IgA

Diagnostic reagent for determination of IgA (Immunoglobulin A) 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
At141	75 mL	HN415	200 mL	MAB140	300 mL	RD140	200 mL
BB230	160 mL	LAB140	160 mL	MAB141	150 mL	TA140N	200 mL
BY141	350 mL	LAB141	160 mL	M3B140	240 mL	TA141N	100 mL
BZ2225	180 mL	LB400	160 mL	M4B140	300 mL	TAB140	203 mL
DAB140	203 mL	LM440	160 mL	M4B141	150 mL	TBAB140	150 mL
DMT140	333 mL	LM441	136 mL			8A141	350 mL

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

INTENDED USE

The test is applied for the quantitative determination of IgA in serum and plasma.

GENERAL INFORMATION

Immunoglobulin A (IgA) has a molecular weight of 160 kDa and contains approximately 10% carbohydrate derived from both N- and O-linked oligosaccharide chains. IgA accounts for approximately 10 to 15% of serum immunoglobulin and has a 6-day half-life. In its monomeric form, its structure is similar to that of IgG, but 10 to 15% of IgA in serum, particularly IgA2, is dimeric and more resistant to destruction by pathogenic bacteria than IgA1. It migrates to the β-γ region on electrophoresis.[1] IgA is an important component of mucosal immunity.[2]

Secretory IgA is found in tears, sweat, saliva, milk, colostrum, gastrointestinal and bronchial secretions. Secretory IgA has a molecular weight of 380 kDa and is composed of two IgA molecules, a secretory component (70 kDa) and a J chain (15.6 kDa). It is synthesized mainly by plasma cells in the mucous membranes of the intestine and bronchi and in the ducts of the lactating breast. Secretory IgA in colostrum and milk is more abundant than IgG and may help protect newborns from intestinal infection. IgA may activate complement via the alternative pathway, but the exact role of IgA in serum is not clear.[1]

Immunodeficiency conditions can be the result of a deficiency of a single factor or combinations affecting multiple immune defense systems. For example, severe combined immunodeficiency (SCID) is a disorder of B cell development or activation that affects 1 in 100,000 newborns and results in broad-spectrum immunoglobulin deficiency. The more common primary deficiencies[3] include only one or two immunoglobulin classes (IgA) or subclasses (IgA or IgG subclasses) or the ability to produce antibodies against polysaccharide antigens. IgA deficiency can lead to false negative test results for the detection of celiac disease, and some affected individuals are at risk of anaphylaxis if they receive IgA-containing blood products.[4]

Polyclonal increases in plasma immunoglobulins are the normal response to infection. IgA is increased in skin, intestinal, respiratory and kidney infections. Chronic bacterial infection may lead to increased concentrations of all immunoglobulins. Monoclonal immunoglobulins, called paraproteins, can be polymers, monomers, individual immunoglobulin chains such as free light chains or heavy chains, or fragments of immunoglobulins. Based on the clinical, epidemiologic and biochemical characteristics of monoclonal paraprotein diseases, IgA-type paraproteins are detected in plasma in approximately 25% of patients. Free light chain paraproteins are seen in the urine of 70% of these patients. Patients are usually predisposed to develop hypercalcemia and amyloidosis. About 60% of paraproteins are associated with plasma cell malignancies (light chain amyloidosis, multiple myeloma or solitary plasmacytoma) and about 15% are due to overproduction of B lymphocytes, mainly in lymph nodes (lymphomas, chronic lymphocytic leukemia, Waldenström macroglobulinemia or heavy chain disease).[1]

TEST PRINCIPLE

Immunoturbidimetric method

IgA in the sample forms a precipitate in the presence of anti-human IgA antibodies. The absorbance value of the turbidity of the precipitate formed by the antigen-antibody complex, measured at a light wavelength of 340 nm, is directly proportional to the concentration of IgA in the measured sample.

REAGENT COMPONENTS

Reagent 1:

Imidazole buffer	≤ 0.1 mol/L
Goat anti-human IgA antibodies,	
Sodium azide	≤ %0.1

REAGENT PREPARATION

Reagent is 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.[5]

SAMPLE REQUIREMENTS

Serum and plasma that are collected with standard procedure can be used. Heparin or EDTA should be used as anticoagulant. Lipemic samples should not be used. Multiple sample freezing and thawing should be avoided.

IgA activity stability in serum and plasma[19]:

8 months at +2/+8ºC
8 months at +20/+25ºC
8 months at -20°C

Annotation:

- Samples should not be refrozen after thawing because repeated freezing and thawing may cause degradation of proteins.[6]

CALIBRATION AND QUALITY CONTROL

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

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

Reference Range[20,21]

Age	Range (mg/dL)
0 to < 1 year	< 14
1 year to < 3 years	< 80
3 years to <6 years	11 – 142
6 years to <14 years	34 – 220
14 years to <19 years	40– 293
>19 years (Adults)	70– 400

Annotation:

- Immunoglobulin concentrations decrease during pregnancy and reach their lowest levels in the early postnatal period. Levels vary greatly in children and adults; as a result of transplacental transmission, immunoglobulins in infants then decrease until about 6 months of age. Levels in children increase from adolescence to adult levels.[7,8]

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 has been verified by using CLSI EP28-A3c protocol.[9]

Unit Conversion:
mg/dL × 0.01 = g/L
g/L × 6.25 = µmol/L

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).[10]

The determined analytic measuring interval for IgA is 3.7 – 650 mg/dL.

Detection Capability

Limit of Detection (LoD): 2.1 mg/dL

Limit of Quantitation (LoQ): 3.7 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.[11]

Linearity

This method shows measurement linearity in the activities up to 650 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 1:5 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.[12]

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.[13]

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

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

Mean Concentration	CV%	%CV	n
75 mg/dL	2.06	2.75	80
372 mg/dL	3.21	0.86	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[14]

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

Mean Concentration	SD*	%CV	n
75 mg/dL	2.57	3.43	80
372 mg/dL	7.37	1.98	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[14]

Prozone Effect: No prozone effect has been observed up to 1300 mg/dL tested for IgA.

Interference

Endogenous interferant and analyte concentrations that have been used in the IgA scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[15,16]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from IgA interference scanning test is appropriate, is determined as ±10%.[17]

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

Bilirubin	: ≤ 20 mg/dL
Rheumatoid Factor	: ≤ 300 IU/mL
Lipemia	: ≤ 7.5 g/L
Hemoglobin	: ≤ 10 g/L

Annotation:

- The presence of rheumatoid factor or heterophilic antibodies binding to reactive antibodies may affect immunoassay.[6]
- In the presence of high levels of immunoglobulins (e.g. in multiple myeloma), prozone or hook effects may lead to falsely low results and require dilution of the sample for accurate quantification.[18]

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.[16]

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 (Occupational Safety and Health Administration) 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.

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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