International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

Memorandum

10 March 2003

To : Dr. Rubhana Raqib
PI of research protocol # 2003-038
Laboratory Sciences Division

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

Sub : Approval of research protocol # 2003-038

Thank you for your memo dated 7th March 2004 with the modified version of your research protocol # 2003-038 titled "Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank", which the ERC considered in its meeting held on 3rd December 2003. The modified version of the research protocol is hereby approved upon satisfactory addressing of the issues raised by the ERC.

You are permitted to send a total of 240 samples to be collected from the study participants of research protocol # 2003-038 to the Tuberculosis Specimen Bank and you shall submit a report to the Committee after completion of dispatching of the samples

You shall conduct the study in accordance with the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the study participants and compliance with the applicable provisions of ERC Guidelines. You shall also submit report(s) as required under ERC Guidelines.

Relevant excerpt of ERC Guidelines and '*Annual/Completion Report for Research Protocol involving Human Subjects*' are attached for your information and guidance.

I wish you all success in running the above-mentioned study.

Thank you once again.

copy: Associate Director
Laboratory Sciences Division

DRAFT

Memorandum

10 March 2003

To : Dr. Rubhana Raqib
PI of research protocol # 2003-038
Laboratory Sciences Division

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

Sub : Approval of research protocol # 2003-038

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I wish you all success in running the above-mentioned study.

Thank you once again.

copy: Associate Director
Laboratory Sciences Division

WHO

MEMORANDUM

To: **Dr. M.A. Quaiyum, Field Coordinator, FHRP**

From: **Dr. David H. Sniadack, Medical Officer-EPI** David H Sniadack 2/6/10/01

Date: **29 October 2001**

Subject: **Study proposal on programmatic and non-programmatic determinants of immunization coverage**

Thank you for asking me to review the proposal referenced above, which is a prospective study of reasons for failure to immunize children. The methodology offers an advantage to the usual retrospective surveys that are prone to recall bias. I am not sure if the precision of the final point estimates of coverage or indicators will be greater than that offered by the weighted average of eleven 30-cluster surveys; it would be nice to include a description of the expected statistical significance/95% CIs of the point estimates. The proposal also includes non-quantitative methodology that may reveal information in addition to what we already know about failure to routinely vaccinate children. These are not only included in the background section of the project proposal but are frequently excised verbatim from our draft CES 2000 report that we shared with you some time ago. The final CES 2000 report includes revised findings from those data that should be used as the source for the project background section instead of what is present in the current draft. According to the CES 2000, major reasons parents gave for nevervaccinating their children included lack of knowledge about immunization (32%), immunization not important (16%), working or too busy (8%), absence of vaccinator or vaccine (7%), child was sick and not taken for vaccination (7%), and vaccination site too far away (6%). Major reasons parents gave for incomplete vaccination (i.e., drop-out children) included lack of knowledge regarding subsequent doses (30%), absence of vaccinator or vaccine (17%), working or too busy (10%), child was sick and therefore not taken for vaccination (10%), and child was away from home (6%). Distance to vaccination site was significantly associated with incomplete or no vaccination in rural areas, but was not often given as a reason for not vaccinating children. Regarding lack of knowledge, parents of never vaccinated children were unaware about the need to vaccinate, and parents of partially vaccinated children were unaware about the need for additional doses of vaccine. We expect the final CES 2000 report to be in print by the end of November and will be happy to share a copy with you.

OBEZ1

International Centre for Diarrhoeal Disease Research, Bangladesh
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Cable : Cholera Dhaka

To: Chairperson, ERC 7 March 2004

Through: Associate Director, LSD

From: Rubhana Raqib, LSD

Subject: Protocol # 2003-038

Thank you for the review of the protocol # 2003-038. The responses to specific comments are given below:

a) The protocol has two objectives: one to validate the ALS assay for diagnosis of active tuberculosis and the second is to collect biological specimens for the TB Specimen Bank activity. The link is that both objectives require collection of specimens from confirmed cases of TB as well as non-TB patients for testing of and validating new diagnostic methods. Thus, the study subjects will be the same for both purposes.

b) The sequence of activity in the protocol has been given in the flow sheet (page 35). However, as suggested we have also included a brief description in the Methods Section (page 14).

c) The sample size for both the Specimen Bank activity and the ALS assay are the same, i.e. n=240. Samples from each patient will be utilized for validation of the ALS assay and for the purpose of storage in the Specimen Bank. Altogether, a total of 240 samples will be collected from both sites combined. This has now been clearly stated in the abstract summary (pages I, 6) and Methods Section (page 19).

d) Definition of high-risk subjects: In this protocol, the term "high-risk subjects" was meant to imply patients presenting to the clinic with symptomatic pulmonary disease who have a high likelihood of having TB. However, since this term is misleading, we have now deleted it from the text (pages 1, 7 and 19).

e) Definition of a non-TB case: A non-TB case is defined as a patient who has chronic cough of ≥3 weeks, fever, repeated sputum smear negative for tuberculosis, repeated sputum culture negative for *M. tuberculosis*, chest x-ray findings and clinical improvement at follow-up without anti-TB treatment with alternative cause of disease may or may not be confirmed. This is shown in table 1, page 12 and has now been incorporated in pages 11 and 19 as suggested.

f) The study aims to store samples from both TB patients as well as non-TB patients who are initially suspected of having TB but later confirmed to be non-TB cases. To test and validate diagnostic methods (to determine sensitivity and specificity), samples from both positive and negative cases are needed and hence the requirement in the present protocol to enroll non-TB patients. Examples of non-Tb diseases: bronchiectasis, pneumonia, lung cancer, lung abscess, aspergillosis etc. However, for our study it is not necessary to confirm such diagnosis; only exclusion of TB is of paramount importance.

g) Enrolment of TB patients will depend upon routine passive case detection and not active case finding. Study physician and consultant involved in the study will select patients from the daily cases attending the TB clinics. This has been mentioned in pages 9 and 14.

h) Blood specimens will be obtained from the patients after initial screening, based on clinical evaluation, sputum microscopy and chest x-ray. Blood will be collected from both TB (n=102), and non-TB patients (n=138) as control subjects who will initially be enrolled as suspected TB cases and later confirmed as non-TB cases. Final diagnosis will be made based on sputum culture report and clinical/CXR changes at follow-up after therapy. This has been shown in the flow sheet (page 35), figure 1 and has now been mentioned in the Design and Methods Section (page 9 & 14).

i) The enclosed documents: An official letter from the WHO/TDR sent by Dr Mark Perkins (Manager, Diagnostic R&D Product Research and Development, WHO/TDR) stating that specimens will be shipped to a central facility under contract to WHO/TDR to receive, cryopreserve and distribute materials in the Bank solely as directed by WHO/TDR. (ii) The letter also mentions that WHO has preliminarily selected ICDDR,B as one of the participating institutes for TB Specimen Bank activity; the final formal letter of acceptance will be provided after their site visit between 8-9 April 2004. (iii) Letter of acceptance of ICDDR,B from the Director for functioning as the collaborating institute of the Bank; (iv) Regarding permission from the GoB: (1) it is a standard practice at ICDDR,B as well as in other research institutes all over the world to send or receive biological samples for various research procedures. This has always been done at ICDDR,B with prior approval of our review boards without seeking additional approval from the GoB. (2) The serum samples that are to be sent to the WHO/TDR Bank will be de-linked when given to investigators and will be used solely for the purpose of testing new diagnostic methods for tuberculosis. (3) No DNA testing will be done in the specimens. (v) WHO is a world-renowned, United Nations specialized agency for health and, therefore, we are confident that the instructions/decisions will be strictly followed and maintained. The terms and conditions for applying to the WHO/TDR to obtain materials for testing have also been mentioned in the letter.

j) Since HIV infection leads to immunosuppression, mostly the immune-based and serological diagnostic methods for TB are affected by HIV co-infection in TB. Even the sputum smear becomes negative and the PPD skin test reactivity decreases due to HIV infection (Bruchfeld J, 2000, Trans R Soc Trop Med Hyg). Some of the relevant references are: Rolfsen N, 1995, STEP Perspect; Somi GR et al, 1999, Int J Tuberc Lung Dis; Ratanasuwan W et al, 1997, Int J Tuberc Lung Dis; Lawn SD et al, 1997, Trop Med Int Health; Villarino ME, 1994, AIDS.

k) We apologize for the unintentional mistake of not providing the consent forms. Bangla version of the main consent form has now been included. The Jagori consent form for HIV testing has also been provided. Since Jagori is a service provision and not a research outfit, it obtains consent for providing the particular counseling service. This is a standard procedure for medical purpose. Therefore, it was not necessary for Jagori to seek approval of the ERC for the HIV-test consent form. Jagori follows the WHO guidelines for HIV testing. As suggested, the main consent form now includes detailed information on HIV testing, and storage of specimens for the TB Specimen Bank. The information that no DNA testing will be done has also been incorporated. A separate consent form has now been included for the patients' willingness to come to ICDDR,B to provide specimens, since counseling for HIV testing and specimen collection will have to be done at the ICDDR,B.

l) Since chest X-ray will be performed, it has been incorporated in the consent form as pointed out. However, Tuberculin skin test will not be performed since it may not be useful in settings of endemic disease.

m) As suggested, the items in ERC Face Sheet have been rectified. However, since we are not obtaining specimens from non-ill subjects, item # 1(b) should be "No".

Some new additions have been incorporated by the WHO/TDR recently (4 March, 2004) in the protocol. (**A**) A flow chart giving an overview of enrolment, clinical and laboratory procedures (figure 1) has been provided. (**B**) From 50 patients who are high volume (5 ml) sputum producers, sputum, urine and blood will be collected and send to the WHO/TDR. From the rest of the patients (n=150), sputum will only be used for AFB microscopy and culture, sensitivity etc and will not be sent. Only sera (20 ml blood) collected from these 150 patients will be sent. (**C**) In the earlier protocol sent by the WHO/TDR, blood volume requirement was flexible (10-15 ml); however, in the present revised protocol, they mentioned the requirement of 20 ml of blood. Together with 5 ml needed for the ALS assay, the total volume of blood now comes to 25 ml.

The necessary documents are attached herewith. We hope that the protocol after incorporation of the suggested points is now acceptable in its present form.

Thanking you.

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Memorandum

8 December 2003

To : Dr. Rubhana Raqib
PI of research protocol # 2003-038
Laboratory Sciences Division

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

Sub : Research protocol # 2003-038

Thank you for your research protocol # 2003-038 titled "Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank", which the ERC considered in its meeting held on 3rd December 2003. After review and discussion, the Committee made following observations on the protocol:

a) The protocol has two distinct objectives, viz, validation of ALS test for diagnosis of active tuberculosis and sending of samples to WHO/TDR specimen bank. The link between these two objectives is unclear to the Committee.

b) The sequence of activities (namely, recruitment of subjects, diagnosis of diseases, obtaining of samples for storage, HIV testing, etc) of the protocol should clearly be described.

c) The sample size to be studied for validation of the ALS test and for storage in the tuberculosis bank should be clearly stated. Further, it is not clear whether the PI plans to collect a total of 240 samples (p9) or 240 samples from each of the sites. It is also not clear whether the PI intends to use the same samples for validation of the ALS test as well as for banking purpose.

d) Definition of 'high-risk subjects' (p17) and the number & types of control be provided.

e) Definition of non-TB cases (p17) should be provided.

f) It is not clear whether the PI intends to store specimens of only confirmed TB cases or not. If other than TB samples are planned to be stored, the purpose and types of diseases (diagnosis) should clearly be defined.

g) The strategy for recruitment of study participants should be explicitly described.

h) It is assumed that the PI intends to collect and store samples from confirmed cases of TB. In that case, collection of large volume of blood (15 ml for bank) from all

cases before diagnosis of TB (either by smear or culture/or x-ray) was considered to be unnecessary. Therefore, the PI should clearly describe how the blood samples would be utilized.

i) The PI should obtain and submit: (i) authenticated statement of the WHO/TDR indicating the establishment of the central WHO/TDR Tuberculosis Specimen Bank/Distributor Impath Inc. in the USA, (ii) selection of ICDDR,B as a participating institution of the Bank, (iii) acceptance of ICDDR,B for functioning as the participating institution of the Bank, (iv) clearance of the GoB for sending Bangladeshi specimens to the Bank, and (v) authorization of the WHO for sending the specimen to the distributor in the USA should be provided for information of the Committee. Further, the terms and conditions for obtaining the stored samples from the Distributor in the USA by Bangladeshi researchers or by researchers from other developing countries in future should also be submitted.

j) The PI has not mentioned which diagnostic test for TB is affected by HIV infection and in which way. The PI is advised to provide supportive references.

k) Consent form: Bangla version of the consent form has not been provided with the protocol. Further, nothing has been mentioned about the HIV test in the English consent form. Also, the PI has not furnished the Jagori's consent form that would be used for HIV testing. It was also unclear to the Committee which Ethical Review Committee has approved that consent form. Further, as the PI intends to do HIV tests in all blood samples to be stored in bank, the consent of the study participants should be obtained at the time of initial enrolment and should be reflected in the main consent form. Also, the storing of samples in a bank for future use has not been reflected in the consent form. Consent should also be obtained for storing the samples in a bank at ICDDR,B and in the USA. It should also be mentioned that the stored samples would not be used for DNA analysis for any reasons other than tuberculosis research.

l) Clinical data will be collected by history, physical examinations and standard laboratory tests. But in the consent form, nothing has been mentioned about the usual clinical work regadring the diagnosis of TB, namely X-ray chest and Tuberculin test.

m) On the ERC Face Sheet, item # 1(b), 2(a, b, c and e) should be marked "YES" instead of 'no".

You are therefore advised to modify the protocol incorporating the above observations and to submit the modified version of the protocol for consideration of the Chair.

Thank you once again.

copy: Associate Director
Laboratory Sciences Division

Attachment 1 Date:

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Dr Rubhana Raqib

Application No. 2003-038

Title of Study: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) WHO/USAID

Project Status: _____

[√] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the 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) Minor or persons under guardianship — Yes / No

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes / No
   - (b) Social risk — 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 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 the study — Yes / No
   - (b) Procedures to be followed including alternatives used — Yes / No
   - (c) Physical risk — 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 parents or guardian (if subjects are minor) — 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 with 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. Example 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 Committee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Rubhana
Principal Investigator

Trainee

Abstract Summary

A major obstacle to the development and testing of new assays for the diagnosis of tuberculosis is the lack of access to good clinical material from well-characterized TB and control patients. The Tuberculosis Diagnostics Initiative (TDI) at the WHO/TDR has developed a global network of six clinical/laboratory sites (Banjul, Kampala, Salvador, Winnipeg and Barcelona) with good diagnostics capabilities and has worked with them to establish a large TB specimen Bank containing meticulously processed serum, sputum and urine collected from well-characterized patients with symptomatic respiratory disease. Additional collaborative sites are now being sought worldwide by the WHO/TDR to represent a geographically diverse population. The WHO/TDR has selected ICDDR,B as a site for this purpose. A total of 240 patients will be recruited for the sample collection. All specimens (blood 25 ml, urine 10 ml and sputum 5 ml) will be collected and processed using a standardized protocol provided by the WHO and will be accompanied by complete clinical information, including the HIV status. Sera, urine and sputum from 50 patients who are high sputum producers and only sera from rest of the 150 patients will sent to the WHO/TDR. When a diagnostic method is shown to work on a HIV-negative population, quite often it fails to perform well in the HIV-infected population. Therefore, HIV testing is required as crucial baseline information in the complete clinical picture. Patients will have to give consent to HIV testing. They will undergo pretest counseling and the results will be provided to them after 3 days during the post-test counseling. This will be entirely a one-to-one interaction between the HIV counselor and the TB patient. Where required, the Voluntary Counseling and Testing (VCT) Services unit will provide counseling. The counselor at VCT will provide the result of the HIV test to the client directly and not to the investigators. It will be up to the client whether or not to disclose the test-results to the investigator. The testing of HIV will be linked. After collection of target number of specimens, these samples will be sent to a WHO-designated Distributor. These specimens will be available to WHO-approved investigators for development and testing of new diagnostic methods. The Distributor will maintain an inventory of specimens received and a record of how these specimens have been stored and distributed. This information will be available to WHO/TDR. WHO/TDR will maintain records of the performance of specimens to help identify potential errors in specimen characterization. The specimens will be blinded when given to investigators for testing of new methods by the WHO/TDR. Also, for each diagnostic test, WHO/TDR will assist with the resolution of discrepancies by facilitating and coordinating exchange of information between the investigator/manufacturer and the Collection Site personnel. No DNA testing will be done on these specimens.

The ALS (antibodies in lymphocyte secretion) assay has been recently studied to investigate its diagnostic performance to detect Tuberculosis (TB)-specific antibodies in patients with active TB. The method was found to be 93 % sensitive and 80% specific in detecting active TB in clinically suspected patients. However, the sample size was quite small (n=49) and was based on specimens from smear positive cases. We therefore aim to validate this method in a larger sample size of 240 suspected patients having illness in which tuberculosis will be part of the differential diagnosis. The validation will be done by comparing the sensitivity and specificity of the ALS assay with the sputum culture method as well as clinical symptoms.

ICDDR,B: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL
Protocol No. 2003-038

FOR OFFICE USE ONLY

RRC Approval:	√ ☐Yes / ☐No Date:
ERC Approval:	☐Yes / ☐No Date:
AEEC Approval:	☐Yes / ☐No Date:

Project Title: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

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: Tuberculosis, ALS assay, specimen bank

Relevance of the protocol:

The ALS (antibodies in lymphocyte secretions) assay has been found to be highly sensitive (93%) and specific (80%) in diagnosing active TB in a small number of patients (n=49). We aim to validate the method in a larger sample size (n=240) among subjects with symptomatic pulmonary disease by comparing it with the sputum culture method. The lack of access to good clinical material from well-characterized TB and control patients has been identified as the major obstacle to the development and testing of new assays for the diagnosis of TB. The Tuberculosis Diagnostics Initiative (TDI) at the WHO/TDR has developed a global network of six clinical/laboratory sites with good diagnostics capabilities and has worked with them to establish a large TB specimen Bank containing meticulously processed serum, sputum and urine collected from well-characterized patients with symptomatic respiratory disease. Additional collaborative sites are now being sought worldwide by the WHO/TDR to represent a geographically diverse population. ICDDR,B has been selected as a site for this purpose by the WHO/TDR. Samples will be collected from the same group of patients as above (n=240). All specimens will be collected and processed using a standardized protocol provided by the WHO and will be accompanied by complete clinical information, including the HIV status. When a diagnostic method is shown to work on a HIV-negative population, quite often it fails to perform well in the HIV-infected population. Therefore, HIV testing is required as crucial baseline information in the complete clinical picture. One set of specimens will be kept in ICDDR,B and one set will be sent to the WHO. These specimens will be available to the WHO-approved/assigned investigators for the development and testing of new diagnostic methods.

Programmes

- ☐ Child Health Programme
- ☐ Nutrition Programme
- ☐ √ Programme on Infectious Diseases & Vaccine Science
- ☐ Health and Family Planning Systems Programme
- ☐ Population Programme
- ☐ Reproductive Health Programme

Principal Investigator: Dr Rubhana Raqib **Division:** LSD **Phone:** 2404

Address: Immunology Laboratory, Laboratory Sciences Division **Email:** rubhana@icddrb.org

Co-Principal Investigator(s): Dr David Sack

Co-Investigator(s): Dr Robert Breiman, Dr Zeaur Rahim, Dr SMM Kamal[1], Dr K Zaman, Dr Tasnim Azim, Dr Jahanara[2]

Student Investigator/Intern:

Collaborating Institute(s): [1]National Institute of Diseases of the Chest and Hospital (NIDCH) & [2]National TB Reference Laboratory in Shyamoli.

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

Gender
- ☐ √ Male
- ☐ √ Females

Age
- ☐ 0 – 5 years
- ☐ 5 – 9 years
- ☐ √ 18 – 19 years
- ☐ √ 20 – 64 years
- ☐ √ 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 NIDCH & Shyamoli TB Hospital
- ☐ 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 For specimen bank & diagnostic purposes

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: 240 ☐

Sub-group 102 TB +ve cases, 138 TB –ve cases ☐ _____ ☐

_____ ☐ _____ ☐

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".

Yes/No

☐ ☐ Is the proposal funded?

If yes, sponsor Name: _____

Yes/No

√☐ ☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1) TDR/WHO

(2) USAID

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.

Dates of Proposed Period of Support	**Cost Required for the Budget Period ($)**
(Day, Month, Year - DD/MM/YY)	*a.* *Ist Year* *2nd Year* *3rd Year* *Other years*
Beginning date December, 2003	92,116 $ _____ _____ _____
End date November, 2004	*b.* ***Direct Cost :*** 92,116 $ ***Total Cost :*** 105,362 $

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 reviewer's comments and is approved.

GB Nair		Oct 23, 2003
Name of the Associate Director	Signature	Date of Approval

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 responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI Rubhana

Date: 23-10-03

Name of Contact Person (if applicable)

Table of Contents

X Check here if appendix is included

PROJECT 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: Rubhana Raqib

Project Name: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

Total Budget 105,362 $ **Beginning Date:** December 2003 **Ending Date:** November 2004

The ALS (antibodies in lymphocyte secretion) assay has been recently studied to investigate its diagnostic performance to detect Tuberculosis (TB)-specific antibodies in patients with active TB. The method was found to be 93 % sensitive and 80% specific in detecting active TB in clinically suspected patients. However, the sample size was quite small (n=49) and was based on specimens from smear positive cases. We therefore aim to validate this method in a larger sample size of 240 suspected patients having illness in which tuberculosis will be part of the differential diagnosis. The validation will be done by comparing the sensitivity and specificity of the ALS assay with the sputum culture method as well as clinical symptoms.

A major obstacle to the development and testing of new assays for the diagnosis of TB is the lack of access to good clinical material from well-characterized TB and control patients. The Tuberculosis Diagnostics Initiative (TDI) at the WHO/TDR has developed a global network of six clinical/laboratory sites with good diagnostics capabilities and has worked with them to establish a large TB specimen Bank containing meticulously processed serum, sputum and urine collected from well-characterized patients with symptomatic respiratory disease. Additional collaborative sites are now being sought worldwide by the WHO/TDR to represent a geographically diverse population. The WHO/TDR has selected ICDDR,B as a site for this purpose. All specimens will be collected and processed using a standardized protocol provided by the WHO and will be accompanied by complete clinical information, including the HIV status. When a diagnostic method is shown to work on a HIV-negative population, quite often it fails to perform well in the HIV-infected population. Therefore, HIV testing is required as crucial baseline information in the complete clinical picture. Samples will be collected from the same set of patients as above (n=240). For this purpose, one set of specimens will be kept in ICDDR,B and one set will be sent to the WHO. These specimens will be available to the WHO-approved investigators for the development and testing of new diagnostic methods.

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

Name	Professional Discipline/ Specialty	Role in the Project
1. Rubhana Raqib	Infectious Diseases / Immunologist	Principal Investigator
2. David Sack	Vaccinology / Microbiologist	Co-Principal Investigator
3. Robert Breiman	Infectious Diseases / Internist	Co-investigator
4. Zeaur Rahim	Microbiologist	Co-investigator
5. K. Zaman	Epidemiologist	Co-investigator
6. SM Mostafa Kamal	Clinician / Microbiologist	Co-investigator
7. Tasnim Azim	Virology / Immunologist	Co-investigator
8. Jahanara	Clinician / Microbiologist	Co-investigator

DESCRIPTION OF THE RESEARCH PROJECT

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).**

1. To evaluate the usefulness of the ALS assay among subjects suspected of having tuberculosis and to compare the sensitivity and the specificity of the ALS assay with the sputum culture for diagnosis of active pulmonary tuberculosis.

2. The purpose of the WHO/TDR Tuberculosis Specimen Bank is to set up the Specimen Bank at the ICDDR,B using clinical materials from well-characterized TB and control patients for the development and testing of new diagnostic methods for TB for use in low-income countries.

   2a. Support the development and evaluation of novel diagnostics
   2b. Limit the need for field trials
   2c. Assist the approval process
   2d. Promote product comparisons
   2e. Facilitate quality control
   2f. Encourage investment in TB diagnostics by providing reliable reference materials.

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).**

Tuberculosis (TB) remains a major global health problem and is the most frequent cause of death from a single infectious agent (5). It accounts for 8 million new cases and 3.8 million deaths annually (3, 5, 21, 32). Eighty percent of the tuberculosis cases involve persons who are in their productive years (15 to 59 years of age) (32). The appearance of multidrug-resistant strains of *Mycobacterium tuberculosis* and the HIV/AIDS epidemic have contributed to the resurgence of active TB in humans. Thus, WHO declared tuberculosis a global emergency in 1993. Surveys carried out in Bangladesh from 1987 to the present suggest the smear positive TB case rate in Bangladesh to be between 1-1.8% (2, 4, 21).

Early diagnosis of TB is crucial to prevent the spread of the disease in the community. However, the clinical and laboratory diagnosis, follow-up of the infection activity and response to the therapy is not always easy to evaluate (7, 33). Although, culture of bacteria is the gold standard in diagnosis and follow-up of disease, it can take up to 6-8 weeks to isolate *M. tuberculosis*. It is estimated that a false negative culture result may be obtained in 10-20% of TB cases (20, 28). A rapid serological test for diagnosis, follow-up of disease activity and response to therapy would be very useful to the clinicians (6, 40). The PPD skin test (Mantoux test) is an important tool for diagnosis of latent TB infection and disease in the developed world but it has low predictive value in Bacillus-Calmette-Guerin (BCG)-vaccinated individuals as well

as in individuals living in areas endemic for TB due to cross-reactivity with BCG and atypical mycobacteria, and false negative reactions in malnourished children (16-18). BCG has been used as an antigen in enzyme immunoassays in *in vitro* studies to determine the disease activity but was aborted due to difficulties in interpretation, or differentiating between active or past disease, and low sensitivity and specificity, respectively (8, 11, 15, 22, 39). With the identification of regions of *M. tuberculosis* genome that are missing in BCG and nontuberculous mycobacterium, new antigens have been identified providing better opportunities for development of novel diagnostic tools (9, 27, 36). The introduction of these antigens resulted in a much higher sensitivity and specificity in cell response assays (37). However, serological tests based on mycobacterial antigens to detect circulating antibodies have been hampered by decreased sensitivity and cross-reactivity with other mycobacteria (14, 24, 29, 30, 35) or have relatively limited utility in the diagnosis of tuberculosis in countries where tuberculosis is endemic (38). Several molecular biological techniques have been proposed as indicators of disease activity in pulmonary and extrapulmonary tuberculosis (13, 23) and are currently the most sensitive and specific diagnostic tests. However, a recent study on inter-laboratory comparisons of PCR-based TB diagnosis have demonstrated the complications of obtaining reproducible results with such sensitive techniques where false positive results can be a major problem (25, 26).

The antibodies from lymphocyte secretion (ALS) assay has earlier been used to detect specific antibody response after oral vaccination with a killed cholera vaccine in healthy adults without any requirement for *in vitro* antigen stimulation (12). We applied the method to investigate the TB diagnostic performance of this assay based on detecting TB-specific IgG antibodies secreted from peripheral blood lymphocytes in supernatant. The patients with active TB had higher BCG-specific antibody responses than patients without TB or healthy subjects (P=0.001). Previous vaccination did not hamper the test for identification of TB and allowed successful differentiation between BCG-vaccinated and *M. tuberculosis*-infected patients. The method was found to be 93% sensitive and 80% specific and the positive predictive value of the test was 97% (31). However, it is expected that the performance characteristics i.e. the positive predictive value of the assay may vary on the basis of the prevalence of TB in the tested population. Considering 2% prevalence of TB in Bangladesh, with a sensitivity of 93% and a specificity of 80%, the positive predictive value of the ALS assay in the population is about 8.6%. Further studies should be carried out to evaluate the validity of the ALS method for diagnosing active tuberculosis in suspected symptomatic patients comprising of a larger sample size by comparing both the smear and culture double positive patients with smear/culture negative symptomatic patients.

Meetings of consultants from industry, academia and public health convened by the Tuberculosis Diagnostics Initiative (TBDI) at WHO/TDR identified lack of access to good clinical material from well-characterized TB and control patients as a major obstacle to the development and testing of new assays. TBDI has since developed a global network of six clinical/laboratory sites with good diagnostics capabilities and has worked with them to establish a large TB specimen Bank containing meticulously processed serum, sputum, urine and saliva collected from well-characterized patients with symptomatic respiratory disease. Additional collaborative sites are now being sought worldwide by the WHO/TDR to represent a geographically diverse population. ICDDR,B has been selected as a site for this purpose by the WHO/TDR. All specimens are collected and processed using a standardized protocol provided by the WHO and are accompanied by complete clinical information, including the HIV status. When a diagnostic method is shown to work on a HIV-negative population, quite often it fails to perform well in the HIV-infected population. Therefore, HIV testing is required as crucial baseline information in the complete clinical picture. The specimen bank enrollment site will be supported by the WHO/TDR for the enrollment and diagnostic characterization of 240 patients and the contribution of specimens to the Central Distribution Facility housing the WHO/TDR TB Specimen Bank. Sputum and other specimens will be collected and processed according to the

existing protocol to assist in confirming a final diagnosis, but only serum will be cryopreserved for submission to the Bank.

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).**

Design

In order to attain the objectives, materials placed into the bank must be carefully collected, and the bank must be organized in a specific way.

1. Microbiologic and clinical data will be collected according to the specific protocol.
2. Complete clinical data will be collected and keyed to biologic specimens.
3. An adequate number of specimens of a variety of types must be available. Initial enrolment will be limited to 240 patients per site, with expansion possible in the future.
4. Adequate volume must be collected so that the specimen bank can be "amplified" by the on-site separation of multiple aliquots per patient.

Patients who have symptoms consistent with pulmonary TB disease will be asked to participate (see inclusion criteria). A consent form to participate and for HIV testing must be signed by the patients. Clinical examination and HIV testing will be performed and the results will be recorded in, in the Case Report Form (sections A, B, Ca, Cb and Cc). The patients will be divided into two groups. The first group will consist of 50 high volume sputum producers (minimum 5ml). They will provide sputum, serum and urine specimens for the bank. The second group, composed of 150 low volume sputum producers, will contribute only serum to the bank. Patients in both groups must provide minimum 2 sputum samples for diagnostic purposes (smear microscopy and culture) and undergo chest radiography. The results of smear microscopy, culture and chest radiography will be entered in the Case Report Form (section D) Follow-up is required after 8-12 weeks in the following situations:

1. Patients who are smear negative, culture negative **and** treated for TB require clinical and radiographic evaluation
2. Patients who are smear negative, culture negative and are NOT treated for TB, require 2 smears and 2 cultures

All patients must be assigned a final diagnosis that matches one of the categories outlined in Table 1. Results must be recorded in Section F of the Case Report Form.

Study Site and Enrolment Criteria

The site for patient selection will be the National Institute of the diseases of the Chest and Hospital (NIDCH) and the National TB Reference Laboratory in Shyamoli. Patients presenting to the hospital and clinic with symptomatic pulmonary disease thought to have a high likelihood of having tuberculosis will be enrolled. Enrolment will depend upon routine passive case detection (i.e. not an active case finding).

Inclusion criteria:

1. Symptoms suggesting pulmonary TB: persistent cough (generally $\geq$3 weeks) and at least one other clinical sign or symptom listed in the Case Report Form.
2. Willingness to give informed consent to sample collection and HIV testing
3. Production of adequate quantity of sputum
4. Adult age ($\geq$ 18 years old)

Exclusion criteria

1. Patients receiving any antituberculosis medication in the previous 60 days
2. Patients without pulmonary disease – those with **only** extra-pulmonary disease will be excluded
3. Children under 18 years of age
4. Asymptomatic patients or those for whom good follow-up and a clear final diagnosis are judged to be difficult.
5. Inability to provide informed consent (e.g. prisoners, mentally impaired).

Patients NOT excluded

1. Those with concomitant disease such as diabetes, HIV, or malignancy. Such findings will be noted on the Case Report Form.
2. Relapsed or re-infected patients with evidence of prior tuberculosis as long as they have received no antituberculosis chemotherapy in the previous 60 days. Diagnosis in this case must be confirmed with smear or culture.

Withdrawal criteria

1. Samples from patients with negative smear microscopy as well as **contaminated** cultures will be considered diagnostically uninterpretable and will not be accepted.
2. Lost or inadequate clinical data – the case report form must be completed and a final diagnosis assigned in all submitted cases.
3. Samples from patients with inadequate microbiologic data will not be admitted. At a minimum, all patients must have **two** sputum specimens examined by AFB smear and culture, Results of examinations that are performed after specimens have been submitted to the bank can consequently be included in the case report form.
4. Samples from patients who do not fit modified ATS/CDC diagnostic criteria (see box insert below).

Clinical Data Collection

Clinical Data will consist of information collected by history, physical and laboratory examination as detailed in the Case Report Form (Annex 1). Case definitions demand that more than one sputum specimen is examined by smear and culture, though data referring to a single sputum (with Unique Sample Number) will be recorded on each Case Report Form (CRF). Data from additional examinations performed on samples from the same patient will be recorded on the CRF and used for assignment of the Final Diagnosis. All essential information must be provided with the specimen for it to be included in the Specimen Bank. Therefore, only specimens meeting protocol criteria and accompanied by a completed CRF will qualify for reimbursement. Annex II: Case Report Form Key provides a detailed explanation of terms and instructions for completion of each data field. For any information on the CRF not considered mandatory, a "not done" option is provided. Two copies of the original Case Report Form should be made. One will be submitted to the central bank facility, and the other to WHO/TDR/CDS/PRD c/o Ms. Izabella Suder.

Specimen Collection:

1. Blood (20ml whole blood to yield 10ml serum)

2. Sputum (5 ml to yield 2.5 ml for storage and an equal volume for processing for 50 patients capable of easily producing sputum. The remaining 150 patients need only to produce an amount adequate to perform smear microscopy and culture).

3. Urine (7.5 ml of clean-catch urine for 5 aliquots of 1.5ml from the same 50 patients that contribute with sputum. From the remaining 150 patients no urine will be collected)

Clinical AIDS: Clinical AIDS will be determined by the presence of an AIDS defining illness in addition to tuberculosis.

HIV Test: The HIV test is not optional. All patients who provide specimen for the WHO/TDR Tuberculosis Specimen Bank will be tested.

CD_4: Determination of CD_4 levels are optional, and though this information may be useful, it is likely to be too expensive to obtain for all HIV^+ patients.

Chest X-ray: Chest x-rays will be optional for patients with microbiologically proven tuberculosis. The diagnosis of culture negative TB must be supported by x-ray evidence. When x-rays are done, they will be scanned to be stored electronically.

Follow-up: All patients who provide specimens for the WHO/TDR Tuberculosis Specimen Bank should receive a final diagnosis that in most cases is based on follow-up exams or visits.

Tuberculosis patients

It is anticipated that all suspects who receive a diagnosis of tuberculosis, will receive follow-up during their course of treatment as part of routine procedure.

Patients without tuberculosis

A non-TB case is defined as a patient who has chronic cough of ≥3 weeks, fever, repeated sputum smear negative, repeated sputum culture negative, chest x-ray findings and clinical improvement at follow-up without anti-TB treatment and alternative cause of disease may or may not be confirmed. If even a few patients who are classified as "non-TB" do, in fact, have tuberculosis, the value of the WHO/TDR Tuberculosis Specimen Bank will be significantly compromised. Patients who do not receive a diagnosis of tuberculosis will, after completion of appropriate treatment for an alternate condition, be asked to return to the clinic after approximately two months. Those in whom symptoms persist should be further evaluated with repeat smear and culture, chest x-ray, and whatever additional exams are thought necessary. It is recognized that some of these patients will not return for follow up.

Final diagnosis: All patients who provide specimens for the WHO/TDR Specimen Bank, whether they are treated for TB, or not, should receive a final diagnosis. Where this diagnosis is tuberculosis, this should be recorded according to category (see Annex I, pg 25).

Table 1: Numbered Final Diagnoses

No.	Diagnosis	Smear	Culture	Caveat/ description	Initial CXR	Microbiological F/U at 2-3 months	Clinical and radiographic response to TB treatment
1	TB, smear positive	pos	pos	must have at least 2 positive smears **and** at least 2 positive cultures or ≥1 culture with ≥ 1+ growth on solid media	irrelevant	NA	Not applicable (NA)
2	TB, smear negative, culture positive	neg	pos	≥ 2 neg. smears and ≥ 2 positive cultures or ≥1 culture with ≥ 1+ growth on solid media	Abnormal except in setting of HIV infection	NA	NA
3	TB, culture negative Treated	neg	neg	neg sms on initial assessment, neg cultures, pos CXR, **and** response to TB Rx	Abnormal AND →	NA →	Both clinical and radiographic improvement
4	Non-TB, untreated	neg	neg	neg sms, neg cultures on initial assessment	irrelevant	Smear and culture negative	NA
9	Indeterminate	Any other combination of results not matching Categories 1-4.					

Patients must have the smear and culture results as listed, **plus** other relevant criteria as noted. Response time for f/u is between 2-3 months.

Collection and Storage of specimens
Blood specimens will be collected after initial screening based on smear and x-ray reports. Serum, urine and sputum specimens will be stored in –70°C before shipment to the WHO/TDR designated Distributor.

WHO/TDR Specimen Bank Distributor
WHO/TDR will contract a commercial organization to provide a central storage and distribution site. This distributor will be responsible for transportation of specimens from the Collection Sites to the central storage facility, maintenance of the frozen specimens and the associated clinical data, and distribution to end-users. Air transportation costs, including shipment of empty Vapor Shippers to and from the airport nearest to the collection sites that can accept cargo shipments, will be paid by the Distributor.

Shipment of Specimens
All specimens will be shipped in specially designed packages containing absorbed liquid nitrogen called Vapor Shippers (also called Dry Shippers), provided by the Distributor. Properly charged with liquid nitrogen, materials contained in a Vapor Shipper will remain at liquid nitrogen temperatures for 25-30 days. Before dispatch from the Collection Sites the following must be verified:

- that the number of vials in the batch corresponds to that on the accompanying list;
- that the ID number of each vial corresponds to the number on the accompanying list;
- that the necessary data for each patient are included; and
- that the collection site details and the date of dispatch are on the accompanying list.

When a total of 350-400 cryotubes have been collected at a given site, they will be shipped by air cargo in Vapor Shippers at -150°C (±10°C) in containers charged with liquid nitrogen to the Distributor's central facility (to be announced). Case Report Forms will be shipped with their associated samples. A summary form (see Annex IV), will accompany each shipment. The costs associated with filling the Vapor Shipper with liquid nitrogen and for transportation to/from the local airport will be paid by the Collection Sites. Other shipment costs will be paid by the Distributor.

Administration of WHO/TDR Tuberculosis Specimen Bank
The WHO/TDR Specimen Bank will be administered by WHO/TDR staff or their designates. Administrative tasks include: triage of requests for specimens; the maintaining of records of specimens collected, stored, and distributed; and evaluation of the performance of both the specimens and new products tested.

Record Keeping
The Distributor will maintain an inventory of specimens received and a record of how these specimens have been stored and distributed. This information will be available to WHO/TDR. WHO/TDR will maintain records of the performance of specimens to help identify potential errors in specimen characterization. The specimens will be blinded when given to investigators for testing of new methods by the WHO/TDR. Also, for each diagnostic test, WHO/TDR will assist with the resolution of discrepancies by facilitating and coordinating exchange of information between the investigator/manufacturer and the Collection Site personnel. It is anticipated that this critical function will increase the value of the Specimen Bank by improving the characterization of specimens through increased follow-up activities.

Communication and Data Management Procedures
The WHO/TDR will be the primary site for communication among the sites, the Distributor, and/or WHO/TDR. The use of E-mail and/or FAX for communication is encouraged. Copies of all communications (including brief written summaries of phone conversations, where required) will be sent to the TDR Medical Officer (Dr. Mark Perkins) who will serve to oversee the day-to-day operations of the Specimen Bank, resolve problems, and assure smooth operations. The

clinical information that will accompany the collected samples will need to be maintained in a dedicated database and shared with the WHO/TDR. All necessary data is noted on the Case Report Form, included below as Annex I. CRFs will be filled out, leaving no blanks, by the Collection Site. Data entry into the database will be performed by the Distributor. The tracking system maintained by the Distributor should make it possible to ascertain, at any given moment, the number of vials of each type remaining in the collection and the number and type disbursed to end-users.

Protocols

The flow sheet (pg 35) gives the sequence of work and specimen collection. In short, a passive selection of patients will be carried out in the Shyamoli TB clinic and in the NIDCH. Signed informed consent will be obtained. Physical and clinical examination, sputum collection microscopy, culture, sensitivity will be carried out. After initial screening (smear, chest x-ray), and before initiation of anti-mycobacterial therapy, blood and urine will be collected. Aliquot of serum samples will be tested for HIV and serum & urine stored for TB Specimen Bank while lymphocytes will be used in the ALS assay and supernatant stored for future testing. Final report of diagnosis will be given to patients after ~2 months. About 138 non-TB and 102 TB patients will be enrolled for this purpose. Samples accompanied by clinical report, collection site information etc will be shipped to the Distributor. Maintenance of inventory, records of specimen storage and distribution, evaluation performance etc will be done by WHO/TDR staff. Detailed protocols for the collection of sputum, serum, and urine are provided as annexed material along with instruction for sample shipment and transmission of clinical and laboratory information. The following three pages, which provide brief overviews of each procedure, are intended to provide a single set of guidelines for use by each specimen collection site, thereby ensuring the quality of all materials in the WHO/TDR Tuberculosis Specimen Bank.

Blood collection:

For serum collection, 20 ml of peripheral blood will be collected in sterile tubes without any additive. For the ALS assay, 5 ml blood from patients will be collected in heparin coated tubes (Vacutainer System; Becton Dickinson, Rutherford, NJ), gently mixed and brought to ICDDR,B in room temperature.

The ALS assay will be performed as described earlier (31). In brief, peripheral blood mononuclear cells (PBMC) will be separated from whole blood by differential centrifugation, and will be suspended in 24-well tissue culture plates (Costar, Cambridge, Mass.) in RPMI 1640 culture medium (GIBCO BRL, Grand Island, NY) containing 10% fetal bovine serum (GIBCO), 2 mM L-glutamine and amphotericin B-penicillin-streptomycin 1%, mixed (Sigma). Cells ($1x10^6$ cells/ml) will be incubated at 37° C with 5% CO_2 and culture supernatants will be collected at 72 hours post incubation. A cocktail of protease inhibitors (4-aminoethyl benzenesulfonyl flouride, 0.2 μm; Aprotinin, 1 μg/ml; Leupeptin 10 μm; sodium azide 1 mg/ml in PBS) will be added to the supernatants and will be stored at –70° C until used for assayed. Using BCG vaccine as antigen (Freeze-dried, glutamate-BCG vaccine for intradermal use, lot # 1861, Japan BCG Laboratories, Japan; no preservatives added, saline used as diluent), IgG titers will be measured in supernatants by the enzyme-linked immunosorbant assay (ELISA) as described earlier (31). Microtitre plates (Nunc-Maxisorp) will be coated with BCG vaccine (1μg/well in carbonate buffer, pH 9.8) and after overnight incubation at 4°C will be blocked with 10% FBS in phosphate buffered saline (pH 7.2) at 37°C for 60 minutes. Following washing, lymphocyte supernatants of appropriate dilutions will be added and incubated for 2 hours at 37°C. After washing, plates will be incubated with rabbit anti-human IgG HRP-conjugate for 2 hours at room temperature. Finally, plates will be developed with substrate (O-phenylenediamine (OPD) and optical density (OD) will be measured at 492 nm after stopping the enzyme reaction. Antigen-specific responses will be expressed as relative titers, which is defined as the optical density multiplied by the dilution factor of the sample.

HIV testing
Sera from patients will be tested for HIV infection by commercially available ELISA kit. When found positive, results will be confirmed by a line immunoassay (LIA) (10).

***Mycobacterial* species identification.**
PCR method will be performed to identify and genotype the mycobacterial spp isolated from sputum culture (19, 34).

Overview of Specimen Collection and Processing Protocols

1) Outline: Urine Collection and Processing

Introduction

Antibodies, human and mycobacterial antigens and other small molecules are shed in the urine of tuberculosis patients. The advantage of urine samples is that, like saliva, they may be non-invasively collected. Urine may be collected and processed by a number of different means; in this protocol they will be briefly sedimented by centrifugation and frozen in aliquots. Antiseptics or other chemicals are not being added to the specimens so it is important that the samples are processed quickly, and frozen before overgrowth of bacterial contaminants erodes the quality of the material.

Collection procedure

Urine should be collected from the 50 high-volume sputum producers. From the remaining 150 patients no urine will be collected. There is no intention to collect sterile, mid-stream specimens, so no special precautions need to be taken in collecting these samples. The patient should be given a centrifuge tube or collection cup and instructed to collect at least 10ml of urine from any portion of his/her stream.

Urine Processing

Safety Issues

Invariably, all urine specimens should be treated as though they were a source of potential hepatitis or HIV infection. Universal precautions should be followed.

- Place urine on ice if there is to be any delay in processing
- Collect urine into 15ml centrifuge tube and sediment at 5 minutes at 400 RCF (400 x g).
- Transfer 1.5 ml aliquots of supernatant into five 2ml prelabeled cryotubes
- Freeze aliquoted urine at -70°C

Transport of Urine Specimens

Urine (1.5 ml aliquots) will be stored in labeled 2 ml cryovials, and frozen at -70°C until the time of shipping. Vials will be shipped at liquid nitrogen temperature in vapor shippers along with other clinical materials.

With each batch of specimens transported, an accompanying list must be prepared with identifies the specimens. Before dispatch from the Collection Sites the following must be verified:

- that the number of vials in the batch corresponds to that on the accompanying list;
- that the ID number of each vial corresponds to the number on the accompanying list;
- that the necessary data for each patient are included; and
- that the collection site details and the date of dispatch are on the accompanying list.

2) Outline: Serum Collection and Transport

Serum Collection, Labeling and Storage

- Specimens will be collected in clinic, at any time after presentation and prior to the initiation of anti-tuberculous treatment.
- Informed consent for blood sampling and HIV testing should be obtained prior to venipuncture.
- A minimum of 20 ml of blood is required for serum collection.
- Do not add any anti-coagulants to the blood collection tubes
- Follow universal blood precautions for safety carefully.
- Use at least a 23 gauge and preferably ≥22 gauge needle.
- Hemolyzed samples will not be acceptable. Avoid hemolysis during venopuncture and separate serum the same day as collection if possible.

Processing Safety Issues

All blood specimens should be treated as though they were a source of potential hepatitis or HIV infection. Universal precautions should be followed.

- Allow blood to clot for 2 hours at 4°C.
- Do not freeze samples prior to separation of serum. More than one sample per patient is acceptable (≤3), but all samples must be collected before the initiation of therapy.
- Centrifuge at 1200 x g for 10 minutes at ambient temperature.
- Do NOT heat-inactivate serum.

Informed Consent ⇓ Venipuncture using ≥23gauge needle ⇓ Additive-free tube ⇓ Allow clot to form for > 2 hours and < 24 hours at 4°C ⇓ Separate serum in centrifuge at ≈1200xg, at ambient temperature for 10 min.	Remove supernatant serum ⇓ Examine for clarity ⇓ Repeat centrifugation if cellular debris or clot remains in serum ⇓ Aliquot serum into 0.5ml in cryovials ⇓ Store cryovials at -70 oC

Storage

- Aliquot serum into 1.0ml cryovials.
- Store serum at –70°C as soon as possible after aliquoting.
- Prenumbered sample labels will be provided by the specimen repository facility.

3) Outline: Sputum Collection and Processing

Sputum Collection, Labeling And Storage

- Specimens to be collected in clinic, over a period of <3 hours, and not pooled overnight.
- A minimum of 5ml of sputum is required.
- Specimens should be refrigerated during any delays before processing, which should happen the day of collection whenever possible.
- More than one sample per patient is acceptable (≤3), but all samples must be collected before the initiation of therapy.
- Prenumbered sample labels will be provided by the specimen repository facility..
- Store sputum in 0.5ml aliquots in cryovials at –70°C.

Processing

- Follow processing protocol precisely.
- Stored specimens will be homogenized but not decontaminated.
- Use refrigerated centrifuge if possible.

Receive and log-in sputum sample
⇓
Homogenize with NALC/Na Citrate and glass beads
⇓
Transfer 2.5ml to #2 centrifuge tube
⇓
For the 50 high volume sputum producers, place rest of tube #1 sample into cryovials in 0.5ml aliquots and store at –70°C
⇓
Add equal volume NaOH to tube #2 for decontamination
⇓

Leave at room temperature for 15 minutes
⇓
Buffer to 45ml with PB
⇓
Sediment at 2400-4000x*g*, 10°C for 15 min.
⇓
Decant supernatant
⇓
Resuspend pellet in 1.0-1.5ml PB
⇓
Make smear for microscopy
⇓
Inoculate cultures and incubate

Smear and Culture

- Microscopy will be performed on resuspended pellet after decontamination.
- Plate to at least 2 media (minimum 2 LJ slants), and in liquid media (Bactec 460 or MGIT).
- Inoculate with a volume of at least 0.2-0.3ml x 2
- Cultures should be read at least weekly.
- Smear and culture results to be recorded in a semi-quantitative fashion. For culture:
- Solid media - Note <10 (exact number), 10-50, >50
- Liquid media - Note days till positive.

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. (TYPE WITHIN THE PROVIDED SPACE).

The tuberculosis lab has the setup for a P3 safety level tuberculosis research at the ICDDR,B equipped with a biohazard safety cabinet and a light microscope. Tissue culture facilities are there at ICDDR,B for carrying out *in vitro* experiments. However, the incubator for *Mycobacterial* culture urgently needs replacement, as the existing incubator has inadequate space for reasonably large sample size. In addition, a light microscope for sputum microscopy is needed for upgrading the set up at the TB lab.

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. (TYPE WITHIN THE PROVIDED SPACE).

Projected Patient Types and Number of Specimens to be Stored per Site

Making assumptions about the prevalence of smear-positive and negative disease among subjects who have a high likelihood of having TB, enrolment of 200 symptomatic patients would yield **approximately** the following distribution of cases and numbers of 0.5ml aliquots.

Diagnosis	Smear	Culture	No. Cases	Sputa	Sera	Urine
TB	+	+	50	250	500	500
TB	-	+	25	125	250	250
TB	+	-	0	0	0	0
TB	-	-	10	50	100	100
Non-TB*	-	-	115	575	1150	1150

Note. Default sample volumes yield 5 aliquots for sputum, 10 for serum, and 10 for urine. Considering 20% attrition, the sample size would be **240** for Specimen Bank. In the earlier study, smear positive symptomatic patients were selected for evaluation of the ALS method. In this proposal, the ALS method will be evaluated for detecting tuberculosis in the above subjects (n=240) with clinical symptoms suggestive of TB, and compared with the combined culture and smear positive as the gold standard. *A non-TB case is defined as a patient who has chronic cough of ≥3 weeks, fever, repeated sputum smear negative, repeated sputum culture negative, chest x-ray findings and clinical improvement at follow-up without anti-TB treatment and alternative cause of disease may or may not be confirmed.

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.

Patient Agreements

Permission to draw 20 ml of blood from adult patients will be required. The ethical implications are outlined below:

1. Patients can participate only after giving informed consent according to the guide lines of the local ethical committee at ICDDR,B.
2. The study will not in any way interfere with the management and standard treatment of the patients. Patients may withdraw from the study at any time point. This decision will not have any influence on the clinical management or therapy of the patients.
3. Participating patients need to agree to provide three types of specimens: sputum (5 ml), blood (20 ml), and urine (10 ml). Approximately 20 ml of venous blood (from median cubital vein) will be taken from adults. There may be a momentary pain and a very small chance of bruising at the site of insertion of the needles. To minimize the chance of infection, aseptic precautions will be taken and disposable, sterile syringes and needles will be used for drawing blood.
4. The data obtained from this study will be kept strictly confidential and will be kept locked in a filing cabinet. Only the investigators will be allowed access to this data.
5. Patients will be tested for HIV infection through the services of "Jagori". The HIV testing will be linked. Only those patients who give consent to test for HIV will be enrolled in the study. The voluntary Counseling and Testing (VCT) Services for HIV patients at ICDDR,B has it own consent form for HIV-testing (enclosed). This consent form will be used together with the consent form for the proposed study. The system followed by VCT (referral system and the OPD system) will also be applicable in the current proposal.

HIV testing

Patients must agree to HIV testing. HIV testing is carried out according to the WHO guidelines. They undergo pretest counseling and the results are provided to them after 3 days during the post-test counseling. This is entirely a one-to-one interaction between the HIV counselor and the TB patient. Where required, on-going counseling is also provided by the VCT unit. The system that is followed in the VCT unit is that the counselor provides the result of the HIV test to the client directly and not to the investigators. It is up to the client whether or not to disclose the test-results to the investigator.

No DNA testing will be done in the stored samples. These specimens will only be used for testing TB diagnostic methods. Moreover, the specimens will be blinded when given to investigators for testing of new methods by the WHO/TDR.

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 study will help in validating the ALS method for diagnosing active pulmonary TB in suspected adult patients. In addition, with the establishment of the enrollment site at ICDDR,B for the TB Specimen Bank, the Centre can collaborate with TDR and TBDI on further diagnostic and operational research in future.

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.

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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.

NAME Rubhana Raqib
PRESENT POSITION Associate Scientist, LSD

ACADEMIC QUALIFICATION:

Degree	Year	Class / Division	Subject	University
PhD	1995	-	Immunology	Karolinska Institute, Sweden
M. Sc.	1988	First Class	Biochemistry	Dhaka University
B. Sc.	1985	First Class	Biochemistry	Dhaka University

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

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TB Specimen Bank, Dec 2003-Nov 2004

Personnel		Mon. Rate	Effort %	WHO 12 months	USAID 12 months	Total US$
Rubhana Raqib	P.I.	1248	30%	0	4,493	
Zeaur Rahim	Co.I.	1522	10%	1,826		
K. Zaman	Co.I.	1744	5%	1,046		
T Azim	Co.I.	1550	5%	930		
Res.Officers: CSA		279	100%	6,686		
Sr Lab Attend		223	100%	2,676		
Field Attend		201	100%	2,412		
Medical Officer		711	20%	1,706		
Subtotal				**17,284**	**4,493**	21,776
Transport						
Local transport				1500	0	
International				0	3,500	
Subtotal				**1,500**	**3,500**	5,000
Supplies and materials						
Vacutainers tubes, cryovials, Nunc vials				3,000	6,000	
Disposable pippets, syringes, needles				1,500	2,500	
Disposables, storage boxes				1,500	2,000	
Sputum collection, Mycobacterial culture				2,000	4,150	
Mycobacterial Spp identification (PCR)				1,200	1,800	
Liq. Nitrogen, transport, personnel handling				1,500	1,000	
Subtotal				**10,700**	**17,450**	28,150
Interdepartmental charges						
Pathol test: X-ray, routine tests				3,500		
HIV testing (serology only)				1,440		
CD4 cell count				1,500		
Computer & Library charges				800		
Subtotal				**7,240**	**0**	7,240
Other contractuals						
Repair/maintain, Commun (email, fax, DHL etc)				1,200		
Utilities, electricity, printing forms, RRC-ERC charges etc				2,500		
Spare parts				750		
Subtotal				**4,450**	**0**	4,450
Capital Equipment:						
Large-CO2 Incubator					13,000	
-70° C freezer					7,500	
Microscope					2,000	
Computer with printer					3,000	
Subtotal				**0**	**25,500**	25,500
Total				**41,174**	**50,943**	**92,116**
26% overhead				**0**	**13,245**	**13,245**
Grand Total				**41,174**	**64,188**	**105,362**

S. Moin
20-Oct-2003

Shamima Moin
Controller, Budget & Costing

Budget Justification

Personnel costs
Salaries and benefits are established according to a uniform salary scale approved by an international Board of Trustees. The salary scales are based on UN scales, however, the actual salaries are about 50% of the UN scale for comparable GS (General staff) and NO (National Officer) levels.

Transportation
Costs for transportation between TB hospitals and laboratory at ICDDR,B has been requested.

Supplies
Vacutainer® Blood Collection Set (glass tubes, tourniquets, safety-gard needle holder, needles, adaptor, needle disposable container) and cryovials are needed for blood collection and storage; Falcon/universal tubes for sputum and urine collection, vials, labels & storage boxes for sample storage; slides & staining reagents for sputum microscopy, liquid and solid media, antibiotics and tubes required for *Mycobacterial spp* culture and sensitivity, strain collection and storage; PCR reagents for *Mycobacterial Spp* identification; disposable tubes/vials, gloves, pipettes, liquid nitrogen for storage, personnel handling, transportation.

Interdepartmental charges
Costs for routine tests including radiography, serology and western blot tests for HIV, CD4 counts by flow cytometry, computer & library use has been requested. Costs for institutional review processes (Research Review Committee (RRC) and Ethical Review Committee (ERC)), printing of forms, electricity, email, courier etc has been requested.

Capital Equipment
A large CO_2 incubator is needed for *Mycobacterial spp* culture. A -70°C freezer is needed for specimen storage. A light microscope is required for sputum smear microscopy. A computer is needed for data storage.

*Funds requested to the TDR/WHO (41,174 US$) for the Specimen Bank are not sufficient and hence costs for the rest of supplies, capital equipment, salary of the PI will be provided by ICDDR,B (50,943 US$) through other sources.

Annex I. Case Report Form

A 1. Site ID#_____ (as assigned by WHO/TDR)
2. **Sample Number:**_____ 3. Sample Date: [____/____/_____](dd/mm/yyyy)

B ***Patient Information***
1. Patient ID#_____ 2. Sex: ☐ Male ☐ Female 3. Age: _____ [Years]

C ***Clinical History***

Ca 1. General Appearance: ☐ Not ill ☐ Mildly ill ☐ Moderately ill ☐ Gravely ill
2. Infection Site: ☐ Pulmonary ☐ pulmonary and _____ (please specify)

Cb **Signs and Symptoms** Duration of symptoms: _____ weeks

		Yes	No			Yes	No
1.	Persistent cough	☐	☐	6.	Contact w/ active case	☐	☐
2.	Fever	☐	☐	7.	Hemoptysis	☐	☐
3.	Malaise	☐	☐	8.	Chest pain	☐	☐
4.	Recent weight loss	☐	☐	9.	Loss of appetite	☐	☐
5.	Night sweats	☐	☐	10.	Other [specify]_____		

Cc **Other Information**

1.	Previous history of TB:	☐ Yes _____years ago		☐ No
2.	BCG (Scar or History):	☐ Yes, estimate years since _____		☐ Scar present
		☐ No BCG	☐ Unknown	
3.	HIV test:	☐ Positive	☐ Negative	
4.	Current steroid use:	☐ Yes	☐ No	☐ Unknown
5.	Pregnant:	☐ Yes	☐ No	☐ Unknown/not relevant
6.	Co-existing illnesses:	☐ Cancer	☐ AIDS	☐ Malaria in the past year
		☐ Diabetes	☐ Other _____	
7.	Chronic Alcoholism:	☐ Yes	☐ No	☐ Not Known
8.	Smoking:	☐ Yes	☐ No	☐ Not Known

D ***Laboratory (at enrolment)***

1.	AFB (score, 0-3+)	☐ 0	☐ 1+	☐ 2+	☐ 3+		
2.	Solid AFB Culture	☐ neg	☐ pos (# col____	<20 ☐ 1+	☐ 2+	☐ 3+	☐ 4+) ☐ contam/lost
3.	Liquid AFB Culture	☐ neg	☐ pos Days till positive _____(pure)				☐ contam/lost
4.	Final culture result	☐ negp MTBcomplex	☐ NTM	☐ contaminated			
5.	CD4 Count	☐ <200	☐ 200-500	☐ >500	☐ Not Done		
6.	PPD	☐ prior pos	☐ current_____mm		☐ Not Done		
7.	Chest X-ray	☐ Normal	☐ Upper zone DZ	☐ Lower zone DZ	☐ Cavities	☐ ND	

E ***Follow-up:*** 1. Received TB Rx? ☐ Yes ☐ No 2. Rcvd routine Abx? ☐ Yes ☐ No
3. Symptoms: ☐ Imp. ☐ Worse ☐ Same ☐ No F/U 4. CXR: ☐ Imp ☐ Worse ☐ Same ☐ ND

F **Final Diagnosis:** _____ (Category #1-8 from chart on page 11) comment:_____
Collector's

Annex II: Key to Case Report Form (CRF)

The CRF is to be completed before submission to the Distributor, accompanying its associated sample. This will require holding the CRF until sputum cultures are finalized and sufficient follow-up has been completed for a final diagnosis to be assigned.

Site ID#: (whole number entry, two digit) 03, 07 - 13 as listed

Unique Sample Number: (whole number entry) A non-repeating number used to identify the material collected for submission to accompany this CRF. This number will be included on preprinted labels provided by the Distributor. This should be the same number used in the microbiology laboratory to denote the smear and culture examinations or should be keyed in the laboratory accessioning process.

Date of Sample: (dd/mm/yy) The date that the clinical material was collected. Normally this would be the same as the culture date and specimen processing date.

Patient information

Patient ID#: (numerical entry, digits not restricted) A non-repeating number used to identify the patient from whom the material was collected. For use associating with other clinical, radiologic or laboratory data obtained on the same patient.

Sex: (check box)

Age: (whole number entry, two digits) Estimate in case of uncertainty.

Clinical History

General Appearance: (check box)
Not ill: Self-explanatory.
Mildly ill: able to carry out routine activities, but symptomatic with fatigue, cough, etc. upon careful inspection.
Moderately ill: some impairment of activities. Visibly ill to a lay person. Still ambulatory and mostly self-sufficient but clearly symptomatic.
Gravely ill: unable to carry out usual activities. Visibly distressed. Requires hospitalization.

Infection Site: (check box plus text entry) Only patients with at least pulmonary disease will be accepted for enrollment. If additional site(s) are involved, specify in text.

Duration of symptoms: (whole number entry) The number of weeks of principal symptom. Symptoms below are checked if they have occurred significantly at any time during the present illness.

1. Persistent cough (check box) – generally defined as three weeks
2. Fever (check box) – subjective or objective
3. Malaise (check box)
4. Recent weight loss (check box)
5. Night sweats (check box)
6. Contact w/ active case (check box) – implies intimate or household contact
7. Hemoptysis (check box)
8. Chest pain (check box) – pleuritic or otherwise (non-cardiac)
9. Loss of appetite (check box)
10. Other (text entry)

Previous history of TB: (check box plus whole number entry if YES) Checking YES implies prior tuberculosis for which the patient received anti-tuberculous chemotherapy for at least one month. Enter "1" for tuberculosis within less than one year. No enrolled patient should have received anti-tuberculous therapy within the previous 60 days.

BCG: (check boxes, whole number entry if YES) Both history and/or scar may be used to determine prior BCG vaccination. Estimate years since vaccination for vacinees. The presence of an identifiable BCG scar is noted separately with a check box on the same line.

HIV test: (check box)

Current steroid use: (check boxes)

Pregnant: (check boxes) Relates to current status, pregnant or not. Check "not relevant" for men, yes or no for all women.

Co-existing illness: (check boxes) Malaria confounds some serologic tests.

Chronic Alcoholism: (check boxes) Generally defined as daily drinking to intoxication.

Smoking: (check boxes) Daily smoking of cigarettes.

Laboratory Results (at enrolment)

AFB (check boxes) Score using IUATLD scale with the exception that all positive smears with <99 AFB/100 immersion fields will be called 1+ (no intermediary "record exact number" value between 0 and 1+)

No AFB/100 fields = 0
1-99 AFB/100 fields = 1+
1-10 AFB/field = 2+
>10 AFB/field = 3+

Solid Culture: (check boxes) Solid culture will be performed using IUATLD/WHO scoring system. This is helpful not only in understanding the burden of infection, but also the chance that an individual culture represented a laboratory cross-contaminant.

No AFB colonies = neg
1-20 AFB colonies = record exact number
20-100 AFB colonies = 1+
100-200 AFB colonies = 2+
200-500 AFB colonies = 3+
>500 AFB colonies = 4+
overgrown with contaminants, lost or otherwise unreadable culture = contam/lost

Liquid Culture: (check boxes and whole number entry) A semi-quantitative method will be used here as well, relying on number of days from inoculation of liquid media until growth detected, by any means, to estimate burden of inoculates. Do NOT fill in this result when culture contains contaminants, as they may speed detection times.

Final culture result: (check boxes) This is a cumulative result. A positive culture in any media type that is considered trustworthy (not cross-contaminated or mislabeled, etc) is considered positive. All positive cultures should be speciated to the MTBcomplex versus NTM (non-tuberculous mycobacteria) level. This may be done using morphology+niacin/nitrate or morphology+NAP or other (molecular) method, but should not rely on morphology alone.

PPD: prior positive (check box) if known to be prior positive, regardless of whether exam will now be repeated. Current ____mm (whole number entry), mm size of induration measured across the forearm at 48-72 hours. This is expected to be most useful in patients from non-endemic areas and in patients thought unlikely to have tuberculosis. PPD testing is not required and may not be useful in settings of endemic disease.

CD4 Count: (check boxes) When available.

Chest X-ray: (check boxes) Some CXR abnormalities are more suggestive of TB than others, however, the check boxes on the CRF do not attempt to discriminate between various types of lesions, only location of disease and the presence or absence of cavities.

Follow-up 2-3 months after enrollment date

Follow-up: Roughly defined as the first 12 weeks after enrolment, whether on TB Rx or not.

On TB Rx: (check box) If patient was started on TB therapy, even if eventually given an alternative diagnosis and TB therapy stopped, this box should be checked YES.

Rcvd routine Abx? (check box) If the patient received antibiotics such as ampicillin or sulfa drugs directed at infections other than tuberculosis, this box should be checked YES.

Symptoms: (check boxes)
Improved (Imp.) = Diminished frequency or severity of symptoms
Worse = Increased frequency or severity of symptoms
Same = No change in frequency or severity of symptoms
No F/U = No evaluation of symptoms during the follow-up period

CXR: (check boxes)

Improved (Imp.) = clearing or healing of CXR findings
Worse = Increased density, distribution or size of CXR pathology
Same = No change in density, distribution or size of CXR pathology
Not Done (ND) = CXR not performed during follow-up

Final culture result: (check boxes) This is a cumulative result. A positive culture in any media type that is considered trustworthy (not cross-contaminated or mislabeled, etc) is considered positive. All positive cultures should be speciated to the MTBcomplex versus NTM (non-tuberculous mycobacteria) level. This may be done using morphology+niacin/nitrate or morphology+NAP or other (molecular) method, but should not rely on morphology alone. Not applicable for subjects who received TB treatment.

Final Diagnosis: (whole number) A whole number should be taken from the chart on page 6 to assign diagnosis. A text comment may be made in the very small space provided.

Collector's Signature: Signature should be of the person assigning the Final Diagnosis

Complete Date: This is the date the final diagnosis is assigned and clinical and laboratory data notation is complete. This date may be 3-6 months after enrollment, but cannot be shorter than the time necessary for sputum culture results to be complete and 12-week f/u.

Annex III: Materials Shipment Summary Form

WHO TB Specimen Bank

Collection site ID# _____ (03= Pretoria, 07=Nairobi, 08= Khartoum, 09=Teheran, 10=Dhaka, 11=Ho Chi Minh City, 12=Medellin, 13=Rio de Janeiro)

Date of loading of Dry Shipper [____/____/____](dd/mm/yy)

Number of aliquots included _____(number of tubes shipped)

Number of patients _____(should equal number of case report forms)

Starting sample (smallest number) _____

Ending sample (largest number) _____

Flow sheet for TB Specimen Bank Activity

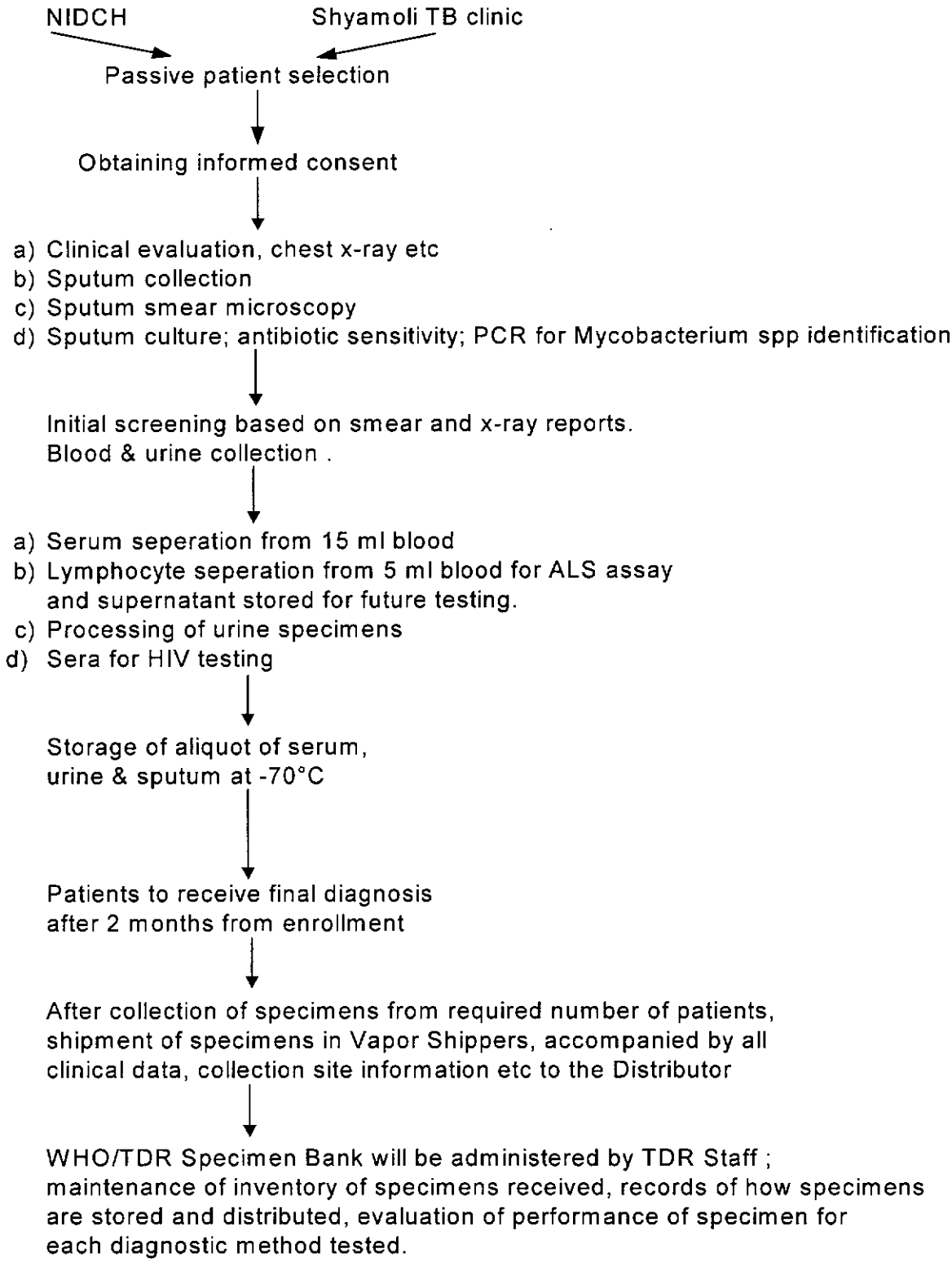
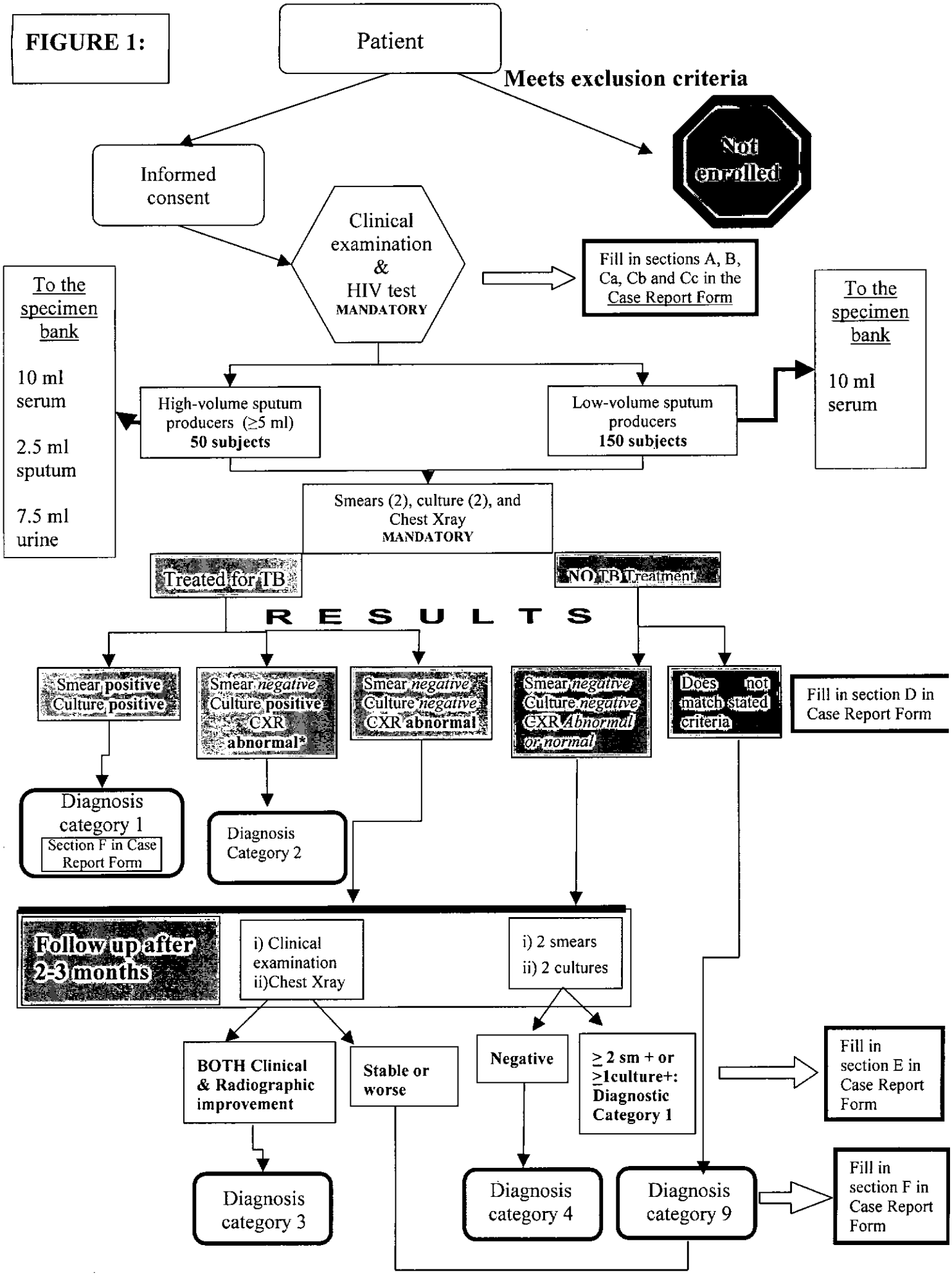


FIGURE 1:



* - An abnormal chest-xray is not required in the setting of HIV infection

Figure 1

Annex Va: Protocol for Serum Collection and Transport

Introduction

New developments in tuberculosis diagnosis require the use of serum or plasma for evaluation of tests based on antibody detection, while sputum specimens are valuable for the evaluation of products based on antigen detection. Most rapid diagnostic tests for tuberculosis currently available or under development are configured for use with either serum or sputum specimens, while a few require plasma or fresh whole blood. The majority of serum antibody-based test target IgG, while a few use IgA or IgM as target immunoglobulins.

Serum is usually preferred to plasma for any immunoassay due to the tendency of clotting factors in plasma to form clots spontaneously and thereby to mimic or mask antigen-antibody reactions.[1,2] Inappropriate collection, handling, and storage of specimens can be the cause of inaccurate results obtained from patient specimens[2]. This protocol therefore focuses on the standard laboratory requirements for diagnostic tests using serum specimens, with IgG and the main target.

Specimen Collection

Collection etiquette

Informed patient consent should be documented before any blood is collected. The patient should be put at ease and submitted to the least possible discomfort. Blood should only be taken by trained staff in designated areas.

Collection procedure

Venous blood (20 ml) is best withdrawn from an antecubital vein.[3] Successful venepuncture may be facilitated by keeping the patient's arm warm, applying to the upper arm a sphygmomanometer cuff kept at approximately diastolic pressure and gently smacking the skin over the site of the vein. In obese patients it may be easier to use a vein on the dorsum of the hand, which is warmed by immersion in warm water. When the hand is dried and the fist clenched, veins suitable for puncture will usually become apparent. If the veins are very small, a 23 SWG (or 0.610mm) needle would be used.[4] Vein punctures in the dorsum of the hand tend to bleed more rapidly than at other sites. The arm should be elevated after withdrawal of the needle and pressure should then be applied for several minutes before an adhesive dressing is placed over the puncture site.

Ideally, congestion should be completely avoided so as to prevent haemoconcentration, although stasis for a period of less than one minute has little effect.[3] In practice, it is usually necessary to use a tourniquet. Ideally, this should be loosened once the needle has been inserted into the vein. If a syringe is used, the piston should be withdrawn slowly and no attempt made to withdraw blood faster than the vein is filling. Allow blood to flow directly in the vacuum system/tube or transfer it immediately into a tube after syringe collection. *Do not add any coagulants when collecting serum.* Care should be taken in re-sheathing needles. They should be inserted into a sheath held in a mechanical holder (not in the hand); commercially available disposal equipment for sharps are recommended.

Technical Notes

- To minimize blood leakage during venepuncture, the use of red top vacutainers or an alternative vacuum-blood collection system is recommended. If these are not available, a sterile syringe and needle may be used. Needles should not be too fine or too long. Those of 19 SWG (or 1.016mm diameter) or 20 SWG (or 0.914mm diameter) are suitable for use with syringes.[3] If a syringe is not being used, needles should be of large bore, e.g.. 16 SWG (or 1.625mm diameter.)[3]
- Haemolysis should always be avoided during specimen collection since some assays may be adversely affected. Haemolysis can be avoided or minimized by using clean apparatus,

withdrawing the blood slowly, not suing too fine a needle, avoiding frothing during withdrawal and by delivering the blood gently into the receiver.

- Blood should not be frozen prior to serum preparation as freezing leads to haemolysis of erythocytes, which may adversely influence immunoassays.
- Blood clots better in glass than in plastic containers.

Serum Preparation

Safety Issues

Invariably, all blood specimens should be treated as though they were a source of potential hepatitis or HIV infection. Universal precautions should be followed and particular attention given to the following:

- Appropriate protective clothing should be worn, including a laboratory coat, latex gloves and eye protection.
- Hypodermic syringes and needles should never be used as substitutes for pipettes.
- Glass Pasteur pipettes should be substituted with plastic whenever possible.
- Pipettes should be drained gently and not blown out violently, otherwise the last drop will form bubbles which burst and create aerosols. Pasteur pipettes are particularly likely to generate bubbles.
- All manipulations should be performed in a Class II biological safety cabinet.
- Creation of aerosols should be limited, especially during centrifugation and serum transfer procedures.
- Sealed centrifuge buckets should always be used. Aerosol-free carriers should be used if possible.
- Appropriate disinfectant should be added to all liquid waste and all materials should be autoclaved for one hour at 121^{o}C.

Preparation procedure

- Allow two hours for the blood to clot at 4^{o}C to enhance natural serum expression This allows for large volumes of serum to be collected after centrifugation.
- Centrifuge tubes at 2000 rpm or 1200 g for 10 minutes at room temperature.
- Transfer the expressed serum (straw-coloured supernatant) to a labeled tube. Observe serum for cellular material and repeat centrifugation if debris is noted.
- Transfer 0.5 ml aliquots of serum into screw-capped, leak proof plastic cryovials.
- Label vials with identification number as well as the date of serum preparation.
- Freeze aliquoted sera at -70^{o}C (long-term.)

Technical notes

- Serum should be prepared on the same day as collection of blood. Adherence to centrifugation speed and duration is necessary since higher centrifuge speed or longer centrifugation periods may result in haemolysis of erythrocytes.
- Historically, serum had been heated at 56^{o}C to inactivate the complement components. This is *not recommended* at Collection Sites.
- Labeled cryovials supplied by the Distributor will be used for freezing serum aliquots.

Storage of Serum Specimens

Serum can safely be stored at 4°C for up to five days, however, it is recommended that specimens be stored at -20°C as soon as possible. However, for long-term storage, -70°C is recommended and for the present protocol, specimens will be stored in -70°C.

Note: IgM is rapidly destroyed at low temperatures and diagnostic tests targeting IgM may be false negative if serum specimens had been stored at -70°C. IgG and IgA are not affected at -70°C. [1]

Some refrigerators have a built-in compartment which will maintain a suitable temperature up to-20°C, but a deep-freeze cabinet designed for this purpose is recommended. Specimens should be packed in such a way inside the freezer to allow for free air circulation and easy identification. A card index, freezer catalogue and boxes with individual holders in rows and columns will facilitate easy identification, particularly in -70°C freezers.

Freeze-drying of serum specimens is not recommended

Thawing and re-freezing of sera should be avoided. Although IgG is fairly stable and can be recovered from thawed and re-frozen specimens (up to a maximum of ten cycles), other immunoglobulins are much more fragile and rapidly lost during thawing/re-freezing.
Finally, it is recommended that freezers be connected to an alarm system and back-up power supply to maintain the required temperature in the case of electrical power failure.

Transport of Serum Specimens

Sera (0.5 ml portions) will be sampled into previously labeled 1 ml cryovials, and frozen at -70°C. Vials will be stored at -70°C until shipped to the Distributor.
With each batch of specimens transported, an accompanying list must be prepared with identifies the specimens. Before dispatch from the Collection Sites the following must be verified:

- that the number of vials in the batch corresponds to that on the accompanying list;
- that the ID number of each vial corresponds to the number on the accompanying list;
- that the necessary data for each patient are included; and
- that the collection site details and the date of dispatch are on the accompanying list.

1. Peacock, JE and RH Tomar. Manual of Laboratory Immunology. Lea and Febiger, Philadelphia: 1980.
2. Hudson, L and FC Hay. Practical Immunology. Third Edition. Blackwell Scientific Publications. London: 1989.
3. Rose, NR, H Friedman and JL Fahey. Manual of Clinical Laboratory Immunology. American Society for Microbiology. Washington, DC: 1986.
4. Dacy, J and FM Lewis. Practical Haemotology. Fourth Edition. J&A Churchill, London: 1968.
5. J. Verschoor, dept. of Biochemistry, Univ. of Pretoria: Personal communication.

Annex Vb: Protocol for Sputum Collection and Processing

Sputum Collection And Labeling

A. PURPOSE

To obtain adequate sputum specimens in a standardized fashion from patients enrolled in the WHO Sputum Sample Bank Collection Protocol and to ensure proper labeling of specimens. Patients are those suspected of having untreated pulmonary tuberculosis on the basis of prior smear, culture, or clinical suspicion.

B. MATERIALS

Sterile screw-capped specimen collection containers	(Baxter C8827-14) OR
Centrifuge tube, 50ml polypropylene	(Baxter C3903-12)
Labels and/or indelible labeling marker	
Disposable gloves	

C: PRINCIPLE

The efficiency of laboratory detection of mycobacteria (accuracy of quantitative smear and culture, other diagnostic tests, and contamination rate) is highly dependent on the availability of high quality specimens for examination. While the methods and instructions involved in sputum collection may appear simple, proper sputum collection is one of the most important procedures performed in TB diagnostics and treatment trials. Sputum intended for this sample bank may be subjected to labor-intensive and expensive scrutiny and may undergo testing using a variety of novel assays. It is imperative that clinical samples be of the highest quality possible and that all errors in transport and identification be scrupulously avoided.

D. PROCEDURE

1. Specimens must be collected in appropriate clean, sterile containers. Wide-mouthed disposable plastic containers or 50ml polypropylene centrifuge tube can be used. For either, the screw caps must fit tightly to avoid leakage.

2. There are two basic types of sputum specimens

   (a) spot specimens - collected at a single time; these are best collected in the early morning when respiratory secretions that have gathered in the lower airways are cleared but may be collected at any time the patient can produce sputum.

   (b) pooled collections - The patient collects all sputum produced over a specific period (usually overnight) in the same container. Pooled specimens have a slightly higher yield on culture than early morning spot specimens but also are more likely to be contaminated.

   In this protocol, spot specimens will be used, though pooled samples will be accepted if the time of pooling has been less than 3 hours unrefrigerated or <12 hours and materials have been refrigerated.

3. Collection must be discussed with the patient in detail. Study personnel (specimen collector, nurse, medical officer) will explain to the patient the nature of the desired specimen.

Patients will be told that nasal secretions and saliva are not sputum. Subjects will be told that the desired sample is a deep-cough sputum consisting of the thick mucoid white-yellow, sometimes blood-tinged material from the lower airways and lung, not saliva or oral secretion). Subjects will be instructed not to touch the inside of the specimen containers or lids with their fingers or other objects.

4. Labeling and notation must be complete.

5. Specimens will be collected in the following manner:

a) The subject will be preferably be seated or standing.
b) Whenever possible, subjects will be instructed to rinse the mouth twice with water before giving the specimen.
c) Subjects will be instructed to inhale deeply, cough vigorously, and expectorate the material produced into collecting receptacle. The subject should be told "to cough the specimen from deep in the chest". If the subject does not cough spontaneously, instruct him/her to take several deep breaths and then hold their breath momentarily; repeating this step several times will often induce coughing.
d) After coughing, the subject will be instructed to hold the sterile specimen container to his/her lower lip and gently release the specimen into the container. Instruct the patient to avoid spills or soiling the outside of the container with the specimen. The lid should be carefully placed on the container without touching the inside of the lid with the fingers and the lid tightened.
e) Lids should be firmly screwed back onto specimen containers to prevent leakage.
f) Specimens visibly contaminated with oral material or food particles will be discarded and the subject will receive a second sterile specimen container and be instructed to try again.
g) The specimen should be of at least 5ml in volume to be acceptable for the banking protocol.
h) Whenever possible, patient samples should include a first-morning specimen.
i) Specimens should be taken to the laboratory for processing as soon as possible after collection.
j) Specimen containers will be labeled with the subject's name or study identification number and the date. Put the identification labeling on the side of the specimen container and not the lid, which could become separated during transport or processing of the specimen. A properly completed laboratory specimen requisition must be attached to each specimen before transport to the laboratory.
k) Throughout collection period and during transport to the laboratory, specimens should be held at 2-8°C whenever possible.

Procedure for Receiving and Storing Sputum Specimens for WHO Specimen Bank

A. PURPOSE

The goal of this contract is to form a specimen bank of meticulously-processed and well-documented samples from patients with suspected tuberculosis. Patients may submit more than sample for diagnostic testing, and multiple samples ($\leq$3) from one patient may be included in the specimen bank, as long as all samples were collected pretreatment. These samples may or may not be independent of those collected to establish the diagnosis. Samples intended for inclusion in the specimen bank protocol may be processed independently from the routine samples received in the laboratory or may be integrated into routine processing, depending upon the workflow of the laboratory. The collection of samples for the sample bank should not interfere with the diagnostic process for any patient.

B. SPECIMEN REQUIREMENTS

At the time of enrollment, all patients must be capable of producing at least 5ml of sputum. Prior to processing, samples should be stored at the site of collection on ice or otherwise refrigerated. A maximum of 20ml of sputum will be processed from each collection.

C. PROCEDURE

1. Sputum samples should be received in a sterile, leak-proof container. Screw-capped centrifuge tubes to be used for processing may be used also for the collection step.

2. Samples should be kept on ice or at 4°C from the time of collection until they are processed. This includes samples obtained in the hospital, clinic and home. If any delay occurs during processing, specimens and aliquots should be refrigerated during that interval.

3. Assign sample accession number as per WHO Case Report Form. Prenumbered specimen labels will be provided by the agent responsible for cataloging and long term specimen storage (BioClinical Partners). If a separate laboratory system of specimen numbering is also used, a key should be maintained to link the two numbers.

4. A unique Patient Number will be assigned on site and noted on the Case Report Form for each enrolled patient.

5. Label the side (not the top) of specimen container with the laboratory accession number. This number is to be used to label all subsequent cryotubes, BACTEC vials, plates, slides, etc. and must be keyed to the Patient Number on the Case Report Form and laboratory computer or registry.

6. All sputum will be aliquoted on site, and stored prior to shipment at –70° C.

Consent form

Voluntary Counselling and Testing Centre
Virology, Laboratory Sciences Division,
International Centre for Diarrhoeal Disease Research, Bangladesh

সম্মতিপত্র

আমি _____ আমার এইচ, আই, ভি, পরীক্ষা করতে আগ্রহী। ইতিমধ্যেই আমি এইচ, আই, ভি, এবং এর পরীক্ষা পদ্ধতি সম্পর্কে বিস্তারিতভাবে জেনেছি। সবকিছু বিবেচনা করে আমি স্বজ্ঞানে, স্ব-ইচ্ছায় ও সম্পূর্ণরূপে নিজের সিদ্ধান্ত অনুযায়ী আই, সি, ডি, ডি. আর, বি, - তে আমার এইচ, আই, ভি, পরীক্ষা করতে সম্মতি দিচ্ছি।

_____

স্বাক্ষর/ বাম বৃদ্ধাঙ্গুলের ছাপ

তাংঃ_____

_____

অভিভাবকের স্বাক্ষর/ বাম বৃদ্ধাঙ্গুলের ছাপ
(সম্মতিদানকারীরর বয়স যদি আঠারোর নিচে হয় তবেই প্রযোজ্য)

তাংঃ _____

Consent form

Voluntary Counselling and Testing Centre
Virology, Laboratory Sciences Division,
International Centre for Diarrhoeal Disease Research, Bangladesh

সম্মতিপত্র

আমি ....., আমার CD4 পরীক্ষা করতে আগ্রহী। ইতিমধ্যেই আমি এইচ, আই, ভি, এবং CD4 এর পরীক্ষা পদ্ধতি সম্পর্কে বিস্তারিতভাবে জেনেছি। সবকিছু বিবেচনা করে আমি স্বজ্ঞানে, স্ব-ইচ্ছায় ও সম্পূর্ণরূপে নিজের সিদ্ধান্ত অনুযায়ী আই, সি, ডি, ডি. আর, বি, -তে আমার CD4 পরীক্ষা করতে সম্মতি দিচ্ছি।

.....

স্বাক্ষর/ বাম বৃদ্ধাঙ্গুলের ছাপ
তাংঃ.....

.....

অভিভাবকের স্বাক্ষর/ বাম বৃদ্ধাঙ্গুলের ছাপ
(সম্মতিদানকারীরর বয়স যদি আঠারোর নিচে হয় তবেই প্রযোজ্য)
তাংঃ .....

REQUEST FOR INCLUSION IN TB PROJECT
CONSENT FORM FOR PATIENTS

Title: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

Investigator: Dr. Rubhana Raqib, ICDDR,B, Mohakhali, Dhaka-1212.

In Bangladesh, tuberculosis (TB) is considered as a major public health problem. Rapid and improved diagnosis and a better understanding of the therapeutic response are very important for its effective control. ICDDR,B is working with the World Health Organization to aid the development of new tests to improve the diagnosis of TB that are appropriate for use in low-income settings. These new tests may use blood, urine or sputum specimens. The specimens will be used both to determine if an individual has tuberculosis and to help evaluate these new diagnostic tests (including the ALS test).

Prolonged cough is one of the important symptoms of TB. You have prolonged cough and we would like to know more about its causes. If you agree to participate, we will ask you some questions regarding your illness. It will take about 15 minutes to answer the questions. Physical examination as well as chest X-ray will be performed. We will culture your sputum samples for isolation of TB bacilli and determine it's sensitivity patterns. This will facilitate to select appropriate drugs against TB. After around 2 months you may have to return to ICDDR,B for follow-up examination. We will request you to give blood, sputum, and urine samples before initiation of treatment. Using 5 ml of blood, ALS test will be performed to diagnose tuberculosis. The rest of the blood specimen will be stored for the WHO/TDR. No DNA analyses will be done on these samples.

There will be very little discomfort or minor pain associated with obtaining blood. Since a new needle will be used for each patient, there is minimal risk of transmitting infection. Some tests will be done using the specimens that you provide to determine if you have tuberculosis and HIV infection. The results of HIV-test will be given to you directly. It will be your decision whether to provide the information to us. As an indirect benefit, your participation may result in the development of new, better and cheaper tests to determine if you or other patients have tuberculosis. You will benefit from participating because more than the routine tests will be done to determine whether or not you have tuberculosis.

Your participation is completely voluntary. You are at liberty to decide not to participate in the study at all or to withdraw from the study at any time without jeopardizing your medical care and treatment. Your identity will remain strictly confidential, but the authorities supporting this study may review the results. If you agree, then we will collect blood (25 ml), sputum (5 ml), and urine (10 ml) samples from you.

যক্ষা প্রকল্পে অন্তর্ভূক্তিকরণের অনুরোধ
সম্মতিপত্র, রোগী

Title: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

গবেষকঃ ডঃ রুবহানা রাকীব, আই.সি.ডি.ডি.আর,বি, মহাখালি,ঢাকা-১২১২।
প্রকল্পের অবস্থানঃ ঢাকা।

বাংলাদেশে যক্ষা একটি অন্যতম প্রধান স্বাস্থ্য সমস্যা। দ্রুত ও উননত রোগ নির্নয় পদ্ধতি এবং ঔষুধের কার্যকারিতা সমপর্কে সঠিক ধারনা যক্ষা নিয়ন্ত্রণে অতীব জরুরী । যক্ষা রোগ নির্ণয় পদ্ধতিকে উন্নত এবং স্বল্প আয়ের মানুষের জন্য উপযোগী করার প্রয়াসে বিশ্ব স্বাস্থ্য সংস্থা এবং আই. সি. ডি. ডি. আর, বি যৌথভাবে নতুন নতুন পরীক্ষা উদ্ভাবনের জন্য কাজ করে যাচ্ছে। এই পরীক্ষা গুলো করার জন্য রক্ত, মূত্র অথবা কফ নমুনা হিসেবে ব্যবহার করা যেতে পারে। এই নমুনা গুলো কোন ব্যক্তির যক্ষা রোগ নির্ণয়ে এবং সেই সাথে নতুন উদ্ভাবিত ALS পদ্ধতির যথার্থতা প্রমানে ব্যবহার করা হবে।

দীর্ঘস্থায়ী কাশি যক্ষার অন্যতম প্রধান উপসর্গ। আপনার দীর্ঘস্থায়ী কাশি আছে এবং আমরা এর কারণ সমন্ধে আরও জানতে চাই। আপনি যদি এই গবেষনায় অংশগ্রহনে ইচ্ছুক থাকেন তাহলে আপনার অসুস্থতা সম্পর্কে আমরা আপনাকে কিছু প্রশ্ন করব । এর উত্তর দিতে আপনার ১৫ মিনিটের মত সময় লাগবে। আপনার শারীরিক পরীক্ষা এবং বুকের এক্সরে করা হবে। যক্ষা জীবানু সনাক্তকরণ এবং ঔষধে উহার সংবেদনশীলতা জানার জন্য আপনার কফের কালচার করা হবে। এই পরীক্ষা যক্ষা নিরাময়ে সঠিক ঔষধ নির্ধারনে সহায়তা করবে। প্রায় ২ মাস পর আপনাকে আপনার পরীক্ষা রিপোর্ট নেয়ার জন্য আই.সি.ডি.ডি.আর,বি তে আসতে হবে । চিকিৎসা শুর্ু করার পূর্বে আমরা আপনাকে নমুনা হিসাবে রক্ত, কফ এবং মূত্র দেয়ার জন্য অনুরোধ করব। ৫ মি.লি রক্ত দিয়ে ALS পদ্ধতিতে যক্ষা রোগ আছে কিনা তা নির্নয় করা হবে। অবশিষ্ট রক্ত নমুনা হিসাবে বিশ্ব স্বাস্থ্য সংস্থার জন্য সংরক্ষন করা হবে। এই সব নমুনায় ডি এন এ পরীক্ষা করা হবে না।

রক্ত সংগ্রহের সময় আপনি সামান্য অস্বস্থি অথবা হালকা ব্যথা অনুভব করতে পারেন। প্রত্যেক রোগীর জন্য আলাদা সূঁচ ব্যবহার করা হবে বিধায় রোগ বিস্তারের ঝুঁকি খুবই কম থাকবে। আপনার যক্ষা এবং এইচ-আই-ভি রোগের জীবানু আছে কিনা তা সনাক্ত করার জন্য আপনার দেওয়া নমুনাগুলো দিয়ে কিছু পরীক্ষা করা হবে। এইচ-আই-ভি পরীক্ষার ফলাফল সরাসরি আপনাকে দেওয়া হবে। আপনি এ সংক্রান্ত কোন তথ্য আমাদের জানাবেন কিনা তা আপনার ব্যক্তিগত ব্যাপার। আপনার এই অংশ গ্রহনে নতুন পদ্ধতি উদ্ভাবিত হতে পারে যা আপনার এবং অন্য রোগীদের যক্ষা রোগ সনাক্তকরনে পরোক্ষ ভাবে সহায়তা করবে। যক্ষা রোগ নির্নয়ের জন্য নিয়মিত পরীক্ষা ছাড়াও অতিরিক্ত কিছু পরীক্ষা করা হবে; ফলে এই গবেষণায় অংশগ্রহনে আপনি উপকৃত হবেন ।

এই গবেষণায় আপনার অংশগ্রহন সম্পুর্ণভাবে স্বেচ্ছামূলক। আপনি এতে অংশগ্রহন নাও করতে পারেন অথবা অংশগ্রহন করার পরেও যে কোন সময় নিজেকে প্রত্যাহার করতে পারেন ; এতে আপনার চিকিৎসার কোন ত্রুটি হবে না। আপনার পরিচয় ও রোগ সংক্রান্ত তথ্য সম্পুর্ণভাবে গোপন রাখা হবে কিন্তু কাজের খাতিরে প্রাপ্ত তথ্যসমূহ সংশ্লিষ্ট প্রতিনিধিগণ পরীক্ষা করে দেখতে পারেন। আপনি যদি রাজি হন তাহলে আমরা আপনার কাছ থেকে ২৫ মি.লি রক্ত, ৫ মি.লি কফ, এবং ১০ মি.লি মূত্র নিব।

আপনি যদি স্বেচ্ছায় এই গবেষণায় অংশগ্রহনে ইচ্ছুক থাকেন তাহলে নিম্নে আপনার স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ দিন।

সম্মতিদান- উপরে বর্ণিত গবেষণা প্রকল্প আমাকে ব্যাখ্যা করা হয়েছে এবং আমি স্বেচ্ছায় এই গবেষণা প্রকল্পে অংশগ্রহন করতে সম্মতিদান করলাম।

If you are voluntarily willing to participate in the study, then please sign your name or give left thumb impression (LTI) below.

Consent: The study described above has been explained to me and I voluntarily consent to participate in it.

Name of the interviewer	Name of the Participant
Signature of the interviewer	Signature or LTI of the Participant
Name of Literate Witness	Signature of the Witness
Date	Date .....

তথ্য সংগ্রহকারীর নাম	অংশগ্রহনকারীর/অভিভাবকের নাম
তথ্য সংগ্রহকারীর স্বাক্ষর	অংশগ্রহনকারীর/অভিভাবকের স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ
শিক্ষিত সাক্ষীর নাম	সাক্ষীর স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ
তারিখঃ-	তারিখঃ-

REQUEST FOR COMING TO ICDDR,B FOR HIV TESTING AND TB DIAGNOSIS IN TB PROJECT
CONSENT FORM FOR PATIENTS

Title: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

Investigator: Dr. Rubhana Raqib, ICDDR,B, Mohakhali, Dhaka-1212.

In Bangladesh, tuberculosis (TB) is considered as a major public health problem. Rapid and improved diagnosis and a better understanding of the therapeutic response are very important for its effective control. ICDDR,B is working with the World Health Organization to aid the development of new tests to improve the diagnosis of TB that are appropriate for use in low-income settings. These new tests may use blood, urine or sputum specimens. The specimens will be used both to determine if an individual has tuberculosis and to help evaluate these new diagnostic tests. One portion of these specimens will be sent to the WHO for evaluation of new diagnostic tests. One portion of the serum specimens will be tested for the presence of HIV infection. The purpose of HIV testing is to be able to determine if the blood samples you provide are infected with HIV. This will allow the laboratories who will use the samples to experiment with new tests to determine if they work well for diagnosing tuberculosis in patients with and patients without HIV infection.

For the above purposes, you will be required to go to the ICDDR,B. Specimens (blood, sputum and urine) will be collected at the ICDDR,B laboratory. Counseling is available to you before you make the decision to participate in this testing.

If you are voluntarily willing to participate in the study and agree to go to the ICDDR,B, then please sign your name or give left thumb impression (LTI) below.

_____	_____
Name of the interviewer	Name of the Participant
_____	_____
Signature of the interviewer	Signature or LTI of the Participant
Date	Date .....
_____	_____
Name of Literate Witness	Signature of the Witness

যক্ষা প্রকল্পে এইচ-আই-ভি পরীক্ষার জন্য আই. সি. ডি. ডি. আর, বি তে যাওয়ার অনুরোধ সম্মতিপত্র, রোগী

Title: Validation of the ALS assay for diagnosis of active tuberculosis and setting up of the Tuberculosis Specimen Bank.

গবেষকঃ ডঃ রুবহানা রাকীব, আই.সি.ডি.ডি.আর,বি, মহাখালি,ঢাকা-১২১২।
প্রকল্পের অবস্থানঃ ঢাকা।

বাংলাদেশে যক্ষা একটি অন্যতম প্রধান স্বাস্থ্য সমস্যা। দ্রুত ও উননত রোগ নির্নয় পদ্ধতি এবং ঔষুধের কার্যকারিতা সমপর্কে সঠিক ধারনা যক্ষা নিয়ন্ত্রণে অতীব জরুরী । যক্ষা রোগ নির্ণয় পদ্ধতিকে উন্নত এবং স্বল্প আয়ের মানুষের জন্য উপযোগী করার প্রয়াসে বিশ্ব স্বাস্থ্য সংস্থা এবং আই. সি. ডি. ডি. আর, বি যৌথভাবে নতুন নতুন পরীক্ষা উদ্ভাবনের জন্য কাজ করে যাচ্ছে। এই পরীক্ষা গুলো করার জন্য রক্ত, মূত্র অথবা কফ নমুনা হিসেবে ব্যবহার করা যেতে পারে। এই নমুনা গুলো কোন ব্যক্তির যক্ষা রোগ নির্ণয়ে এবং সেই সাথে নতুন উদ্ভাবিত পদ্ধতি গুলোর যথার্থতা প্রমানে ব্যবহার করা হবে। যক্ষা নির্ণয়ের নতুন পদ্ধতি যাচাই করার জন্য আপনার নমুনার কিছু অংশ বিশ্ব স্বাস্থ্য সংস্থায় প্রেরণ করা হবে। আপনার এইচ-আই-ভি রোগের জীবানু আছে কিনা তা নির্নয়ের জন্য আপনার দেয়া রক্ত পরীক্ষা করা হবে। পরীক্ষাগারে যক্ষা এবং এইচ-আই-ভি রোগের জীবানু সংক্রমিত ব্যক্তির নমুনা ও শুধু যক্ষা রোগের জীবানু সংক্রমিত ব্যক্তির নমুনা পরীক্ষা করা হবে, যা নতুন উদ্ভাবিত পদ্ধতি গুলোর যথার্থতা প্রমানে সহায়ক হবে।

উপরের পরীক্ষা গুলো করার জন্য আপনাকে আই.সি.ডি.ডি.আর,বি যেতে হবে। নমুনা হিসাবে আপনার রক্ত, কফ এবং মূত্র আই.সি.ডি.ডি.আর,বি-র পরীক্ষাগারে সংগ্রহ করা হবে।

আপনি যদি স্বেচ্ছায় এই গবেষণায় অংশগ্রহনপূর্বক আই.সি.ডি.ডি.আর,বি-তে যেতে ইচ্ছুক থাকেন তাহলে নিম্নে আপনার স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ দিন।

সম্মতিদান- উপরে বর্ণিত গবেষণা প্রকল্প আমাকে ব্যাখ্যা করা হয়েছে এবং আমি স্বেচ্ছায় এই গবেষণা প্রকল্পে অংশগ্রহন করতে সম্মতিদান করলাম।

তথ্য সংগ্রহকারীর নাম	অংশগ্রহনকারীর/অভিভাবকের নাম
তথ্য সংগ্রহকারীর স্বাক্ষর	অংশগ্রহনকারীর/অভিভাবকের স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ
শিক্ষিত সাক্ষীর নাম	সাক্ষীর স্বাক্ষর অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ
তারিখঃ-	তারিখঃ-

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR, B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

14 December 2003

Dr. Mark D. Perkins
Manager,
Diagnostics WHO-TDR
World Health Organization
20 Avenue Appia
Geneva CH 1211
Switzerland

Dear Mark,

I am happy to learn that our application in response to WHO/TDR's TB diagnostic Initiative (TBDI) Specimen Bank Activity RFA has been considered and ICDDR,B has preliminarily been selected as site for Specimen Bank Activity pending a site visit. The ICDDR,B is happy to participate with WHO as a collaborator in this project. The most appropriate time for this visit would be sometime between 28-30 January 2004. Meanwhile we are in the process of getting approval from our institutional review boards for the proposal that includes some critical issues such as HIV testing, shipment of samples to the WHO designated Distributor etc.

As I have mentioned earlier, I want to clarify that we plan to collect the specimens from the ALS assay since we have an interest in this assay. Collection of these specimens will in no way detract from the specimens that are needed for the specimen bank. If in future, others might like to use these ALS specimens, they would approach us through your office.

With best wishes.

David A Sack, MD
Director