Interferences in Immunological Assays: Causes, Detection, and Prevention

ABSTRACT

Immunological assays are fundamental tools in modern diagnostics, providing indispensable insights vital for clinical decisions and scientific inquiries. However, the reliability of these assays is at times compromised by various interferences, casting doubt on the authenticity of results. This abstract explores the origins, detection methods, and potential solutions concerning interferences in immunological assays.

Various factors, such as cross-reactivity, sample matrix effects, endogenous interferences, and test-specific nuances, can introduce deviations in immunological assessments. Understanding these interference mechanisms is crucial for devising effective countermeasures. A range of approaches, from implementing interference controls and serial dilution analyses to utilizing specific tests susceptible to interference, have been employed for detection. Additionally, advancements in technology have enhanced detection capabilities by introducing tools resistant to interference.

To address anomalies in immunological assays, a comprehensive approach is essential. Implementing rigorous quality standards during assay design and execution is paramount. Furthermore, documenting interference incidents and establishing guidelines for disclosing such occurrences promote transparency in academic and clinical settings. Collaborative efforts involving researchers, assay manufacturers, and regulatory agencies are integral to driving progress and ensuring result accuracy.

This paper emphasizes the importance of identifying, characterizing, and mitigating the diverse interferences present in immunological assays. Tackling the complexities of these interferences and embracing innovative strategies are central to refining the precision and utility of diagnostic immunology.

Keywords: (Interferences, Immunological tests, Cross reactivity, Antibody-related interferences, Binding proteins, Matrix effect)

1. INTRODUCTION

Alterations in immunological assay outcomes, known as interferences, significantly impact the accuracy and precision of diagnostic results.

Such interferences can either enhance or diminish the perceived concentration of a targeted molecule, often skewing clinical interpretations and research conclusions. Specifically, when an interference leads to an outcome that appears greater than the genuine value, it's designated as a 'positive' interference(1)(2). On the contrary, 'negative' interferences denote values that are perceived to be lower than they truly are.

The biomedical domain, inclusive of diagnostic and research sectors, holds deep concerns regarding these interferences. Essentially, they represent potential pitfalls that might distort the perceived levels of crucial biomolecules, including antigens, antibodies, and cytokines, among others. These quantifications play indispensable roles in diagnosing ailments, gauging therapeutic impacts, and navigating the realms of basic research.

Interferences, with their dualistic influence, either amplifying or diminishing results, accentuate the need for a comprehensive understanding of their origins and manifestations. It is paramount for professionals in the field to both

anticipate and address these interferences adeptly to safeguard the integrity of assay outcomes. This exposition delves into the multifaceted nature of interference sources, their mechanistic underpinnings, detection techniques, and counter-strategies. By navigating these challenges, we aspire to bolster the confidence in immunological assay results, ensuring their clinical and research relevance remain uncompromised

2. TYPES OF INTERFERENCES IN IMMUNOLOGICAL ASSAYS

Interferences in immunological assays encompass a diverse range of factors and phenomena that can affect the accuracy and reliability of results. These interferences can be categorized into several types, each with its own unique characteristics(3)(4).

One common type of interference is cross-reactivity. This occurs when antibodies, designed to target specific antigens, exhibit a lack of specificity, leading them to interact with unintended molecules. Such cross-reactivity can arise due to structural similarities between the target antigen and other molecules, resulting in a false signal.

Another type of interference involves the presence of co-reactants and the improper specificity of antibodies. Co-reactants present in the sample can interfere with the assay, leading to erroneous results. Additionally, antibodies used in the assay may not be perfectly specific, causing them to interact with molecules other than the intended target.

Interference can also occur through the presence of anti-analyte antibodies, anti-reagent antibodies, or anti-animal protein antibodies. These antibodies can cross-react with the assay components, affecting the assay's performance.
Proteins that bind specifically to hormones, such as albumin and pre-albumin, can introduce interference in hormone assays. These binding proteins may compete with the antibodies used in the assay, leading to inaccurate results.

The effect of the sample matrix, known as the matrix effect, can also be a source of interference. Conditions such as hemolysis, lipemia, icterus, and others can alter the properties of the sample, potentially affecting the assay outcome.

Lastly, the "hook effect" is an interference type that occurs when there is an excess of antigen relative to antibodies in the sample. This excess can saturate the binding sites of the antibodies, preventing accurate measurement.

Understanding these various types of interferences is crucial for assay development, optimization, and interpretation, as they can impact the reliability of immunological assays in clinical diagnostics and research. Researchers and healthcare professionals must carefully consider and address these interferences to ensure accurate and meaningful results.

2.1 CROSS-REACTIVITY

The phenomenon of interference occurs when an antibody fails to accurately distinguish its intended target antigen, resulting in a lack of specificity. This inherent limitation in antibody specificity can have notable implications, especially in competitive assays, where only a single type of antibody is employed. In such cases, interference tends to be more frequent. This interference often takes the form of what is referred to as a cross-reactant, which competes with the marked antigen (AG) for antibody binding sites(5)(6). As a consequence, interference in competitive assays typically leads to a positive interference effect, causing a distortion in the assay results(1).

In non-competitive assays, such as sandwich assays, interference can occur due to the neutralization of one of the antibodies, whether it's the capture antibody or the labeled antibody. However, this type of interference is less common in non-competitive assays(7). This is because two different epitopes are generally recognized by excess monoclonal antibodies, providing a higher degree of specificity and reducing the likelihood of interference.

Consider, for instance, the context of steroid assays where these interferences can originate from various sources. Physiologically, conditions like pregnancy can introduce interference, altering the accuracy of steroid measurements. Pathological factors, such as renal insufficiency, can also contribute to interference, posing challenges in diagnostic settings. Additionally, therapeutic interventions like corticosteroid or estrogen therapy can introduce exogenous elements that interact with the assay components, further complicating the interpretation of results. Diagnostic considerations should also be taken into account when addressing interferences, as they can vary widely in origin and impact.

In conclusion, the lack of specificity in antibody-antigen interactions is a key factor contributing to interference in immunoassays, whether they are competitive or non-competitive. Understanding the origins and implications of these interferences is crucial for researchers and healthcare professionals alike. It underscores the importance of rigorous assay design, optimization, and the need to consider potential interfering factors, whether they are of physiological, pathological, therapeutic, or diagnostic origin, in order to ensure the accuracy and reliability of immunological assay results.

2.2Antibody-Related Interferences in Assay

2.2.1 Anti analytes antibodies

Antibodies against analytes, referred to as anti-analyte antibodies, can be found in the serum of patients with autoimmune disorders, such as anti-thyroglobulin antibodies in thyroid disorders or anti-insulin antibodies in diabetes. These autoantibodies arise as a result of the immune system mistakenly targeting the body's own molecules, including hormones or proteins, and can interfere with diagnostic assays designed to measure these molecules accurately(8)(9). Furthermore, in some cases, anti-analyte antibodies can develop in response to specific treatments, such as the presence of anti-insulin antibodies in patients receiving insulin therapy. Additionally, there are instances where antibodies against certain analytes are present without a known etiology, with one common example being macro-prolactin, a complex formed between antibodies and prolactin. Depending on the specific assay used, these interferences caused by anti-analyte antibodies can lead to results that are either overestimated or underestimated. This underscores the importance of recognizing and addressing such interferences in clinical diagnostics, as they can impact the accuracy of test results and, consequently, patient care and treatment decisions.

2.2.2Heterophilic antibodies

The serum of certain individuals may contain heterophilic antibodies, which are antibodies directed against animal immunoglobulins (Ig) known as anti-idiotypes or anti-isotypes (IgG or IgM). These antibodies can develop as a result of various factors, including contact with animals, accidental exposure, medical treatments, or certain diseases(10).

One example of heterophilic antibodies is rheumatoid factor (RF), an autoantibody that targets the Fc portion of IgG antibodies. RF is commonly found in patients with rheumatoid arthritis and in approximately 5% of healthy individuals. Its presence in serum can lead to falsely elevated test results(11)(12)(13). Heterophilic antibodies like RF can introduce significant challenges in diagnostic testing, potentially leading to misinterpretation of results and clinical decisions. Therefore, their detection and appropriate management are crucial for accurate laboratory analyses and reliable healthcare outcomes.

2.2.3Anti-reagent antibodies

Interference caused by antibodies targeting specific reagents used in assays has also been documented. These antibodies, known as anti-reagent antibodies, can pose challenges in immunoassays. One example is the presence of anti-avidin or anti-streptavidin antibodies, particularly relevant when the assay methodology relies on the interaction between biotin and avidin or streptavidin(14). In such cases, these antibodies can disrupt the binding between biotin and the respective protein, potentially leading to inaccurate test results. Detecting and addressing anti-reagent antibodies is critical in the field of diagnostic testing, as they have the potential to introduce variability and errors into the assay outcomes. Developing strategies to mitigate the impact of such interference is essential to ensure the reliability and precision of immunoassays, which play a pivotal role in clinical diagnostics and biomedical research.

2.3 INTERFERENCES CAUSED BY BINDING PROTEINS

Some analytes exist in the serum in both free (active) and bound forms, with the latter typically binding to transport proteins. This phenomenon is particularly relevant for molecules like steroid hormones, thyroid hormones, and vitamin D.

The transport proteins involved often include albumin, pre-albumin, and specific hormone-binding proteins. The combination of the free and protein-bound forms constitutes the total form of the analyte.

In many cases, the measurement of the free (active) fractions, such as free T3 and free T4, is crucial for obtaining accurate results. Maintaining consistent in vitro (laboratory) conditions with those found in vivo (within the organism) is essential for precision. It's also important to consider potential sources of interference, such as free fatty acids, which can displace T4 from its binding protein(15)(16).

To measure these analytes accurately, indirect methods can be employed to isolate the free form through processes like equilibrium dialysis or ultrafiltration. These methods are known for their precision and are not affected by anomalies in transport proteins, whether quantitative or qualitative. They are also unaffected by the presence of autoantibodies in the serum or by abnormal forms of albumin with increased affinity for T4, such as familial dysalbuminemic hyperthyroxinemia.

When analyzing total forms, it is necessary to completely displace the bound form and prevent the tracer from binding to the binding proteins. This can be achieved through solvent extraction, denaturation of the transport protein, or the addition of a competitor that displaces the analyte without being recognized by the assay's antibodies(17). These considerations are critical for obtaining accurate and reliable measurements of analytes present in both free and bound forms in serum.

2.4 MATRIX EFFECT

The majority of immunoassays are conducted on serum, and these tests are typically minimally impacted by hemolysis, lipemia, or icterus.

Biochemical Interferences Significant hemolysis, characterized by the release of proteases, can have pronounced effects on analytes sensitive to proteolysis, such as insulin, ACTH, PTH, glucagon, or calcitonin, leading to an underestimation of results(18). Hemolysis, the breakdown of red blood cells and release of their contents into the serum, can disrupt the integrity of these analytes, thus highlighting the importance of maintaining sample quality for accurate immunoassay results.

2.4.1Immunological Interferences

Excessive lipid presence can interfere with and alter the affinity of certain antibodies in assays that rely on antigen-antibody interactions, consequently leading to inaccurate results. One such example is the measurement of free thyroxine.

When lipids are in excess in a blood sample, they can disrupt the reaction between antibodies and antigens, especially in immunoassays. In the measurement of free thyroxine, for instance, an excessive amount of lipids can alter the binding between the specific antibody and thyroxine, resulting in erroneous results. This immunological interference underscores the importance of carefully preparing serum samples to ensure the reliability of assays and to avoid biases stemming from external factors such as lipemia. Therefore, it is crucial to follow proper sample collection and preparation procedures to minimize these interferences and obtain accurate laboratory data

2.4.2Optical Interferences

Many assays rely on a colorimetric principle: measuring a specific color at a defined wavelength corresponding to the parameter being assayed. Any alteration in the color of plasma or serum, such as that caused by hemolysis, lipemia, or icterus, can potentially interfere with these measurements.

Colorimetry, a widely used analytical technique, is based on the absorption of light by a particular substance, resulting in a distinct color. In clinical diagnostics, colorimetric assays are employed to quantify a wide range of analytes, from glucose and cholesterol to enzymes and hormones. The accuracy of these assays depends on the precise measurement of the color produced, typically at a specific wavelength, which is then correlated with the concentration of the target substance.

However, the presence of optical interferences, such as hemolysis (the breakdown of red blood cells), lipemia (excess fat in the blood), or icterus (jaundice), can significantly affect the color of the serum or plasma. This alteration can lead to inaccurate colorimetric measurements, thereby compromising the reliability of the assay results. Therefore, it is crucial to carefully assess and address potential optical interferences when conducting colorimetric assays, particularly in clinical laboratories where precise and dependable results are essential for patient care and medical decision-making.

2.4.2Other factors

The interaction between an antigen and an antibody can be influenced by various other factors beyond those previously discussed. These additional factors include pH, protein concentration, and ionic strength of the sample.

pH: The pH level of the sample can significantly impact the binding affinity between antigens and antibodies. Different antibodies may have optimal binding capabilities within specific pH ranges, and deviations from these ranges can alter the binding kinetics. Researchers and assay developers must carefully consider and control the pH conditions to ensure accurate and reproducible results.

Protein Concentration: The concentration of proteins in a sample, particularly those unrelated to the target antigen, can affect the availability of binding sites and, consequently, the assay's sensitivity and specificity. High concentrations of non-specific proteins can lead to increased background noise and reduced assay precision. Proper sample preparation techniques, including protein removal or dilution, are essential to mitigate these interferences.

Ionic Strength: The ionic strength, determined by the concentration of ions in the sample, can influence the electrostatic interactions between antigens and antibodies. Changes in ionic strength can affect the stability of antibody-antigen complexes. Researchers often use buffer solutions to control and maintain the ionic strength within an optimal range for the assay.

Considering these factors is vital when designing, optimizing, and performing immunoassays to ensure reliable and meaningful results. By carefully managing pH, protein concentration, and ionic strength, researchers can minimize potential interferences and enhance the accuracy and reproducibility of immunological measurements."

2.5 THE HOOK EFFECT

The 'hook effect,' a phenomenon observed in certain immunoassays, occurs when there is an excessive amount of the target antigen present in the sample(1). This excess antigen saturates the binding sites of both the capture and detection antibodies, preventing the formation of a stable three-dimensional network in the assay.

In typical immunoassays, the concentration of antigens is expected to fall within a linear range, allowing for a proportional response in the test signal. However, when the antigen concentration exceeds this linear range, the 'hook effect' comes into play. In this situation, the signal produced by the assay plateaus or decreases as the excess antigen interferes with the binding of antibodies, particularly in sandwich-type assays.

This interference can lead to a significant underestimation of results because the assay no longer accurately reflects the actual concentration of the analyte in the sample. Researchers and clinical laboratory professionals must be aware of the 'hook effect' and take measures to address it when designing and performing immunoassays. Strategies for mitigating this effect may include sample dilution or modifying the assay procedure to accommodate high antigen concentrations. Properly managing the 'hook effect' is crucial for obtaining accurate and reliable results, particularly in clinical diagnostics and research applications.

3. IMPLICATION OF INTERFERENCES

Medical Consequences for the Patient: An erroneously abnormal immunoassay result, whether it is falsely elevated or lowered, may suggest a pathological condition and can have regrettable consequences for the patient. Similarly, a falsely normal result can be detrimental to the proper management of a medical condition(19).

Unjustified Therapeutic Decisions: Initiating, adjusting, or discontinuing drug therapy; surgical procedures; initiating or excluding a medication protocol (such as renal transplantation, chemotherapy, etc.) can all be influenced by these erroneous results.

Consequences in Clinical Research: As insidious as they may be, interferences in immunoassays often eventually come to light. In some cases, they have only been recognized after the results they influenced were already published."

Interferences in immunoassays can have far-reaching implications, affecting both individual patient care and the reliability of scientific research. Falsely abnormal results can lead to unnecessary medical interventions or the omission of necessary treatments, potentially impacting patient health. In clinical research, the discovery of interferences, although often subtle, is essential to maintain the integrity and validity of research findings. Researchers must remain vigilant in identifying and addressing these interferences to ensure the accuracy of both diagnostic tests and scientific studies(20)(21).

4. INTERFERENCE DETECTION AND PREVENTION

Biologists play a critical role in ensuring the accuracy of laboratory test results, particularly when there is a discrepancy between expected clinical outcomes and laboratory findings. In such cases, several strategies can be employed to detect and mitigate potential interferences(22).

One approach is to repeat tests using different sample dilutions. If the results vary depending on the dilution level, it may suggest the presence of interference. Additionally, employing different assay methods can be a valuable tool. If different results are obtained using various methods for the same sample, this can signal potential interference(23)(24)(25).

Maintaining effective communication between biologists and clinicians is equally essential. Clinicians must provide relevant clinical information to aid in the interpretation of results. Biologists, in turn, have a responsibility to educate clinicians about the limitations of immunoassays and the risks associated with obtaining false results. Collaboration between these two professional groups is crucial, especially when reported results do not align with the clinical context.

Furthermore, manufacturers have a role to play in ensuring the quality of reagents used in laboratory tests. Rushing new immunoassays to market without thorough evaluation of their sensitivity to heterophilic antibodies on a sufficient sample size can lead to issues. Continual improvement and diligence in assessing and addressing potential interferences in assay development are essential(26).

5. CONCLUSION

In the pursuit of accurate and reliable laboratory results, the vigilance of biologists stands as a crucial safeguard. When discrepancies arise between expected clinical outcomes and laboratory findings, biologists employ various strategies to

detect and prevent potential interferences. The use of different sample dilutions and assay methods aids in uncovering anomalies, ensuring that results truly reflect the underlying biological reality.

Equally vital is the seamless communication between biologists and clinicians. Clinicians' provision of pertinent clinical information facilitates a more precise interpretation of results. Meanwhile, biologists must educate clinicians about the limitations of immunoassays and the risks associated with obtaining false results. This collaborative effort ensures that laboratory findings align harmoniously with the clinical context, thereby enhancing patient care.

Manufacturers, too, bear responsibility in this quest for accuracy. The rushed introduction of new immunoassays without rigorous sensitivity testing can introduce unwanted complications. To maintain the integrity of laboratory tests, manufacturers must continuously strive for improvement and diligently assess potential interferences.

In conclusion, the triad of biologists' vigilance, effective communication among healthcare professionals, and the commitment of manufacturers is paramount to ensuring the accurate and reliable interpretation of laboratory results. The collective effort of these stakeholders not only safeguards patient well-being but also upholds the integrity of scientific research, marking a resounding commitment to the pursuit of knowledge and healthcare excellence.

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