BÜHLMANN GanglioCombi® *Light* ELISA

Pre-Analytics

|	
Specimen	Serum
Specimen collection	Use conventional plain tubes to collect blood. Process to serum according to manufacturer's instruction.
Storage of Serum	• Prepare aliquots • 2-8°C (up to 16 days) • -20°C (up to 12 months) • Freeze/thaw cycles not recommended

Number of Analyses

- Kit allows for full flexibility regarding:
  - number of samples to be analyzed
  - use and arrangement of enzyme conjugates on microtiter plates
- Generally, a sample can be investigated for 3 different antibodies with IgG and IgM and/or with IgG/IgM mix conjugate.
- Each kit contains 1 microtiter plate.

Enzyme Conjugate	Number of Analyses per Kit	Number of Analyses per individual Sample
IgG & IgM	12 + 12	2 x 3 (2 conjugates/3 gangliosides)
IgG/IgM Mix	24	1 x 3 (1 mix conjugate/3 gangliosides)

Literature References

Kuijf M et al., Journal of the Neurological Sciences, 2005
Spatola M et al., Neurology, 2016
Franciotta D et al., Clin Chem Lab Med, 2018

Microtiter Plate Layout
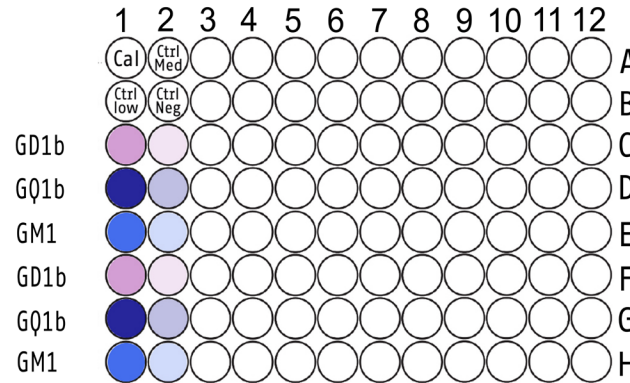
- Rows coated with 3 different gangliosides
- Break-away strip convenience

Reagent Preparation

Reagent	Temperature °C
Wash buffer	2-8
Incubation Buffer	2-8
Enzyme conguate	2-8
TMB substrate	18-28
Stop solution	18-28

Sample Preparation

1. Dilute serum samples 1:50 with incubation buffer and vortex gently.

2. Leave diluted samples, reconstituted calibrators and controls at 2-8°C for 30 minutes prior pipetting (for targeted investigation).

ELISA Procedure*

Precoated Microtiter Plate

wash 2x (≥ 300 µL wash buffer)

Calibrators, Controls, Serum (1:50) (100 µL)

incubate (2 hr ± 5 min)

wash 3x (≥ 300 µL wash buffer)

Enzyme Label (100 µL)

incubate (2 hr ± 5 min)

wash 3x (≥ 300 µL wash buffer)

(2 – 8°C)

TMB Substrate (100 µL)

incubate (30 min ± 2 min)
plate shaker: (400–600 rpm)

Stop Solution (100 µL)
≤ 30 min

Absorbance (450 nm)

(18 – 28°C)

*Please see the full instructions for use (IFU) on buhlmannlabs.com for more detailed information.
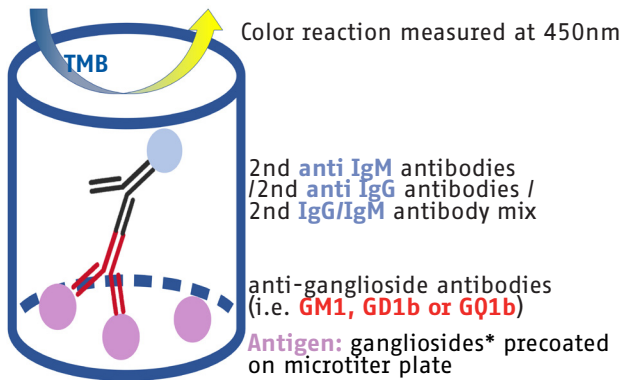
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Methodology

Method	ELISA for Research Use Only
Analyte	anti-ganglioside antibodies: GM1, GD1b, GQ1b
Test	multi-parametric, semi-quantitative
Suggested Use	Targeted investigation of anti-ganglioside antibodies with IgG, IgM, and/or IgM/IgG Mix.

Principle of the Assay

Color reaction measured at 450nm

2nd **anti IgM** antibodies /2nd **anti IgG** antibodies / 2nd **IgG/IgM** antibody mix

anti-ganglioside antibodies (i.e. **GM1, GD1b or GQ1b**)

Antigen: gangliosides* precoated on microtiter plate

*We use double-quality control-checked gangliosides for the coating of the microtiter plates.

Reference Interval
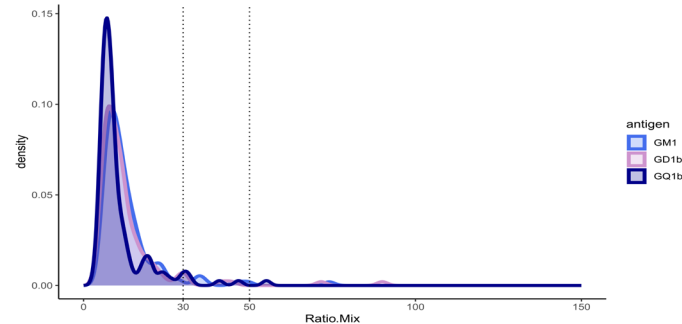
Healthy Individuals: N = 120 (m = 60; f = 60)

Negative: 95.0%; Grey zone: 3.9%; Positive: 1.1%

Performance Date

Crossreactivity:	No (incl. AIDs[1] and ONDs[2])
Reproducibility (%CV):	7.7-19.1%
Specificity (95 %CI):	79.8% (66.8-93.2%)
Sensitivity (95 %CI):	57.0% (38.6-75.4%)

[1] Autoimmune diseases (N = 50): Vasculitis and ANCA positive denoted: 3 and 10; SLE: 5; RA: 9 Sjögren Syndrome: 6; Others (ANA positive denoted): 3; Autoimmune Thyroïditis: 5; MCTD: 1; Autoimmune peripheral neuropathies (GM1 and GQ1b pos): 1; MG: 7

[2] Other neurological diseases (N = 34): Alcoholic: 1; diabetic: 5; ALS: 15; Sarcoïdosis: 4;WM: 4; Chagas Disease: 5

Results

- The measurement of absorbance is proportional to the titer of ganglioside antibodies in a given sample.
- Titers are expressed as a % ratios of the calibrator

Handling of Titer levels

Titer levels [%Ratio]	Interpretation [titer]
< 30	negative
30 - 50	grey zone (equivocal)
> 50	positive

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