Enteric fever and Diagnostic Challenges

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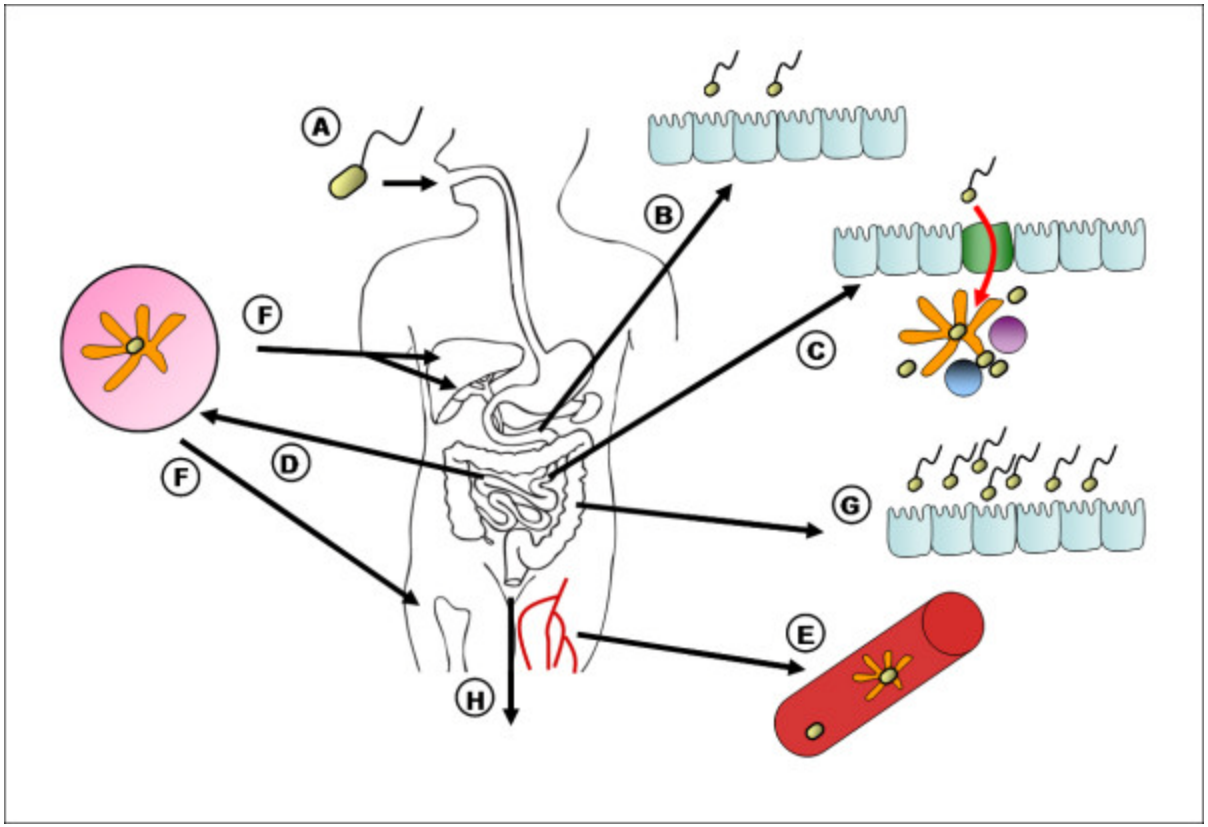
Diagnosis of enteric fever

- Undiagnosed and maltreated cases of enteric fever may result in severe complications and increased morbidity
- The development of a reliable and rapid diagnostic methods for enteric fever is urgently needed



The lifestyle of *Salmonella* Typhi in the human host and implications for diagnostics

http://www.biomedcentral.com/1471-2334/10/45/figure/F2

Baker *et al. BMC Infectious Diseases* 2010

Diagnostics tests available for enteric fever

Bone marrow-

Culture- this invasive procedure is not routinely used

Blood-

Culture: sensitivity 20-70%,takes 3-7 days

PCR: non-specific amplification and contamination

Serum/ Plasma-

Widal test: less specificity in endemic areas

Rapid Immunological tests:

Tubex - 60% sensitive and 58% specific

Typhidot- 67% sensitive and 54% specific

Stool-

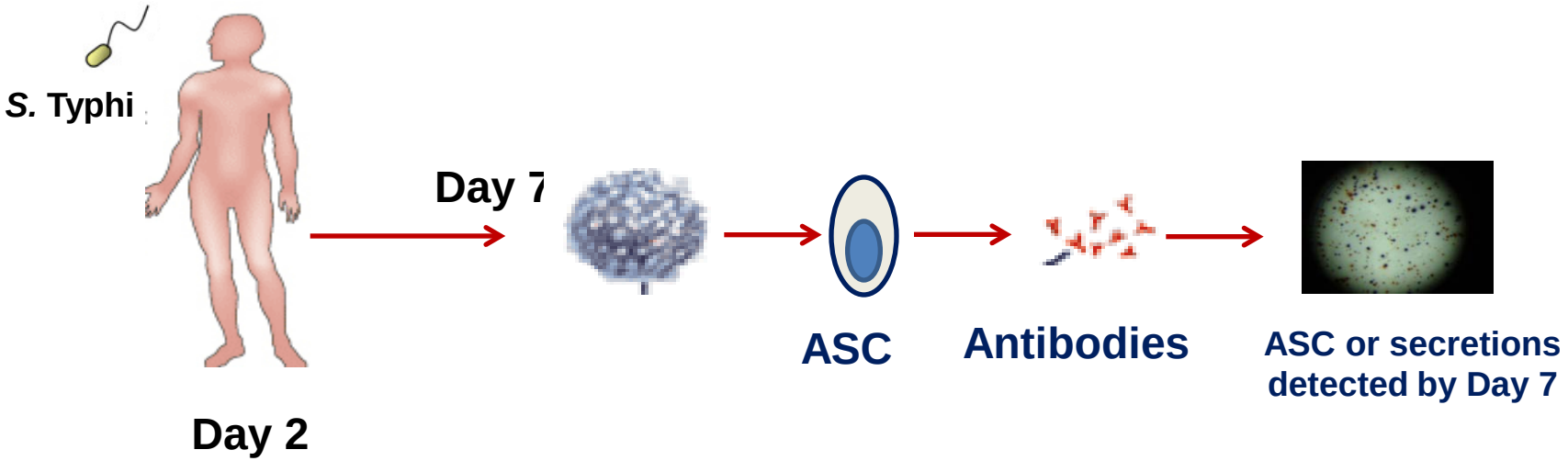
Poor sensitivity at the acute phase of disease

Urine-

Culture- Sensitivity very low

PCR- report of flagellin gene (*fliC)* for diagnosis of *S.* Typhi

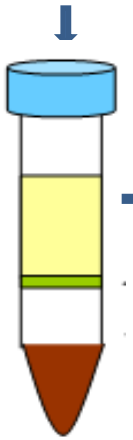
Mucosal response and surrogate markers of protective immunity



- Activated mucosal lymphocytes migrate from intestinal tissue and circulate within peripheral blood before re-homing to mucosal tissues
- This migration peaks around 5-7 days after intestinal infection
- The immune response can be measured in peripheral blood mononuclear cells (PBMC) in lymphocyte secretions and responses measured using enzyme immunoassays

Activated mucosal lymphocytes migrate from intestine to the circulation and best depicts a mucosal response and a RECENT exposure

Blood
(1 ml)

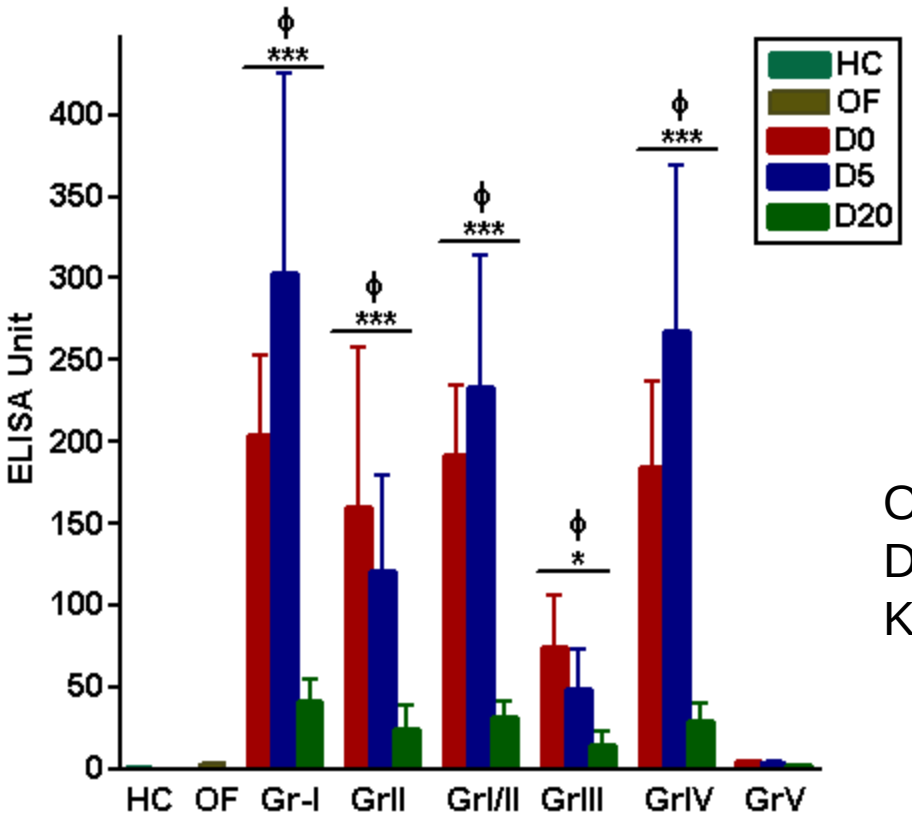


Differential centrifugation

Incubation of cells at 37^0 C for 18-48 hours

Kinetic ELISA readout
>10 ELISA Unit is taken as positive

S. Typhi specific IgA antibody responses in secretions of lymphocytes



Other febrile illnesses
Dengue, Leptospirosis
Kala azar, TB, others

Patients with febrile illnesses

Gr-I, blood culture positive;

GrII, fourfold change in Widal

GrIII, Widal titer of 320

GrIV, negative culture and Widal but an anti-serovar Typhi IgA titer of >10 ELISA units in the assay

GrV, all assays negative; OF, other febrile illness; HC, healthy controls

Sheikh et al. 2009

This method is also useful for diagnosis of patients with *S.* Paratyphi infections

TPTest (Typhoid and Paratyphoid test)

TPTest results in study participants

Characteristics	No. of individuals	TPTest	
		Positive	Negative
Patients with *S.* Typhi bacteremia	27	27	0
Patients with *S.* Paratyphi A bacteremia	12	12	0
Clinically suspected enteric fever but blood culture negative	204	44	160

The TPTest was also tested in patients with confirmed illness other than enteric fever
The sensitivity and specificity of the TPTest are 100% and 91% respectively

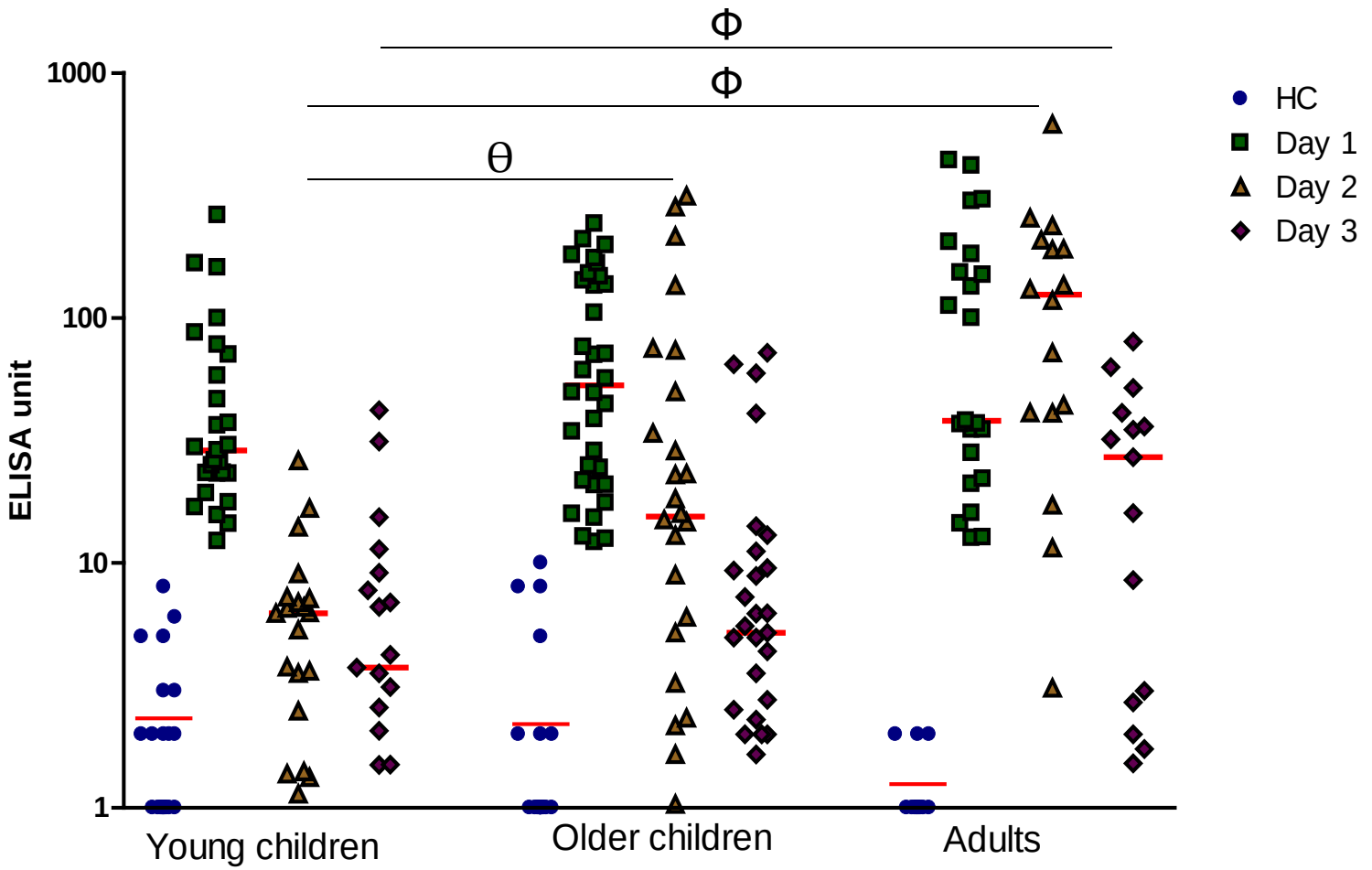
Patients with suspected enteric fever have been recruited from different field areas:

- Kamalapur field site
- icddr,b hospital
- Mirpur field site

Based on the reproducibility of the test, the TPTest was introduced at the Clinical Diagnostic Services of the icddr,b from May 2012. This was based on results of over 1000 specimens tested in febrile patients and healthy controls.

Utilizing the TPTest we can detect 60% of cases within 14-18 hours of receiving a specimen and the remainging at around 48 h of culture of cells

TPTest results in different age groups of typhoid patients



No difference was found at the acute stage of disease among young children, older children and adults showing the utility of the TPTest in all age groups

The method can be used even in young children for detection of enteric fever using 1 ml of blood only

Technology Transfer to the Microbiology Department of the Dhaka Medical College and Hospital (DMCH)

From the Outpatient Department of DMCH

- 152 suspected enteric fever patients enrolled

- *S*. Typhi and *S*. paratyphi A isolated from 22 and 7 patients respectively
  -all were positive by TPTest

-Additional 38 patients were detected by TPTest in patients negative by culture

Simplification of the TPTest for use in laboratories lacking facilities

Our existing method uses the following:

Density gradient centrifugation on ficoll-hypaque for separation of peripheral blood lymphocytes

37°C incubator with a constant 5% CO_2 supply

ELISA reader for readouts

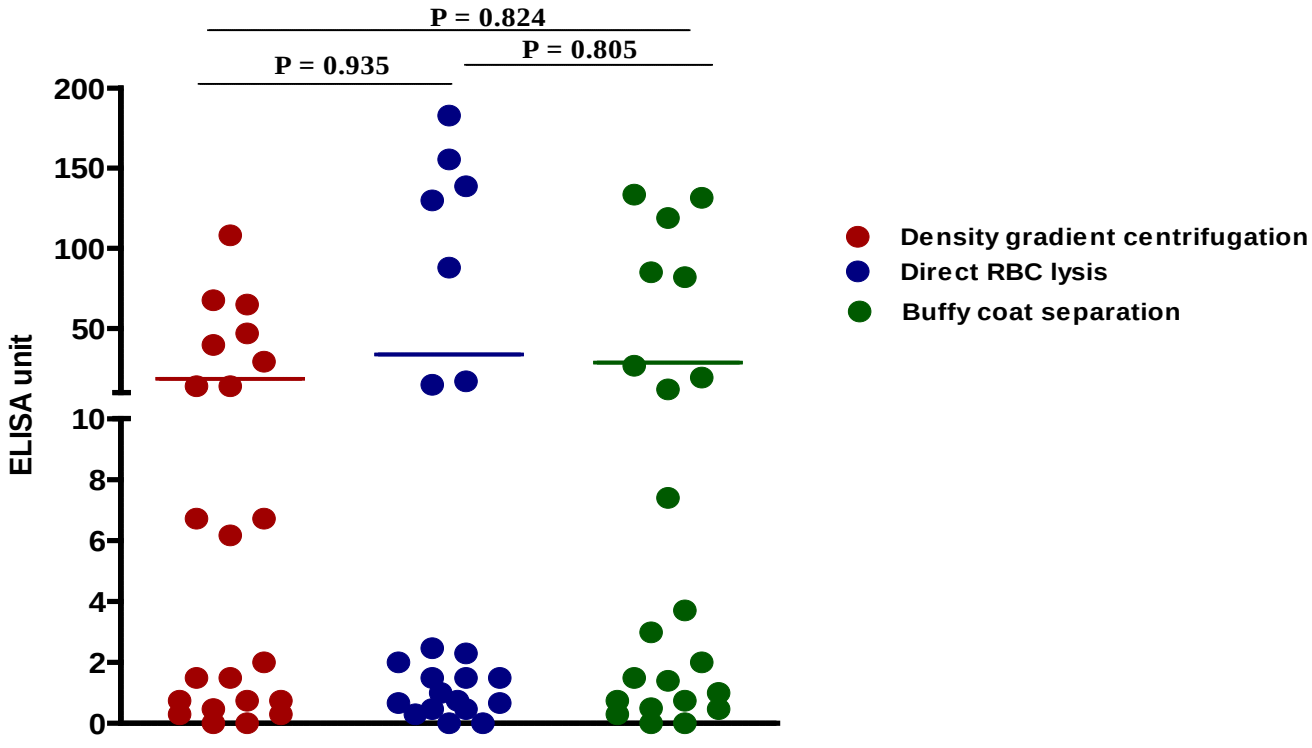
We are working on procedures to make the TPTest simpler

Simple cell separation procedure i.e. buffy coat preparation or RBC lysis

Incubation of cells at 37°C without CO_2 incubation

Immunodot blot assay for detection of the antibody response in cell culture secretions

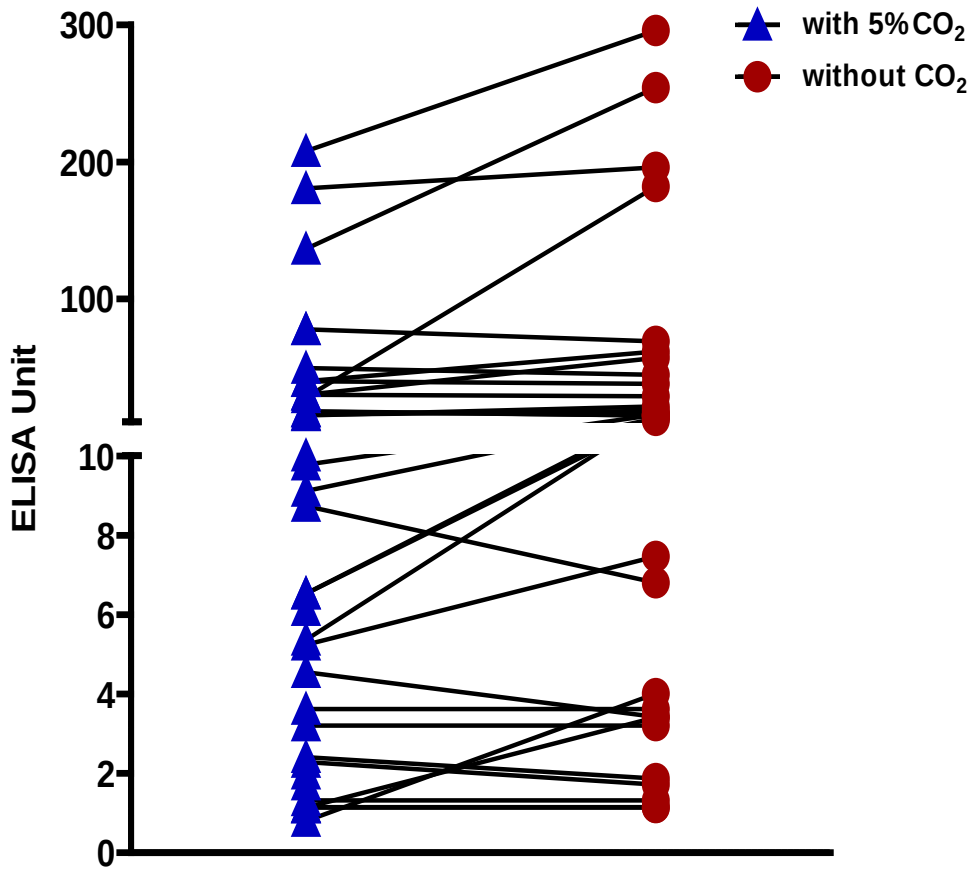
Comparison of TPTest results using leukocytes recovered by various techniques



No significant differences in antibody responses were seen when cells were separated by different methods

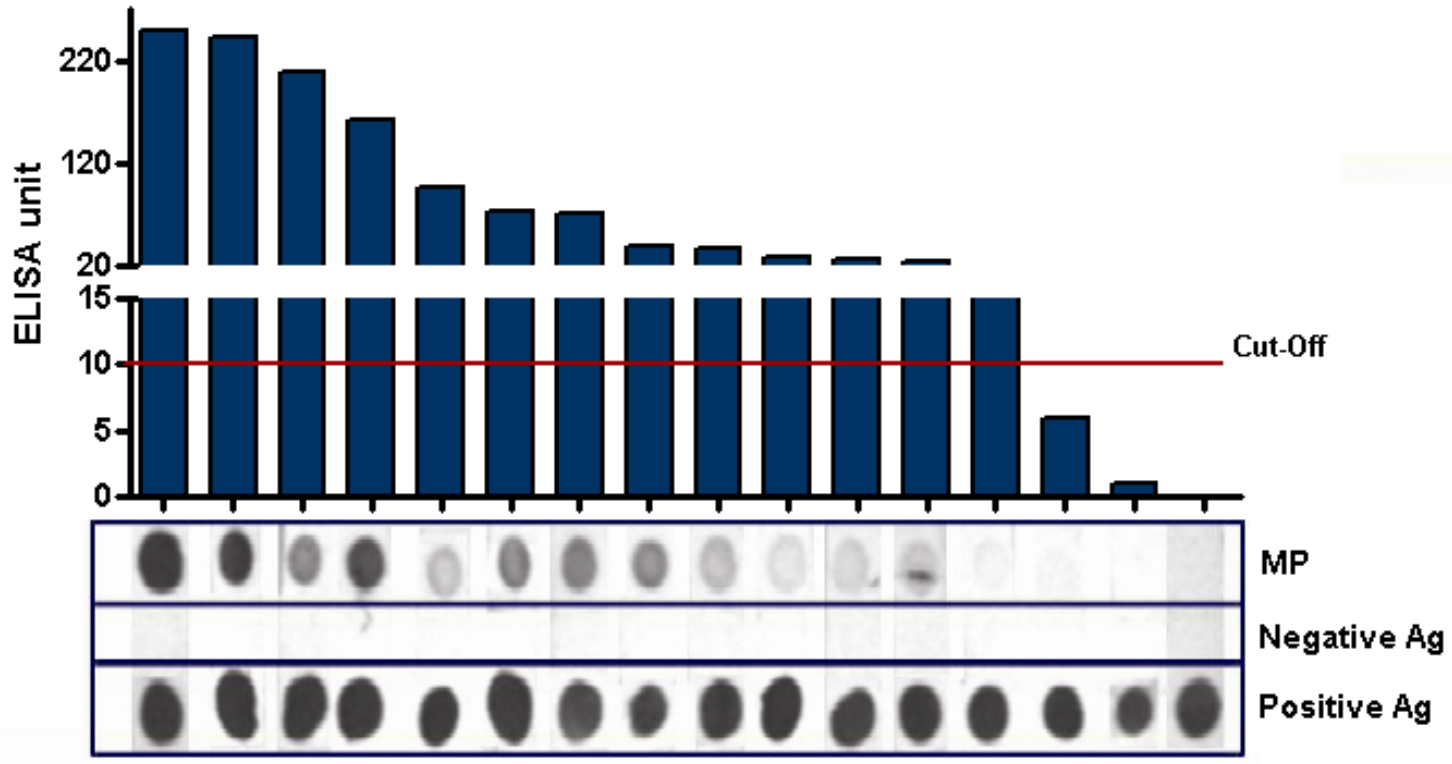
Farhana, Shahnaz et al. ongoing studies

Comparison of TPTest results using peripheral blood mononuclear cells incubated at 37ºC in presence or absence of supplemental CO_2



No significant difference in antibody response was found

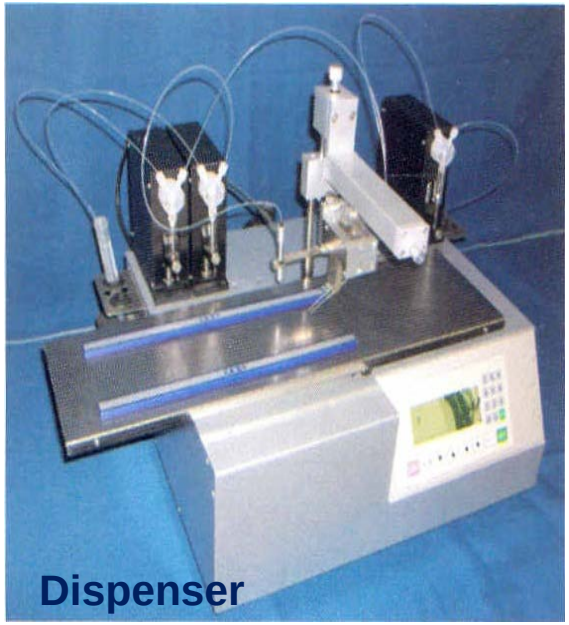
ELISA and immunodot blot approaches



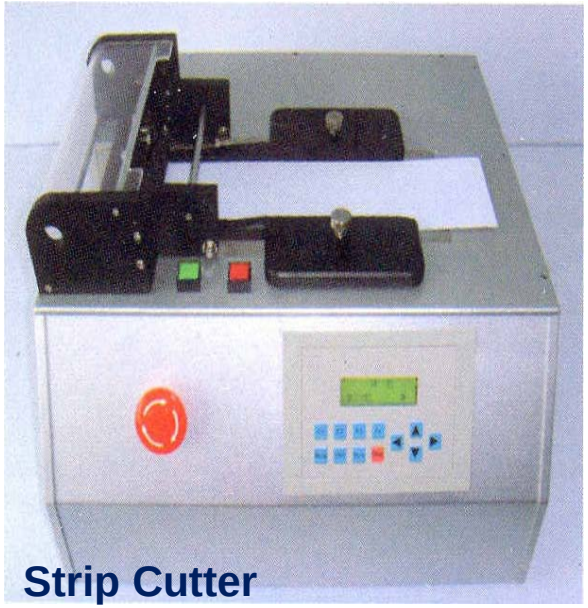
Immunodot blot sensitivity ≥16 ELISA units

Initiated development of lateral flow device for rapid test with industrial partner

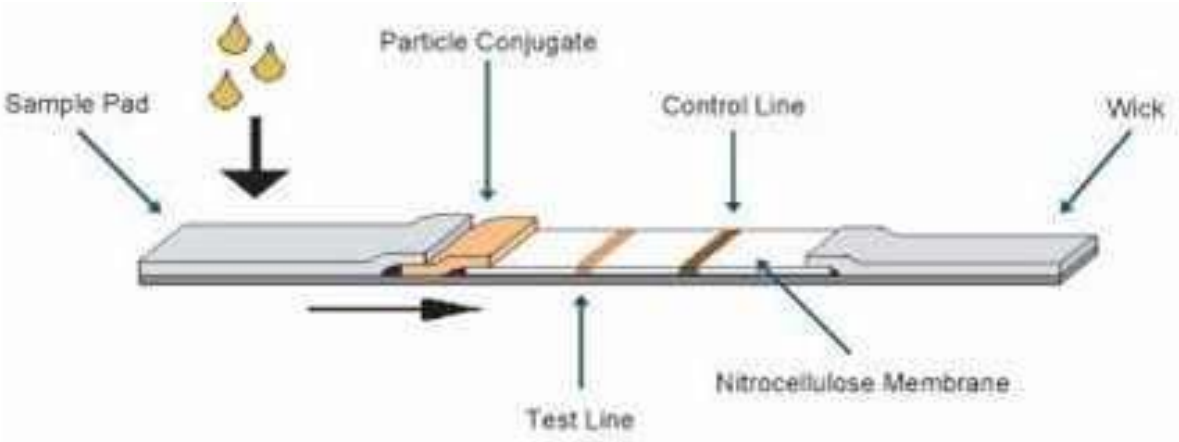
Biotechnology Derived Product Facility (**BDPF**), **Incepta** Pharmaceuticals Ltd



Dispenser



Strip Cutter



Immunochromatography strip test/ lateral flow test

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