Challenges and Learnings from Validation of AAV Neutralizing Antibody (NAb) Assays under IVDR

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6th June, 2024

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Introduction

Cell-based Neutralizing Antibody Assay (Nab) against AAVx capsid

- Compound type: AAV-based Gene Therapy
- Viral vectors effectively interact with human cells, deliver their genetic material, and express their proteins.
- Previous exposure to virus may compromise safety and/or efficacy of the therapy.
- Antibody titers have been assigned as inclusion or exclusion criteria for gene transfer therapy protocols.

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Introduction

Scope	1) **Screen patients for neutralizing antibodies for inclusion/exclusion purposes → IVDR** 2) Assess neutralizing activity as clinical endpoint → **GCP/IVDR**

- Assay already validated according to GCP requirements in the USA
- Assay currently in validation phase to fulfill IDE requirements in the USA

→ Best approach for both GCP and IVDR compliance in Europe?

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Approach: Hybrid validation

Regulatory Requirements for IVDR (In-house test approach)

- GCP compliance
- Single health institution
- ISO 15189 compliant processes
- Compliance with IVDR Annex I General Safety and Performance Requirements (GDPR), e.g. by setting up a Technical Documentation according to Annex II of IVDR

Assay Requirements

- Ability to detect neutralizing antibodies against AAVx capsid
- Analytical and clinical performance demonstrated during validation (extent of clinical performance depends on the claimed intended purpose)
- Validation setup should follow CLSI and/or EMA/FDA guidelines

→ How to address validation parameters (full-blown CLSI guidelines?)
→ What is different compared to a GCP validation? What can be done the same way?

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Validation Parameters

GCP Validation (FDA/EMA)	IVDR Validation (CLSI)
Cut point	Limit of Blank
Sensitivity	Limit of Detection
Selectivity	Limit of Quantification
Target Interference	Measuring Range
Drug Tolerance	Cross-reactivity
Precision	Interfering substances
Stability	Precision
	Stability
	Trueness
	Carry-Over
Testing a surrogate positive control is accepted	**Actual samples should also be tested**

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Limit of Blank

According to CLSI EP17-A2

- **Classical approach**
  - Run negative samples over multiple days by multiple operators (at least 60 datapoints)
  - Set a 95% threshold using a non-parametric (e.g. 95th percentile) or parametric approach (e.g. Mean Signal +/- 1.645 x SD) on normalized signals
  - same approach to cut point approach in clinical assays

→ LoB was assessed like a cut-point for clinical programs

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Limit of Detection

According to CLSI EP17-A2

- Run low positive samples over multiple days by multiple operators (at least 60 datapoints)
- Calculate pooled standard deviation (SD_L) of all results
- Calculate LoD as follows: LoD = LoB + 1.645 x SD_L
- **Final Result: Normalized Signal**

According to GCP guidelines

- **Calculation of assay sensitivity**
  - Spike negative pool/samples with surrogate positive control and run dilution curves (at least 6 curves)
  - Interpolate concentrations to the cut point for each curve
  - Calculate sensitivity as follows: Mean Sensitivity + t0.05 x SD, where t0.05 = one-sided confidence interval
  - **Final result: concentration of surrogate positive control**

→ LoD was assessed like sensitivity for clinical programs and reported as concentration

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Measuring Range

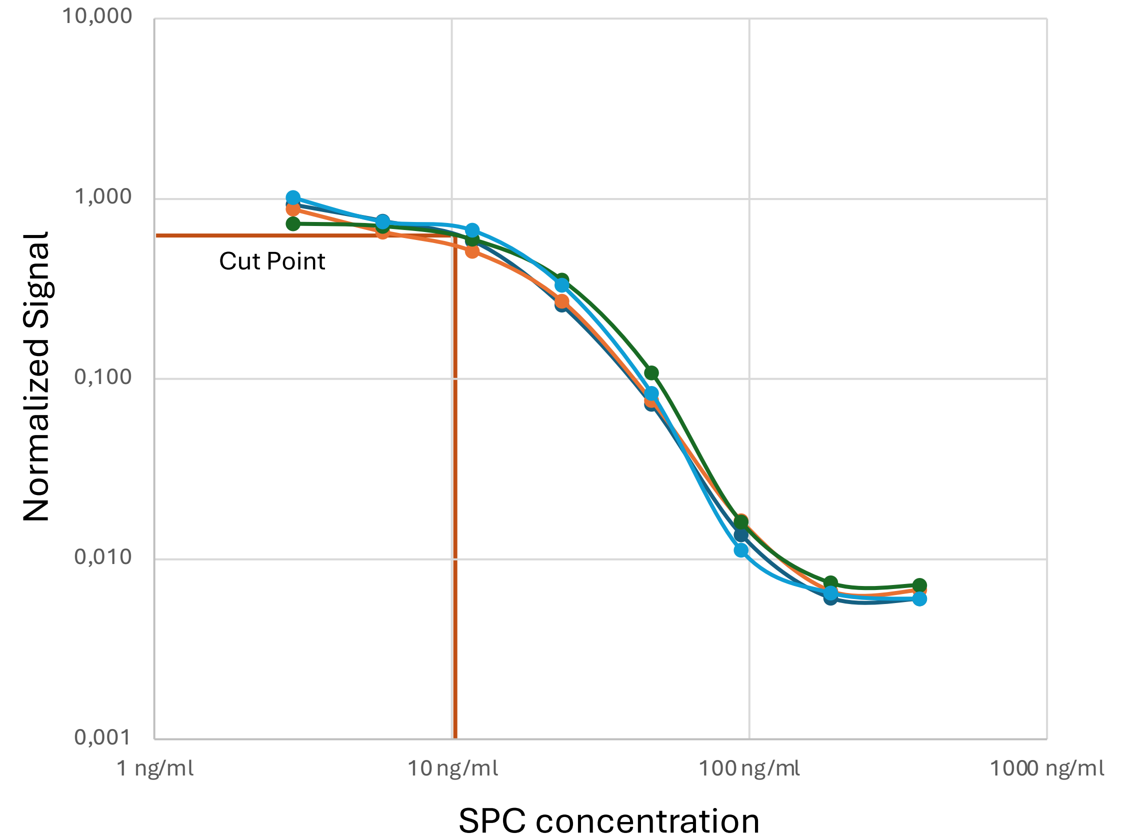
- **According to CLSI EP-6A**

- At least 5 concentrations should be tested in 2 replicates at each level.

➢ The surrogate antibody positive control (SPC) was tested at high concentrations and diluted in series in negative pool/samples.

➢ Hook effect at high antibody concentration was tested.

→ Measuring range was assessed as sensitivity and hook effect



Simulated data based on real case studies

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Cross Reactivity

- **Cross reactivity to other AAV serotypes**

  - ➢ An antibody against a different AAV serotype was tested in the assay
  - ➢ The antibody tested negative

  - ➢ QCs are spiked with capsids of other AAV serotypes
  - ➢ Capsids of 2 other serotypes were tested
  - ➢ No difference in the signal was observed

→ No cross reactivity was observed

→ Cross reactivity between AAV serotypes has been previously reported. Is there a need to test cross-reactivity?

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Interfering Substances

- **CLSI EP07 Section 5: Paired difference testing**
  - Samples spiked with interferent should be tested against unspiked sample
  - %difference of response is calculated to assess interference (should be below 20%)

Interfering Substances
Bilirubin conjugated
Bilirubin unconjugated
Cholesterol total
Glucose
Paracetamol
Triglyceride-rich lipoproteins
Heparin
Others

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Interfering Substances

- **Results**

Substance	Test concentration	Reference interval	Sample	Qualitative Interference (Pos/Neg)	Quantitative Interference (Interference >20%)
Bilirubin	0.4 mg/mL	0-0.002 mg/mL	NC	No	No
			LPC 1		
			LPC 2		
Glucose	10 mg/mL	0.74-1 mg/mL	NC	No	No
			LPC 1		
			LPC 2		
Cholesterol, total	4 mg/mL	<2 mg/mL	NC	No	No
			LPC 1		
			LPC 2		Yes

NC= negative control, LPC= low positive human serum sample

→ Quantitative interference was observed for 1 out of 3 samples for 4 substances.

→ Is the detected quantitative interference an artifact? Should this be assessed?

→ Qualitative interference was not seen. Interference was accepted.

Simulated data based on real case studies

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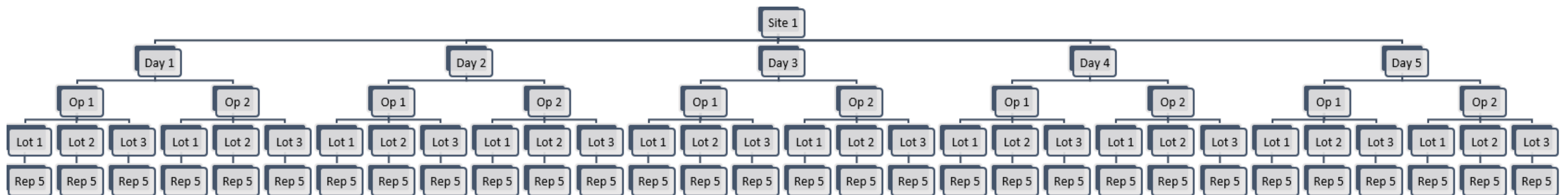
Precision

- **GCP Validation**
  - Assess precision of all QCs after cut point experiments in at least 6 runs (12 replicates per sample)
  - Involvement of several analyst and analytical instruments recommended
- **CLSI EP05-A3 Section 3 (Quantitative Measurements)**
  - For each sample: 20 days, 2 runs per day, 2 replicates per runs (80 replicates per sample)
  - Run similar setups with multiple instruments, multiple lots and multiple operators



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Precision

- **Hybrid solution**
  - 3 Samples were tested in a non-balanced approach: 10 days, 2 operators per day, 2 lots of cells, 3 replicates per run = 120 replicates per sample
  - 4 operators and 3 instruments were involved

		Between Run	Between Day	Between Operator	Between Instrument	Total (within Laboratory)	Acceptance Criterion
Sample	N	%CV	%CV	%CV	%CV	%CV	%CV
NC	120	7.2	6.1	5.8	3.4	8.6	20.0
LPC	120	12.4	8.2	7.8	10.1	16.6	20.0
HPC	120	16.8	15.2	14.6	18.1	17.9	25.0

NC= negative control, LPC/HPC= low/high positive human serum sample

- **Results from the GCP validation**

→ Precision with less replicates showed comparable results

→ Do more replicates add any value to the assay?

		Total (within Laboratory)	Acceptance Criterion
Sample	N	%CV	%CV
NC	12	13.8	20.0
LPC	12	16.1	20.0
HPC	12	21.7	25.0

Simulated data based on real case study

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Stability

CLSI EP25-A

Samples Short-Term and Freeze/Thaw Stability

- Assess stability in terms of measurand drift and other metrics (positive/negative testing)
- ➢ 3 samples (2 sets each) and 3 timepoints were tested

In-Use Reagent Stability

- Period of time that a regent remains viable once placed into use
- ➢ Critical reagents were left for longer periods at RT or ice. Freeze/thaw stability of the AAVx vector was tested

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Stability

Sample transportation stability

- ➢ Mimicked in the lab by storing the samples in dry ice

Reagent transportation stability

- Transportation stability of reagents is an essential parameter to be assessed for diagnostic products as they are shipped to multiple sites
- As we follow the in-house test approach, reagents are not intended to be shipped off-site

➢ Transportation stability was not assessed

→ Stability was assessed based on the drift analysis and qualitative results
→ Testing in-use reagent stability for IVDR requires additional efforts
→ Is it necessary to test sample transportation stability? LTS?

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Conclusions

- Clear guidance on expected scope for IVDR-compliant validations for bioanalytical assays is missing.
- A GCP validation can be considered state of the art. The extent of an IVDR validation probably should be more comparable to a GCP validation.
- Setup of the validation should be well aligned with all relevant stakeholders.

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Acknowledgements

BioAgilytix Colleagues	For the open discussions and efforts on implementing IVDR In Hamburg and for the support in preparation of this presentation.
Sponsors	For the projects and the collaborative work.
Tobias Bartl, CE Plus- regenold GmbH	For the great support in implementing IVDR and ISO.
European Bioanalysis Forum (EBF)	For organizing this workshop and for the opportunity to present.

Thank You!

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