REGULATORY CONFORM REPLACEMENT OF TRITON-X IN AN ANTI-MAG ANTIBODIES ELISA - THE IMPACT OF DIFFERENT DETERGENTS ON PERFORMANCE AND STABILITY

Sara V. Bantleon[1], M.-E. Überschlag[1], S. Deutschmann[1], G. Miljojkovic[1], X. Dervillez[1], C. Bauer[1], K. Ammann[1], R. Cotti[1], T. Schuster[1] and C.-B. Gerhold[1,*]

*correspondence to: cbg@buhlmannlabs.ch; +41 61 487 12 52

[1] BÜHLMANN Laboratories AG - 4124 Schönenbuch, Switzerland

IFCC WORLDLAB DUBAI 2024 MAY 26-30

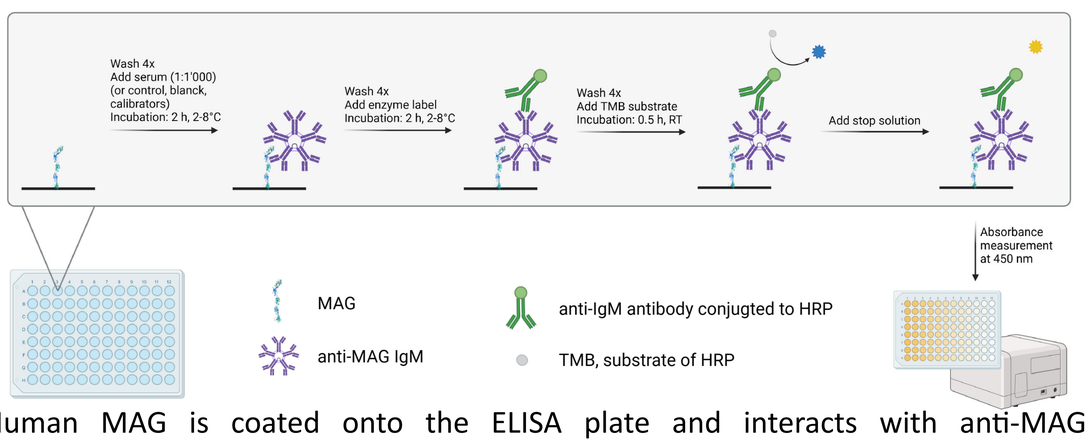
P0701

Background

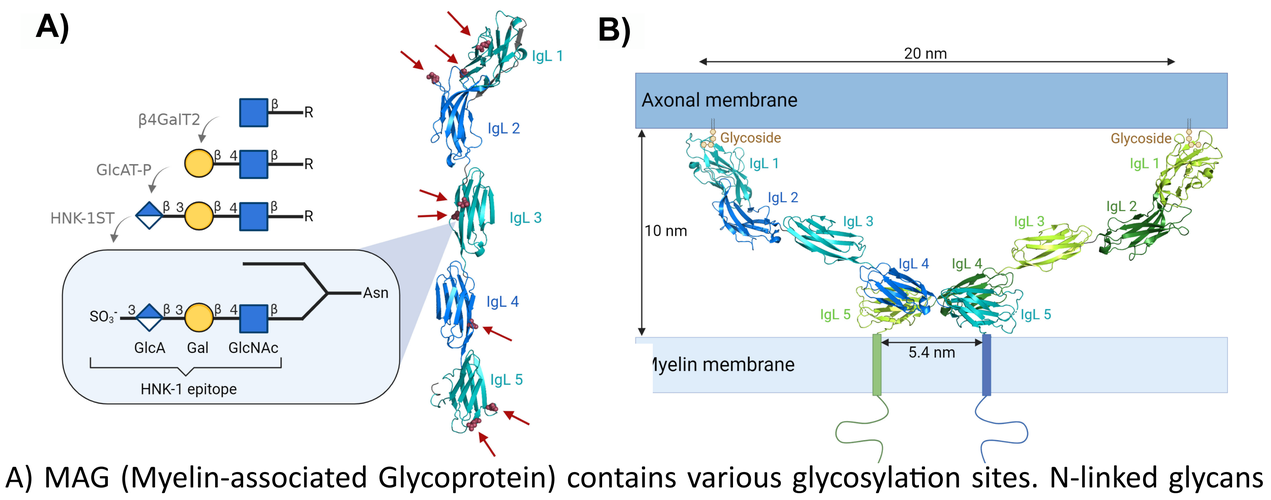
ELISA is a common, sensitive and reliable method for the detection of autoantibodies in inflammatory neuropathies.

The use of detergents is of particular importance for removal of unspecific binding events, ensuring the specificity of the assay. In Anti-MAG (Myelin associated Glycoprotein) Neuropathy, the BÜHLMANN Anti-MAG Antibodies ELISA (EK-MAG) is the accepted gold standard. However, in its current form, it contains the detergent Triton X-100, which has recently been identified as an environmental toxin, particularly affecting aquatic life forms.

Principle of the assay



Human MAG is coated onto the ELISA plate and interacts with anti-MAG autoantibodies from human serum samples. Detergent containing wash buffer eliminates unspecific interactions while specific interactions with anti-MAG IgM autoantibodies remain bound to the plate. HRP labelled anti IgM allows for photometric detection via TMB.



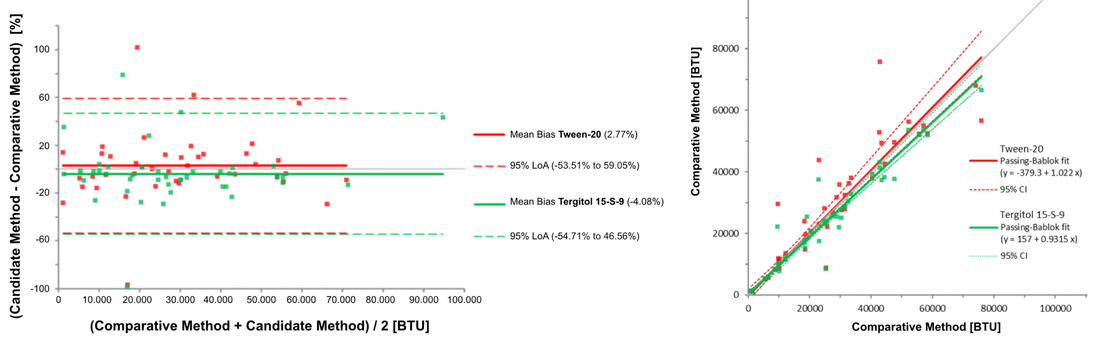
A) MAG (Myelin-associated Glycoprotein) contains various glycosylation sites. N-linked glycans contain the HNK-1 epitope that itself contains a sulfated glucuronic acid, which is the major epitope that is recognized by autoimmune antibodies.

B) schematic representation of the MAG structure (PDB: 5LF5) depicts how MAG contributes to the integrity of the mylein sheath of axons.

Methods

Here, we present performance and stability data on the Anti-MAG Antibodies ELISA containing different eco-friendly detergents. For a method comparison, we included 41 samples across the measuring range and performed Bland-Altman and Passing-Bablok Regression Analysis, to compare reagents containing either Tergitol 15-S-9 or Tween-20 with the Triton-X-100 containing standard. Moreover, specificity was assessed with healthy and, more importantly, differential autoimmune diagnosis patient samples. These experiments served as a decision point for detergent selection for Triton-X replacement. A newly produced and released lot will undergo the full verification studies and real-time stabilities will be assessed with three lots.

Method comparison

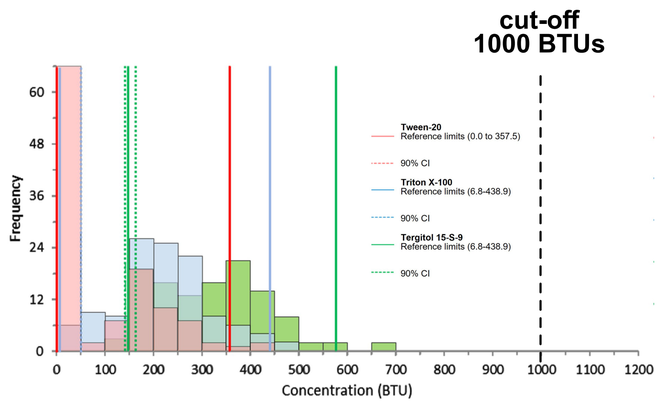


Method Comparison shows very comparable values across the measuring range of the assay with the different detergent set-up. Compared to the Triton-X assay, the simultaneously produced Tween-20 assay shows a mean bias of 2.77%, while Tergitol was measured with a mean bias of -4.08%.

Compared to an existing Triton-X lot on the market, these lots exhibited a mean bias of -17.4% and -10.4%, respectively.

This initial method comparison served as a decision point for detergent replacement.

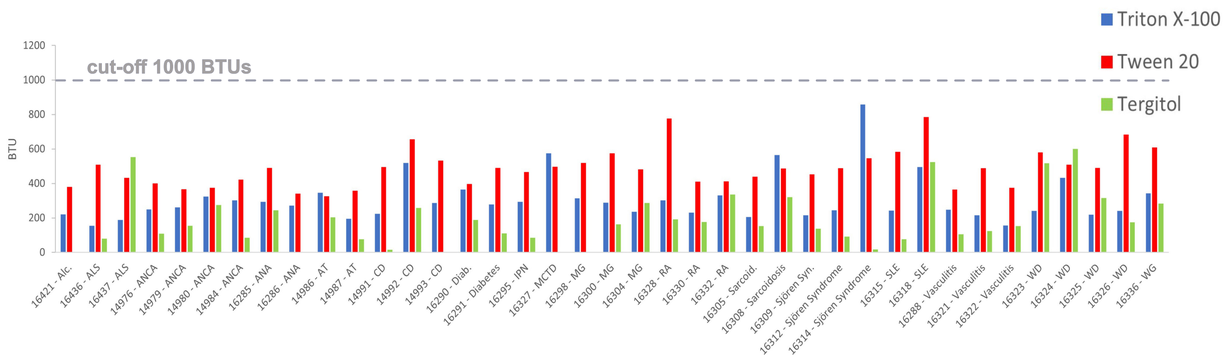
Reference Interval



Description	N	Mean (BTU)	Median (BTU)	Reference Interval 2.5 – 97.5 Percentil (BTU)	90% Confidence Interval (BTU)			
					Lower reference limit		Upper reference limit	
Triton	116	217.6	216.5	6.8-438.9	0.0	to 50.0	-	to -
Tween	116	90.0	0.0	0-357.5	0.0	to 0.0	-	to -
Tergitol	116	320.6	322.5	147.8-576.4	143.0	to 163.0	-	to

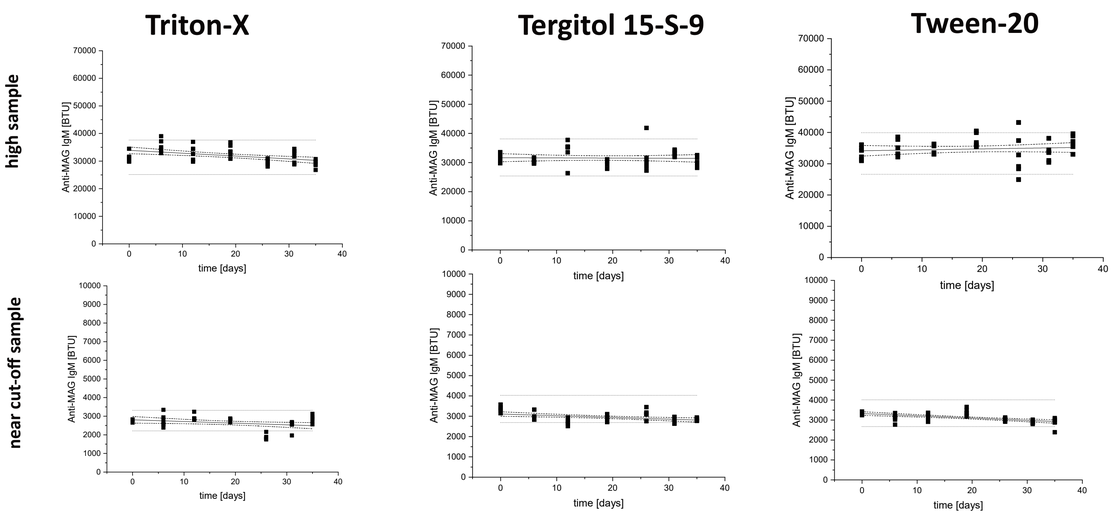
116 healthy controls were measured well below the cut-off of 1000 BTU for Triton-X and alternative detergents suggesting that non-specific interactions can be efficiently abrogated by all tested detergents. In fact, the overall reference intervall of Tween-20 performed slightly better than Triton-X or Tergitol in this experiment.

Cross-reactivity



A sample set of 39 sera with 15 different pathologies was evaluated as the discrimination of MAG Neuropathy from other autoimmune diseases as well as other neuropathies is essential for the specificity of the assay. All tested 39 differential diagnosis samples did not give rise to results above the 1000 BTU cut-off for any of the used detergents in this set-up. Overall, BTU signals are comparable for all detergents with Tergitol 15-S-9 performing slightly better than Triton-X or Tween-20. In summary, all assay set-ups gave rise to low & acceptable cross-reactivities below the cut-off value and thus all three detergents allow for the required specificity of the BÜHLMANN anti-MAG Antibodies ELISA.

Accelerated Stability



Accelerated stability of the assay at 45°C shows very comparable values for high and near-cut-off samples for all detergent compositions.

Results - Summary

Overall agreement between the different detergents was good, with a mean bias of -17.4% and -10.4%, respectively compared to a released Triton-X EK-MAG. The reference interval was determined using sera from 116 healthy controls, while 40 sera from patients with a differential autoimmune diagnosis, e.g. Myasthenia Gravis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, and others were analyzed for cross-reactivity. All measured negative controls were well below the cut-off of 1000 BTU for both alternative detergents. Accelerated stability studies at elevated temperatures do not indicate a negative effect of the choice of detergent on reagent stability and kit shelf life.

Conclusions

In conclusion, our results demonstrate that Triton X-100 can be fully replaced by more ecofriendly detergents in the Anti-MAG Antibodies ELISA, without affecting assay performance or reagent stability. Importantly, the high specificity of the assay remains intact, ensuring reliable diagnosis of anti-MAG neuropathy indicated by the presence of specific IgM autoantibodies directed against the patient MAG. A fully verified IVDR conform assay will be available after completion of necessary change management procedures.