Unique Commercially Available Multiparametric Anti-Ganglioside Antibodies ELISA Complying With New Regulatory IVDR Standards

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Valeria Eckhardt[1], Renato Cotti[1], Christina Bauer[1,2], Marie-Eve Ueberschlag[1], Hüseyin Ilgü[1], Johannes Schneider[1], Stefan Neu[1], Sabine Kräuchi[1], Benjamin Ricken[1], Thomas Schuster[1], Christian-Benedikt Gerhold[1,2]

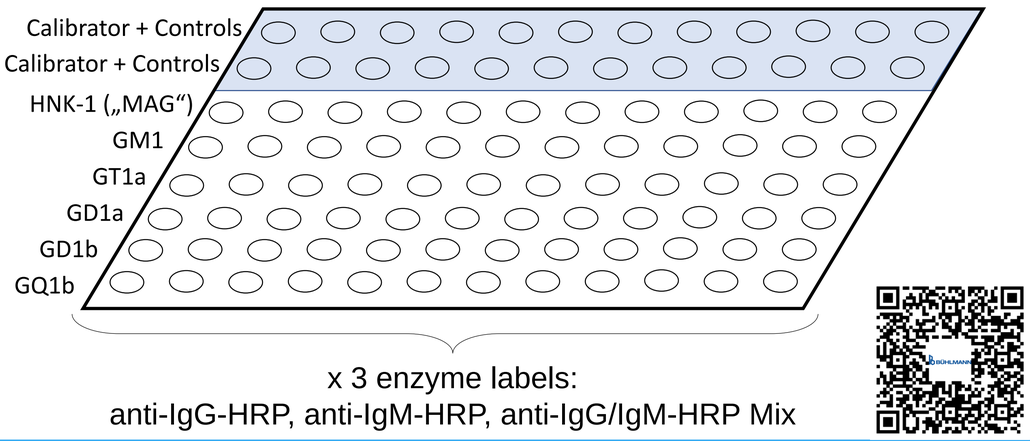
[1] BÜHLMANN Laboratories AG, Schönenbuch, Switzerland
[2] to whom correspondence shall be addressed: cba@buhlmannlabs.ch, cbg@buhlmannlabs.ch

INTRODUCTION

In Vitro Diagnostic Device Regulation (IVDR) is the new harmonized EU regulatory framework to ensure safety and performance of *in vitro* diagnostics (IVD). Prominent IVDR aspects include scientific validity of the analyte and traceability. The BÜHLMANN GanglioCombi® MAG ELISA (aka GanglioCombi) is the only commercially available multiparametric IVD-test to determine antibodies (IgG and IgM) causing autoimmune-related neuropathies. To achieve a high degree of comparability, OD values for the individual ganglioside antibodies are normalized to a standardized and traceable calibrator. The results are expressed as "%Ratio" and can be attributed to one of three relevant titer categories:

NEGATIVE (< 30 %Ratio), GREYZONE (30-50 %Ratio), and POSITIVE (> 50 %Ratio).

Here, we present the adapted and recently validated GanglioCombi adhering to the stringent IVDR guidelines.



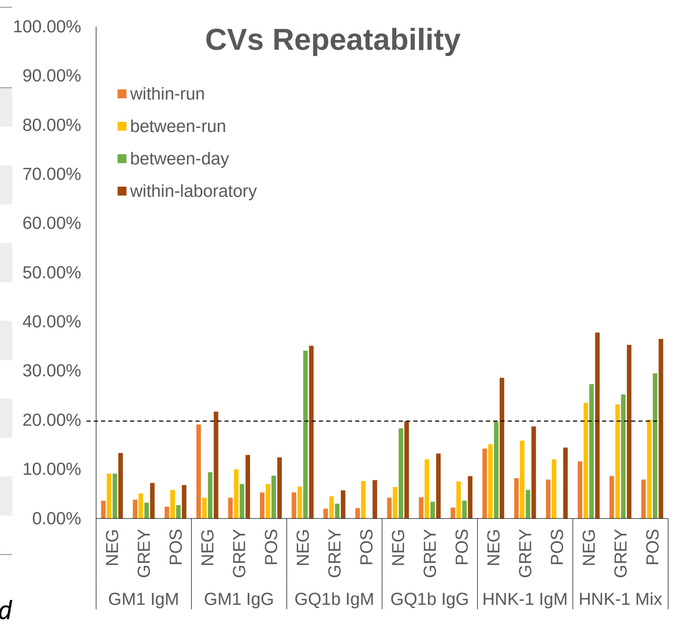
Repeatability

3 samples at different target values were measured in duplicates on 20 days in 2 runs per day (20 x 2 x 2 study design). The following gangliosides/antigens and enzyme labels were used and regarded as representative for the entire assay:

- HNK-1 („MAG“) with anti-IgM and anti-IgG/M-Mix enzyme labels
- GM1 with anti-IgG and anti-IgM enzyme labels
- GQ1b with anti-IgG and anti-IgM enzyme labels

Antigen	Sample type	enzyme-label	% within category
HNK-1	Negative	IgM	100%
	Positive	IgM	100%
HNK-1	Negative	IgG/IgM Mix	100%
	Positive	IgG/IgM Mix	95%
GM1	Negative	IgG	100%
	Positive	IgG	100%
GM1	Negative	IgM	100%
	Positive	IgM	100%
GQ1b	Negative	IgG	100%
	Positive	IgG	100%
GQ1b	Negative	IgM	100%
	Positive	IgM	100%

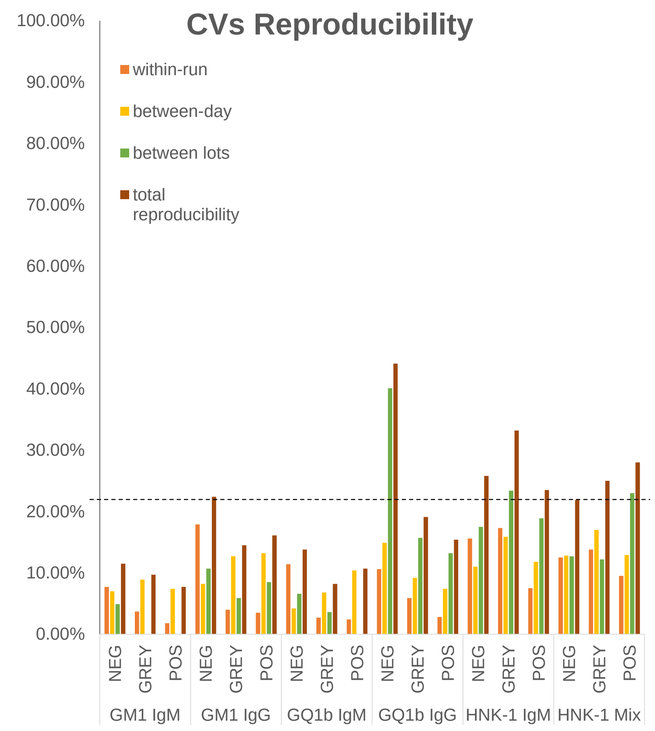
Note: Greyzone values were not regarded as category changes in this analysis as they should be re-tested at a later timepoint



Reproducibility

Three operators, each using a different plate reader and kit lot, performed the reproducibility studies with 5 replicates per sample on 5 days (3 x 5 x 5 study design).

Antigen	Sample type	enzyme-label	% within category
HNK-1	Negative	IgM	100%
	Positive	IgM	100%
HNK-1	Negative	IgG/IgM Mix	100%
	Positive	IgG/IgM Mix	100%
GM1	Negative	IgG	100%
	Positive	IgG	100%
GM1	Negative	IgM	100%
	Positive	IgM	100%
GQ1b	Negative	IgG	100%
	Positive	IgG	100%
GQ1b	Negative	IgM	100%
	Positive	IgM	100%



Qualitative analysis of the reproducibility shows that all negative and positive samples stay within category.

CONCLUSION

The GanglioCombi is a robust, standardized, multiparametric ELISA test that has been renovated and validated in line with the current high IVDR standards. GanglioCombi results reliably support the diagnosis of autoimmune-mediated polyneuropathies. The assay uniquely combines several clinically relevant ganglioside antigens and the primary MAG epitope HNK-1.

BÜHLMANN GanglioCombi® is a registered trademark in many countries.

Clinical Performance

Overview of systematic literature analysis:

Study	Positive controls (Cases)	Negative controls	Epitope	Cutoff	Sensitivity	Specificity
Hashemilar et al., 2014	Pediatric GBS (n = 45)	DC (n = 35)	GM1 GQ1b	1200 BTU* 2400 BTU*	0.51 0.56	0.89 0.74
Sharma et al., 2011	Pediatric GBS (n = 57)	NC (n = 42) DC (n = 35)	GM1	800 BTU*	0.82	0.33 0.83
Khandelwal et al., 2006	GBS (n = 13)	HC (n = 19)	GM1	800 BTU*	0.31	0.74
Uetz-von Allmen et al., 1998	GBS, CIDP (n = 19, 14))	NC (n = 100) HC (n = 110)	GM1	35 AU**	0.30	0.93 0.95
Spatola et al., 2016	GBS (MFS) (n = 12)	DC (n = 34)	GQ1b	50% ratio	0.92	0.97
Delmont et al., 2019	MAG-neuropathy (n = 41)	NC (n = 112) HC (n = 6)	HNK-1 (MAG)	50% ratio	0.98	0.99

HC: Healthy control – DC: Disease control (non-neurological) – NC: Neurological control
** BTU: BÜHLMANN Titer Units – **AU: Arbitrary Units*

Based on a bivariate analysis, autoimmune peripheral neuropathy was detected with a sensitivity of 68.1 % (95% CI: 39.6 – 87.5 %) and specificity of 88.0 % (95% CI: 72.3 – 95.3%). The area under summarizing receiver operating characteristic curve (AUC-SROC) was 0.85.

1. Hashemilar, M. *et al.* Evaluating the status of antiganglioside antibodies in children with Guillain-Barré syndrome. *Neuroimmunomodulation* **21**, 64–68 (2013).
2. Sharma, M. B. *et al.* The presence of Mycoplasma pneumoniae infection and GM1 ganglioside antibodies in Guillain-Barré syndrome. *J. Infect. Dev. Ctries.* **5**, 459–464 (2011).
3. Uetz-von Allmen, E. *et al.* Antiganglioside GM1 antibodies and their complement activating capacity in central and peripheral nervous system disorders and in controls. *Eur. Neurol.* **39**, 103–110 (1998).
4. Spatola, M., Du Pasquier, R., Schluep, M. & Regeniter, A. Serum and CSF GQ1b antibodies in isolated ophthalmologic syndromes. *Neurology* **86**, 1780–1784 (2016).
5. Khandelwal, D. *et al.* IgM anti-GM1 antibody titers in patients with monomelic amyotrophy. *Neurol. India* **54**, 399–401 (2006).
6. Delmont, E. *et al.* Relevance of anti-HNK1 antibodies in the management of anti-MAG neuropathies. *J. Neurol.* **266**, 1973–1979 (2019).

Analytical Sensitivity

Limit of Blank (LoB): determined based on normal donor sera (95% confidence interval), 4 samples x 5 replicates x 5 days
Limit of Detection (LoD): determined based on patient sera diluted with normal sera, 4 samples x 5 replicates x 5 days

Results are shown as %Ratio:

Antigen	Enzyme label	Effective LoB	Effective LoD
HNK-1	IgM	12	26
HNK-1	IgG/IgM Mix	14	27
GM1	IgG	6	15
GM1	IgM	5	21
GQ1b	IgG	8	18
GQ1b	IgM	3	16

Cross-Reactivity

Cross-Reactivity has been tested with 85 samples of patients with different disorders: