RUBELLA IgM

"Capture" Enzyme Immunoassay for the determination of IgM Class antibodies to Rubella virus in human serum or plasma

REF **GD7712 00**

IVD

INDICATION

Infection with Rubella virus in children and adults is a self-limited, mild disease characterized by an erythematous rash, mild upper respiratory symptoms and suboccipital lymphadenopathy. After recovery, the individual is immune to subsequent infection with Rubella virus. Primary infection of a pregnant woman however, particularly in the first trimester of pregnancy, may result in a high risk of fetal infection with severe complications. Cataracts, deafness, congenital heart disease and other malformations, which may occur singularly or in combination, characterize congenital rubella. It is extremely important therefore to identify those women who are not immune to Rubella and to immunize them well before they become pregnant. This can be achieved by screening the serum for the presence of antibodies to Rubella: a positive result is indicative of immunity. The ELISA has been shown to be a sensitive and reliable procedure for detection of antibodies to Rubella. Recently developed, IgM "capture" assays for Rubella provide the clinician with a powerful and reliable diagnostic test, not affected by rheumatoid factor, for the monitoring of "risk" population.

PRINCIPLE OF THE ASSAY

Microplates are coated with an anti-human IgM monoclonal antibody, which, in the first incubation, captures specifically this class of antibody present in the sample. After washing out all the other components of the sample, in the second incubation bound anti Rubella IgM specific antibodies are detected by the addition of recombinant Rubella antigen conjugated with horseradish peroxidase (HRP). The enzyme captured on the solid phase, acting on the TMB-Substrate, generates an optical signal that is proportional to the amount of IgM antibodies present in the sample and can be detected by an ELISA reader.

KIT CONTENT

1. **Reagent A – Microplate**
   12x8 strips.
   8 wells breakable strips, coated with monoclonal antibody anti-human IgM. The strips are assembled on a plastic frame and contained in a sealed bag with desiccant. Bring the strips to room temperature before use, to prevent any moisture formation inside the bag.

2. **Reagent B – Antigen Conjugate**
   1 vial of 13.5 ml.
   Rubella virus recombinant antigen conjugated with Horseradish peroxidase (HRP). Transparent or slightly opalescent liquid, colorless or pale yellow colored.

3. **Reagent C – Washing Solution 25x**
   1 bottle of 50 ml.
   Concentrated solution to be diluted 1:25 with distilled water. Transparent or slightly opalescent liquid, colorless or pale yellow.

4. **Reagent D – Chromogen 11x**
   1 vial of 1.5 ml.
   Transparent colourless liquid containing Tetramethyl-benzidine (TMB).
   Avoid light exposure.

5. **Reagent E – Substrate Buffer**
   1 vial of 15 ml.
   Transparent colourless liquid containing, in Citric acid and Sodium acetate solution pH 4.1-4.3, H_2O_2. Preserving agent: 0.05% ProClin 300.

6. **Reagent F – Stop Solution**
   1 vial of 25 ml.
   It contains 0.2 mol/l Sulphuric acid solution. Transparent colourless liquid.

7. **Reagent G1 – Preliminary Sample Diluent**
   1 vial of 12.5 ml.
   Solution for preliminary dilution of sera. Transparent, dark blue colored. Preserving agent: 0.05% ProClin 300.

8. **Reagent G – Sample Diluent**
   1 vial of 12.5 ml.
   Transparent liquid, colorless. Preserving agent: 0.05% ProClin 300.

9. **Negative Control**
   1 vial of 1.5 ml.
   Inactivated human serum negative for p24 HIV-1, HBsAg and antibodies to Rubella-IgM, HIV1,2, HCV. Transparent or slightly opalescent liquid, green colored. Preserving agent: 0.05% ProClin 300, 0.05% Sodium azide.

10. **Positive Control**
    1 vial of 1.2 ml.
    Inactivated human serum positive for antibodies to Rubella-IgM, negative for p24 HIV-1, HBsAg, anti-HIV-1,2 and anti–HCV. Transparent or slightly opalescent liquid, red colored.

11. **Cardboard sealers**
    2 cardboard sealers to be used to cover the plate during the incubations.

12. **Package insert:** instruction for use GD7712 00 it/ing.

13. **Additionally Provided items:** plate for preliminary dilution of samples.

MICROBIOLOGICAL STATE AND CLEANING GRADE

1. All the materials of human origin resulted negative to HbsAg, HIV 1&2 and HCV FDA approved tests. Anyhow, as no test can guarantee the absolute absence of infective agents, handle reagents as potentially infected, especially standards, controls and samples.
2. Avoid any contact with skin and mucous membrane, in particular for Substrate Buffer, Chromogen and Stop Solution.
3. Use protective disposable talk-free gloves.
4. Avoid contaminating reagents when taking them from the vials. We recommend to use automatic pipettes with disposable tips. When dispensing reagents, do not touch with tips the wall of wells in order to avoid cross-contaminations.
5. For the washing step, use only the Washing Solution provided in the kit and follow carefully the indications reported.
6. Avoid the substrate/chromogen to come in contact with oxidizing agents or metallic surfaces; avoid intense light exposure during incubation or reagent preparation.
7. Do not forget to neutralize and/or autoclave the solution or washing wastes or any fluids containing biological samples before discarding them into the sink. Solid waste (used plates, tips, vials, glassware, etc.) should be disinfected by 6% peroxide of hydrogen with 0,5 % synthetic washing-up liquid or 3% of chloramin B solutions. Total time of deactivation should be no less than one hour. Another resolved to application disinfectant is possible to use. Also solid waste should be disinfected by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1,5 kHz/sm2 (0,15 mPa). Liquid waste (washing water) should be disinfected by dry chloramin B adding in concentration 30g/l (deactivation time – no less than 2 hours). Also liquid waste can be disinfected by boiling treatment for 30 min or by autoclaving for 1 hour at temperature 124-128 ºC and pressure 1,5 kHz/sm2 (0,15 mPa). Tools and equipment should be wiped 2 times by 70 % ethanol before and after work.
8. **Xi** Some reagent contain ProClin 300. ProClin 300 0.05%: **Irritant**. May cause sensitization by skin contact. After contact with skin, wash immediately with plenty of soap and water.

STORAGE AND STABILITY

1. The kit has to be stored at 2-8 °C and used before the expiry date stated on the label.
2. Unused strips have to be placed in the bag containing the desiccant and firmly sealed before re-store at 2-8 °C. After opening the strips are stable up to the expiry date stated on the label.
3. The diluted washing solution can be stored 3 days at 2-8 °C when stored in a tightly sealed vial.
4. All other reagents can be repeatedly used up to exhaustion if stored at 2-8 °C, provided that they are handled carefully to avoid any environment contamination. Under these conditions the reagents are stable up to the expiry date stated on the labels.

AUXILIARY MATERIALS

- Semi automatic pipettes able to dispense of 10, 50, 90 and 100 µl and disposable pipette tips.
- Plate for preliminary dilutions of samples (provided).
- Graduated cylinders of 25, 100, 1000 ml.
- Disposable gloves.
- Chronometer.
- Purified water.
- Microplate reader equipped with 450 nm and 620-680 nm filters.
- Microplate incubator set at 37 (±0.5) °C.
- Automatic microplate washer.

SAMPLES

Collect a blood sample according to the current practices. The test should be performed on serum or plasma. Separate the serum or plasma from the clot or red cells as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Specimens with observable particulate matter should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield falsely positive results. Do not heat the samples. The specimens can be stored at 2-8 ºC if screening is performed within 3 days or they may be deep-frozen at -20 ºC. The plasma must be quickly thawed by warming for a few minutes in a water bath at 40 ºC (to avoid fibrin precipitation). Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrosted more than 1 time cannot be used. Do not use contaminated, hyperlipaemic or hyperhemolysed sera or plasma.

REAGENTS PREPARATION

- WORKING WASHING SOLUTION
  The concentrated solution may present a sediment that can be dissolved at 35-39 °C and shaking.
  Dilute the required volume of concentrated solution with the corresponding volume of purified water (see table below) and mix carefully before use.
- CHROMOGEN/SUBSTRATE
  Dilute the required volume of Substrate Buffer with the corresponding volume of Chromogen (see table below) and mix carefully before use.
  Substrate/Chromogen mixture should be colourless.

Consumption of reagents for the manual test procedure

Used strips n°	Working Washing Solution		Chromogen/Substrate	
	Washing Sol. (ml)	Purified water (ml)	TMB (ml)	Substrate Buffer (ml)
1	2.0	48.0	0.1	1.0
2	4.0	96.0	0.2	2.0
3	6.0	144.0	0.3	3.0
4	8.0	192.0	0.4	4.0
5	10.0	240.0	0.5	5.0
6	12.0	288.0	0.6	6.0
7	14.0	336.0	0.7	7.0
8	16.0	384.0	0.8	8.0
9	18.0	432.0	0.9	9.0
10	20.0	480.0	1.0	10.0
11	22.0	528.0	1.1	11.0
12	24.0	576.0	1.2	12.0

SAMPLE PREPARATION

Preliminary dilution

Dilute samples with Preliminary Sample Diluent in the plate for preliminary dilution of samples: pipette 90 µl of Preliminary Sample Diluent and add 10 µl of the tested sera samples to the wells (serum dilution ratio is 1:10). Carefully mix. Dark blue color should change to blue-green. If you don't observe change of the color then test results may be false, or there is no serum added to the well.

ASSAY PROCEDURE

1. At least 30 minutes before use, bring all reagents, controls and samples to room temperature (18-24 °C), mixing them carefully on vortex.
2. Do not mix reagents from different lots.
3. We recommend to distribute the controls in duplicate.
4. Distribution and incubation times must be the same for all wells in the same analysis.
5. Avoid long interruptions between each step of the assay procedure.
6. It is suggested to eliminate the excess of washing solution from the microplate after washing by blotting it gently on an absorbent paper pad.
7. We recommend to read the plate with an ELISA automatic reader able to subtract the background at 620-680 nm and to read the absorbance of samples and controls at 450 nm. Reading the absorbance at 450 nm only is possible.

ASSAY SCHEME

1. Put the desired number of microstrips into the frame.
2. Follow the scheme:

<table>
<tr><td rowspan="5">First incubation</td><td></td><td colspan="2">Anti IgM antibody coated wells</td></tr>
<tr><td>REAGENTS</td><td>Controls</td><td>Sample</td></tr>
<tr><td>Sample Diluent (Reagent G)</td><td>-</td><td>90 µl</td></tr>
<tr><td>Controls</td><td>100 µl</td><td>-</td></tr>
<tr><td>Preliminarly diluted samples</td><td>-</td><td>10 µl</td></tr>
<tr><td></td><td colspan="3">- Carefully mix fluids in wells.
- Cover the strips with cardboard sealer.
- Incubate 30 minutes at 37 (± 0.5) °C.</td></tr>
<tr><td>Wash</td><td colspan="3">- Peel out the cardboard sealer and aspirate the reaction solution from all wells.
- Add into each well 380-400 µl of working washing solution. Allow a soak time at least 40 seconds and aspirate. Repeat this procedure 4 times. Incomplete washing will adversely affect assay precision.</td></tr>
<tr><td rowspan="2">Second incubation</td><td>Antigen conjugate (Reagent B)</td><td>100 µl</td><td>100 µl</td></tr>
<tr><td colspan="3">- Cover the strips with cardboard sealer.
- Incubate 60 minutes at 37 (± 0.5) °C.</td></tr>
<tr><td>Wash</td><td colspan="3">- Peel out the cardboard sealer and aspirate the reaction solution from all wells.
- Add into each well 380-400 µl of working washing solution. Allow a soak time at least 40 seconds and aspirate. Repeat this procedure 4 times. Incomplete washing will adversely affect assay precision.</td></tr>
<tr><td rowspan="4">Colorimetric reaction</td><td>Chromogen-Substrate mixture (Reagent D+E)</td><td>100 µl</td><td>100 µl</td></tr>
<tr><td colspan="3">- Cover the strips with cardboard sealer.
- Incubate 30 minutes at room temperature (20-24 °C), avoiding light exposure.</td></tr>
<tr><td>Stop Solution (Reagent F)</td><td>50 µl</td><td>50 µl</td></tr>
<tr><td colspan="3">Read the optical density at least 3-4 min after stopping of the reaction. Read the optical density at 450 nm with 620-680 nm as reference.</td></tr>
</table>

VALIDITY OF THE ASSAY

The assay is to be considered valid if:

1. The mean OD value for the Negative Control is less than 0.200.
2. The mean OD value of the Positive Control is greater than 0.600.

If these criteria are not met the test should be considered invalid and should be repeated.

CALCULATION OF RESULTS

The presence of detectable Rubella IgM antibodies is determined by comparing the absorbance measured for each sample to the cut-off value calculated according to the following formula:

Cut-off = mean OD value of Negative Control + 0.350

0.350 is a coefficient defined by manufacturer during statistical processing.

INTERPRETATION OF RESULTS

Positive: if the OD value is > Cut-off x 1.1
Negative: if the OD value is < Cut-off x 0.9
Undefined: if the OD value is in range
Cut-off x 0.9 < OD < Cut-off x 1.1
Undefined samples should be retested.

Note:

- Serological assays alone can only determine exposure to the virus and should not be used as the sole basis for a diagnosis of infection. All other appropriate clinical findings and laboratory data must be considered in order to establish a diagnosis.
- Even if "capture" tests guarantee a higher specificity when compared with traditional methods, false-positives can be found in about 1-2% of the IgM positive population.

PRECAUTIONS IN USE

Refere to Safety Data Sheet.
The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management
Please refer to local legal requirements.

AMS S.p.A. – Registered Office and Plant
Via E. Barsanti 17/A
00012 Guidonia (Rome) – Italy
Ph. + 39 0774 354441 r.a.
Fax + 39 0774 578035
www.ams-analyzers.com
info@ams-analyzers.com

Diagnostics Manufacturing
Via Galileo Galilei, 38
Seggiano di Pioltello (MI) - Italy
Ph. + 39 02 929189.1
Fax + 39 02 929189.39
www.gdsrl.com
infodiagnostics@ams-analyzers.com

Met. GD771200.0 eng
Edition: 2012/02/27