BÜHLMANN GanglioCombi™ MAG ELISA

with enzyme labels IgG/IgM Mix, IgG and IgM

Detection of anti-ganglioside and -MAG auto-antibodies by ELISA

("MAG", GM1, GM2, GD1a, GD1b, and GQ1b)

For research use only.
Not for use in diagnostic procedures.

EK-GCM-U 2 x 96 wells

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ENGLISH

INTENDED USE

BÜHLMANN GanglioCombi™ MAG ELISA is an immunoassay intended to detect auto-antibodies against defined relevant neural antigens / epitopes in serum samples.
For research use only. Not for use in diagnostic procedures.

INTENDED APPLICATION

With regard to the three different enzyme labels, the device components allow three application options:

1. Testing with the IgG/IgM Mix conjugate allows to screen for the presence of auto-antibodies.

2. Testing with individual IgG and/or IgM conjugates for auto-antibody isotype determination.

3. For laboratory work-up we suggest combining both procedures: sample screening using the Mix (option 1), followed by differentiation of Mix-positive samples by separate IgG and IgM enzyme labels (option 2).

PRINCIPLE OF THE ASSAY

BÜHLMANN GanglioCombi™ MAG ELISA is based on the enzyme-immunometric assay technique. The wells of the provided microtiter plate are coated with gangliosides: GM1, GM2, GD1a, GD1b and GQ1b as well as with a synthetic MAG (Myelin Associated Glycoprotein) “mimotope”. The MAG “mimotope” is a synthetic sulphated disaccharide. It mimics a MAG carbohydrate epitope, HNK-1, recognized by anti-MAG auto-antibodies.
Calibrator, controls and sera are incubated in the microtiter wells and anti-ganglioside and/or -MAG auto-antibodies present in the samples bind to the immobilized gangliosides or MAG-analogue. After washing off unbound substances, the antibodies are detected with horseradish-peroxidase (HRP) labelled antibodies against human IgG and/or IgM. Following a second washing step in which unbound enzyme label is removed, a substrate solution containing tetramethylbenzidine (TMB) is added. A blue colour develops in proportion to the amount of antibodies bound to the immobilized gangliosides or MAG-analogue. Colour development is stopped by adding an acidic stop solution (diluted sulphuric acid) which turns the blue solution into yellow. The intensity of the colour is measured at 450 nm.

The measured absorbance is proportional to the titre of auto-antibodies present in a given sample. The titres of auto-antibodies are expressed as % ratios of the calibrator.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Microtiter Plate precoated with gangliosides and MAG analogue	2 x 12 x 8 wells	B-GCM-MP	Ready to use
Plate Sealer	6 pieces		
Wash Buffer Concentrate (10x) with preservatives	2 bottles 100 mL	B-GCO-WB	Dilute with 900 mL of deionized water
Incubation Buffer with preservatives	1 bottle 100 mL	B-GCO-IB	Ready to use
Calibrator Lyophilized with preservatives	1 vial	B-GCO-CA	Add 1.5 mL of Incubation Buffer
Negative, Low and Medium Control Lyophilized with preservatives	3 vials	B-GCO-CONSET	Add 1.5 mL of Incubation Buffer
Enzyme Label IgG/IgM Mix Anti-human IgG and IgM Ab conjugated to HRP in a protein-based buffer with preservatives	2 vials 11 mL each	B-GCO-ELGM	Ready to use
Enzyme Label IgG Anti-human IgG Ab conjugated to HRP in a protein-based buffer with preservatives	1 vial 11 mL	B-GCO-ELG	Ready to use
Enzyme Label IgM Anti-human IgM Ab conjugated to HRP in a protein-based buffer with preservatives	1 vial 11 mL	B-GCO-ELM	Ready to use
TMB Substrate TMB in citrate buffer	2 vials 11 mL	B-TMB	Ready to use
Stop Solution 0.25 M sulfuric acid	2 vials 11 mL	B-STS	Ready to use **Corrosive agent**

Table 1

STORAGE AND SHELF LIFE OF REAGENTS

Sealed / Unopened Reagents	
All sealed / unopened kit components are stable at 2-8 °C until the expiration date printed on the labels.	
Opened / Reconstituted Reagents	
Microtiter Plate	Return unused strips immediately to the aluminium pouch containing the desiccant packs and reseal along the entire edge of zip-seal. Store for up to 4 months at 2-8 °C.
Diluted Wash Buffer	Store for up to 4 months at 2-8 °C.
Calibrator	Store for up to 4 months at 2-8 °C. **Do not freeze!**
Controls	
Incubation Buffer	Store at 2-8 °C until expiration date printed on the labels.
Enzyme Labels	
TMB Substrate	
Stop Solution	Store at 18-28 °C until expiration date printed on the labels.

Table 2

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes with disposable tips: 20 µL, 100 µL and 1000 µL pipettes
- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions
- 1000 mL cylinder for the reconstitution of the wash buffer
- Squeeze bottle for wash buffer or automatic microtiter plate washer
- Blotting paper
- Orbital shaker for microtiter plates
- Microtiter plate reader for the measurement of absorbance at 450 nm

PRECAUTIONS

Safety precautions

- Both, calibrator (B-GCO-CA) and controls (B-GCO-CONSET) of this kit contain components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with good laboratory practices using appropriate precautions.
- Stop solution: The stop solution (B-STS) contains sulfuric acid (0.25 M). The reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothes. After contact with eyes or skin, wash immediately with plenty of water.
- Reagents: Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation / burns can occur.
- Unused solution should be disposed of according to local state and federal regulations.

Technical precautions

- Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected, if reagents are incorrectly diluted, modified or stored under conditions other than those as detailed in this instruction for use.
- Residues in the microtiter plate wells result from the production process. They are removed in the washing step (assay procedure step 3) and do not affect the results.
- Prepare reagents before starting the assay procedure. Reagents used in steps 3-9 must be cold (2-8 ˚C) and kept cold while pipetting and washing. Put the TMB substrate at room temperature (18-28 ˚C).
- Steps 3-9: Use cold (2-8 °C) reagents for all these steps and keep them cold while pipetting. Recommendation: Prepare the wash buffer the evening before performing the assay and place it into the fridge overnight.
- Wash steps 3, 6 and 9: The wash steps are crucial for removing residues in the microtiter plate wells resulting from the production process (step 3) as well as any unbound antibodies (steps 6 and 9).
  → Always perform the wash steps with cold (2-8 ˚C) wash buffer.
  → Make sure that all wells are completely empty after the last washing cycle.
- Step 9: Adjust TMB substrate to room temperature (18-28 °C) before using it.
- Step 11: Shake the microtiter plates during the incubation with substrate. Depending on the orbital plate shaker, we recommend 400-600 rpm. The solution should move in the wells but must not spill over.
- If an automated washer is used, “plate mode” should be chosen so that dispensing is performed sequentially on all strips before aspirating.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Every effort should be made to ensure that no cross contamination occurs between reagents, samples or between wells.
- Microwells cannot be re-used.

SPECIMEN COLLECTION AND STORAGE

- The procedure requires <0.1 mL of blood or <50 µL of serum, respectively.
- Refer to page 7 to learn about the interference of haemolyzed, lipemic or icteric samples.
- Collect blood into plain tubes (no anti-coagulant), avoid haemolysis, leave to clot for one hour, centrifuge for 10 minutes at approximately 1500 x g at room temperature (18-28 °C), collect the serum.
- We recommend freezing aliquots of samples if you need to store samples in order to avoid repeated freezing / thawing.
- Store serum samples at ≤–20 °C up to 4 months. For long-term storage we recommend -70 °C (samples are stable for >1 year). Frozen samples should be thawed and vortexed thoroughly prior to use.

ASSAY PROCEDURE

You can choose between three basic options:

(1) Detection of IgG/IgM mix-isotypes: step 4a-4e and 7

(2) Detection of IgG and IgM isotypes: step 4a'-4f'and 7'

(3) Two-step approach: measurement of all samples with option 1 and post-measurement of the positive samples with separate enzyme conjugates (IgG and IgM) as option 2.

Note: Equilibrate TMB substrate to room temperature (18-28 ˚C).

1. Dilute all samples to be investigated 1:50 with incubation buffer. Use 30 µL of serum + 1470 µL (cold: 2-8 °C!) incubation buffer. Mix by vortexing and leave diluted samples as well as reconstituted calibrator and controls for 30 minutes at 2-8 °C prior to pipetting (refer to step 4a and b).
2. Prepare a plate-frame with the required number of strips to test the samples. Reseal the remaining strips in the foil pouch together with the desiccant packs immediately. Store refrigerated.

Note: Use cold reagents in steps 3 to 9.

3. Wash coated wells twice using at least 300 µL of cold! wash buffer per well. Empty wells and tap plate firmly onto blotting paper to remove remaining liquid completely.

Note: Immediately proceed to the next steps.

Option 1: Detection of IgG/IgM mix-isotypes

4a. Calibrator: Pipet 100 µL of calibrator into the well A1 (refer to figure 1A).

4b. Controls: Pipet 100 µL of medium control into well B1, of low control into well A2 and of negative control into well B2 (refer to figure 1A).

Note: If more than three strips per run are used, calibrator and controls can be tested in duplicates (see figure 1A).

4c. Serum: Pipet 100 µL of diluted serum 1 into wells C1-H1 (refer to figure 1A).

4d. Serum: Pipet 100 µL of diluted serum 2 into wells C2-H2 (refer to figure 1A)

4e. Pipet 100 µL of diluted sera 3-24 into subsequent wells (refer to figure 1A).

Option 2: Detection of IgG Isotypes

4a'. Calibrator: Pipet 100 µL of calibrator into the well A1 (refer to figure 1B).

4b'. Controls: Pipet 100 µL of medium control into well B1, of low control into well A2 and of negative control into well B2 (refer to figure 1B).

Note: If more than three strips per isotype are used, calibrator and controls can be tested in duplicates (see figure 1B).

4c'. Serum: Pipet 100 µL of diluted serum 1 into wells C1-H1 (refer to figure 1B).

4d'. Serum: Pipet 100 µL of diluted serum 2 into wells C2-H2 (refer to figure 1B).

4e'. Pipet 100 µL of diluted sera 3-12 into subsequent wells.

Detection of IgM isotypes.

4f'. Repeat steps 4a'-4e' using subsequent wells or a new microtiter plate if necessary (refer to figure 1B).

For options 1 and 2: Sample incubation and washes

5. Cover the plate with a plate sealer and incubate for 2 hours ±5 minutes at 2-8 °C (do not shake the plate).
6. Remove plate sealer. Empty the wells and wash three times using at least 300 µL of cold wash buffer (2-8 °C) per well. Empty the wells and strike the plate firmly onto blotting paper in order to remove washing buffer completely.

For option 1: Detection of IgG/IgM mix-isotype

7. Add 100 µL of enzyme label IgG/IgM mix to the wells.

For option 2: Detection of IgG and IgM isotypes

7'. Add 100 µL of enzyme label IgG or IgM to the respective wells (refer to figure 1B).

For option 1 and 2: Incubation with enzyme labels, washes, and detection

8. Cover the plate with a plate sealer and incubate for 2 hours ±5 minutes at 2-8 °C (do not shake the plate).
9. Remove plate sealer. Empty the wells and wash three times using at least 300 µL of cold wash buffer (2-8 °C) per well. Empty the wells and strike the plate firmly onto blotting paper.

Note: Adjust TMB substrate solution to room temperature (18-28 °C).

10. Add 100 µL of TMB substrate solution to each well.
11. Cover plate with a plate sealer, incubate plate on an orbital plate shaker at 400-600 rpm for 30 ±2 minutes at 18-28 °C. Protect the plate from direct light.
12. Add 100 µL of stop solution to all wells. Proceed to step 13 within 30 minutes.
13. Read absorbance at 450 nm in a microtiter plate reader.

QUALITY CONTROL

A good understanding of this instruction for use is necessary to obtain reliable results. These will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following the instruction for use.
Since there is no control serum for anti-ganglioside antibodies commercially available, we recommend using a anti-ganglioside positive, and negative serum pool for internal quality control.
A minimal OD value of 1.2 is recommended for the calibrator. All controls must be within the established expected ranges (% ratio). The expected ranges of the controls are lot-specific and indicated in the QC data sheet.
Performance characteristics should be within established limits. If these characteristics are not in conformity with established limits and repetition excludes handling failures, check the following issues: i) Have all reagents, used in steps 3-10 been kept at 2-8 °C? ii) accuracy of the pipets, thermometers, and timers, iii) settings of ELISA washer and reader, iv) expiration date of the reagents v) storage and incubation conditions vi) colour of the TMB substrate solution (should be colourless) and vii) purity of the water.

STANDARDIZATION

The calibrator included in this kit has been calibrated against internal reference material. It has been adjusted to 100 % ratio**.**

RESULTS AND CALCULATION

Calculation of results:

1. Record absorbance (OD) at 450 nm for each well (calibrator, controls and samples).
2. Average the duplicate calibrator and control values (if available).
3. Results are expressed as ratio of absorbance of samples and the (averaged) absorbance of the calibrator.

IgG/IgM Mix isotypes

$$\%\ \text{Ratio:} \quad \frac{\text{absorbance of samples or controls}}{\text{absorbance of calibrator}} \times 200$$

IgG and IgM isotypes

$$\%\ \text{Ratio:} \quad \frac{\text{absorbance of samples or controls}}{\text{absorbance of calibrator}} \times 100$$

Programs to calculate results as % ratio are available on most microplate readers.

Note: Results presented in tables 3 and 4 are examples. Calibrator and controls must be used in each individual assay.

LIMITATIONS

1. The BÜHLMANN GanglioCombi™ MAG ELISA has not been validated for plasmapheresis samples.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (within-run):

"MAG": 3.4-15.0 % CV	**GM1: 1.4-5.7 % CV**
GM2: 2.0-14.2 % CV	**GD1a: 1.5-7.7 % CV**
GD1b: 2.3-4.4 % CV	**GQ1b: 2.6-5.2 % CV**

For each of the five gangliosides coated on the microtiter plate two anti-ganglioside positive sera were selected. The serum samples were assayed in twelve replicates in a single run for each of the enzyme labels: IgG/IgM Mix, IgG and IgM with one reagent lot. For the synthetic "MAG" epitope coated on the microtiter plate, four anti-MAG positive sera were selected and were assayed in twelve replicates in a single run with enzyme label IgM with two reagent lots. The results are summarized in table 5, 6, 7 and 8.

Inter-Assay Precision (between-run):

"MAG": 5.6-15.1 % CV	**GM1: 9.0-21.0 % CV**
GM2: 5.0-16.5 % CV	**GD1a: 11.1-24.1 % CV**
GD1b: 8.0-13.2 % CV	**GQ1b: 8.2-19.6 % CV**

For each of the five gangliosides coated on the microtiter plate two anti-ganglioside positive sera were selected. The serum samples were assayed in single replicates in 20 independent runs, with one run per day, for each of the enzyme labels: IgG/IgM Mix, IgG and IgM. For the synthetic "MAG" epitope coated on the microtiter plate, four anti-MAG positive sera were selected and were assayed in duplicates in ten independent runs, with one run per day, with enzyme label IgM. The inter-assay precision studies were performed with one reagent lot. The results are summarized in table 9, 10, 11 and 12.

Limit of Blank (LoB): ≤6.1 % ratio

The LoB was established according to the CLSI guideline EP17-A. Twelve blank replicates (incubation buffer) per ganglioside were essayed in a single run for all three enzyme labels: IgG/IgM Mix, IgG and IgM. As MAG auto-antibody testing can only be interpreted in the context of IgM detection, the "MAG" epitope was assayed with IgM enzyme label only. The Limit of Blank (LoB), expressed as the % ratio to the calibrator absorbance, was calculated to be ≤6.1 % for enzyme label IgG/ IgM Mix, ≤3.5 % the enzyme label IgG and ≤5.3 % the enzyme label IgM. The highest LoB obtained with the three different enzyme labels was taken to determine the overall Limit of Blank (LoB). The LoB was calculated using parametric analysis.

Limit of Detection (LoD): ≤8.1 % ratio

The LoD was established according to the CLSI guideline EP17-A. For each of the five gangliosides as well as the synthetic "MAG" coated on the microtiter plate a single sample representing low antibody concentration was selected. The low-level samples were measured in twelve replicates, in a single run for each of the three enzyme labels: IgG/IgM Mix, IgG and IgM. As MAG auto-antibody testing can only be interpreted in the context of IgM detection, the "MAG" epitope was assayed with IgM enzyme label only. The LoD, expressed as the % ratio to the calibrator absorbance, was calculated to be ≤8.1 % for enzyme label IgG/ IgM Mix, ≤6.3 % for enzyme label IgG, and ≤6.9 % for enzyme label IgM. The highest LoD obtained with the three different enzyme labels was taken to determine the overall Limit of Detection (LoD).

Functional Sensitivity: ≤8.1 % ratio

Precision values obtained for serum samples in the inter-assay precision study were plotted against their mean % ratio values. A cubic polynomial fit was applied to the data points to obtain a precision profile. As an intersection of the one-sided 95 % confidence interval of the fit with the 20 % CV acceptance criterion was not observed, the functional sensitivity was determined as equal to the LoD. The results are summarized in figure 2.

Linearity

The linear range of the BÜHLMANN GanglioCombi™ MAG ELISA was determined according to the CLSI guideline EP06-A. Multiple sera with concentrations over the entire measuring range of the test, allowing the evaluation of the majority of ganglioside/enzyme label combinations, were used. Serum samples were diluted according to the instruction for use. Subsequently dilution series from each sample were prepared in graduations of 10 % using incubation buffer as the diluent. Linearity was defined as the interval in which the relative difference between the linear and, if significant, higher order polynomial fit was below 20 %. For ratios ≤25 % an absolute difference of below 5 % ratio was allowed. The results are summarized in table 13.

Method Comparison for the MAG "mimotope":

Anti-MAG Autoantibodies ELISA (EK-MAG): κ = 0.85

Anti-SGPG Autoantibodies ELISA (EK-SGPG): κ = 0.77

Eighty (80) serum samples with anti-MAG IgM autoantibody signals over the entire measuring range were assayed in singlets with IgM enzyme label detection on "MAG" coated microtiter plates and with the anti-MAG Autoantibodies ELISA (EK-MAG) and the anti-SGPG Autoantibodies ELISA (EK-SGPG) test. Measurements were performed using two "MAG" coated microtiter plate lots. The results were analysed with Kappa statistics. The correlation data is illustrated in figure 3A and 3B.

INTERFERING SUBSTANCES

No interference is detected with the following substances up to the following concentrations: unconjugated bilirubin (icteric sera): 40 mg/d; conjugated bilirubin (icteric sera): 60 mg/dL; hemoglobin: 400 mg/dL; hemolyzate (hemolyzed blood): 400 mg/dL and triglycerides (Intralipid®): 2000 mg/dL.

APPENDIX I

TABLES AND FIGURES

Microtiter plate set-up: IgG/IgM-Mix label

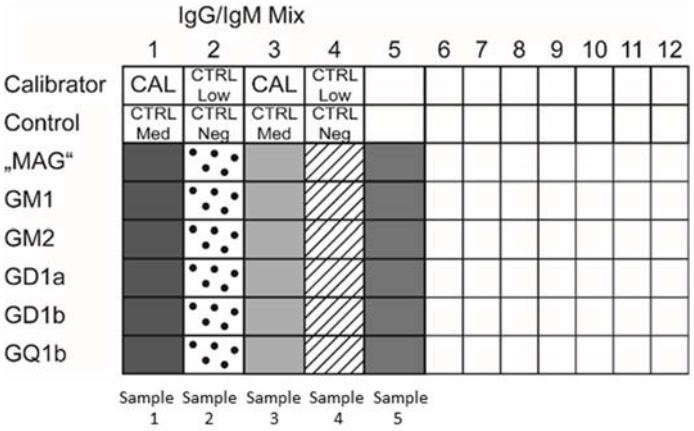


Figure 1A: ≤ 24 samples / Kit (2 MP / Kit)

Microtiter plate set-up: IgG & IgM labels

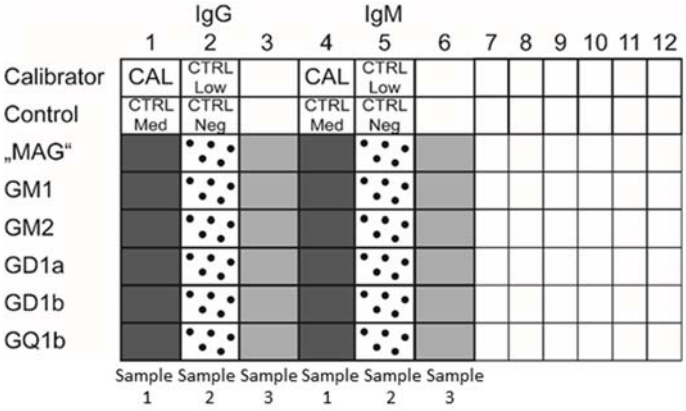


Figure 1B : 2 profiles / sample, ≤ 12 sample / Kit (2 MP / Kit)

Example of Results

A IgG/IgM-Mix label

B-GCO-ELGM	Absorbance (OD450)	Ratio [%]
Calibrator	1.415	
	1.445	
Calibrator Avg.	1.430	200
Medium Control	0.498	69
	0.482	67
Med. Control Avg.	0.490	68
Low Control	0.195	27
	0.191	26
Low Control Avg.	0.193	27
Negative Control	0.090	12
	0.100	14
Neg. Control Avg.	0.095	13
Sample 1 “MAG”	0.300	42
Sample 1 GM1	0.544	76
Sample 1 GM2	0.106	15
Sample 1 GD1a	0.162	45
Sample 1 GD1b (w	0.745	104
Sample 1 GQ1b	0.090	13

Table 3

Example of Results

B IgG & IgM labels

Enzyme label	Absorbance (OD450)		Ratio [%]	
B-GCO-ELG/ B-GCO-ELM	IgG	IgM	IgG	IgM
Calibrator	1.789	2.576		
	1.833	2.527		
Calibrator Avg.	1.836	2.551	100	100
Medium Control	1.267	1.743	69	68
	1.237	1.764	67	69
Med. Control Avg.	1.252	1.753	68	69
Low Control	0.567	0.938	30	37
	0.584	0.942	32	37
Low Control Avg.	0.571	0.940	31	37
Neg. Control	0.061	0.098	3	4
	0.051	0.095	3	4
Neg. Control. Avg.	0.056	0.097	3	4
Sample 1 “MAG”	0.532	0.208	29	8
Sample 1 GM1	0.171	3.814	9	150
Sample 1 GM2	0.116	0.095	6	37
Sample 1 GD1a	1.117	0.574	61	23
Sample 1 GD1b	1.021	0.354	56	14
Sample 1 GQ1b	0.378	0.208	21	8

Table 4

Intra-Assay Precision (Within-Run)

Enzyme label IgM: synthetic “MAG”								
	Lot L15AB				**Lot L24AD**			
Sample	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Mean [Ratio]	65.8	78.9	155.0	152.9	68.6	88.4	182.6	171.1
SD [Ratio]	4.5	7.6	5.2	7.3	10.3	7.2	17.2	19.4
CV [%]	**6.9**	**9.6**	**3.4**	**4.7**	**15.0**	**8.2**	**9.4**	**11.3**

Table 5

Enzyme label IgG/ IgM										
Ganglio sides	**GM1**	**GM1**	**GM2**	**GM2**	**GD1a**	**GD1a**	**GD1b**	**GD1b**	**GQ1b**	**GQ1b**
Sample	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Mean [Ratio]	86	156	72	192	124	264	110	310	286	84
SD [Ratio]	1.2	2.5	5.2	1.2	4.8	2.0	2.0	4.0	4.6	1.9
CV [%]	**2.9**	**3.2**	**14.2**	**1.2**	**7.7**	**1.5**	**3.6**	**2.6**	**3.2**	**4.5**

Table 6

APPENDIX I

TABLES AND FIGURES

Intra-Assay Precision (Within-Run)

Enzyme label IgG										
Ganglio sides	**GM1**	**GM1**	**GM2**	**GM2**	**GD1a**	**GD1a**	**GD1b**	**GD1b**	**GQ1b**	**GQ1b**
Sample	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Mean [Ratio]	59	82	49	115	72	153	139	111	89	222
SD [Ratio]	1.6	4.7	1.8	6.9	4.4	7.2	4.0	2.8	4.5	5.8
CV [%]	**2.7**	**5.7**	**3.7***	**6.0***	**6.1**	**4.7**	**2.9**	**2.5**	**5.1**	**2.6**

Table 7

Enzyme label IgM										
Ganglio sides	**GM1**	**GM1**	**GM2**	**GM2**	**GD1a**	**GD1a**	**GD1b**	**GD1b**	**GQ1b**	**GQ1b**
Sample	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Mean [Ratio]	79	87	43	152	122	103	56	142	72	74
SD [Ratio]	1.8	1.2	0.9	4.8	3.6	3.9	2.5	3.3	3.8	2.6
CV [%]	**2.3**	**1.4**	**2.0**	**3.1**	**3.0****	**3.8****	**4.4**	**2.3**	**5.2**	**3.5**

Table 8

Inter-Assay Precision (Between-Run)

Enzyme label IgM: synthetic "MAG"				
	Lot 3317N			
Sample	**1**	**2**	**3**	**4**
Mean [Ratio]	106.0	115.1	176.1	173.6
SD [Ratio]	11.7	17.4	12.6	9.7
CV [%]	**11.0**	**15.1**	**7.1**	**5.6**

Table 9

Enzyme label IgG/IgM										
Ganglio sides	**GM1**	**GM1**	**GM2**	**GM2**	**GD1a**	**GD1a**	**GD1b**	**GD1b**	**GQ1b**	**GQ1b**
Sample	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Mean [Ratio]	74	184	138	176	224	92	96	306	92	262
SD [Ratio]	3.5	13.9	11.3	7.8	14.2	7.5	4.8	13.4	3.8	13.3
CV [%]	**9.5**	**15.2**	**16.5**	**8.9**	**12.7**	**16.2**	**10.1**	**8.8**	**8.2**	**10.1**

Table 10

Enzyme label IgG										
Ganglio sides	**GM1**	**GM1**	**GM2**	**GM2**	**GD1a**	**GD1a**	**GD1b**	**GD1b**	**GQ1b**	**GQ1b**
Sample	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Mean [Ratio]	52	84	45	118	44	139	109	140	42	204
SD [Ratio]	6.4	17.6	6.4	21.5	10.6	15.5	14.2	18.5	8.3	23.6
CV [%]	**12.4**	**21.0**	**14.1***	**18.2***	**24.1**	**11.1**	**13.0**	**13.2**	**19.6**	**11.6**

Table 11

Inter-Assay Precision (Between-Run)

Enzyme label IgM										
Ganglio sides	**GM1**	**GM1**	**GM2**	**GM2**	**GD1a**	**GD1a**	**GD1b**	**GD1b**	**GQ1b**	**GQ1b**
Sample	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Mean [Ratio]	102	63	47	120	112	65	154	46	171	63
SD [Ratio]	10.9	5.7	2.4	8.8	35.2	14.6	19.1	3.7	16.2	9.9
CV [%]	**10.7**	**9.0**	**5.0**	**7.3**	**31.5****	**22.6****	**12.4**	**8.0**	**9.5**	**15.6**

Table 12

Functional Sensitivity

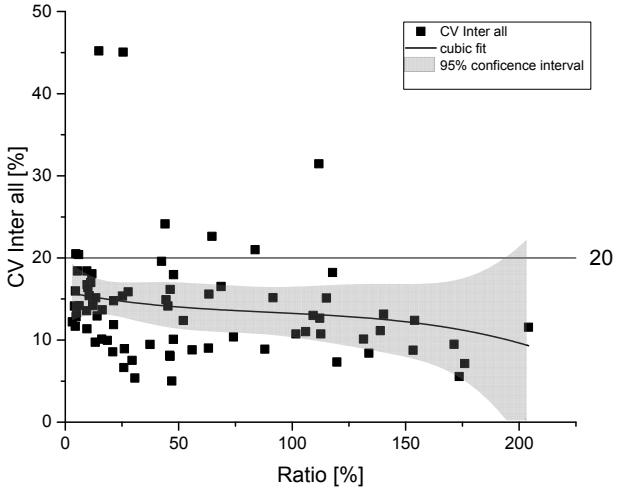


Figure 2

Linearity

Ganglio-sides	Enzyme label	Serum	Range measured	Linear range
"MAG"	IgM	Reg 83	3-81	3-75
		Reg 54	22-57	22-57
GM1	IgG	SP69	7-94	7-94
		P II	9-181	17-175
		JJ	6-127	6–127
	IgM	SP69	10-93	21-79
		SM1	14-148	26-114
			9-138	9-89
			16-173	30-128
	Mix	P II	16-302	16-302
		SP69	14-218	22-178
GM2	IgM	RL1739	8-110	13-102
GD1a	IgG	EP4076	4-88	4-88
	IgM	68-MA	14-111	14-111
		MS	7-106	17-103
GD1b	IgG	581-SP	5-99	5-99
	IgM	72 BR	14-163	14-148
	Mix	581-SP	18-264	18-176
			12-198	34-166
GQ1b	IgG	MG	18-228	18-228

Table 13

APPENDIX I

TABLES AND FIGURES

Method comparison for MAG “mimotope”

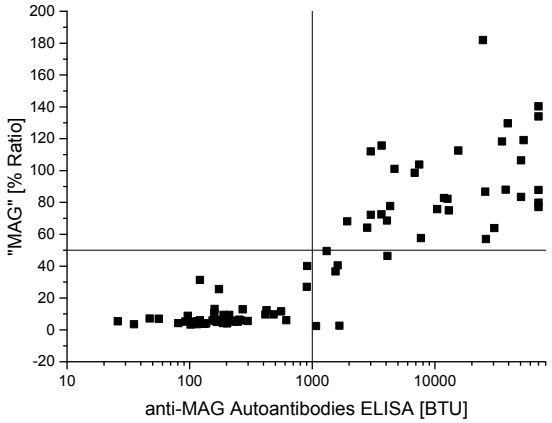


Figure 3A: The horizontal line indicates the 50 % ratio cut-off applied in the BÜHLMANN GanglioCombi™ ELISA tests. The vertical line indicates the 1000 BTU cut-off applied in the anti-MAG Autoantibody ELISA.

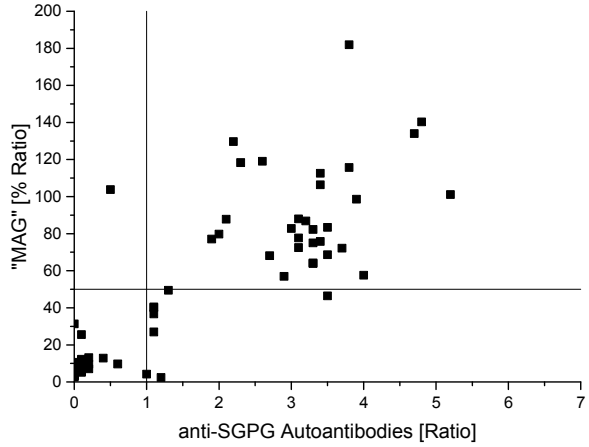
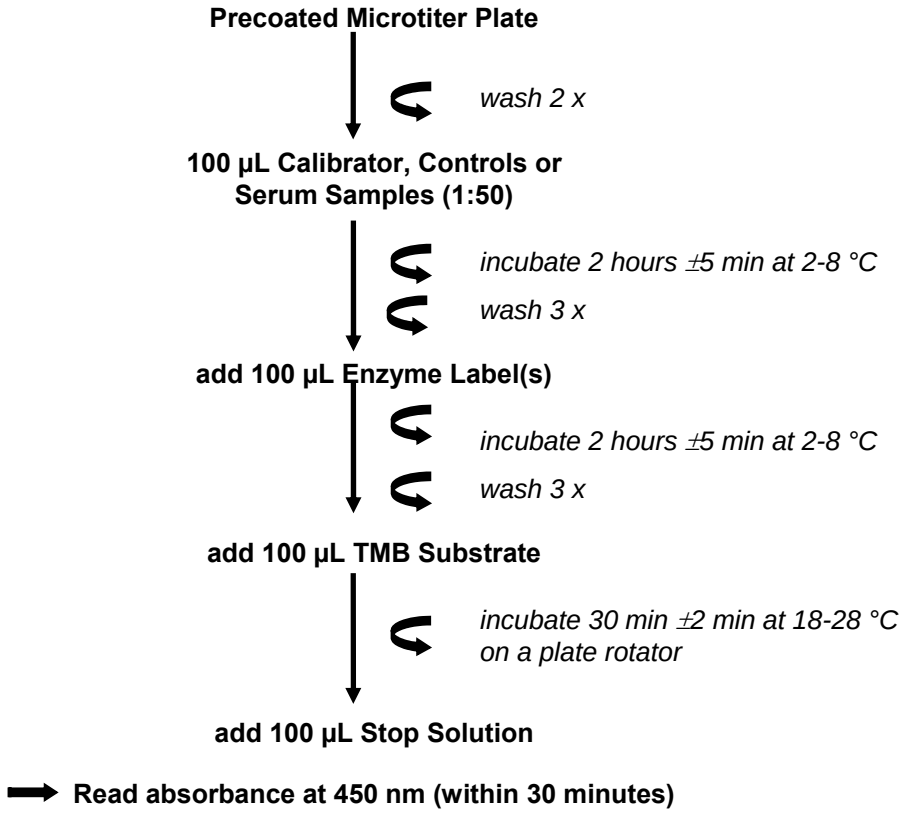


Figure 3B: The horizontal line indicates the 50 % ratio cut-off applied in the BÜHLMANN GanglioCombi™ ELISA tests. The vertical line indicates the cut-off value of 1 applied in the anti-SGPG Autoantibody ELISA.

APPENDIX II

SHORT PROTOCOL

BÜHLMANN GanglioCombi™ MAG ELISA



TIME TO RESULT: 4.5 HOURS

APPENDIX III

SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Contains sufficient for <n> tests
	Consult Instructions for Use-
	Temperature limitation
MP	Microtiterplate
BUF WASH 10X	Wash Buffer Concentrate (10x)
BUF INC	Incubation Buffer

Symbol	Explanation
CONTROL -	Negative Control
CONTROL L	Low Control
CONTROL M	Medium Control
CAL	Calibrator
EL IgG	Enzyme Label IgG
EL IgM	Enzyme Label IgM
EL MIX	Enzyme Label IgG/IgM Mix
SUBS TMB	TMB Substrate
SOLN STOP	Stop Solution

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