Multiplex immunogenicity assay for a multicomponent vaccine: focus on specificity

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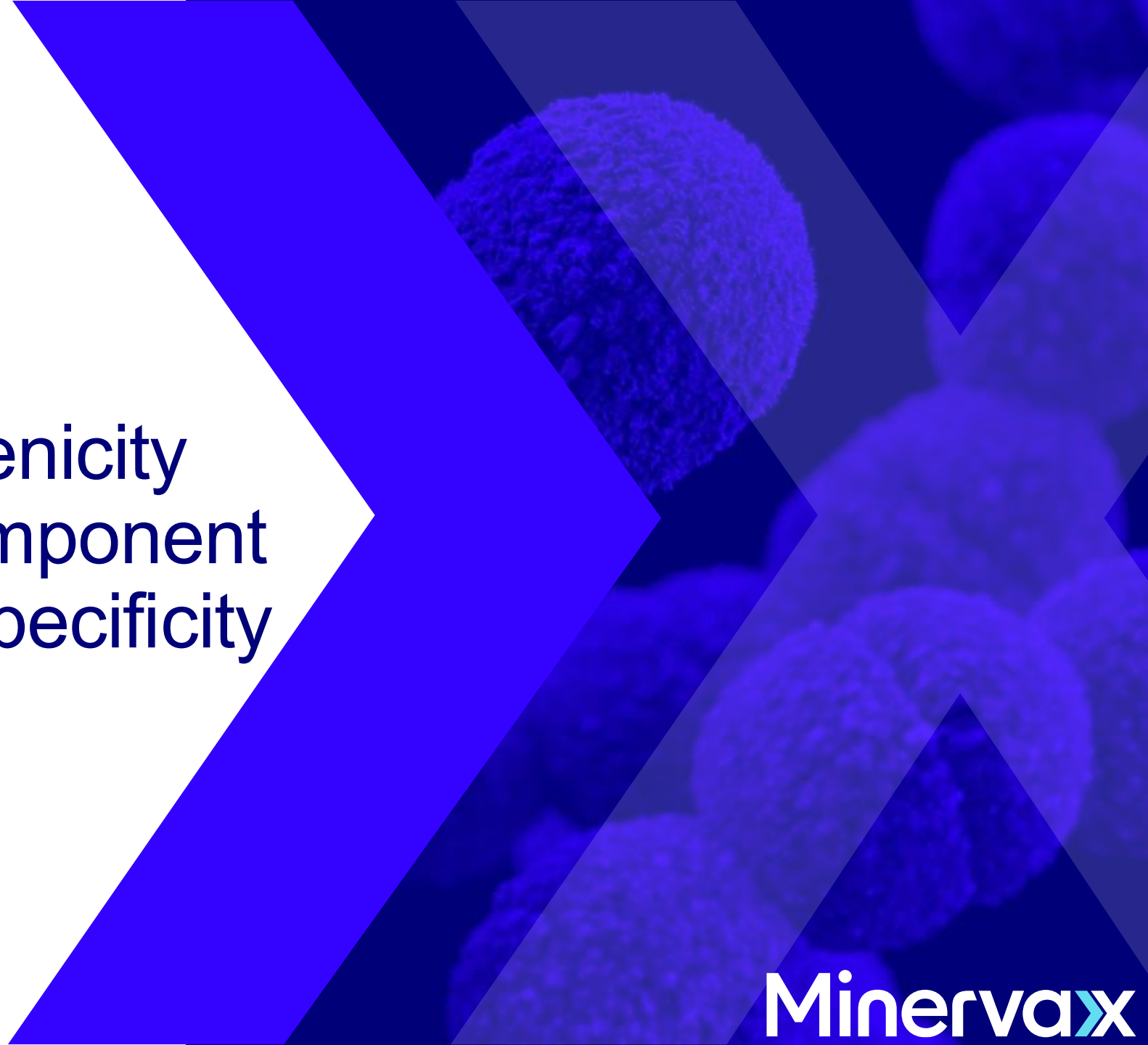

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Towards a lifesaving vaccine

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AGENDA

1. Intro to GBS infection and MinervaX protein vaccine
2. Bioanalytical strategy
3. The assay and context of use
4. What is the focus regarding specificity in vaccine assays and multiplex?
5. Method Development to Validation with focus on Specificity
6. Conclusions

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FOCUS ON GROUP B STREPTOCOCCUS (GBS) INFECTION

High prevalence

15-30%

of individuals globally have GBS in their gastrointestinal / urogenital tract

1 in 4

Adults carry GBS

Most

Adults are non-symptomatic

…with significant medical complications

In pregnant women and newborns

Adverse pregnancy outcomes (stillbirths and preterm delivery)

Responsible for 50% of neonatal life-threatening infections and 80% of meningitis in newborns

Responsible for stillbirths, premature births, newborn deaths and babies with long term disabilities

Neonatal infections in 0.5-2 in 1,000 live births (sepsis, pneumonia & meningitis)

- Early Onset Disease (EOD) at 0-6 days of age
- Late Onset Disease (LOD) at 7-90 days of age

In older adults (obese/diabetic)

Bacteraemia, SSTI 3, osteomyelitis, pneumonia and abscesses

28,000 cases in US alone

- 4 days hospitalization, 30% ICU, 5-10% fatal

… and need for a vaccination

In pregnant women and newborns

Maternal vaccination enable passive transfer of IgG to babies over the placenta

In older adult (obese/diabetic)

At risk

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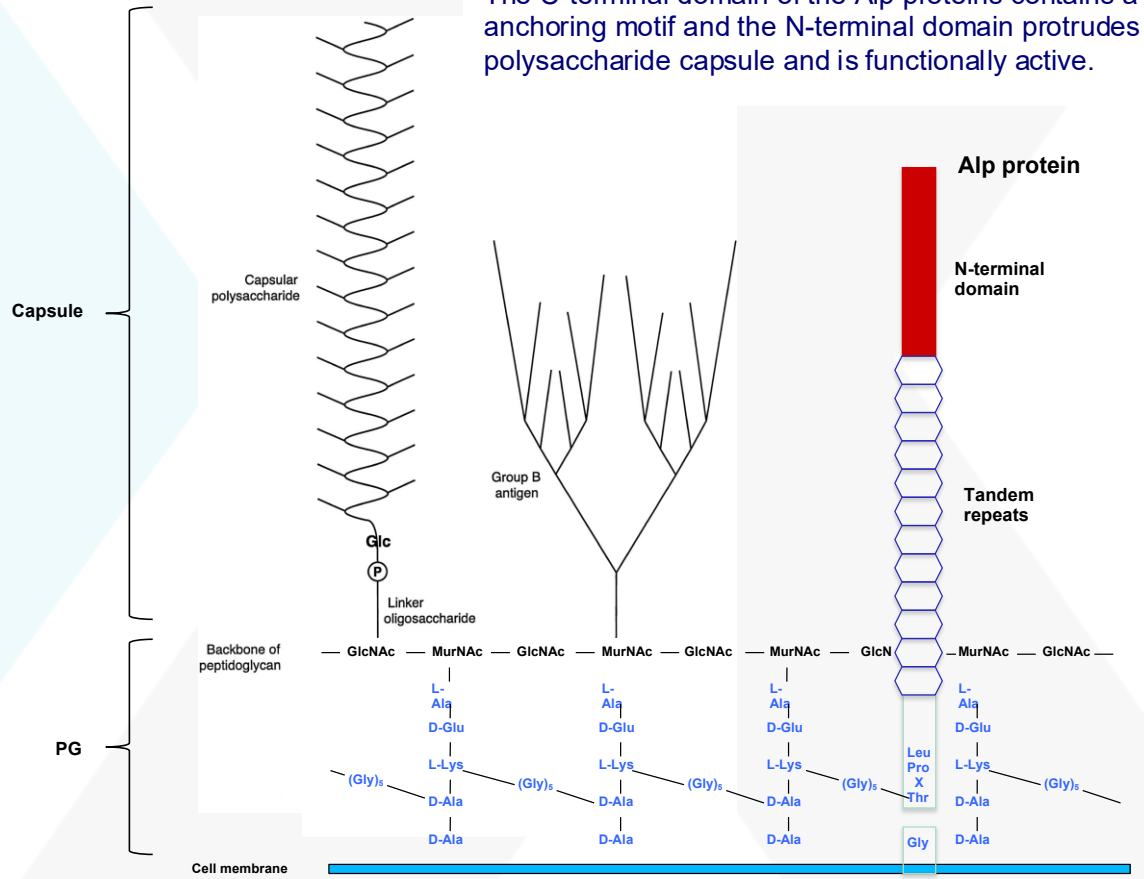
GBS CELL WALL AND ALP PROTEINS

- MinervaX GBS vaccine is protein-only based on the Alp-proteins
- Past/Competitor vaccines are based on Capsular polysaccharide (CPS)
- Six GBS Alp variants: AlphaC (AlpC), Rib, Alp1, Alp2, Alp3, and Alp4. Alp4 is extremely rare.
- Extracellular exposure and exceptionally broad coverage of clinical isolates, makes Alp N-terminal domains (AlpNs) highly relevant as vaccine candidates.

GBS Capsule and Cell membrane

The C-terminal domain of the Alp proteins contains a cell wall-anchoring motif and the N-terminal domain protrudes from GBS' polysaccharide capsule and is functionally active.



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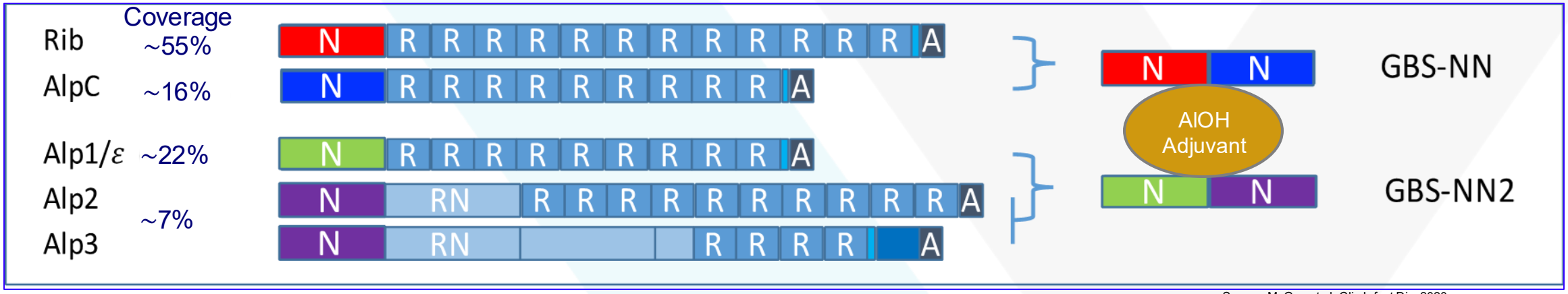
ALPN PROTEIN-BASED GBS VACCINE



Source: McGee et al. Clin Infect Dis. 2020 Feb 15

Vaccine Target

- N-terminal domain of highly conserved Alpha-like **GBS surface protein** family, AlpN
- Limited antigenic drift despite presence of natural protective immunity

Vaccine Composition

- AlpN vaccine consists of two fusion proteins, GBS-NN and GBS-NN2
- $Al(OH)_3$ adjuvant
- *AlpN cover >99% of all clinical isolates, both invasive and colonizing isolates

Functional Antibodies

- AlpN domains induce protective antibodies
- Killing GBS via opsonophagocytosis
- And blocking entry of GBS across epithelial cell barriers

Dual Mode of Action

- Direct protection of immunized individual (pregnant person and older adult)
- Transfer of antibodies across placenta protects infants for first 3 months of life

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CONTEXT OF USE

- IgG concentration assay against each vaccine AlpN protein.
- Results of future sample analysis in future studies will be used to compute population Geometric Mean Concentration (GMC) and maternal transfer of anti-AlpN IgG against each individual vaccine protein components from the vaccinated mother to the infant.
- Supportive to surrogate endpoint marker (SEM) for natural protection (natural history of GBS infection in mothers and infants) and vaccine efficacy for protection of disease in parallel to functional immunogenicity assays.
- Range of assay is on a log scale ranging from approximately 0.010-150 µg/mL (or above) according to results using ELISA in Phase 1 and Phase 2.

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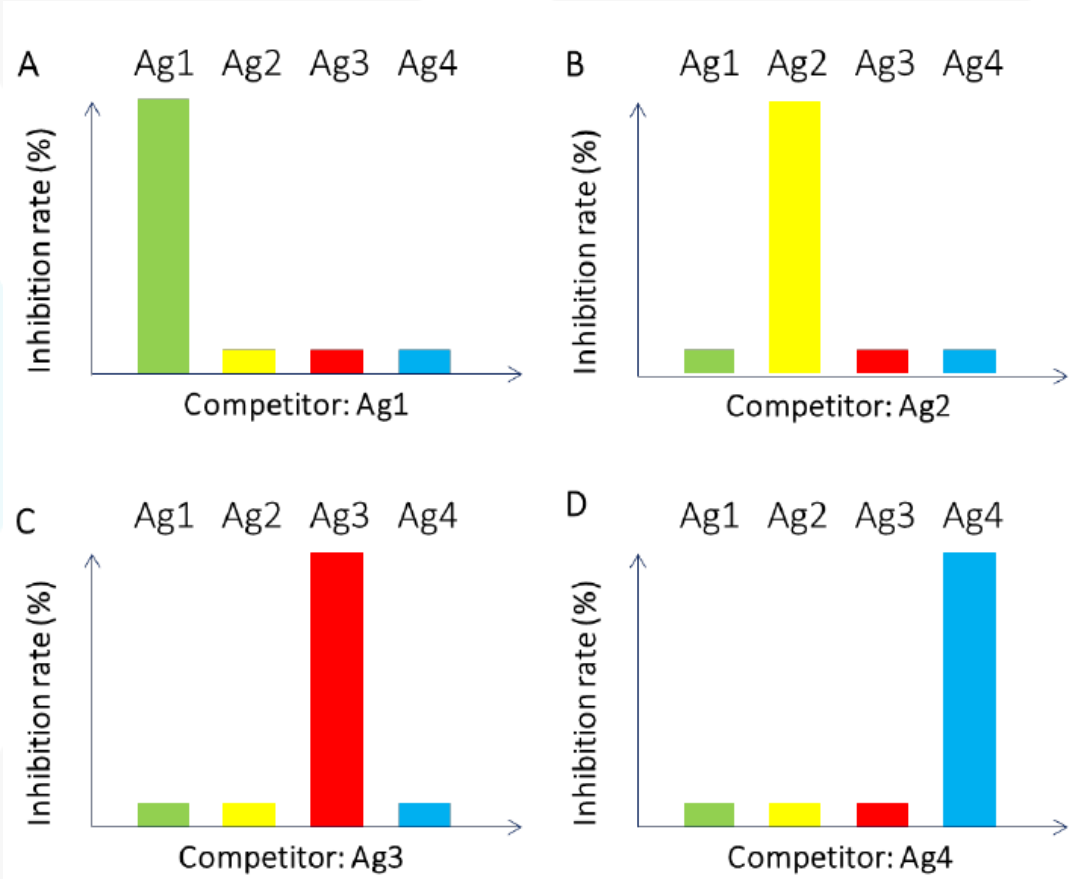
FOCUS ON SPECIFICITY IN MULTIPLEX VACCINE ASSAYS?

- **Dessy et. al. 2024 (WRIB paper)**
- Specificity is one of the most important assay parameters of vaccine immunogenicity
- Specificity is usually shown by:
  Antigen-specific antibodies, without cross-reactivity by the simultaneous presence of other antibodies or antigen-antibody complexes.
  - ≥ 80% inhibition is expected using homologous antigen inhibition.
  - For a multiplex assay (e.g. Luminex, MSD platform), e.g, for a 4-plex; four different competition conditions are required.
  - In the example, each competition condition only impacts the antibody titer of the homologous antigen.
- WRIB paper summary: Multiplexed assay can be considered specific when the results demonstrate no cross-reactivity for the coated antigens.
- MinervaX note: The paper does not include discussion regarding expectations when the target infection for vaccine have a high degree of natural history with various clinical isolates, both invasive and colonizing isolates.



Dessy et al. Harmonization of Vaccine Ligand Binding Assays Validation. Bioanalysis 2024

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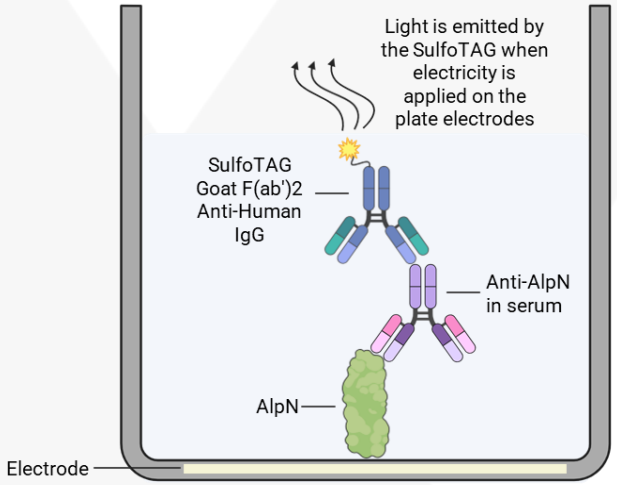
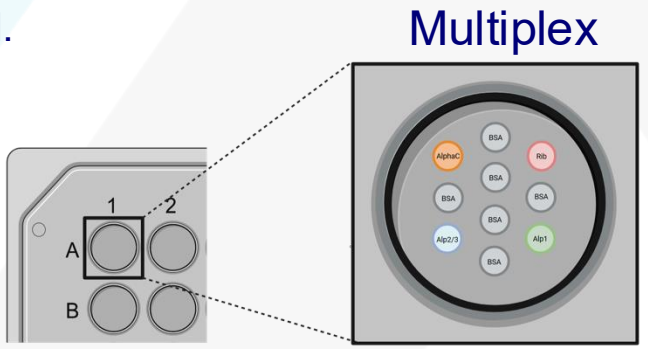
THE MULTIPLEX ASSAY

- Method was transferred to electrochemiluminescence format from ELISA (direct coating)
- Multiplex and Singleplex formats was developed at Meso Scale Discovery (MSD) and then transferred for further extensive optimization.
- The reference serum is a pool of multiple sera collected from vaccinated non-pregnant females after administration of a booster vaccination with GBS-AlpN vaccine (ie Phase 2 follow-up study). Used for calibration standards and quality control samples (QC).
- Concentration of reference serum was based on bridging to previous ELISA reference serum.
- Calibration standards, QCs and serum samples are added to the plates, each in single wells at a minimal required dilution (MRD) of 1000-fold.

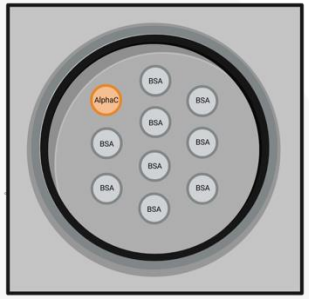


Antigen	Spot position
Alp1N	10
Alp2/3N	3
AlphaCN	1
Rib	8



Multiplex

Singleplex



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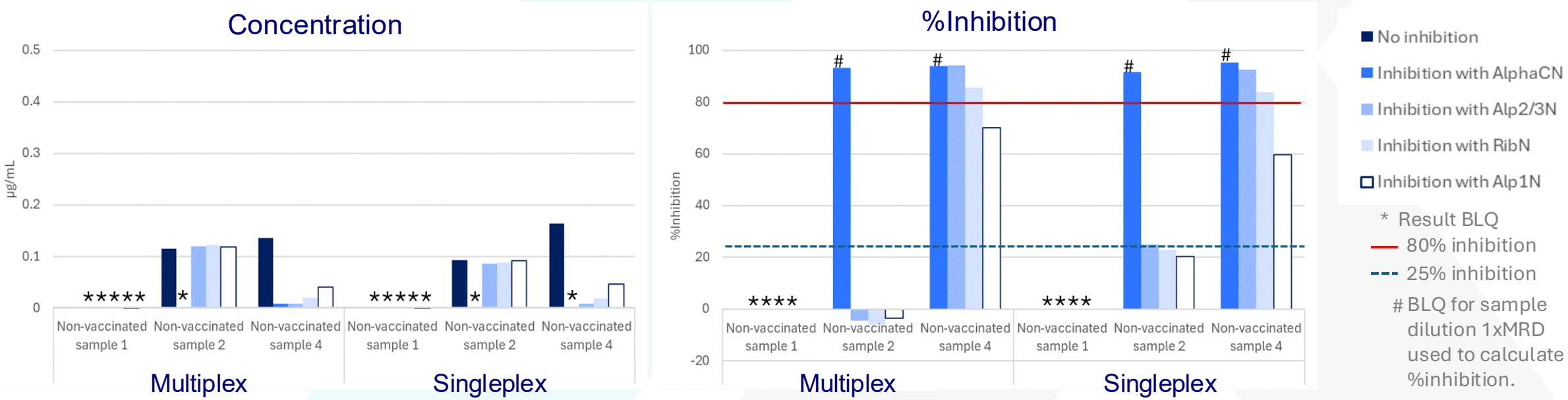
SPECIFICITY DATA DURING ASSAY DEVELOPMENT

- Samples from non-vaccinated (natural history of infection) show different degree of cross-reactivity.
- Similar pattern observed across coated antigen and in samples from vaccinated.
- No difference between singleplex and multiplex.

AlphaCN as coated antigen as an example



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PROOF OF CONCEPT FOR ANALYTICAL SPECIFICITY IN ALPN MULTIPLEX ASSAY USING MABS

The mAbs are fully human and were cloned and produced internally at MinervaX from vaccine-specific memory B cells sorted by FACS from PBMC obtained from MinervaX clinical phase 1 and 2 studies.

	Alp1N [Kd/NEG]	**Alp2/3N [Kd/NEG]**	**AlphaCN [Kd/NEG]**	**RibN [Kd/NEG]**
mAb 44	NEG	NEG	$3.9x10^{-10}$	NEG
mAb 74	NEG	$3.5x10^{-09}$	NEG	$6.0x10^{-08}$
mAb 103	NEG	NEG	NEG	$4.3x10^{-10}$
mAb 123	$4.8x10^{-10}$	$4.7x10^{-10}$	$1.1x10^{-9}$	$2.2x10^{-09}$
mAb 148	$4.1x10^{-10}$	NEG	NEG	NEG
mAb 154	NEG	$2.5x10^{-10}$	NEG	NEG
mAb 166	$2.7x10^{-10}$	$8.0x10^{-10}$	NEG	NEG
mAb 177	$6.6x10^{-10}$	$2.6x10^{-10}$	$2.4x10^{-09}$	NEG

Single cell antibody variable gene (VDJ) was sequenced and mAbs were developed for research purposes.
The mAbs have been characterized for reactivity by AlpN IgG ELISA and SPR Biacore technology.
Total protein concentration of each purified mAb was also determined by SPR, using a Mabtech calibrator standard.

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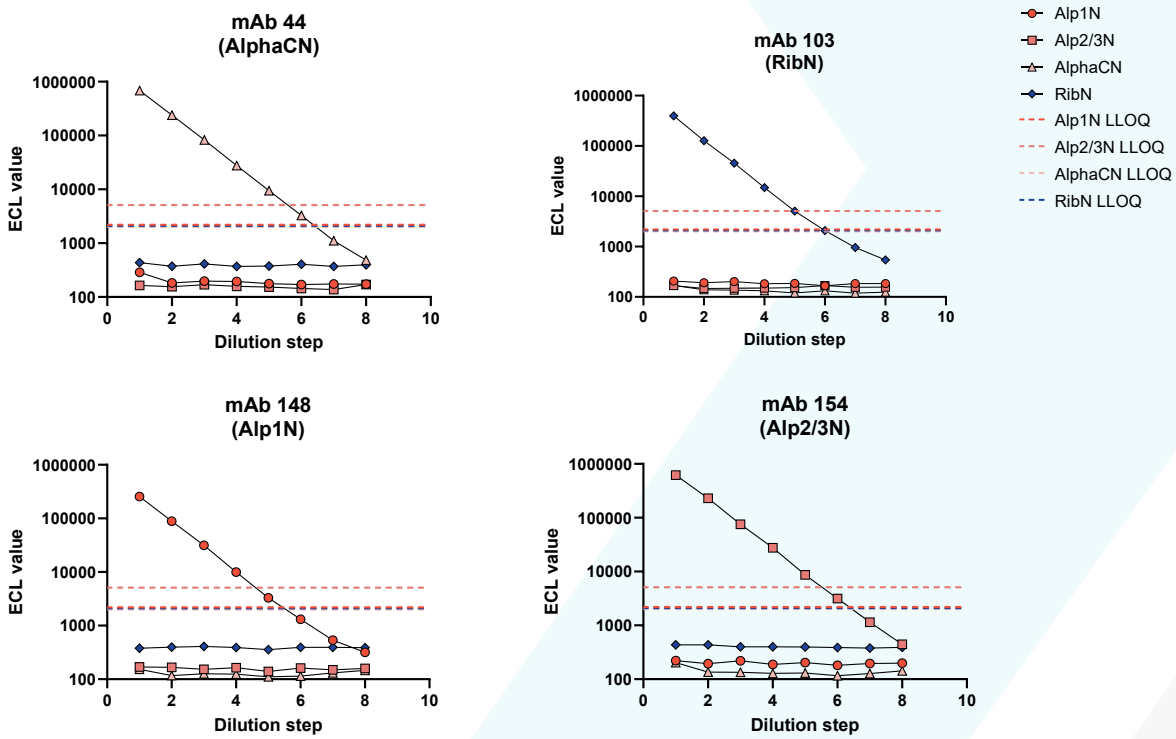
MONO-SPECIFIC MABS ARE DETECTED/INHIBITED BY SINGLE ALPN PROTEIN

Serial dilution vs Signal

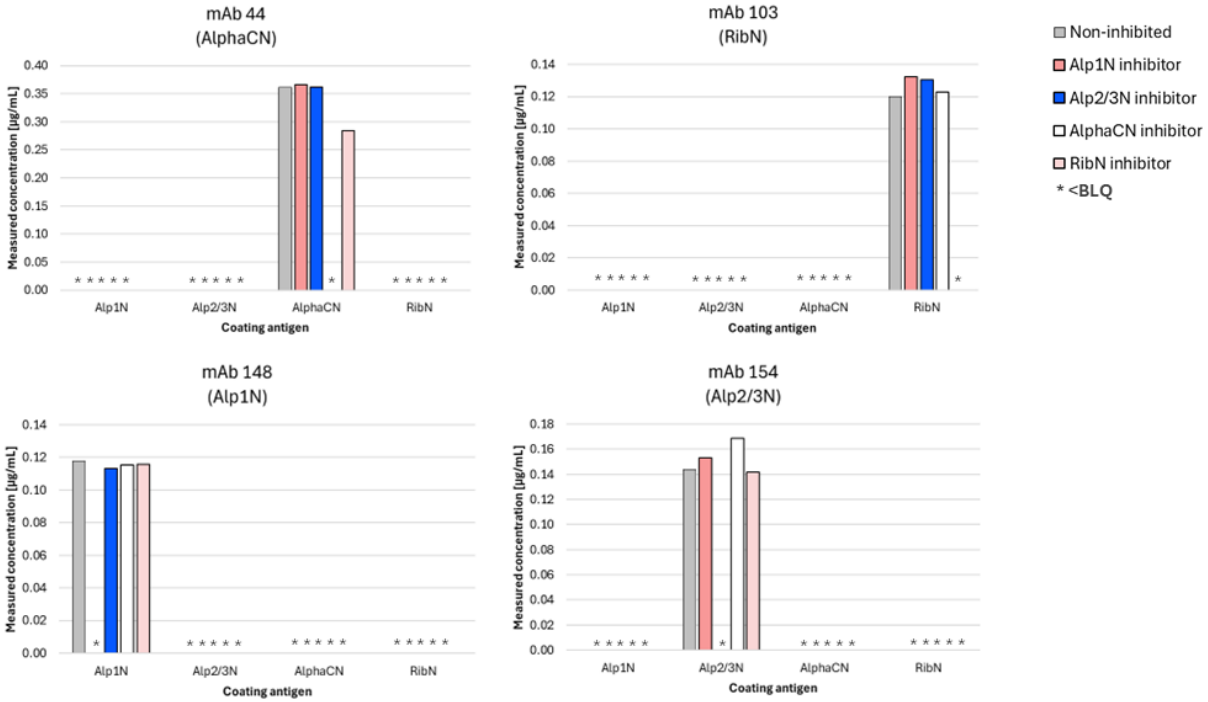
mAb Concentration range: 0.002-5 µg/mL

Solid lines represent antigen coating
Stippled lines represent LLOQ



Concentration without and with inhibition

mAb Concentration: ~0.5 µg/mL



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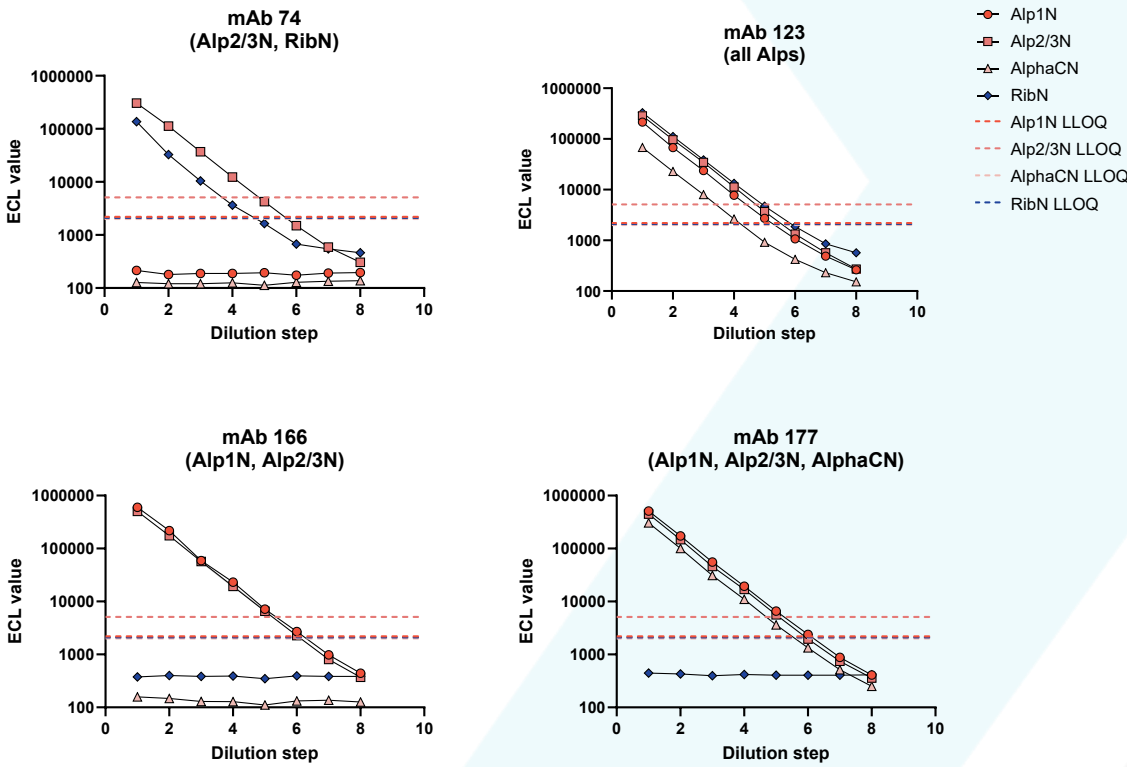
MULTI SPECIFIC MABS ARE DETECTED/INHIBITED BY MORE THAN ONE ALPN PROTEIN

Serial dilution vs Signal

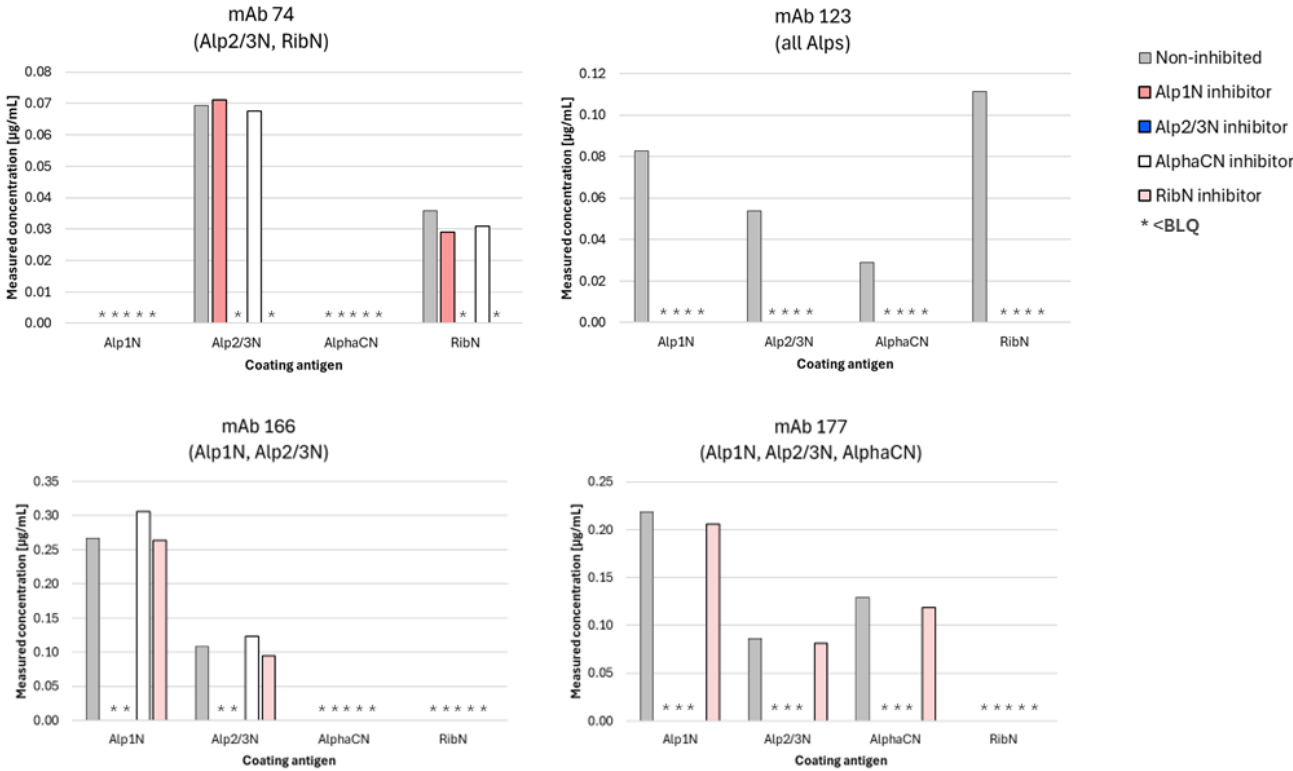
mAb Concentration range: 0.002-5 µg/mL

Solid lines represent antigen coating
Stippled lines represent LLOQ



Concentration without and with inhibition

mAb Concentration: ~0.5 µg/mL



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ASSAY VALIDATION WITH APPROVED RESULTS

- Tested in both singleplex and multiplex
- Analytical Range/Limit of Quantification (LLOQ and ULOQ)
- Precision
- Relative accuracy
- Dilutional Linearity
- Dilutability/Parallelism
- **Specificity: today's focus**
- Sample stability (short term only in multiplex)

- Reference serum (pool of multiple sera collected from vaccinated non-pregnant females) as calibrator/QCs and for Precision/Relative Accuracy/Linearity in 8 different dilutions.
- 20 samples of 4 different sample types, all tested in Precision/Relative Accuracy/Linearity in 5 different dilutions within analytical range. Each of the 20 samples in one dilution was used for specificity.
  - Infant, unvaccinated mother (n=5)
  - Infant, unvaccinated mother (n=5)
  - Unvaccinated mother (n=5)
  - Vaccinated mother (n=5)
- 5 vaccinated adult samples were used for Dilutability into analytical range and Parallelism, since these samples are the highest antibody concentration samples available.

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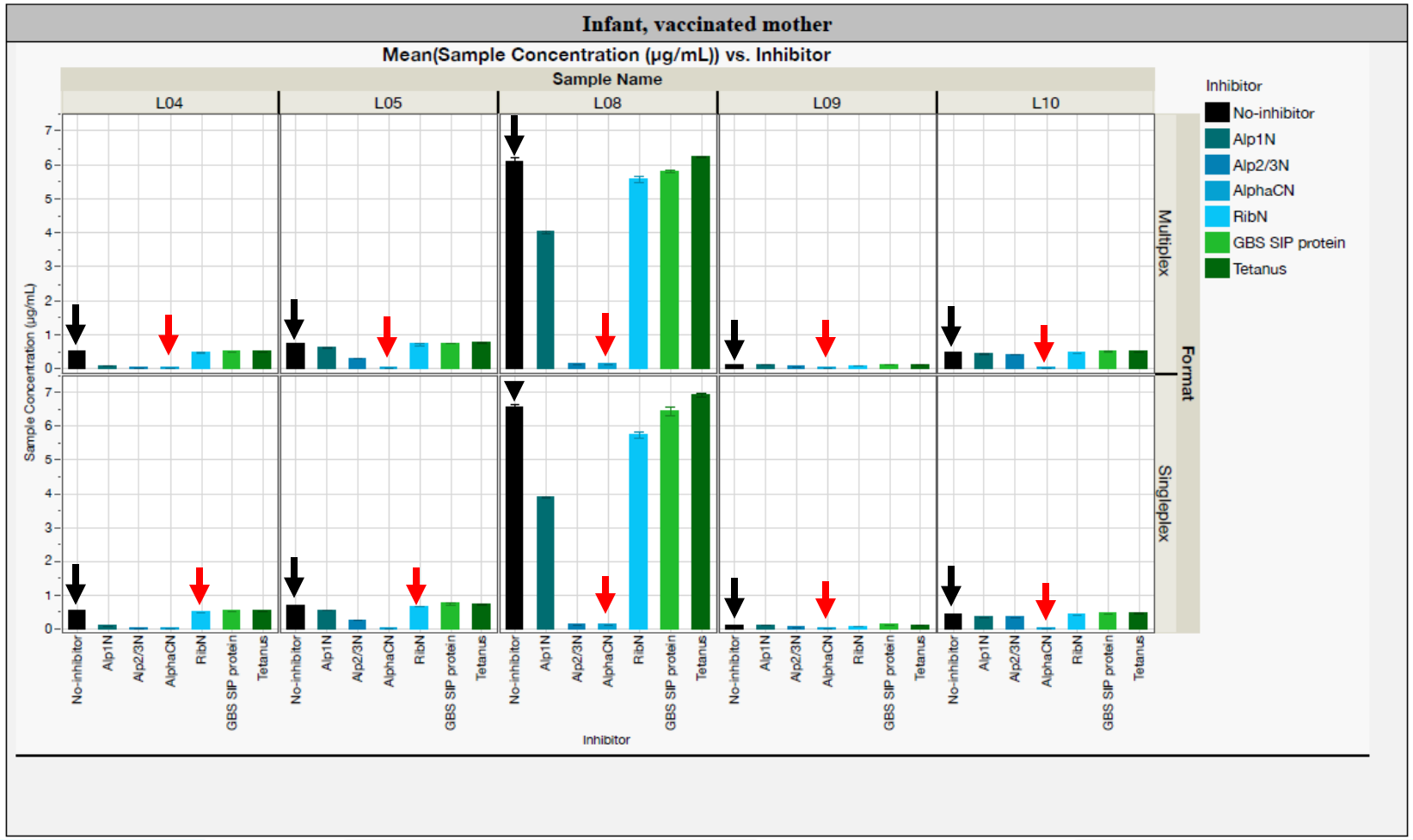
SPECIFICITY WAS CONFIRMED DURING METHOD VALIDATION

Anti-AlphaC (coated antigen) used as an example

- 5 infant samples (babies born to vaccinated mothers) as example show varying concentration (↓) and varying inhibition.
- Homologous specificity between identical AlpN protein coating and inhibition was ≥ 80% (↓).
- There was no cross-reactivity to heterologous non-AlpN bacterial proteins (SIP/Tetanus).
- No difference between singleplex and multiplex ECLIA plates.

Concentration with and without competition antigens against AlphaCN coated spots



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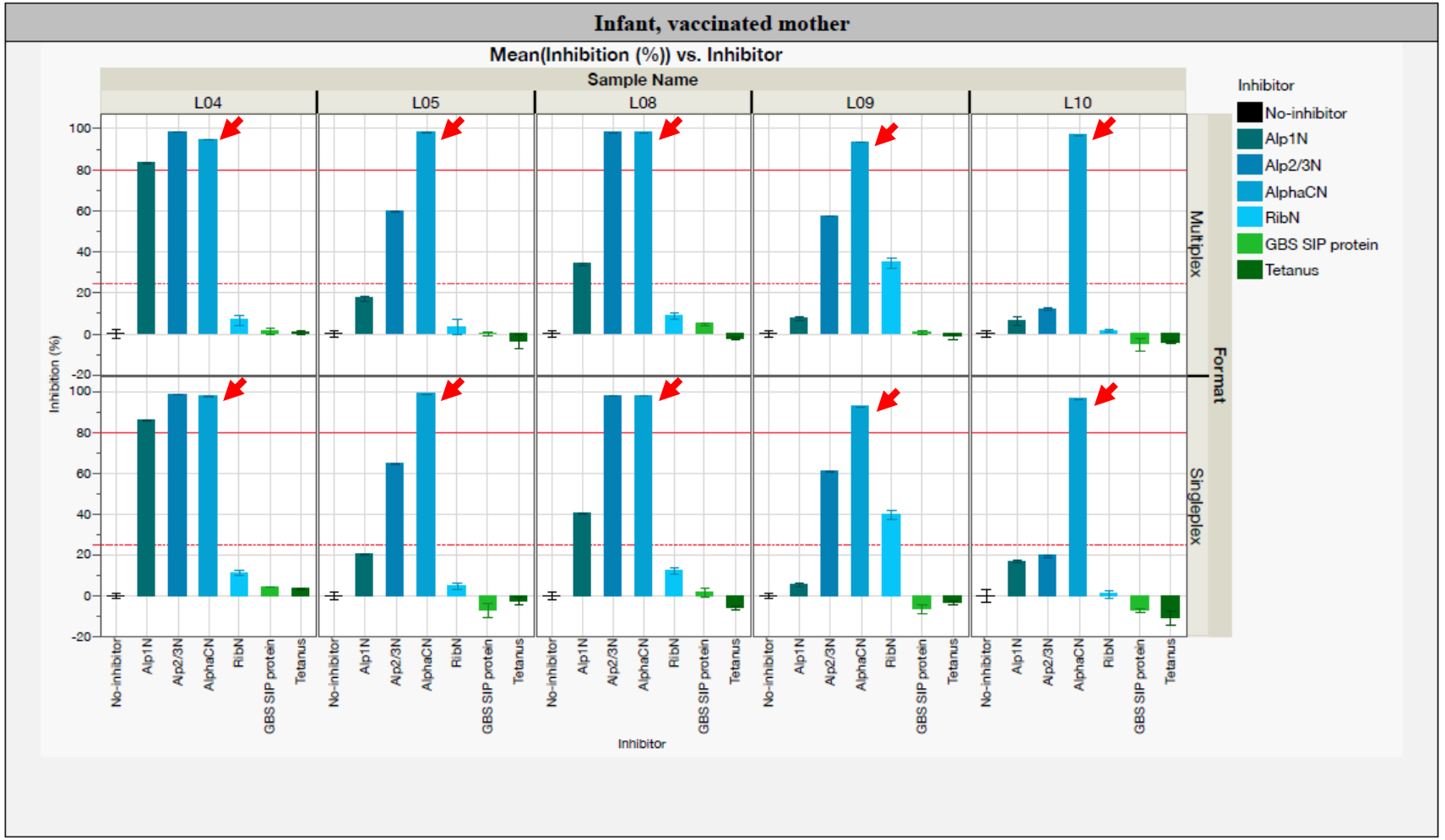
%Inhibition with and without competition antigens against AlphaCN coated spots



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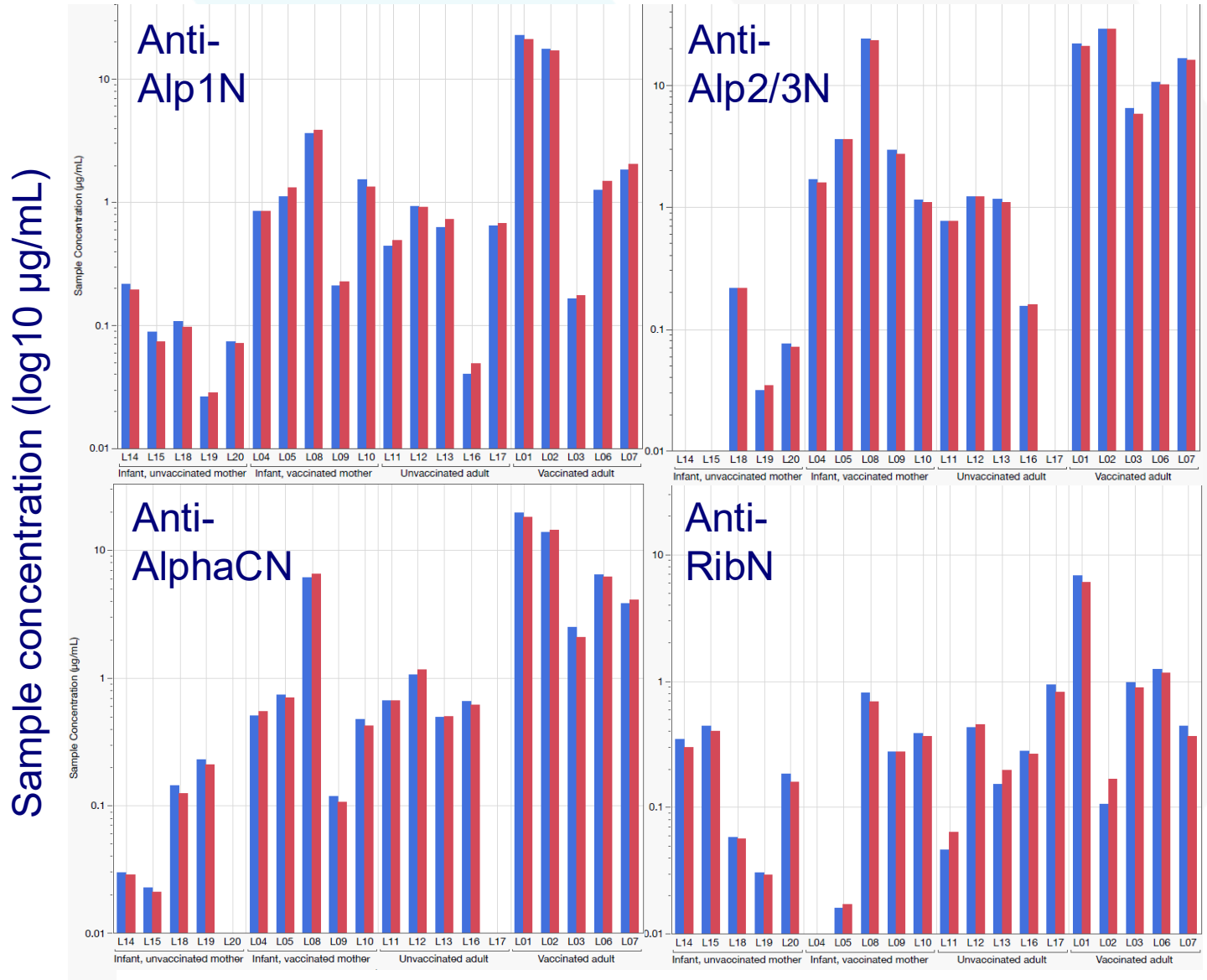
MULTIPLEX VS SINGLEPLEX IN 20 SAMPLES (4 SAMPLE TYPES)



Red bars: Singleplex
Blue bars: Multiplex

All concentrations in the figure as non-inhibited samples.

Analyte	Median Ratio between singleplex and multiplex (%CV)
Anti-Alp1N	94.9%
Anti-Alp2/3N	102.3%
Anti-AlphaCN	105.1%
Anti-RibN	105.6%

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SPECIFICITY WAS ACCEPTABLE IN THE 4 VACCINE ANTIGENS AND THE 4 SAMPLE TYPES

- Homologous and heterologous specificity was confirmed across sample types and across antigens.
  - 89.3% of evaluable individual samples had ≥ 80% inhibition with homologue inhibitor for both Multiplex and Singleplex, while 100% of evaluable individual samples had ≤ 25% inhibition with heterologous inhibitors for both Multiplex and Singleplex
  - 20 samples of 4 different sample types:
    - Infant, unvaccinated mother (n=5)
    - Infant, unvaccinated mother (n=5)
    - Unvaccinated mother (n=5)
    - Vaccinated mother (n=5)
- Cross-reactivity showed varying degree between AlpN proteins and between samples as expected due to history of natural infection.

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CONCLUSION

- MinervaX AlpN ECLIA demonstrates:
  - Comparison between singleplex and multiplex have a neglectable and non-systematic difference between the measurements, and the formats can be used interchangeable.
  - Good assay performance with specific measurement of individual AlpNs in both multiplex and singleplex format.
  - Cross-reactivity between anti-Alp1N, anti-Alp2/3N, anti-AlphaCN, anti-RibN antibodies are expected due to natural history of infection.
  - Specificity and cross-reactivity has been confirmed in >20 samples from 4 sample types and 8 mAbs.
- Recommendation for the vaccine community: Harmonize on expected specificity in immunogenicity assays when the target infection for vaccine has a high degree of natural history with various clinical isolates.

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ACKNOWLEDGEMENTS

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Thank you!

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