



Instruction Manual

REF 5002

March 23, 2007

INTENDED USE

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

Anti-Gangliosid Dot

- 5 x 11 determinations -

IVD *In vitro* diagnostic device



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

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:

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

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

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.



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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 or cerebrospinal fluid (CSF).

Anti-Gangliosid Dot includes 5 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 11 lines are coated with one of the highly purified gangliosides GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b and GQ1b, 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. 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 5 x 11 determinations

A	Dot strips	5 dot strips
Ag	5 strips with 12 test dot lines - 11 test lines coated with highly purified human gangliosides GM1, GM2, GM3, GM4, GD1a, GD1b, GD2, GD3, GT1a, GT1b, and GQ1b - Positive control	
B	Buffer, 10-fold	15 ml
BUF	sufficient for 150 ml	concentrate capped white
C	IgG conjugate, 21 fold	0.3 ml
CONJ G	Anti-human IgG (rabbit) coupled with horse radish peroxidase	ready to use
D	IgM conjugate, 21 fold	0.3 ml
CONJ M	Anti-human IgM (rabbit) coupled with horse radish peroxidase	ready to use
E	Substrate	11 ml
SOLN TMB	3,3',5,5'-Tetramethylbenzidine	ready to use capped blue
F	Incubation tray for 10 dot stripes	1 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 5 x 11 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 distilled water (1+9).

For each test strip 10 ml of buffer solution are requested

Example:

15 ml concentrated buffer + 135 ml distilled 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.

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. Place the stripes with the reactive side down into the respective wells and dispense 1 ml of buffer solution (made of B).
3. Add patient samples to the buffer solution
serum: 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 3 times 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 (**Caution:** Turn over carefully the incubation tray and gently decant the buffer solution, any remaining solution has to be removed with an absorbent paper). Wash 3 times 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 and wash 2 times for 5 min with 1 ml buffer solution (made of B) at RT in order to **stop** the **substrate reaction** (**Caution:** Turn over carefully the incubation tray and gently decant the buffer solution, any remaining liquid has to be removed with an absorbent paper).
12. 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

Results should be interpreted only after dot stripes have been dried for at least 30 min and glued onto the analysis scheme (see kit content).

The **positive control line** must be positive in all cases. The colouration 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 human antigens and detect specific antibody binding of the sample in the test.

REFERENCE VALUES

Anti-Gangliosid Dot	Result
	positive
GM1	
GM2	
GM3	positive
GM4	
GD1a	
GD1b	
GD2	
GD3	
GT1a	
GT1b	
GQ1b	

Positive result:

A sample is considered to be **positive** in respect to one of the gangliosides if the colouration of the test line is **visible**.

Negative result:

A sample is considered to be **negative** in respect to one of the gangliosides if the colouration of the test line is **uncoloured**.

Validation:

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

Limitations of Method

Healthy individuals should be tested negative by the Anti-Gangliosid Dot. However, IgM antibodies to GM1 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.

Comments

INCUBATION SCHEME

Anti-Gangliosid Dot (5002)

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.	Place the stripes with the reactive side down into the respective wells; dispense 1 ml of buffer solution (made of B)
3.	Pipette neat patient sample <div style="float: right; text-align: right;"> serum: 10 µl (resulting dilution 1+100) CSF: 50 µl (resulting dilution 1+20) </div>
4.	Incubate 120 minutes, 4°C while shaking
5.	Wash Decante, dispense 1 ml buffer solution (made of B) 3 x 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) 3 x 5 minutes at RT while shaking
9.	Pipette 0.5 ml substrate (E)
10.	Incubate 10 minutes, RT while shaking
11.	Wash Decante, dispense 1 ml buffer solution (made of B) 2 x 5 minutes at RT while shaking
12.	Dry line dot stripes 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) as a preservative. 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.