BECKMAN COULTER

IMMAGE® Immunochemistry Systems Chemistry Information Sheet

IGG Immunoglobulin G

REF 446400 (300 tests)

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

IGG reagent, when used in conjunction with IMMAGE® Immunochemistry Systems and Calibrator 1, is intended for quantitative determination of immunoglobulin g (IGG) in human serum or cerebrospinal fluid (CSF) by rate nephelometry.

CLINICAL SIGNIFICANCE

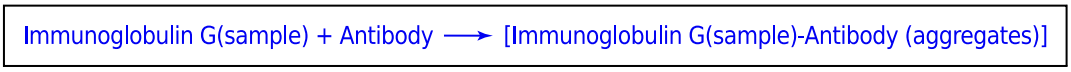
The measurement of gamma globulin in serum and other body fluids aids in the diagnosis of autoimmune diseases, sarcoidosis, chronic liver disease, chronic and recurrent infections, lymphoid malignancies, multiple myeloma and severe combined and variable immunodeficiencies.[1]

Should a paraprotein be identified in blood or urine or both, its heavy and light chains should be typed and the concentrations of polyclonal IgG, IgA, and IgM determined. These studies confirm whether the spike on the electrophoretic pattern is indeed a paraprotein, they help to decide the probable prognosis, and they show whether the polyclonal immunoglobulins are so low that they leave a patient vulnerable to infections.[2]

METHODOLOGY

The IGG test measures the rate of increase in light scattered from particles suspended in solution as a result of complexes formed during an antigen-antibody reaction.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Serum and Cerebrospinal Fluid (CSF) samples are the recommended specimens.

Serum

Serum samples should be collected in the manner routinely used for any clinical laboratory test.[3] Freshly drawn serum from a fasting individual is preferred.

CSF

CSF samples must be centrifuged prior to analysis to remove possible cellular and bacterial contaminants.

SPECIMEN STORAGE AND STABILITY

Serum

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum be physically separated from contact with cells within two hours from the time of collection.[4]
2. If serum samples are not assayed within 8 hours, samples should be stored at +2°C to +8°C. If samples are not assayed within 72 hours, samples should be stored frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.[4]

CSF

CSF specimens not analyzed upon receipt should be refrigerated at +2°C to +8°C. Samples may be frozen.[1]

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

For sample volumes refer to the Sampling Template.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

KIT COMPONENTS	QUANTITY
IGG Cartridge	1
Antibody	
Antigen Excess Solution (AGXS)	
Evaporation Caps	2
IGG Reagent Bar Code Card	1

INITIAL VOLUMES OF SAMPLE AND REAGENTS IN THE CUVETTE

	Serum	CSF
Sample Volume	0.10 µL	21 µL
Total Reagent Volume	341.90 µL	321 µL
Antibody	21 µL	21 µL
Buffer 1	300 µL	300 µL
Diluent 1	20.90 µL	

REACTIVE INGREDIENTS

REAGENT CARTRIDGE CONSTITUENTS	VOLUME
IGG Antibody (processed goat sera)	7.5 mL
IGG Antigen Excess Solution (processed diluted human serum)	2.2 mL
Sodium Azide (used as a preservative)	< 0.1% (w/w)

Also non-reactive chemicals necessary for optimal system performance.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[5]

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

IMMAGE Immunochemistry Systems Wash Solution
IMMAGE Immunochemistry Systems Buffer 1
IMMAGE Immunochemistry Systems Diluent 1
Calibrator 1
Centrifuge capable of 5,000 x g
At least two levels of control material

REAGENT PREPARATION

1. Invert cartridge gently before removing screw caps.
2. Remove screw caps from reagent cartridges. Check each cartridge for bubbles and remove any bubbles present.
3. Place evaporation caps on both reagent cartridge compartments before loading the cartridge on the instrument. See Appendices for evaporation cap directions.
4. Reagent cartridges should be stored upright and can be removed from the refrigerator and used immediately.
5. Mix all buffers and diluents thoroughly by inversion. Remove screw cap from container. Check each container for bubbles and remove any bubbles present. Place evaporation cap on container before loading the container on the instrument. See Appendices for evaporation cap directions.

ACCEPTABLE REAGENT PERFORMANCE

Acceptability of a reagent is determined from the successful performance of quality control testing, as defined in the QUALITY CONTROL section of this chemistry information sheet.

REAGENT STORAGE AND STABILITY

Storage conditions other than those recommended may cause erroneous results.

Reagent Cartridges

1. Return all reagent cartridges to the refrigerator (+2°C to +8°C) upon completion of the daily workload.
2. The IGG reagents are stable for 30 days with the evaporation caps in place. Alternatively, reagent life can be maximized by replacing evaporation caps with screw caps and storing at +2°C to +8°C upon completion of the daily workload.
3. The IGG reagent is stable until the expiration date on the label if stored at +2°C to +8°C with the screw caps in place.

Diluent 1 and Buffer 1

1. Diluent 1 and Buffer 1 are stable on the system for 30 days with the evaporation caps in place.

2. Diluent 1 and Buffer 1 are stable until the expiration date on the label if they are stored at room temperature with the screw caps in place.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

Calibrator 1

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

The calibrator is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

Calibrator storage location:

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[5]

IMMAGE IMMUNOCHEMISTRY SYSTEM CALIBRATION INFORMATION

1. The IMMAGE® Immunochemistry Systems calibration is reagent lot specific.
2. The IGG reagent lot should be recalibrated when changing Buffer 1 lot or following specific part replacements or maintenance procedures as defined in the IMMAGE *Operations Manual*.
3. The IMMAGE Immunochemistry System is designed for minimum calibration. Calibrations retained in system memory should be monitored by the performance of quality control procedures on each day of testing.

4. Calibration for IGG is stable for 30 days.
5. The system will automatically perform a verification check during calibration and produce a calibration report. The system will alert the operator of a failed calibration. An explanation of any accompanying error message can be found in the TROUBLESHOOTING Section of the IMMAGE® Immunochemistry Systems *Operations Manual*.
6. Calibration verification information can be found in the CALIBRATION VERIFICATION section of the IMMAGE® Immunochemistry Systems *Chemistry Reference Manual*.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

It is recommended that at least two levels of control material, normal and abnormal, be analyzed daily. Refer to the CALIBRATORS AND CONTROLS section of the IMMAGE® Immunochemistry Systems *Chemistry Reference Manual*, for a list of Beckman Coulter controls. Controls should also be run with each new calibration, with a new lot of reagent or buffer, and after specific maintenance or troubleshooting as detailed in the IMMAGE® Immunochemistry Systems *Operations Manual*. More frequent use of controls or the use of additional controls is left to the discretion of the user based on work load and work flow.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. After setup, load reagents onto the system as directed in the IMMAGE *Operations Manual*.
2. Select chemistries to be calibrated, if necessary. Load bar coded calibrators, controls, and samples or program and load non-bar coded controls and samples for analysis as directed in the IMMAGE *Operations Manual* .
3. Follow the protocols for system operation as directed in the IMMAGE *Operations Manual*.

CALCULATIONS

The IMMAGE Immunochemistry System will automatically calculate results.

REPORTING RESULTS

REFERENCE INTERVALS

The reference interval values for serum IGG were established using an Array® 360 System, for a population of 205 apparently healthy male and female adults from the U.S.A. and were verified on the IMMAGE Immunochemistry System. The IGG reference interval values for CSF IGG were determined by turbidimetric methodology.[6]

Table 2.0 Reference intervals[a]

	SAMPLE TYPE	REFERENCE INTERVALS
Beckman Coulter	Serum	751 – 1,560 mg/dL
Literature[6]	CSF	0.48 – 5.86 mg/dL

a Each laboratory should establish its own reference interval(s) based on its patient population.

	SAMPLE TYPE	REFERENCE INTERVALS
Laboratory		

Refer to References (1,7,8,9) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

UNITS AND CONVERSION FACTOR

Results for the IGG test are reported in default units of mg/dL. Metric conversion within the same unit category will occur automatically if a new unit is selected. A conversion factor must be entered when selecting a unit category different from the default.

Refer to the System Setup section of the IMMAGE *Operations Manual* for more detailed information on units and conversion factors.

PROCEDURAL NOTES

LIMITATIONS

1. Samples containing a monoclonal immunoglobulin may result in a condition of antigen excess and artificially decreased values. Since the presence of an M-protein can normally be detected using protein electrophoresis, the validity of immunochemical results should be determined by observing consistency with an electrophoretic pattern. If a sample is in antigen excess at the starting dilution, reassay at the next higher dilution to provide a result more consistent with the electrophoretic pattern.
2. With CSF testing, the immunochemical results should be validated by observing consistency with an electrophoretic pattern for IgG bands that may indicate disease processes.

INTERFERENCES

1. The following substances were tested in serum for interference with this methodology at the initial dilution:

Table 3.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT
Bilirubin	Porcine	5 – 30 mg/dL	None
Lipid	Human Triglyceride	150 – 1,000 mg/dL	None[a]
Hemoglobin	Human	100 – 500 mg/dL	None

a Quantitation of specific proteins by nephelometry may not be possible in lipemic samples due to the extreme light scattering properties of the sample.

2. Nonspecific interference can occur between less dilute serum samples and polymer-enhanced buffer when off-line dilutions less than 1:36 are assayed.
3. Dust particles or other particulate matter (i.e. debris and bacteria) in the reaction solution may result in extraneous light-scattering signals, resulting in variable sample analysis.
4. CSF samples contaminated with blood may show significant error in protein analysis.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The IGG test is designed to detect concentrations of this analyte using an initial 1:216 serum sample dilution and undiluted (neat) CSF samples.

Table 4.0 Analytical Range

SAMPLE TYPE	BECKMAN COULTER ANALYTICAL RANGE
Serum	Initial: 200 – 3,600 mg/dL Extended: 33.3 – 21,600 mg/dL
CSF	Initial: 0.93 – 16.7 mg/dL Extended: 0.93 – 3,600 mg/dL

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 5.0 Reportable Range

SAMPLE TYPE	LABORATORY REPORTABLE RANGE

Refer to the IMMAGE® Immunochemistry Systems *Chemistry Reference Manual* section on CALIBRATION VERIFICATION, for more details on laboratory reportable range.

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for IGG in serum determination is 33.3 mg/dL and sensitivity for IGG in CSF determination is 0.93 mg/dL.

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of samples to an accepted clinical method. Values obtained for IGG using the IMMAGE IGG test were compared to the values obtained using an Array® 360 System. Both normal and abnormal serum samples were included in the analysis.

Table 6.0 Equivalency Values

	SERUM Array 360 SYSTEM	CSF Array 360 SYSTEM
N	323	100
Slope	1.037	1.037
Intercept	-16.3	0.11
Mean (IMMAGE)	1,750	5.89
Mean (Array 360)	1,703	5.58
Correlation Coefficient (r)	0.971	0.985

The equivalency values were determined using patient serum samples ranging from 75.9 to 8,920 mg/dL and patient CSF samples ranging from 0.94 to 41.7 mg/dL. Refer to References (10,11) at the end of this chemistry information sheet for guidelines on performing equivalency testing.

PRECISION

A properly operating IMMAGE® Immunochemistry Systems should exhibit imprecision values less than or equal to the maximum performance limits listed below. Maximum performance limits were derived by an examination of the precision of various methods, proficiency test summaries, and literature sources.

Table 7.0 Maximum Performance Limits

TYPE OF PRECISION	SAMPLE TYPE	SD (mg/dL)	% CV	CHANGEOVER VALUE (mg/dL)[a]
Within-run	Serum	20	4.0	500
Total	Serum	20	6.0	333
Within-run	CSF	0.20	5.0	4.00
Total	CSF	0.20	8.0	2.50

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative serum performance data for the IMMAGE Immunochemistry System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[12] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 8.0 Typical Imprecision Values

TYPE OF PRECISION	SAMPLE	Data Points[a]	Test Mean Value (mg/dL)	SD (mg/dL)	% CV
Within-run	Serum Level 1	80	549	10.8	2.0
	Serum Level 2	80	1,293	33.3	2.6
	Serum Level 3	80	2,362	51.2	2.2
Total	Serum Level 1	80	549	13.7	2.5
	Serum Level 2	80	1,293	39.1	3.0
	Serum Level 3	80	2,362	89.6	3.8

a The serum point estimate is based on the data from 1 system, run for 20 days, 2 runs per day, 2 observations per run on an instrument operated and maintained according to the manufacturer's instructions.

Comparative CSF performance data for the IMMAGE Immunochemistry System evaluated using the NCCLS Proposed Guideline EP10-T2 appears in the table below.[13] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 9.0 Typical Imprecision Values

TYPE OF PRECISION	SAMPLE	Data Points[a]	Test Mean Value (mg/dL)	SD (mg/dL)	% CV
Within-run	CSF Level 1	30	1.62	0.086	5.3
	CSF Level 2	30	7.85	0.104	1.3
	CSF Level 3	30	13.5	0.52	3.9
Total	CSF Level 1	30	1.62	0.124	7.6
	CSF Level 2	30	7.85	0.203	2.6
	CSF Level 3	30	13.5	0.58	4.3

a The CSF point estimate is based on the data from 1 system, run for 5 days, 2 runs per day, 3 observations per run on an instrument operated and maintained according to the manufacturer's instructions.

Refer to References (10,12,13) for guidelines on performing precision testing.

NOTICE

These degrees of precision were obtained in typical testing procedures and are not intended to represent performance specifications for this test procedure.

ADDITIONAL INFORMATION

For more information, refer to the IMMAGE Immunochemistry Systems *Operations Manual*.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

1. Tietz, N. W., *Clinical Guide to Laboratory Tests*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1990).

2. Burtis, C. A., Ashwood, E. R., *Tietz Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).

3. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, pp 478 518, W. B. Saunders, Philadelphia, PA (1986).

4. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).

5. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).

6. Ritchie, R. F., et al., "Automated Quantitation of Proteins in Serum and Other Biological Fluids", *Amer. J. Clin. Path.* , 59:151 (1973).

7. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1992).

8. Henry, J. B., ed., *Clinical Diagnosis and Management by Laboratory Methods*, 17th Edition (1984).

9. Statland, Bernard E., "Clinical Decision Levels for Lab Tests", *Medical Economic Book*, Oradel, New Jersey (1983).

10. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).

11. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Tentative Guideline, NCCLS publication EP9-T, Villanova, PA (1993).

12. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).

13. National Committee for Clinical Laboratory Standards, *Preliminary Evaluation of Quantitative Clinical Laboratory Methods*, Tentative Guideline, 2nd Edition, NCCLS publication EP10-T2, Villanova, PA (1993).

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