Instruction Manual

REF 5003

April 01, 2014

Anti-Gangliosid Dot

- 20 x 12 determinations -

IVD *In vitro* diagnostic device

CE

Enzyme immunodot for the determination of IgG and/or IgM antibodies to gangliosides in human serum, plasma or cerebrospinal fluid

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Anti-Gangliosid Dot is used for the qualitative detection of IgG or IgM antibodies to gangliosides in human serum, plasma or cerebrospinal fluid (CSF) for the diagnosis of autoimmune neuropathies. Performing an IgG/IgM antibody screening is also possible.

Inflammatory neuropathies of the peripheral nervous system are characterized by numerous clinical symptoms ranging from slight weariness and uncharacteristic indisposition to neuromuscular disorders and functional deficiency like respiratory paralysis and cardiac arrest.

Recently autoantibodies to gangliosides have been identified in patients suffering from disorders of the peripheral nervous system. Gangliosides belong to the group of acid glycolipids containing a lipid (ceramide), oligosaccharide and sialic acid. Gangliosides are components of cell membranes and especially found in the central and peripheral nervous system. Ganglioside-like structures also appear on the surface of microorganisms. Inflammatory neuropathies often occur following an infection with Campylobacter jejuni, Cytomegalovirus, Epstein-Barr virus, Mycoplasma pneumoniae or Haemophilus influenzae. Antibodies to ganglioside structures of the microorganisms may cross-react to gangliosides of the myelin sheath or neurofibre and induce inflammation processes with subsequent demyelination.

Following ganglioside antibodies were described to be specific for neuropathies of the peripheral nervous system:

Guillain-Barré syndrome	GM1, GD1a, GD1b, GT1a, GT1b, GQ1b	IgG (IgM)
Miller-Fisher syndrome	GQ1b, GT1a	IgG
Multifocale musculare neuropathy	GM1, GM2, GM3, GD1a, GD1b	IgM
Chronic inflammable demyelinated polyn.	GM2, GM3, GD1a, GD1b	IgM
Chronic-atactic neuropathy (CANOMAD)	GM3, GD1b, GD2, GD3, GT1b, GQ1b	IgM
Acute atactic-sensoric neuropathy	GD1b, GD3	IgG
Acute musculare axonal neuropathy	GM1, GD1a	IgG
IgM paraproteinemia, demyelinating neuropathy	Sulfatide	IgM (IgG)

As a result of the cross-reactivity with microbial structures anti-GM1 IgM antibodies might be found in healthy people, too. A single incidence of these antibodies is not pathognomonic for a neuropathy.

Willison HJ, Yuki N: Peripheral neuropathies and anti-glycolipid antibodies. Brain, 2002, 125, 2591-2625

Khalili-Shirazi A, Gregson N, Gray I, Rees J, Winer J, Hughes R: Antiganglioside antibodies in Guillain-Barre syndrome after a recent cytomegalovirus infection, J Neurol Neurosurg Psychiatry, 1999, 66, 376-9

Schwerer B, Neisser A, Bernheimer H: Distinct immunoglobulin class and immunoglobulin G subclass patterns against ganglioside GQ1b in Miller Fisher syndrome following different types of infection. Infect Immun, 1999, 67, 2414-201

Alaniz ME, Lardone RD, Yudowski SL, Farace MI, Nore GA: Normally occurring human anti-GM1 immunoglobulin M antibodies and the immune response to bacteria. Infect Immun, 2004, 72, 2148-51

PRINCIPLE OF THE TEST

Anti-Gangliosid Dot is a sensitive immunodot assay for the qualitative determination of IgG and/or IgM antibodies to gangliosides in human serum, plasma or cerebrospinal fluid (CSF).

Anti-Gangliosid Dot includes 20 numbered test stripes (line dot stripes). The stripes consist of a membrane where different autoantigen lines are sprayed on. One line serves as a positive control and the other 12 lines are coated with one of the highly purified gangliosides GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, GQ1b and sulfatide, respectively.

During the first incubation autoantibodies of the patient sample bind to the target antigens immobilized on the solid phase (membrane). Following an incubation period of 120 minutes at 4° C while shaking, unbound sample components are removed by a wash step.

Bound antibodies react specifically with anti-IgG or anti IgM conjugated to horse radish peroxidase (POD) in a second step. Performing an IgG/IgM antibody screening using both conjugates in one tray is also possible. Following an incubation period of 60 min at 4°C excessive conjugate is separated from the solid-phase immune complexes by an additional washing-step.

The horse radish peroxidase converts the colourless substrate solution into a dark purple precipitating line on the membrane. After 10 min while shaking the reaction is stopped by a wash step.

Stripes can be read off after a drying step.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma and cerebrospinal fluid (CSF) can be used, too.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

Preparation before use

Samples to be assayed are used at 4°C. Take care to agitate samples gently in order to ensure homogeneity.

TEST COMPONENTS for 20 x 12 determinations

A **Ag**	**Dot strips** 20 strips with 13 test dot lines - 12 test lines coated with highly purified gangliosides GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, and GQ1b (human), sulfatide (bovine) - Positive control	20 dot strips
B **BUF**	**Buffer, 10-fold** sufficient for 150 ml **10x**	15 ml concentrate capped white
C **CONJ** **G**	**IgG conjugate, 20 fold** Anti-human IgG (rabbit) coupled with horseradish peroxidase	1.2 ml ready to use capped red
D **CONJ** **M**	**IgM conjugate, 20 fold** Anti-human IgM (rabbit) coupled with horseradish peroxidase	1.2 ml ready to use capped green
E **SOLN** **TMB**	**Substrate** 3,3`,5,5`-Tetramethylbenzidine	11 ml ready to use capped blue
P **CONTROL**	**Positive Control** human serum or plasma, positive for antibodies to gangliosides (see leaflet enclosed) **+**	0,1 ml ready to use
N **CONTROL**	**Negative Control** human serum or plasma, negative for antibodies to gangliosides (see leaflet enclosed) **-**	0,1 ml ready to use
F	**Incubation tray for 12 dot stripes**	2 x

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- pipette tips
- refrigerator or a cold room to perform the first two incubation and washing steps
- shaker (rocking shaker recommended)
- graduated cylinders
- distilled or de-ionized water
- plastic pincers
- paper towel

Size and storage

The Anti-Gangliosid Dot has been designed for 20 x 12 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the Anti-Gangliosid Dot have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

The first two incubation steps are performed at 4°C with precooled reagents (buffer solution, conjugate). The second wash step is performed at room temperature (RT). Therefore, the reagents (buffer solution, substrate) must have RT in time.
The dot stripes are sealed in a plastic foil bag. A sufficient number of dot stripes has to be cut off with a scalpel or a cutter from the retaining membrane. Unused dot stripes have to be kept dry and stored in the plastic foil bag.

Dilute the 10 fold concentrated buffer with de-ionized or destilled water (1+9).

For each test strip 5 ml of buffer solution are requested

Example:
15 ml concentrated buffer + 135 ml destilled water.
The prepared solution is stable at 2 - 8 °C up to 30 days.

All other components are ready for use and stable until the expiry date.

Avoid exposure of the substrate to light.

The positive Control P is to be handled like a normal serum sample.

Cleaning procedure of the incubation tray

After application incubate the incubation tray for 30 min with a detergent and rinse with water subsequently.

In the following step fill in any type of alcohol (methanol, propanol or ethanol), incubate on the rocking shaker for 30 min and subsequently rinse with water.

Clean the incubation tray with a cotton bud, rinse with water, and let it dry.

ASSAY PROCEDURE

- **Follow the instruction strictly and avoid any time shift.**
- **The whole assay has to be performed on a shaker (rocking shaker recommended)**
- **Until the substrate reaction all reagents are incubated at 4°C. Keep the required reagents refrigerated.**
- **After the conjugate reaction the assay is run at RT. Ensure that the required reagents (buffer solution, substrate) have RT (18°-25°C).**

1. Take the reagents and sufficient number of dot stripes out of the box, mix the reagents gently.
2. Dispense **1 ml** of buffer solution (made of B) in each well. Place the strips with the reactive side down into the respective wells.
3. Add patient samples / positive control (P) and negative control (N) to the buffer solution:
   serum/plasma: **10 µl** (resulting dilution 1+100)
   CSF: **50 µl** (resulting dilution 1+20)
4. Incubate **120 min at 4 °C** while shaking.
5. Decant (**Caution:** Turn over carefully the incubation tray and gently decant the buffer solution, any remaining liquid has to be removed with an absorbent paper). Wash **5 min at 4°C** with **1 ml** buffer solution (made of B) while shaking.
6. Pipette **1 ml** buffer solution (made of B) and add into the respective wells.
   IgG determination: **50 µl** conjugate C
   IgM determination: **50 µl** conjugate D
   IgG/IgM screening: **50 µl** of conjugates C and D each,
7. Incubate for **60 min at 4° C** while shaking.
8. Decant as above. Wash **5 min at RT** with **1 ml** buffer solution (made of B) while shaking.
9. Pipette **0,5 ml** substrate (E) into the respective wells
10. Incubate for **10 min** at **RT (18-25°C)** while shaking.
11. Decant as above and wash **2 min at RT** with **1 ml** buffer solution (made of B) while shaking.
12. Decant as above and wash **2 min at RT** with **1 ml distilled water** while shaking to stop the reaction.
13. Collect the dot stripes from the wells and dry the membranes by pressing the reactive side of the stripe onto absorbent paper briefly. After approximately **30 min** the stripes are to be interpreted.

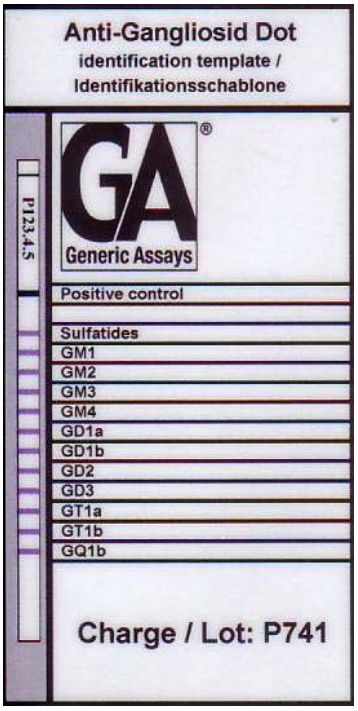
DATA PROCESSING

The evaluation of the test results will be performed by means of the provided lot specific evaluation template. For doing this the strips must have been dried and glued onto the template.

The **positive control line** must be positive in all cases. The coloration of the line ensures that the test has been run correctly and the kit components are not degraded. If the positive control line shows no coloration the results **cannot** be interpreted.

The test lines are coated with highly purified antigens and detect specific antibody binding of the sample in the test.

The intensity of the bands given on the lot-specific evaluation template (see picture below) serves as cut-off for the decision of positive and negative results.



Positive result:

A sample is considered to be **positive** in respect to one of the antibodies if the colouration of the test line shows a more intense coloration then the band on the identification / evaluation template.

Negative result:

A sample is considered to be **negative** in respect to one of the antibodies if the colouration of the test line shows the same or less intense coloration then the band on the identification / evaluation template.

Validation:

In order to interpret the results the test line of the positive control has to show a clear colouration.

Additional validation can be done by processing the positive control (P). Target results of this control are stated at the leaflet enclosed in the kit.

Limitations of Method

Healthy individuals should be tested negative by the Anti-Gangliosid Dot. However, IgM anti-ganglioside antibodies can be found in healthy people because of the cross-reactivity to microbial antigens. Furthermore, asymptomatic individuals can show a positive antibody reaction.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

INCUBATION SCHEME

Anti-Gangliosid Dot (5003)

Up to step 7 all reactions are performed at 4 °C; the required reagents (dot stripes, buffer solution, conjugate) and patient samples must be refrigerated. The following steps have to be performed at RT (18-25°C): Ensure that the needed reagents have RT!

1.	Mix required reagents gently.	
2.	Dispense 1 ml of buffer solution (made of B) into each well of the tray, place the strips with the reactive side down into the buffer solution;	
3.	Pipette neat patient sample / positive control (P)/ negative control (N)	serum/plasma: 10 µl (resulting dilution 1+100) CSF: 50 µl (resulting dilution 1+20)
4.	Incubate	120 minutes, **4°C** while shaking
5.	Wash	Decante, dispense 1 ml buffer solution (made of B), 5 minutes at **4°C** while shaking
6.	Pipette 1 ml buffer solution (made of B) and add: IgG determination: **50 µl** conjugate C IgM determination: **50 µl** conjugate D IgG/IgM screening: **50 µl** of conjugates C and D each,	
7.	Incubate	60 minutes, **4°C** while shaking
8	Wash	Decante, dispense 1 ml buffer solution (made of B), 5 minutes at room temperature (**RT)** while shaking
9.	Pipette 0.5 ml substrate (E)	
10.	Incubate	10 minutes, **RT** while shaking
11.	Decant, washing of the strips	While shaking, RT, 2 minutes with 1 ml buffer solution (diluted from B)
12.	Decant, washing of the strips, stop of the reaction	While shaking, RT, 2 minutes with 1 ml distilled water
13.	Dry test strips on paper towel for 30 minutes, read out results	

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for re-constituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C prior use in the original shipping container.
- Some of the reagents contain small amounts of kathon (1% v/v) or sodium azide (< 0,1%) as a preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the kit contains potentially hazardous materials the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.