

INSTRUCTIONS FOR USE

IgM

VITROS Chemistry Products IgM Reagent

Immunoglobulin M

REF 680 1734

Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products IgM Reagent is used on the VITROS 5,1 FS Chemistry System, the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System to quantitatively measure immunoglobulin M (IgM) concentration in human serum and plasma.

Summary and Explanation of the Test

IgM is the first immunoglobulin synthesized in response to antigenic challenge and accounts for 5 to 10% of the total circulating immunoglobulins. The role of IgM in activating the complement cascade and promoting phagocytosis makes it an important factor in eliminating particulate antigens and microorganisms from the circulation.¹ IgM levels are used to evaluate monoclonal proteins seen on serum electrophoresis, and to monitor the progression or therapeutic response of patients with macroglobulinemia. Levels are frequently increased in viral infections, rheumatoid arthritis, and chronic hepatocellular disease, active sarcoidosis, Waldenström's macroglobulinemia, and malignant lymphoma. Decreased levels are seen in association with recurrent, chronic, or severe infections, multiple myeloma (IgA or IgG), and protein-losing enteropathy (but not nephrotic syndrome).²

Principles of the Procedure

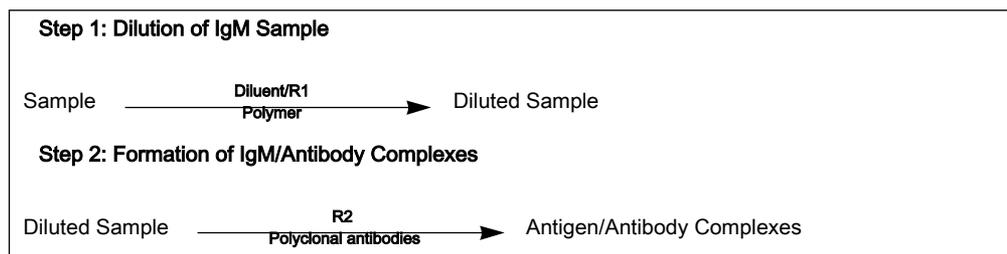
The quantitative measurement of immunoglobulin M (IgM) is performed using the VITROS Chemistry Products IgM Reagent in conjunction with the VITROS Chemistry Products Calibrator Kit 20 and VITROS Chemistry Products FS Diluent Pack 2 on VITROS 5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS IgM Reagent is a dual chambered package containing ready-to-use liquid reagents. Samples, calibrators and controls are automatically diluted in saline and mixed with Reagent 1 containing a polymer. Addition of antisera specific for human IgM (Reagent 2) produces an immunochemical reaction yielding antigen/antibody complexes. The light scattering properties of the antigen/antibody complexes increase solution turbidity proportional to IgM concentration in the sample. The turbidity is measured spectrophotometrically at 340 nm. Once a calibration has been performed for each reagent lot, the IgM concentration in each unknown sample can be determined using the stored calibration curve and the measured absorbance obtained in the assay of the sample.

Test Type and Conditions

Test Type	VITROS System	Approximate Incubation Time	Temperature	Wavelength	Reaction Sample Volume
Blanked Endpoint	5600, 4600, 5,1 FS	Incubation 1: 5 minutes	37 °C (98.6 °F)	340 nm	6.7 µL
		Incubation 2: 5 minutes			

Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

For *in vitro* diagnostic use only.

WARNING: *This product contains sodium azide. Disposal of reagents into sinks with copper or lead plumbing should be followed with plenty of water to prevent formation of potentially explosive metallic azides.*

WARNING: *Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29³ or other published biohazard safety guidelines.*

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer's product literature.

Reagents

Reactive Ingredients

Reagent 1 (R1): None

Reagent 2 (R2): Goat antisera to human IgM 1mL/mL

Other Ingredients

Reagent 1 (R1): Preservative, polymer, buffer, inorganic salt

Reagent 2 (R2): Preservative, buffer, inorganic salt

Reagent Handling

Caution: Do not use reagent packs with damaged or incompletely sealed packaging.

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.
- Reagents are supplied ready for use.
- Avoid agitation, which may cause foaming or the formation of bubbles.

Reagent Preparation

1. Remove from refrigerated storage.
2. Immediately load into Supply 3.

IMPORTANT: Do not loosen or remove caps prior to loading.

Reagent Storage and Stability

VITROS Chemistry Products IgM Reagent is stable until the expiration date on the carton when it is stored and handled as specified. Do not use beyond the expiration date.

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	Until expiration date
Opened	On-analyzer	System turned on	≤ 4 weeks
	On-analyzer	System turned off	≤ 30 minutes

Verify performance with quality control materials after reloading reagents that have been removed from Supply 3 and stored for later use.

Specimen Collection, Preparation and Storage

Specimens Recommended

- Serum

- Plasma:
 - Heparin
 - EDTA

IMPORTANT: *Certain collection devices have been reported to affect other analytes and assays.⁴ Owing to the variety of specimen collection devices available, Ortho-Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this assay.*

Specimens Not Recommended

None.

Serum and Plasma

Specimen Collection and Preparation

Collect specimens using standard laboratory procedures.^{5, 6}

Note: For details on minimum fill volume requirements, refer to the operating instructions for your system.

Patient Preparation

No special patient preparation is necessary.

Special Precautions

- Liquid EDTA plasma specimens collected in partially filled sample tubes may result in negative prediction biases.
- Centrifuge specimens and remove the serum or plasma from the cellular material within two hours of collection.⁷
- Refer to "Sample Dilution" for dilution instructions.

Specimen Handling and Storage

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Mix samples by gentle inversion and bring to room temperature, 18–28 °C (64–82 °F), prior to analysis.

Specimen Storage and Stability

Storage	Temperature	Stability
Room temperature	18–28 °C (64–82 °F)	≤ 1 day
Refrigerated	2–8 °C (36–46 °F)	≤ 7 days
Frozen	≤ -18 °C (≤ 0 °F)	≤ 4 weeks

IMPORTANT: *Avoid repeated freeze-thaw cycles.*

Testing Procedure

Materials Provided

VITROS Chemistry Products IgM Reagent

Materials Required but Not Provided

- VITROS Chemistry Products Calibrator Kit 20
- Quality control materials, such as VITROS Chemistry Products Protein Performance Verifiers I, II, and III
- VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline)

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
- For additional information, refer to the operating instructions for your system.

IMPORTANT: *Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.*

Sample Dilution

IMPORTANT: *Samples with IgM concentration that exceed the system's measuring (reportable) range may be diluted with an equal volume of saline using VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline) by the VITROS Integrated and 5,1 FS/*

4600 Chemistry Systems. Higher dilution ratios are not recommended when using the On- Analyzer Dilution mode.

On-Analyzer Sample Dilution:

Refer to the operating instructions for more information on the On-Analyzer Dilution Procedure. Use VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline) for the dilution.

Manual Sample Dilution:

If IgM concentrations exceed the system’s measuring (reportable) range after 1:2 dilution (1 part sample + 1 part saline) using the On Analyzer Dilution Mode on the VITROS Integrated and 5,1 FS/4600 Chemistry Systems:

1. Manually dilute the original sample with saline from VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline). Do not exceed a maximum dilution of 1:10 (1 part sample + 9 parts saline).
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain an estimate of the original sample’s IgM concentration.

IMPORTANT: *If using the system in On-Analyzer Dilution Mode, do not manually dilute samples for analysis. Refer to the operating instructions for more information on the On-Analyzer Dilution Procedure.*

Calibration

Required Calibrators

VITROS Chemistry Products Calibrator Kit 20

Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Calibrator 20.

Calibration Procedure

Refer to the operating instructions for your system.

When to Calibrate

Calibrate:

- When the reagent lot number changes.
- When critical system parts are replaced due to service or maintenance.
- When government regulations require.

For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

The VITROS IgM assay may also need to be calibrated:

- If quality control results are consistently outside acceptable range.
- After certain service procedures have been performed.

For additional information, refer to the operating instructions for your system.

Calculations

Absorbance is measured at 340 nm after each incubation step (blank, endpoint) and the response calculated from the difference in absorbance values. Once a calibration has been performed for each reagent lot, IgM concentration in the unknown samples can be determined using the stored calibration curve and the calculated response obtained in the assay of each sample.

Validity of a Calibration

Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the Review Assay Data screen (found via Options → Review/Edit Calibrations → Review Assay Data). Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

Measuring (Reportable) Range

Conventional Units (mg/dL)	SI Units (g/L)
25.00–400.00	0.25–4.00

For out-of-range samples, refer to “Sample Dilution”

Traceability of Calibration

Values assigned to the VITROS Chemistry Products Calibrator Kit 20 for IgM are traceable to the BAM-IRMM-LGC (Bundesanstalt für Materialforschung und -prüfung/Institute for Reference Methods and Materials/Laboratory of the Government Chemist) ERM-DA470 Reference Material.⁸

Quality Control

Quality Control Material Selection

IMPORTANT: VITROS Chemistry Products Protein Performance Verifiers are recommended for use with the VITROS Integrated and 5,1 FS/4600 Chemistry Systems. Evaluate the performance of other commercial control fluids for compatibility with this assay before using for quality control.

Control materials other than VITROS Chemistry Products Protein Performance Verifiers may show a difference when compared with other IgM methods if they:

- Depart from a true human matrix.
- Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.

Quality Control Procedure Recommendations

- Choose control levels that check the clinically relevant range.
- Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.
- To verify system performance, analyze control materials:
 - After calibration.
 - According to local regulations or at least once each day that the assay is being performed.
 - After specified service procedures are performed. Refer to the operating instructions for your system.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- For general quality control recommendations, refer to CLSI *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition*⁹ or other published guidelines.
- For additional information, refer to the operating instructions for your system.

Quality Control Material Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Protein Performance Verifier I, II, and III or to other manufacturer's product literature.

Results

Reporting Units and Unit Conversion

The VITROS 5,1 FS/4600 Chemistry and VITROS Integrated Systems may be programmed to report IgM results in conventional or SI units.

Conventional Units	SI Units
mg/dL	g/L (mg/dL x 0.01)

Limitations of the Procedure

No antigen excess effect was observed for samples with IgM concentration up to 10,000 mg/dL (100 g/L).

An abnormally elevated level of a monoclonal immunoglobulin, as in cases of macroglobulinemia, multiple myeloma, amyloidosis, or other lymphoproliferative diseases, may interfere with the quantification of IgM.¹¹

Known Interferences

None identified.

Other Limitations

- For samples that generate a Sample Integrity T-index flag where the T-Index value is between 150 and 300 and the observed IgM concentration is >50 mg/dL, dilute the sample and re-analyze (refer to the Sample Dilution section).
- Samples that generate a Sample Integrity T-index flag where the T-Index value is between 150 and 300 and the observed IgM concentration is ≤50 mg/dL, may be manually diluted two parts sample and one part saline. Obtain the original sample concentration by multiplying by 1.5.

- Samples that generate a Sample Integrity T-index flag where the T-Index value is >300 should be re-drawn or measured by an alternative method.
- Certain drugs and clinical conditions are known to alter IgM concentration *in vivo*. For additional information, refer to one of the published summaries.^{12, 13}

Expected Values

Reference Interval

This reference interval is based on an external study.¹⁰

Conventional Units (mg/dL)	SI Units (g/L)
40–230	0.40–2.30

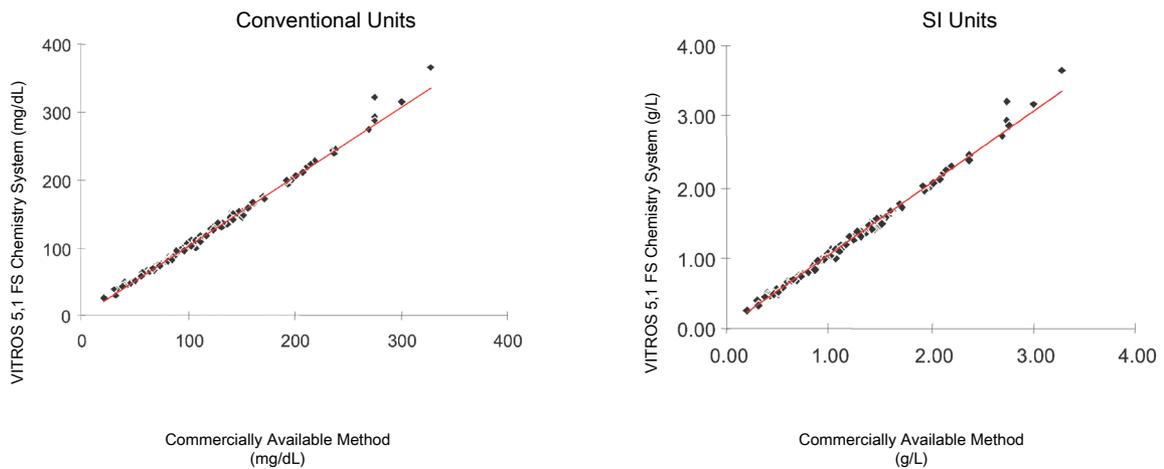
Each laboratory should confirm the validity of these intervals for the population it serves.

Performance Characteristics

Method Comparison

The plots and data below show the results of a method comparison study with serum samples analyzed on the VITROS 5,1 FS Chemistry System and a commercially available system, based on NCCLS Protocol EP9.¹⁴

The table also shows the results of comparisons of serum samples on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System. The testing followed NCCLS Protocol EP9.¹⁴



	n	Slope	Correlation Coefficient	Conventional Units (mg/dL)			SI Units (g/L)		
				Range of Sample Conc.	Intercept	Sy.x	Range of Sample Conc.	Intercept	Sy.x
5,1 FS [†] vs. Commercially Available method [†]	124	1.02	0.998	25.23–365.00	+1.10	5.52	0.25–3.65	+0.01	0.06
5600 vs. 5,1 FS [†]	115	1.01	0.999	36.98–394.43	-0.80	5.31	0.37–3.94	-0.01	0.05

[†] Dade Behring Human IgM assay

[†] Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

Precision

Precision was evaluated with quality-control materials on the VITROS 5,1 FS System following NCCLS Protocol EP5.¹⁵

Precision was also evaluated with quality control materials on the VITROS 5600 Integrated System following NCCLS Protocol EP5.¹⁶

These results are guidelines. Variables such as instrument maintenance, environment, reagent storage/ handling, control material reconstitution, and sample handling can affect the reproducibility of test results.

	Conventional Units (mg/dL)			SI Units (g/L)			Within Lab CV%*	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD*	Mean Conc.	Within Day SD*	Within Lab SD*			
5,1 FS†	46.04	1.47	2.86	0.46	0.01	0.03	6.21	88	22
	78.27	1.56	3.24	0.78	0.02	0.03	4.14	86	22
	193.69	2.43	4.59	1.94	0.02	0.05	2.37	88	22
5600	40.84	0.80	1.02	0.41	0.01	0.01	2.50	88	22
	79.69	1.11	1.45	0.80	0.01	0.01	1.82	88	22
	172.32	2.72	3.45	1.72	1.72	0.03	2.00	88	22

* Within Day precision was determined using two runs per day with two replications per run.

* Within Lab precision was determined using a single lot of reagents with one analyzer and at least four calibrations.

† Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

Specificity

Substances that Do Not Interfere

The substances listed in the table, at the concentrations shown, were tested according to NCCLS Protocol EP7. ¹⁷ Bilirubin and hemoglobin were tested at IgM concentrations of approximately 25 mg/dL (0.25 g/L) and 190 mg/dL (1.90 g/L) and found not to interfere, bias <4.5 mg/dL (<0.05 g/L) at 25 mg/dL IgM and bias <22.5 mg/dL (<0.23 g/L) at 190 mg/dL IgM. The remaining compounds listed in the table were tested at an IgM concentration of approximately 80 mg/dL (0.80 g/L) and found not to interfere, bias <12 mg/dL (<0.12 g/L).

Compound	Concentration	
Acetaminophen	20 mg/dL	1324 µmol/L
Acetyl-L-cystein (N)	100 mg/dL	6.13 mmol/L
Amoxicillin	20 µg/mL	55 µmol/L
Ascorbic acid (L)	3 mg/dL	0.17 mmol/L
Bilirubin	60 mg/dL	1.03 mmol/L
Carbamazepine	120 µg/mL	507.6 µmol/L
Dipyron	30 mg/dL	0.85 mmol/L
Ethamsylate	3 mg/dL	0.11 mmol/L
Gentamicin sulfate	120 µg/mL	251 µmol/L
Hemoglobin	500 mg/dL	5 g/L
Ibuprofen	400 µg/mL	1940 µmol/L
Lidocaine	60 µg/mL	256 µmol/L
Methotrexate	90.9 mg/dL	2 mmol/L
Procainamide	100 µg/mL	423 µmol/L
Propranolol	5 µg/mL	19.3 µmol/L
Rantidine	200 µg/mL	637 µmol/L
Salicylic acid	50 mg/dL	3.6 mmol/L
Simvastatin	16 µg/mL	1.2 mmol/L
Theophylline	25 mg/dL	1388 µmol/L
Valproic acid	500 µg/mL	3465 µmol/L

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Glossary of Symbols

The following symbols may have been used in the labeling of this product.

	Do Not Reuse		Upper Limit of Temperature		Range
	Use by or Expiration Date (Year-Month-Day)		Lower Limit of Temperature		Range of Means
	Batch Code or Lot Number		Temperature Limitation		Midpoint
	Serial Number		Consult Instructions for Use		Revised
	Catalog Number or Product Code		Attention: The Instructions for Use (IFU) has been updated		Supersedes
	Caution		For use in Slide Supply 1		Irritant
	Manufacturer		For use in Slide Supply 2		Harmful
	Date of Manufacture		SI Units		Toxic
	Authorized Representative in the European Community		Conventional Units		Corrosive
	Contains Sufficient for "n" Tests		Value		Flammable
	In vitro Diagnostic Medical Device		Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations		Estimated within-lab SD

Revision History

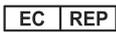
Date of Revision	Version	Description of Technical Changes*
2014-09-05	5.0	Glossary of Symbols: added Date of Manufacture
2012-02-28	4.0	Glossary of Symbols: updated
2010-11-01	3.0	Added information for the VITROS 4600 Chemistry System
2009-03-12	2.0	<ul style="list-style-type: none"> • Added information for the VITROS 5600 Integrated System • Test Type and Conditions – Added statement • Traceability of Calibration– Updated • Method Comparison – Added information on sample types • References – Updated • Glossary of Symbols – Updated • Minor wording and formatting changes
2004-09-08	Version 1.0	First release of document

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

 Signature

 Obsolete Date



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