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Bringing innovation to global health

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Challenges of Wanted and Unwanted Immunogenicity Assays

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Clinical Immunology, Crucell

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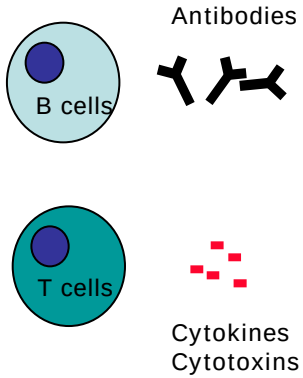
Imunogenicity: wanted or unwanted

- Vaccines
  - Wanted immunogenicity:
    - Neutralization and elimination of pathogens / malignancies
  - Unwanted immunity:
    - Enhancement of infection
    - Cross reactive antibodies to self proteins
    - Vector neutralization



- Protein therapeutics
  - Unwanted immunogenicity:
    - Adverse events: autoimmunity, hypersensitivity
    - Drug neutralization

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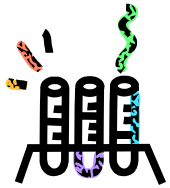
Challenges of immunogenicity assays

- True references and surrogate references



- Relevant and irrelevant immune responses

- Existing and non-existing guidelines

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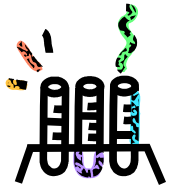
Challenges of immunogenicity assays

- True references and surrogate references



- Relevant and irrelevant immune responses
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Positive controls for new biologics

- Humans have generally not been exposed to new biologics
- True reference or positive control for immune responses against new biologics are not existing
- Surrogate antibody controls:
  - Animal serum, affinity purified antibodies, idiotypic moabs
  - No true assay sensitivity, no assay comparison possible
  - ➢ Generally accepted by regulatory authorities

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Positive controls for new vaccines

Pathogen	Antibody assays		T cell assays	
	+ serum	Antigen	**+ T cells**	Antigen
Malaria	√ donors from endemic regions	√ recombinant protein / peptide	- Single sample Not in sufficient quantity	√ peptides
Tuberculosis	√ donors from endemic regions	√ recombinant protein	- Single sample Not in sufficient quantity	√ peptides
HIV	√ donors from endemic regions, cohort studies	√ recombinant protein	~ cohort studies Not in sufficient quantity	√ peptides
Ebola	- Not existing	√ recombinant protein	- Not existing	√ peptides
Pandemic Influenza	- Not existing	- Not existing	- Not existing	~ consensus peptides

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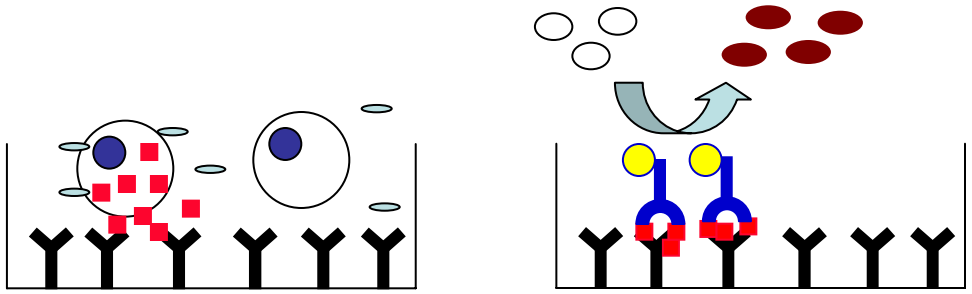
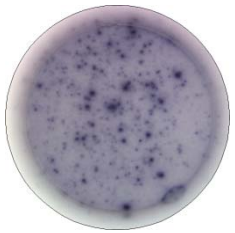
Enzyme Linked ImmunoSpot assay (ELISPOT)





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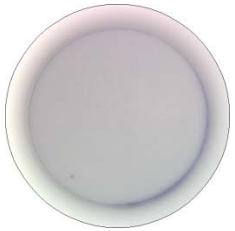

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T cells stimulated with a pool of 15mer peptides (⬭) of the Malaria antigen (11 aa overlapping)

Specific T cells secrete IFNγ (■) and are defined as Spot Forming Units per million cells (SFU/10^6)

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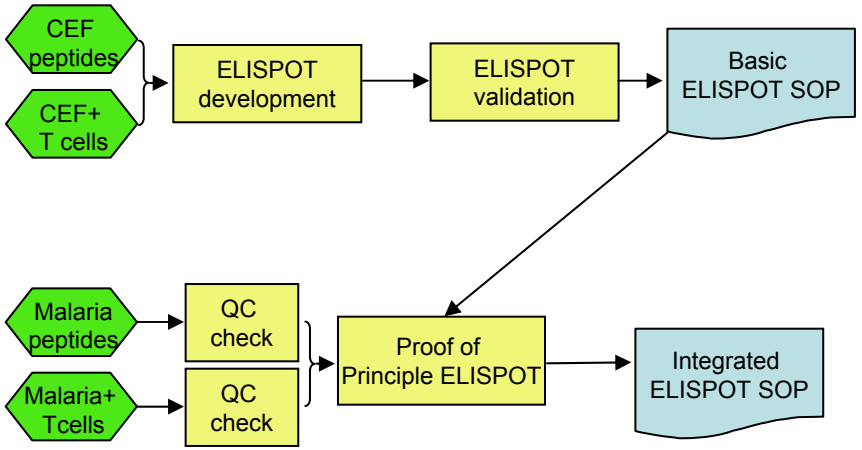
Elispot development and validation strategy

- Positive control in Elispot: CEF response
  - CMV/EBV/Flu T cell epitope mix. Most donors are positive for at least one of those peptides
  - Prepare and freeze large batch of healthy donor T cells at -160°C
  - Determine CEF response of donor → set acceptance criteria



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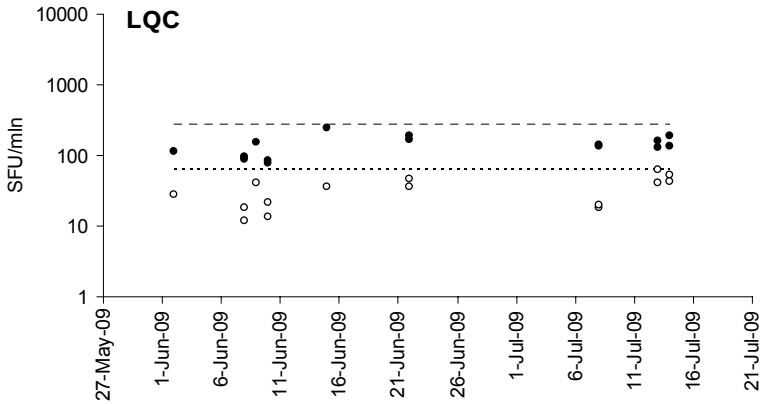
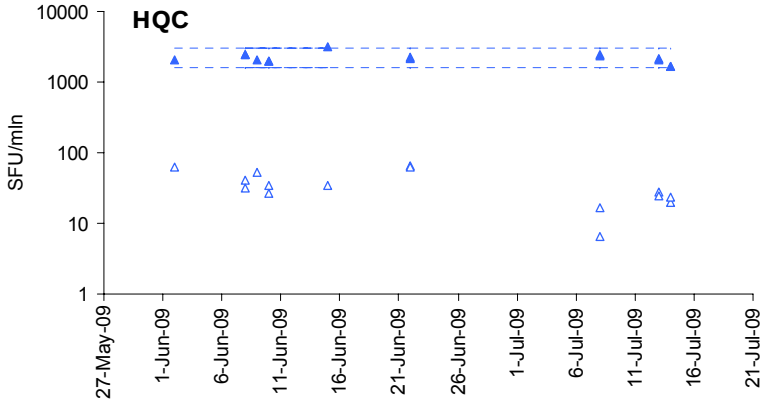
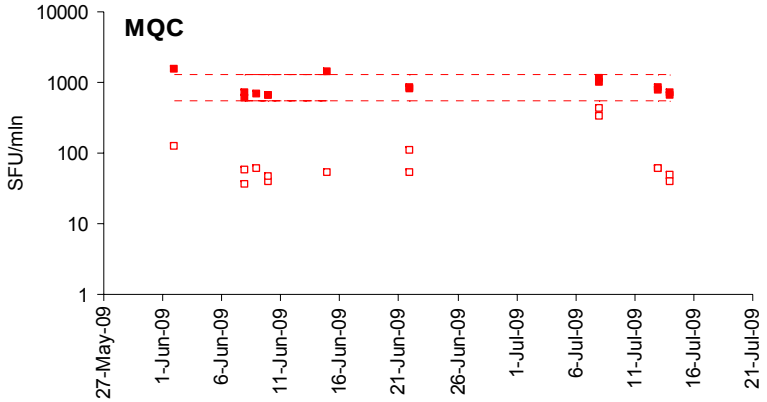
Elispot validation performance

- CEF+ controls used as validation samples and assay controls
- Overall intermediate precision = 29% for CEF positive samples







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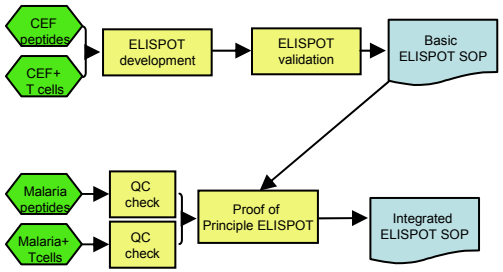
Alternative control sample strategy

T cell assays

- *Assumption: Malaria-ELISpot and CEF ELISpot behave similarly*
  - *Do the controls accurately reflect the clinical trial samples?*

    (Spot size, stimulus threshold, background)



Serology assays

- *Assumption: reference serum and trial samples behave similarly*
  - *Do the controls accurately reflect the clinical trial samples?*

    (Paralellism, stability, affinity, isotype composition)

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Challenges of immunogenicity assays

- True references and surrogate references
- Relevant and irrelevant immune responses

- Existing and non-existing guidelines

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Which immune responses are clinically relevant

- Unwanted immunogenicity:
  - Which immune response, and what level causes adverse events (autoimmunity, hypersensitivity, etc)
  - Do antibodies neutralize the drug/vector

- Wanted immunogenicity:
  - Which immune response is effective against the pathogen / malignancy
  - What level of immune response confers protection against infection / disease

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Which immune responses are clinically relevant

- Safety aspect:

  First goal is to detect any anti drug immune response
  → qualitative assay, sensitivity

  - *How to investigate clinical relevance of observed immunogenicity?*

- Efficacy aspect:

  Aim is to investigate mode of action and protective level
  → functional assays, assay range

  - *How to investigate which immune responses are protective?*

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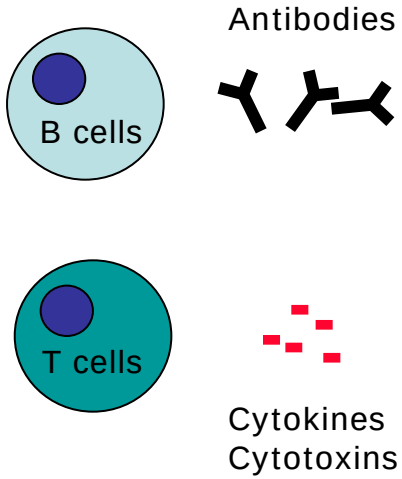
Correlates of protection for vaccines



- Antibodies capture the pathogen
  - Opsonisation
  - Neutralization
  - Can prevent infection
- T cells kill infected cells
  - Eliminate infected or malignant cells
  - Cytokines inhibit replication/proliferation
  - Can clear the infection /

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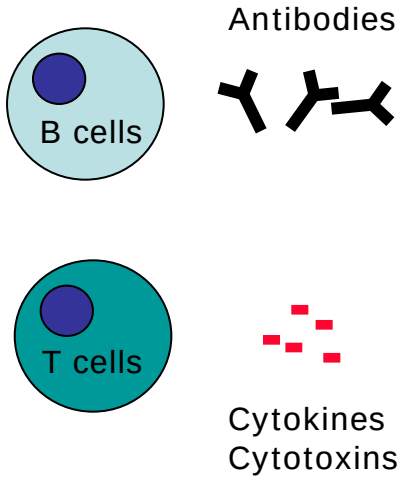
Correlates of protection for infectious diseases



- Most current vaccines are based on protective antibody titers
  - Influenza: 1/40 HI
  - Hepatitis B: 10 mIU/ml
  - Haemophilus Infl B: 0.15 ug/ml

Vaccines need to reach the protective antibody titer to get registration

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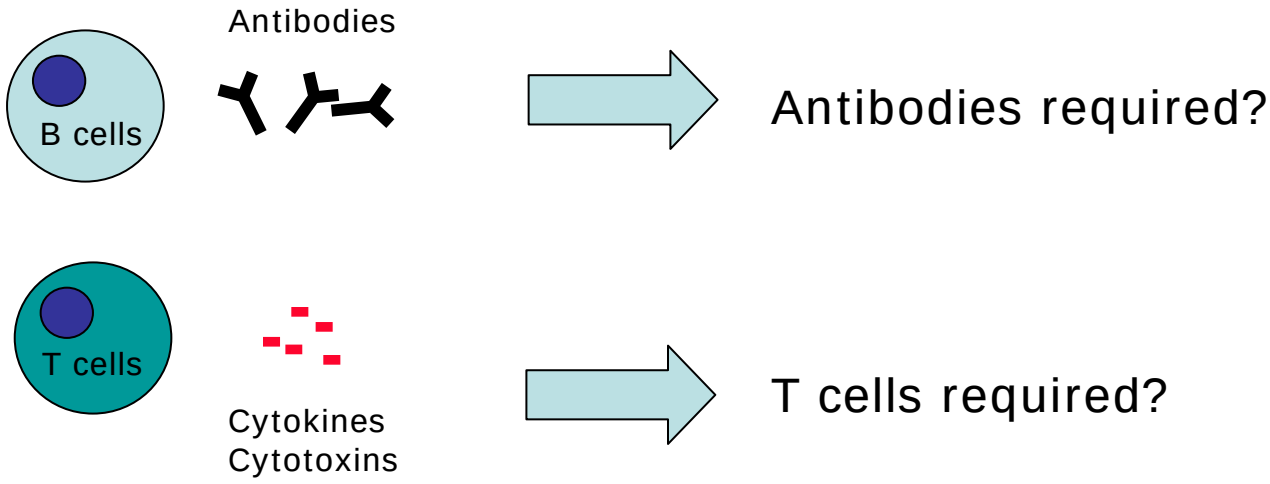
Correlates of protection:
one or multiple immune mechanisms

- Several pathogens lack a correlate of protection
  - HIV
  - Tuberculosis
  - Malaria



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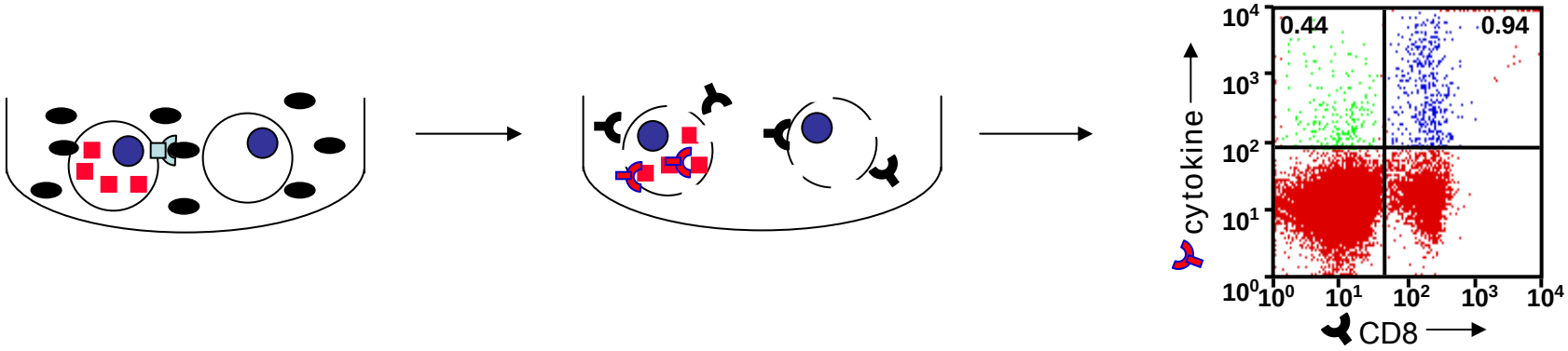
Intracellular Cytokine Staining (ICS)



- Antigen specific T cell detection and characterization at single cell level
- Simultanous measurement of 18 parameters possible
- Different marker panels:
  - Activation: CD3, CD8, CD4, HLA-DR, Ki67, BcL-2, CCR5, CCR7, CD38, CD27, viability
  - Memory: CD3, CD8, CD4, CD57, CD103, CD45RO, CD28, CCR5, CCR7, CD27, viability
  - Effector: CD3, CD8, CD4, CD57, CD107, CD62L, Perforin, Granzyme B, IL-2, IFNγ, TNFα

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Investigation of protective immune response

Choices between assays, functionality, location

- Antibodies
  - Quantity (ELISA)
  - Quality (Neutralization assay)

Too many variables to validate

- T cells:
  - Quantity (tetramers)
  - Quality (Elispot, ICS)
  - Characteristics (phenotype, activation, maturation markers)

- Location:
  - Peripheral blood
  - Target tissue

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Immuno assay endpoint strategy
wanted immunogenicity

- Selection and validation of 1 antibody assay and 1 T cell assay as standard read out of immunogenicity: secondary end point

- Selection of several exploratory assays to investigate correlate of protection: exploratory end point

→ challenge studies, field studies

- If an exploratory assay proves to be correlate of protection

→ CoP immunoassay validated as primary endpoint

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Challenges of immunogenicity assays for vaccines

- True references and surrogate references
- Relevant and irrelevant immune responses
- Existing and non-existing guidelines

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existing Guidelines for assay validation

- ICH (analytical assays for product testing)

- FDA (bioanalytical method validation)
- FDA (immunogenicity of protein therapeutics)

- EMA (immunogenicity of protein therapeutics)
- EMA (bioanalysis of drug concentrations)

- White papers on unwanted immunogenicity
- White papers on bioanalysis, biomarkers

➢ Vaccine immunogenicity = biomarker? (fit for purpose)

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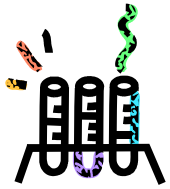
Challenges and Opportunities

- ✓ Alternative references and positive controls



- ✓ Investigation into the Correlates of Protection

- ✓ Unwanted Immunogenicity guidelines

- ❑ Write our own white paper on vaccine immunogenicity

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EBF Topic Team on Vaccines

- Discussions on immunogenicity for vaccines are ongoing
- Other EBF members working on vaccines are invited to join
- White paper planned for 2013

TT 34: Standardization of bioassays for vaccines

EBF

TT lead: Jasja Wolthoorn

Background and Aim

- ❑ In contrast to the unwanted immunogenicity field, regulatory papers and recommendations on how to perform and validate assays for wanted immunogenicity of vaccines are not published yet.
- ❑ Aims:
  - Organize break-out session on wanted and unwanted immunogenicity (this conference)
  - Publish White Paper on how to perform and validate assays for the immunogenicity of vaccines

Team Members

- Kevin Maskell, Merck Millipore
- Dorte Komerup Dittevsen, Lundbeck
- Jenny Hendriks, Crucell
- Stefan Kostense, Crucell
- Arjen Companjen, Crucell
- Melody Sauerborn, TNO Triskelion
- Jasja Wolthoorn, TNO Triskelion

Ongoing Activities and Current Results

- ❑ Organization EBF 2012 break-out session on wanted and unwanted immunogenicity on Thursday 15th from 14.00-15.30h: welcome to join !
- ❑ Current team discussion on White Paper:

Immunogenicity assays for vaccines compared to related assay types:

Ligand-binding assays for PK study	Immunogenicity assays for unwanted immunogenicity	Immunogenicity assays for vaccines
- Quantitative (mass/ international units) - True assay control - Assay: sensitive, accurate, precise, specific.	- Qualitative/ semi-quantitative (titer) - No true assay control - Focus on lowest possible detection limit - Clinical relevance immunogenicity: functional assays like cell-based assays	- Semi-quantitative (titer) - No true assay control - Focus on correlate of protection - Clinical relevance immunogenicity: functional assays involving viruses

Future Plans

❑ Publish White Paper on this topic in 2013:

Key terms: antibody serology assays, validation, correlate of protection, neutralization antibody assays, therapeutic vaccines, regulation.

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