillance of kala azar in Bangladesh in collaboration with GoB institutions including IEDCR, NIPSOM, and DGHS. Thus, following publications only show the recent work of the PI, and are not related to kala azar.)

1. Biofortification for alleviation and prevention of vitamin A deficiency in Bangladesh. **Kazi M. Jamil**, Marjorie Haskell, Ana Perez-Exposito, Mohammad Hossain, M K R Bhuiyan, Sreekanth Attaluri, and Kenneth H. Brown. 14th Triennial Symposium of the International Society of Tropical Root Crops, 2006, (Abstract).
2. Daily consumption of Indian Spinach (*Basella alba*) or sweet potatoes has a positive impact on total body vitamin A stores of Bangladeshi men. Marjorie J. Haskell, **Kazi M. Jamil**, Ferdaus Hassan , Janet M. Peerson, M. Iqbal Hossain, George J. Fuchs, and Kenneth H. Brown. *Am J Clin Nutr*. 2004 Sep;80 (3):705-14.
3. Human milk as a source of ascorbic acid: no enhancing effect on iron bioavailability from a traditional complementary food consumed by Bangladeshi infants and young children. Lena Davidsson, **Kazi Asif Jamil**, Shafiqual Alam Sarker, Christophe Zeder, George Fuchs, Richard Hurrell. *Am J Clin Nutr.* 2004 Jun;79(6):1073-7.
4. Detection of endotoxin in sera from children hospitalized for treatment of diarrhea in Bangladesh. Ahmed T, Azam MA, Armed N, **Jamil KM**, Hassan F, Ogura N, Tamura H, Yokochi T. *J Endotoxin Res*. 2004;10(4):223-8.
5. Antioxidant status in children with edematous malnutrition. **KM Jamil,** T Ahmed, J Ensunsa, J Peerson, C Keen, and KH Brown. 10th Asian Conference on Diarrhoeal Diseases and Nutrition (ASCODD), 2003 (Abstract).

Biography of the Investigators:

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

(Note: Biography of the external Investigators may, however, be submitted in the format as convenient to them)

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1. Name: Dr. Rashidul Haque

2. Present Position: Scientist & Head, Parasitology Laboratory, Laboratory Sciences Division, ICDDR,B

3. Educational background (last degree and diploma & training relevant to the present research proposal)

M.B - Sofia Medical Academy, Sofia , Bulgaria, 1985

Ph.D - Institute of Parasitology, Bulgarian Academy of Sciences, Sofia, Bulgaria, 1988

Certificate Course on Laboratory Diagnosis of Parasitic Diseases, London School of Hygiene of Tropical Medicine, London, 1991.

4. List of ongoing research protocols
(start and end dates; and percentage of time)

4.1 As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2003-010	01/07/03	31/12/07	40%
2003-022	01/10/03	30/03/08	15%
2003-021	02/07/03	01/07/08	10%
2004-021	01/09/04	01/08/09	5%

As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2006-027	01/10/06	31/03/09	5%
2006-023	09/05/06	09/04/07	10%
2006-049	01/10/06	30/09/07	5%

5. Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	52
b) Peer reviewed articles and book chapters	4
c) Papers in conference proceedings	21
d) Letters, editorials, annotations, and abstracts in peer-reviewed journals	5
e) Working papers	0
f) Monographs	0

6. Five recent publications including publications relevant to the present research protocol:

Haque R, Roy S, Kabir M, Stroup SE, Mondal D, Houpt ER. 2005. *Giardia Assemblage* A infection and Diarrhea in Bangladesh. *J Infect Dis* 192: 2171-2173.

Kurkjian KM, Vaz LE, Haque R, Cetre-Sossah C, Akther S, Roy S, Steurer F, Amann J, Ali M, Chowdhury R, Wagatsuma Y, Williamson J, Crawford S, Breiman RF, Maguire JH, Bern C, Secor WE. 2005. Application of an improved method for the recombinant rK39 enzyme-linekd immunosorbant assay to detect visceral leishmaniasis disease and infection in Bangladesh. *Clin Diagn Lab Immunol* 12 : 1410-1415.

Haque R, Mondal D, Duggal P, Kabir M, Roy S, Farr BM, Sack RB, Petri WA Jr. 2006. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. *Infect Immun* 74: 904-909.

Bern C, Amann J, Haque R, Chowdhury R, Ali M, Kurkjian KM, Vaz L, Wagatsuma Y, Breiman RF, Secor WE, Maguire JH. 2006. Loss of leishmania skin test antigen sensitivity and potency in a longitudinal study of visceral leishmaniasis in Bangladesh. *Am J Trop Med Hyg* 75 (4): 744-748.

Thriemer K, Haque R, Wagatsuma Y, Salam MA, Akther S, Attlmayr B, Fukuda M,Schaecher K,
Miller RS, Noedl H. 2006. Therapeutic efficacy of qunine plus Sulfadoxine –pyremethamine for the treatment of uncomplicated falciparum malaria in Bangladesh. Am J Trop Med Hyg. 2006 75(4):645-649.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

(Note: Biography of the external Investigators may, however, be submitted in the format as convenient to them)

1 Name : Stephen Luby

2 Present position : Head, Programme on Infectious Diseases and Vaccine Sciences

3 Educational background :
(last degree and diploma & training relevant to the present research proposal)

University of Texas Southwestern Medical School at Dallas
MD 1986

University of Rochester Strong Memorial Hospital
Internship and residency in Internal Medicine.

Centers for Disease Control -- Epidemic Intelligence Service 1990
Completed Preventive Medicine Residency 1993

List of ongoing research protocols
(start and end dates; and percentage of time)

4.4. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2005-026	1 Oct 2005	31 Dec 2007	5
2005-023	1 Feb 2006	30 Jun 2008	10
2006-043	1 Nov 2006	31 July 2007	5

4.5. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2003-024	1 Sep 2003	31 Dec 2007	5
2003-002	June2003	Dec 2006	5

4.6. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2006-054	21 Jan 2006	21 Jan 2007	2

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	100
b) Peer reviewed articles and book chapters	9
c) Papers in conference proceedings	1
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	2
d) Working papers	0
b) Monographs	0

6 Five recent publications including publications relevant to the present research protocol

1.) Luby S, Qamruddin C, Shah A, Omair A, Pasha O, Khan AJ, Hoodbhoy F, McCormick J, Fisher-Hoch S. The relationship between therapeutic injections and high prevalence of hepatitis C infection in Hafizabad, Pakistan. *Epidemiology and Infection*. 1997, 119:349-356.

2.) Luby S, Zaidi N, Rehman S, Northrup R. Improving private practitioner sick-child case management in two urban communities in Pakistan. Tropical Medicine & International Health. 2002 March; 7(3):210-219.

3.) Luby S, Hoodbhoy F, Jan A, Shah A, Hutin Y. Long term improvement in unsafe injection practices following community intervention. *International Journal of Infectious Diseases*. 2005 Jan;9(1):52-59.

4.) Luby SP, Agboatwalla M, Feikin DR, Painter J, Billhimer W, Altaf A, Hoekstra RM. Effect of handwashing on child health: a randomised controlled trial. *Lancet*. July 15, 2005; 366:225-33.

5.) Luby SP, Rahman M, Hossain MJ, Blum LS, Husain NM, Gurley E, Khan R, Ahmed B, Rahmin S, Nahar N, Kenah E, Comer JA, Ksiazek TG. Foodborne Transmission of Nipah Virus, Bangladesh. *Emerging Infectious Diseases*. 2006 Dec 12(12):1888-1894.

Budget Justifications

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

Dr. Steve Luby will provide overall supervision of the project. Dr. Kazi Jamil, the PI of the project, will be mainly responsible for obtaining scientific and ethical approval, supervision of all the clinical activities of the project in the fields, and coordination among other partners of the project, including the Upazilla Health Complexes, the regulatory government agencies, and the Community-based Medical College Hospital in Mymensigh. Dr. Rashidul Haque will provide laboratory support to the project and will also share other major responsibilities of the project with Dr. Jamil. Both Dr. Jamil and Dr. Haque will be supported by ICDDR,B funds. Two medical officers will be trained on the safe procedure for splenic aspiration, requirement for GSK-funded drug trials on kala azar. It is expected that they would be sent to a kala azar treatment centre in India to obtain hands-on training on the procedures, especially splenic aspiration under ultrasonographic guidance and management of kala azar patients. These physicians are expected to work for the Phase-III trial of sitamaquine expected to be conducted in Bangladesh at the end of 2007. Laboratory investigations, including splenic aspiration with parasitological examination and PCR will be conducted for correct diagnosis and monitoring of response to treatment with the most commonly used treatment regimen for kala azar in Bangladesh, namely 28 intramuscular injections of SAG. Some laboratory investigations will be carried out to monitor drug toxicity or other complications. Other aspects of medical treatment and care have been planned to conform with 'good clinical practice' for the drug trial. Funding for all investigations, treatment, and staff development have been sought from GSK.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration.

This is mentioned under biography of the investigators.