IgM Turbidimetric 5+1

IVD CE

mtd DIAGNOSTICS

Quantitative determination of IgM in human Serum and Plasma. Turbidimetric method.

REF **TB1073** R1: 2x25 mL + R2: 1x10 mL

METHOD AND PRINCIPLE

Anti-human IgM antibodies, added to samples containing IgM (Antigen), bind by developing insoluble complexes, increasing the absorbance which is photometrically measurable at 340 nm. The increase is directly proportional to the amount of IgM present in the sample. The use of a calibration curve at known values makes it possible to determine the concentration of IgM in the unknown sample.

CLINICAL SIGNIFICANCE

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells, in response to bacteria, viruses and other microorganisms, as well as exposure to other substances that are recognized by the body as foreign ("non-self") and dangerous antigens. There are five classes of immunoglobulins and many subclasses. Each class represents a group of antibodies and has a slightly different role.

Immunoglobulin M (IgM). They are produced as the body's first response to a foreign antigen, providing short-term protection. The IgM concentration increases for a few weeks and then decreases when IgG production begins.

Immunoglobulin G (IgG). About 70-80% of the immunoglobulins of the blood. They are produced during a first infection or exposure of antigens, increasing concentration after a few weeks from contact, and then decrease and stabilize. The body keeps the memory of the different IgG, which can be reproduced at each exposure to the same antigen. IgG antibodies are the basis of long-term protection against microorganisms. In those who have a normal immune system, sufficient IgG is produced to prevent re-infection. IgG is the only immunoglobulin that can pass through the placenta, providing protection to the fetus during pregnancy and to the newborn during the first month of life.

Immunoglobulin A (IgA). They make up about 15% of the total immunoglobulins in the blood but are also present in saliva,

tears, gastric and respiratory secretions and breast milk. IgAs provide protection against mucosal infections (respiratory tract, upper and lower pathways and gastrointestinal tract, stomach and intestine). Passing with breastfeeding from the mother to the newborn, they provide protection for the baby's gastrointestinal tract. Significant concentrations of IgA are produced from the sixth month of the child onwards, therefore every IgA present in the infant's blood is derived from the mother's milk.

Congenital and acquired immunodeficiencies cause a deficiency of all immunoglobulins whose dosage is important for the monitoring of immunoglobulin therapy and of the clinical course of multiple myeloma.

IgA increases in responses to infectious renal, skin, respiratory diseases, cirrhosis, myeloma and plasma cell proliferative disorders.

IgG increase in responses to infectious diseases, in the course of hepatitis, cirrhosis, myeloma, plasma cell proliferative disorders, autoimmune diseases. In particular, increases in monoclonal IgG (paraproteins) have been found in multiple myeloma, lymphoid leukemia, Waldenstrom macroglobulinemia.

IgM increase in response to viral infectious diseases, in the course of malaria, chronic hepatitis, biliary cirrhosis, myeloma, and proliferative plasma cell disorders. In particular, increases in monoclonal IgM (paraproteins) are found in multiple myeloma, lymphoid leukemia, Waldenstrom macroglobulinemia.

REAGENT COMPOSITION

Reagent (R1):

Tris Buffer, pH 7,5	100 mmol/L
Sodium Chloride	150 mmol/L

Reagent (R2):

Tris Buffer, pH 7,5	100 mmol/L
Sodium Chloride	150 mmol/L
Anti-human IgM Antibody (Goat)	

REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Absorbance of Blank Reagent >0.300 at 340 nm in cuvette 1 cm against water. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

Antibody Start (two-Reagents)

The Reagents are liquid and ready-to-use.

Sample Start (Mono-Reagent)

Mix 5 parts of **R1** + 1 part of **R2.** (e.g. 10 mL of R1 + 2 mL of R2) to obtain the Working Solution. Avoiding foaming, shake gently before to use.

Stability: 6 mounths at +2° to + 8°C; 5 days at +15° to + 25°C, 30 days on board (cooled rack).

SPECIMEN

Serum, Li-Heparin or EDTA Plasma.

Avoid hemolysate or lipemic samples. Separate the serum from the clot quickly. Defrost only once. Stability in serum or plasma:

4 days at +2 to +8 °C, 1 day at 20-25° C; 3 months at -20°C.

PROCEDURE

Wavelength: 340 nm

Temperature: +37°C

Measurement: against distilled water

Antisera Start procedure:

Reagent (R1)	700 µL
Sample / Calibrator /H_2O	10 µL

Mix and after 30" read Absorbance (Abs1). Then add:

Reagent (R2)	140 µL

Mix, after other 300" read Absorbance again (Abs 2).

Calculate ΔAbs (Abs 2 – Abs 1) for samples and calibrators.

Sample Start procedure:

Working Solution	700 µL
Sample / Calibrator/H_2O	8 µL

Mix, read Absorbance (Abs 1) after 30". After other 300" read Absorbance again (Abs 2). Calculate ΔAbs (Abs 2 – Abs 1) for samples and calibrators.

CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

Use **MTD Diagnostics** Plasma Protein Multicalibrator

Plasma Protein Multicalibrator REF TUC1030 (3x1 mL)

Dilute calibrator in NaCl 9 g/L as follows:

Dilution	1	2	3	4	5	6
CAL (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	--
Factor	0.0	0.1	0.25	0.5	0.75	1.0

Multiply the concentration of Protein Calibrator by the corresponding factor to obtain the concentration of each dilution.
To avoid to dilute the Multicalibrator, it is possible to use:

Plasma Protein Multicalibrator Set REF TUC1035 (5x1 mL)

a multipoint calibration curve in pre-filled vials, each with a specific concentration. The values are shown on the label of each vial.

CALCULATION

The analytical session can not be validated if the Δ Abs (Abs2 - Abs1) of the Blank Reagent is > 0.300 at 340 nm in a 1 cm cuvette of optical path.
Plot the different ΔAbs (Abs2-Abs1) absorbances against the concentration of each calibrator dilution. The concentration of the sample is calculated by interpolation of its ΔAbs (Abs2-Abs1) value on the calibration curve. For automatic calculation, use the SPLINE curve but other mathematical method can be used (Point-Point; Logit-Log 4P, etc..).
Conversion Factor: mg/dL x 0,01 = g/L ; mg/L x 0,1 = mg/dL

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

Plasma Protein Control Level 1 REF: TUC1040 (3 x 1 mL)
Plasma Protein Control Level 2 REF: TUC1050 (3 x 1 mL)

The range of the values of the controls must be evaluated as a guideline, since it can be determined by the application of the method or by the user's manual skills or by other factors. The values obtained must be used for the evaluation of the Precision of the method (Repeatability). For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

EXPECTED VALUES

Adults	40 - 230 mg/dL
Children <1 month	10 - 30 mg/dL
Children 1-3 months	10 -70 mg/dL
Children 3 – 12 months	20 -100 mg/dL
Children 1- 2 years	40 -140 mg/dL
Children 2 - 5 years	40 - 180 mg/dL
Children 5 - 9 years	40 - 160 mg/dL
Children 9 - 13 years	40 - 150 mg/dL

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:
Low Level: Samples (n) = 20; Average = 81; S.D. = 1.420; CV = 1.73%
High Level: Samples (n) = 20; Average = 138; S.D. = 1.76; CV = 1.27%
ACCURACY (CORRELATION): A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

y = 0.99 x + 3.5 r = 0.99

SENSITIVITY: 3 mg/dL.
LINEARITY: 3 – 300 mg/dL.

SPECIFICITY / INTERFERENCES

No interferences was observed by Bilirubin up to 20 mg/dL, Hemoglobin up to 1000 mg/dL, Triglycerides up to 800 mg/dL. Other substances may interfere.

PRECAUTIONS

R1 and R2 contain TRIS BUFFER 100 mmol/L – pH 7.5 - CAS 1185-53-1
H315: Causes skin irritation
H319: Causes serious eye damage
H335: May cause respiratory irritation

The antibody present in the preparation are of animal origin and are not capable of transmitting infectious diseases to humans. However, since there are no methods to ensure the total absence of such infectious agents or of other microbes, this product must be handled as if it were risky and potentially capable of transmitting infectious diseases of any kind, in accordance with Good Laboratory Practice standards.
The products do not contain other dangerous substances or mixtures, according to the EC Regulation n ° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.
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However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:
S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.
FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
IVD	In Vitro Diagnostic		Temperature Limitation
i	Consult instructions for use	Σ	Contains sufficient for <n> test
REF	Catalog Number		Use By
LOT	Batch Code		Manufacturer

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