DRG® Meningitis IgG (EIA-4918)

Revised 16 Sept. 2010 rm (Vers. 3.1) **USA: RUO**

INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgG antibodies to groups ACWY Meningococcus in human plasma and sera.

THIS KIT IS FOR RESEARCH USE ONLY.

PRINCIPLE OF THE TEST

Microplates are coated with a preparation of purified capsular polysaccharides formed by serogroups A, B, C, Y and W135. In the 1st incubation, the solid phase is treated with diluted samples and anti-Meningococcus IgG are captured, if present, by the antigens. After washing out all the other components of the sample, in the 2nd incubation bound anti-Men IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP). The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-Men IgG antibodies present in the sample. A cut-off value permits to transform the optical density values detected in positive or negative results due to the presence of absence of anti-Men IgG.

COMPONENTS

Each kit contains sufficient reagents to perform 96 tests.

1. **Microplate:** **MICROPLATE**
   12 strips x 8 breakable microwells coated with polysaccharides derived from Meningococcus ABCYW. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.
2. **Negative Control:** **CONTROL -**
   1 x 2.0 mL/vial. Ready to use and pale yellow color coded.
   Contains human serum negative for IgG anti Meningococcus, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.
3. **Positive Control:** **CONTROL +**
   1 x 2.0 mL/vial. Ready to use and dark green color coded.
   Contains human serum positive for IgG anti Meningococcus, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. .
4. **Wash buffer concentrate:** **WASHBUF 20X**
   1 x 60 mL/bottle. 20x concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.1% Kathon GC.

5. **Enzyme conjugate:** **CONJ**
   1 x 16 mL/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.
6. **Chromogen/Substrate:** **SUBS TMB**
   1 x 16 mL/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).
   Note: To be stored protected from light as sensitive to strong illumination.
7. **Stop solution, Sulphuric Acid:** **H_2SO_4 0.3 M**
   1 x 15 mL/vial, contains 0.3 M H_2SO_4 solution.
   Attention !: Irritant (Xi R36/38; S2/26/30)
8. **Specimen Diluent:** **DILSPE**
   2 x 60 mL/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.
9. Plate sealing foils n°2
10. Package insert n°1

MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (1000, 100 and 10 µL) and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

SPECIMEN: PREPARATION AND WARNINGS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.

4. Sera and plasma can be stored at +2°C - 8°C for u p to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8 µ filters to clean up the sample for testing.

PREPARATION OF COMPONENTS AND WARNINGS

Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storage. In this case call the customer service. Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at +2°C - 8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Controls:
Ready to use. Mix well on vortex before use.

Wash buffer concentrate:
The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2°C - 8°C.

Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes.
If this component has to be transferred use only plastic, possibly sterile disposable containers.

Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong illumination, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, possible sterile disposable container.

Sample Diluent:
Ready to use component. Mix carefully on vortex before use.

Stop Solution/Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Legenda: R 36/38 = Irritating to eyes and skin.
S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µL/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section O "Internal quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of +5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450 nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to > 2.0; (c) linearity to > 2.0; repeatability > 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of an ELISA automated work station is recommended when the number of samples to be tested exceed 20-30 units per run.

PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.

3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dilute all the content of the 20x concentrated Wash Solution as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer's instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Dilute samples 1:101 into a properly defined dilution tube
   (example: 1000 µL Sample Diluent + 10 µL sample).
   Do not dilute the Controls as they are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µL of Negative and Positive Controls in proper wells according to the schemes proposed at page 6. Then dispense 100 µL of diluted samples in each properly identified well.
4. Incubate the microplate for **60 min at +37°C**.
   Important note: *Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.*
5. Wash the microplate with an automatic washer as reported previously (section 9.3).
6. Pipette 100 µL Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1.
   Important note: *Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.*
7. Incubate the microplate for **60 min at +37°C**.

8. Wash microwells as in step 5.
9. Pipette 100 µL Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate **at room temperature (18-24°C) for 20 minutes.**
   Important note: *Do not expose to strong direct illumination. High background might be generated.*
10. Pipette 100 µL Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive control and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, as described in section 9.5, at 450nm filter (reading) and possibly at 620-630nm (background subtraction), blanking the instrument on A1.

General Important notes:

1. *If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.*
2. *Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.*

ASSAY SCHEME

Method	Operation
Controls	100 µL
Samples diluted 1:101	100 µL
1st incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
Enzyme conjugate	100 µL
2nd incubation	**60 min**
Temperature	+37°C
Wash step	4-5 cycles
TMB/H2O2	100 µL
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450 nm

An example of dispensation scheme is reported below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S 4										
B	NC	S 5										
C	NC	S 6										
D	PC	S 7										
E	PC	S 8										
F	S 1	S 9										
G	S 2	S 10										
H	S 3	S 11										

Legenda: BLK = Blank, NC = Negative Control, PC = Positive Control, S = Sample

CUT-OFF CALCULATION

If data are valid, calculate the mean OD450 nm value of the Negative Control (or NC) and then apply the following formulation to calculate the cut-off value:

NC + 0.250 = Cut-Off

Important Note: *When the calculation of results is made by an automatic work station, assure that the system has been loaded with the right formulation.*

REFERENCES

1. Howitz M et al.. Surveillance of bacterial meningitis in children under 2 y of age in Denmark, 1997-2006 Scand J Infect Dis. 2008 Aug 14:1-7. PMID: 18720256
2. [No authors listed] Meningococcal B vaccine: new drug. The only vaccine against some serogroup B meningococci. Prescrire Int. 2008 Jun;17(95):95-7. PMID: 18623907
3. Trotter CL et al.. Optimising the use of conjugate vaccines to prevent disease caused by Haemophilus influenzae type b, Neisseria meningitidis and Streptococcus pneumoniae. Vaccine. 2008 Aug 1 8;26(35):4434-45. Epub 2008 Jun 17. PMID: 18617296
4. Inés Agudelo C et al.. Serogroup Y meningococcal disease, Colombia. Emerg Infect Dis.2008 Jun;14(6):9901. PMID: 18507927
5. van Alphen L et al.. Meningococcal B vaccine development and evaluation of efficacy. Hum Vaccin. 2008 Mar-Apr;4(2):158-61. Epub 2007 Aug 14. PMID: 18388494
6. Pedersen MØ et al.. Neisseria meningitidis. The pathophysiological role of lipopolysaccharides in association with meningococcal disease and septic shock. Ugeskr Laeger. 2008 Feb 4;170(6):421-6. Review. Danish. PMID: 18252172.
7. Zughaier SM et al.. Physicochemical characterization and biological activity of lipooligosaccharides and lipid A from Neisseria meningitidis. J Endotoxin Res. 2007;13(6):343-57. PMID: 18182462

8. Yeh SH et al.. Update on adolescent immunization: pertussis, meningococcus, HPV, and the future. Cleve Clin J Med. 2007 Oct;74(10):714-6, 71 9-27. Review. PMID: 17941292
9. Pace D et al.. Meningococcal A, C, Y and W-135 polysaccharide-protein conjugate vaccines. Arch Dis Child. 2007 Oct;92(10):909-15. Review. PMID: 17895339
10. Cartwright K et al.. Meningococcal disease in Europe: epidemiology, mortality, and prevention with conjugate vaccines. Report of a European advisory board meeting Vienna, Austria, 6-8 October, 2000. PMID: 11534497
11. Riddell A et al.. Vaccines against meningococcal disease: current and future technologies. PMID: 11727513
12. Giardina PC et al.. Effect of antigen coating conditions on enzyme-linked immunosorbent assay for detection of immunoglobulin G antibody to Neisseria meningitidis serogroup Y and W135 capsular polysaccharide antigens in serum. Clin Diagn Lab Immunol. 2003 Nov;10(6):1136-40. PMID: 14607879
13. Plested JS et al.. Enzyme linked immunosorbent assay (ELISA) for the detection of serum antibodies to the inner core lipopolysaccharide of Neisseria meningitidis group B. J Immunol Methods. 2000 Apr 3;237 (1-2):73-84. PMID: 10725453
14. Colino J et al.. A quantitative ELISA for antigen-specific IgG subclasses using equivalence dilutions of anti-kappa and anti-subclass specific secondary reagents. Application to the study of the murine immune response against the capsular polysaccharide of Neisseria meningitidis serogroup B. J Immunol Methods. 1996 Apr 19;190(2):221-34. PMID: 8621957
15. Sippel JE et al.. Detection of Neisseria meningitidis cell envelope antigen by enzyme-linked immunosorbent assay in donors with meningococcal disease.Trans R Soc Trop Med Hyg. 1980;74(5):644-8. PMID: 6782717
16. Gheesling L et al.. Multicenter comparison of *Neisseria meningitidis* serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. J of Clinical Microbiology. 1994 Jun Vol 32 n°6.
17. Cheryl M et al.. Assignment of additional anti capsular antibody concentration to the *Neisseria meningitidis* group A, C, Y, and W-135 meningococcal Standard Reference Serum CDC1992. Clinical and Diagnostic Laboratory Immunology. 2002-May Vol. 9 n°3.
18. Akinwolere O A O et al.. Two enzyme immunosorbent assays for detecting antibodies against meningococcal capsular polysaccharides A and C. J Clin Pathol. 1994; 47:405-410.