UNIT NO. 1

PRE AND POST ANALYTICAL FACTORS AFFECTING LABORATORY RESULTS

A/Prof Robert Hawkins

ABSTRACT

The results of laboratory tests have a substantial role in the diagnosis and management of disease. However, laboratory results do not always correspond with the patient's clinical status. Unexpected results may arise from factors other than disease. The total testing process consists of 3 separate phases — pre-analytical, analytical, and post-analytical. The accuracy and reliability of a laboratory result depend on the quality of each phase. In this article, the common variables in the pre-analytical and post-analytical phases and their effect on test result quality in primary care are reviewed.

Keywords: Laboratory Tests; Quality; Pre-analytical; Post-analytical;

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INTRODUCTION

Laboratory results play a key role in patient care. It is estimated that around two-thirds of important clinical decisions about admissions, discharge, and medication are based on laboratory test results.[1] However, laboratory results do not always correspond with the patient's clinical status. Although laboratory results are usually valid, unexpected and unreliable results may derive from factors other than disease. The total laboratory testing process (or total testing cycle) was described by George Lundberg as a "brain-to-brain loop" comprised of a series of activities, starting with the clinical question in the clinician's mind, leading to test selection, sample collection, transport to the laboratory, analysis, reporting back to the clinician, and final interpretation and decision-making by the clinician.[2] These activities have traditionally been separated into 3 phases (pre-analytical, analytical and post-analytical). The various activities in each phase of the total testing process are shown in Table 1. Errors that may affect the accuracy and reliability of results can occur in any of the phases. Typically, it is the analytic type of errors that most frequently come to mind as the reason for an incorrect result. However, a number of studies have shown that analytic errors are often not the problem. A study of 49 primary care clinics showed that 56 percent of detected errors were attributable to test requesting and specimen handling (pre-analytic phase), 13 percent to test analysis (analytic phase), and 27 percent to the post-analytic phase.[3] In the judgement of the practice staff, 27 percent of the reported problems had an impact on patient care. In this paper, I review the common pre-analytical and post-analytical factors relevant to primary care which most often affect laboratory results in healthy individuals and which explain unexpected results.

Healthcare is a relatively high-risk area and the overall defect rate in healthcare in the United States is estimated as 31–69 percent.[4] Error rates are often described using the sigma concept, which refers to the number of standard deviations which lie between the process mean and the specification limit. As the process standard deviation becomes smaller, more standard deviations will fit between the mean and the specification limit, increasing the sigma number and decreasing the likelihood of items exceeding the specification limit. Using this measure, healthcare performs at a 1–2 sigma level, which compares poorly with non-healthcare industries such as airline baggage handling (approximately 4 sigma). Performance varies in different areas of healthcare, with values of 1 sigma (e.g. use of beta-blockers post myocardial infarction, detection and management of depression) to 3 sigma (e.g. adverse drug events, hospital-acquired infections). The analytical phase of laboratory medicine is arguably the best-performing sector in healthcare, with close to 5-sigma performance (0.002%).[4,5] This is more than 3000 times lower than the rates of infection and medication errors and reflects the standardised quantitative nature of much of laboratory medicine testing. However, the accomplishments of laboratory medicine drop when errors in all phases of the total testing process are considered.[6,7] The proportion of errors associated with the 2 extra-analytical phases is 4–5 times that seen in the analytical phase, with the pre-analytical phase consistently representing over half of all errors in published studies.[6–12]

How important are laboratory errors? Errors in healthcare are of concern when they lead to actual or potential adverse outcomes to patients. Given the complex nature of healthcare and the difficulty in assessing the effect of a specific laboratory error on patient management, the prevalence of proven patient harm is difficult to assess. Obvious extreme errors in qualitative results with clear links to therapy or management decisions (e.g. histopathology, blood transfusion, microbiology, virology, genetic testing) are easiest to measure but assessing the effect of quantitative errors in clinical biochemistry and haematology results is much harder. Such difficulties mean that present measurements probably significantly underestimate the size of the problem in light of the high volume of quantitative testing performed in clinical laboratories. Published data suggest that 24-30 percent of laboratory errors have an effect on patient care while actual or potential patient harm occurs in 3-12 percent.[6-8] Some areas,

ROBERT HAWKINS
Senior Consultant, Laboratory Medicine, Tan Tock Seng Hospital.
Visiting Consultant, Pathology and Laboratory Medicine,
KK Women's and Children's Hospital.
Adj Associate Professor, Lead for Chemical Pathology,
Lee Kong Chian School of Medicine.

such as molecular genetics testing, can have actual harm rates of up to 100 percent.[6,13]

PRE-ANALYTICAL FACTORS

1. Diet and Fasting

In response to the ingestion of food, marked metabolic and hormonal changes occur, principally due to the absorption of fluids (water and/or alcohol), lipids, proteins, carbohydrates, and other food constituents. Most pronounced and clinically significant biochemical changes are observed up to 4 hours after a meal for triglycerides, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, sodium, magnesium, potassium, C-reactive protein (CRP), uric acid and total bilirubin.[14] As little as 1 hour after a meal, the lymphocyte count shows significant decreases and this effect is even more pronounced 2 hours afterwards. The largest clinically significant variations in neutrophils, eosinophils, red blood cells, haematocrit and mean corpuscular haemoglobin (MCH) occur up to 4 hours after ingestion of food.[15] The issue of diet relates to not only the distinction between fasting and non-fasting status but other factors such as type of diet (e.g. high-fat, low-fat, vegetarian), length of time since the last meal (e.g. 2 hours, 12 hours, 48 hours, or more), and test-specific dietary concerns. The effects on postprandial glycaemic reactions of adding a glass of water (300 mL) to a meal after a 12 hour fast were found to increase the peak blood glucose and serum insulin concentrations in healthy subjects, and the blood glucose concentration in well-controlled diabetic patients. In practice it is suggested that the volume of water which an individual may drink on the day preceding the phlebotomy should mirror the usual daily ingested water volume of each individual.[16] Chronic and acute smoking, along with alcohol consumption are associated with changes in postprandial response. A marked increase in the triglyceride-rich lipoprotein metabolic rate has been observed immediately after smoking a single cigarette.[17] Coffee has also been shown to acutely increase glucose concentration. Fasting blood glucose increases by almost 12 percent within 1 hour after the consumption of one 12 oz (about 350 mL) café latte. [18] This effect is more evident among females and overweight individuals.[19]

Is there a need for fasting to be overnight? Moreover, if there is no diurnal variation, can the collection be performed at any time, as long as the patient fasts the recommended time period (e.g. 06:00–18:00 with collection at 18:01)? In theory, fasting need not be overnight if there is no diurnal variation, as long as the fasting time recommendation is followed. It is however inconvenient to fast during the daytime and to draw blood at other times as other analytes are often included with the blood request. Many analytes with diurnal variations have their reference values set at 7 to 9 a.m. and their reference value changes need to be calculated for samples drawn at the same time of day. It is recommended that all blood tests should be drawn preferably in the morning from 7 to 9 a.m. Fasting should last for 12 hours, during which water consumption is permitted. Alcohol should be avoided for 24 hours before blood sampling. In the morning before blood sampling, patients should refrain from cigarette smoking and caffeine-containing drinks (tea, coffee, etc.).[16]

Medications also cause a wide range of effects on laboratory tests, including their intended (therapeutic) effects, side effects, and analytic interferences. Interested readers are referred to a compendium of such information by Young.[20]

2. Exercise

A number of analytes, including aspartate aminotransferase, bilirubin, creatine kinase, high-density lipoprotein cholesterol, lactate dehydrogenase, neutrophils, and uric acid, can increase either transiently or long term after exercise. The magnitude of change depends on the person's fitness, muscle mass, duration and intensity of exercise, and amount of time that has elapsed between the exercise event and collection of the blood specimen. Exercise during specimen collection (i.e. pumping of the fist) can produce increased lactic acid concentration and decreased pH, which may, in turn, result in increased concentrations of ionised calcium and potassium.[21] The increased pressure in the veins during fist clenching may result in pressure-induced changes similar to those seen with tourniquet placement.

3. Posture

As the body changes from a lying to a standing position, blood pressure-sensitive hormones increase in concentration to raise the blood pressure to maintain cerebral perfusion. As the blood pressure increases, there is an efflux of water from the intravascular space into the interstitium with a decrease in blood volume and an increase in concentration of analytes that are too large to pass through the vascular wall. In general, these are analytes that are bound to large proteins (e.g. albumin, bilirubin, calcium, cholesterol, many drugs and enzymes, total protein and triglycerides). Such changes may be significant, e.g. cholesterol concentrations may rise by up to 15 percent. It is recommended that patients sit for 5 minutes before collection of specimens to minimise this posture-related change.[22]

4. Tourniquet Application

Tourniquet application can change analyte concentrations in a similar manner to fist clenching through increasing pressure below the tourniquet. Protein-bound non-diffusible analytes increase in concentration during the time of tourniquet application.[21,23,24] The venous stasis that results from prolonged tourniquet application promotes anaerobic glycolysis, with the accumulation of plasma lactate and a reduction in blood pH. The hypoxic effect drives potassium out of the cell, causing a spurious increase in the serum potassium level. This effect can be accentuated if the phlebotomist requests the subject to repeatedly clench the fist to permit visualisation of the vein. The contraction of forearm muscles during repeated fist clenching causes the release of potassium, since there is a reduction in the intracellular electronegativity during the depolarisation of muscle cells, which favours the release of potassium rather than its uptake.[25] Repeated fist clenching during phlebotomy, after application of the tourniquet, can cause a 1–2 mmol/L increase

in potassium. The Clinical and Laboratory Standards Institute guideline for collection of blood specimens by venepuncture states that the tourniquet should be released as soon as blood flow is established to minimise the time the tourniquet is in place (<1 minute).[26] If a tourniquet has been in place for longer than 1 minute, it should be released and reapplied after 2 minutes. Occlusion time should be minimised and pumping of the fist should be avoided. Tourniquet application for longer than 1 minute is also associated with a greater risk of sample haemolysis.[27]

5. Time of Day

The timing of the collection is important for some analytes which show predictable changes in concentration during a 24-hour period in response to factors, including light and dark, meals, sleep, posture, and stress. These include acid phosphatase, adrenocorticortrophic hormone, aldosterone, bilirubin, catecholamines, cortisol, follicle-stimulating hormone, growth hormone, iron, luteinising hormone, progesterone, prolactin, testosterone, thyroid-stimulating hormone, triglycerides, urea, and uric acid. Laboratories often provide different time-related reference intervals (e.g. morning and evening cortisol) for some analytes but not all (e.g serum iron concentrations may be 30 percent lower in the evening than in the morning).

For many drug measurements, timing of the collection is crucial. This is especially true for drugs in which the therapeutic window (i.e. the difference between subtherapeutic and toxic levels) is relatively small. Trough drug levels are typically collected immediately before the next dose of medication is given. Peak levels are drawn at a specific time after the last dose, determined by the drug pharmacokinetics and the route of administration.

6. Blood Collection Tube Anticoagulants

Selecting the correct anticoagulant is critical for many tests because anticoagulants designed to optimise one test method may interfere with another test method.[28] For example, anticoagulants containing fluoride, designed for glucose measurements, may interfere with haematologic and electrolyte studies by altering blood cell membrane permeability and morphologic features. EDTA designed for maintenance of blood cell membranes chelates calcium and will result in low calcium measurements. In general, venepuncture using a needle and syringe should be avoided for safety reasons. Evacuated venous blood collection tube systems with needle holder are preferred. When performing a multi-tube collection using evacuated blood tubes, the potential for anticoagulants in one evacuated blood tube to contaminate blood collected into a subsequent evacuated blood tube necessitates adherence to a standard order of collection. The following order of draw is recommended:

(1) Blood culture tubes;
(2) Coagulation tube (e.g. blue stopper);
(3) Serum tube with or without clot activator, with or without gel (e.g. red, yellow stopper);
(4) Heparin tube with or without gel plasma separator (e.g. dark green, light green stopper);
(5) EDTA tube (e.g. purple stopper); and
(6) Glycolytic inhibitor (e.g. grey stopper).

Allow the tube to fill until the vacuum is exhausted and blood flow ceases. For tubes that contain additives, this will ensure there is a correct ratio of blood to additive. This is particularly important for coagulation testing. Immediately after drawing each tube, mix the blood gently and thoroughly by inverting the tube 4 to 6 times, or following the specific indications provided by the different manufacturers of blood tubes.[29]

7. Specimen Processing

Allowing the cells to remain in contact with the plasma or serum for long periods allows the concentration of some analytes to increase (e.g. potassium, creatine kinase, lactate dehydrogenase, phosphate, and ammonia) while others decrease (e.g. glucose, bicarbonate, and acid phosphatase). Tubes should be held upright at room temperature while clotting. Although sample refrigeration will preserve some analytes (e.g. glucose), the low temperature will inhibit the red cell Na-K ATPase and will allow potassium to immediately diffuse out of the red cells and into the serum and plasma, increasing the potassium measurement even in the absence of haemolysis.

POST-ANALYTICAL FACTORS

In the post-analytical phase of the testing process, results are released to the clinician who interprets them and makes diagnostic and therapeutic decisions accordingly. Various steps in the process can be identified — communication of the result by the laboratory, result received by the correct provider, result reviewed, result interpreted correctly, result follow-up appropriate to patient. Special consideration should be given to critical value reporting. A laboratory critical value is any result for which an immediate, life-saving action is available and required. Clinicians and laboratory staff should understand the difference between critical values, critical tests and abnormal test results. The system must allow the responsible clinician to be notified promptly so that treatment can be started. Responsibility for the various steps lies with both the laboratory and the clinician as shown in Table 2.

Increasing use of electronic result routing systems and medical records systems is associated with increasing problems of test results sent but not received by the correct caregiver. Causes can include logic errors in interface and results routing, provider record issues, electronic health record system settings, and system maintenance–related errors. Results routing in the electronic health records system is a complex proposition and sometimes implemented without sufficient knowledge of laboratory or clinician work processes. Despite the theoretical possibility of customising critical result definitions for individual providers, the potential for errors, particularly during IT system downtime, necessitates use by most laboratories of universal cut-offs across all providers. Clinicians should work with the laboratory to ensure the right laboratory result gets to the right person at the right time in an interpretable format. Failure to follow up on test results is an underlying factor in many medical

malpractice lawsuits. The frequency of the failure to follow up on laboratory test results in an ambulatory setting varies from 2 percent to 50 percent among primary care providers.[30]

In terms of result interpretation, a significant issue well recognised by laboratories but often overlooked by clinicians is inter-laboratory variability in methodology and numeric results, and application of inappropriate reference intervals.[31–34] The laboratory community has made great progress over recent decades in standardising measurements of many analytes, including electrolytes and lipids. However, some analytes, particularly those measured using antibodies, remain difficult if not impossible to standardise. Such tests include tumour markers, hormones, and proteins. Different manufacturers use different antibodies looking at different epitopes on the same analyte as part of the measurement process. Although similar results may be obtained in health, a predictable relationship between different methods breaks down in patients with elevated and atypical populations of the analyte, e.g. tumour-marker follow-up where intact and molecular fragments may be present. It is important to use a single consistent laboratory method when monitoring laboratory results and for laboratories to highlight in their reports any changes in methodology or equipment. Use of inappropriate reference intervals are also a source of confusion — many laboratories report age- and sex-stratified reference intervals for many analytes, including creatinine, uric acid, and alkaline phosphatase. Provision of the correct patient demographics (age and sex) at the point of requesting is essential to ensure appropriate reference intervals are included in the laboratory report.

SUMMARY

Issues arising outside the laboratory, i.e. during the pre-analytical and post-analytical phases of the total testing cycle, account for the majority of problematic samples and results encountered by clinicians. Prevention is the key to reducing the effect of these variables and improved knowledge by clinical staff of the many pre-analytical and post-analytical variables that can adversely affect laboratory results. The phlebotomy staff is a key component of the control process. Phlebotomists should understand the rationale behind collection and processing requirements and must adhere to the requirements for all specimens collected. Prevention also requires education of and cooperation among clinicians, nurses, clerical staff, phlebotomists, and laboratory professionals to ensure that the right specimen is drawn under the right set of circumstances. In terms of post-analytical processes, care must be taken to ensure that the right report goes to the right clinician within the right timeframe, particularly for critical results, and that a consistent laboratory methodology is used for serial laboratory test monitoring, especially for tumour-marker follow-up. Improving the quality of the laboratory medicine service requires increased focus on error in the pre- and post-analytical phases of testing. Correcting such problems requires increased cooperation between laboratory and non-laboratory personnel to improve the quality of specimen collection and data dissemination.[35]

Table 1: Total Testing Process

Pre-analytical Phase	Analytical Phase	Post-analytical Phase
Requesting Collecting Processing Transporting	Testing	Recording Reporting Interpreting

Table 2: Post-analytical Processes

Post-analytical Factors	Responsibility	
	Laboratory	Clinician
Communication of result by laboratory	X	
Result received by correct provider	X	X
Result reviewed		X
Result interpreted correctly	X	X
Result follow-up appropriate to patient		X

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LEARNING POINTS

- **The commonest cause of laboratory errors in primary care is errors arising during the pre-analytic phase of the total testing cycle.**
- **It is recommended that all blood tests should be drawn preferably in the morning, from 7 to 9 a.m. Fasting should last for 12 hours, during which water consumption is permitted. The tourniquet should be released as soon as blood flow is established to minimise the time the tourniquet is in place. Pumping of the fist should be avoided.**
- **Ensure that a consistent laboratory methodology is used for serial laboratory test monitoring, especially for tumour-marker follow-up.**