IgG (Immunoglobulin G)

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

Ref No	Pack
MH-392	75 mL
MH-393	50 mL

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

INTENDED USE

The test is applied for the quantitative determination of IgG in serum and plasma. Only 35% is in plasma and the remaining 65% is in the extravascular space.

GENERAL INFORMATION

Immunoglobulin G (IgG) accounts for 70 to 75% of the total immunoglobulins in plasma. IgG consists of two γ-heavy and two light chains linked together by disulfide bonds. The molecular weight of IgG is approximately 150 kDa, including one N-linked oligosaccharide in each heavy chain. The oligosaccharide structure can change in inflammatory states and affect interactions with receptors. In agarose gel electrophoresis, IgG migrates broadly into the γ- and slow β-regions as a result of charge heterogeneity due to sequence variation.[1,2]

There are four subclasses of IgG: IgG_1, IgG_2, IgG_3 and IgG_4. IgG_1, IgG_2 and IgG_4 have a circulating half-life of about 22 days. IgG_3 has a half-life of 7 days. IgG_1 and IgG_3 strongly activate complement through the classical pathway, IgG_2 weakly activates complement and IgG4 does not activate complement. More than one IgG molecule must cluster to activate complement. Both IgG_1 and IgG_3 bind to Fc receptors on phagocytic cells, activate killer monocytes and cross the placenta via receptor-mediated active transport. IgG_1 is the major IgG that crosses the placenta and neonatal concentrations are similar to maternal concentrations. Neonates have low IgG production due to immaturity of their immune system and IgG concentrations decrease in infancy as the maternal antibody repertoire is cleared.[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.[1] The more common primary deficiencies include only one or two immunoglobulin classes (IgA) or subclasses (IgA or IgG subclasses) or the ability to produce antibodies against polysaccharide antigens.

Selective deficiency of IgG subclasses is not uncommon, but it is unclear whether it is a significant risk for infection. IgG_2 deficiency may be associated with a poor response to polysaccharide antigens and an increased risk of infection with encapsulated organisms.[1,3]

Diagnosis of major deficiencies in immunoglobulin production, especially in newborns, is clinically important to prevent infection as maternally acquired antibodies are reduced. Infants have a transient physiologic IgG deficiency, with the lowest level seen at around 3 months of age. Prolonged or severe physiologic deficiency may be associated with increased infection rates, especially with encapsulated bacteria. Maternal IgG concentrations, transmitted through the placenta, rise rapidly in the fetus during the last half of pregnancy but fall several months after birth. Two groups of newborns are at risk for clinically significant IgG deficiency: premature infants who start life with less maternal IgG and infants with delayed onset of IgG synthesis. Monitoring IgG concentrations can identify this problem. By 6 weeks of age, increased IgM and normal salivary IgA concentrations indicate a favorable prognosis. Contact of the newborn with environmental antigens normally causes B lymphocytes to proliferate and IgM concentrations to begin to rise, followed weeks to months later by elevated IgA and IgG.[1]

Polyclonal increases in plasma immunoglobulins are the normal response to infection. IgA increases in skin, intestinal, respiratory and kidney infections. Chronic bacterial infection may cause all immunoglobulin concentrations to increase. Monoclonal immunoglobulins, called paraproteins, can be individual immunoglobulin chains or fragments of immunoglobulins, such as polymers, monomers, free light chains or heavy chains. Based on the clinical, epidemiologic and biochemical characteristics of monoclonal paraprotein diseases, IgG-type paraproteins are detected in plasma in approximately 50% of patients. Free light chain paraproteins are seen in the urine in 60% of these patients. Patients are generally more susceptible to infection.

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

IgG in the sample forms a precipitate in the presence of anti-human IgG antibodies. The absorbance value of the turbidity of the precipitate formed by the antigen-antibody complex, measured at a light wavelength of 540 nm, is directly proportional to the IgG concentration in the sample.

REAGENT COMPONENTS

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

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagent is 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.[4]

SAMPLE REQUIREMENTS

Serum or plasma collected by standard procedure can be used. Li-heparin heparin or K2-EDTA should be used as anticoagulant for plasma. Multiple sample freezing and thawing should be avoided.

IgG activity stability in serum and plasma[19]:

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

Annotation:

- Samples should not be refrozen after thawing because repeated freezing and thawing may cause degradation of proteins.[5]
- Lipemic samples should not be used.

CALIBRATION AND QUALITY CONTROL

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

Protein Calibrator- Lyophilized
Ref.No: VT-012

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: VT-013

Protein Control Level II- Lyophilized
Ref.No: VT-014

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 14 days	320 – 1205
15 days to < 1 year	148 – 631
1 year to < 4 years	317 – 994
4 years to <10 years	501 – 1165
10 years to <19 years	595 –1308
>19 years (Adults)	700 – 1600

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 decline until they reach about 6 months of age. Levels in children increase from adolescence to adult levels.[5,6,7]

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.[8]

Unit Conversion:
mg/dL x 0.01 = g/L
g/L x 6.67 = 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).[9]

The determined analytic measuring interval for IgG is 100-3500 mg/dL.

Detection Capability

Limit of Detection (LoD): 50 mg/dL

Limit of Quantitation (LoQ): 100 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.[10]

Linearity

This method shows measurement linearity in the activities up to 3500 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.[11]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatibility 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.[12]

Repeatibility (Within Run) and Repeatibility (Day to Day) SD (standard deviation) and CV% values of Cholesterol have been given in the table 1 and 2 respectively.

Table 1. IgG Repeatibility (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
713 mg/dL	4.85	0.68	80
1712 mg/dL	11.0	0.64	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[13]

Table 2. IgG Repeatibility (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
713 mg/dL	9.41	1.32	80
1712 mg/dL	12.9	0.75	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[13]

Method Comparison

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

Passing-Bablock equation:[14]
y= 1.027x + 0.55 mg/dL
r=0.985

Interference

Endogenous interferant and analyte concentrations that have been used in the IgG 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 IgG interference scanning test is appropriate, is determined as ±10%.[17]

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

Hemoglobin	: ≤ 1000 mg/dL
Bilirubin	: ≤ 20 mg/dL
Rheumatoid factors	: ≤ 300 IU/mL
Lipemia	: ≤ 1500 mg/dL

Annotation:

- The presence of rheumatoid factor or heterophilic antibodies binding to reactive antibodies may affect immunoassay.[5]
- 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

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