PERIPHERAL NERVE SOCIETY
2022 ANNUAL MEETING
14-17 MAY INTERCONTINENTAL MIAMI
MIAMI

Standardization and Metrological Traceability of a Commercial anti-Ganglioside Antibodies ELISA

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INTRODUCTION

BÜHLMANN GanglioCombi™ ELISA is a multiparametric test to determine anti-ganglioside antibodies in a semi-quantitative way. It allows for a targeted investigation of immune-mediated neuropathies. There are no recognized reference materials nor reference measurement procedures for anti-ganglioside antibodies. To ensure consistency of results over time, we guarantee a transparent traceability chain (Fig. 5). This is achieved by using an internal reference material (IRM) to produce standardized calibrators. Due to the similar structure of gangliosides on our ELISA, anti-GM1 antibodies can serve as a surrogate standardization.

METHODS

Sialic acid residues on gangliosides used in BÜHLMANN GanglioCombi™ ELISA determine fine specificities of anti-ganglioside antibodies. GM1 represents the antigenic core structure of four C6 sugars and one sialic acid, that is commonly shared among different gangliosides (Fig. 1).

Therefore, anti-GM1 antibodies were used exemplarily to standardize multiparametric anti-ganglioside antibodies ELISAs (Fig. 2).

RESULTS

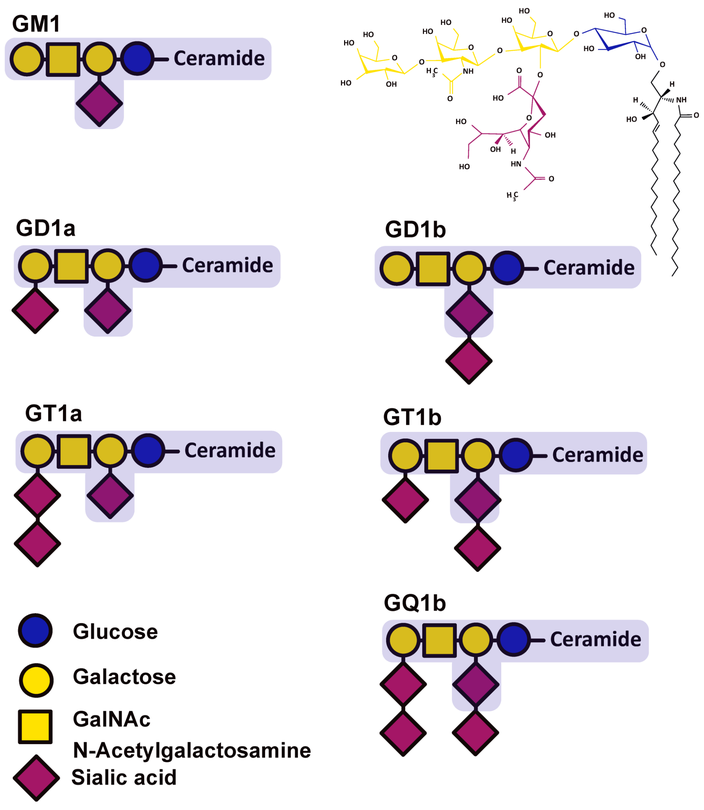
An IRM was generated from monoclonal anti-GM1 IgG and IgM antibodies (Fig. 4). Following the protocol by Blirup-Jensen et al.,2008, the value of the IRM is assigned to a calibrator stock, which is subsequently gravimetrically diluted into calibrators (Fig. 5). Based on the anti-GM1 antibodies standardization, the combined relative uncertainty of the calibrators of the BÜHLMANN Ganglio Combi™ ELISA is calculated. The IRM traceable Ganglioside-ELISAs will be compared to the current version in a validation stage. The assays will be submitted to IVDR, the new European regulatory basis for in vitro diagnostic medical devices.

FIGURES

Figure 1

Ganglioside structures

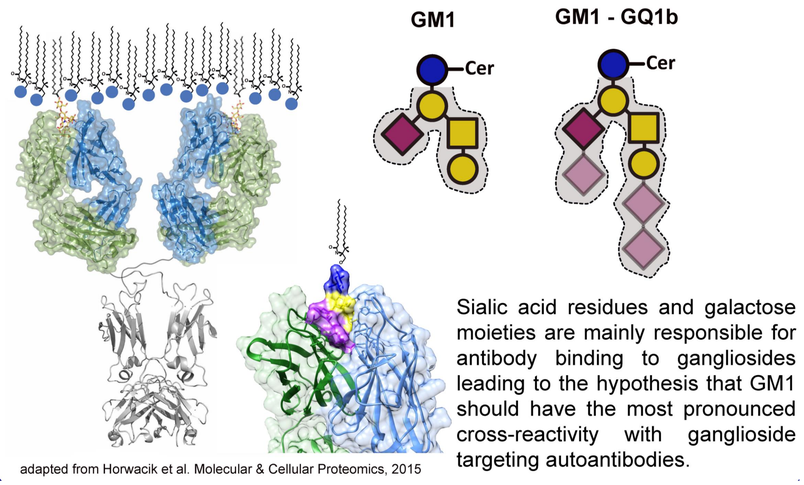
Glycolipid structures of gangliosides involved in human neuropathies with GM1 as major backbone component



Adapted from Goodfellow & Willison, 2018, Progress in Molecular Biology and Translational Science ISSN 1877-1173; Jerkovic & Kalanj-Bognar, 2010, DOI: 10.2478/v10134-010-0043-6

Figure 2

Accessibility GM1 by ganglioside binding antibodies



Sialic acid residues and galactose moieties are mainly responsible for antibody binding to gangliosides leading to the hypothesis that GM1 should have the most pronounced cross-reactivity with ganglioside targeting autoantibodies.

adapted from Horwacik et al. Molecular & Cellular Proteomics, 2015

Figure 3

Principle of the assay: sandwich ELISA

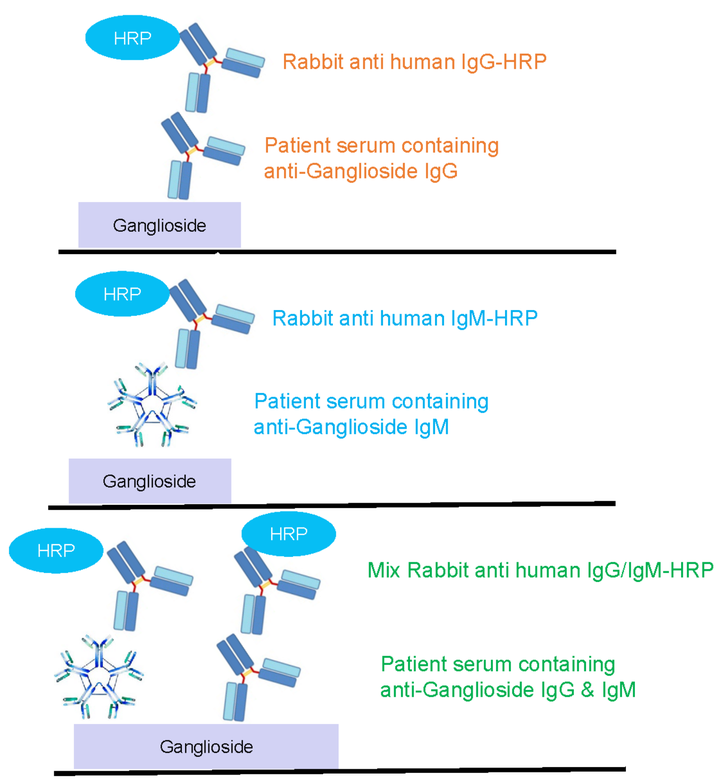


Figure 4

Reference material components

Two human, monoclonal anti-GM1 antibodies

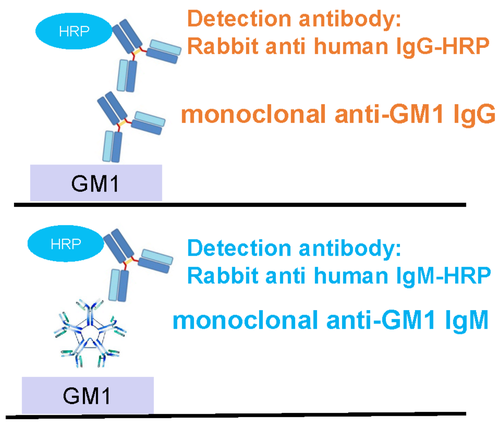
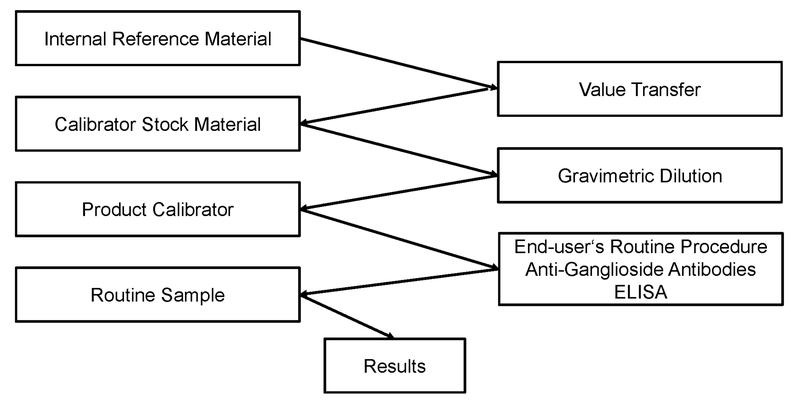


Figure 5

Metrological Traceability & Standardization



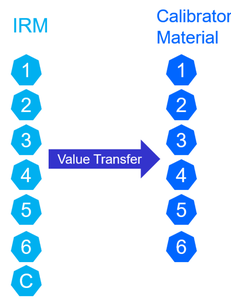
$$U_{comb\ of\ Calibrator\ at\ 95\%\ CI} = 2x\sqrt{u^2_{Value\ Transfer} + u^2_{CASET\ Dilution}}$$

$$2x\sqrt{14.64^2 + 0.41^2} = 29.3\ \%$$

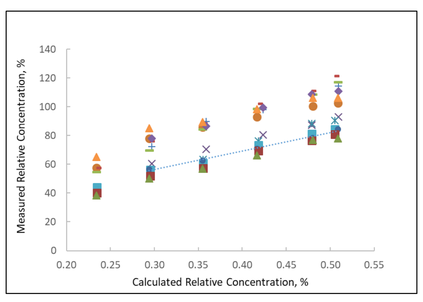
The combined uncertainty of the calibrator at the 95% CI is 29.3%.

Value Transfer Protocol

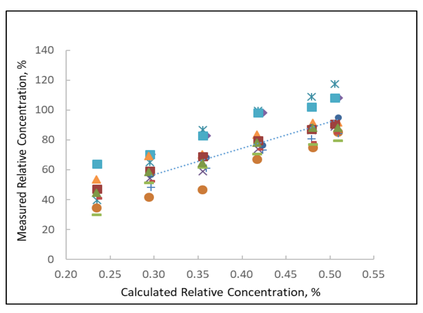
	# dilutions	# runs	# replicates	# days
IRM	6	3	2	4
Calibrator Material	5 - 6	3	2	4
IRM Mastercontrol	1	3	2	4



Value Assignment Calibrator IgG



Value Assignment Calibrator IgM



CONCLUSIONS

Based on the modularity and structural similarity of various gangliosides, that are targeted by autoantibodies in neuropathies, anti-GM1 antibodies can serve as a surrogate standardization. The transparent anti-GM1 antibodies-based traceability chain of the anti-ganglioside antibodies ELISAs truly display the uncertainty of the assays.
This concept not only fulfills the requirements of IVDR regulations, but also guarantees long-term robustness of these state-of-the-art commercial assays for the detection of anti-ganglioside antibodies.