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MOST COMMON INTERFERENCES IN IMMUNOASSAYS

MIHAELA GAĆE, ZVJEZDANA ŠPACIR PRSKALO, SANJA DOBRIJEVIĆ and LJILJANA MAYER

Department of Medical Biochemistry in Oncology, University Hospital for Tumors,
University Hospital Center Sestre milosrdnice, Zagreb, Croatia

Summary

The development of technology has enabled fast and easy determination of tumor markers and hormones at all levels of medical and biochemical diagnostics. Markers and hormones are most commonly determined by immunoassays and methods of molecular diagnostics. Immunoassays are based on the specific antibody-antigen reaction. Numerous factors during analysis may affect the measurement of tumor markers and hormones in patient samples (1). Although immunoassays are sensitive and reproducible they can miss the specificity and accuracy. Immunoassays are influenced by various interferences and most common of them will be explained in text below.

KEY WORDS: *interferences, immunoassay, heterophilic antibodies, human anti-mouse antibodies*

NAJČEŠĆE INTERFERENCIJE KARAKTERISTIČNE ZA IMUNOKEMIJSKE METODE

Sažetak

Razvoj tehnologije je omogućio brzo i jednostavno određivanje hormona i markera. Najčešće se određuju imunokemijskim i metodama olekularne biologije. Imunokemijski testovi se temelje na specifičnoj reakciji protutijelo-antigen. Brojni faktori mogu utjecati na određivanje tumorskih markera i hormona u uzorku krvi. Iako su imunokemijske metode osjetljive i reproducibilne može im nedostajati specifičnosti i točnosti. Najčešće interferencije su opisane u tekstu.

KLJUČNE RIJEČI: *interferencije, imuno testovi, heterofilna protutijela, ljudska protumišja protutijela*

INTRODUCTION

Interference is caused by interaction with one or more steps in the immunoassay procedure and the analyte concentration or the antibody binding is influenced (2). Interference may increase (positive interference) or decrease (negative interference) the measured result and can be analyte-dependent or analyte-independent.

Analyte-dependent interferences in immunoassays are caused by interaction between components in the sample with one or more reagent antibodies. The common interferences of hemolysis, icterus, lipemia, effects of anticoagulants and simple storage are independent of the analyte concentration.

Endogenous interfering substances can occur in both healthy and pathological patient samples. Each patient has his own unique sample properties and interference results from an interaction with one or more steps in the immunoassay procedure. The measurable analyte concentration in the sample or antibody binding is altered by many factors (Table 1) (3).

Exogenous interferences are any interference caused by the introduction of external factors or conditions. Any external factors, in vivo or in vitro, which are not normally present in native,

Table 1.

NATURE OF INTERFERENCES.

1. Interferences that alter the measurable analyte concentration in the sample
• Hormone binding proteins • Pre-analytical factors, e.g., anticoagulants, sample storage • Autoanalyte antibodies
2. Interferences that alter antibody binding
• Heterophile antibodies • Human anti-animal antibodies • High-dose hook effect

properly collected and stored samples can introduce interference. For example, hemolysis, lipemia, icterus, blood collection, tube additives, administration of radioactive or fluorescent compounds, drugs, herbal medicines, nutritional supplements, sample storage and transport are all exogenous interferences that can adversely affect immunoassays (4).

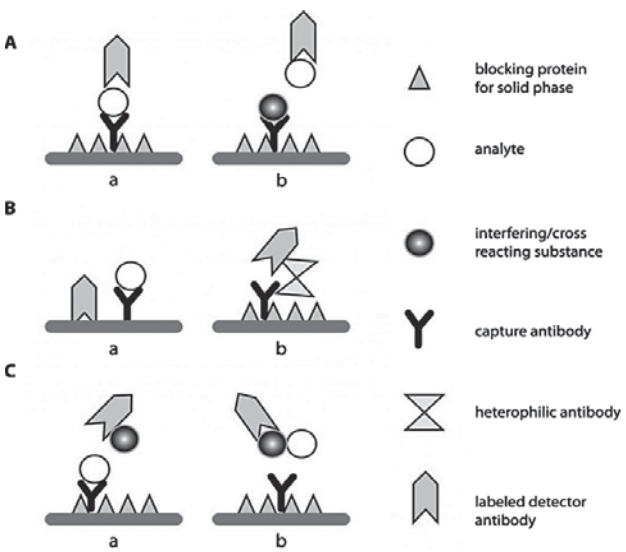

Figure 1. Different interfaces in immunometric immunoassays (5):
Aa – assay without any interference;
Ab – cross-reactivity of an interfering substance with capture antibody, resulting in false negative results
B – positive interference:
Ba – unspecific binding of labelled detector antibody to a not blocked solid phase;
Bb – "bridge" binding by heterophilic antibodies or HAMA, respectively;
C – negative interference:
Ca – change or sterical confirmation after binding or interfering protein to Fc fragment of detector antibody
Cb – masking of the epitope on analyte surface by a protein of the sample

A choice of different interference effects which may appear in immunoassays are schematically shown in Figure1. Different interference effect can yield false positive or in some case false negative results

One of the most common interference in immunoassays is a cross-reaction. Cross-reaction is a problem in diagnostic immunoassays where endogenous molecules with a similar structure to the measured analyte exist of where metabolites of the analyte have common cross-reacting epitopes, and where there is administration of structurally similar medications (7).

Pre-analytical factors

Blood collection tubes are not inert containers. Silicone oils present in some blood collection devices or tubes can physically interfere with antigen-antibody binding.

For the **sample type**, according to the NACB (National Academy of Clinical Biochemistry) recommendations of quality requirements in the preanalytical phase (9), serum or plasma are usually equally appropriate for immunochemistry analysis.

Hemolysis and icterus do not affect immunoassays as other analytes measured by spectral or chemical means. Samples with any sign of hemolysis are not acceptable for immunoassays of relatively labile analytes due to the release of proteolytic enzymes from erythrocytes that degrade these analytes (calcitonin, parathyroid hormone, gastrin). **Lipemia** can interfere in some immunoassays may produce erroneous results in some assays by interfering with antigen binding, even when antibodies are linked to a solid support (6).

Stability of the tumor markers are generally stable, but serum or plasma should be separated from the clot and stored at 4° C (short-term) or -30° C (long-term) as soon as possible.

Carryover due to inadequate washing or failure to detect a sample clot can result in over- or under-estimation of values, but with fully automated process carryover factor is reduced to minimum.

Hormone binding globulins such as albumin (because of its large concentration), sex hormone binding globulin (SHBG), thyroid binding globulin (TBG) and cortisol binding globulin (CBG) can alter the measurable analyte concentration in the

sample either by removal or blocking of the analyte (6).

Autoantibodies have been described that can cause interference for a number of analytes including thyroid hormones in both free and total forms (10), thyroglobulin (11). Positive or negative influence may occur, depending on whether the autoantibody-analyte complex partitions into the free or the bound analyte fraction. Interference from autoantibodies can occur in both immunoassay formats (6).

Antibody interference

There are two types of endogenous antibodies in patient´s specimen, heterophilic antibodies and human anti-animal antibodies.

Heterophilic antibodies are antibodies produced against poorly defined antigens. They are multi-specific antibodies of the early immune response and generally show low affinity and weak binding (8). Human anti-animal antibodies (HAAA) are high-affinity, specific polyclonal antibodies generated after contact with animal immunoglobulin (12).

The most common anti-animal antibodies are human anti-mouse antibodies (HAMA). Mouse monoclonal antibodies are applying mostly for a therapy and diagnostic purposes. HAMA can interfere with mouse monoclonal antibodies that are the main component of the reagent.

Heterophilic antibodies interfere with immunoassays and can bind to the conjugate, enzyme, or other parts of the detection system in reagent-limited assays. The same heterophile may react differently for different antibody combinations hence giving rise to a falsely elevated result in one assay but a lower result in another assay. Reagent manufactures routinely add blocking agent to their assay formulations in order to reduce interference.

High-dose hook effect

The hook effect is caused by excessively high concentrations of analyte simultaneously saturating both capture and detector antibodies (Figure 2).

In immunoassays with very large analyte concentration ranges antigen-antibody reactions can go into antigen excess and result in falsely decreased results and potential misdiagnosis (6). To avoid high/dose hook effect the quantity of the reagent antibodies must be increased and/or reduce the amount of sample required for analysis. Design assay should ensure that the concentrations of both capture and detector antibodies are sufficiently high to manage with levels of analytes over the entire pathological range.

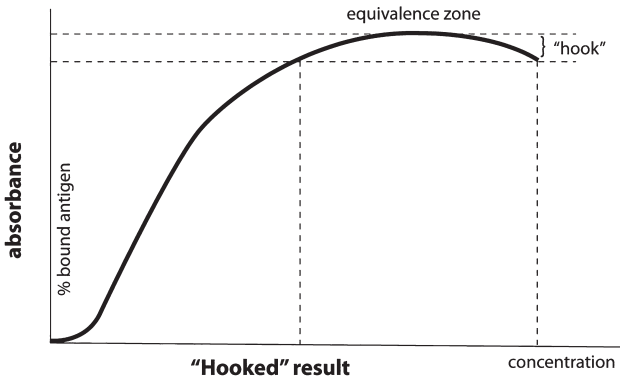

Figure 2.The hook effect - An excessive amount of analyte overwhelms the binding capacity of the capture antibody. This results in an inappropriately low signal that causes erroneous low or normal result ("hooked" result) for a patient with an excessively elevated serum analyte concentration (5).

CONCLUSION

Interference in immunoassays is a serious problem which can have important clinical consequences and may lead to unnecessary clinical investigation as well as inappropriate treatment with potentially unfavorable outcome for the patient. There is no single procedure that can rule out all interferences. It is important to recognize the potential for interference in immunoassay and to put procedures in place to identify them wherever possible. Process need to be in place in order to make both laboratories and physicians aware of the potential for immunoassay interference, which can lead to clinical misinterpretation. In table 2. are shown some of the most common interferences (11–14).

Most important is a consideration of the clinical picture. If there is any suspicion of discordance between the clinical and the laboratory data an attempt should be made to reconcile the difference. Constant dialogue is required between physician and laboratory about unexpected immunoassay results. Physicians should be encouraged to communicate specifically with the laboratory

Table 2.

MOST COMMON INTERFERENCES AND OTHER SOURCE OF VARIATION IN IMMUNOASSAYS

Analyte	Possible interference and other sources of variation		
	Increased result value	Decreased result value	Increased or decreased value
α-1-fetoprotein AFP	Benign disease (alcohol hepatitis, cholestasis), pregnancy).	Long-term sample preservation.	HAMA and heterophile antibodies.
Carcinoembryonic antigen CEA	Benign disease (chronic hepatitis, renal disease, gastrointestinal diseases), smoking, Li and Na-heparin plasma.	Long-term sample preservation.	HAMA and heterophile antibodies.
Carbohydrate Antigen 15-3 CA 15-3	Benign disease (chronic hepatitis, autoimmune disease, gynecologic diseases), other cancer diseases.	High-dose hook effect.	HAMA and heterophile antibodies.
Carbohydrate Antigen 125 CA 125	Benign disease (endometriosis, ovarium cysts, liver diseases, pregnancy, hart failure), other cancer diseases, menstruation period.	Long-term sample preservation.	HAMA and heterophile antibodies.
Carbohydrate Antigen19-9 CA 19-9	Menstruation period, pregnancy, contamination of sample with secretion (saliva, etc.), benign disease (chronic hepatitis, cholestasis, gynecologic diseases).	Patients with blood group Levis(a) and Levis(b).	HAMA and heterophile antibodies.
Human epididymis protein 4 HE4	Menopause, renal disease	Pregnancy, High BMI	
Total specific prostate antigen tPSA	Hormonal therapy, prostate manipulation and ultrasound examination, ejaculation, other cancer disease (breast cancer, salivary gland neoplasms).	Prolonged time to sample spin, thermal sample manipulation.	HAMA and heterophile antibodies.
Human chorionic gonadotropin total hCG	Heterophile antibodies, non-specific protein binders, Renal disease, menopause, cannabis consummation.	Hook effect, thermal sample manipulation.	
Thyroid Stimulating Hormone TSH	AntiTPO, HAMA and auto antibodies Pregnancy, Cardio vascular risk		
Thyroxine T4 and free form fT4	T4 autoantibodies, HAMA Hashimoto's thyroiditis, Graves' disease		Non-esterified fatty acids, thyroid binding globulin (TBG)
Triiodothyronine T3 and free form fT3	T3 autoantibodies. HAMA Hashimoto's thyroiditis, Graves' disease		Non-esterified fatty acids, thyroid binding globulin (TBG)

about discordance between results and clinical findings.

The process should also include on-going education, review of patient results in the clinical setting, protocols for the testing of suspected interference, and notification of interferences both to the physician and to the diagnostic manufacturer.

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Author's address: Mihaela Gaće, Department of Medical Biochemistry in Oncology, University Hospital for Tumors, University Hospital Center Sestre milosrdnice, Ilica 197, 10000 Zagreb, Croatia. e-mail: mihaelagace@yahoo.com