Bringing innovation to global health
Binding and activity of Anti-Vaccine Antibodies in short and long term stability studies

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Vaccines vs Protein therapeutics

- Protein therapeutics
  - Unwanted immunogenicity

- Adenovirus–based vaccines
  - Unwanted immunogenicity
    - Responses against the vector
  - Wanted immunogenicity
    - Serology
    - Cell Mediated Immunity
Unwanted Immunogenicity
Immunogenicity induced by therapeutic proteins

- Therapeutic proteins may induce immunogenicity

- Immunogenicity against these proteins can hamper their effect
  - Neutralization of product or endogenous counterpart by anti-drug-antibodies (ADA)

- Therapeutic protein types:
  - Fusion proteins such as EPO:
    - Neutralization of product or endogenous counterpart (red-cell aplasia, EPO); accelerated clearance
  - Antibodies (humanized or fully human):
    - Neutralization by binding to idioype (human-anti-human antibodies (HAHA)); accelerated clearance
Wanted vs Unwanted Immunogenicity

- Unwanted immunogenicity
  - Semi-Quantitative assays: screening + confirmatory assays
  - FDA and EMA guidelines for assessment of immunogenicity of therapeutic proteins mainly focus on antibody responses

- Wanted immunogenicity
  - Quantitative assays: correlate of protection
Immunogenicity of Vaccines
Anti-Vaccine-Antibodies (AVA)

- Unwanted immunogenicity
  - Anti-Vaccine-Antibodies (AVA) to the vector may inhibit the efficacy of the vaccine
    - Functional cell-based assay

- Wanted immunogenicity
  - Anti-Vaccine-Antibodies (AVA) to the insert may indicate protective level of the vaccine
    - ELISA for antibody binding to the antigen
Unwanted Immunogenicity
AVA induced by vaccines

- Assay utilized to assess unwanted immunogenicity to viral vaccines (neutralizing antibody responses):
  - Neutralization assay
    - Assay based functionally inhibiting viral infection
Monitoring of Adenovirus neutralizing antibodies

- Pre-screening / selection of subjects for vaccine trials
- Verification of correct vaccine administration
- Monitoring of antibody associated AE’s

→ Validated adenovirus neutralization assay required in support of clinical trials
**CS specific antibody ELISA**

Well coated with CS repeat peptide (NANP)$_6$C

Human serum is incubated, specific antibodies bind to the CS peptide

Human IgG specific antibody conjugated with HRP is added

HRP converts substrate resulting in colorimetric reaction

<table>
<thead>
<tr>
<th>Reference serum dilution</th>
<th>OD 492nm</th>
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<tbody>
<tr>
<td>75</td>
<td>1.6</td>
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<tr>
<td>150</td>
<td>1.4</td>
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<td>38400</td>
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</table>

- Reference serum = 2300 ELISA Units (EU/ml) titer, defined as the dilution at which 50% of the OD value is reached (ED50).
- Sample titer is calculated relative to the reference serum, expressed in relative ELISA Units/ml
Monitoring of *P. Falciparum* CS protein-specific antibodies

- CS ELISA monitors serum antibodies to CS peptide repeat
- Level of Abs may be related to protection

→ Validated CS ELISA required in support of clinical trials

→ 2 assays to monitor AVA in serum:
  - assessing binding and functional Abs
Stability studies: Guidelines of FDA and EMA

• FDA:

The stability of the analyte in biological matrix at intended storage temperatures should be established. The influence of freeze-thaw cycles (a minimum of three cycles at two concentrations in triplicate) should be studied.

The stability of the analyte in matrix at ambient temperature should be evaluated over a time period equal to the typical sample preparation, sample handling, and analytical run times.

• EMA:

Stability studies should investigate the different storage conditions over time periods that equal or exceed those applied to the actual study samples.
Stability Studies

• Stability testing separately addressed in assay Validation and expanded on if required

• Layout stability studies
  • Freeze/thaw cycles
  • Impact of Heat Inactivation
  • Short term stability
  • Stability around Heat Inactivation
  • Long term Stability
  • Shipment
Freeze/thaw stability

Ad neutralization assay

- Stability of High, Intermediate, Low and Negative Internal Controls after 3 freeze thaw cycles
- Now expanded on with more freeze/thaw cycles
Impact of Heat Inactivation I

Ad neutralization assay

- SOP requires Heat Inactivation (HI)
- 60 min at 57°C
- Heat Inactivation required before shipment for safety reasons of recipient

-> impact of twice HI
Impact of Heat Inactivation II

CS ELISA

- SOP requires Heat Inactivation (HI)
- 30 min at 56°C

-> Compare with 60 min HI
Stability around Heat Inactivation

Short term stability is confirmed before and after HI.
Limited short term stability:

Stability of short term storage for 2 hours on the bench (3 replicates):

Accuracy:
IC1: 82.6%
IC3: 87.2%
IC5: 90.3%

- No significant change from baseline
- Difference of the means is smaller than the variation of the assay (clue 1)
Preparative stability

*Ad neutralization assay*

- The titer increases with incubation time, which may indicate a more effective neutralization.
- Alternatively, the virus may start to degrade, resulting in an overestimation of the titer.
- Preparation time was limited to 1 hour.
Long term Stability
Trending of internal control results I

- Trending of internal control results over time to identify performance issues, sample instability or other issues

**Ad neutralization assay**
Long term Stability
Trending of internal control results II

CS ELISA

Cruccell
**Shipment stability**

**Ad neutralization assay**

- No significant change in titers if samples are shipped
- Difference of the means is smaller than the variation of the assay (clue 2)

**CS ELISA**

- p=0.0507
- p=0.30

**Graphs:**
- Log10 Titer
- IC90 log2
- Sample
- Shipped
- not Shipped
Specific recommendations and considerations

- Ongoing expansion of stability studies:
  - Matrix: Serum vs Plasma
  - Align Heat Inactivation protocols
  - Test more freeze thaw rounds

- Consider specific circumstances: power loss/RT at (sub-)tropical locations/LN2 storage

- Always trend for long term stability and control of reagents

- Statistical considerations: test for difference vs test for equivalence
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  - Jerome Custers

- QA
Combating infectious diseases

by bringing innovation to global health