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Exogenous sample contamination. Sources and interference

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ABSTRACT

Clinical laboratory medicine is involved in the vast majority of patient care pathways. It has been estimated that pathology results inform 60–70% of critical patient care decisions. The primary goal of the laboratory is to produce precise and accurate results which reflect the true situation in vivo. It is not surprising that interference occurs in laboratory analysis given the complexity of some of the assays used to perform them.

Interference is defined as "the effect of a substance upon any step in the determination of the concentration or catalytic activity of the metabolite". Exogenous interferences are defined as those that derive from outside of the body and are therefore not normally found in a specimen and can cause either a positive or negative bias in analytical results. Interferences in analysis can come from various sources and can be classified as endogenous or exogenous. Exogenous substances could be introduced at any point in the sample journey.

The laboratory must take responsibility for the quality of results produced. It has a responsibility to have processes in place to identify and minimise the occurrence and effect contamination and interference. To do this well the laboratory needs to work with clinicians and manufacturers. Failure to identify an erroneous result could have an impact on patient care, patient safety and also on hospital budgets. However it is not always easy to recognise interferences. This review summarises the types and sources of exogenous interference and some steps to minimise the impact they have.

1. Introduction

Clinical laboratory medicine is involved in a large proportion of patient care pathways [1] and it is the responsibility of the laboratory to take ownership of the quality of results produced. It has been estimated that pathology results have a direct influence in 60–70% of critical decisions [2–4]. The primary goal of the laboratory is to produce precise and accurate results with reflect the true situation in vivo. It is not surprising that interference occurs in laboratory analysis given the complexity and range of measurable substances in the body and the complexity of some of the assays used to perform them. This is especially the case with immunoassays [5]. Interferences in analysis can come from various sources and can be classified as endogenous or exogenous. As defined below these are substances that cause the result to be erroneous as a result of another substance being present. This other substance could be introduced at any point in the sample journey.

It is very important to recognise that interferences exist and as far as possible to put in place measures to mitigate the risk. Failure to identify an erroneous result could result in an impact on patient care, patient safety and also on hospital budgets due to unnecessary further clinical investigations or therapy [6–9]. However it is not always easy to recognise all interferences, especially when clinical decisions are based solely on laboratory analysis as can be the case with some tumour markers [5].

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2. Exogenous interferences

2.1. Definition of exogenous interference

In 1995 Kroll and Elin [10] defined interference as "the effect of a substance present in the sample that alters the correct value of the result, usually expressed as concentration or activity, for an analyte". Prior to this, in 1986, Letellier defined it as "the effect of a substance upon any step in the determination of the concentration or catalytic activity of the metabolite" [11]. Both of these suitably define the interferent but Letellier's definition fits well the total testing process as it hints at "any step in the process".

Exogenous interferences are defined as those that derive from outside of the body and therefore would not normally be present in a specimen. This is the opposite of endogenous interferents which occur naturally e.g. lipaemia or haemolysis. They can cause either a positive or negative bias in analytical results. The interferences will not usually be obvious and it is therefore essential to know how the assay works to understand how the interferent may be causing an erroneous result.

2.2. Proposed approach to detection

The laboratory has a responsibility to have processes in place to identify and minimise the occurrence and effect contamination and interference. The Clinical and Laboratory Standards Institute (CLSI) has a

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standard for interference testing which demonstrates when and how you should perform interference testing [12]. To do this well the laboratory needs to work with clinicians and manufacturers. Clinicians must help by asking questions and informing the laboratory if the results don't fit with the clinical picture. Likewise if an interference is identified the laboratory should inform manufacturers so they can further optimise their assays to decrease the chance of a recurrence [5]. Most laboratories will have delta checks in place with rules to flag significant changes in results between samples on the same patient. Most analytical platforms will also have alerting systems put in place by the manufacturers to flag when any abnormalities in the reactions are detected.

If interference is suspected there are various processes that can be performed in the investigation process. These include

- Processing the sample on a different platform using different reagents (and antibodies if an immunoassay).
- Processing the sample by a reference method.
- Perform serial dilutions. These will likely be non-linear if there is interference present.
- In immunoassay blocking reagents can be used to investigate for heterophillic antibodies [5,13].
- Removal of the interferent e.g. chromatography or polyethylene glycol precipitation.
- Spiking in more of the suspected interferent (if known) to analyse the effect on the analyte of interest.

When setting up an assay it is also important that, as part of the validation and/or verification procedure, any possible interferences are tested for to establish an acceptable level and to put in place procedures to highlight and mitigate any potential erroneous results [14]. This will include not just interfering substances but also carryover effects.

If contamination has occurred it is important that there are appropriate algorithms in place to investigate possible erroneous results and feedback to the requester to ensure an appropriate patient pathway is followed [15]. In the case where a patient is known to have something circulating (e.g. an antibody) that interferes in an assay this should be logged as an alert on their file.

2.3. Drug interferences

There are many drugs that interfere in laboratory analysis to give falsely high or low results via various different mechanisms, which can either cause interference physiologically or analytically [16]. Physiological effects can be either intended effects or due to a side effect of the drug. There have been a couple of attempts to establish a database of these over the years [17,18] but the best of these is the 'Effects of drugs on clinical laboratory tests' establish by a collaboration between Donald Young and his team and the American Association for Clinical Chemistry [19,20]. As the data here is so comprehensive this review will not look at drug interferences in any detail. However although the database is comprehensive patients are often on more than one drug and as such the database cannot accurately reflect the real world situation, merely provide a starting reference point of what may be happening. It is therefore essential that if interference is suspected it is investigated following the CLSI guidelines for the investigation of interference described above [12].

2.4. Herbal interferences

Like prescribed drugs, herbal remedies can also interfere in laboratory analysis. The problem with herbal remedies is that they are commonly used and without any regulation from healthcare professionals. This can lead to misleading results not being understood as the patients are unaware they may be an issue and the healthcare team are not informed that the patient is taking a supplement. In fact herbal remedies are well documented to interfere in laboratory analyses. For example St. Johns Wort (*Hypericum perforatum*) and *Ginkgo biloba* can both induce enzymes and therefore affect circulating levels in the body [21,22]. The drug interferences in laboratory analysis database compiled by Young and his team also includes herbal remedies [14].

Foods can also interfere with laboratory analysis in the same way as drugs and herbal remedies. Foods like avocado, broccoli, cabbage and cranberry are all enzyme inductors, whereas soya and grapefruit juice are inhibitors [23–25]. As with the herbal remedies anything that inducts or inhibits enzymes can cause levels of any circulating drugs to be affected. Food has also been well documented to interfere with urine assays, in particular in the mass spectrometric analysis for 5 hydroxyindoleacetic acid (5-HIAA) which can be affected by avocados, bananas, pineapples, plums, walnuts, tomatoes, kiwi fruit, and eggplant [26].

2.5. Skin disinfectants

Prior to blood collection the venepuncture site is cleaned using a disinfectant. If this is not allowed to dry it can have an effect on analytical results. Alcohol can cause haemolysis or affect blood alcohol levels [3]. The oxidative effects of betadine (iodine) can cause elevations in phosphate, urate and potassium levels [27]. Alternatives to iodine include chlorhexidine gluconate or benzalkonium chloride but the latter has been shown to interfere in electrolyte test results [4,28].

2.6. Tube and their additives

2.6.1. Additives

Additives are an essential part of modern blood collection as they help preserve the analytes as close to the in vivo situation as possible. Often these are anticoagulants such as ethylenediaminetetraacetic acid (EDTA), heparin or citrate. Additives can be in powder form or liquid. When in liquid form there is a risk of a dilution effect when tubes are incompletely filled. Contamination of an additive from one tube to another can occur by three possible mechanisms and is only possible if the correct order of draw is not followed. Direct tipping of samples into other tubes causes the most significant, obvious and easily detectable route of contamination but also there is the possibility of back flow into the needle when following an incorrect order of draw. Or direct syringe contamination when using a needle and syringe rather than a closed loop system and transferring to collection tubes via an incorrect order of draw. Further details of these mechanisms have been discussed elsewhere by Cornes et al. and therefore will not be further elaborated upon here [15,29,30].

EDTA is a widely used sample tube anticoagulant, normally occurring in the form of a potassium salt, kEDTA [29,31,32]. It chelates divalent cations including zinc, calcium and magnesium [33,34]. Contamination of other samples with kEDTA via the mechanisms discussed above can lead to artefactually high potassium concentrations and low magnesium, calcium, zinc and alkaline phosphatase [32,35–37]. Of these spurious hyperkalaemia is the most clinically serious and most likely to lead to patient mismanagement [35,38]. Minor contamination is also easy to miss unless EDTA is directly measured in the sample [29,37].

Sodium citrate tubes are used for most coagulation samples and as an anticoagulant in some cannulas (e.g. Citra-Lock™). It is usually in the form tri-sodium citrate. As this is usually a liquid additive it is essential that tubes are properly filled to avoid a dilution effect. Contamination of a sample with sodium citrate causes raised sodium results with a disproportionally low chloride and a negative osmolar gap [39–41]. The negative osmolar gap and low chloride is due to the anion:cation dissociation ratios which are 3:1 for sodium citrate and 1:1 for sodium chloride [7].

Heparin is extensively used as an anticoagulant and acts via the inhibition of thrombin which in turn stops the generation of fibrin from fibrinogen [13]. Exogenously administered heparin has been shown to

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