anti-Ganglioside M1 (-GM1) Antibodies ELISA

Pre-Analytics

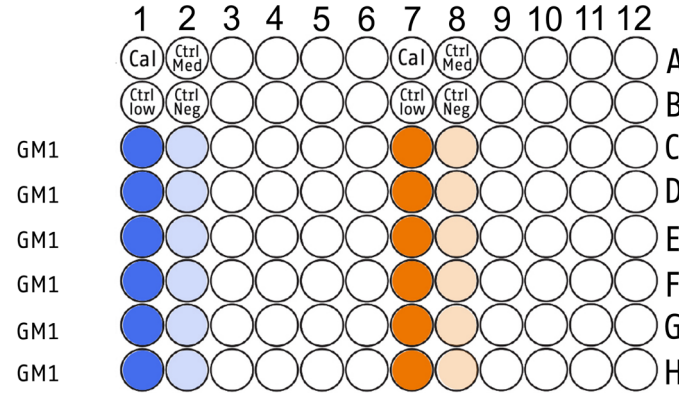|	
Specimen	Serum
Specimen collection	Use conventional plain tubes to collect blood. Process to serum according to manufacturer's instruction.
Storage of Serum	• Prepare aliquots • Freeze/thaw cycles not recommended • 2-8°C (up to 16 days) • -20°C (up to 12 months)

Number of Analysis

- Kit allows for full flexibility regarding:
  - number of samples to be pipetted
  - use and arrangement of enzyme conjugates on microtiter plate
- Generally, a sample can be investigated for IgG and IgM.
- Each kit contains 1 microtiter plate.

Enzyme Conjugate	Number of Analysis per Kit	Number of Analysis per individual Sample
IgG & IgM	21 -42	2 x 1 (2 conjugates / GM1)

Literature References

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

Microtiter Plate Layout

- Rows coated with GM1
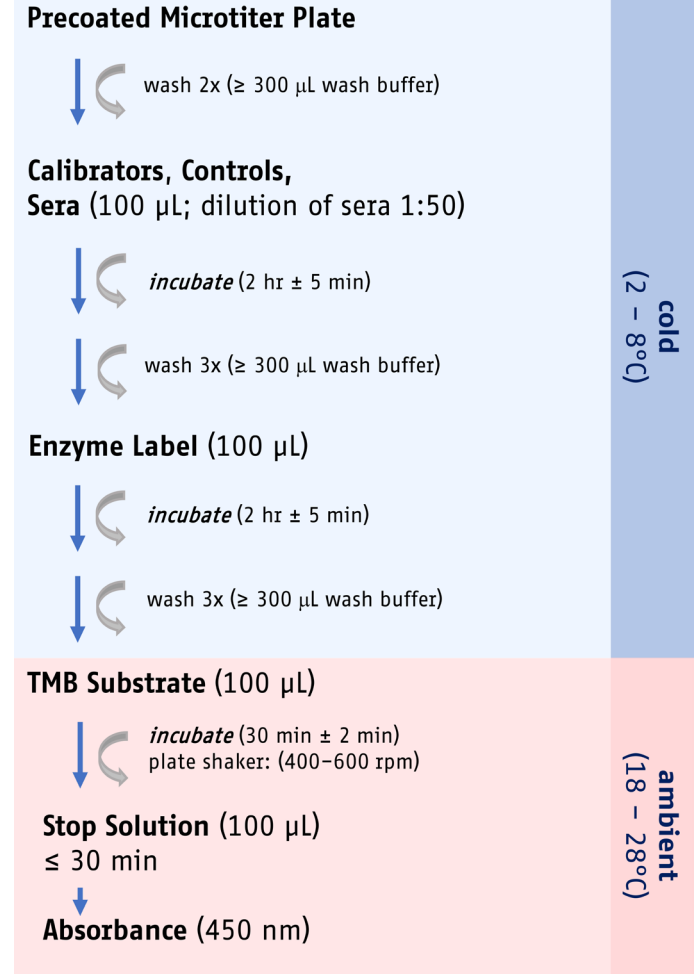- Break-away strip convenience

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 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).
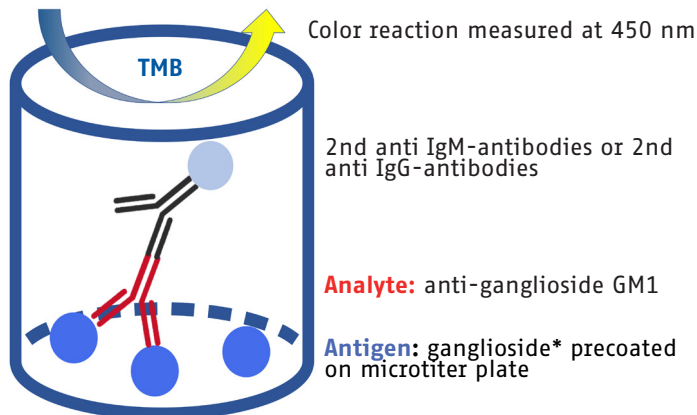
ELISA Procedure

cold (2 – 8°C)

Precoated Microtiter Plate

↓ wash 2x (≥ 300 µL wash buffer)

Calibrators, Controls, Sera (100 µL; dilution of sera 1:50)

↓ *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)

ambient (18 – 28°C)

TMB Substrate (100 µL)

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

Stop Solution (100 µL) ≤ 30 min

↓

Absorbance (450 nm)

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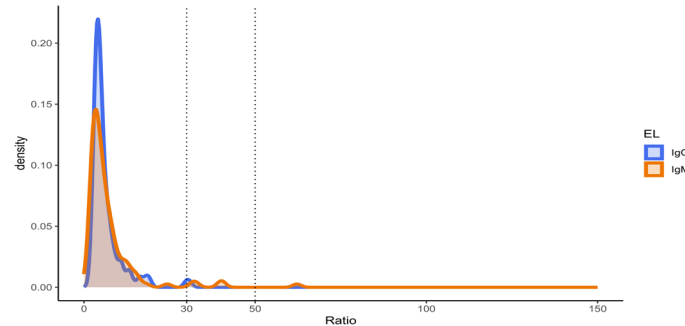## Methodology

Method   ELISA
For laboratory use only

Analyte   anti-ganglioside M1 (-GM1) antibodies.

Test   Semi-quantitative ELISA

Suggested Use Targeted investigation of anti-GM1 antibodies with IgG and IgM.

Reference Interval

Healthy Individuals: N = 120 (m = 60; f = 60)
IgG: Negative: 99.2%; Grey zone: 0.8%; Positive: 0%
IgM: Negative: 95.8%; Grey zone: 3.3%; Positive: 0.8%

Results

- The measurement of absorbance is proportional to the titer of anti-GM1 antibodies present in a given sample.

- Calibrators (GM1)

- Titers are expressed as %Ratio of anti-GM1 Antibodies

Handling of Titer levels

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

Ordering information

anti-GM1 Antibodies ELISA   1 ganglioside   EK-GM1-GM

Variants:

·BÜHLMANN GanglioCombi® MAG ELISA   5 gangliosides, 1xMAG   EK-GCM

·BÜHLMANN GanglioCombi® *Light* ELISA   3 gangliosides   EK-GCL-S

Principle of the Assay

TMB

Color reaction measured at 450 nm

2nd anti IgM-antibodies or 2nd anti IgG-antibodies

Analyte: anti-ganglioside GM1

Antigen: ganglioside* precoated on microtiter plate

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

Performance Data

Crossreactivity: No (incl. AIDs[1] and ONDs[2])

Reproducibility (%CV):   7.7-16.1%

Specificity (95 %CI):   77.8% (60.9-97.4%)

Sensitivity (95 %CI):   48.7% (27.9-69.5%)

[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

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