Actualitati in diagnosticul si monitorizarea imunologica in boli infectioase emergente

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Outline

1. Challenges and successes of measuring antigen-specific T cell responses to vaccines

2. ELISPOT technique – elevating it to an exact science

3. Revealing cell-mediated immune responses in a preclinical study that assesses the efficacy of a promising dengue vaccine

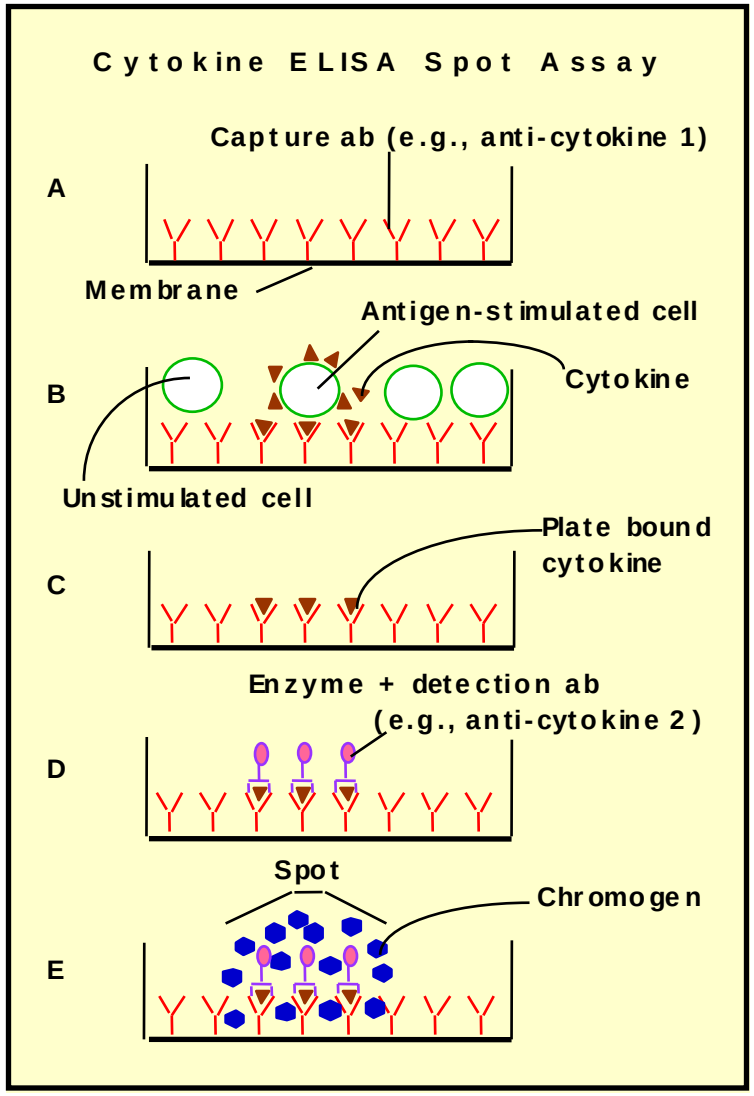
1. Challenges of measuring antigen-specific T cell responses to vaccines

- Measuring T-cell immunity has proven to be a challenge due to the need to test live cells in functional assays *ex vivo*.
  - For the reliable measurement of T cell functions it is imperatively necessary that the test conditions don't change the functionality of T cells *in vitro* as compared to the one *in vivo*.
  - Antigen-specific T cells of interest typically occur at very low frequencies in test samples, such as peripheral blood.
  - The many variables that can affect T cell functionality have earned T cell assays the reputation of being rather fragile, with even minor changes of test conditions potentially having a major impact on the test results.

Successes of measuring antigen-specific T cell responses to vaccines

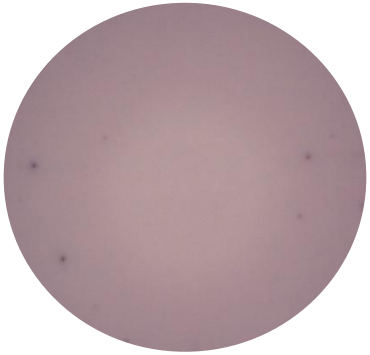
- The ELISPOT technique measures cytokine production at a single-cell level and has a sensitivity of 1 in 1,000,000 cells.
- Our company Cellular Technology Limited (C.T.L.) has perfected the ELISPOT technique and has elevated it to an exact science.
- C.T.L. has been on the forefront of introducing protocols for cryopreservation of peripheral blood mononuclear cells (PBMC) in serum-free medium such that, upon thawing, the cells retain their full functionality.
- This has enabled the generation of "reference PBMC" as ideal tools for assay development and standardization, and use in vaccine trials for the evaluation of cell-mediated immunity.

Principle of the cytokine ELISPOT assay

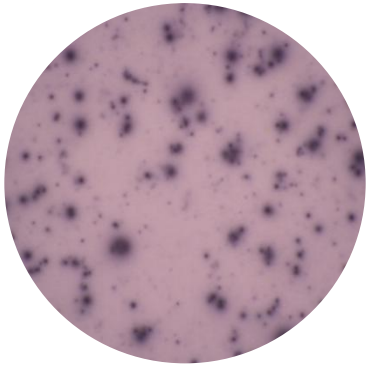


Single-color IFN-γ ELISPOT assay

Medium



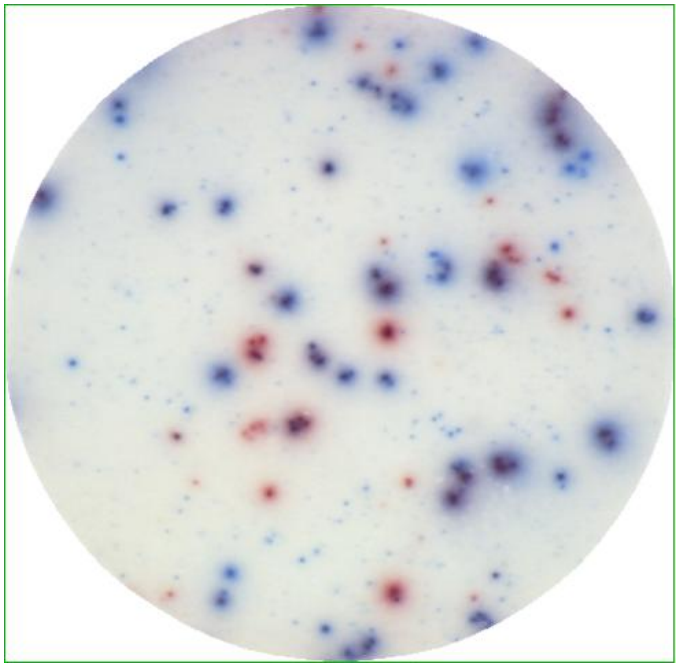
CEF peptide pool



ELISPOT – single cell cytokine secretion

Double-color ELISPOT assay

IFN- γ/IL-4



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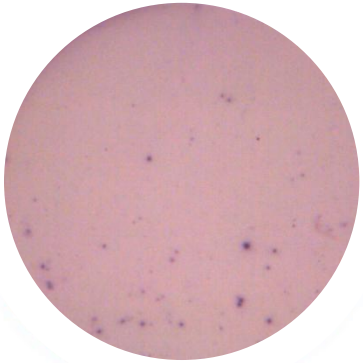
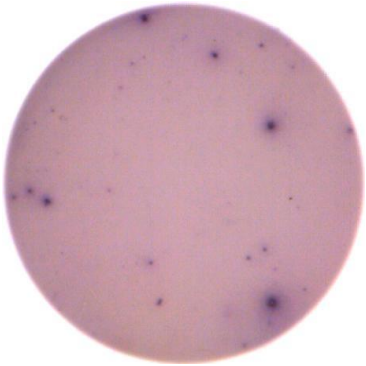
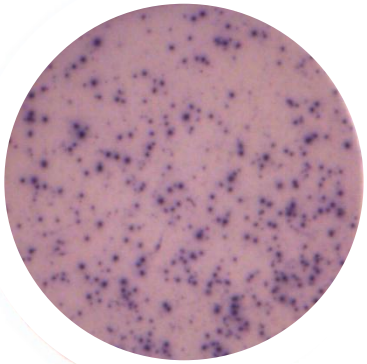
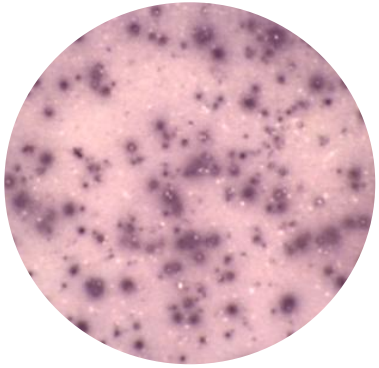
Medium		
CEF peptide pool		

Obtaining reference PBMC

Whole blood processing conditions in serum-free medium (CTL Test™) in healthy donors

4 conditions:

- Fresh = within 2 hours of blood draw
- 4°C = after keeping whole blood for 24 h at 4°C
- RT on rocker = after keeping whole blood for 24 h at room temperature on a rocker
- RT = after keeping whole blood for 24 h at room temperature without rocking it

Parameters evaluated comparatively for the 4 separation conditions (performed on thawed PBMC)

- Determination of cell viability
- Determination of cell recovery
- Determination of cell functionality in an IFN-γ ELISPOT assay by stimulation with mosquito antigen and CEF peptide pool

Conclusions

- Keeping whole blood for 24 hours at 4°C has a negative impact on cell viability, recovery and functionality:
  - In serum-free medium the cell viability, cell recovery and responses to mosquito antigen and CEF pool were drastically reduced in tested donors.
- Keeping whole blood for 24 hours at room temperature (RT) is the better approach:
  - In serum-free medium the cell viability, recovery and responses to mosquito antigen and CEF pool were similar to those obtained for fresh processed blood in tested donors.

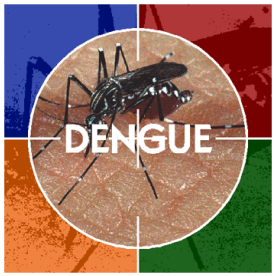
PANDORA opening her box – release of infectious diseases into our world!

painting by John William Waterhouse (1896)

"...the woman opened up the cask,
And scattered pains and evils among men."
Works and Days, *Hesiod*

What is dengue?

- Dengue (dengue fever, "break-bone fever") is an infectious disease caused by any one of four closely related viruses (DEN-1, DEN-2, DEN-3, or DEN-4).
- The viruses are transmitted to humans by the bite of an infected mosquito.
- Dengue is the most widespread vector-borne viral disease of humans - over 100 million cases/year worldwide.
- The more severe forms of dengue are called dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). They can be fatal if unrecognized and not properly treated.



How long do the symptoms of the disease last?

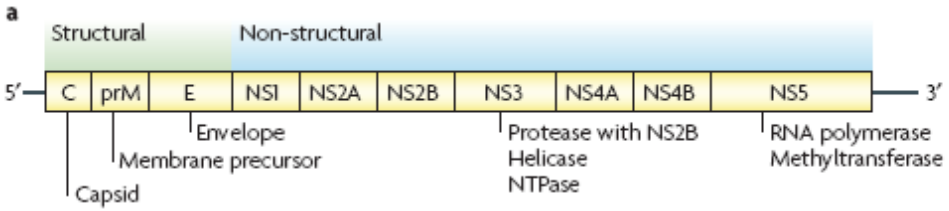
- The fever lasts from 2 to 7 days. Usually the disease is mild.
- The non-neutralizing antibodies from a previous infection or maternally acquired antibodies are thought to form complexes with a different serotype during a subsequent infection and cause DHF or DSS, which can be fatal.
- Hemorrhagic manifestations, tendency to bruise easily, bleeding nose or gums, and possibly internal bleeding. The smallest blood vessels (capillaries) become excessively permeable ("leaky"), and this may lead to failure of the circulatory system and shock, followed by death, if the circulatory failure is not corrected.
- No specific treatment, but it can however be effectively treated by fluid replacement therapy if an early clinical diagnosis is made.

Where can outbreaks of dengue occur?

- Endemic in most regions of the tropics
- Most cases reported in Asia, India, Caribbean, Africa, Central and South America (Brazil), Mexico
- In the USA – Texas, Arizona, Florida, Hawaii

Structural proteins of the dengue virus

- **Capsid (C):** Basic protein involved in packaging the viral genome into a nucleocapsid core.
- **M protein (M):** Membrane glycoprotein expressed as a precursor (prM). prM is cleaved during particle maturation.
- **E protein (E):** Membrane glycoprotein containing receptor binding site and fusion peptide; is major target of protective antibodies.



Whitehead et al.(2007), Nature Rev., 5, 518-528.

Determination of cell-mediated immunity (CMI) by ELISPOT in a dengue vaccine trial

Efficacy of a Tetravalent Chimeric Dengue Vaccine (DENVax) in Cynomolgus Macaques

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Results of dengue study

Three tetravalent formulations of chimeric dengue (DENVax) viruses containing the pre-membrane (prM) and envelope (E) genes of serotypes 1-4 into the attenuated DENV-2 PDK-53 genome were tested for safety, immunogenicity and efficacy in cynomolgus macaques (*Macaca fascicularis*).

Subcutaneous injection of the DENVax formulations was well-tolerated with low levels of viremia detected, and virus neutralizing antibody titers were induced against all four dengue virus serotypes (DENV-1, -2, -3, -4) after one or two administrations.

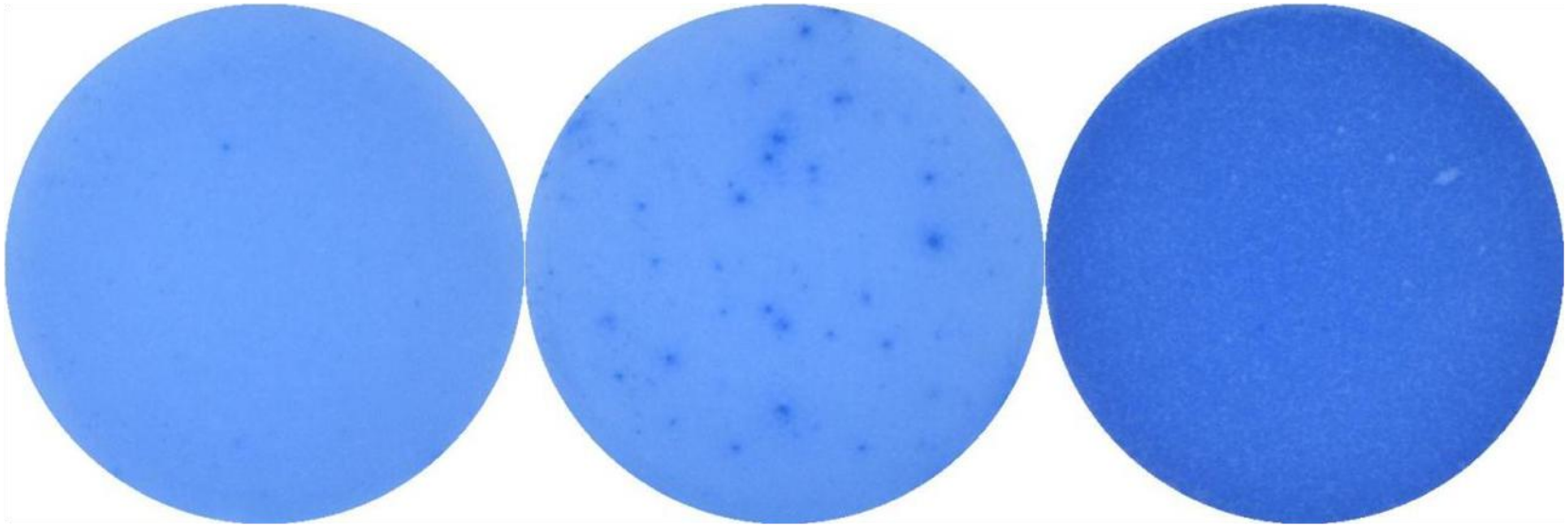
All animals immunized with the high dose formulation were protected from viremia after challenge with all four DENVs, and all immunized animals, regardless of formulation, were completely protected from DENV-3 and DENV-4 challenge.

Some animals that received a low dose of DENVax were only partially protected from challenge with DENV-1 or DENV-2.

In contrast, all control animals developed high levels of viremia for multiple days following challenge with wild-type DENV-1-4.

This study highlights the safety and efficacy of the tetravalent DENVax formulations.

IL-2 spot morphology induced by live DEN-2 virus



Conclusions

- ELISPOT assays offer unique qualification opportunities for drug and vaccine development using cytokine-based immune monitoring and standardization strategies.
- ELISPOT's sensitivity is orders of magnitude above other methods, such as ELISA,CBA and ICS.
- ELISPOT assays have a high resolution and are GLP- and CLIA-compliant.
- ELISPOT assays are extremely informative in the immune monitoring and biomarker screening during pre-clinical and clinical phases of drug and vaccine development.

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