

Modified - 8/5/90

Date 6-3-90

8-3-90

Attachment 1.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator DILARA ISLAMTrainee Investigator (if any) 226Application No. 90-004Supporting Agency (if Non-ICDDR,B) SABECTitle of Study Development of an

Project status:

immunodiagnostic assay for the detection of

() New Study

Shigella and identification of species/

() Continuation with change

serotype specificity directly from human samples

No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population: NA

5. Will signed consent form be required: NA

(a) Ill subjects Yes No

(a) From subjects Yes No

(b) Non-ill subjects Yes No

(b) From parent or guardian

(c) Minors or persons under guardianship Yes No

(if subjects are minors) Yes No

Does the study involve: NA

6. Will precautions be taken to protect NA

(a) Physical risks to the subjects Yes No

anonymity of subjects Yes No

(b) Social Risks Yes No

7. Check documents being submitted herewith to Committee:

(c) Psychological risks to subjects Yes No

— Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).

(d) Discomfort to subjects Yes No

✓ Protocol (Required)

(e) Invasion of privacy Yes No

— Abstract Summary (Required)

(f) Disclosure of information damaging to subject or others Yes No

— 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)

Does the study involve: NA

— Informed consent form for subjects

(a) Use of records, (hospital, medical, death, birth or other) Yes No

— Informed consent form for parent or guardian

(b) Use of fetal tissue or abortus Yes No

— Procedure for maintaining confidentiality

(c) Use of organs or body fluids Yes No

— Questionnaire or interview schedule *

Are subjects clearly informed about: NA

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

(a) Nature and purposes of study Yes No

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.

(b) Procedures to be followed including alternatives used Yes No

2. Examples of the type of specific questions to be asked in the sensitive areas.

(c) Physical risks Yes No

3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(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

(PTO)

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

Dilara Islam
Principal Investigator

Trainee

PROPOSAL FOR M.Sc./Ph.D. RESEARCH PROJECT

(ICDDR,B in collaboration with Karolinska Institute, Sweden)

1. STUDENT : Ms. Dilara Islam
2. TECHNICAL GUIDANCE :
 - a) Dr. Tasnim Azim
 - b) Dr. Firdausi Qadri
 - c) Dr. Sayedul Islam
3. TITLE OF PROJECT : Development of an immunodiagnostic assay for the detection of *Shigella* and identification of species/serotype specificity directly from human samples
4. STARTING DATE : April, 1990
5. COMPLETION DATE : October, 1991
6. TOTAL BUDGET REQUESTED: \$23,000
7. FUNDING SOURCE : SAREC
8. PROJECT SUPERVISORS :
 - a) Dr. Saul Tzipori, ICDDR,B
 - b) Prof. Alf Lindberg, Karolinska Institute
9. AIMS OF THE PROJECT
 - a) General aim
To develop an immunodiagnostic assay using species/serotype specific monoclonal antibodies for the detection of *Shigella* antigen directly from stool, blood and possibly urine. The assay will utilize immunomagnetic polymer or latex particles and will be compared with immunofluorescence and bacterial culture for speed, cost, sensitivity and specificity.

b) Specific aims

- 1) Development of latex agglutination assay using Mabs produced against serospecific lipopolysaccharides (LPS) to detect *Shigella* species O-antigen directly from faeces, blood and urine of infected patients.
- 2) Using Mabs-coated immunomagnetic particle (IMP) to detect bacterial antigens as above.
- 3) Compare above methods with immunofluorescence assay and conventional culture methods for sensitivity and specificity.

c) Significance

The development of a rapid sensitive and specific assay that will detect *Shigella* with species/serotype specificity, directly from stool, blood and possibly urine, will be of tremendous benefit. Such an assay will facilitate rapid diagnosis and therefore improve patient care, and will be a very useful tool in epidemiological, clinical and future vaccine studies. Currently there are at least 3 studies at ICDDR,B which could benefit from an assay that identifies patients with shigellosis shortly after admission with species/serotype specificity.

10. ETHICAL IMPLICATIONS

Blood, stool and urine will be collected from 60 adult humans who will be selected for a study (also supported by SAREC) (Local and

systemic immune response to shigellosis in adult humans, P.I. Ms. Rubhana Raqib, Protol. No. 90-005). Small amounts of blood/plasma (200 µl) collected from these individuals will be used in the immunodiagnostic assay for detection of bacteremia by the immunodiagnostic assay. In addition, stool (and urine samples if proved useful) will be collected from 30 individuals with diarrhoea of various causes from the ICDDR,B surveillance system, and from 30 age-matched control individuals without diarrhoea (volunteers from ICDDR,B).

11. BACKGROUND, RESEARCH PLAN AND BIBLIOGRAPHY

a) Background

Monoclonal antibodies to Shigella species

Monoclonal antibodies have been prepared against the LPS of *S. flexneri* which are species and serotype specific (Carlin and Lindberg, 1983, 1986, 1987). These Mabs have been used successfully to serotype clinical isolates cultured from stool obtained from patients with shigellosis (Carlin *et al.*, 1989), in contrast to polyclonal immune sera which are cross reactive within *S. flexneri* species. In addition to the monoclonal antibodies against LPS, Mabs against the B subunit of shiga toxin has been used to detect nanogram levels of the toxin from stool of patients with *S. dysenteriae* type 1 infection (Donohue-Rolfe *et al.*, 1986). Other Mabs have been prepared and characterized against the A subunit of shiga toxin and the LPS of *S. flexneri* types 1a, 2a, 2b (Islam and Stimson, 1987).

Coagglutination assays using Mabs absorbed to sensitized *Staphylococcus aureus* Cowan 1 cells have been successfully used for the detection of *Shigella* antigen (Carlin *et al.*, 1989) and may also be used in direct or indirect immunofluorescence assays for detection of shigellae as has been used for *V. cholerae* 01 (Brayton *et al.*, 1987).

Rapid identification of bacteria based on the presence of cell surface adhesin such as *E. coli* containing the K88⁺ pili has been accomplished using specific Mabs linked to superparamagnetic polymer particles coated with antibody (Lund *et al.*, 1988). These methods can be applied for the detection of shigellae or shigellae antigen from stool, blood and possibly urine. Rapid detection of shigellae will benefit patient care and perhaps reduce risk of complication.

Rapid assays with high degree of specificity and sensitivity will also benefit a considerable number of studies currently being conducted at the Centre. These include:

- a) The therapeutic value of hyperimmune bovine colostrum against shigellosis (a quick identification of the causative bacteria will allow registration of patients in the study shortly after admission concurrently with the onset of conventional treatment.

- b) Cohort study in which some 200-800 faecal specimens will need to be cultured for *Shigella* each week. An immunodiagnostic assay will reduce costs, time, labour and be more direct in identifying serotype and group specificity.
- c) An immunological study on peripheral blood which requires enrollment of confirmed patients with shigellosis shortly after admission (Protocol No. 89-014).
- d) An immunological study on local and systemic immune response of adults to shigellosis is planned in conjunction with this study (Protocol No. 90-005). A rapid test will allow an immediate enrollment in the study of confirmed shigellosis cases.

b) Research plan

Development of immunodiagnostic assays

The development of an assay will largely be conducted at the Karolinska Institute, using locally produced Mabs against LPS of *S. flexneri* which are type- and group-specific (Carlin *et al.*, 1983-1989). Hybridoma cell lines producing these Mab will be brought to ICDDR,B (please see Appendix 1).

Isolation of shigellae using latex agglutination assay

Latex particles (0.8 μ m). will be coated with Mabs (Appendix 2) and used in slide agglutination assays to detect shigellae. Results will be recorded within 5 minutes and scored from negative to positive on a scale of 0 to 4+.

Comparison with other methods

Isolation of Shigellae using immunomagnetic particles

Superparamagnetic, monosized polymer particles coated with sheep anti-mouse immunoglobulin (Dynabeads M-450 SAM, Dynal A/S, Oslo, Norway) will be coated with Mabs (Appendix 3) as described earlier (Lund *et al.*, 1988) and used for detection of shigellae antigen directly from stool, blood and urine. Bacteria attached to Mab coated beads will be detected by staining with acridine orange/ethidium bromide and observation under fluorescence microscopy. The sensitivity of immunoassays will be determined by testing different concentrations of bacterial suspension with different concentrations of Mab coated beads. Specificity will be determined by testing Mab coated particles with different enteric bacteria. Such an assay will help for quick identification of *Shigella* strains from patients. It will also be particularly useful in the selection of patients for ongoing studies as mentioned in the previous page.

Conventional bacterial culture

Results will be compared with routine microbiological culture methods used for isolation of shigellae which will be performed at the same time.

Immunofluorescence

An indirect immunofluorescence (IF) assay with monoclonal antibody and a fluorescent dye labelled mouse antiantibody will also be applied directly to identification of shigellae directly from stool for comparison.

Direct slide agglutination assay

Mabs will be absorbed to *S. aureus* Cowan 1 as has been described for *S. flexneri* earlier (Carlin *et al.*, 1989) and used in slide agglutination.

The above methods will be compared for speed, cost, sensitivity, specificity and simplicity.

Biological samples from patients

Stool, blood and urine will be collected from 60 adults comprising 15 patients with *S. dysenteriae* type 1 and 15 with *S. flexneri* infections, and 30 from non-diarrhoea adult controls. These will be the same individuals who will be enrolled for the study on "Local and systemic immune response to shigellosis in humans" also supported by SAREC. Three samples of blood, stool and urine will be obtained

from each of the 45 persons. In addition, urine and stool samples will be collected from 30 patients from the ICDDR,B surveillance system, and 30 from age-matched control individuals (volunteers from ICDDR,B) without diarrhoea.

12. SEQUENCE OF WORK

September, 1989 - April, 1990

Will be spent at the Karolinska Institute in the development of an immunodiagnostic assay under the supervision of Professor Alf Lindberg.

April, 1990 - April, 1991

Will be spent at ICDDR,B in Dhaka under the supervision of Dr. S. Tzipori. The student will continue the development of the assay and apply it to stool, blood and urine specimens as outlined above. The assay will also be compared with other existing methods of culture, IF and agglutination tests.

May, 1991 - October, 1991

Will be spent at the Karolinska Institute to complete analysis of data and prepare dissertation for a M.Sc./Ph.D. degree.

14. BIBLIOGRAPHY

- Brayton, P.R., Tamplin, M.L., Huq, A. and Colwell, R.R. 1987. Enumeration of *Vibrio cholerae* O1 in Bangladesh waters by fluorescent-antibody direct viable count. Appl. Environ. Microbiol.
- Carlin, N.I.A., Rahman, M., Sack, D.A., Zaman, Z., Kay, B. and Lindberg, A.A. 1989. Use of monoclonal antibodies to type *Shigella flexneri* in Bangladesh. J. Clin. Microbiol. 27:1163-1166.
- Carlin, N.I.A. and Lindberg, A.A. 1987. Monoclonal antibodies apecific for *Shigella flexneri* lipopolysaccharides: clones binding type IV, V and VI zantigens, group 3, 4 antigen and an epitope common to all *Shigella flexneri* and *Shigella dysenteriae* type 1 strains. Infect. Immun. 53:110-115.
- Carlin, N.I.A. and Lindberg, A.A. 1986. Monoclonal antibodies specific for *Shigella flexneri* lipopolysaccharide: clones binding to type I and type III: 6,7,8 antigens, group antigen 6 and a core epitope. Infect. Immun. 53:103-109.
- Carlin, N.I.A. and Lindberg, A.A. 1983. Monoclonal antibodies specific for O-antigenic polysaccharides of *Shigella flexneri* clones binding to II, II:3,4 and 7,8 epitopes. J. Clin. Microbiol. 18:1183-1189.

- Donohue-Rolfe, A., Kelley, K.A., Bennish, M. and Keusch, G.T.
1986. Enzyme-linked immunosorbent assay for *Shigella* toxin.
J. Clin. Microbiol. 24:65-68.
- Islam, M.S. and Stimson, W.H. 1987. Production of monoclonal
antibodies to *Shigella*. Enzyme-linked immunosorbent assay
for screening hybridoma antibodies with intact bacteria.
Letter. Appl. Microbiol. 4:85-81.
- Levinsky, R.J. and Soothill, J.F. 1977. A test for antigen
antibody complexes in human sera using IgM of rabbit
antisera to human immunoglobulin. Clin. Exp. Immun. 29:428.
- Lund, A., Hellemann, A.L. and Vartdal, F. 1988. Rapid isolation
of K88⁺ *Escherichia coli* by using immunomagnetic particles.
J. Clin. Microbiol. 26:2572-2575.
- Zola, H. 1987. Monoclonal antibodies: A manual of techniques.
CRC Press, Inc. Boca Raton, Florida.

13. BUDGET

1989-90	:	Studentship	...	\$ 2,000	
		Supplies	...	8,000	10,000
1990-91	:	Studentship	...	3,000	
		Supplies	...	10,000	13,000

TOTAL			...		\$ 23,000

NOTE

It is felt that since this protocol has been approved by the WHO Working Group, no external review was sought.

APPENDIX-1

Preparation of Mabs

BALB/C mice or LOU/C rats primed by intraperitoneal injection of ~0.5 ml Pristane. After 10-12 days hybridoma cells will be inoculated intraperitoneally. Ascitic fluid will be extracted from animals before they start with respiratory difficulties; and centrifuged and stored in aliquots at -20°C .

Purification of antibodies

For $(\text{NH}_4)_2\text{SO}_4$ purification, 50% saturated salt solution in PBS was used. Pellet dissolved in 0.1-0.3% of initial volume, and dialyzed against PBS. Sephacryl S-300 packed in Pharmacia column (100 x 15 cm). Column equilibration and elution buffer is 0.1 M Tris-HCl with 0.5% NaCl pH 8.0 and column flow rate set at 15 ml/hr. Protein A sepharose CL-4B powder dissolved in PBS (wet column volume 1 ml). Column washed with PBS, and equilibrated with 0.1 M Tris-HCl pH 8.0. Sample will be eluted as follows:

- 1) 7-8 ml of binding buffer
- 2) 7-8 ml of citric acid buffer, pH 4.0
- 3) 7-8 ml of citric acid buffer, pH 3.0

Fraction collected in tube containing 0.1 ml of 1 M Tris-HCl, pH 8.0 to adjust the pH immediately.

APPENDIX-2

Preparation of latex reagent

Latex particle will be washed with Glycine buffered saline (GBS) pH 8.2 (0.1 M glycine, 0.15 M NaCl), and diluted to 1:5 dilution. Mabs will also be diluted with GBS. Procedure of reagent preparation is:

- 1) One part of diluted latex mixed with one part of Mab and incubated at 37°C for 2 hours
- 2) After incubation two part of GBS containing 1% BSA + 0.01% NaN₃ added and stored at 4°C

APPENDIX-3

Coating of Mabs With immunomagnetic particle

Dynabeads M-450 (uncoated) washed with 0.05 M Tris-HCl pH 9.5 and resuspended in same buffer. Beads diluted from 30 mg/ml to 20 mg/ml.

Antibody mixed with beads and incubated on a rotator with mild rotation. After incubation, tube or vial containing mixture is placed on magnet and supernatant removed, and resuspended in PBS containing 0.5% BSA. Mab coated beads washed three times, and finally resuspended in PBS supplemented with 0.1% BSA and 0.01% NaN_3 . Final concentration of beads made to 10 mg/ml and stored at 4°C.