CE

Quantia® IgM

TURBIDIMETRIC IMMUNOASSAY FOR ESTIMATION OF IMMUNOGLOBULIN IgM IN HUMAN SERUM

SUMMARY

The absolute levels of immunoglobulin concentration are a measure of the severity of the inflammatory process, especially in virally induced chronic liver disease, chronic bacterial infections, collagen vascular diseases and other autoimmune diseases. In diseases that cause hypergammaglobulinemia quantitative determination in conjunction with the clinical presentation as well as analysis of serological and clinical chemistry parameters or test results may not only help in diagnosis, differential diagnosis, but may also prove to be useful for disease monitoring and prognosis.
Immunoglobulin IgM comprises approximately 7 – 10 % of normal serum immunoglobulins and are the prominent antibody class in primary response to most antigenic stimuli. As IgM does not cross the placenta presence of virus specific IgM in cord blood or neonatal serum is indicative of congenital infection. IgM levels may be increased in chronic liver disease, infections, Waldenstrom's macroglobulinemia, and malignant lymphoma. Decreased levels are observed in immune deficiency states, non-IgM myeloma, infancy and early childhood lymphoma.
Quantified IgM measurements are useful for the detection of frequent chronic or acute infections, suspected immunodeficiency, screening for congenital infections and monitoring patients with Waldenstrom's macroglobulinemia.
Information regarding the concentration of IgM can be obtained by using **Quantia®-IgM** reagents.

REAGENT

1. **Quantia®-IgM** Activation buffer (R1): Ready to use.
2. **Quantia®-IgM** Anti-human IgM Reagent (R2): Ready to use solution of anti-human IgM antibody.
3. **Quantia®-IgM** Calibrator: Lyophilized preparation of serum equivalent to the stated amount of IgM on a mg/dl basis, when hydrated appropriately. The **Quantia®-IgM** calibrator is traceable to CRM 470.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2 - 8°C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and calibrator is as per the expiry date mentioned on the respective vial label.
3. The reconstituted calibrator is stable for 7 days at 2 - 8°C and 48 hours at 25 - 30°C (RT.).

PRINCIPLE

Quantia®-IgM is a turbidimetric immunoassay for the detection of immunoglobulin IgM in human serum and is based on the principle of agglutination reaction. The test specimen is mixed with the Activation buffer (R1). Anti-human IgM reagent (R2) is then added and allowed to react. Presence of IgM in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength 340 nm. The extent of turbidity corresponds to the concentration of IgM in the test specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. All the reagents derived from human source have been tested for HBsAg and HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as a preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. The **Quantia®-IgM** reagents should be mixed well before use to improve test performance.
6. Do not use vortex mixers for mixing reagents. Gently mix the reagents and samples during test procedures.
7. Calibrators of different manufacturers must not be used with **Quantia®-IgM** reagents.

8. The calibration curve must be validated periodically with known controls.
9. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
10. The **Quantia®-IgM** assay is recommended only for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
11. The procedures mentioned in this pack insert are based on a minimum reading volume of 500 µl (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection by approved techniques.
Only serum should be used for testing. Should a delay in testing occur, store the samples at 2 - 8°C. Samples can be stored for upto a week at 2 - 8°C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid sera. Turbid or particulate serum samples must be clarified by centrifugation at 2000 rpm for 15 minutes prior to testing. Use the clear supernatant for testing.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 340 nm wavelength filters and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, optically clean disposable cuvettes such as **Quantiamate semi micro cuvettes**/ glass cuvettes.

TEST PROCEDURE

Bring reagents and samples to room temperature before use.
Assay conditions:

Wavelength	340 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

Method for preparation of IgM Calibration Curve

The **Quantia®-IgM** calibrator must be reconstituted exactly with 1.0 ml of distilled water, wait for five minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for preparing the IgM calibration curve. The Concentration (S) of IgM in the reconstituted calibrator is as mentioned at the end of this package insert.
Prepare 1.0 ml of 80 mg/dl IgM **working standard (Sw)** from the reconstituted calibrator as mentioned at the end of this package insert. The working standard (Sw) is stable for eight hours at 2-30°C.
Prepare dilutions of the working standard (Sw) as mentioned below for preparation of calibration curve.

Test Tube No.	**1**	**2**	**3**	**4**	**5**
Calibrator dilution No.	D1	D2	D3	D4	D5
Vol. of Working standard (Sw)	400 µl	200 µl D1	200 µl D2	200 µl D3	10 µl D1
Vol. of Saline	-	200 µl	200 µl	200 µl	190 µl
Conc. of IgM in mg/dl	S	S/2	S/4	S/8	S/20

The above five dilutions of the calibrator including the highest 80 mg/dl (D1) and lowest 4 mg/dl (D5) concentrations of measuring range **must** be used for the preparation of the calibration curve.

Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.

Test procedure for preparation of calibration curve

1. Zero the instrument with distilled water
2. Pipette 500 µl of **Quantia®-IgM** activation buffer (R1) and 25 µl of working standard (D1) in the measuring cuvette. Mix well and incubate for five minutes at 37°C.
3. Read absorbance (A1).

4. Add 50 µl of **Quantia®-IgM** reagent (R2), mix gently and start the stopwatch simultaneously.
5. Read absorbance (A2) at the end of **exactly** five minutes.
6. Repeat steps No. 2 – 5 for each diluted working standard (D2 to D5) for preparing calibration curve.
7. Calculate ΔA (A2 – A1) for each dilution of the working standards (D1 to D5). Plot a graph of ΔA versus concentration of IgM on the graph paper provided with the kit.

"The calibration curve" so obtained is valid only for the same lot of Quantia®-IgM reagents.

Test procedure for specimen

For determination of IgM concentration in the test specimen, dilute the test specimen 1:10 with normal saline.

1. Follow steps 2 – 5 as mentioned in above procedure for calibration curve using the diluted test specimen in place of the working standard.
2. Calculate ΔA (A2 – A1) for the diluted test specimen.

VALIDATION CRITERIA

If the ΔA of the diluted test specimen is less than ΔA obtained for the standard of highest concentration (D1) then proceed directly for calculations of results.

If the ΔA of the diluted test specimen is higher than ΔA of the standard with highest concentration (D1) then the test has to be rerun by carrying out further dilution of test specimen such as 1:20, 1:40, 1:60 etc., till the ΔA of the diluted specimen is less than ΔA of D1.

CALCULATIONS

For determination of IgM concentration in the test specimen :

1. Interpolate ΔA of the diluted test specimen on the calibration curve and obtain the IgM concentration 'C' of the diluted test specimen.
2. Multiply the IgM concentration 'C' with the dilution factor (F) of the test specimen for obtaining the concentration of IgM in the neat test specimen.

Concentration of IgM in the neat test specimen in mg/dl = C x F

(Where 'F' is the dilution factor of the test specimen i.e. 10 for 1:10 dilution of test specimen and so on)

SPECIFIC PERFORMANCE CHARACTERISTICS

Measuring range

The **Quantia®-IgM** reagent has been designed to measure serum IgM concentrations in the range of about 40 – 800 mg/dl, and is linear between the measuring range. The exact range is dependent on the Calibrator value, which is lot specific.

Detection limit / Analytical Sensitivity

Detection limit : 4 mg/dl

The detection limit represents the lowest measurable IgM concentrations that can be distinguished from zero.

Prozone limit

No prozone effect was observed upto an IgM concentration of 3200 mg/dl.

Precision

Intra-assay precision	n	Mean mg/dl	SD	CV (%)
Sample 1	10	74	4	5.6
Sample 2	10	200	10	4.9
Sample 3	10	710	30	4.2

Inter-assay precision	n	Mean mg/dl	SD	CV (%)
Sample 1	10	45	4	9
Sample 2	10	200	11	5.4
Sample 3	10	810	25	3

Interference
No interference was observed with:

Interference factor	No interference upto
Glucose	500 mg/dl
Albumin	10 g/dl
Bilirubin	50 mg/dl
Haemoglobin	500 mg/dl
Triglycerides	1000 mg/dl

REFERENCE VALUES

The interim reference values for immunoglobulin IgM in normal populations are 40 - 230 mg/dl.
The reference values are related to age, geographical and methodological differences and vary widely. Each laboratory should define its own reference range for the relevant population.

REMARKS

1. Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results.
2. When ΔA obtained for the test specimen is greater than the ΔA of the standard with highest concentration then, it indicates that the concentration of IgM in the test specimen is beyond the measuring range of the **Quantia®-IgM** assay. Such specimens should be rerun with further dilutions.
3. Markedly lipemic, hemolysed, and contaminated serum samples could produce non-specific values.
4. Use of plasma rather than serum can lead to erroneous values.
5. Do not read results beyond five minutes.
6. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. Clinical Laboratory Diagnostics, Edited by Lothar Thomas, M.D., 1st Ed., 1998, TH-Books Verlagsgesellschaft mbH, Frankfurt, Germany Pg 667-678.
2. Plasma proteins Clinical Utility and Interpretation, Foundation for Blood Research.
3. Tietz textbook of Clinical Chemistry Edited by Carl A Burtis, 2nd Ed., Pg 678-685.
4. Interpretation of Diagnostic tests, Jacques Wallach M. D., 5th Ed., Pg 68-71.
5. Dati et al., Interim reference ranges for 14 serum proteins based on standardization with CRM 470, Eur. J. Clin. Chem. Clin. Biochem. 1996; 34: 517-520.
6. Data on file: Tulip Diagnostics (P) Ltd.

Quantia®-IgM Calibrator

Lot No.:		
Conc. (S) on reconstitution with 1.0 ml distilled water		**mg/dl**
To prepare 1.0 ml working standard (Sw)	**Vol. of normal saline**	**µl**
	+	
	Vol. of reconstituted calibrator	**µl**