



NOTE CHANGES:

Limitations of the Procedure - added Limitation #2 (Ref. - Bibliography #7 & #8)

SYMBOL DEFINITIONS

- = Consult Directions for Use
- = *In Vitro* Diagnostic Reagent
- = Storage Temperature
- = Code Number
- = Expiration Date
- = Lot Number
- = Amount
- = Contains biological material of animal origin

PRODUCT AVAILABILITY

The following is available from Bion Enterprises, Ltd. (Bion):

Description	Code No.	
IgG Removal Reagent, 2.5 ml	GBR-9983	
IgG Removal Reagent, 20.0 ml	GBR-9981	

INTENDED USE

The Bion IgG REMOVAL REAGENT is designed to functionally remove potentially interfering immunoglobulin IgG antibodies from human serum prior to testing for IgM. This product is for *In vitro* diagnostic use.

SUMMARY AND EXPLANATION

The early and accurate diagnosis of many infectious diseases has become increasingly dependent upon the measurement of pathogen-specific IgM antibodies in human serum^{1,2,3,4}. IgM antibodies generally appear 7-10 days after primary infection, peak 2-3 weeks, and decline to undetectable levels in 3-6 months. Therefore, the identification of pathogen-specific IgM in human serum suggests a current or recent infection. In addition, since maternal IgM does not cross the placenta under normal physiological conditions, the presence of pathogen-specific IgM in cord blood or neonatal serum is highly suggestive of a congenital infection⁵.

The identification of pathogen-specific IgM is contingent upon the removal of pathogen-specific IgG for accuracy. Specific IgG in a patient specimen may compete with specific IgM antigenic sites on the substrate. Depending upon the ration of IgG to IgM, false negative IgM results may be obtained. In addition, RF-IgM (IgM class IgG) if present in conjunction with specific IgG, may interact with antigen bound IgG resulting in a false positive IgM test.

Therefore, the accurate measurement of pathogen-specific IgM is highly dependent upon the effective removal or neutralization of specific IgG.

Removal of pathogen-specific IgG and RF-IgM is most easily accomplished by treating the patient specimen with species (normal goat) anti-human IgG. This treatment will result in the formation of immune complexes containing human IgG, effectively reducing the ability of the pathogen-specific IgG to interact with the substrate. In addition, the aggregated IgG contained in the immune complexes provides excellent substrate for RF-IgM binding. Therefore, the treated sample will be free of specific IgG and RF-IgM, and can be used for specific IgM measurements.

PRINCIPLE OF THE TEST PROCEDURE

Patient specimens to be tested for pathogen-specific IgM are first diluted in the IgG Removal Reagent (goat anti-human IgG). Immune complexes containing patient IgG are formed during the subsequent incubation period. RG-IgM, if present, will bind to the immune complexes resulting in the removal of both pathogen-specific IgG and RF-IgM.

REAGENTS

2.5 ml or 20 ml ready to use dropper vial containing goat anti-human IgG. Ready to use.

STORAGE AND STABILITY

Stable at 2-8°C until stated expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of *in vitro* diagnostics may perform the test.
2. All reagents should be brought to room temperature (20-25°C) prior to use.
3. Do not freeze. Specific activity of the reagent may be compromised by freezing and thawing.
4. The IgG Removal Reagent should be used only as a diluent for patient specimens.
5. Do not use IgG Removal Reagent to dilute Controls or Conjugate.
6. Do not use IgG Removal Reagent in any of the wash steps.
7. Reagents should not be used beyond stated expiration date.
8. Avoid microbial contamination of reagents or incorrect results may occur.
9. Care should be taken to avoid splashing and generation of aerosols.
10. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

MATERIALS PROVIDED

Bion IgG REMOVAL REAGENT

MATERIALS REQUIRED BUT NOT PROVIDED

1. Vortex Mixer
2. Centrifuge (3000 rpm)
3. Disposable 10 X 75 mm test tubes with stoppers
4. Calibrated pipette to deliver 5-10 µl, with disposable pipette tips
5. Felt tip marking pen

TEST PROCEDURE

1. Allow IgG Removal Reagent, all serums and reagents to equilibrate to room temperature (20-25°C).
2. Holding dropper vial in vertical position, dispense appropriate amount of the IgG Removal Reagent into 10 X 75 test tubes. Identify each tube using a felt tip marking pen.

<u>Usage:</u>	<u>IgG Binding Reagent</u>	+	<u>Serum</u>
	1 drop (0.05 ml)	+	5 µl
	2 drops (0.1 ml)	+	10 µl

3. With the calibrated pipette, add appropriate amount of serum and mix well on the vortex mixer (1:10 dilution).
4. The sample is now ready for testing and can be used as the first dilution in the IgM assay. No incubation is required. Alternately, if testing is not going to be done immediately, the sample may incubate for ≥ 15 minutes.

NOTE: During this incubation period, the IgG Removal Reagent may become cloudy as immune complex formation progresses.

5. Flocculent material will not interfere with Bion IgM IFA assays.

Optional: Stopper test tube and centrifuge at 3000 rpm for 30 minutes to completely remove flocculent material. This precipitate sometimes makes it difficult to read antigen substrates. The supernate is now ready for use as the first dilution in the IgM assay.

6. To assay the mixture, follow the instructions for the standard IgM IFA test procedure.

LIMITATIONS

1. When used according to this procedure, Bion's IgG Removal Reagent has been shown to neutralize up to 21 mg/mL of IgG. Normal adult IgG levels may range from 8-16 mg/mL⁶. Patients with an IgG level exceeding 21 mg/mL may require additional treatment to neutralize all IgG.

2. IgG removal reagents may lower the total amount of IgM present in serum after treatment. The amount removed can be as high as 25% of total IgM present⁷. The effects of IgG removal on the sample may vary by assay type performed (IFA, ELISA, etc.). The specific IgM may vary by assay specificity of related organism as well. Inherent cross-reactivity between related viruses, previous infections with related viral species, and bacterial infections is also possible⁸. It is suggested that paired serums be treated and processed for antibody titers at the same time to minimize processing differences. Serums tested for acute infection should be checked for the patient's clinical history and repeated if indeterminate. Paired samples are recommended for analysis.

PERFORMANCE CHARACTERISTICS

The Bion IgG REMOVAL REAGENT was evaluated for the removal of IgG using a turbidimetric methodology. Twenty samples were tested for IgG before and after treatment with the IgG REMOVAL REAGENT. The results of this evaluation demonstrated a 95.5% $\pm$ 1.3% Efficiency in the reduction of IgG⁹.

The functional removal of IgG was demonstrated by testing 48 samples using the IgG Removal Reagent and comparing them with a commercially available reagent. There was 100% agreement between the two IgG removal reagents⁹.

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9. Data on file, Bion, Des Plaines, Illinois.

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