Attachment 1.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 25/09/88

Principal Investigator Dr. S. TZIPORI

Application No. 88-026

Title of Study Hyperimmune Bovine Colostrum (HBC): Evaluation of its therapeutic value in diarrhoeal disease

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

Project status:
(x) New Study
( ) Continuation with change
( ) 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:
(a) Ill subjects Yes (No)
(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)

Does the study involve:
(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)

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

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 N/A

6. Will precautions be taken to protect anonymity of subjects Yes No N/A

7. Check documents being submitted herewith to Committee:
___ 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.

No human subject is involved.

(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.

S. TZIPORI
Principal Investigator

Trainee

88-026
25/9/88

SECTION-I RESEARCH PROPOSAL

TITLE	:	Hyperimmune Bovine Colostrum (HBC): Evaluation of its therapeutic value in diarrhoeal disease
PRINCIPAL INVESTIGATORS	:	Dr. S. Tzipori Dr. D. Mahalanabis Prof. R. Eeckels
COINVESTIGATORS	:	
STARTING DATE	:	January 1989
COMPLETION DATE	:	December 1989
TOTAL PROJECT COST	:	$ 47,164
SCIENTIFIC PROGRAM HEAD	:	Dr. S. Tzipori

This protocol has been approved by the Laboratory Sciences Division.

Signature of the Scientific Program Head: S. Tzipori

Date : 25/9/88

ABSTRACT SUMMARY

Preliminary studies have indicated that HBC may be effective in the treatment of several specific enteric infections. The purpose of this study is to produce HBC against specific enteric infections and test its effectiveness in patients infected with the corresponding pathogen. Initially, HBC will be produced against *Shigella dysenteriae*, *S. flexneri*, rotavirus and *Giardia lamblia*. The therapeutic value of the HBC will then be tested under clinical conditions using bovine colostrum from unimmunized cows as control. The gammaglobulin will be extracted from the colonstrum and will be given orally over 2-4 consecutive days.

The HBC will be prepared by vaccinating pregnant cows with the relevant purified antigen mixed with adjuvant. The colostrum will be collected immediately after calving over two days, analyzed in terms of bacterial counts, etc., and amount of specific anbitody. This portion of the work will be carried out in Australia, where the procedure has been developed and is in place. The actual application of the treatment to patients at the Clinical Research Centre, with the appropriate clearance from ERC, will be submitted under a separate protocol.

REVIEWERS:

a) Ethical Review Committee _____

b) Research Review Committee _____

c) Director _____

SECTION-II RESEARCH PLAN

A. INTRODUCTION

1. Objectives

a) To establish whether antibody from cows can be tailored to act as specific antimicrobial agents for enteric infections against which there is no treatment.

b) To develop this theme to produce a multivalent "drug" which will be: (i) cheap (colostrum is not normally used for human consumption), (ii) nutritious, nontoxic and without side-effects, (iii) no problems of drug resistance by bacteria, and (iv) can be made effective against persistent diarrhoea when more information is generated regarding aetiology.

c) Investigate optimal vehicles for delivery when objectives a and b have been achieved; for instance, freeze-dried, purified Ig given in enteric-coated capsules. Such capsules can have activity against a single or multiple infections.

This protocol deals specifically with objective a; objectives b and c will be considered after the outcome of the first one has been fully evaluated.

2. Background

Results from numerous studies in animals and humans have shown that the presence of specific antibody in the lumen of the

intestine is of major importance for protection against infections of the gastrointestinal tract. In contrast, antibodies in the serum although may modify an infection, do not prevent or protect against diarrhoea. The use of vovine colostral antibody prophylactically for treatment of enteric infections in humans is a new concept. HBC was tested successfully as an antimicrobial agent on human volunteers challenged with enterotoxigenic *E. coli* (ETEC) with a view to providing prophylaxis for travellers diarrhoea (1). It was also shown that it can be an effective treatment against persistent diarrhoea due to cryptosporidiosis in immune deficient individuals (2,3).

The ue of HBC is an attractive option in the management of enteric infections against which there is no treatment, e.g. viruses. There are, however, other added advantages in using HBC in the management of diarrhoea; there are no risks of serious side-effects often associated with the use of drugs--although it may cause gastrointestinal symptoms of hypersensitivity--and will overcome the problem of development of drug resistance. This is particularly relevant to shigellosis in which the only effective treatment is antimicrobial agents. If it can be shown to be effective against individual infections (rotavirus, Campylobacter, enteroadherent *E. coli*, *Giardia*, *Cryptosporidium*, *Shigella* spp. and possibly others), a "cocktail," made of HBC produced against several of the above, could prove to be an effective treatment against some or most cases of persistent

diarrhoea in children. Colostrum is available in large quantities (25-30 liters per cow) in high milk producer countries. Colosltrum is not used for human consumption and can be made available for this purpose fairly cheaply once a system has been established. The HBC can be used for treatment either as whole-milk which has an added nutritional benefit, or as separated and freeze-dried Ig, given as enteric-coated capsules, or as whey. The amount of milk and the length and frequency of treatment will be determined under clinical conditions.

The major immunoglobulin isotype of bovine colonstrum is IgG1, followed by IgG2, IgA and IgM. Unlike monogastric mammals in which IgA plays the major role, in rumminants IgG1 provides the principle lactogenic immunity to the newborn which, like IgA, is somewhat resistant to acid and to proteolytic enzymes.

3. Method

Production of HBC

Ten to twelve weeks prior to parturition, pregnant cows in nominated dairy herds in Australia, will be vaccinated with antigens prepared from enteric pathogens. Six cows will be used for each of the 4 pathogens which include rotavirus, 2 *Shigella* spp. and *Giardia* in the first year. Six cows will be used to obtain normal milk for control. Later, when more information becomes available with regard to persistent diarrhoea, other antigens, for instance enteroadherent *E. coli*, will also be included to vaccinate cows. It is

thought that HBC can be used in enteric infections against which there is no treatment (e.g. viruses) or the treatment may become ineffective because of development of antimicrobial resistance.

a) Production of HBC against rotarivus: Pregnant cows will be vaccinated with each of the 4 cell culture adapted human rotavirus serotypes. They are now available at the Centre and cell culture facilities will be used to produce high titered rotaviruses. Viral antigen (titre 10^8) will be mixed with equal volume of Fruend's Incomplete Adjuvant (total volume 8 ml) which will be given parenterally followed two weeks later by intramammary infusion of the same preparation via the teat canal. A third injection will be given two weeks later (4-6 weeks prior to calving) in the same site as the first injection. After calving the colostrum will be collected during the first 2 days which should yield 15-20 liters of antibody-rich colonstrum per cow. The half-life of bovine gamma globulin is short and although secretion of specific antibody in the colostrum and in the milk will continue for sometime, its level may be too low for therapeutic purposes. The therapeutic half-life will need to be determined later and will vary according to the antigen used. Initially, we plan to collect the colostrum produced during the first two days only. The milk will

be frozen at -20°C in 5 liter containers. Representative samples for testing sterility and amount of specificity of Ig will be taken before freezing each container. The ingredients present in bovine colostrum are included in Appendix A. Appendix B illustrates levels of specific antibody in HBC produced against cryptosporidium sporozoites, the infective stage of the parasite. The milk will be shipped in bulk to Dhaka. Thirty cows derived from 5 herds will be involved during 1989 and trained part-time technician is available in Australia to carry out the activities required to produce the HBC. The antigen will be produced at the Centre, but the amount and specificity of Ig will be done at the Royal Children's Hospital in Melbourne, to make sure that only products with high titres will be shipped.

b) Production of HBC against *Shigella*: A mixture of heat-killed (60°C over 30 minutes) and formalin-killed (0.2%) of 10^{11} organisms (dysenteriae or flexneri) will be mixed in equal volume with adjuvant (to5tal of 8 ml) which will be administered to cows as described for rotaivrus. The procedure for inactivation was the same as that carried out for the cholera vaccine (4).

c) Production of HBC against *Giardia*: Trophozoites grown in culture (10^8) will similarly be mixed with adjuvant (8 ml) and administered to pregnant cows.

ELISAs to test amount and determine the specifities of the corresponding antibody are available in Melbourne and will be carried out by the part-time technician.

There are two calving seasons in Australia, mid-April and in September. In January the PI will travel to Melbourne to set-up the first vaccination schedule which will include 15 cows to be vaccinated. The first study will include production of HBC against the above-planned 4 pathogens. The second calving will be a repeat of the first, unless the earlier studies suggest otherwise.

d) Clinical studies: Four groups of patients, each with proven infections with either rotavirus, *Giardia*, *S. dysenteriae*, or *S. flexneri*, respectively, will be enrolled in the study. Each group will be treated with the corresponding HBC, and a few in each group will act as conrol and will be given non-immune colostrum. Patients will be treated 3 times a day with 400 ml over two days. They will be observed during this period. A detailed protocol outlining the clinical study will be submitted separately by the three PIs.

REFERENCES

1. Tacket *et al.* 1988. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. New Engl. J. Med.; 318:1240-1243.

2. Tzipori *et al.* 1986. Remission of diarrhoea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. Br. Med. J.; 293:1276-1277.

3. Tzipori *et al.* 1987. Chronic cryptosporidial diarrhoea and hyperimmune cow colostrum. Lancet; ii:344-345.

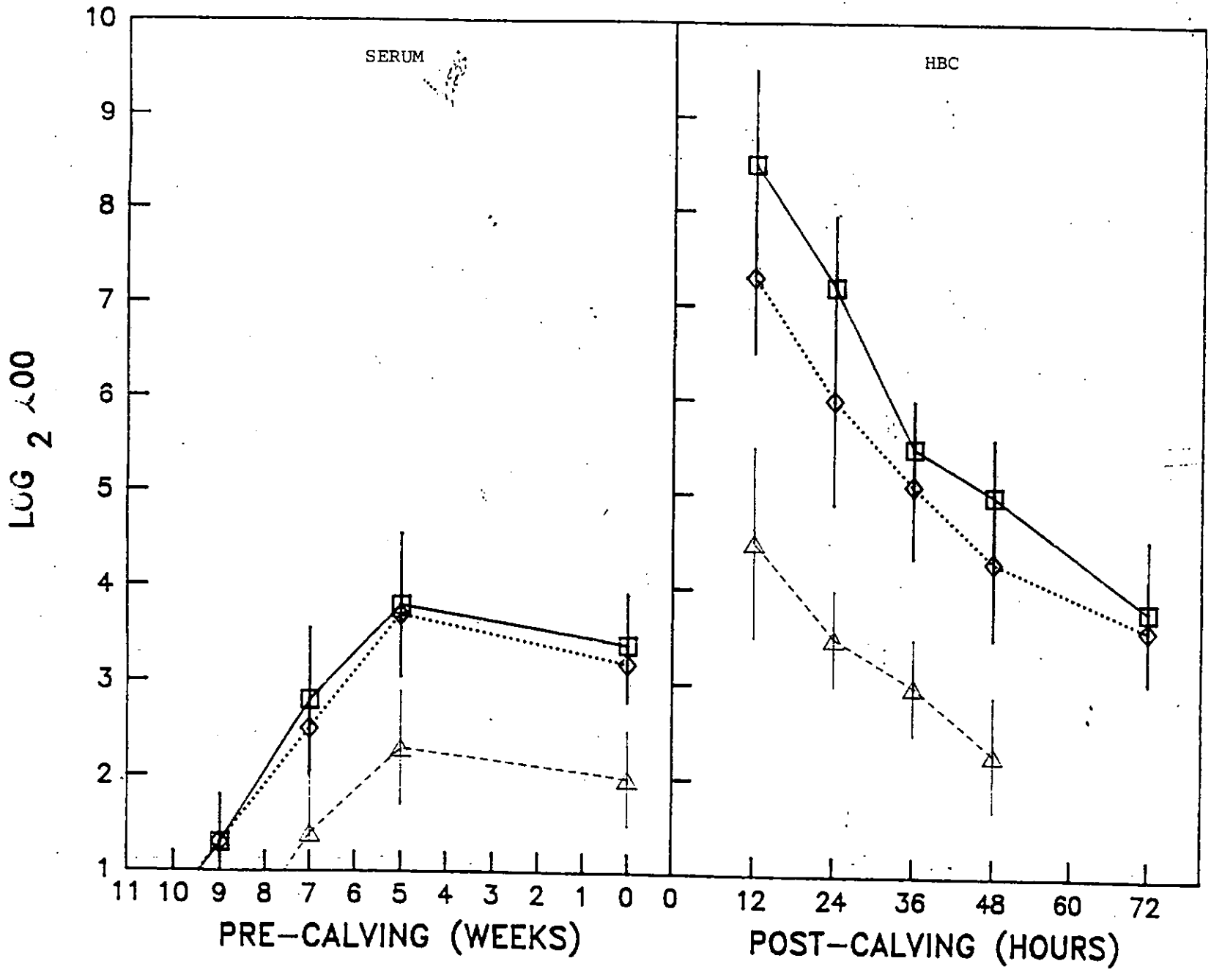
4. Clements *et al.* 1987. Subunit-whole cell and whole cell oral vaccines against cholera; studies on reactogenicity and immunogenicity. J. Infect. Dis.; 155:79-85.

APPENDIX-A

Composition of the Colostrum

Constituents	Colostrum 0 hr	Colostrum 12 hr	Colostrum 24 hr	Normal milk
Total solids(%)	24.75	20.71	17.09	12.86
Lactose(%)	3.10	3.10	3.10	4.60
Ash(%)	1.12	1.04	0.96	0.72
Choline (PPM)	370	320	690	130
Protein(%)	11.35	9.60	7.07	3.25
Casein(%)	5.2	5.31	5.2	3.1
Albumin(%)	1.5	1.2	1.0	1.5
Immunoglobulin (mg/ml)	38.23	32.22	21.52	-

Antibody response to vaccination of cows with Cryptosporidium antigen



ICDDR,B
BUDGET PROPOSAL
(In US $)

PARTICULARS

Program name: LABORATORY SCIENCES DIVISION Protocol title: Hyperimmune Bovine Colostrum (HBC): Evaluation of its therapeutic value in diarrhoeal disease

P. I's name: Dr. S. Tzipori

Protocol no: Starting date: January 1889

Budget code: Completion date: December 1989

EXPENSE CATEGORY A/C No.	Description	Refer Page	Column A 1st year Jan.-Dec.	Column B 2nd year Jan.-Dec.	Column C 3rd year Jan.-Dec.	Column D Total Project Cost
3100	Local Salaries	2				
3200	Intl. Salaries	8				
3300	Consultants	14				14,200
3500	Travel Local	15				
3600	Travel Intl.	16				5,000
3700	Supplies & Mat.	18				9,200
3800	Other Costs	19				
4800	Inter Deptl. Ser.	20				6,000
	Total Direct cost					34,400
0000	Indirect cost = 31% of total direct cost					10,964
	TOTAL OPERATING COST					45,364
0300	Capital expenditure Refer page no. 21					1,800
	TOTAL PROJECT COST					47,164

S. Tzipori
P.I.'s signature

Reviewed by Budget & Finance

Budget Code: _____

CONSULTANTS 1988
(Local and International)

Name & Job Title	Travel From-To-From	No. of days	** Perdiem Rate	** Perdiem Total	***Honorarium Rate	***Honorarium Total	**** Travel cost	Total $ Amount
Part-time Technician (in Australia)	-			-		13,000	1,200	14,200
							TOTAL	*14,200

** Check with Finance Ext. 214 for perdiem rate.
*** Check with Personnel Tel. 601100 for honorarium rate.
**** Check with Travel Tel. 602836 for ticket cost. Travel cost includes transit perdiem, ticket cost and ground transportation.

*AGREES WITH PAGE 1 A/C 3300 COLUMN 2

Budget87.14

Budget Code: _____

TRAVEL PLAN (INTERNATIONAL)-1988

Name & Job Title	Travel From-To-From	Estim. days of travel (A)	** Perdiem Rate (B)	** Perdiem Amount (C=AxB)	*** Transportation Air (D)	*** Transportation Ground (E)	**** Other Cost (F)	Total $ Amount (G= C+D+E+F)
Dr. S. Tzipori P.I.	Dhaka-Melbourne-Dhaka							
Dr. S. Tzipori, PI	Dhaka-Melbourne-Dhaka							2,500
								2,500

TOTAL * 5,000

** Check with Finance Ext. 214 for perdiem rate.
*** Check with Travel Tel. 602836 for transportation cost.
**** Includes excess baggage, embarkation fee, travel tax etc.

*AGREES WITH PAGE. 1 A/C 3600 COLUMN C

Budget Code: _____

SUPPLIES AND MATERIALS-1988

A/C Code	Item Description	$ Amount
3701	Drugs (used for medication in the hospitals and field stations)	
3702	Glassware (Bottle, beaker, cylinder, petridish, aluminium seal, slides, stopper, tube etc.)	
3703	Hospital supplies (bandage, gauze, blade, bowl, catheter, cotton, needle, syringe, solution, leukoplast, towel etc.)	
3704	Stationery and office supplies (Battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)	
3705	Chemicals and media (Acid, reagent, dextrose, sodium, bactoagar etc.)	1,750
3706	Materials for uniform (Cloth, button etc. required for making uniforms)	
3707	Fuel, oil and lubricants (Diesel, mobil, petrol, kerosene etc.)	
3708	Laboratory supplies (Aluminium foil, bag, blade, brush, cap, container, film X-Ray etc.)	
3709	Housekeeping supplies (Aerosol, battery, wiping cloth, duster, lock and key etc.)	
3710	Janitorial supplies (Bleaching powder, brush, detol, detergent, insecticide, soap etc.)	
	Page total (balance c/f)	1,750

(Contd. to page No. 18)

Budget Code: _____

SUPPLIES AND MATERIALS-1988

(Contd. from Page No. 17)

A/C Code	Item Description	$ Amount
	Page total from page No.17 (balance b/f)	1,750
3711	Tools and spares (Automobile spares, tyres, tubes, battery, stores required for maintenance services etc.)	
3712	Non-stock supplies (Materials not normally kept in stock and purchased only against specific requisitions)	4,690
	Sub-Total	6,440
3713	Freight and other charges Add 30% to above sub-total for imports.	2,760
	TOTAL	* 9,200

*AGREES WITH PAGE 1 A/C 3700 COLUMN C

Note: For rates please contact Supply Ext.260.
Add 10% for inflation

Budget Code: _____

**INTERDEPARTMENTAL SERVICES-1988

A/C Code	Service Area	$ Amount
4801	Computer	
4802	Transport Dhaka	
4803	Transport Matlab	
4804	Water transport-Matlab	
4805	Transport Teknaf	
4806	Xerox and mimeograph	
4807	Pathology	
4808	Microbiology tests	
4809	Biochemistry	
4810	X-Ray	
4811	I.V. fluid	
4812	Medis	
4813	Patient hospitalisation study	
4814	Animal research (in Australia)	6,000
4815	Medical illustration	
4817	Telex	
4818	Out patient care	
4819	Maintenance charges	
4820	Vehicle maintenance charges	
4821	Library service charges	
4822	Staff Clinic Charges - Dhaka	
	Page total (balance c/f)	6,000

(Contd. to page No. 21)

Budget Code: _____

**INTERDEPARTMENTAL SERVICES-1988

(Contd. from Page No. 20)

A/C Code	Service Area	$ Amount
	Page total from page # 20 (balance b/f)	6,000
4823	Staff Clinic Charges - Matlab	
4824	Bacteriology Test	
4830	Transport Subsidy	
	TOTAL	* 6,000

*AGREES WITH PAGE 1 A/C 4800 COLUMN C

** See Annexure-B for rates.

Budget Code: _____

CAPITAL EXPENDITURE-1988

Item description	Country of manufacture	No. of units	Cost+freight $ amount **
Freezers			1,800
	TOTAL	*	1,800

** For cost & freight please contact Supply (Ext.260).

*AGREES WITH PAGE 1 A/C 0300 COLUMN C