Application Note

Controlling Interfering Antibodies in Diagnostic Assays

Introduction

It is well recognized that immunoassays, particularly in sandwich formats, are subject to interference from a range of endogenous antibodies. These include anti-animal antibodies, rheumatoid factor, other auto-antibodies and heterophilic antibodies. They can be from all classes (IgG, IgA, IgM or IgE) and can bind to antibodies from other species, as well as other cell fractions or assay components. These endogenous antibodies can create both false positive and false negative results by changing the binding ability of the target analyte or assay antibodies, giving an analytical response that is inconsistent with a patient's true clinical condition. It can be difficult to recognize and resolve these spurious results. Moreover, there are numerous reports where patients were incorrectly diagnosed and unnecessarily or incorrectly treated.[1]

The occurrence of antibody-associated interference is highly dependent on the specific assay used, with published estimates on prevalence varying widely in the literature.[2] It is acknowledged by the Federal Drug Administration (FDA) and other regulatory bodies[3] that clinical laboratories and immunoassay manufacturers must be aware of the potential for erroneous results and take adequate precautions to develop robust assays and recommend retesting procedures.

The many technical challenges to control interfering antibodies and ensure correct assay results are further complicated by such factors as antibodies persisting in a patient for months to years and varying in affinity and concentration over time, their reactivity may differ substantially from assay to assay and from manufacturer to manufacture, causing erroneous results in one assay while having no effect in another along with the question of how to resolve a true test result from a false one.

Immunoassay interference from human anti-animal and other antibodies represents a true antigen/antibody reaction. It is the specific reactivity of these antibodies with those in immunoassays that can cause false positive or false negative results. This specific interaction is distinct from non-specific binding (NSB) that generally causes high background signals. In the case of NSB, proteins including antibodies and target analytes, can stick to the solid support or other components of the assay. Inert or irrelevant proteins, detergents or substances such as dried milk or bovine serum albumin (BSA) can be used to block the reactive sites on the substrates thereby preventing NSB. In contrast, interfering antibodies need to be blocked using specific antibodies and/or normal antibodies from the same species as the assay, thus distinguishing these two types of immunoassay interferences.

Types of Interfering Antibodies

Human Anti-Animal Antibodies (HAAA)

Human anti-animal antibodies result from a true immune response that occurs after exposure to animal proteins. The most widely recognized of this group and probably the most commonly interfering is Human Anti-Mouse Antibody (HAMA). However, human antibodies are produced against a variety of species including rabbit, dog, goat, sheep, cattle, or rat. HAMAs can cause interference in sandwich assays comprised of mouse monoclonals, and other HAAAs may interfere with assays designed with polyclonal antibodies or antibodies from two or more species. There are many factors influencing the development of these HAAAs or HAMAs in an individual, exposure to the animal species through food handling, pet, domestic or laboratory animal care. They also can occur as a direct result of monoclonal imaging or antibody therapy, factors that are becoming of increasing importance as applications of these modalities are becoming more frequent. These antibodies can be high-affinity and reactive to a range of immunoglobulin domains and any classes or subclasses of antibodies. Since antibodies are highly conserved across species, an antibody to immunoglobulins of one species may cross-react with immunoglobulins from another species. Strategies for controlling HAAA typically include sera or purified antibody classes of the same species and class as contained in the immunoassay. Blocking reagents provide antibodies that occupy binding sites on the HAAA thus, preventing interference.

How Interfering Antibodies Impact Results

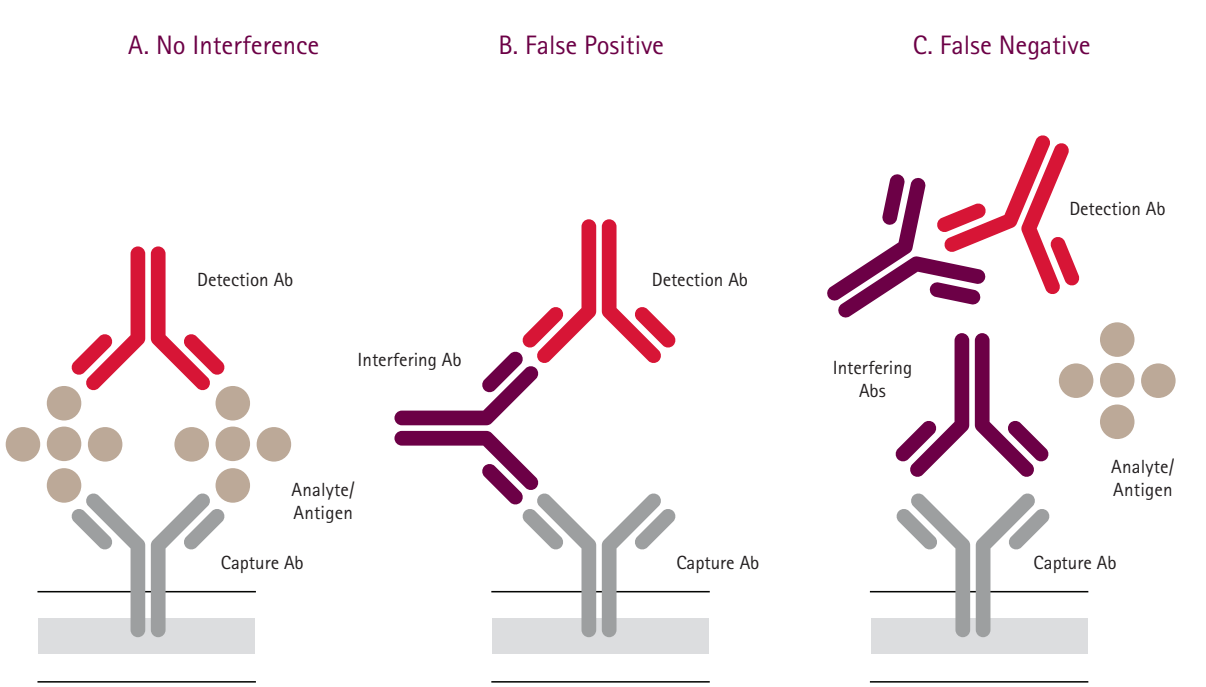

Figure 1.

A. A normal sandwich assay with no antibody interference.

B. A false positive result with the interfering antibody (Ab) forming a bridge between the detection and capture Abs instead of the analyte/antigen.

C. A false negative result where the interfering Ab binds to the detector or capture Abs, prohibiting the target analyte from binding correctly.

Rheumatoid Factor (RF)

RF is typically an IgM class antibody that recognizes the Fc region of IgG. RF can bind not only to human IgG but also immunoglobulins from other species. It is found in many rheumatoid arthritis patients, some normal populations and other patients with a variety of auto-immune diseases. An effective approach to controlling the influence of RF is by including an anti-RF antibody as a component in the blocking system.

Heterophile or Heterophilic Antibodies

This description can refer broadly to human antibodies with the ability to bind to animal antibodies, antigens or some auto-antigens but is used more accurately to describe poly-reactive antibodies against poorly defined or unknown antigens. They are found in about 40% of the population.[4] These are typically low affinity, and weakly reactive, but can still bridge the detector and capture antibodies in the absence of analyte and effectively compete for binding sites with the analyte. Interference can be minimized with the addition of normal sera or purified antibodies from the same species used in the assay. These normal antibodies saturate the binding sites of the heterophilic antibodies and prevent interference from occurring.

How Blocking Reagents Prevent False Results

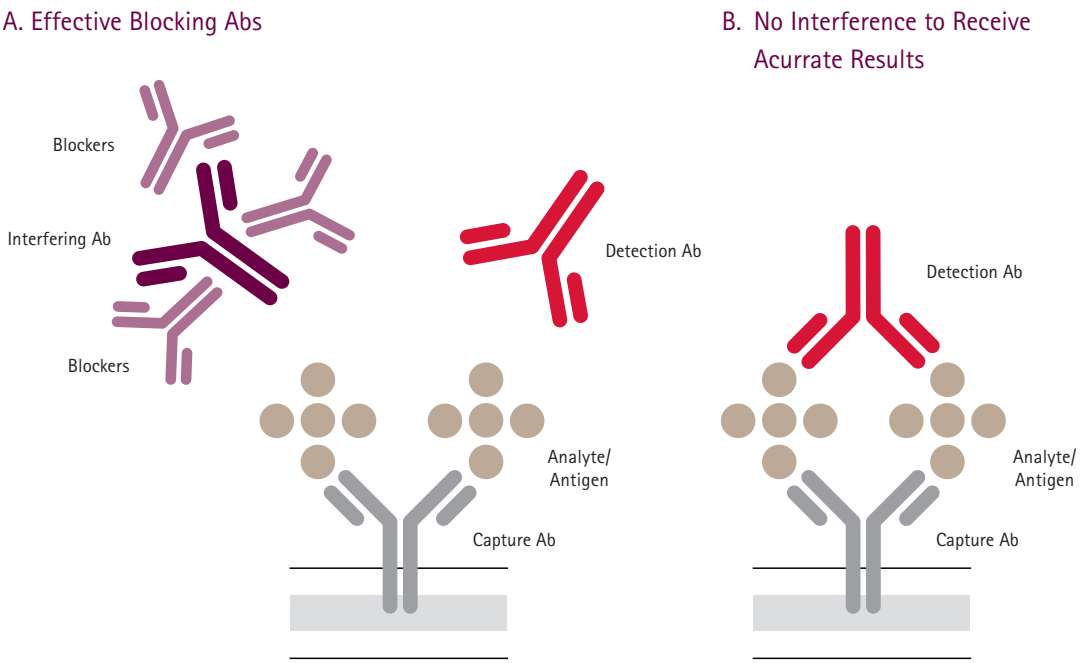

Figure 2.

A. Blocking Abs neutralize the interfering Abs.

B. With neutralized interfering Abs, an accurate result is achieved through correct binding of the analyte/antigen between the capture and detection Abs.

How Blocking Reagents work

It is important to consider how to control interfering anti- bodies during the development phase of any immunoassay. There is no universal approach to controlling interferences for all assay systems, and development of a blocking system must be considered with respect to all features of the specific assay under development including antibodies used, the target analyte and the target patient population. Blocking strategies are typically based on addition of species-specific, non-immune normal sera, polyclonal IgG, non-immune mouse monoclonal antibodies, anti-human immunoglobulins, or fragments of IgG. For some assays, passive blocking is effective and can be accomplished by the simple addition of normal serum or purified normal immunoglobulins or subunits from the same species as the detector and capture antibodies. This overwhelms the interfering antibodies and reduces the probability that they will bind to the detector or capture antibodies. A passive approach may not be sufficient in many cases due to factors such as the presence of high concentrations or high affinity interfering antibodies which can overwhelm the blocking system, and necessitate a more targeted approach. In this case, an active blocking strategy may be required using targeted antibodies that bind to and neutralize specific components of potential interferences.

Blocking formulations may need to incorporate a multi-tiered approach and include purified antibodies of the same subclass, subunits, polymers, specific antibodies or a cocktail of several components that may neutralize such factors as HAMA and RF at the same time. In some assays, low concentrations of blocking agents may be effective while other assays require much higher concentrations and/or a tiered approach to be effective.

The formulation must be considered with respect to the specific assay and developed with positive and negative patient samples and those known to contain interfering components. While manufacturers of blocking reagents, can try to provide relevant data on blocker efficiency, blocker evaluation and optimization must be done on the immunoassay it will be used in. A sandwich assay with two mouse monoclonals may have very different requirements than an assay with antibodies from two different species. The demands of a one-step assay may also be more sensitive to interfering antibodies than a two-step assay where they may be diluted or removed by washing. All of these factors must be considered to develop effective formulations for controlling antibody-associated interference and achieve an optimized system for a specific assay.

Merck Millipore's ChemiBlock™ Reagents for Interfering Antibodies

Chemicon® International Inc., now part of Merck Millipore Corporation, has worked closely for over 20 years with commercial diagnostics manufacturers to develop effective strategies for minimizing and controlling antibody-associated interferences. As both an *in vitro* diagnostic developer and manufacturer, Merck Millipore understands the needs of kit and reagent manufacturers and the benefits of effective blocking reagents. While no single formulation can control interferences in all immunoassays, Merck Millipore offers several effective broad spectrum formulas, in addition to a range of animal sera and purified immunoglobulins, so that the final formulation can be easily optimized for best kit performance. Merck Millipore's product offering reflects a continued commitment to improve the quality and accuracy of immunoassay results.

Key Features:

- We offer large lot sizes (up to Kg quantities of most blocking reagent formulations) reducing the frequency of quality control.
- Our automated equipment and processes ensure our blocking reagents perform consistently and reliably in your applications.
- Our wide array of blocking reagents includes successful formulations to control HAAA, HAMA, RF, auto-antibody and heterophilic antibody interference.
- We manufacture a broad range of immunoassays and can understand your manufacturing standards and supply specifications.
- Reagents can be configured and developed to fit your specific assay needs.

Product List

Merck Millipore's ChemiBlock reagents are well recognized for their ability to reduce interference-associated antibodies such as human anti-animal antibodies (HAAA) or human anti-mouse antibodies (HAMA) and other similar agents in patient samples. Merck Millipore provides a broad range of specialized blocking reagents to minimize the effects of interfering antibody on immunoassays. These reagents contain animal sera and purified components that actively bind and neutralize HAAA, HAMA, Heterophilic, Rheumatoid Factor and other interfering antibodies.

Ordering Information

Active Blocking Reagents

Designed to actively block interfering antibodies (HAAAs, RF, Heterophiles) allowing the binding of analyte/antigen to the capture and detection antibodies. Since active blocking reagents contain specific antibodies they may typically be used in lower concentrations than passive blocking reagents.

Description	Catalogue No.
Super ChemiBlock Heterophile Blocking Agent Super ChemiBlock is a highly effective broad spectrum blocking reagent containing highly purified mouse immunoglobulins combined with other proprietary immunoreagents. Designed for use in classic mouse sandwich assays where RF and HAAA interference are of particular concern. It may be used in combination with other reagents. It is not recommended for serological assays for human antibodies to infectious disease agents.	CBS-K
Rheumatoid Factor Removal Reagent Sheep anti-human IgG Fc fragment whole serum from which labile components have been removed. Designed for the removal of IgM class rheumatoid factor and IgG from serum and plasma prior to testing for specific IgM antibodies in ELISA or other immunoassays.	Inquire
Goat anti-Human IgG Fc Fragment (Gamma Globulin Fraction) Purified goat anti-human IgG Fc fragment adsorbed against human Fab and IgM. Similar reactivity to RFRR-K.	AB728-K
Goat anti-Human IgG Fc Fragment (Gamma Globulin Fraction). Affinity purified. Highly purified goat anti-human IgG Fc fragment adsorbed against mouse IgG. For use in mouse monoclonal based assays.	AP113-K

Passive Blocking Reagents

Passive blocking reagents are comprised of normal animal immunoglobulins and other components. These blockers deplete and reduce the concentration of interfering antibodies allowing analyte/antigen to bind to capture and detection antibodies. Passive blocking reagents may be combined and/or used with active blockers to optimize formulations for specific assays.

Description	Catalogue No.
ChemiBlock II Heterophile Blocking Agent Highly purified mouse immunoglobulins with other components for optimal multi-species effectiveness.	CBII-K
Mouse IgG (Purified) Protein A purified polyclonal Mouse IgG. Cost effective HAMA Blocker with high level purity and broad reactivity.	PP54-K
Rabbit IgG (Gamma Globulin Fraction) Purified polyclonal rabbit IgG obtained from normal rabbit serum. For immunoassays based on rabbit antibodies and other cross reactive species.	PP63-K
Rabbit IgG (Purified) Highly purified polyclonal rabbit IgG obtained from normal rabbit serum. For immunoassays based on rabbit antibodies and other cross-reactive species.	PP64-K
Goat IgG (Gamma Globulin Fraction) Purified polyclonal goat IgG obtained from normal goat serum. For immunoassays based on goat antibodies and other cross-reactive species	P41-K
Sheep IgG (Gamma Globulin Fraction, Lyophilized) Purified polyclonal sheep IgG obtained from normal sheep serum. For immunoassays based on sheep antibodies and other cross-reactive species. Lyophilized powder.	PP441-K
Bovine IgG (Gamma Globulin Fraction) Purified polyclonal bovine IgG obtained from normal bovine serum. For immunoassays based on bovine antibodies and other cross-reactive species.	PP031-K
Equine IgG (Gamma Globulin Fraction) Purified polyclonal equine IgG obtained from normal horse serum. For immunoassays based on equine antibodies and other cross-reactive species.	PP301-K

Isotype Specific Mouse IgG

Description	Catalogue No.
Purified Mouse IgG1. Affinity purified. Protein A purified mouse hybridoma IgG1 subclass antibody for use in assays employing mouse IgG1 antibodies.	PP100-K
ChemiBlock Blend Heterophile Blocking Agent. Affinity purified. A blend of Protein A purified mouse hybridoma IgG1, IgG2a, IgG3a subclasses. Broad spectrum effectiveness for mouse monoclonal sandwich assays.	PP1230-K

References

1. Tate J, Ward G. *Interferences in Immunoassay.* **Clin Biochem Rev 25:1–16** (2004)
2. Kricka, LJ. *Human anti-animal antibody interferences in immunological assays.* **Clin Chem 45:942–956** (1999)
3. Clinical and Laboratory Standards Institute. **Immunoassay interference by endogenous antibodies; proposed guideline 27(9)1–27** (2007)
4. Levinson SS, Miller JJ. *Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays.* **Clin Chim Acta 325:1–15** (2002)

To Place an Order or Receive Technical Assistance

Europe

Christine Bolsinger (Germany, Switzerland, Austria)
Sales Manager Europe
Tel: +49 171 5340735
Email: christine.bolsinger@merckgroup.com

Mark McCurley
Business Development Manager
Tel: +44 7525 732769
Email: mark.mccurley@merckgroup.com

Japan

Kazuhisa Kameyama
Business Development Manager
Tel: +81-90-7424-1147
Email: kazuhisa.kameyama@merckgroup.com

Daigo Kanai
Business Development Manager
Tel: +81-80-1260-4412
Email: daigo.kanai@merckgroup.com

China

Wei Zhu
Business Development Manager
Tel: +86-13788954409
Email: wei.zhu@merckgroup.com

Rest Of The World

Peter McGregor
Business Development Manager
Tel: +44 7775 79 44 23
Email: peter.mcgregor@merckgroup.com

Email: Diagnostic_Technical_Group@merckgroup.com

MERCK MILLIPORE

www.merckmillipore.com/diagnostics