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Title: The investigation of interferences in immunoassay

Running title: Immunoassay interference investigations

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Abstract

Immunoassay procedures have a wide application in clinical medicine and as such are used throughout clinical biochemistry laboratories both for urgent and routine testing. Clinicians and laboratory personnel are often presented with immunoassay results which are inconsistent with clinical findings. Without a high index of suspicion interferences will often not be suspected. Artifactual results can be due to a range of interferences in immunoassays which can include cross reacting substances, heterophile antibodies, autoantibodies and the high dose hook effect. Further, pre-analytical aspects and certain disease states can influence the potential for interference in immunoassays. Practical solutions for investigation of artifactual results in the setting of the routine clinical laboratory are provided.

Key words: immunoassay interference; heterophile; high-dose hook; artifactual results

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The investigation of interferences in immunoassay

Introduction

Most clinicians accept laboratory results at face value and do not query the validity of that result. For this reason the laboratory has a particular responsibility to ensure the validity of the results they release.

Immunoassay is an important part of the diagnostic pathology laboratory, and because of the relatively low concentrations of analyte being measured and because of the complexities of the antigen-antibody interaction, this technique is relatively susceptible to interferences.

In some areas, possible interferences may be highly problematic and highly visible. A good example is with the use of troponin in assessing the person with possible acute coronary syndrome. The presence of troponin is a core part of the diagnosis of myocardial infarction and results are frequently required urgently. We have had patients present with a relatively constant concentration of troponin regardless of whether they are experiencing chest pain, suggesting that the apparent troponin result is artifactual. With such patients it is of considerable importance to sort out what is happening – is an interferent present or is the troponin result real?

However, with many assays there may never be reason to suspect the presence of an interferent as the results may be open to interpretations that can handle a variety of results. An example of this is serology looking at possible infections, where either a negative or a positive result can be rationalised on clinical grounds.

The purpose of this review is not so much to identify the mechanisms of immunoassay interferences – there have been many excellent reviews published on this topic [1- 8] – but rather to discuss ways of becoming aware of possible interferences and how to investigate them. We have deliberately concentrated on relatively simple procedures that will be available in non-specialist laboratories.

The nature of interferences

An interference is defined as the effect of a substance present in the sample that alters the correct value of the result [9]. There are many possible reasons for false results to be obtained during an immunoassay procedure and these are considered briefly below.

Interferences in immunoassay fall in to 2 broad categories – analyte-independent and analyte-dependent, as shown in Table 1. Analyte-independent problems are considered below in the section on the investigation of possible interferences. A summary of analyte-dependent interferences is presented below.

Analyte-dependent interferences

Cross-reacting substances

One of the beauties of immunoassay is the unique specificity of the antibody for the analyte. One of the problems of immunoassay is that where structural differences are very small, e.g. cortisol and prednisolone, there may be substantial cross-reactivity. To demonstrate this point, in Table 2 we have assembled data on cross-reactivity for cortisol assays from different manufacturers in 2017. It is apparent that some important potential interferences such as prednisolone are detected to a very variable extent between different assays and further that assays from the same manufacturer may show marked changes in cross-reactivity over time. For example the Roche cortisol assay had 171% cross-reactivity with prednisolone in 2014 but an assay with new antibodies in 2017 had only 8%

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cross-reactivity. While Roche did not comment on cross-reactivity of 5α-tetrahydrocortisol in 2017, in 2014 they acknowledged that it had a very high cross-reactivity in cortisol assays (165%). While 5α-tetrahydrocortisol is predominantly a urinary metabolite (as a glucuronide), in persons with renal failure it might be anticipated to be in relatively high concentrations in plasma and discordant results have been observed in cortisol assays in patients with renal failure [10, 11]. Manufacturers are constantly changing their assays and it is essential to be aware of these potential interferences.

Another example of significant cross-reactivity is with the immunosuppressant compound cyclosporine A. This compound is metabolised to a large number of metabolites with varying biological activity and varying cross-reactivity to the antibodies used in the assay, and for this reason some specialist centres use HPLC or LC-MS to assay the parent compound [12].

Heterophile antibodies

Heterophile antibodies are naturally occurring, poly-specific, usually low affinity antibodies which after antigen exposure are replaced by high affinity antibodies [3]. Because of their relatively low affinity they usually interfere in assays to a lesser extent than human anti-animal antibodies (see below). Diagnostic companies now use a number of approaches to eliminate or minimise interference which include: addition of trace amounts of animal serum of the same animal species as used to raise the antibodies in the assay to assay reagents; addition of non-specific animal immunoglobulin to reagents ; addition of heat-aggregated non-specific murine monoclonal antibody MAK33 to reagents; the use of F(ab')2 fragments for the solid phase; and this is usually sufficient to block the interference by heterophile antibodies [7].

Human anti-animal antibodies (HAAA)

In contrast to heterophile antibodies, HAAA are high affinity antibodies directed against specific animal immunoglobulins. Exposure to animals which are used for the preparation of the antibodies used in immunoassays is the trigger. Mice are of particular importance as murine antibodies are the most widely used in commercial immunoassay, and mice are ubiquitous in the environment.

Figure 1 shows a potential mechanism of interference by antibodies in the typical 2-site assays used for protein analysis in the immunoassay laboratory.

Autoantibodies

Autoantibodies are endogenous antibodies directed against the body's own components. Well known examples are macro-prolactin (macro-PRL) which is a complex of IgG and PRL [13], and anti-thyroid peroxidise (anti-TPO) commonly found in autoimmune thyroiditis [14]. Autoantibodies have been described to many analytes including enzymes [15], TSH [16], thyroglobulin [17], insulin [18], thyroid hormones [19] and testosterone [20, 21], to name a few examples.

Rheumatoid Factor (RF) is an autoantibody directed against the Fc portion of an individual's IgG. It is common with up to 25% of older individuals having RF present in their blood [22]. RF has been identified as an interferent in several assays including cTnI [23] and thyroid hormones [24]

Prolactin (PRL) exists primarily as a 23kD monomer, but may exist both as dimers and bound to an immunoglobulin, usually IgG. The immunoglobulin-bound form is known as macro-prolactin. Macro-PRL is considered biologically inert, probably because its large molecular size prevents it from crossing capillary walls to exert its biological effects [25]. Thus macro-prolactinemia is effectively a benign condition.

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However, all immunoassays for PRL recognize macro-PRL to some extent, and this can be problematic as women with benign macro-prolactinemia may be confused with women with true hyper-prolactinemia and be inappropriately treated. In some studies, macro-PRL may account for around 25% of cases of hyper-prolactinemia [13, 26]. Thus finding some way of identifying macro-PRL from true hyper-prolactinemia was important. Lindstedt identified PEG precipitation as a possible means of rapidly and reliably separating monomeric- and macro-PRL in the diagnostic laboratory [27]. The use of PEG precipitation for identifying macro-PRL and other analytes is considered in the investigation of interferences below.

IgG autoantibodies against cTnI have been known for many years. Interestingly one of the first reports was of a negative interference [28]. It appears that the autoantibodies are directed against cTnI but only when it is in the circulating cTnI-cTnC-cTnT (I-T-C) complex [29]. The prevalence of circulating antibodies to cTnI have been described as between 5% [30] and 12.5% [31]. This is much higher than seen in routine clinical practice and implies we are missing a substantial number of cases. The presence of cTnI appears to be associated with myocardial inflammation [32], so the presence of antibodies is a logical defence mechanism and perhaps explains why the prevalence of troponin auto-antibodies is so high.

High dose hook effect

The high dose hook effect can occur when an analyte being measured is present in very high concentrations. Whilst it can occur with 2-site immunometric assays, it is particularly a problem with nephelometric assays.

In 2-site assays the excess antigen may prevent “sandwich” formation with capture and signal antibodies both being bound separately to the analyte, and signal antibody being washed away and a resultant apparent low concentration of analyte being recorded [2, 33].

Nephelometric assays rely on the formation of Heidelberger-Kendall complexes which are large and scatter light [34]. In the presence of antigen excess these large complexes do not form and the concentration of the analyte is under-estimated as shown in Figure 2.

Any analyte which can be present in very high concentration, in particular tumour markers including prolactin and hCG may be prone to the high dose hook effect.

Clues as to the presence of a possible interference

There are 2 usual ways in which the possibility of an interferent may be suspected.

1. ***Discordant results***

An example of a result that would raise suspicion of an interference would be if thyroid function tests were requested and the usual relationship of thyroid hormone (TT4 or fT4) and TSH was disturbed. Because of the sensitive negative feedback relationship between thyroid hormones and the secretion of pituitary TSH, these should exist in either high/low, euthyroid/euthyroid or low/high combinations. While for example a high/high relationship may indicate thyroid hormone resistance, an interference is far more likely by orders of magnitude. A low/low combination may reflect a sick euthyroid state but again an interference should be considered.

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Some substances measured by immunoassay maintain a fairly constant concentration eg TSH. Others however, such as troponin are expected to show a distinct rise and fall. The presence of a constant moderately increased troponin does not fit an expected pattern and an interference may be suspected. Similarly, a failure of troponin to rise when an acute coronary syndrome is highly likely, may indicate a negative interference.

Total β-hCG is used to identify pregnancy and to identify malignancies in both females and males. The presence of detectable total β-hCG in a situation where pregnancy is unlikely needs to be viewed very cautiously as life-changing treatments may result. Any suggestion of discordance with the clinical setting should lead to a vigorous search for a possible interference.

2. Clinical interaction

If the laboratory is lucky, it will have good relations with clinical staff and they will be aware of the possibility of interferences as they view the results they receive. Communication between clinicians and the laboratory is a most important way of minimizing the release of bad results.

The investigation of possible interferences

A proposed sequence of investigations of possible interferences is shown in Table 3 and expanded below.

1 High index of suspicion

There have been a number of published studies looking objectively at the incidence of interferences in immunoassay, and these have shown that there is an underlying incidence of 3-4% of significant interferences [35, 36].
The problem is largely managed by diagnostic companies by the addition of animal serum or immunoglobulins from the same animal species as the antibodies used in the assay [1]. However, these additions will not handle large amounts of heterophile antibody or HAAA. The prevalence of false results with these modified assays may still be as high as 0.53% [37]. While 2-site immunometric assays appear to be more prone to interferences than assays with other architecture, any form of immunoassay may be affected. Some areas of the clinical laboratory show much greater awareness of the problem of interferences. For example looking at PubMed in December 2016 for “immunoassay interferences”, of the most recent 200 articles only 6 related specifically to microbiology or serology with the majority relating to drugs and hormones. The implication is that in some areas of the lab many false results are being released because the index of suspicion is low.

2 ***Exclude pre-analytical* problems**

There are many potential pre-analytical causes of false results in immunoassay as shown in Table 1, and these should be methodically excluded.

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While for some assays, only specific sample types are recommended – for example EDTA for ACTH estimation [38]– for most immunoassay analytes, a variety of matrices are acceptable. For convenience and to speed turn-around times, we often use Li-heparin samples on our main-frame analyzers. Heparin is a relatively poor anticoagulant and inadequate initial centrifugation, or extended time before sampling, can result in the formation of micro-clots which are notorious for generating spurious results in immunoassay. Re-centrifugation of samples may be necessary.

Significant lipemia and hemolysis may be problematic, particularly if nephelometric assays are used. We always check lipemic and haemolytic indices off our main frame analyzers to ensure this is not a problem as defined for each individual analyte.

While it should not be a problem with modern instruments, the potential for carry-over should be considered [39]. Occasionally there will be extremely high concentrations of tumor markers or β-hCG and if there is inadequate washing of samples, the following sample may be contaminated and give an erroneously high result.

Some analytes in blood will be highly protein-bound eg thyroxine and cortisol. Reliable measurement of total T4 or cortisol is dependent upon assay conditions ensuring total separation of the small hormone from its binding protein.

Most immunoassays now use chemiluminescence as the signal in immunoassays utilising either acridinium esters or luminol derivatives and paramagnetic particles as a separation tool. Some use biotin-streptavidin as the separation tool [40] and ruthenium [41] for an electrochemiluminescent signal. Antibodies to ruthenium have been reported [42] and also biotin [43]. Further, the therapeutic use of biotin [44] has caused false results on immunoassay utilising biotin-streptavidin [45, 46].

Some disease states can cause artifactual changes in measured substances. For example during diabetic ketoacidosis, concentrations of free fatty acids rise in blood and these can cause displacement of thyroxine from its binding proteins with an artifactual increase in measured fT4.

3 Repeat analysis on another instrument from a different manufacturer

Most immunoassays nowadays use monoclonal antibodies (or affinity purified antibodies) directed against specific epitopes. Assays from different manufacturers are likely to be directed against different epitopes, potentially using antibodies originating from different animal species. If an assay for a particular analyte is run on a different instrument with a different assay design, significant discordance in results between the 2 assays may result and give the clue of an interference.

A variant to this principle can be used with cardiac troponin assays. Whilst measuring quite different chemical entities, both cardiac troponin T (cTnT) and cardiac troponin I (cTnI) rise in similar fashion following acute myocardial infarction. Our routine assay is for cTnI and when we suspect an interference we run cTnT to see if the clinical profile is similar.

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As discussed above under analyte-independent interferences, antibodies to ruthenium and therapeutic use of biotin may cause problems in assays which use these substances as part of their assay construction. In such a setting, repeating the assay on another instrument with different assay architecture is of great value.

Occasionally use of monoclonal assays (or highly specific antibodies/assays) can be a problem. Monoclonal antibodies used for a particular assay are directed against a specific epitope, whilst polyclonal antibodies between them recognise many epitopes on that antigen. It is possible to have a benign mutation which does not affect biological function but which causes loss of one or more epitopes. If the monoclonal antibody used in a particular assay is directed against this missing epitope, a false low result will be obtained. Examples of these missing epitopes include LH [47] and PTH [48]. It is important where such an interference is suspected, to use a different assay with antibodies directed against a different epitope. Best of all is to use an assay with polyclonal antibodies but these are no longer widely available.

4 Use of heterophile blocking tubes (HBTs)

It is popularly assumed that use of heterophile blocking tubes will reliably identify the presence of an interfering substance. This is not necessarily so. There have been a number of studies reporting failure of HBTs to identify proven interferences [37, 49-52]. With one of the more commonly used HBTs from Scantibodies, it is acknowledged by the company that this HBT will not reliably work where the assay uses material derived from mice [53], and murine elements are very common in commercial immunoassays. When incubation with an HBT causes a significant change in result, it is likely that an interferent is present. However, lack of change is not evidence that there is no interference.

5 Polyethylene glycol (PEG) precipitation

The use of polyethylene glycol (PEG) for separating native prolactin from its macro- form has been well described [13]. This utilizes the principle that PEG acts as a solvent sponge, causing the concentration of proteins in the water space to rise until their solubility is exceeded and they precipitate. Generally, larger proteins have lower solubility than smaller proteins, and macro-PRL is precipitated at a PEG concentration of 25% while the monomeric form remains in solution.

The general principles of PEG precipitation of macro-complexes can be applied to any smallish molecule bound to an immunoglobulin. There have been reports of macro-TSH being investigated with PEG precipitation [16, 54] and also troponin [30, 55]. However, the behaviour of any assay used in conjunction with PEG precipitation needs to be carefully validated before use.

6 Serial dilutions

Serial dilutions (1:2, 1:4, 1:8 etc) are a possible means of looking for interferences. The simple concept is that in the absence of an interferent, as a sample is progressively diluted, the measured concentration of that analyte should progressively fall in linear/parallel fashion. In the presence of an interferent, it would be anticipated that there would be non-

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linearity and non-parallelism. However, with immunoassay there are many potential confounders such as assay configuration, relative affinity of the assay and interfering antibodies, antibody valency and steric hindrance to identify a few [56].

Consideration must be given as to whether dilution studies should be performed with a zero diluent or a patient sample containing zero concentration of the analyte in question as the matrix may be more or less important in different assays with items such as immunoglobulins in patient material having an effect.

Significant deviation from linearity can be taken as supporting the presence of an interfering substance but apparent parallelism does not prove the absence of an interferent.

7 Check using another matrix

Some analytes such as hCG (and metabolites) are small enough to be readily filtered by the renal glomerulus and appear in substantial quantities in the urine in conditions such as early pregnancy. Indeed this formed the basis for pregnancy testing [57].

Immunoglobulins are large molecules and not filtered by the glomerulus, so if serum hCG appears to be inappropriately detected, then hCG analysis of a urine sample collected at the same time may determine whether the raised hCG is real or the result of an interference. Similarly, an apparently inappropriately low serum hCG can be checked with a urine sample and a high urine hCG would support the presence of a negative interference [58].

8 Dosing for suspected negative interference

Negative interference has been detected by dilution of the sample using assay diluent [59, 60]. An alternate suggested approach is as follows. If a result is suspected of being falsely low, i.e. a negative interference is suspected, then doing serial dilutions upwards can be performed using a patient sample with a high concentration of the analyte under investigation. At a 1:1 dilution an intermediate concentration should be obtained. Linearity and parallelism can be assessed with serial dilutions but the same provisos as for Serial Dilutions above hold.

All of the above techniques can be utilised in small non-specialist laboratories. A combination of these different procedures will usually identify that a positive interference is present. Where uncertainty exists, especially if a false negative interference is suspected, then referral to, or discussion with a specialist laboratory is indicated.

Specialised investigations

Extraction may be performed with organic solvents to denature possible interfering proteins and separate charged molecules (eg steroid glucuronides) from relatively uncharged molecules such as cortisol.

Potentially interfering immunoglobulins may be extracted by incubation with an immunoglobulin-binding molecule such as protein A or protein G [61].

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Gel filtration column chromatography using an agent such as Sephacryl 300 which has a separation range of approximately 10,000 – 1,500,000 daltons MW enables the molecular weight associated with the analyte to be defined [30].

Measurement of steroid hormones by LC-MS/MS is now performed routinely in reference laboratories. These procedures include sample extraction to remove proteins, and the ability of the technique to separate and identify structurally similar molecules overcomes the specificity problems of direct immunoassay [62].

Conclusions

Immunoassay is no place for optimists. Interferences can and do occur and they will not be identified unless analysts are alert and suspicious regarding the results they put out.

While tools such as heterophile blocking tubes are widely believed to be all the investigation that is needed for possible interferences, as indicated in the text above, it appears that they do not work reliably in assays using murine antibodies and these are the commonest antibodies used in immunoassay laboratories.

It is quite feasible to have a small armamentarium of secondary investigations available to investigate possible interferences as outlined above, and all immunoassay laboratories should have some or all of these set up.

When all else fails, ensure you have a contact in a sophisticated laboratory where a variety of more sophisticated procedures are available.

As a final note of caution, remember that positive interferences are frequently more obvious than negative interferences.

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References

1 Kricka LJ. Human anti-animal antibody interferences in immunological assays. Clin Chem 1999; 45: 942-56.

2 Selby C. Interference in immunoassay. Ann Clin Biochem 1999; 36: 704-21.

3 Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta 2002; 325: 1-15.

4 Klee GG. Interferences in hormone immunoassays. Clin Lab Med 2004; 24: 1-18.

5 Tate J, Ward G. Interferences in immunoassay. Clin Biochem Revs 2004; 25: 105-120.

6 Sturgeon CM, Viljoen A. Analytical error and interference in immunoassay: minimizing risk. Ann Clin Biochem 2011; 48: 418-32.

7 Bolstad N, Warren DJ, Nustad K. Heterophilic antibody interference in immunometric assays. Best Prac Res Clin Endocrinol Metab 2013; 27: 647-61.

8 Ismail AAA. Idenitfying and reducing potentially wrong immunoassay results even when plausible and “not unreasonable.” Adv Clin Chem 2016; 66: 241-94.

9 Kroll MH, Elin RJ. Interference with clinical laboratory analyses. Clin Chem 1994; 40: 1996-2005.

10 N'Gankam V, Uehlinger D, Dick B, Frey BM, Frey FJ. Increased cortisol metabolites and reduced activity of 11beta-hydroxysteroid dehydrogenase in patients on hemodialysis. Kidney Int 2002; 61:1859-66.

11 Dodd AJ, Ducroq DH, Neale SM, Wise MP, Mitchem KL, Armston A, Barth JH, El-Farhan N, Rees DA, Evans C. The effect of serum matrix and gender on cortisol measurement by commonly used immunoassays. Ann Clin Biochem 2014 ;51:379-85.

12 Andrews DJ, Cramb R. Cyclosporin: revision in monitoring guidelines and review of current analytical methods. Ann Clin Biochem 2002; 39: 424-35.

13 Fahie-Wilson MN, John R, Ellis AR. Macroprolactin; high molecular mass forms of circulating prolactin. Ann Clin Biochem 2005; 42: 175-192.

14 Sinclair D. Clinical and laboratory aspects of thyroid autoantibodies. Ann Clin Biochem 2006; 43: 173-83.

15 Remaley AT, Wilding P. Macroenzymes: biochemical characterization, clinical significance, and laboratory detection. Clin Chem 1989; 35: 2261-70.

16 Sakai H, Fukuda G, Suzuki N, Watanabe C, Odawara M. Falsely elevated thyroid-stimulating hormone (TSH) level due to macro-TSH. Endocr J 2009; 56: 435-40.

17 Spencer CA. Clinical review: clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). J Clin Endocrinol Metab 2011; 96: 3615-27.

18 Eisenbarth GS, Jackson RA, Pugliese A. Insulin autoimmunity: the rate limiting factor in pre-type 1 diabetes. J Autoimmun 1992 Apr; Suppl A: 241-6

19 Sakata S, Matsuda M, Ogawa T, Takuno H, Matsui I, Sarui H, Yasuda K. Prevalence of thyroid hormone autoantibodies in healthy subjects. Clin Endocrinol 1994; 41: 365–370.

20 Torjesen PA, Bjøro T. Antibodies against [125I] testosterone in patient's serum: a problem for the laboratory and the patient. Clin Chem. 1996;42:2047-8

21 Kuwahara A, Kamada M, Irahara M, Naka O, Yamashita T, Aono T. Autoantibody against testosterone in a woman with hypergonadotropic hypogonadism. J Clin Endocrinol Metab. 1998;83:14-6

22 Mierau R, Genth E. Diagnosis and prognosis of early rheumatoid arthritis, with special emphasis on laboratory analysis. Clin Chem Lab Med 2006; 44: 138-43.

23 Dasgupta A, Banerjee SK, Datta P. False-positive troponin I in the MEIA due to the presence of rheumatoid factors in serum. Am J Clin Pathol 1999;112:753-6.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

24 Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem 1998; 44: 440-54.

25 Andersen AN, Pedersen H, Djursing H, Andersen BN, Friesen HG. Bioactivity of prolactin in a woman with an excess of large molecular size prolactin, persistent hyperprolactinaemia and spontaneous conception. Fertil Steril 1982; 38: 625-8.

26 Gibney J, Smith TP, McKenna TJ. Clinical relevance of macroprolactin. Clin Endocrinol 2005; 62:633-43.

27 Lindstedt G. Endogenous antibodies against prolactin – a “new” cause of hyperprolactinaemia. Eur J Endocrinol 1994; 130: 429-32.

28 Bohner J, von Pape K-W, Hannes W, Stegmann T. False-negative immunoassay results for cardiac troponin I probably due to circulating troponin I autoantibodies. Clin Chem 1996; 42: 2046.

29 Vylegzhanina AV, Kogan AE, Katrukha IA, Antipova OV, Kara N, Bereznikova AV et al. Anti-cardiac troponin autoantibodies are specific to the conformational epitopes formed by cardiac TnI and TnT in the ternary troponin complex. Clin Chem 2017; 63: 343-50.

30 Warner JV, Marshall GA. High incidence of macrotroponin I with a high-sensitivity troponin I assay. Clin Chem Lab Med 2016; 54: 1821-9.

31 Adamczyk M, Brashear RJ, Mattingly PG. Circulating cardiac troponin I autoantibodies in human plasma and serum. Ann NY Acad Sci 2009; 1173: 67-74.

32 Goser S, Andrassy M, Buss SJ, Leuschner F, Volz CH, Ottl R et al. Cardiac troponin I but not troponin T induces severe autoimmune inflammation in the myocardium. Circulation 2006; 114: 1693-702.

33 Smith TP, Kavanagh L, Healy ML, McKenna TJ. Technology insight: measuring prolactin in clinical samples. Nat Clin Pract Endocrinol Metab 2007; 3:279-89.

34 Jacobs JFM, van der Molen RG, Bossuyt X, Damoiseaux J. Antigen excess in modern immunoassays: to anticipate on the unexpected. Autoimmun Revs 2015; 14: 160-7.

35 Ward G, McKinnon L, Badrick T, Hickman PE. Heterophilic antibodies remain a problem for the immunoassay laboratory. Am J Clin Pathol 1997; 108: 417-21.

36 Emerson JF, Ngo G, Emerson SS. Screening for interference in immunoassays. Clin Chem. 2003;49:1163-9.

37 Ismail AAA, Walker PL, Barth JH, Lewandowski KC, Jones R, Burr WA. Wrong biochemistry results: two case reports and observational study in 5310 patients on potentially misleading thyroid-stimulating hormone and gonadotropin immunoassay results. Clin Chem 2002; 48: 2023-9.

38 Evans MJ, Livesey JH, Ellis MJ, Yandle TG. Effect of anticoagulants and storage temperatures on stability of plasma and serum hormones. Clin Biochem 2001; 34:107-12

39 Gould MJ, Wilgen U, Pretorius CJ, Ungerer JPJ. Probing indiscretions: contamination of cardiac troponin reagent by very high patient samples causes false-positive results. Ann Clin Biochem 2012; 49: 395-8.

40 Diamandis EP, Christopoulos TK. The biotin-(strept)avidin system: principles and applications in biotechnology. Clin Chem 1991; 37:625-36.

41 Blackburn GF, Shah HP, Kenten JH, Leland J, Kamin RA, Link J, Peterman J, Powell MJ, Shah A, Talley DB, et al. Electrochemiluminescence detection for development of immunoassays and DNA probe assays for clinical diagnostics. Clin Chem 1991; 37:1534-9.

42 Gessl A, Blueml, Bieglmayer C, Marculescu R. Anti-ruthenium antibodies mimic macro-TSH in electrochemiluminescent immunoassay. Clin Chem Lab Med 2014; 52: 1589-94.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

43 Chen T, Hedman L, Mattila PS, Jartti L, Jartti T, Ruuskanen O, Söderlund-Venermo M, Hedman K. Biotin IgM antibodies in human blood: a previously unknown factor eliciting false results in biotinylation-based immunoassays. PLoS One. 2012;7:e42376. doi: 10.1371/journal.pone.0042376. Epub 2012 Aug 3

44 Sedel F, Papeix C, Bellanger A, Touitou V, Lebrun-Frenay C, Galanaud D, Gout O, Lyon-Caen O, Tourbah A. High doses of biotin in chronic progressive multiple sclerosis: a pilot study. Mult Scler Relat Disord 2015; 4:159-69.

45 Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Factitious Graves' Disease Due to Biotin Immunoassay Interference-A Case and Review of the Literature. J Clin Endocrinol Metab 2016; 101:3251-5

46 Trambas CM, Sikaris KA, Lu ZX. More on Biotin Treatment Mimicking Graves' Disease. N Engl J Med 2016; 375:1698

47 Haavisto AM, Pettersson K, Bergendahl M, Virkamaki A, Huhtaniemi I. Occurrence and biological properties of a common genetic variant of luteinizing hormone. J Clin Endocrinol Metab 1995; 80: 1257-63.

48 Odell WD, Hobbs MR, Benowitz B. An immunologically anomalous parathyroid hormone variant causing hyperparathyroidism. Clin Endocrinol 2001; 55: 417-20.

49 Plebani M, Mion M, Altinier S, Girotto MA, Baldo G, Zaninotto M. False-positive troponin I attributed to a macrocomplex. Clin Chem 2002; 48: 677-9.

50 Ellis MJ, Livesey JH. Techniques for identifying heterophile antibody interference are assay specific: study of seven analytes on two automated immunoassay analyzers. Clin Chem 2005; 51: 639-641.

51 Imperiali M, Jelmini P, Ferraro B, Keller F, della Bruna R, Balerna M, Giovanella L. Interference in thyroid-stimulating hormone determination. Eur J Clin Invest 2010; 40: 756-8.

52 Giovanella L, Imperiali M, Verburg FA, Ceriani L. Evaluation of the Brahms Kryptor thyroglobulin minirecovery test in patients with differentiated thyroid carcinoma. Clin Chem Lab Med 2013; 51: 449-53.

53 Cantor D. Reason for limitations of heterophilic blocking tube use on certain Beckman Coulter Access assays. Clin Chem 2005; 512: 1311.

54 Mills F, Jeffery J, Mackenzie P, Cranfield A, Ayling RM. An immunoglobulin G complexed form of thyroid-stimulating hormone (macro thyroid-stimulating hormone) is a cause of elevated serum thyroid-stimulating hormone concentration. Ann Clin Biochem 2013; 50: 416-20.

55 Michielsen EC, Bisschops PG, Janssen MJ. False positive troponin result caused by a true macrotroponin. Clin Chem Lab Med 2011; 49: 923-5.

56 Ismail AAA. On detecting interference from endogenous antibodies in immunoassays by doubling dilutions test. Clin Chem Lab Med 2007; 45: 851-4.

57 Chard T. Pregnancy tests: a review. Hum Reprod 1992; 7: 701-10.

58 Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. Lancet 2000; 355: 712-5.

59 McClaren M, Yen T, Price L, Ward G. Negative interference in an insulin assay. (2009) Clin Biochem Revs 2009; 30 Suppl: S13.

60 Ward G, Andrew M, Freemantle M, Deakin H, Kanowski D, Price L. Negative interference in the Kryptor free beta hCG immunoassay. Clin Biochem Revs 2011; 32 Suppl: S24.

61 Moser AC, Hage DS. Immunoaffinity chromatography: an introduction to applications and recent developments. Bioanalysis 2010; 2: 769-790

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62 Grebe SKG, Singh RJ. LC-MS/MS in the clinical laboratory – where to from here. Clin Biochem Revs 2011; 32: 5-31.

63 Emerson JF, Lai KKY. Endogenous antibody interferences in immunoassays. Lab Med 2013; 44: 69-73.

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Table 1: Interferences in immunoassay

Analyte-dependent interferences

- Cross reacting substances (lack of specificity)
    - Pharmacy
        - eg prednisolone cross-reacting in cortisol assays
    - Metabolic
        - Metyrapone therapy and 11-deoxycortisol cross-reacting in cortisol assays
    - Disease states
        - CKD – conjugates of 5alpha-tetrahydrocortisol cross reacting in serum cortisol assays in persons with renal disease
        - CKD C-terminal fragments interfering in2-site immunometric PTH assays
- Endogenous antibodies
    - Reagent antibodies (heterophile; HAAA)
    - Analyte autoantibody (macro complexes)
- Hook effect in 2-site immunometric and nephelometric assays for tumour marker markers
- Binding proteins
    - OCP and TBG or CBG
- Idiopathic
- Pre-analytical
    - Renin cryoactivation

Analyte-independent interferences

- Pre-analytical
    - Inadequate centrifugation with microclots
    - Haemolysis, lipemia, icterus
    - Carryover from very high concentration analyte eg hCG or tumour markers
- Analytical
    - Inadequate separation from binding proteins
    - Antibodies directed against labels eg ruthenium
    - Interference with signal generation by therapeutic ingestion of similar agent eg biotin
    - Disease states may cause artifactual changes in analytes eg FFA displacing T4 from binding proteins

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Table 2: Changing specificity of cortisol assays

Specificity of Cortisol Assays (serum steroids) 2014					
	Cross Reactivity (%)				
	Roche	Centaur	Immulite	DXi	Architect
Cortisol	100	100	100	100	100
Corticosterone	5.8	5.3	1.2	2.1	0.9
Cortisone	0.3	31.1	1.0	8.1	2.7
l l-deoxycortisol	4.1	23.3	1.6	17.8	1.9
17-α-OH-progesterone	1.5	1.2	0.2	5.3	0.6
Progesterone	0.4	<0.1	<0.1	0.50	<0.1
l l-deoxycorticosterone	0.7	1.8	<0.1	0.9	<0.1
Prednisolone	171	109	62	7.6	12.3
5α-tetrahydrocortisol	165	6.5		??	??
Fludrocortisone	Not tested	Not tested	??	Not tested	36.6
Dexamethasone	<0.1	0.2	<0.1	<0.1	<0.1

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Specificity of Cortisol Assays (serum steroids) 2017					
	Cross Reactivity (%)				
	Roche	Centaur	Immulite	DXi	Architect
Cortisol	100	100	100	100	100
Corticosterone	2.5	5.3	1.2	2.1	0.9
Cortisone	6.6	31.1	1.0	8.1	2.7
l l-deoxycortisol	4.9	23.3	1.6	17.8	1.9
17-α-OH-progesterone	<0.1	1.2	0.2	5.3	0.6
Progesterone	0.4	<0.1	<0.1	0.50	<0.1
l l-deoxycorticosterone	0.6	1.8	<0.1	0.9	<0.1
Prednisolone	8	109	62	23.9	12.3
5α-tetrahydrocortisol				<0.1	0.5?
Fludrocortisone	0.2				36.6
Dexamethasone	<0.1	0.2	<0.1	<0.1	<0.1

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Table 3: Proposed procedures to follow when an interference is suspected

	The investigation of possible interferences
1	A high index of suspicion
2	Exclude pre-analytical problems
3	Repeat analysis on another instrument from a different manufacturer
4	Use of heterophile blocking tubes
5	PEG precipitation
6	Serial dilutions for positive interference
7	Serial dilutions for negative interference
8	Check using a different matrix eg urine for hCG
9	Solvent extraction
10	Selective removal of immunoglobulins
11	Column chromatography
12	Tandem-mass spec

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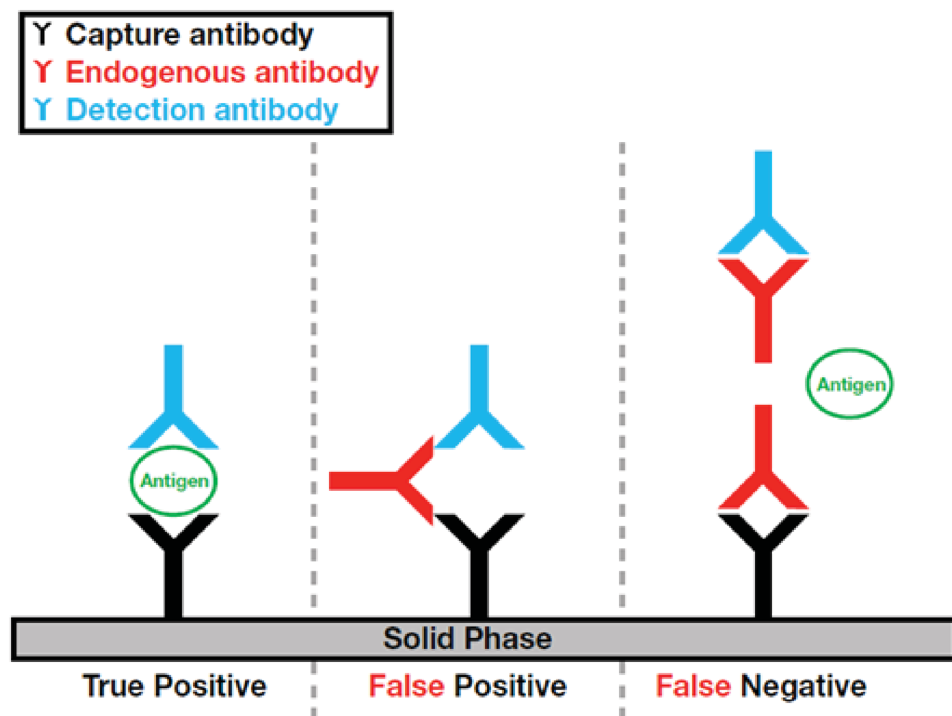

Figure 1

Schematic of how endogenous antibodies can interfere in immunoassays to produce false-positive or false-negative results.

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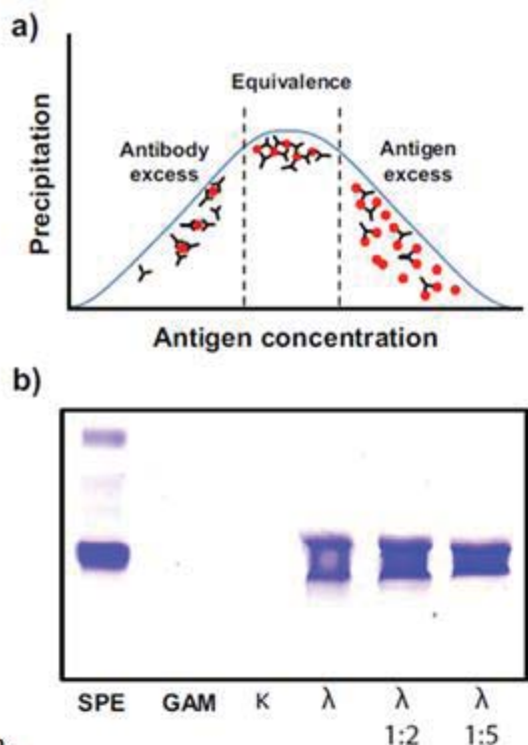

Figure-2-

Principle of antigen excess. (a) Relation between the number and size of immunoprecipitates (measured signal) and the antigen concentration (curve according to Heidelberger and Kendall [1]). (b) Antigen excess clearly visualized. The immunofixation electrophoresis of urine from a patient with large quantities of Bence Jones lambda shows central clearing of the immunoprecipitate in lane 4, where the patient's undiluted urine reacted with anti-λ. In lanes 5 and 6, the patient's urine was diluted 1:2 and 1:5, respectively, and no antigen excess effect was seen. SPE, serum protein electrophoresis; GAM, antisera against IgG, IgA and IgM; κ, kappa; λ, lambda.

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Highlights

- Procedures for investigation of unusual or unexpected immunoassay results
- Practical approach based on personal experiences for routine non specialised laboratories
- Summary of common interferences

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