IgM

Diagnostic reagent for determination of Immunoglobulin M (IgM) concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
BB240	160 mL	LAB160	160 mL	M3B160	240 mL	TA160N	200 mL
BY161	350 mL	LAB161	160 mL	M4B160	300 mL	TA161N	100 mL
BZ2235	180 mL	LB410	160 mL	M4B161	150 mL	TAB160	203 mL
DAB160	203 mL	LM444	160 mL	RD160	200 mL	TBAB160	150 mL
DMT160	333 mL	MAB160	300 mL			8A161	350 mL
HN419	200 mL	MAB161	150 mL				

Changes made in the instructions for use are marked as grey.

INTENDED USE

This test is applied for the quantitative determination of IgM in human serum or plasma.

GENERAL INFORMATION

Immunoglobulins (antibodies) are produced against foreign immunogens and initiate clearance of the foreign molecule or organism. Human immunoglobulin molecules are composed of one or more basic units consisting of two identical heavy (H) chains and two identical light (L) chains. L chains have one variable and one constant domain, while H chains have one variable and three to four constant domains; the variable region is known to play a role in antigen recognition and binding. The wide diversity of variable domains is caused by somatic recombination and mutation of immunoglobulin genes. The constant domains are the same for every immunoglobulin molecule in a given subclass and have sites for binding to complement receptors and complement activation. The variable domains contain antigen binding sites and the constant domains of the heavy chains have sites for complement activation and receptor binding. Treatment of immunoglobulins with pepsin or papain can produce antigen binding fragments (Fab) and constant region fragments (Fc). Variations in the constant domains of the heavy chains (Fc region) result in classes and subclasses into which immunoglobulins are grouped: IgM, IgG (four subclasses), immunoglobulin A (IgA) (two subclasses), immunoglobulin D (IgD) and immunoglobulin E (IgE) respectively.[1]

Light chains, produced independently and in slightly larger quantities than heavy chains, are of two types: kappa (K) and lambda (λ). Whereas the heavy chain genes are located on chromosome 14, the K light chains are encoded by a gene on chromosome 2 and the λ chain gene is located on chromosome 22.

Immunoglobulins are synthesized in the bone marrow by plasma cells that are derived from B lymphocyte stem cells. More mature B lymphocytes which mainly found in lymph nodes and blood develop receptor immunoglobulins on their surface membranes. After binding to a target antigen, these B lymphocytes reproduce and transform into a plasma cell clone that produces antibodies against the target antigen. B lymphocytes initially have IgM surface receptors and secrete IgM as an initial or "primary" response to an antigen.[1]

IgM is produced in the early stages of B cell development. The major immunoglobulin synthesized in the immature immune system of newborns is IgM. It is the third most abundant immunoglobulin in adult serum and usually accounts for 5% to 10% of total circulating immunoglobulins. As a membrane receptor molecule, IgM is monomeric, but most serum IgM is a pentamer containing five monomers linked to the small J (junction) chain via disulfide bonds. Plasma cell malignancies may secrete monomeric IgM in addition to or instead of pentamers. IgM does not cross the placenta and therefore has no role in the etiology of hemolytic disease of newborns. It activates complement more effectively than IgG. Binding of an IgM molecule may be sufficient to activate complement CP.[1] In rare hyper-IgM syndromes, class switching to IgG and IgA is inadequate. Affected patients have IgG and IgA deficiency and increased susceptibility to infection.[2]

Polyclonal increases in plasma immunoglobulins are a normal response to infection. As IgG predominates in autoimmune responses and IgA is increased in skin, intestinal, respiratory and renal infections, IgM is increased in primary viral infections and bloodstream infection with parasites such as malaria. Chronic bacterial infection can lead to increased concentrations of all immunoglobulins.[1]

TEST PRINCIPLE

Immunoturbidimetric measurement

IgM in the sample aggregates in the presence of anti-human IgM antibodies. The absorbance of the resulting antigen-antibody complex, measured turbidimetrically at

340 nm, is directly proportional to the IgM concentration in the sample.

REAGENT COMPONENTS

Imidazole buffer	: ≤ 0.2 mol/L
Goat anti-human IgM antibodies	
Sodium azide	: ≤ %0.1

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[3]

SAMPLE REQUIREMENTS

Serum and Li-heparine or K_2 EDTA plasma can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

IgM activity stability in serum and plasma: [4]

7 days at +20/+25 ºC
7 days at +2/+8ºC
6 months at -20ºC

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Protein Calibrator. We recommend:

Protein Calibrator- Lyophilized
Ref.No: PC30
Ref.No: PC31
Ref.No: PC32 (for Olympus AU series)
Ref.No: PC33 (for Olympus AU series)

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Protein Control Level I- Lyophilized
Ref.No: PCN01
Ref.No: PCN02
Ref.No: PCN03 (for Olympus AU series)
Ref.No: PCN04 (for Olympus AU series)

Protein Control Level II- Lyophilized
Ref.No: PCN05
Ref.No: PCN06
Ref.No: PCN07 (for Olympus AU series)
Ref.No: PCN08 (for Olympus AU series)

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Adults	: 40-230 mg/dL

Children and Youth

0-14 day-old	: 3-32 mg/dL
15 day-<13 week-old	:10-67 mg/dL
13 week-<1 year-old	:14-82 mg/dL
1-<19 year-old	: 45-178 mg/dL

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval data have been verified by using CLSI EP28-A3c protocol.[5]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[6]

The determined analytic measuring interval for IgM is 2-300 mg/dL.

Detection Capability

Limit of Detection (LoD): 0.5 mg/dL

Limit of Quantitation (LoQ): 2.0 mg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[7]

Linearity

This method shows measurement linearity in the activities up to 300 mg/dL.Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[8]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[9]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of IgM have been given in the table 1 and 2 respectively.

Table 1. IgM Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
55 mg/dL	1.30	2.36	80
193 mg/dL	2.05	1.06	80

***SD: Standard Deviation**

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[10]

Table 2. IgM Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
55 mg/dL	1.77	3.22	80
193 mg/dL	2.45	1.27	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[10]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

r=0.985
Passing-Bablock equation:
y= 1.007x + 0.3 mg/dL

Prozone Effect: No prozone effect has been observed up to 1400 mg/dL tested for IgM.

Interference

Endogenous interferant and analyte concentrations that have been used in the IgM scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[11,12]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from IgM interference scanning test is appropriate, is determined as ±10%.[13]

In IgM test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Hemoglobin	: ≤ 20 g/L
Bilirubin	: ≤ 20 mg/dL
Rheumatoid factors	: ≤ 150 IU/mL
Lipemia	: ≤ 4.40 g/L

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[12]
These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.

Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

REFERENCES

1. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 31: Amino Acids, Peptides, and Proteins, p.349-e42, Elsevier, St. Louis, Missouri 63043
2. Durandy, A., S. Peron, and A. Fischer, Hyper-IgM syndromes. Curr Opin Rheumatol 2006. 18(4): p. 369-76.
3. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
4. Guide to Laboratory Tests. 4th ed. St. Louis, MO: Elsevier Saunders; 2006
5. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
6. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018
7. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
8. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
9. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
10. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
11. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
12. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry - Third Edition. CLSI Document EP07. Wayne, PA: CLSI; 2018.
13. CLIA proficiency testing criteria for acceptable analytical performance, as printed in the Federal Register July 11, 2022;87(131:41194-242.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests