Instructions for Use

BIOSORB® ABSORBENTS CE

Isolation of IgM and IgA by separation of IgG using anti-human IgG antiserum suitable to all IgM or IgA determinations:
Biosorb® IgG Absorbent, 48 separations, Cat.No. 90-1048
Biosorb® IgG Absorbent, 120 separations, Cat.No. 90-1120

Separation of group-specific antibodies to spirochaetes using Treponema phagedenis homogenate suitable to Borrelia and Leptospira IgG determinations:
Biosorb® Reiter Absorbent, Cat.No. 36-6105

INTENDED USE
The Biosorb® Absorbents remove immunoglobulins disturbing assays of IgM or IgA or assays of spirochetal antibodies in human serum. They are designated for the in vitro diagnostic use only.

INTRODUCTION
Isolation of IgM and IgA
Assaying serum directly for IgM or IgA activity has several drawbacks if pathogen specific IgG is also present. This is mainly true if the concentrations of antigen specific IgM and IgA are low compared to the antigen specific IgG. The IgG may interfere with IgM and IgA antigen interaction by competing for binding sites on the antigen (steric hindrance). This may result in reduction or inhibition of IgM/IgA binding and so in false negative results. On the other side IgM rheumatoid factor present in patient serum can cause false positives in IgM assays. IgM rheumatoid factor is a IgM autoantibody directed against IgG antibodies (in immune complexes). If specific IgG is bound to the antigen IgM rheumatoid factors may bind to the “activated” IgG and detected by the specific IgM conjugate. By analogy IgA rheumatoid factors can affect IgA assays.
To eliminate these problems IgG must be removed from the sample. The isolated IgM and/or IgA are then tested for specifity to the pathogens. Treating the samples with anti-human IgG antiserum (**Biosorb® IgG Absorbent Cat.No. 90-1048** and **90-1120**) provides a simple, rapid and efficient method. Human IgG molecules are bound in stable immune complexes (no centrifugation is necessary to remove these complexes) which do no longer block the IgM/IgA binding sites, thus avoiding false negative results. Indirectly also rheumatoid factors present will no more lead to false positive results.

Serology of Spirochaetes
Spirochaetal serology (Treponema pallidum, Borrelia burgdorferi, Leptospira interrogans) is complex. Sera of patients infected with one of these pathogens or which only came into contact with apathogenic members of spirochaete family often show cross reactions (also with high titers) when tested with a heterologous antigen from the spirochetal group. Obviously the crossreacting (common) spirochaetal antigens (epitopes) are strong immunogens, i.e. antibodies are regularly formed especially against these antigens/epitopes. Differentiation of the involved pathogen only by serological means therefore is difficult to impossible.
To increase the specifity of serotests on spirochaetal antibodies patient's sample should be pretreated with absorbent (**Biosorb® Reiter Absorbent Cat.No. 36-6105**) which removes cross reacting antibodies to a high extent. However, sensitivity of spirochaetal antibody assays is reduced by this measure. Titers of specific antibodies will be more or less decreased by serum treatment with Reiter spirochaetes.

CONTENTS AND PRINCIPLE OF THE ASSAY
Biosorb® IgG Absorbent:
Anti-human IgG based system: serum dilution after treatment is 1:5. Suitable to all IgG/IgM or IgG/IgA separations for all methods (IFA, Elisa, Blot etc.)
Cat.No. 90-1048: for 48 patient samples (separations), ready to use; 2 ml.
Cat.No. 90-1120: for 120 patient samples (separations), ready to use; 5 ml.
Treating patient's sample with Biosorb® reagent human IgG molecules from the serum sample will be complexed with anti-IgG antiserum. Fixed IgG molecules cannot disturb the reactivity of IgM or IgA in the assay. After treatment with Biosorb® IgG Absorbent serum can be used directly or diluted with PBS for the IFA or Elisa dilution buffer etc. to the final test dilution.

Biosorb® Reiter Absorbent
Cat.No. 36-6105: Treponema phagedenis homogenate based system; ready to use; 1 ml.
Dilution of serum sample after application of the absorbent is 1:5;
Treatment of patient's sera with Biosorb® Reiter Absorbent removes antibodies directed to common antigens on spirochaetes.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
Appropriate containers to perform absorption
Precision pipettes and pipette tips to deliver 1-1000 µl
Vortex mixer
PBS buffer
Timer

STORAGE AND STABILITY
Store absorbents at the temperature specified on the label. The **unopened** reagents are stable up to the expiry date indicated on the label if the recommendations are strictly followed. Do not use any of these reagents after they have expired.
After first use the reagents have to be well closed and stored at the temperature specified on the label. These reagents must be consumed as soon as possible. Stability on reuse does not necessarily correlate with expiry date.

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SAFETY PRECAUTIONS

1. The Absorbents are for in vitro diagnostic use only.
2. Absorbents contain 0.09% sodium azide. Sodium azide is poisonous. Don't swallow and avoid any contact with skin and mucous membranes. Azide containing reagents must not be brought into contact with any copper or lead containing objects, for example certain drain pipes, as this could lead to formation of explosive metal azides.
3. The safety regulations of trade associations and of the respective institute (laboratory) should be strictly followed (see notices, laboratory guidlines, safety instructions etc.).
4. Actual Good Laboratory Practice rules (GLP guide lines) should always be followed.
5. Materials and reagents used in the test must be disposed off in compliance with the applicable legal regulations and the workplace has to be disinfected.

MATERIAL FOR TESTING

Serum and plasma are both suitable for testing. Serum and plasma samples are stable for about 1 week if held at 5-10°C. If storage or repeated testing of samples is required for longer periods, samples should be subdivided into small portions (50 µl), snap-frozen in liquid nitrogen and stored at or below -20°C. Larger volumes of serum or plasma should not be exposed to repeated freeze-thaw cycles because this can cause aggregation of proteins and degradation of some serum and plasma components. Because azide does not interfere with the assay, serum and plasma samples also may be stabilized with 0.09% azide. Such samples can be stored for prolonged periods (up to 1 year) at 5-10°C without loss of analyte.

QUALITY CONTROL AND TROUBLE SHOOTING

Sorbents should not be temperated to room temperature by heating.
Treatment of sera with Reiter Absorbent takes away crossreacting antibodies (antibodies to genus-specific antigens of spirochaetes). Test specifity will be increased by this procedure but test sensitivity will be decreased. This should be taken into account when results of absorbed sera are compared with results of non-absorbed sera.
Preparing appropriate test dilution of sera the former sera dilution following the absorbtion has to be considered.
Assay sensitivity and specificity are continually monitored in Bios® control laboratory to assure consistent test performance. Bios® applies all serum and control standards available from WHO or other official institutions for assay standardization.
Warranty by Bios is extended only if directions of use are strictly followed, if solely Bios products are verifably applied in the test, and if the test is accomplished by qualified personnel.

PROCEDURE OF THE ABSORPTION

Allow all reagents and serum samples to reach room temperature before absorption (about 10 minutes). After worming up gently put Biosorb® bottle upsite-down for 2 or 3 times before use to mix the protein suspension.

Application of Biosorb® IgG Absorbent for the removal of IgG in IgM or IgA assays:
1. For absorption mix 1 part of patient serum with four parts of Biosorb® IgG Absorbent (for example 10 µl of serum sample and 40 µl Biosorb® IgG Absorbent). Put Biosorb® IgG Absorbent immediately back into the refrigerator.
2. Carefully vortex the mixture
3. Incubate the mixture for 10-15 minutes at room temperature.
4. Serum dilution in the mixture is **1:5**. The mixture may be used directly for IgM/IgA testing or diluted with PBS for IFA, Elisa diluent buffer or other appropriate buffer to the final test dilution. **No additional centrifugation step is required.**
5. Do not apply Biosorb® IgG Absorbent to Bios® positive IgM/IgA controls. Titers, indicated on the label of these controls could vary. Our positive controls have been sufficiently pretreated in our laboratory.
6. When planning serum dilutions the dilution of patient sample by the absorption step (1:5) must be taken into account.
7. Biosorb® IgG Absorbent is suitable for the removal of IgG independent of method (IFA, Elisa, Blot etc.) or manufacturer.

Application of Biosorb® Reiter Absorbent for absorption of genus-specific antibodies to spirochaetes:
1. For absorption mix 1 part of patient serum with four parts of Biosorb® Reiter Absorbent (for example mix 10 µl of serum sample with 40 µl Biosorb® Reiter Absorbent). Put Biosorb® Reiter Absorbant immediately back into the refrigerator.
2. Carefully vortex the mixture
3. Incubate the mixture at room temperature for 10-15 minutes.
4. Serum dilution in the mixture is **1:5.** The mixture may be used directly for testing or diluted with PBS for IFA, Elisa diluent buffer or other appropriate buffer to the final test dilution. **No additional centrifugation step is required.**
5. Do not apply Biosorb® Reiter Absorbent to Bios® positive spirochete controls. Titers, indicated on the label of these controls could vary. Our positive controls have been sufficiently pretreated in our laboratory.
6. When planning serum dilutions the dilution of patient sample by the absorption step (1:5) must be taken into account.
7. Biosorb® Reiter Absorbent is suitable for all antibody assays in spirochaete serology independent of method (IFA, Elisa, Blot etc.) or manufacturer.

If IgG/IgM, IgA separation using Biosorb® IgG Absorbent is carried out prior to absorption (of the IgM/IgA fraction) with the Reiter absorbent a smaller amount of Biosorb® Reiter Absorbent is required (for example 1 part of IgM fraction and 1 part of Biosorb® Reiter Absorbent). The overall antibody concentration in the sample is already lowered by the first absorption step. Sample dilution after both absorption steps (1:5 and 1:2) is **1:10**. Also in this case, **no additional centrifugation step is required** and the absorbed serum can be used directly in the test.

REFERENCES

1. Feldner J.: RF-Absorbens: IgM-Antikörperbestimmung ohne Rheumafaktor-Interferenz. Lab.med. 14, 1990, 283-288
2. Hunter E.F., Deacon W.E., Meyer P.F.: An Improved Test for Syphilis, the Absorption Procedure (FTA-ABS). Publ. Health Rep. 79, 1964, 410-412

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