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SCAN-BRIEF

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In This Brief...

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Scantibodies Heterophilic Blocking Reagent (HBR)

Introduction

The existence of heterophilic antibodies and their potential for causing interference in immunoassays has been known for many years. The potentially devastating effects of false positive assay results on the patient and the medical community have more recently been delineated. The increasing use of the susceptible 2-site immunometric ("sandwich") assay format has led to growing concern over the problem. For this reason, Scantibodies Laboratory has developed a unique heterophilic blocking reagent (HBR) that minimizes the occurrence of heterophilic antibody interference.

***Presenting*...Scantibodies' HBR**

A Qualification Test for the Application of HBR

1. Do you develop sandwich immunoassays?
2. Have you ever tested any samples which have caused a false positive result?
3. How is it possible to confirm that a sample is a false positive sample and not a true positive sample?
4. Is your assay subject to HAMA or heterophilic interference?
5. Have you identified the category of false positive samples that affects your assay?
6. Does your assay require a blocking reagent? If so, should the blocking reagent be a HAMA blocking reagent or a heterophilic blocking reagent?
7. Do you require a universal blocking reagent which can be added to all assays?
8. What criteria have you established to evaluate a heterophilic blocking reagent?

HeterophilicAntibody Interference—What is It?

Heterophilic antibodies are endogenous antibodies found in patients' serum/plasma which can bind to immunoglobulins of other species, including the species used to generate the antibodies used as reagents for immunoassays. These antibodies can interfere in immunoassay, causing a spurious elevation of measured value that is independent of the true analyte concentration, thus potentially misclassifying samples. Although they can affect various assay formats, their main effect is on 2-site immunometric assays.

These "sandwich" assays use at least two antibodies directed

against different epitopes of an antigen; one antibody is bound (or becomes bound) to a solid-phase, while the other is in solution and tagged with a signal moiety such as 125-I, enzyme, fluorophore, CLIA label, etc. Normally, antigen present in the sample "bridges" the two antibodies so that the amount of labeled antibody which becomes bound to the solid-phase is proportional to the antigen concentration in the sample. *(See figure 1)*.

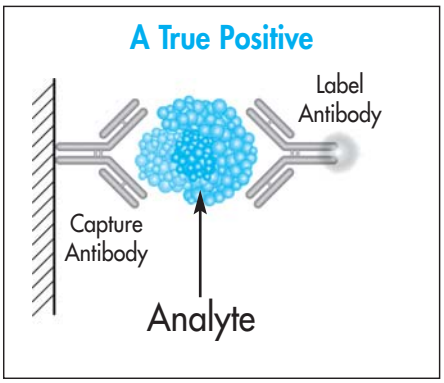

Figure 1

However, heterophilic antibodies can also "bridge" the two antibodies independently of antigen, resulting in an increase in bound labeled antibody concentration. *(see Figure 2)*.

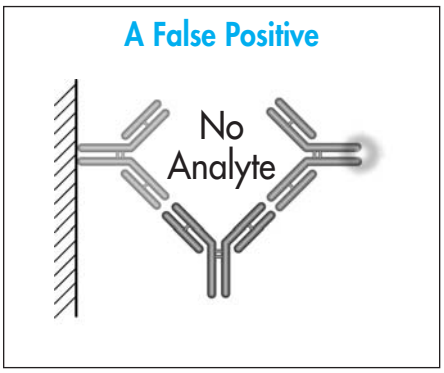

Figure 2

The Unique Aspects of HBR over Conventional Blocking Methods

Conventional, passive blocking methods use nonspecific substances (mouse IgG, mouse serum, nonspecific monoclonal antibodies, aggregated IgG, etc.) to block the binding of the human heterophilic antibody. All of these approaches rely on the affinity of the human heterophilic antibody to affect the blocking. The affinity of the human heterophilic antibody is typically in the K-value range of 10^5–10^6.

HBR accomplishes its binding by a totally different approach. The HBR is a specific binder that is directed against the human heterophilic antibody. When HBR binds to the human heterophilic antibody, the blocking is accomplished by steric hinderance. The HBR blocking is effected by the specific binder which has an affinity in the range of K = 10^9.

The specific binding action of the HBR, coupled with the thousand times higher affinity in the reaction, results in the following advantages of HBR over conventional blocking methods:

1. With HBR, less protein is required for blocking (no decrease in assay signal).
2. HBR blocks more false positives than are blocked with conventional methods.
3. HBR blocks all anti-species (anti-rabbit, anti-goat, anti-sheep, as well as anti-mouse).
4. HBR blocks all anti-subtypes of mouse monoclonals (example: anti-mouse IgG_1, anti-mouse IgG_2, etc.); whereas, the use of one monoclonal antibody such as IgG_1, will only block human antibodies to that subspecies of monoclonal antibodies.
5. The use of HBR does not have to be avoided in certain tests, as in the case in which an analyte-specific monoclonal antibody is used for blocking.

Heterophilic Interference —What is Known?

1. The interfering factor is an immunoglobulin, and both IgG and IgM heterophilic antibodies have been reported.
2. They occur at a high incidence. Depending upon patient population, up to 40% incidence has been reported, and the existence of at least 10% incidence has been documented.
3. The magnitude of the interference varies from sample to sample, and may vary within a patient over time.
4. The heterophilic antibodies are not species specific, but can bind to a variety of animal antibodies. Thus, the interference is not limited to monoclonal antibody-based assays.
5. The interference is probably mediated via the Fc region of the antibodies used in the assay.
6. Heterophilic antibodies are not a single, specific entity, but are a multi-component phenomenon. They comprise a mixed population of antibodies which can cause interference, some or all of which may be present in a particular sample.
7. EIA's appear to be more susceptible than RIA's, possibly because of the increased modification of the Fc region during conjugation.

Heterophilic Antibodies— Sources

There is a variety of possible causes for inducing heterophilic

antibodies in patients, including:

- Exposure to animals (e.g. animal technicians, veterinarians, animal handlers)
- Alternate animal contact therapy (e.g. thymic cells, sheep cells, embryonic cells)
- Exposure to animal products (e.g. food preparation)
- Special diets (e.g. cheese)
- Deliberate immunization (e.g. therapies, vaccinations, certain imaging treatments).
- Rheumatoid factors can also act as heterophilic antibodies.
- Blood transfusions.
- Autoimmune diseases
- Dialysis
- Patent medicines (OKT3)
- Maternal transfer
- Cardiac Myopathy
- G.I. Disease (E. Coli)

Heterophilic Interference—How is it Detected?

A variety of methods have been proposed:

1. Discordant values from the clinical picture or another reference assay.
2. Poor dilution performance of certain samples in an assay with normally satisfactory performance.
3. Removal of specific analyte from sample by affinity chromatography to see if signal is abolished (if not, then it is a false positive).
4. Addition of heterophilic blocking reagent to see if observed value is decreased.

What is the Difference Between Heterophilic Antibody and HAMA?

Heterophilic antibody is a generic term used to describe all human antibodies which can bind to animal antibodies and cause interference in immunoassays.

HAMA (human anti-mouse antibody) is one type of heterophilic antibody which can bind to mouse antibodies.

What is a Blocking Reagent?

A blocking reagent is a preparation which, when added to immunoassay reagents, prevents non-analyte mediated bridging of antibodies by heterophilic interference.

There are two main types of blocking reagent:

1. "HAMA" type.

These block only one specific human anti-species antibody activity (e.g. human anti-mouse), and are typically normal serum, normal IgG, monoclonal antibody not directed against the target analyte, etc.

They are "passive blocking agents" in that they are added in excess concentration so that any specific anti-species antibodies present in the sample bind to these in preference to the specific immunoreactants present in low concentrations.

Such reagents are of limited use as they only remove one component of the heterophilic interference, which is a multi-component phenomenon. It is frequently observed that addition of mouse IgG (for example) to a double-monoclonal sandwich assay will only correct a portion of the heterophilic interference.

2. True Heterophilic Blocking Reagents

These are formulations which have the ability to remove all types of heterophilic interference. Some are "active blocking reagents" in that they are directed specifically against the interfering heterophilic antibodies, allowing them to be used at lower concentrations, (thus minimizing any adverse effects on the immunoassay reaction), and with enhanced effects on blocking kinetics.

Characteristics of an Ideal Heterophilic Blocking Reagent

1. It should have the ability to correct interference from all samples, irrespective of whether it is caused by specific anti-species activity, rheumatoid factor, etc.
2. It should be effective at low concentrations so as not to interfere with the dose-response curve.
3. It should not interfere with spike and recovery of the analyte in human serum.
4. It should not interfere with linearity of dilution of a true positive patient sample.
5. It should exhibit reproducible performance with no lot-to-lot variation.
6. It should have long term stability.
7. Its cost should be such that incorporation is economically viable.
8. It should be suitable for incorporation into one of the immunoreagents to obviate the requirement for an additional reagent or a separate sample pretreatment step.

The Scantibodies Heterophilic Blocking Reagent (HBR)

Scantibodies HBR is a novel reagent which has been specifi-

cally designed to combat the problems of heterophilic antibody interference in immunoassays. It is a unique formulation of immunoglobulins targeted specifically against heterophilic antibodies to neutralize their interference. HBR is a defined reagent with a purity >95%.

Unlike most of the non-specific "passive blockers" which are available from other suppliers (which need to be added in vast excess to ensure that heterophilic antibodies will bind to them in preference to assay-specific components), Scantibodies' HBR is an "active blocker". This formulation of immunoglobulins is targeted specifically against heterophilic antibodies and is therefore able to neutralize their interference.

Performance Characteristics

1. HBR blocks false positive reactions from a panel of:
   a. human anti-mouse antibodies (HAMA)
   b. human anti-goat antibodies
   c. human anti-sheep antibodies
   d. human anti-rabbit antibodies
   e. rheumatoid factor

   The broad specificity means that Scantibodies' HBR is suitable as a "Universal Reagent" for 2-site immunometric assays using any of the commonly employed antibodies as solid-phase or labeled reagents.
2. Scantibodies' HBR, being a specific, highly purified reagent, can be used at very low concentrations (sometimes 100x or 1,000x's less than normal mouse IgG) so that the assay signal is not adversely affected.
3. Scantibodies' HBR does not interfere with spike and recovery of analyte in human serum.
4. Scantibodies' HBR does not interfere with linearity of dilution of a true positive patient sample.
5. Scantibodies' HBR is highly reproducible from lot to lot.
6. Scantibodies' HBR is stable for up to 5 years.
7. The price of incorporation of Scantibodies' HBR in an assay is less than the cost of using mouse IgG or mouse serum.
8. Scantibodies' HBR can be added directly to assay buffers, conjugates, etc.

False Positive Identification

Scantibodies has identified donors which elicited false positive results. These donors were the most difficult to block with conventional methods (mouse IgG, etc.) These samples may be used to help identify false positives and evaluate the vulnerability of an assay format against heterophilic interaction.

Please note that false positive samples are very often assay specific. Therefore, these samples may not show false positives in certain assays.

Availability

Scantibodies' HBR is supplied with a data sheet detailing the following:

i. Description: IgG
ii. Purification Method
iii. Purity by SDS-PAGE
iv. Buffer composition
v. Storage conditions
vi. Stability
vii. Appearance
viii. No preservatives

HBR is supplied in liquid form.

For pricing details or to arrange for a FREE sample of HBR, please contact Scantibodies Laboratory at the numbers given below.

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