

# RaSP Project

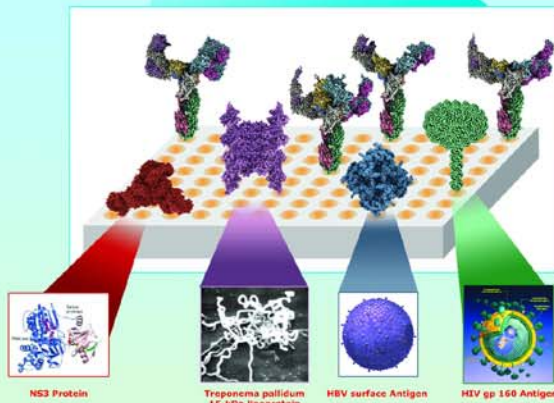
Rapid Surface Plasmon Resonance for parallel detection of pathogens in blood



## Introduction

- Biological materials like blood, tissues and organs are now representing a daily routine material that our hospitals and research institutes are dealing with for many preventive, therapeutic, and research applications. They represent the hope for many medical situations but at the same time they pose a risk of transmitting diseases via blood transfusion, tissues transplantation or organ transplantation.
- Thus, there is an urgent need to improve the technologies used for screening diseases such as HIV and hepatitis C. The same need exists to test donor blood in a cheap and fast way, especially in less developed countries where the rate of infected blood is high due to high prevalence of diseases.
- In this project we will develop a very fast, cheap and at the same time very sensitive method which has the potential to detect more than 100 blood pathogens simultaneously.

### RASP System Overview



## Methodology

HIV\HCV

HBV\ Treponema pallidum

### A- Preparation of plasmid DNA



### B -Immunization Protocol for Monoclonal Antibody Production:

Plasmid DNA was diluted in Ringers' solution, and a volume equal to 10% of the body weight was injected into the lateral tail vein in 6 to 7 seconds, the Balb/c Mice were immunized 5 times with 50 µg pCMV6-XL4 (delivered on days 0, 14, 21, 28, and 35).

### A -Immunization Protocol for Monoclonal Antibody Production:

Injection of 15-kDa lipoprotein of Treponema pallidum in mammalian cells for the immunization. In addition to the injection of recombinant Hepatitis B surface antigen protein for the immunization against HBV.

### C -Fusion technique:

The animals were sacrificed (under sterile conditions) and spleens are extracted. Then the myeloma cells prepared and the fusion technique is done (using PEG method). The final fused cells then plated on 96 well plates and incubated for 5-7 days to give the cells the chance to grow and divide.

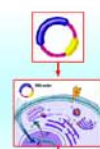
### D - Clonal isolation of hybridomas:

Detection of the HIV in the samples by using ELIZA kit for detection of anti HIV antibodies then the sample was diluted to single cell in 95 well using serial dilution.

### E -Transferring clones:

The selected clones of Hybrid cells that are producing antibodies in satisfactory concentrations are then transferred to a 24 well tissue culture plates, then to 6 well tissue culture plate and then to a small tissue culture flask. The selected clones that secrete considerable amounts of specific antibodies are preserved under liquid Nitrogen in a Mab. Cell Bank. From each clone a colony is prepared, tested for subculture, specificity of antibody and level of antibody production. The selected antibodies are then purified from the culture media and prepared for immobilization.

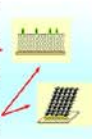
### Recombinant expression in mammalian cells



### Antibody Immobilization

#### Preparation of the Slide Surface:

- 1- A Sensor chip is a glass surface coated with an inert metal (gold).
- 2- Linker layer is deposited on the metal, a self-assembled monolayer (SAM).
- 3- The  $(HS-(CH_2)_n-CH_3, n > 10)$  will be used in producing the (SAM) surface.
- 4- On the linker layer a matrix of (dextran hydrogel) is deposited which provides a hydrophilic layer to covalently bind the ligand via aldehyde, or aldehyde chemistry.



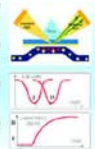
## The Result:

Following dispensing the fusion product into the 96 well T.C. plates immediately after fusion. Splenocytes and myeloma cells were seen adhered in most of the wells. Splenocyte-Splenocyte and Myeloma-Myeloma hybrids were also present. By the time of first screening 70% of the wells showed the presence of living hybrid cells. There was few wells contaminated with fungi, the contaminated wells were treated with copper sulfate, then the total media with copper sulfate were aspirated to prevent contamination between adjacent wells. Many wells showed hybridoma growth, thus the fusion was judged as successful. The first screening done 10 days after the fusion experiment, revealed the presence of a total of 90 wells showing different degrees of reactivity.

The cloning procedures performed twice for some clones and three times for others, resulted in the isolation of pure clones of hybrid cells producing antibodies directed against the glycoprotein gp 160. Out of the 90 wells that gave positive reaction in the preliminary screening using ELIZA, 22 strongly reaction clones were selected for cloning using limiting dilution method. At the end of the cloning procedures 6 clones were selected for their high levels of antibody production, the 6 clones were subjected to successive cultures and all of them are preserved under liquid nitrogen as the time and resources are not enough for characterization and only the highest two clones in the culture ratio and the antibody production titers were allowed for scaling up cultivation at the laboratory.

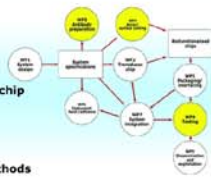
## Principle of SPR

- The sensor surface coated with receptors specific to the analyte, the presence of the analyte can be detected.
- Fabricating the sensor surface will change the nature of its surface electrons and also change the measured SPR.
- Fortunately, the change in refractive index on the surface is linear to the amount of molecules bound (quantitative analysis).
- The change of the refractive index is measured as a change in resonance angle or resonance wavelength.
- Most of the SPR-machines convert the actual measured values (angle or wave length) into an arbitrary one e.g. Resonance Unit (RU).



## Project Details

- WP 1 Design of the system
- WP 2 Development of the transducer chip
- WP 3 Preparation of specific monoclonal antibodies
- WP 4 Immobilisation of antibodies on the transducer chip
- WP 5 Transducer chip packaging
- WP 6 Instrument hardware and software
- WP 7 System integration
- WP 8 Clinical Testing / comparison with standard methods
- WP 9 Dissemination/Exploitation



## The Consortium

Participant no.	Participant organization name	Participant org. short name
1 (Coordinator)	FraunhoferGesellschaft e. V., Institut für Biomedizinische Technik, Germany	IBMT
2	University of Budapest, Hungary	Un-B
3	University of Birmingham, UK	UnB
4	Leuven University of Applied Sciences	ULAS
5	The Holding Company for Biological Products and Vaccines, Egypt	VACSERA
6	MilkiMikMed Ltd., Hungary	MMM
7	Institute for Analytical Sciences, Dortmund Germany	IAS
8	Milvex GmbH, Germany	Milvex
9	ISP NASU Ukraine	ISP NASU