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IgG BINDING REAGENT

CE

NOTE: Changes highlighted

SYMBOL DEFINITIONS

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

PRODUCT AVAILABILITY

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

Description	Code No. REF
IgG Binding Reagent, 2.5 ml	GBR-9982
IgG Binding Reagent, 20.0 ml	GBR-9980

INTENDED USE

The Bion IgG BINDING REAGENT is designed to remove IgG and rheumatoid factor of the IgM class from human serum prior to performing the indirect fluorescent antibody assay for the determination of specific IgM antibodies in this human serum.

SUMMARY AND EXPLANATION

The greatest source of error in IgM testing is interference by the presence of pathogen-specific IgG. This can occur in one of two ways:[1,2,3]

1. False Negative Reactions may occur due to high levels of specific IgG blocking the IgM in the competition for antigenic sites during the first step of the staining reaction. The IgG, being more avid than the IgM, will react with the antigenic sites first, blocking the IgM from participating in the reaction. The IgM will then be washed away during the first wash, leaving nothing for the anti-IgM conjugate to react with in step two of the staining reaction resulting in a false negative reaction in the presence of specific IgM.

2. False Positive Reactions may occur when Rheumatoid Factors are present along with IgG antibodies. Some Rheumatoid Factors can be immunoglobulin M anti-IgG. When IgG reacts with the antigenic sites during the first step of the staining reaction, the Rheumatoid Factor anti-IgG will then react with the bound IgG. Rheumatoid Factor being an antibody of the IgM class will then react with the anti-IgM conjugate in step two of the staining reaction, resulting in a false positive reaction in the absence of specific IgM.

It is, therefore, strongly recommended that each serum specimen be pre-treated to remove any IgG interference by separating the IgM from the IgG using any of the standard methodologies.[4,5,6] One such methodology is IgG immunoprecipitation.[5,6]

PRINCIPLE OF THE TEST PROCEDURE

The IgG binding reagent consists of anti-human IgG antibody. When incubated with serum, it forms an immunoprecipitation reaction of IgG complexes which in turn combine with IgM class rheumatoid factor, thus eliminating these interfering substances prior to performing the IFA assay for IgM on patients' serums.

REAGENTS

2.5 ml or 20 ml ready to use dropper vial containing whole sheep serum in glycine/saline at pH 7.4 with 1 mM EDTA, 0.1% Sodium Azide, 0.1% E-amino-n-caproic acid and 0.01% benzamidine.

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. Refrigeration (2-8°C) of reagents immediately upon arrival will insure stability until labeled expiration date.
4. Reagents should not be used beyond stated expiration date.
5. Avoid microbial contamination of reagents or incorrect results may occur.
6. Care should be taken to avoid splashing and generation of aerosols.
7. 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.
8. The preservatives used are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize build up of azide and metal compounds.

MATERIALS PROVIDED
Bion IgG BINDING 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 all serums and reagents to equilibrate to room temperature (20-25°C).

2. Holding dropper vial in vertical position, dispense appropriate amount of the binding reagent into 10 X 75 test tubes. Identify each tube using a felt tip marking pen.

Usage:	IgG Binding Reagent	+	Serum
	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 mixture can be used, after mixing, as the first dilution in the IgM assay.

5. 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
At a ratio of 4 to 1 (reagent to serum), this reagent will precipitate up to 15 mg/ml of IgG antibody from human serum. Sera with IgG levels greater than this should be treated with a proportionally increased amount of the binding reagent.

PERFORMANCE CHARACTERISTICS
The Bion IgG BINDING REAGENT was evaluated for the removal of IgG using a nephelometric methodology. Samples were tested for IgG, IgA and IgM before and after treatment with the IgG BINDING REAGENT. There was a 96% to 100% reduction in the serum IgG content with no reduction in either the IgA or IgM.[7]

The functional removal of IgG was demonstrated by testing samples for IgG antibodies by immunofluorescence assay (IFA) before and after treatment with Bion IgG BINDING REAGENT. All samples demonstrated serum IFA IgG titers before treatment and were negative for IgG antibodies after treatment.[7]

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

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