



Instruction Manual

REF 5005

January 30, 2009

Anti-Gangliosid 7 Dot

- 20 x 7 determinations -

IVD *In vitro* diagnostic device



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



GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telefon: +49 (0) 33708-9286-0
Fax: +49 (0) 33708-9286-50

www.genericassays.com

INTENDED USE

Anti-Gangliosid 7 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, GD1a, GD1b	IgM
Chronic inflammable demyelinated polyn.	GM2, GD1a, GD1b	IgM
Chronic-atactic neuropathy (CANOMAD)	GD1b, GT1b, GQ1b	IgM
Acute atactic-sensoric neuropathy	GD1b	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.

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 7 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 7 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 7 lines are coated with one of the highly purified gangliosides GM1, GM2, GD1a, GD1b, 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, 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 the reaction is stopped by a wash step.

Stripes can be read off after drying.

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 7 determinations

A	Dot strips	20 dot strips
Ag	20 strips with 8 test dot lines - 7 test lines coated with highly purified gangliosides GM1, GM2, GD1a, GD1b, GT1a, GT1b, GQ1b (human) - Positive control	
B	Buffer, 10-fold	2 x 15 ml concentrate capped white
BUF	10x	
C	IgG conjugate, 20 fold	1.2 ml ready to use capped red
CONJ G	Anti-human IgG (rabbit) coupled with horseradish peroxidase	
D	IgM conjugate, 20 fold	1.2 ml ready to use capped green
CONJ M	Anti-human IgM (rabbit) coupled with horseradish peroxidase	
E	Substrate	11 ml ready to use capped blue
SOLN TMB	3,3',5,5'-Tetramethylbenzidine	
F	Incubation tray for 12 dot stripes	2 x

Available in addition: REF 50031

P	Positive Control Serum	0.1 ml ready to use
CONTR	human serum or plasma, positive for antibodies to gangliosides (see leaflet enclosed)	+

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 7 Dot has been designed for 20 x 7 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 7 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 have 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.

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/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 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.

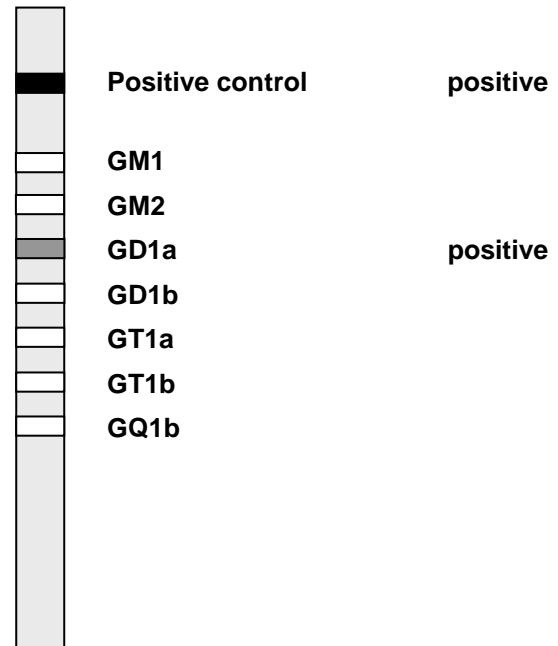
DATA PROCESSING

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** 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 shows no coloration the results **can not** 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



Positive result:

A sample is considered to be **positive** in respect to one of the gangliosides if the colouration of the test line is clearly **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 it has to show a clear colouration.

Limitations of Method

Healthy individuals should be tested negative by the Anti-Gangliosid 7 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 7 Dot (5005)

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. Steps 8 – 12 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="display: flex; justify-content: space-between; margin-left: 150px;"> serum/plasma: 10 µl (resulting dilution 1+100) </div> <div style="display: flex; justify-content: space-between; margin-left: 150px;"> 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.