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Binding and activity of Anti-Vaccine Antibodies in short and long term stability studies

Jenny Hendriks

Clinical Assays, Crucell

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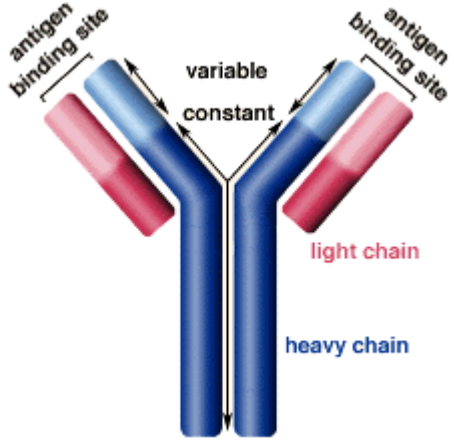
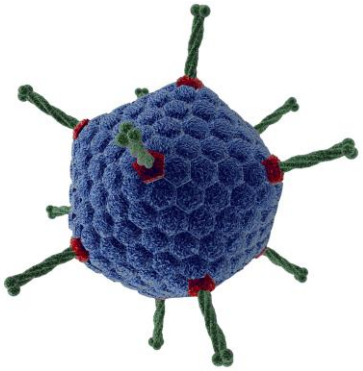
Vaccines vs Protein therapeutics

- Protein therapeutics
  - Unwanted immunogenicity
- Adenovirus–based vaccines
  - Unwanted immunogenicity
    - Responses against the vector
  - Wanted immunogenicity
    - Serology
    - Cell Mediated Immunity





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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 idiotype (human-anti-human antibodies (HAHA)); accelerated clearance

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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

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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

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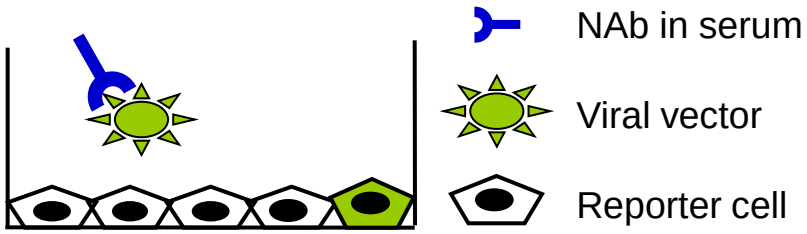
Unwanted Immunogenicity
AVA induced by vaccines

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



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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

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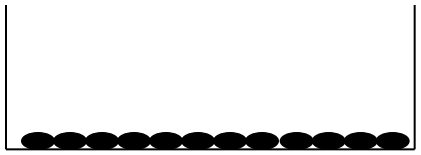
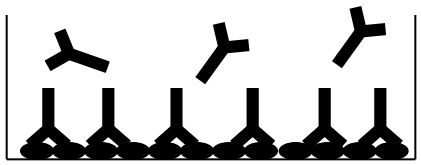
Wanted Immunogenicity

AVA induced by vaccines

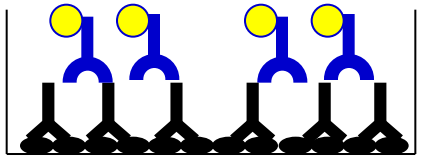
CS specific antibody ELISA



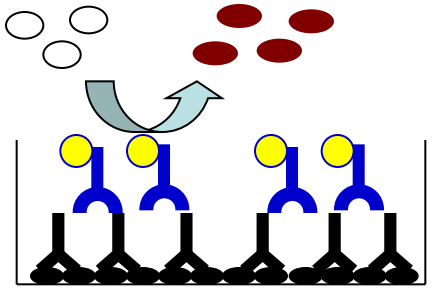
Well coated with CS repeat peptide $(NANP)_6C$



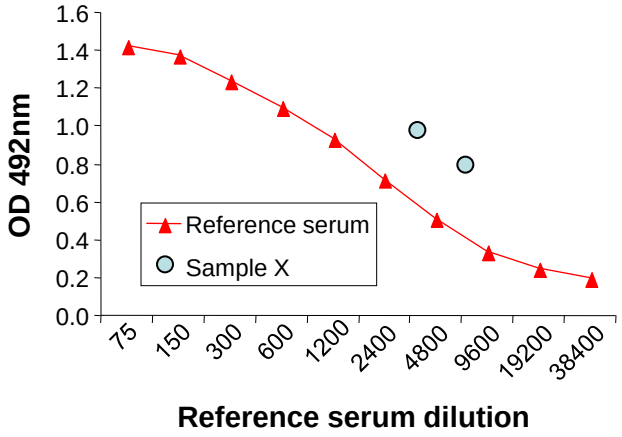
Human serum is incubated, specific antibodies bind to the CS peptide



Human IgG specific antibody conjugated with HRP is added



HRP converts substrate resulting in colorimetric reaction



- 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

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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

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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.

intended storage temperature) and short-term (bench top, room temperature) storage, and after going through freeze and thaw cycles and the analytical process. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. The procedure should also include an evaluation of analyte stability in stock solution.

- EMA:

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

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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

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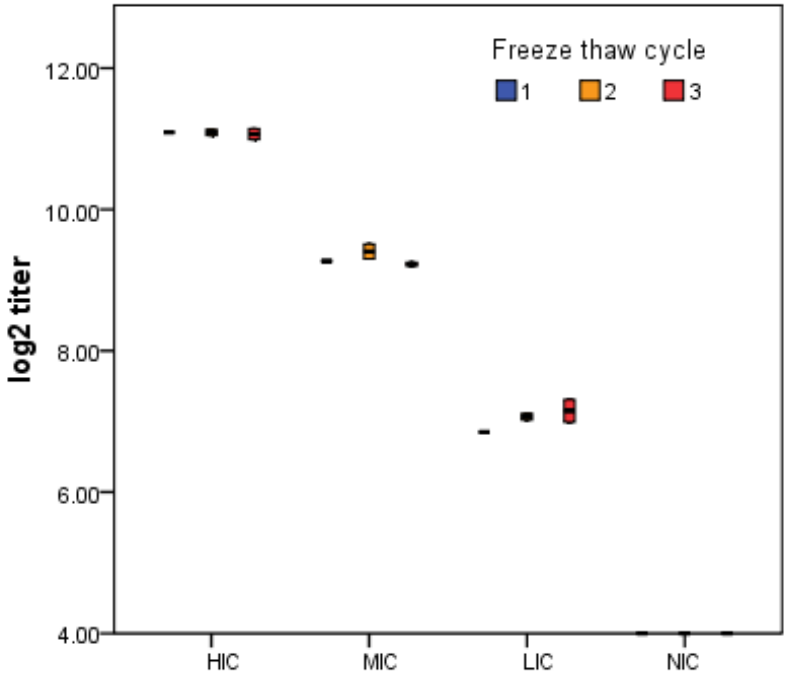
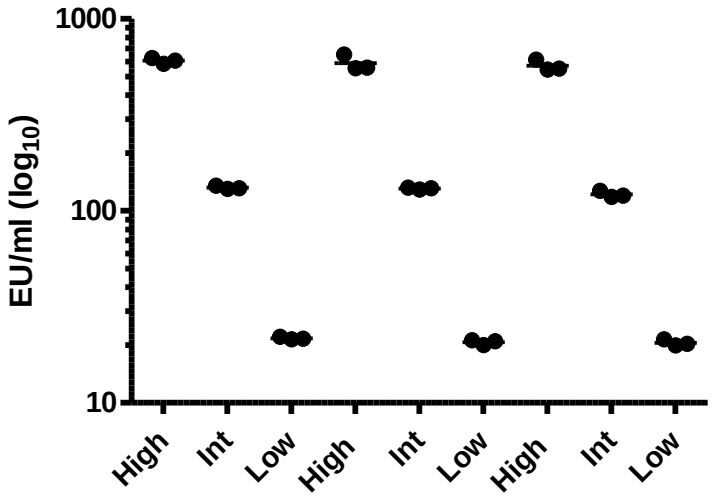
Freeze/thaw stability

Ad neutralization assay



CS ELISA



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

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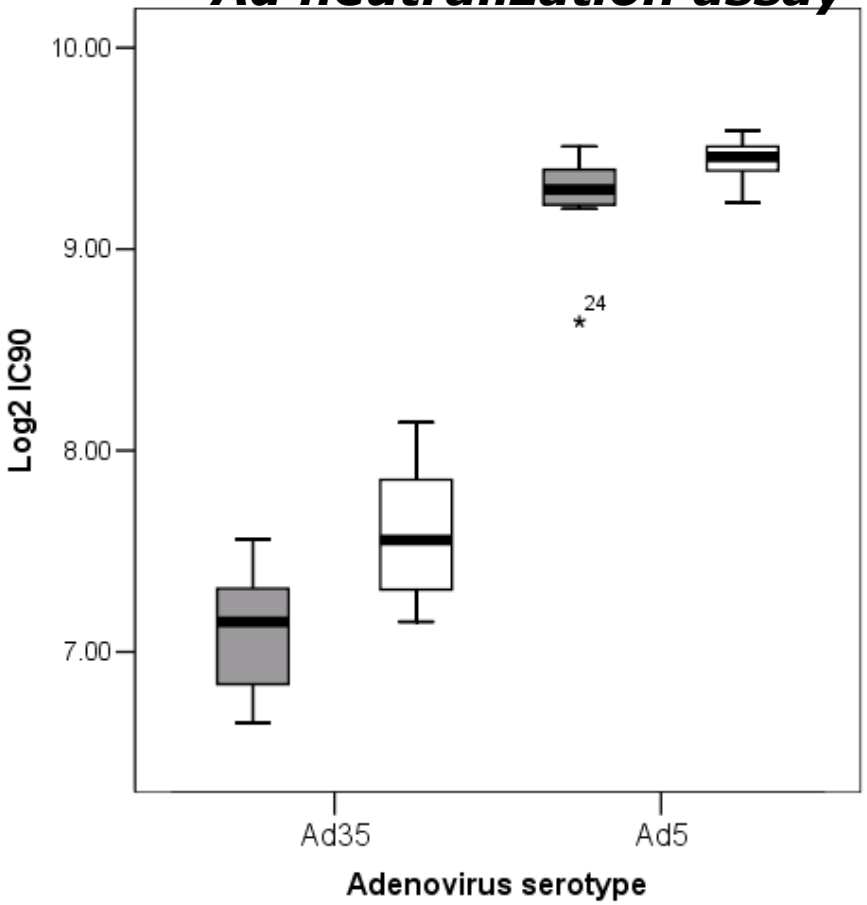
Impact of Heat Inactivation I

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

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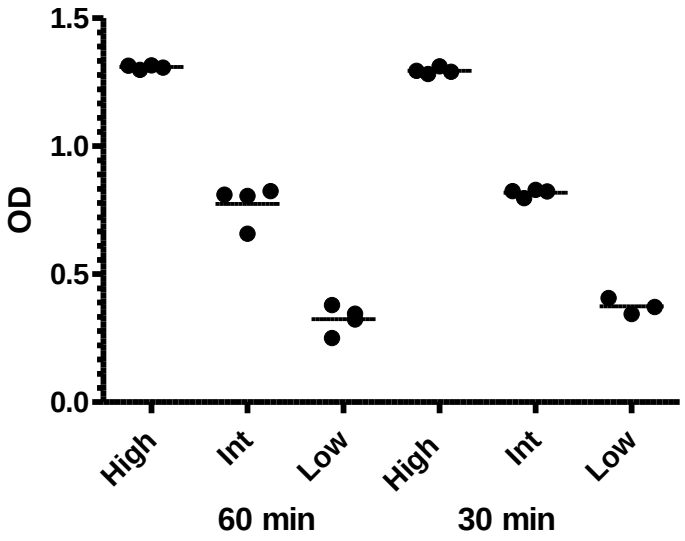
Impact of Heat Inactivation II

CS ELISA



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

->Compare with 60 min HI

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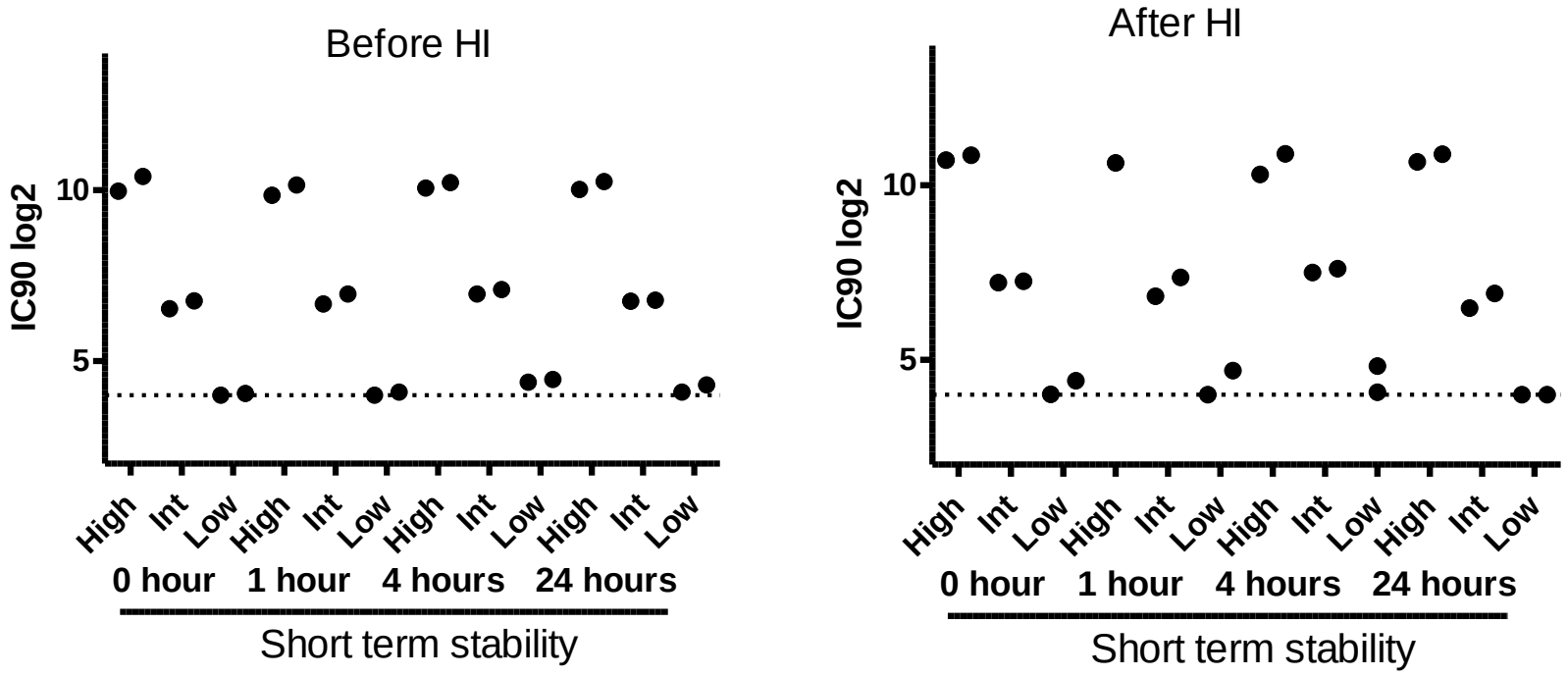
Stability around Heat Inactivation



->Short term stability is confirmed before and after HI

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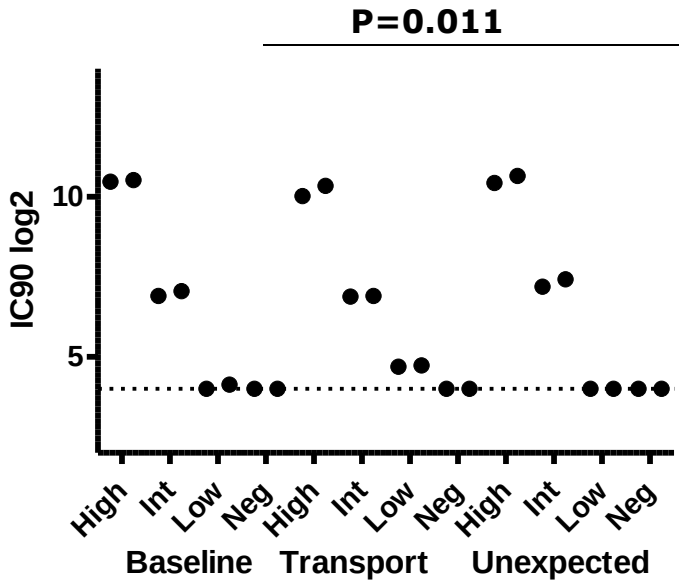
Short term Stability

Ad neutralization assay



CS ELISA

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)

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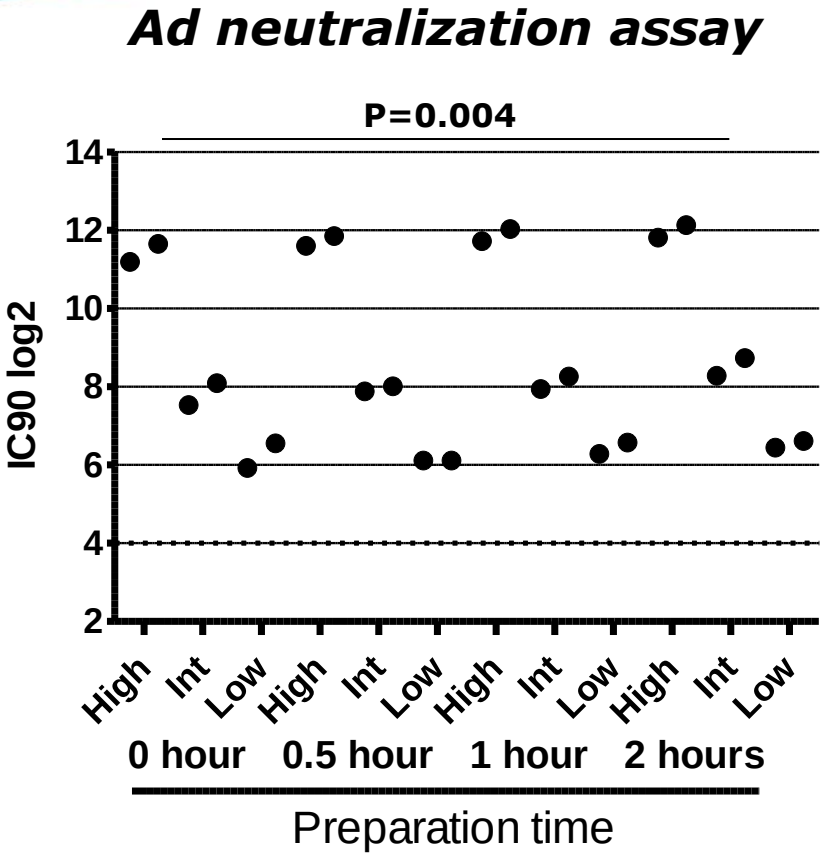
Preparative stability



- 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.

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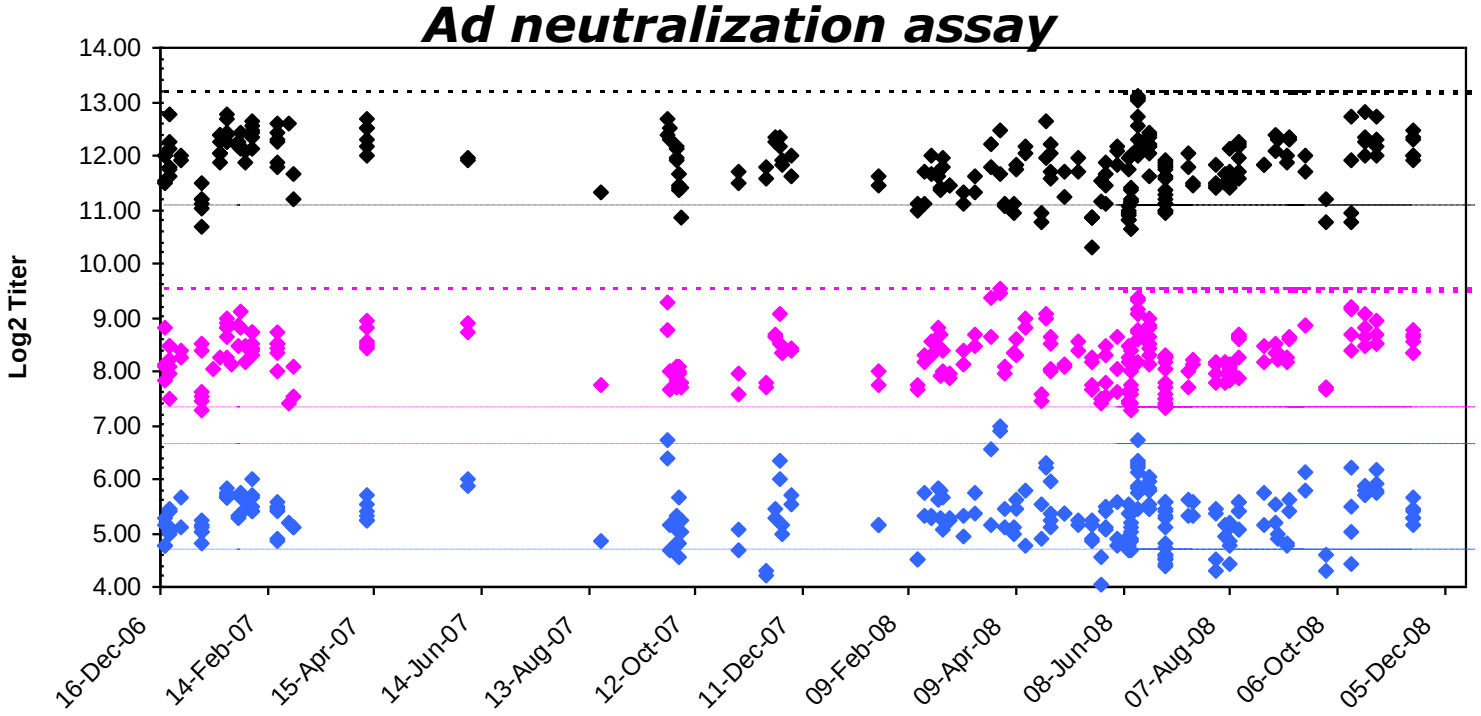
Long term Stability
Trending of internal control results I

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



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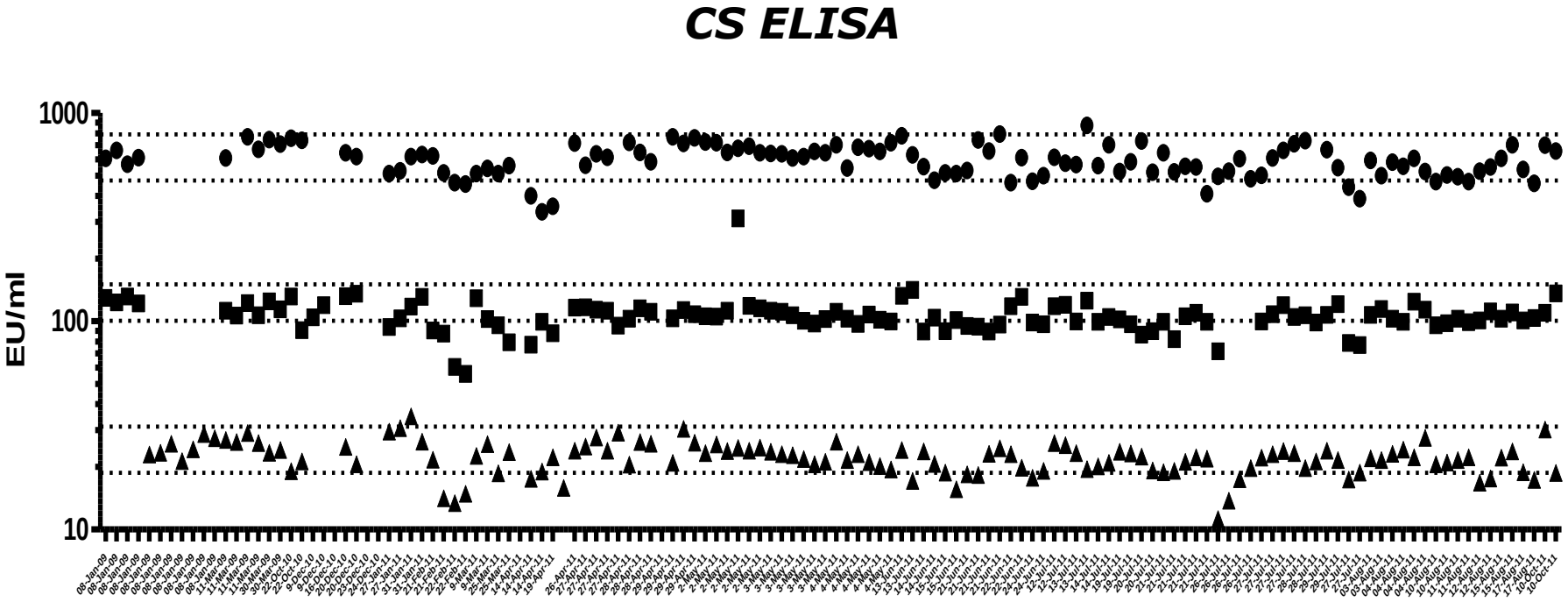
Long term Stability
Trending of internal control results II



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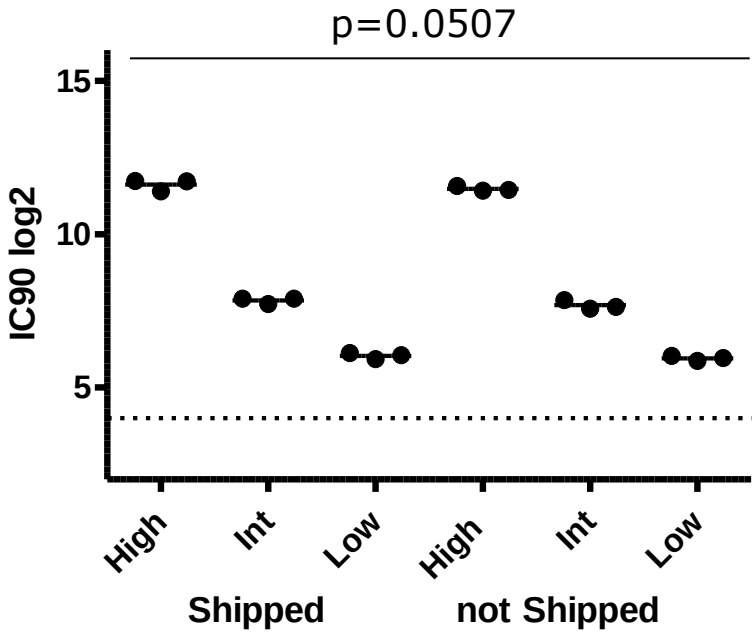
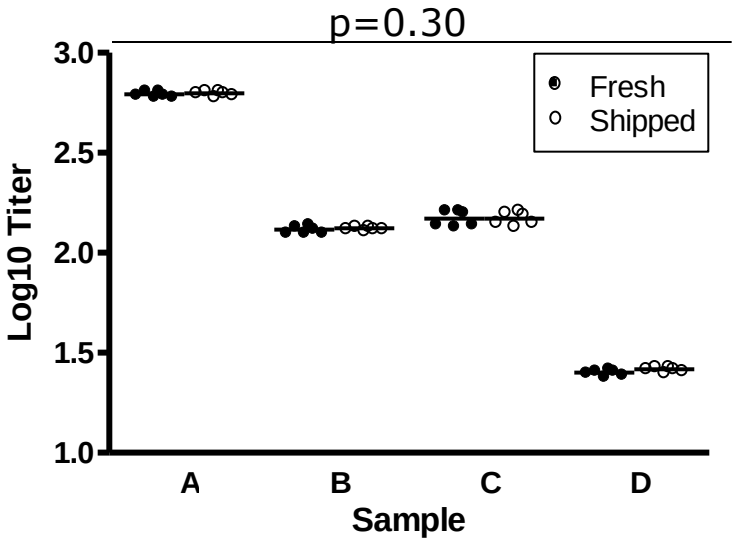
Shipment stability

Ad neutralization assay



CS ELISA



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

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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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  - Stefan Kostense
- Vector generation
  - Jerome Custers
- QA

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