Date 06.06.90

ETHICAL REVIEW COMMITTEE, ICDDR,B.

cipal Investigator FIRDAUSI QADRI
ication No. 90-010

e of Study : Hemagglutination (HA) ity and adhesiveness of Shigella spp. rt 2): Characterization of the adhesin/ agglutinin and other outer membrane ponents, including the use of mono- nal antibodies (mabs).

Trainee Investigator (if any)

Supporting Agency (if Non-ICDDR,B)

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

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

Source of Population:
(a) Ill subjects — Yes No N/A
(b) Non-ill subjects — Yes No
(c) Minors or persons under guardianship — Yes No

Does the study involve:
(a) Physical risks to the subjects — Yes No
(b) Social Risks — Yes No
(c) Psychological risks to subjects — Yes No
(d) Discomfort to subjects — Yes No
(e) Invasion of privacy — Yes No
(f) Disclosure of information damaging to subject or others — Yes No

oes the study involve: N/A
a) Use of records, (hospital, medical, death, birth or other) — Yes No
b) Use of fetal tissue or abortus — Yes No
c) Use of organs or body fluids — Yes No

re subjects clearly informed about: N/A
a) Nature and purposes of study — Yes No
b) Procedures to be followed including alternatives used — Yes No
c) Physical risks — Yes No
d) Sensitive questions — Yes No
e) Benefits to be derived — Yes No
f) Right to refuse to participate or to withdraw from study — Yes No
g) Confidential handling of data — Yes No
h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes No

5. Will signed consent form be required:
(a) From subjects — Yes No N/A
(b) From parent or guardian (if subjects are minors) — Yes No

6. Will precautions be taken to protect anonymity of subjects — Yes No

N/A 7. Check documents being submitted herewith to Committee:

NO Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
___ Protocol (Required)
___ Abstract Summary (Required)
___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
___ Informed consent form for subjects
___ Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

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

Firdausi Qadri
Principal Investigator

Trainee

APPLICATION FOR PROJECT GRANT

1. PRINCIPAL INVESTIGATOR: Dr. Firdausi Qadri

   OTHER INVESTIGATORS : Dr. Tasnim Azim
   Mr. Shaikh Abu Hossain
   Dr. M. Sayedul Islam
   Mr. Gabriel Mondol
   Dr. Dilara Islam (Ph.D. Student)

2. TITLE OF PROJECT : Hemagglutination (HA) ability and adhesiveness of *Shigella* species (Part 2): Characterization of the adhesin/hemagglutinin and other outer membrane components, including the use of monoclonal antibodies.

3. STARTING DATE : June, 1990

4. COMPLETION DATE : July, 1993

5. TOTAL BUDGET REQUESTED US$ 201,900

6. FUNDING SOURCE :

7. HEAD OF PROGRAMME : Dr. Saul Tzipori

8. AIMS OF PROJECT :

a) General aim:

To determine the contribution of HA and/or adhesins to cellular attachment of *Shigella* species, and develop immunodiagnostic and seroepidemiologic assays using monoclonal antibodies.

b) Specific aims:

1) Purification of outer membrane components, which include; lipopolysaccharides (LPS) and capsular polysaccharides from strains of *S. dysenteriae* type 1 and *S. flexneri* to determine their chemical, adhesive and antigenic nature.

2) Production of mabs against the above purified antigenic components to help determine their contribution to the adhesion of *Shigella* species to cultured cells and to mammalian erythrocytes.

3) Development of rapid immunodiagnostic assays using the above produced mabs, and mabs previously developed in Glasgow, to detect *Shigella* species in stool, blood and possibly in urine.

4) Development of seroepidemiological assays using immunochemically defined LPS and synthetic, glycoconjugates as antigens in enzyme immunoassays to measure species and serotype - specific *Shigella* antibody in body fluids of patients.

c) Significance:

These studies will generate a decisive information on the chemical biological and antigenic nature of the hemagglutinating activity of strains of *S. dysenteriae* and *S. flexneri* demonstrated in the previous protocol, which will help define their role in bacterial attachment to colonocytes and hence to the pathogenesis of shigellosis. The use of Mabs for the development of rapid immunodiagnostic and seroepidemiologic assays will be of benefit for the identification of *Shigella* strains and measurement of antibody in clinical studies, in epidemiological investigations, and in future vaccine trials.

9. ETHICAL IMPLICATIONS

Not applicable, laboratory bacterial isolates will be studied in a laboratory-based investigation.

10. BACKGROUND, RESEARCH PLAN AND BIBLIOGRAPHY

a) Background

1) Virulence of Shigellae

Studies carried out on shigellae in the past few years have shown that outer membrane proteins (OMPs), LPS, and toxin when present, contribute to the virulence of these organisms (Levine *et al.* 1983). The outer membrane proteins have been shown to be important for the penetration of shigellae into host cells (Hale *et al.*, 1983) and smooth LPS found to be necessary for virulence (Watanabe *et al.*, 1984).

Adherence of bacteria to host cell surfaces is a primary requisite for pathogenesis of many bacteria (Beechey, *et al.*, 1981). The adherence of some strains of *S. flexneri* to guinea pig colonic cells (Izhar *et al.*, 1982) and HeLa cells (Pal and Formal, 1989) demonstrate that invasive shigellae too have a capacity for binding to cell surfaces. Recently we have shown that strains of *S. dysenteriae* type 1 and *S. flexneri* (types 1a, 1b, 2a, 2b) adhere to mammalian erythrocytes (Qadri *et al.*, 1989) causing their agglutination, a phenomenon not observed previously (Mulczyk, 1967). Strains of *S. dysenteriae* type 1 and *S. flexneri* also adhered to cultured intestinal cells (Henle 407) before invading them.

2) LPS and HA

Bacteria attach to host cell surfaces through fimbriae, or flagella, or by non fimbrial components such as surface proteins, LPS, the bacterial capsule or glycocalyx (McSweegan and Walker, 1986). Fimbriae have been detected in strains of *S. flexneri* (Mulczyk, 1967) whereas others

failed to observe them on strains of *S. dysenteriae* type 1 (Qadri *et al.*, 1989) In the search for bacterial component(s) which is/are responsible for the hemagglutinating activity (ongoing protocol No. 88-006, "hemagglutinating ability and adhesiveness of *Shigella* species"), it was demonstrated that, at least in *S. dysenteriae* type 1, HA is mediated by the lipopolysaccharide (manuscript in preparation). The culture supernatant of bacteria grown in Casamino Acids-yeast extract (CYE) broth medium contained hemagglutinating activity from which extracted lipopolysaccharide (Westphal and Jann, 1965) or O-polysaccharide side chains (Moll, 1986) agglutinated erythrocytes. Rabbit antibody raised against *S. dysenteriae* type 1 LPS inhibited adhesion of shigellae to human intestinal Henle 407 cells and to erythrocytes.

3) Capsular and Glycocalyx Components

Adhesins that have been characterized in enteric pathogens are proteins, either fimbrial (e.g. K-88, K-99, CFA-1 and CFA-2 antigens, Evans *et al.*, 1980) or non fimbrial in nature (Goldhar *et al.*, 1987). In some cases however attachment is mediated by LPS, examples include *Campylobacter jejuni* (McSweegan, 1986) and *E. coli* F-18 (Cohen, 1985). Studies in *S. flexneri* suggest that either LPS (Izhar *et al.*, 1982) or protein adhesins (Pal and Formal, 1989) may be involved. It has also been observed that bacteria which express HA or adherence ability are surrounded by capsule or glycocalyx (Goldhar, 1987; Knutton, 1984; Kuesecek, 1984). It is believed that both bacterial and eukaryotic cells *in vivo* are covered by a glycocalyx which are composed of a matrix of polysaccharides and the actual interacting surfaces between the two

cell types are usually the respective glycocalyces (Costerson *et al.*, 1981). *In vitro* cultures are usually artificial and hence the glycocalyx is often absent. We have detected capsule and slime in *S. dysenteriae* type 1 cultured in the CYE broth medium (Qadri *et al.*, 1989). Although capsules have been detected earlier in shigellae (Edwards and Ewing, 1972) they have not been chemically and antigenically characterized. Our preliminary results suggest that the capsular polysaccharide in *S. dysenteriae* type 1 may be similar to the O-antigenic polysaccharide of the LPS since the agglutination of the bacteria in type specific antiserum is not reduced in the presence of the capsule. A detailed analysis of the chemical and antigenic composition of the capsule needs to be carried out. For this purpose mabs will be particularly suitable as probes to investigate the antigenic determinants present on the hemagglutinin, the capsule and glycocalyx. These mabs can also be used to study the protective role of the antibody in neutralizing bacterial adhesion to cell surfaces. To rule out non-specific effects of Mabs in inhibiting bacterial adherence control Mabs of same isotype and of unrelated specificity will be used. Further Fab fragments of Mabs will be separated from Fc portions and only the Fab portions used for adherence assays. It is the Fc portion which may give rise to non-specific steric hindrance.

The characterization of the adhesin from strains of *S. flexneri* (types 1a, 1b, 2a, 2b) also need to be carried out which will help determine whether protein and/or non proteinaceous components are involved.

4) Detection of antigens and serospecific antibody in *Shigella* infection

i) *Monoclonal antibodies in Shigella species*

Monoclonal antibodies have been produced against the LPS of *S. flexneri* which are type and group specific (Carlin and Lindberg, 1983, 1986, 1987). These mabs have been used successfully to type clinical isolates obtained from stool samples of patients (Carlin *et al.*, 1989). They have been found to be highly specific for identification of the different serotypes of *S. flexneri* since polyclonal antisera are cross reactive and results obtained with these reagents are often questionable. In addition to the monoclonal antibodies against LPS, mabs against the B subunit of shiga toxin has been used to detect nanogram levels of toxin from stools 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 as well as the LPS of *S. flexneri* types 1a, 2a, 2b (Islam, and Stimson, 1987; Islam 1988 Ph.D. dissertation). Mabs against *Shigella* antigens can be used in immunoassays which will improve their sensitivity and specificity. These include coagglutination assays using mabs absorbed to sensitized *Staphylococcus aureus* Cowan 1 cells (Carlin *et al.*, 1989). Mabs can also be used in direct or indirect immunoflourescence assays for detection of shigellae as has been used for *V. cholerae* O1 (Brayton *et al.*, 1987).

Rapid identification of bacteria based on the presence of cell surface adhesins such as *E. coli* containing the $K88^+$ pili was accomplished with specific mabs linked to superparamagnetic polymer particles coated with antibody (Lund *et at.*, 1988). These methods can be applied for the

detection of shigellae from stool blood and possibly urine in which these bacteria or bacterial antigen are present in very low numbers. Rapid detection of shigellae from patients with dysentery will help in early institution of treatment in order to minimize complications. Early detection of infection will be very helpful in several studies currently being conducted or planned for the future.

The rationale for the development of an immunodiagnostic assay and the methodology are presented in a separate supplementary research protocol (title: Development of an immunodiagnostic assay for the detection of *Shigella* and identification of species/serotype specificity directly from human samples, Protocol No. 90-004, P.I. Ms. Dilara Islam. This supplementary protocol is funded separately by SAREC.

ii) *Purified LPS and synthetic glycococaguluates in enzyme immunoassays*

O-antigen specific ELISAs have beeen used for the detection of specific serum antibodies against *S. dysenteriae* type 1 (Lindberg, 1984) and *S. flexneri* (Ekwall, 1988). Since purified LPS obtained from different *S. flexneri* serotypes show cross reactions, chemical and immunochemical characterization of these LPS have been carried out to increase the specificity of these assays. Synthetic oligosaccharides representing the O-antigenic side chains of LPS of *S. flexneri* have been coupled to bovine serum albumin and used as serospecific antigens in ELISA (Lindberg, 1988). Development of tests which will measure specific *Shigella* antibody in body fluid will be very helpful in assessing the antibody response to infections.

Such an assay will be useful in a number of studies currently conducted at the Centre and for future studies. These include:

1) Local and systemic immune response to shigellosis in adult humans. (P.I. Ms. Rubhana Raqib, Ph.D. student, Protocol No. 90-005).

2) Study of the immune response to *S. dysenteriae* type 1 in an effort to identify abnormalities leading to the development of complications. (Ongoing protocol No. 90-014, P.I. Dr. T. Azim).

3) A longitudinal study on diarrhoea of infants with particular reference to homotypic and heterotypic reinfections and cross protection among rotavirus serotypes and *Shigella* serotype/serogroups, respectively. (To be developed)

b) Research plan

i) *Extraction of LPS, O-antigenic polysaccharides and capsular polysaccharide*

LPS will be extracted from *S. dysenteriae* type 1 using the hot phenol water procedure (Westphal and Jann, 1965). Further purification will be carried out using nucleases and proteases (Cryz *et al.*, 1984). O-antigenic polysaccharides will be separated from LPS by refluxing in 1% acetic acid solution (Moll, 1986).

Hemagglutinin will be extracted from culture supernatants of bacteria grown in CYE medium then filtered; dialysed, freeze-dried and purified by chromatography on a column of Sepharose 4B. Polysaccharides eluting in the void volume will be used for further characterization. LPS will

also be separated from the culture supernatants by the phenol water procedure (Westphal and Jann, 1965).

Capsular polysaccharides will be extracted from culture supernatant of bacteria by precipitation with cetylpyridinium chloride (Kato *et al.*, 1981). Using this procedure the acidic polysaccharides which represent the capsular material will be precipitated and separated from the neutral polysaccharides which usually represent the O-antigenic side chains of LPS (Hasegawa *et al.*, 1983). The carbohydrates will be further fractionated using gel filtration and characterized using analytical methods such as ultraviolet absorption spectroscopy, infrared spectroscopy and chemical procedures generally used for such polysaccharides (Hasegawa *et al.*, 1983). Extensive chemical analysis of the structure of the polysaccharides will be carried out with the help of Prof. Moshiuzzaman at the Department of Chemistry, University of Dhaka. Immunochemical analysis will be carried out using crossed immunoelectrophoresis (Kroll, 1973), Western blotting (Towbin *et al.*, 1979) and rocket immunoelectrophoresis (Kusecek, 1984).

ii) *Extraction and Purification of adhesins from S. flexneri*

Hemagglutinating strains of *S. flexneri* (Table 1) will be used for extraction and purification of potential protein adhesins using methods such as those described earlier for *E. coli* (Salit and Gotschlich, 1977; Goldhar *et al.*, 1987). The procedure in brief will involve extraction of adhesin from bacterial cell surface with buffer and further purification using acetate precipitation, ammonium sulfate fractionation etc. The dialyzed material will be separated into components using gel

filtration as well as ion exchange chromatographic techniques utilizing the Mono Q or Mono S adsorbents of the Fast Protein Liquid Chromatographic System (FPLC) of Pharmacia. In addition to these methods culture supernatant of bacteria will be processed and tested for hemagglutinating activity and chemically analysed as described above for *S. dysenteriae* type 1. Adhesins will be analysed by polyacrylamide gel electrophoresis both under denaturing (Laemmli, 1970) and non denaturing conditions (Davis, 1964). Immunochemical analysis will be carried out using crossed immunoelectrophoresis (Weeke, 1973) and Western blotting techniques (Towbin, 1979).

iii) *Production of Mab*

BALB/c mice will be injected with antigens (De. St. Groth *et al.*, 1980) and spleen cells of the immunized mice will be fused with myeloma cells (X63-Ag.653 or NS1) according to the method of Kohler and Milstein (1976) using polyethylene glycol 1500 as the fusing agent. Hybrids will be screened for recognition of the hemagglutinin by ELISA. Detection of hemagglutinin of *S. dysenteriae* type 1 detection will be based on ELISA using purified LPS (Lindberg *et al.*, 1984), purified polysaccharides or the antigen used for immunization. Hybrids reactive with specific antigens will be cloned at least twice by limiting dilution and grown as ascites-producing tumors in the peritoneal cavities of pristane-primed BALB/c mice as well as *in vitro* culture. Polyclonal antisera will also be prepared in rabbits for comparisons.

iv) *Purification and characterization of mabs*

Antibodies will be purified from the ascites fluid by chromatography on protein A Sepharose column and then kept frozen (Abe *et al.*, 1989). The isotype of the mabs will be determined by double diffusion in gel analysis or by ELISA using isotype-specific antimouse immunoglobulin (obtained from Sigma). Monoclonality will be tested by isoelectric focusing procedures (Awdeh *et al.*, 1968).

Characterization of activity of mabs: Purified and characterized antibodies prepared against adhesins will be used in adhesion assays to test for inhibition of binding both to cultured intestinal cells (Henle 407) and to mammalian erythrocytes. In other cases mabs will also be screened against specific and non-specific antigens using ELISA and Western blotting procedures (Towbin *et al.*, 1979).

v) *Application of mabs in diagnostic procedures*

These procedures are being developed in a separate protocol (P.I. Ms. Dilara Islam; copy attached). Other Mabs specific for *S. dysenteriae* type 1 or *S. flexneri* antigens (Table 2) as well as those that will be prepared at ICDDR,B will be used to detect bacteria or bacterial antigens. Samples will be obtained from the clinical laboratory at ICDDR,B. For this purpose procedures which will be used have been described in protocol attached (P.I. Ms. D. Islam).

vi) *Lipopolysaccharides and glycoconjugates as antigens in specific enzyme immunoassays*

Purified LPS and synthetic glycoconjugates will be used in ELISA to develop tests at ICDDR,B capable of measuring serotype and group specific serum and secretory antibody against shigellae in patients.

Dr. A. Weintraub from the Karolinska Institute will be spending 3-4 weeks to help develop these assays. His contribution will be mostly in establishing techniques required for the purification of *Shigella* LPS and glycoconjugates. This will allow serotype and group-specific differentiation of serum and secretory antibody in patients previously exposed to shigellosis as outlined in the 3 studies mentioned above. His visit will be funded by SAREC.

c) Bibliography

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11. BIBLIOGRAPHY OF INVESTIGATORS (last 5 years)

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b) Dr. Firdausi Qadri

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c) M. S. Islam

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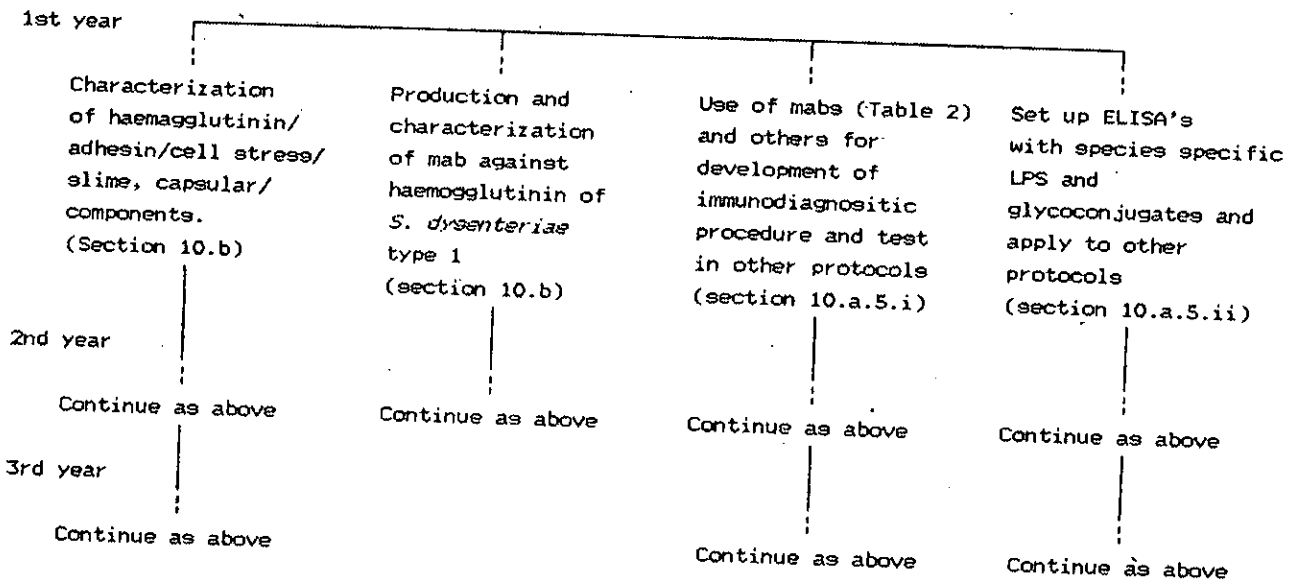
2) Islam, M.S. and Stimson, W.H. Production and characterisation of monoclonal antibodies with therapeutic potential against Shiga toxin. Clin. Exp. Immunol. (in presss).

3) Islam, M.S. and Stimson, W.H. Production and characterization of monoclonal antibodies with diagnostic potential against *Shigella flexneri*. J. Clin. Lab. Invest. (in press).

12. FLOW CHART



Sequence of work in the next 3 years:

First year

1. Preparation of mabs against the adhesin of *S. dysenteriae* type 1, characterization of the mabs and use it to study the process of adhesion. This in turn will result in optimization of procedures of preparation of mabs in the tissue culture laboratory at ICDDR,B.

2. Characterization of the adhesins from strains of *S. flexneri* and if possible start preparation of mabs.

3 Use of previously prepared mabs against antigens of *Shigella* species (Table 2) to set up immunodiagnostic procedures.

Second and third year

1. Continue work on characterization of adhesins and using biochemical and immunological procedures.

2. Preparation of mabs.

3. Use of mabs in immunodiagnostic procedures and compare these methods with existing micrbiological techniques used for routine diagnosis at the clinical research centre at ICDDR,B.

13. ITEMIZED SPECIFIC TASKS FOR EACH LISTED INVESTIGATOR

a) Dr. Tasnim Azim

Preparation and characterization of mabs against specific adhesin, LPS, capsular polysaccharides, etc. Standarization of procedures in the mab laboratory (20%).

b) Gabriel Mondol

Assistance in preparation of mabs (100%).

c) Ms. Dilara Islam (Ph.D. student)

Use of mabs in immunodiagnostic procedures.

d) Dr. M. Sayedul Islam

Preparation of mabs, application of previously prepared mab (Table 2) in immunodiagnostics (as above) (100% of part-time consultancy, 12 hr/week).

e) Dr. A. Weintraub (Karolinska Institute, Sweden)

Will help with the development of ELISA with glycoconjugates to measure serotype specific and species-specific serum and secretory antibody against shigellae in humans. He will be spending 3-4 weeks at ICDDR,B and is funded by SAREC.

f) Dr. Firdausi Qadri

Characterization of the hemagglutinin from *S. dysenteriae* type 1. Extraction and analysis of capsular polysaccharides. Characterization of the hemagglutinins adhesins from strains of *S. flexneri*. Characterize mabs produced against antigens of shigellae. Use mabs to characterize the process of adhesion to host cell surfaces (100%).

g) Dr. S. M. Faruque

As in (f) (100%).

14. BUDGET

A. OPERATING COST

a) Personnel

	Year 1	Year 2	Year 3	Total
Firdausi Qadri, NO-C	15,400	16,900	18,600	50,900
M. Sayedul Islam (Consultant)	2,000	2,000	2,000	6,000
Gabriel Mondal, GS-6	8,400	9,300	10,200	27,900
Sk. Abu Hossain, GS-5	6,500	7,200	7,900	21,600
Laboratory Attendant, GS-2	3,600	4,000	4,400	12,000
Total	35,900	39,400	43,100	118,400

B. CAPITAL QUIPMENT

Incubator (with carbon dioxide attachment)			5,000	5,000
Liquid nitrogen jar, and accessories			6,500	6,500
Total			11,500	11,500

C. OPERATING COSTS

a) Plastic/glassware/ membrane/etc.	7,500	6,500	6,500	20,500
b) Chemicals & reagents	7,000	6,500	6,500	20,000
c) Media & serums	6,000	6,000	6,000	18,000
Total	20,500	19,000	19,000	58,500
D. SUPPLIES & MISCELLANEOUS	2,000	2,000	2,000	6,000
E. ANIMAL RESOURCES	2,500	2,500	2,500	7,500
GRAND TOTAL	60,900	62,900	78,100	201,900

FQ:mh/B4:HABILITY.PRT

Table 1

*Strains to be used in the study

Species	Strain	Characteristics
S. dysenteriae type 1	14731	HA+
	12588	HA+
	3351	HA+
S. flexneri type 1a	18818	HA+
type 1b	613	HA+
type 2a	4986	HA+
type 2b	24470	HA+

*These strains have been obtained from patients at the Clinical Research Centre at ICDDR,B and have been characterized (Qadri *et al.*, 1989) and at present stored at -70°C.

Table 2

Monoclonal antibodies available for immunodiagnostic assays

Monoclonal antibodies	Source	Reference
[1]Mabs specific for (a) A subunit of Shiga toxin and (b) LPS of *S. flexneri* (type 1a, 2a and 2b)	Prepared at University of Strathclyde, Glassgow	Islam, 1989

[1]Hybridoma cell line secreting these mabs are at present stored in liquid nitrogen in the Tissue Culture Laboratory at ICDDR.B

FQ:MH/B4:HABILITY.PRT

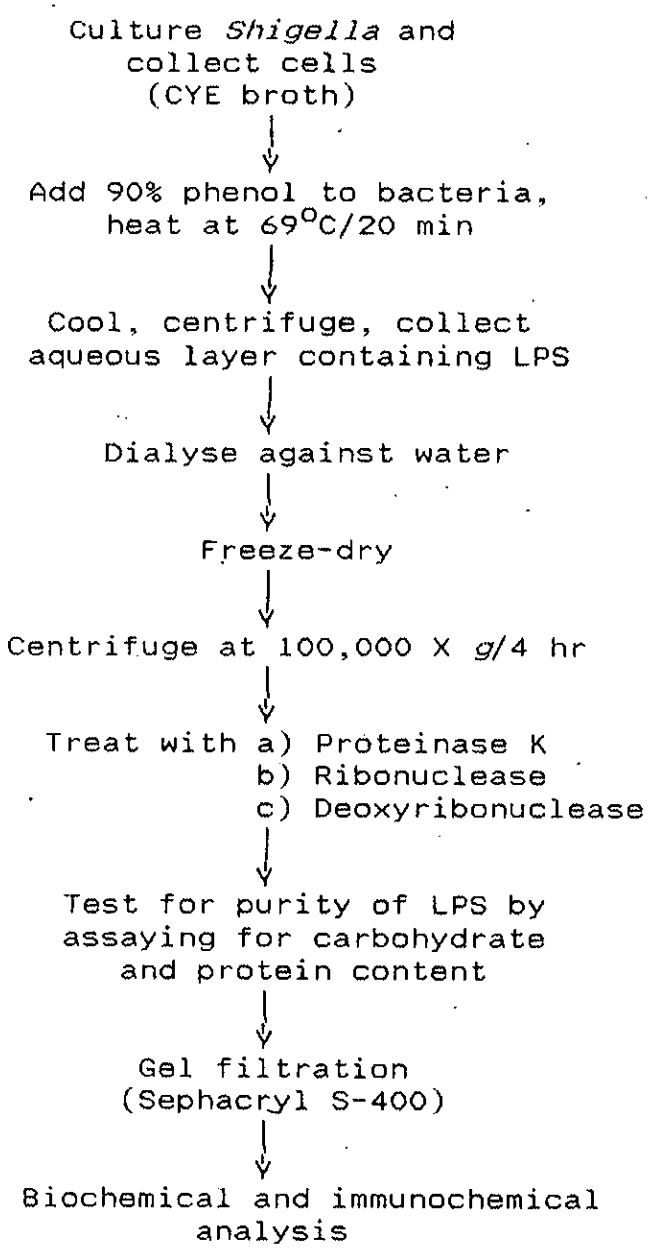
Table 3

Evaluation of immunodiagnostic assays
(immunomagnetic bead and latex particles)

Immunomagnetic bead	Latex particles
Specificity a) Use specific mab as well as mab produced against unrelated antigen b) Use no mab in assay c) Use samples from age-matched controls as well as patients	
Sensitivity a) Compare with conventional bacterial culture methods and other diagnostic procedures for detecting shigellae	
Accuracy and precise efficiency of test to detect both bacteria or released bacterial-LPS or protein	Efficiency of test to detect both live or non-culturable bacteria

FLOW CHART I

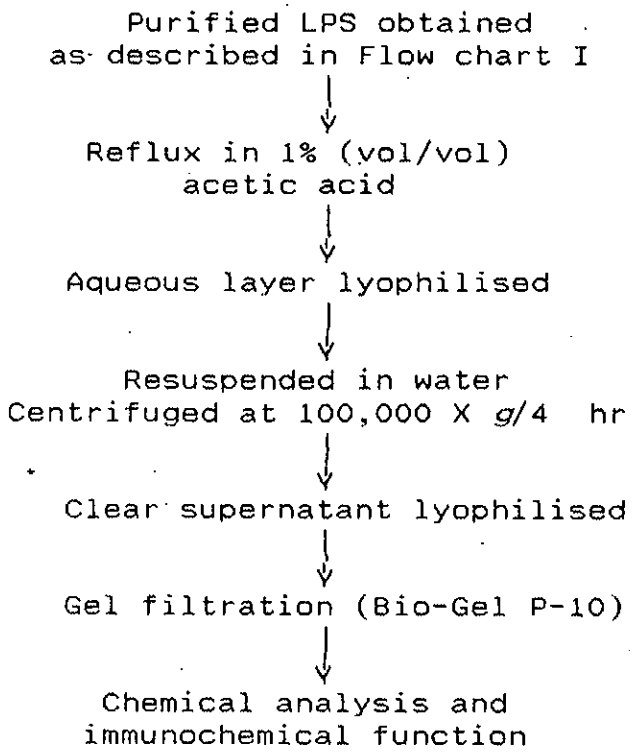
EXTRACTION AND PURIFICATION OF LPS



Reference: Westphal and Jann, 1965

FLOW CHART II

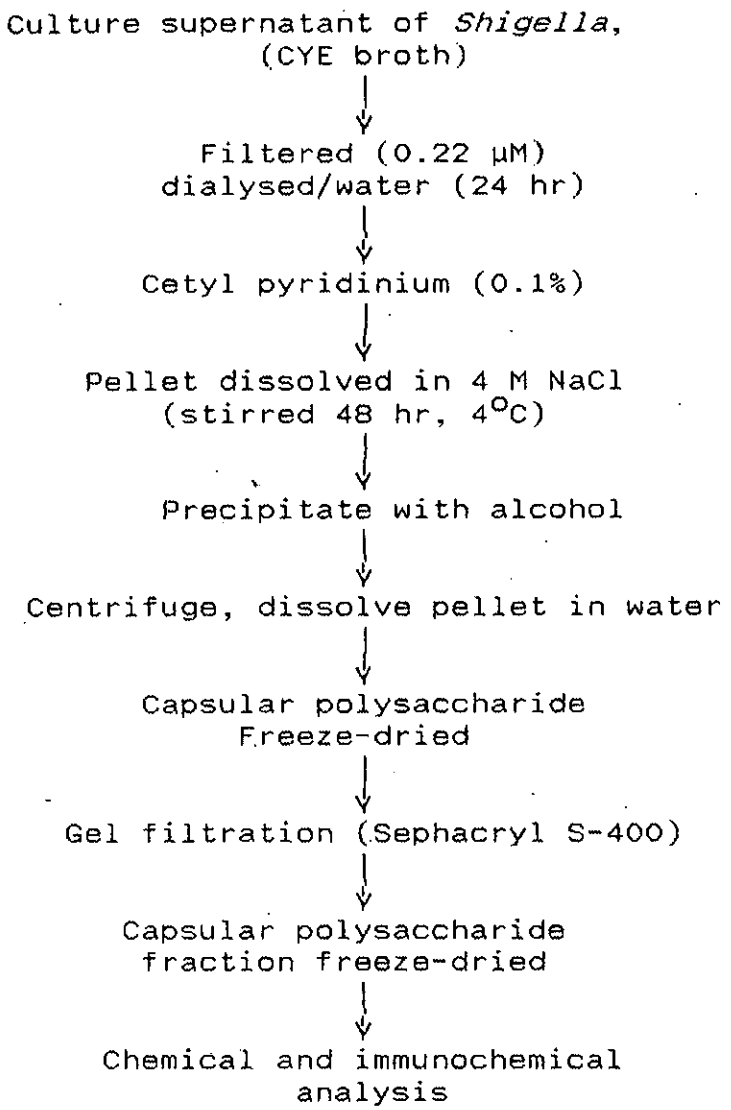
SEPARATION OF O-ANTIGENIC POLYSACCHARIDE FROM LPS



Reference: Molla *et al.*, 1986

FLOW CHART III

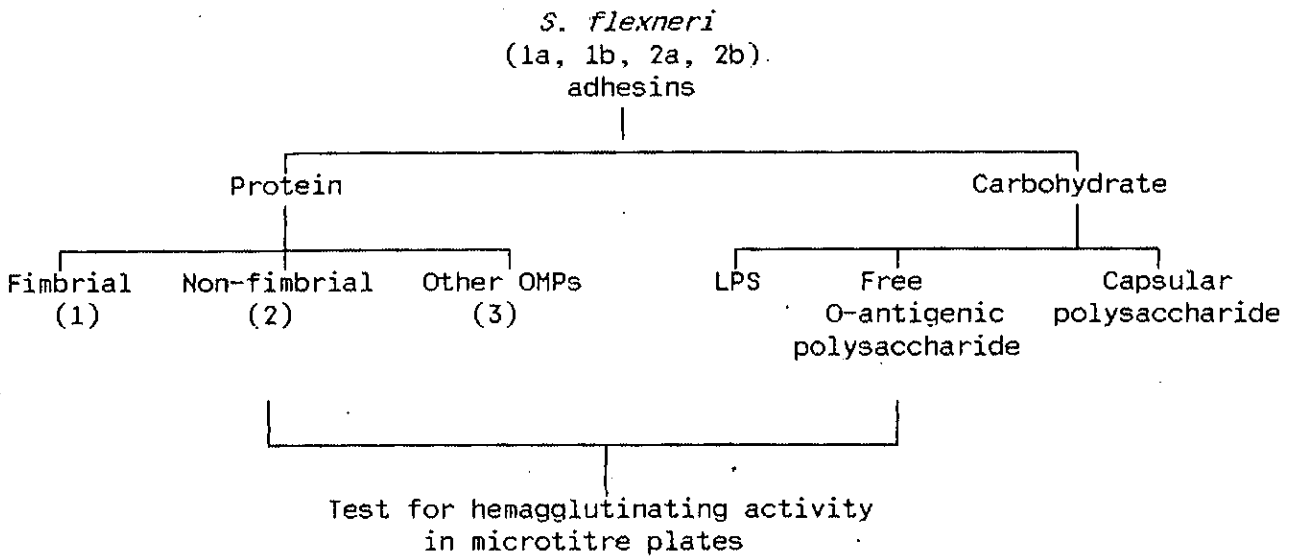
EXTRACTION AND PURIFICATION OF CAPSULAR POLYSACCHARIDES



Reference: Kato *et al.*, 1981

FLOW CHART IV

METHODS TO BE TESTED FOR EXTRACTION OF ADHESIN FROM STRAINS OF *S. flexneri*



References:

(1) Salit and Gotschlich, 1977

(2) Goldhar *et al.*, 1987

(3) a) Johnston and Gotschlich, 1974
b) Oaks *et al.*, 1985

ject title: Hemagglutination(HA) ability and adhesivenocc of Shigella species (Part 2) components including the use of monoclonal antibodies

ncipal Investigator(s): Dr. Firdausi Qadri et al.

mary of Referee's Opinions: Please use the following table to evaluate the ious aspects of the propocal by chooking the appropriate boxes. Your detailed ments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
ity of Project	X		
uacy of Project Design	X		
bility of Methodology	X		
hility within time period		H	
priatonocc of Budgot	X		
tial value to field of knowledge	X		

USIONS

port the application:

a) without qualification ☒

b) with qualification:
- on technical grounds ☐
- on level of financial support ☐

not support the application ☐

Referee: Dr. Arthur Donohue-Rolfe

on: Assistant Professor of Medicine

ation: New England Medical Center Hospitals - Tufts Univ. Sch. of Medicine

Arthur Donohue-Rolfe
ature

April 14, 1990
Date

DETAILED COMMENTS

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Adherence of a *Shigella* bacterium to colonic cells is certainly a necessary step in the invasion process. One would expect that interference with this process would protect the host from developing clinical illness. Despite the obvious importance of adherence, relatively little is known about the mechanism(s) involved in adherence of *Shigella* to colonocytes. An understanding of *Shigella* attachment mechanisms potentially will allow the development of effective therapeutic intervention protocols and vaccine candidates.

In light of the above, I consider the work outlined in the proposal of Dr. Qadri and her co-workers to be of tremendous potential value to the field. Considering the project's importance to developing vaccines and treatment regimens, it is an appropriate project for the ICDDR,B to undertake. Dr. Qadri's group has made significant contributions to the understanding of Shigella adherence. This proposal is a logical extension of what has already been done.

A particular strength of the project is that the antigens and monoclonal and polyclonal antibodies generated from the research will have a dual purpose. The antigens and antibodies will be used to study adhesion and, regardless of their importance to adherence, will also be used in the development of diagnostic procedures. These procedures may be of great value in understanding the role of the host immune response in the prevention of shigellosis and in the development of the severe complications of shigellosis.

I have always been somewhat concerned about growing *Shigella* strains in rich liquid medium and then studying a process such as adherence or invasion. The conditions in the gut are clearly dramatically different. The addition of certain antibiotics to *E. coli* strains which produce Shiga-like toxins result in a significant increase in toxin production. Thus, the cell stress experiments outlined in the proposal are of significance and may reveal the coordinate regulation of several virulence factors. Although the investigators propose to characterize the gene(s) for stress factors, realistically this is beyond the time frame of the proposal.

The adhesion studies using the antibodies should be interpreted with extreme caution by the investigators. Binding a relatively large molecule to the bacterial cell surface could easily inhibit adherence by steric hindrance. Any antibody which gives an adherence inhibitory effect should be retested using Fab fragments of the antibody, Clear proof of an adhesin must depend on a combination of biochemical, immunological, and genetic experiments.

~~Considering the amount of work proposed, the budget seems very reasonable. Overall this is an outstanding proposal by a very capable group.~~

Considering the amount of work proposed, the budget seems very reasonable. Overall this is an outstanding proposal by a very capable group.

ject title: Hemagglutination (HA) ability and adhesiveness of Shigella species (Part 2): characterization of the adhesin/hemagglutinin and other outer membrane components, including the use of monoclonal antibodies.

cipal Investigator(s): Drs. Tasnim Azim, S.M. Faruque, M.S. Islam, F. Qadir, Mr. G. Mondol

mary of Referee's Opinions: Please see the following table to evaluate the ous aspects of the proposal by checking the appropriate boxes. Your detailed ents are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
ity of Project	✓		
uacy of Project Design		✓	
ability of Methodology		✓	
ibility within time period		✓	
ppriateness of Budget	No comments		
ntial value to field of knowledge	✓		

LUSIONS

pport the application:

a) without qualification ☐

b) with qualification:
- on technical grounds ☑
- on level of financial support ☐

not support the application ☐

of Referee: Major General M R Choudhury

ion: Commandant

tution: AFIP&T, Dhaka Cantt

gnature

01/6/90
Date

DETAILED COMMENTS

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

I have gone through the protocol & I have the following comments to offer:

1. General

1.1. The protocol lists several interesting areas of investigations covering immunology, stress biology & hybridoma technology. It also proposes collaboration with Karolinska Institute to test some of the reagents.

1.2. It is indeed reassuring to note that the protocol reflects availability of certain useful expertise in LSD of ICDDR,B.

2. Specific

2.1. The title of this protocol is rather vague and does not indicate some of the important and wider areas of investigations mentioned in the corpus of the protocol.

2.2. In the 'Research Plan', extraction of LPS, O-antigenic polysaccharides and capsular polysaccharides has been mentioned but the procedures for their chemical analysis which the investigators plan to adopt have not been spelt out clearly.

2.3. Details of the method for extraction, purification & characterization of adhesins from S.flexneri should be mentioned. Similarly 'step-by-step' procedures which will be adopted in cell stress experiments with shigella species should have been described.

2.4. It will be clear from the protocol that most of the tests mentioned have already been carried out by other workers. There are also some new tests proposed by the investigators. Evidently there must be an efficient built-in mechanism in the proposed studies so that all the tests and the experiments that this protocol plans to develop must pass the critical evaluations for 'specificity', 'sensitivity', 'accuracy' and 'precision'. An out-line of such a plan should be incorporated in the protocol.

2.5. It will definitely be interesting and useful to develop specific monoclonals against the selected components of LPS & OMP.

2.6. With regard to purification and characterization of monoclonal antibodies ref should have been made to the work of Carlin et al (1989) on the use of monoclonal antibodies to type of Shigella flexneri in Bangladesh.

REF: Carlin NIA et al, 1989 : Use of monoclonal antibodies to type Shigella flexneri in Bangladesh, J Clin Microbiol 27 No. 6; 1163-1166

roject title: Hamagglutination (HA) ability and adhesiveness of Shigella species (Part 2): Characterization of the adhesin/hemagglutinin and other outer membrane components, including the use of monoclonal antibodies.

rincipal Investigator(s): Dr: Firdausi Qadri et al.

ummary of Referee's Opinions: Please see the following table to evaluate the arious aspects of the proposal by checking the appropriate boxes. Your detailed omments are sought on a separate, attached page.

	Rank Score			
	High	Medium	Low	
uality of Project		✓ →	←	Not Enough Detail To Judge.
dequacy of Project Design			✓ Poor	
uitability of Methodology		✓		
easibility within time period			Poor ✓	Trying To Do Too Much!
ppropriateness of Budget		✓		
otential value to field of knowledge	✓			

ONCLUSIONS

support the application:

a) without qualification ☐

b) with qualification:
- on technical grounds ☑
- on level of financial support ☑

do not support the application ☐

me of Referee: DR. DENNIS J. KOPECKO

sition: ASST. PROF., DEPT. BACTERIAL IMMUNOLOGY

stitution: WALTER REED ARMY INSTITUTE OF RESEARCH, WASH, DC

D. J. Kopecko
Signature

May 13, 1990
Date

DETAILED COMMENTS

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

The investigators have chosen some very important questions to address, the answers to which may have great significance to the field of *Shigella* research. However, the general aims seem overly ambitious. The investigators would be better off choosing one of the three major objectives and focusing on that one for 3 years. The immunodiagnostic and seroepidemiologic approach is very worthwhile. The hemagglutinin/adhesion approach is fraught with serious problems. Does human erythrocyte agglutination have anything to do with adhesion to colonic epithelial cells? Many M-Ags or surface proteins may sterically interfere with adhesion to HeLa cells but [redacted] the proteins may not actively be involved in adhesion. How will the investigators separate the real from artificial phenomena? It is clear that growth under "stress" conditions causes the synthesis of new proteins (see Nature — : , 1990) but how will the investigators show the specific virulence functions are induced? Why characterize the adhesin from 4 different serotypes of *S. flexneri*? Do one strain first and if time allows, look at one other.

In summary, this ~~is~~ proposal is aimed at some interesting questions, but ~~the~~ far too many approaches are planned to be successful and not enough thought has gone ~~in~~ into the design of the experiments. I would have to give this a low priority on their basis.

Protol No. 90-010

Title: Hemagglutination (HA) ability and adhesiveness of *Shigella* species (Part 2): Characterization of the adhesin/hemagglutinin and other outer membrane components, including the use of monoclonal antibodies (mabs)

P.I.: Dr. F. Qadri *et al.*

Clarification on comments made by reviewers:

Changes have been made in the protocol based on comments made by the reviewers.

Points raised by Dr. Arthur Dononue-Rolfe, Tufts University, School of Medicine

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Non-specific effects of mabs in neutralizing adherence will be detected using methods described above. The reviewer's comment that characterization of genes for stress factors may require more time and is beyond the time-frame of the present protocol has been accepted. We have decided to omit these experiments for the time being and develop it later on.

Points raised by Major General M.R. Chowdhury, Armed Forces Institute of Pathology and Transfusion, Dhaka, Bangladesh

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2.1. The title now better reflects the nature of the investigations that will be carried out since the section on "cell stress" has been omitted.

2.2. A more detailed plan on the extraction of LPS, O-antigenic polysaccharide and capsular polysaccharides has now been incorporated in the protocol (Flow Chart I, II and III).

2.3. Different methods will be used to extract adhesins from *S. flexneri*. Since either polysaccharide or protein antigens may be involved in adhesin, a number of procedures may have to be tested. The different methods that may need to be used are now shown in Flow Chart IV.

2.4. The immunodiagnostic tests will be critically evaluated for specificity, sensitivity, accuracy and preciseness. These points are mentioned in the protocol on development of immunodiagnostic assay (Prtocol No. 90-004) and are included in Table 3 in the present protocol.

2.5. Reference has been made to Carlin *et al.*, 1989 (Page 6) in the protocol.

Points raised by Dr. Dennis Kopecko

1. The point has been raised as to whether *in vitro* hemagglutination can be related to adherence of *Shigella* to colonic epithelial cells. Binding of bacteria to erythrocytes resulting in their agglutination has been successfully used as a simple assay to understand the ability of pathogenic bacterial strains to adhere for the last 30 years or so. In studies carried out by us over the last two years, we have been able to show that all strains of *S. dysenteriae* type 1 tested (Qadri *et al.*, 1989, see list of references) and different serotypes of *S. flexneri* agglutinate erythrocytes. We have extended this study further and shown that strains of shigellae also adhere to cultured intestinal epithelial cells before invading them. Hence, adherence ability of strains of shigellae is based on data obtained from hemagglutination assays as well as binding to human cultured intestinal cells.

2. The reviewer has cautioned that mabs may inhibit adhesion due to nonspecific steric effects. To rule out nonspecific effects of monoclonal antibodies in neutralizing adherence different approaches will be used which include:

   a) Control mabs of same isotype and of unrelated specificity will always be used in all studies.

b) If mabs are found to inhibit adherence $F(ab)_2$ and Fab fragments will be prepared in order to ensure that inhibition is not due to steric hindrance (which is usually caused by the Fc fragment). These points have been clarified in the protocol (page 5).

c) The rationale behind studying four different serotypes of *S. flexneri* (1a, 1b, 2a, 2b) is that all of them have been shown to have HA ability. In *S. dysenteriae* type 1, we have found that LPS is the adhesin. It is possible that either LPS or protein may be responsible for adherence in the different serotypes of *S. flexneri*. It would actually be simple to study only one, however, that will leave the study incomplete.

3. Based on the comments raised by the reviewers that the work involved is too diverse and is beyond the time-frame. We have decided to focus the work on the hemagglutination and adhesion of shigellae and monoclonal antibodies and omit the part on cell stress experiments.

We wish to point out that each of the investigators will be involved with different aspects of the work. Ms. D. Islam will develop immunodiagnostic assays based on mabs. Dr. T. Azim will mainly deal with supervision of the mab, laboratory, as well as preparation of new mabs against adhesin and other surface components of shigellae. Dr. S. Islam will develop mab as well as characterize and use

previously prepared mabs (Table 2) in diagnostic assays. The biochemical and immunochemical characterization of hemagglutinin, adhesin and other components will be carried out by Dr. F. Qadri. Mr. G. Mondol and Mr. Abu Hossain will give technical support to the work.

FQ:mh/86:CLARIF