Interference and Uncertainty in Biochemistry Assays

At BSPS we are proud to offer a service and use equipment that is accredited to international standards to give you confidence that the results that you receive are reliable and robust.

There are some factors which are beyond the control of the laboratory which may cause problems with blood tests, including medications and supplements. In addition, a very small minority of individual patients may react atypically to particular assays. Some examples of known technical issues are listed below, along with the assays most likely to be affected. This list is not intended to be exhaustive; new interferences are discovered regularly and individual patients may react atypically to particular assays in ways not seen previously.

We rely on our users to notify us of any results which are clinically unlikely – as the healthcare professional ordering the test you may be in possession of clinical information which makes a result dis-believable. In the laboratory we have access to a very limited amount of clinical information and while we will always do our best to identify any incorrect results before they reach you, we may have no reason to be suspicious of a result which appears very wrong to you.

We would always ask that in the event that you receive an unexpected result or a result which does not fit with the clinical picture, you contact the Duty Biochemist to discuss the result. We may be able to offer an interpretation of the result, or to suggest a possible interferent which could be causing a spurious result.

We are always grateful for feedback on any aspect of our service, including the technical and clinical service we offer.

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BIOTIN

Biotin is widely available as an over the counter (OTC) supplement and is commonly included in multivitamins as well as supplements marketed for hair and nails. Biotin is occasionally prescribed in pregnancy and renal failure, as well as being used at high doses in the treatment of multiple sclerosis, mitochondrial energy disorders and various inborn errors of metabolism.

With increasing numbers of people taking high dose OTC biotin supplements, it has become clear that this can cause inaccurate results in a large number of immunoassay tests.

The immunoassay instruments used at the St Peter's Hospital, Royal Surrey County Hospital, and Frimley Park Hospital sites of BSPS are Centaur XP instruments supplied by Siemens Healthineers Ltd. Tests including hormones, tumour markers, antibiotics, some drugs, haematinics, cardiac markers, bone markers and PIIINP are measured on these platforms. Some of these immunoassay tests use biotin as an integral part of the assay and therefore high levels of biotin in a patient's blood sample can cause these assays to give false results.

In patients taking supra-physiological doses of biotin, serum biotin levels may be as high as 1160ug/L one hour after taking a single oral dose of 300mg biotin.

All of our assays which use biotin as a component have been tested for interference with biotin concentrations of up to 1500ug/L, and the table below lists the assays we currently offer, along with the magnitude of the possible effect, and the amount of biotin in the patient's blood at which an effect is seen.

We suggest that you ask patients whether they are taking biotin and bear it in mind when interpreting their test results. The advice from the manufacturer for patients taking biotin is that samples should be collected just before a dose (i.e. at trough level).

Please contact the Duty Biochemist via the GP Helpdesk if you have any questions or if you have a particular set of results that you would like to discuss.

Effect of High Dose Biotin Supplements on Biochemistry Tests

Test	Effect of biotin	Biotin concentration at which effect seen
BNP	reported results may be up to 12% lower than true result	moderate
DHEAS It is recommended that DHEAS is not measured in patients taking biotin	reported results may be up to 16% higher than true result	low
	reported results may be up to 4000% higher than true result	very high
Folate	reported results may be up to 300% higher than true result	moderate
	reported results may be up to 460% higher than true result	very high
PIIINP	reported results may be up to 33% lower than true result	moderate
	reported results may be up to 100% lower than true result	very high
SHBG	reported results may be up to 25% lower than true result	very high
Testosterone	reported results may be up to 108% higher than true result	moderate
	reported results may be up to 7000% higher than true results	very high
Troponin I	reported results may be up to 40% lower than true result	low
	reported results may be up to 100% lower than true result	very high

Assays Which Contain Biotin But Are Unaffected By Biotin Supplements

fT4	no effect
Oestradiol	no effect
PTH	no effect
Troponin I (high sensitivity)	no effect

HETEROPHILE ANTIBODIES

Immunoassay tests use antibodies to detect biological molecules. The antibody binds to the molecule of interest and then goes on to interact with other antibodies or chemicals in the assay reagents to produce a signal which we can measure. Immunoassays are extremely powerful and sensitive tests due to the highly specific binding properties of antibodies and can detect tiny concentrations of the target molecule.

Immunoassays are used widely throughout laboratory medicine and within biochemistry we use them to perform a large number of tests, including hormones, some drugs, tumour markers, cardiac markers, haematinics, antibiotics and nutritional and bone markers.

Some patients possess heterophile antibodies – these are antibodies directed against animal antigens which will bind weakly but non-specifically to a wide variety of antigens. These antibodies may react in immunoassays and block the proper binding of the antibodies within the assay. The can result in falsely low or falsely high results (depending on the type of immunoassay).

Heterophile antibodies (sometimes called “human anti animal antibodies” or HAAA), can be found in up to 30% of the population and can arise following significant exposure to animals, including keeping pets, although in some cases the cause of their presence is unknown.

If you have some results that are clinically unlikely and suspect that a patient may have heterophile antibodies, please contact the Duty Biochemist via the GP Helpdesk to discuss whether we can do further investigations to determine whether this is the case (we may need a repeat sample to do this).

MONOCLONAL GAMMOPATHIES AND PHOSPHATE, UREA

In certain disease states where significantly elevated paraprotein (M-protein) levels occur, there is a potential for interference in our phosphate and urea assays from specific monoclonal proteins, resulting in falsely elevated results. In addition, for particular patients we may be unable to measure phosphate at all due to the interference from the paraprotein.

If you note any unexpected results for patients with myeloma, Waldenstroms macroglobulinaemia, MGUS, POEMS, or any other condition associated with a monoclonal gammopathy, please contact the laboratory to discuss. On a case by case basis we may be able to arrange for analysis of these analytes by an alternative method although this cannot be guaranteed.

DRUG EFFECTS

Sulfasalazine, Sulfapyridine and ALT and Ammonia

ALT and ammonia testing may give false results in patients taking sulfasalazine for the treatment of e.g. ulcerative colitis, Crohn's disease or rheumatoid arthritis.

The affected ammonia method is used at the Frimley Park Hospital and St Peter's Hospital sites. The affected ALT method is used at all three Surrey BSPS sites - St Peters Hospital, Royal Surrey County Hospital, and Frimley Park Hospital. If you are unsure where your blood sample has been analysed, please contact the laboratory who will be able to clarify this for you.

- In patients taking sulfasalazine, ALT results could be falsely low. The reported result could be up to 26% lower than the true result.
- In patients taking sulfasalazine, ammonia could be falsely elevated; the reported result could be up to 76% higher than the true result.

An effect on test results is only likely to be seen if a blood sample is taken soon after a dose. We recommend that in patients taking sulfasalazine, blood samples should be taken just before a dose which will minimise the possibility of obtaining a false result.

In addition, ammonia results may be falsely low in patients taking sulfapyridine (up to 18% lower than the true result). This drug is no longer prescribed in the UK but the same advice applies and we would recommend taking blood for ammonia measurement just before a dose of sulfapyridine to minimise the effect of the drug.

Paracetamol, N-Acetylcysteine and Creatinine

Paracetamol overdose can lead to high levels of a metabolite called NAPQI when the usual pathways for detoxification are overwhelmed. High levels of NAPQI can cause interference in our method for measuring creatinine, and cause results to be reported as being lower than the true value. This may falsely reassure the clinician about the patient's renal function.

N-acetylcysteine (NAC) is the treatment of choice for paracetamol overdose. It is used only in patients at risk of hepatotoxicity according to the treatment nomogram available in the BNF.

NAC can interfere with our method for measuring creatinine and cause results to be reported as being lower than the true value. This may falsely reassure the clinician about the patient's renal function. In addition, once the patient finishes their course of NAC and we report true creatinine results, this apparent rise in creatinine may trigger Acute Kidney Injury alerts.

If you are treating a patient with NAC, please ensure you record this in the clinical details on the request. For patients where we detect paracetamol, we will add a comment to the creatinine results to remind you of this possible interference.

Eltrombopag and Bilirubin

In August 2018 the MHRA issued an alert noting that Eltrombopag may interfere with creatinine and bilirubin test results. Eltrombopag is prescribed only very rarely in the UK to patients with immune thrombocytopenic purpura (ITP). ITP has a prevalence is about 3 in 100,000 adults per year. Eltrombopag is used only as a second line option and is associated with a high cost – funding approval is required for this drug, which is usually only prescribed by haematology consultants.

Eltrombopag is a highly coloured reddish-brown drug, which may cause serum discolouration. The creatinine method in use in Berkshire and Surrey Pathology Services is not affected by this discolouration, but our bilirubin method may give false results in patients being treated with Eltrombopag.

In these patients, both total bilirubin and conjugated bilirubin results may be reported as being either lower or higher than the true value.

If you are sending blood samples for analysis for a patient being treated with Eltrombopag, please ensure that you clearly state the drug therapy in the clinical details to ensure that we can identify and comment appropriately on the results.

Phenindione and Creatinine

Phenindione is a vitamin K antagonist similar to warfarin. It is used rarely in the UK as is associated with a higher rate of toxic side effects and hypersensitivity than warfarin.

Phenindione can interfere with our method for measuring creatinine and cause results to be reported as being lower than the true value. This may falsely reassure the clinician about the patient's renal function.

If you are treating a patient with phenindione, please ensure you record this in the clinical details on the request to ensure that we can identify and comment appropriately on the results.

Etamsylate and Creatinine, Lactate, Triglycerides

Etamsylate is a haemostatic drug given for prophylaxis and control of haemorrhages from small blood vessels. It is very rarely used in the UK.

Etamysylate can interfere with our method for measuring creatinine and cause results to be reported as being lower than the true value. This may falsely reassure the clinician about the patient's renal function. It can also cause lactate and triglyceride results to be falsely low.

If you are treating a patient with etamsylate, please ensure you record this in the clinical details on the request to ensure that we can identify and comment appropriately on the results.

Metyrapone and Cortisol

Metyrapone is an inhibitor of 11b-hydroxylation in the adrenal cortex, used to treat Cushing's syndrome. Due to the action of this drug, patients treated with metyrapone typically have significant concentrations of 11-deoxycortisol circulating in their blood. This metabolite cross reacts with the assay for cortisol in use at Frimley Park, St Peters Hospital and the Royal Surrey County Hospital causing the results to be falsely high.

The cortisol assay in use at our Berkshire sites is much less subject to interference from 11-deoxycortisol, and we have shown that the Berkshire results correlate well with mass spectrometry methods and can be used for clinical management of patients on metyrapone.

If you are treating a patient with metyrapone and need to assess the efficacy of the treatment, please contact your local laboratory and we can arrange for samples to be sent to Berkshire for analysis on a case by case basis.

BIOLOGICAL VARIATION AND UNCERTAINTY OF MEASUREMENT

While we do ask you to be critical of any result that does not fit the clinical picture, it is important to bear in mind that normal biological and analytical variability can have significant effects on patient results.

For example, the intra-individual biological variation of serum ALT can be up to 19% - that is to say, when taking two samples from the same patient on the same day, the ALT results could vary by up to 19%.

"Analytical variation" means the extent to which our assays can vary. Although the vast majority of our assays are highly automated in order to minimise human error, there are still tiny variations in temperature, instrument movement, reagents, light scatter and other components of the assay which means that if we measure the same sample twice we will not get exactly the same result. Our typical analytical variability for ALT is around 4%.

We also have many instruments performing the same tests across our network. This gives us resilience and allows us to continue processing samples if one of the instruments requires maintenance and repairs, but it does introduce further variation as slight differences in the instruments can lead to differences in the results.

We can combine these variabilities to look at the total effect on a patient result – the above example would mean that if you send two samples on the same patient on the same day, you could get ALT results of **18** IU/L and **22** IU/L, and this difference would be entirely down to the biological and analytical variability.

What Do BSPS Do To Minimise Variation?

We have a number of approaches to minimise variation. All of our instruments are regularly maintained and serviced by our staff and by the manufacturer. We have long term contracts with our suppliers to ensure continuity of service and of supply of reagents and materials that we need. We take steps to ensure that the samples we receive are of high quality, by giving clear instructions on how they should be collected, stored and transported to us, on any relevant patient preparation, and by following standard procedures for processing samples when they arrive at the laboratory.

We regularly run quality control samples through our instruments. These are samples with known concentrations of analyte, so we know before analysing them what the result should be.

If an instrument does not give a result within a set target range, we do not analyse patient samples on that instrument until we have taken steps to improve the performance and repeated the quality control analysis to ensure that the instrument is working properly. Because we have large volumes of these quality control samples, we run them several times a day over long periods of time (months in most cases), so we also monitor our instruments performance over time and can see any long-term changes in the results they produce.

We participate in External Quality Assurance (EQA) schemes. These are national or international programmes. The organisers send out samples to all of the participating laboratories, we measure the analytes and report our results back to the organisers. The scheme organisers then prepare a report for us, giving details of how our result compared to other laboratories. This allows us to determine whether our instruments are performing as well as, or better than other similar laboratories, and also to see how our particular instrument type compares to other instruments.

Want to know more?

If you would like to learn more about this area of laboratory medicine, the most prominent expert in the field is an American scientist called Professor James Westgard. He has published many papers and books in this area, and his website is a good place to start: www.westgard.com

If you would like information about the variability of the assays used within BSPS, which is also referred to as “Uncertainty of Measurement”, please contact the Duty Biochemist via the GP Helpdesk who will be able to direct you to the most relevant person.