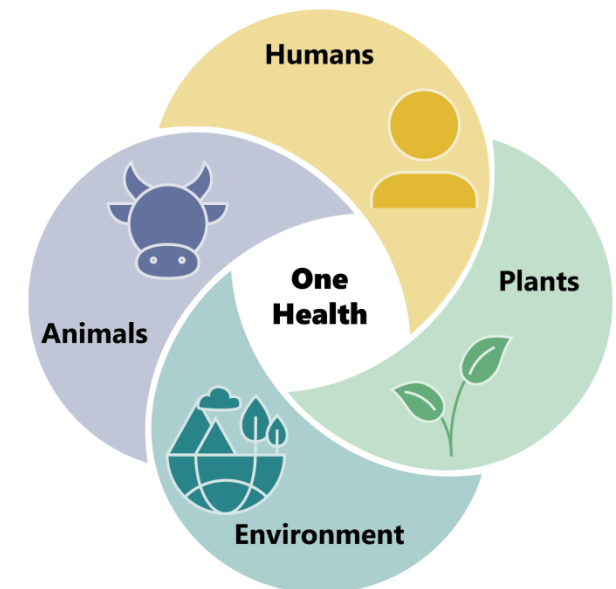


Potency testing of aluminium adjuvanted tick borne encephalitis virus vaccines

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Tick borne encephalitis virus



- Flavivirus, mainly transmitted by ticks
- Endemic in Middle, Northern and Eastern Europe and North Asia (european subtype)
- Many cases undetected, but in cases of clinical manifestation causes meningitis, encephalitis, myelitis, radiculitis with fatality rate 0.5 – 2 % and often permanent neurological disorders (european subtypes)
- 3 structural proteins (M, C and E), E is main target for antibodies

Tick borne encephalitis virus vaccines



- 2 vaccines licensed in the European Union
- inactivated whole virus vaccines, aluminium adjuvanted
- good protection: 99 % with regular and > 90 % with irregular vaccination schedules (Raffl et al, Vaccine 2025)

Development of ELISA methods for potency assays

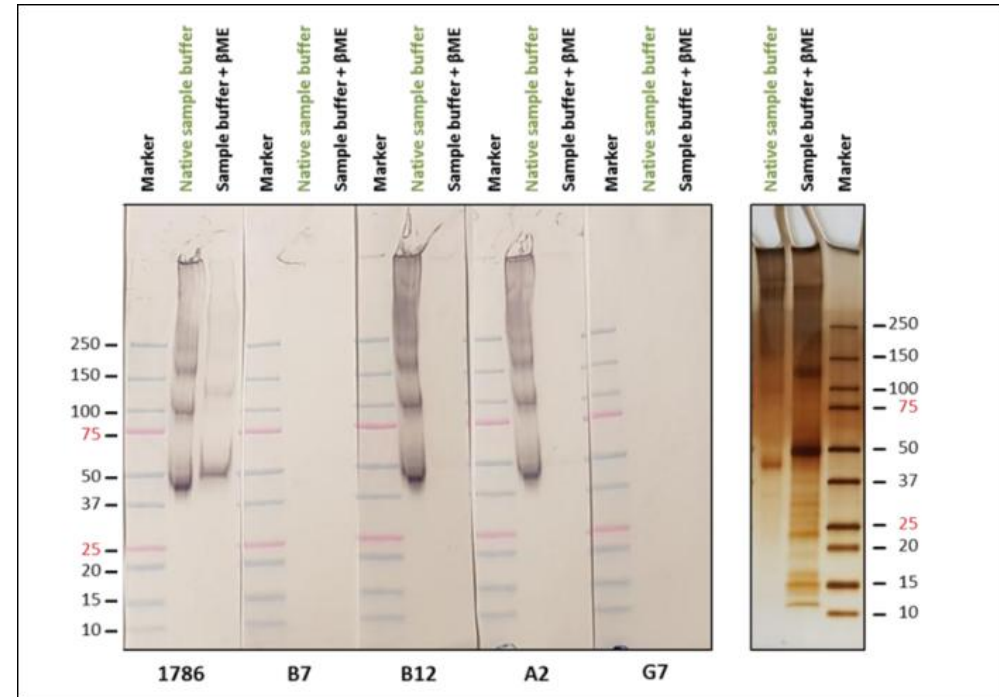
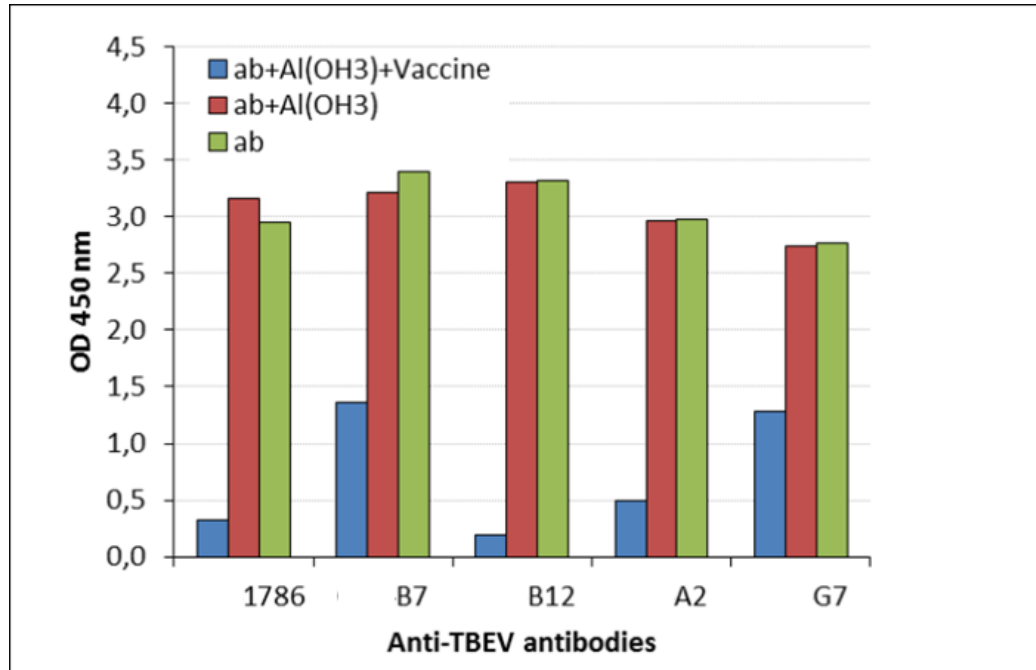


Selection of antibodies

- Independent method development for the two vaccines (VAC2VAC project)
- Antibodies were provided by the manufacturers
- Characterisation of antibodies was based on different methods may including Western Blotting, interaction of antibody with degraded antigen (eg. pH, detergents, heat stress / accelerated temperature), epitope mapping and neutralization assays

Method development was partially carried out as part of the VAC2VAC project. The VAC2VAC project received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement Nr 115924. This Joint Undertaking received support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

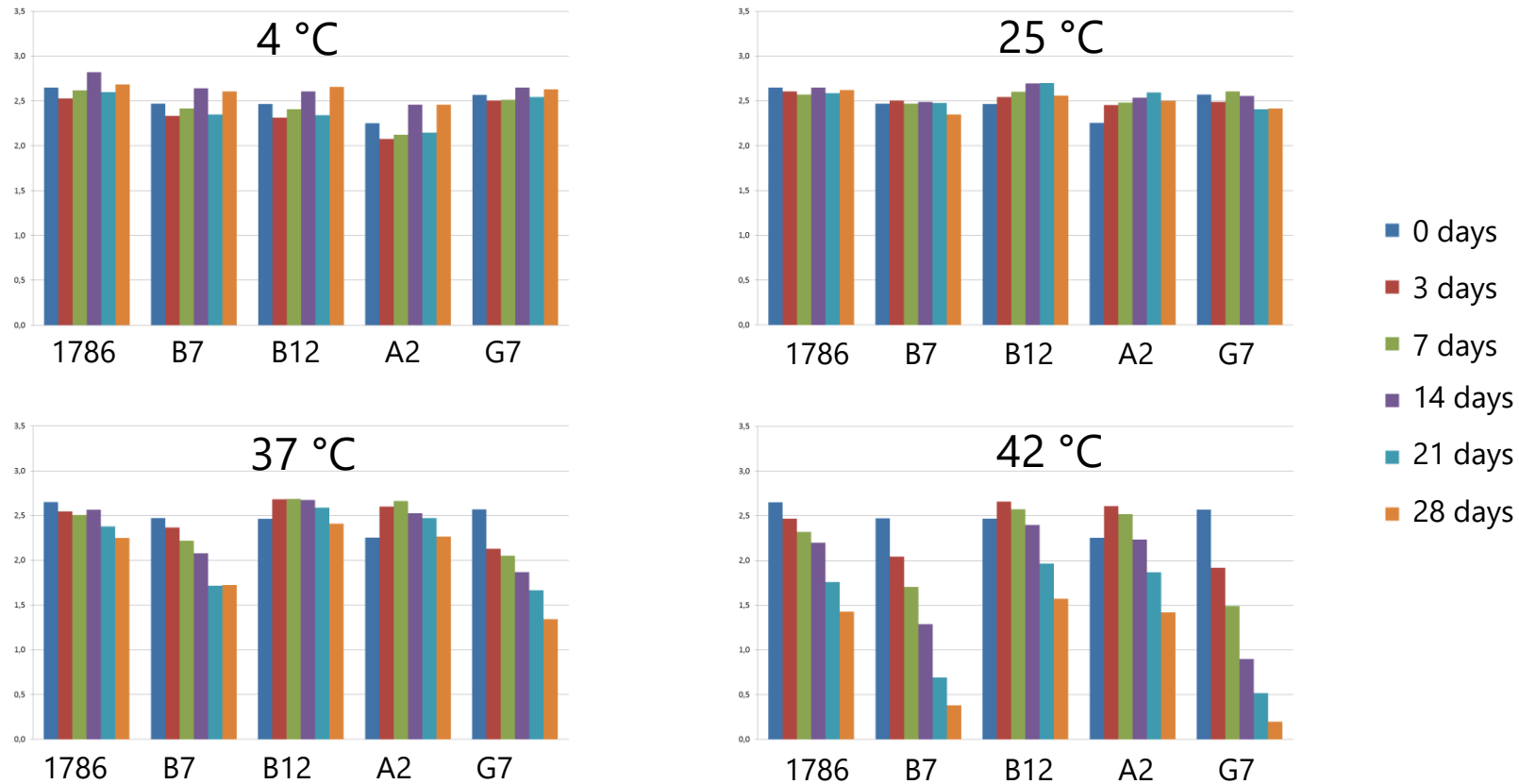
Binding of antibodies to adjuvanted antigen and Western Blotting



Antibodies bind to adjuvanted TBEV in solution (depletion of free antibodies in solution)

Different binding of antibodies to TBEV after SDS-PAGE and Western blotting indicating differences in sensitivity of binding to denaturation and/or SDS treatment of samples

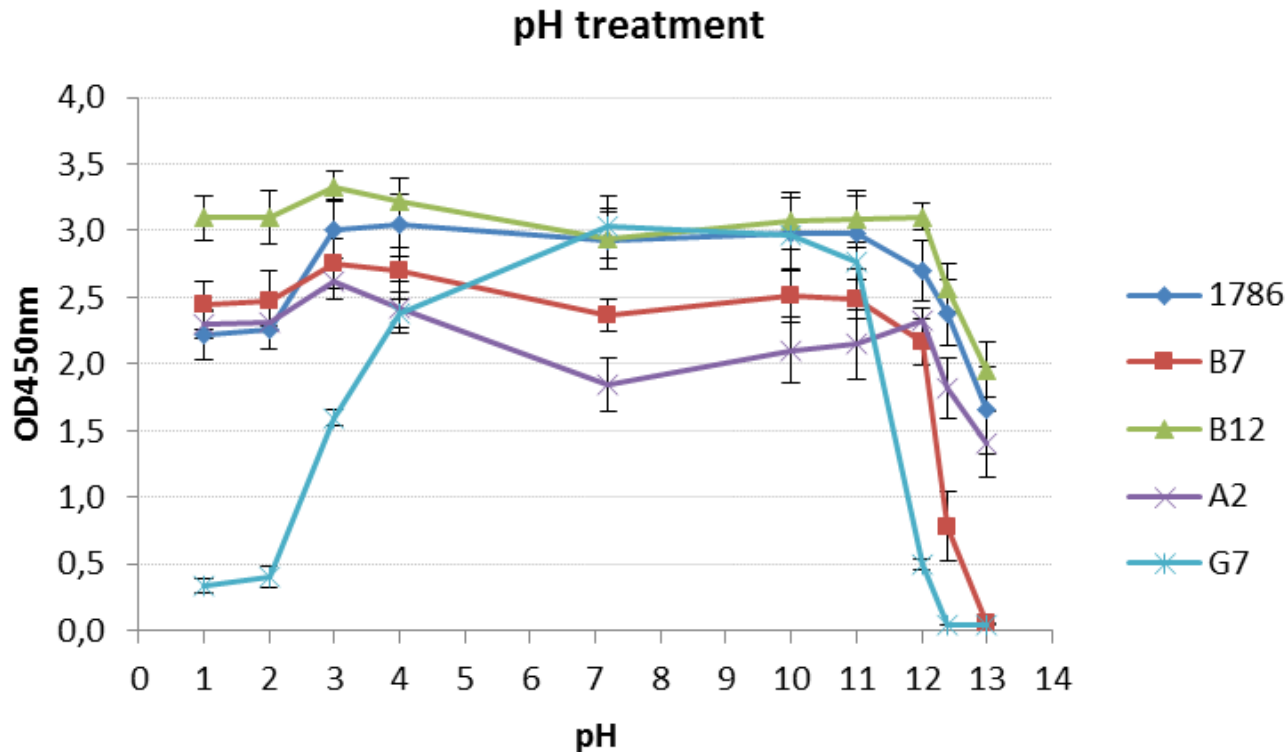
Heat stress



ELISA plates coated with antigen (inactivated virus, non-adsorb), stored at the indicated temperature for the indicated time

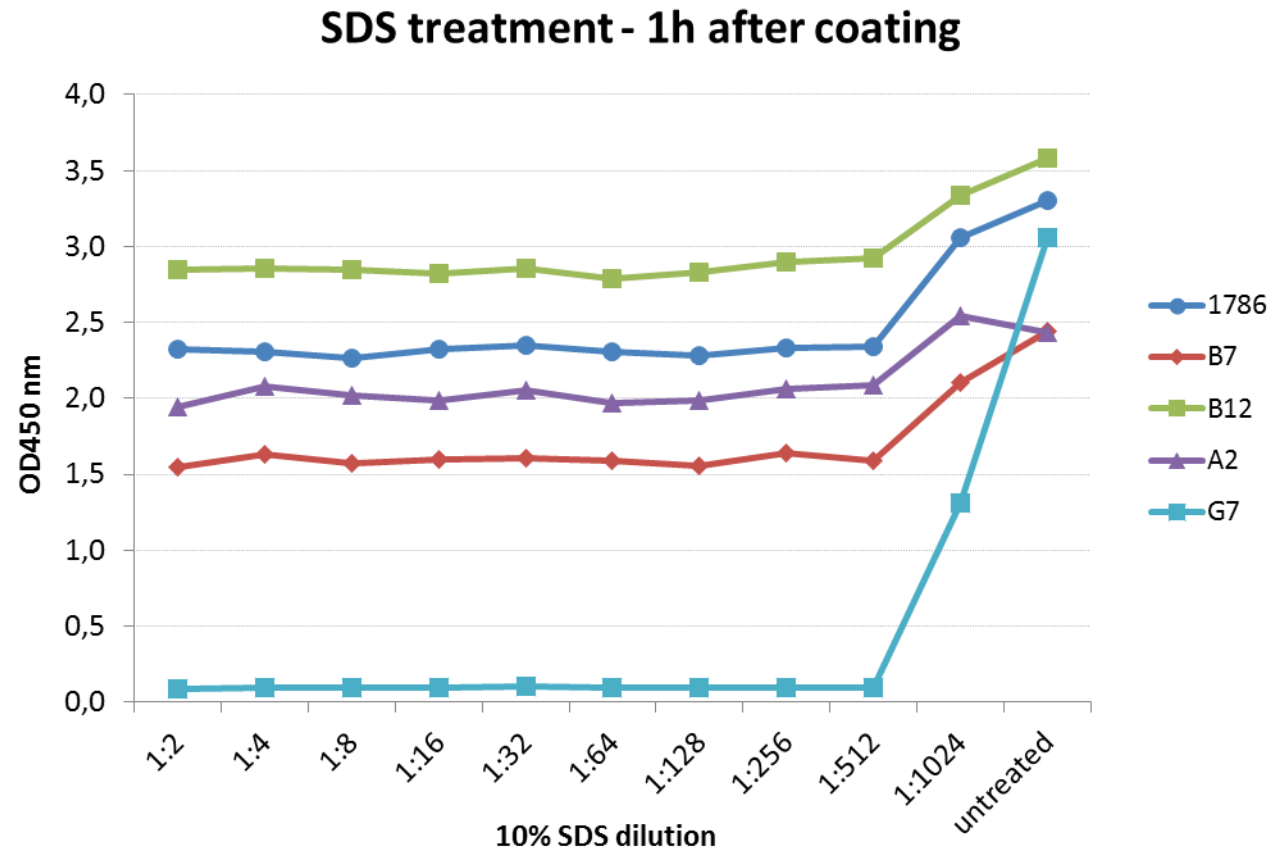
pH treatment

Coated antigen treated with different pH before direct ELISA



ELISA plates coated with antigen (inactivated virus, non-adsorb) and treated with different pH for 1 h, RT

SDS treatment



Selection of antibodies - conclusions

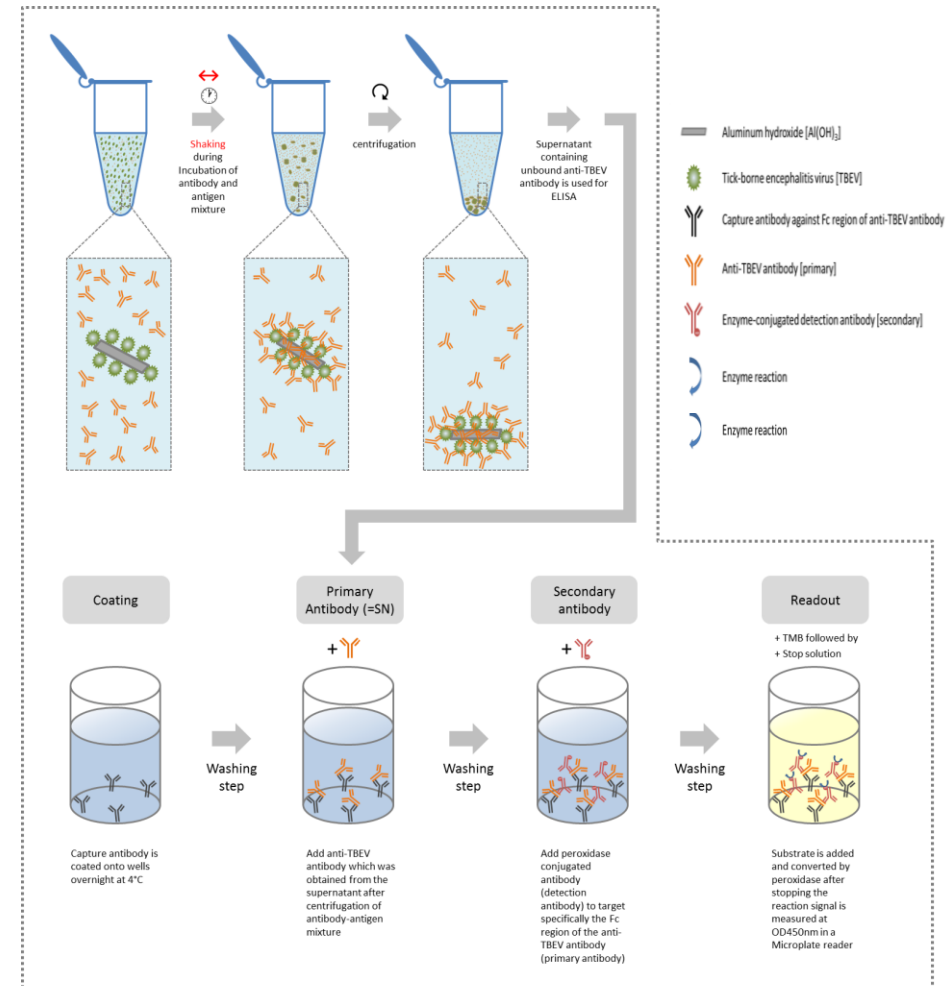


- mAb 1 (G7): detection and quantification of intact antigen (conformational antigen)
- mAb2 (A2): quantification of available antigen
- The binding of mAb1 is a surrogate for potency

Development of ELISA methods in the presence of aluminium hydroxide

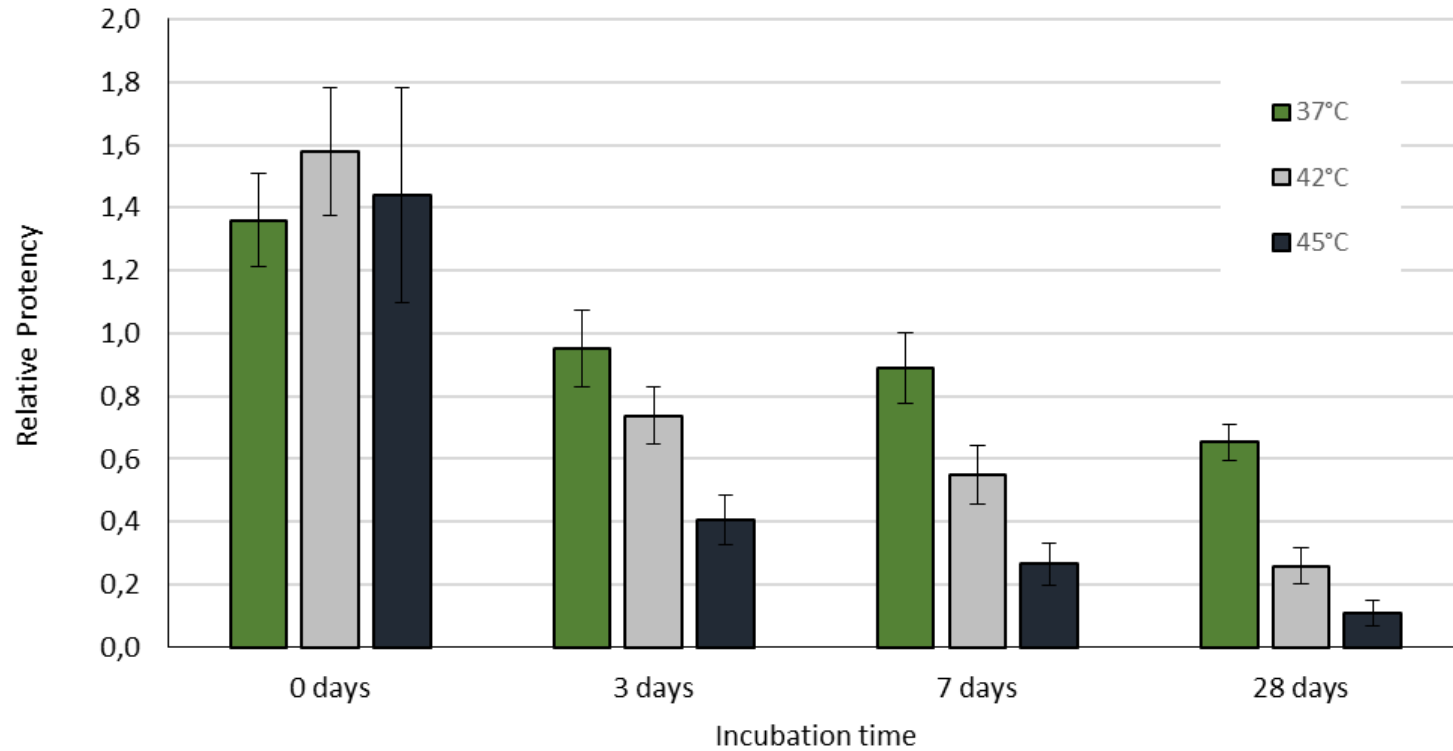
Principle of the methods

- Desorption is problematic for this type of vaccines
- Selection of antibodies capable of binding antigen adjuvanted to aluminium hydroxide
- Competition format: measurement of unbound antibodies



Heat stress

Confirmation in formulated vaccines / bulks



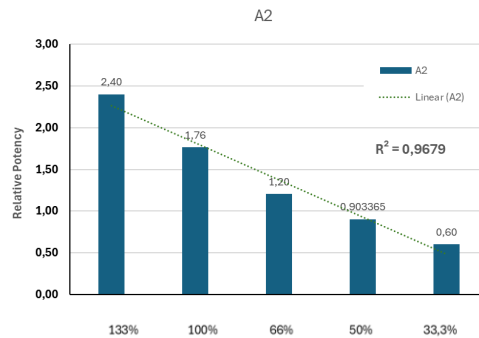
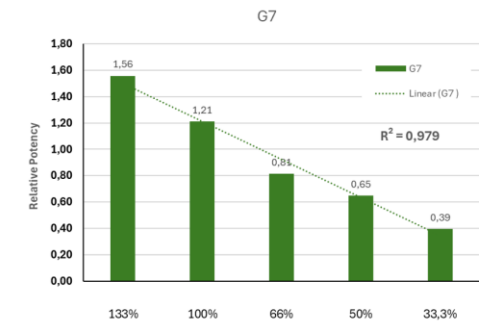
Optimization of assay and validation according to ICH Q2 for mAb1 and mAb2



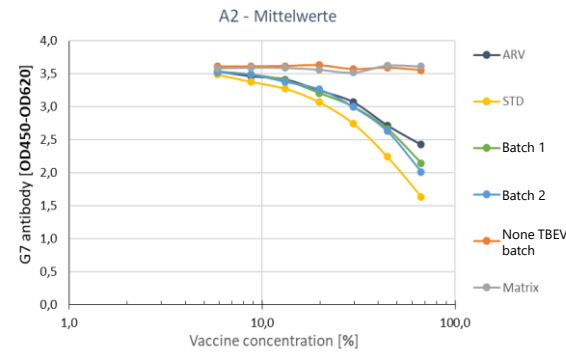
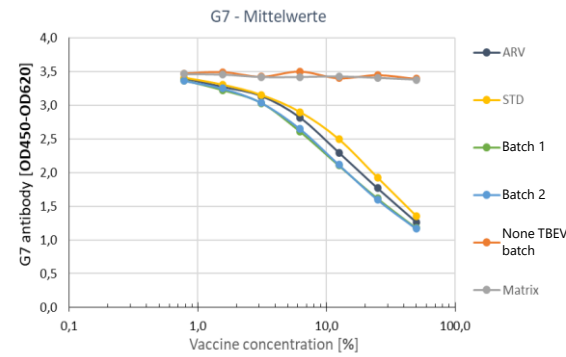
- Accuracy
- Linearity
- Precision
- Intermediate Precision
- Repeatability
- Range
- Robustness

Validation of the method

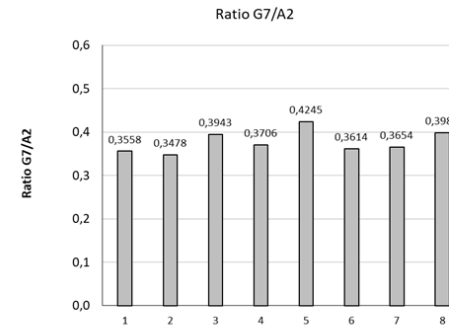
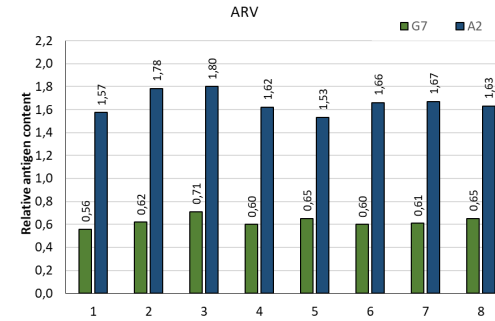
Linearity



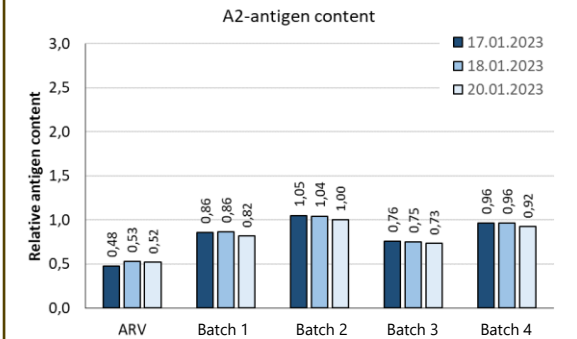
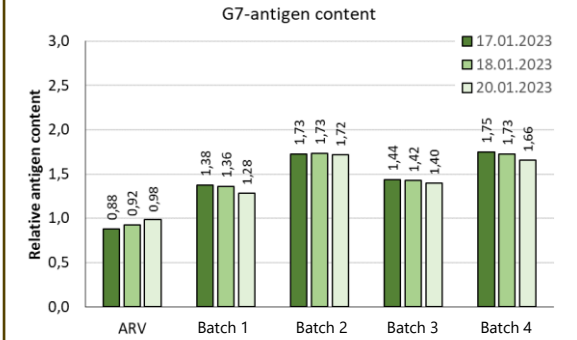
Specificity



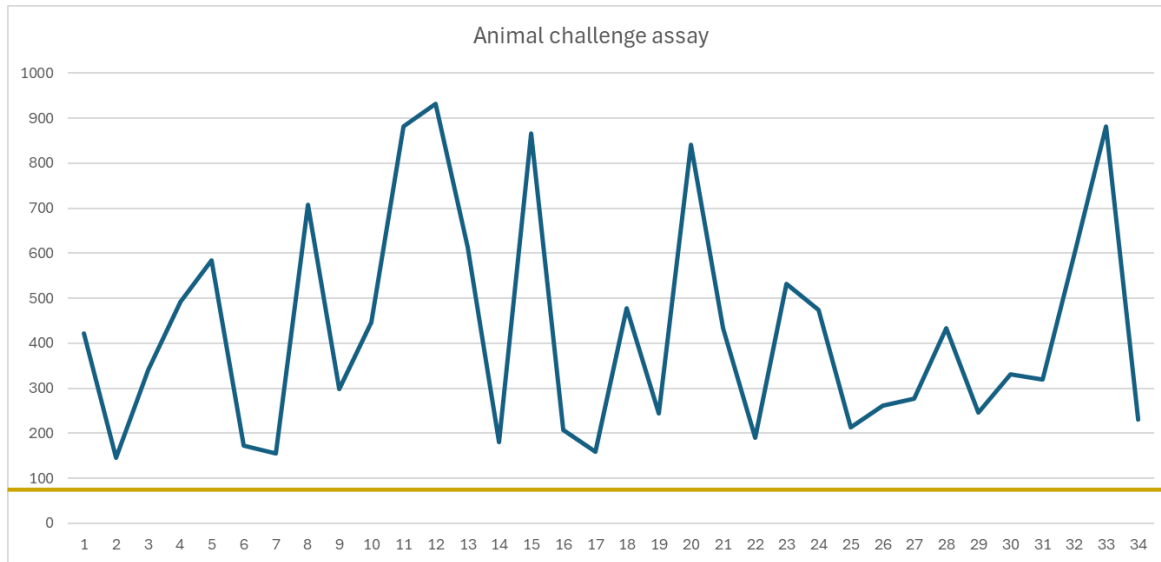
Precision



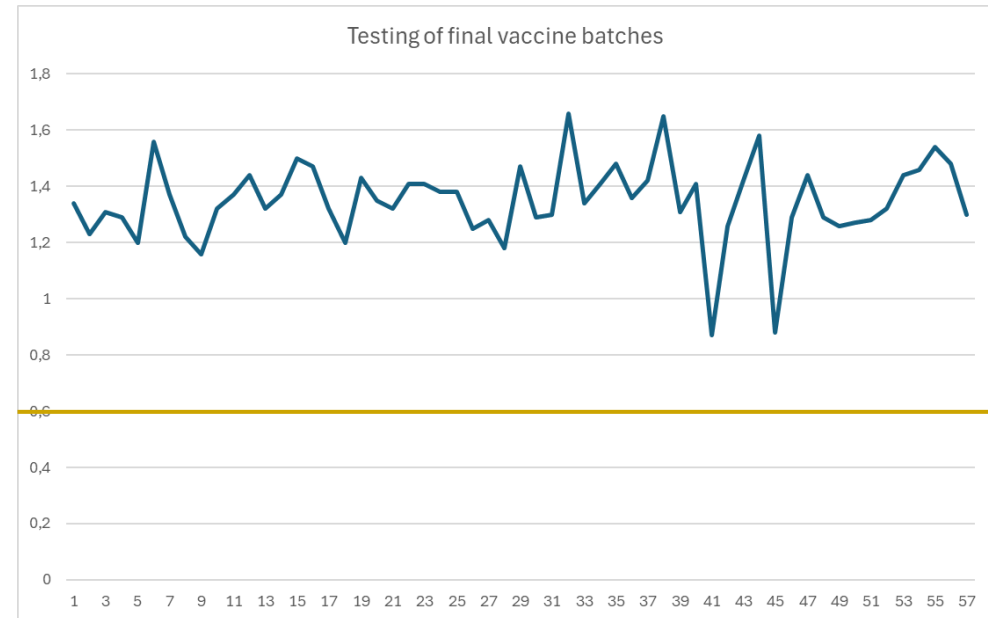
Repeatability



Batch testing



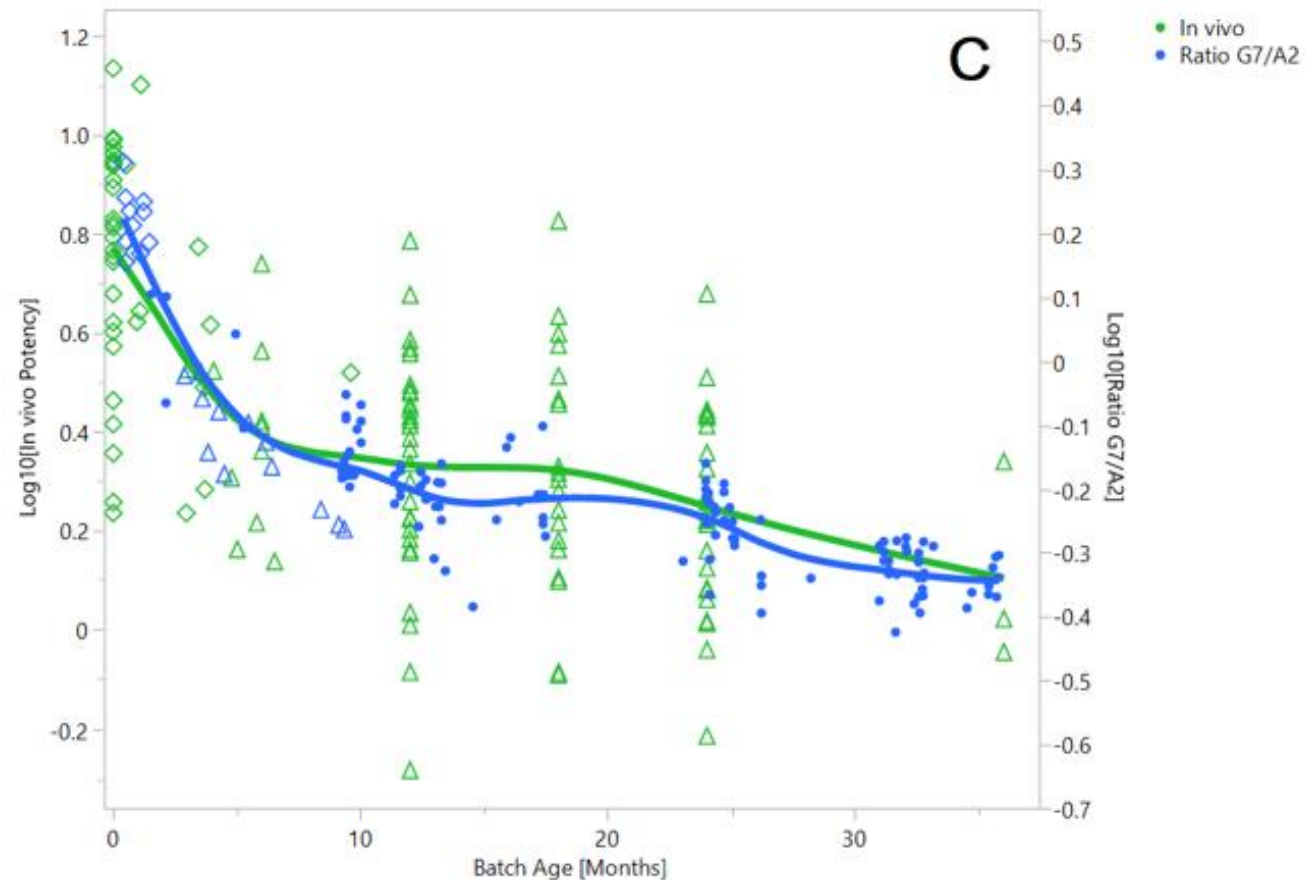
Bulks tested by classical animal challenge assays



Final batches tested by G7 ELISA

Stability

Correlation between mAb1/mAb2 ratio and in vivo assays (figure provided by manufacturer)



Conclusions



- ELISA with mAb1 is a surrogate for potency at release with superior precision to the animal challenge test; measures amount of conformational antigen
- Additional ELISA with mAb2 and calculation of the Ab1/Ab2 ratio enables monitoring of the amount of conformational antigen in relation to the overall structure and/or age of the antigen / aluminium hydroxide complexes
- Overall strategy similar for both vaccines, different sets of antibodies are used for the two manufacturers

Acknowledgements



- VAC2VAC Team within AGES
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