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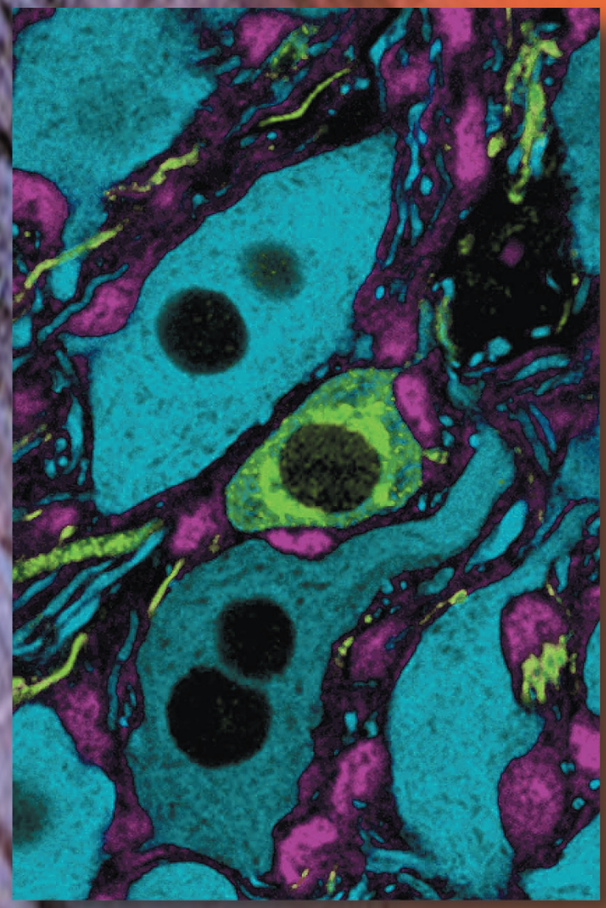

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Heparin resistance – a clinical and laboratory conundrum

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Abstract

Unfractionated heparin (UFH) is a sulphated glycosaminoglycan used as an immediate acting anticoagulant in acute thrombosis. Safe and effective management of UFH anticoagulation is dependent on a reliable measure of its anticoagulant effect. UFH therapy is most commonly monitored by the activated partial thromboplastin time (APTT), with prolongation of the APTT within a relatively narrow therapeutic range indicating therapeutic anticoagulation. There are some situations in which even with very high doses of heparin, the APTT fails to show prolongation within the therapeutic range and this is commonly termed heparin resistance. Heparin resistance is defined as the requirement of high doses of UFH (>35000 IU/day) to achieve therapeutic APTT values. Heparin resistance, both true and apparent, can complicate monitoring of heparin, particularly in acutely ill patients. This paper will explore the structure and action of heparin, discuss laboratory testing methods in heparin monitoring, investigate causes of both true and apparent heparin resistance, and discuss the recent evidence of heparin resistance in SARS-Cov-2 infections.

Keywords: ***SARS-Cov-2 infections, APTT, UFH therapy***

Introduction

Unfractionated heparin (UFH) is the most common anticoagulant used in the hospital setting worldwide, being used perioperatively, in intensive care units, for prophylaxis and treatment of venous thromboembolism and in cardiac surgery. It is considered safe for use in all patient populations including neonates, children and in pregnancy. UFH is administered either intravenously or subcutaneously as it is not readily absorbed by the gastrointestinal tract. UFH has a short half-life of approximately 90 minutes when administered subcutaneously and 30 minuntes when administered intravenously (McRae *et al* 2021). UFH has a narrow therapeutic window with the risk of bleeding in overdosing and risk of thrombosis in underdosing (Smahi *et al* 2020). UFH anticoagulation can be easily reversed by the use of protamine sulfate, or simply by discontinuing administration (McRae *et al* 2021). There is a high degree of variability in patient anticoagulant response to heparin, due in part to considerable non-specific binding of UFH to various cells and proteins (Smahi *et al* 2020; Finley and Greenberg 2013). Careful laboratory monitoring of UFH therapy is therefore necessary to ensure optimal patient care. The most common method of monitoring UFH therapy is the APTT (Smahi *et al* 2020). The APTT is a global coagulation test that reflects both the intrinsic and common coagulation pathways (McLaughlin *et al* 2019). It is simple to perform and readily available in most coagulation laboratories, however there are several factors which complicate the use of the APTT for UFH monitoring (McRae *et al* 2021). Heparin resistance is defined as a dose requirement of greater than 35000 units of heparin within a 24h period to reach the therapeutic range as determined by APTT measurement (Thota *et al* 2012). True heparin resistance reflects *in vivo* blunting of the anticoagulant effect of heparin, while apparent heparin resistance is demonstrated where a subtherapeutic APTT persists despite therapeutic anticoagulation *in vivo* (Downie *et al* 2019). Heparin resistance is commonly observed in acutely ill patients, with relatively high rates of heparin resistance being observed in the current SARS-Cov-2 pandemic (White *et al* 2020). It is important for both clinicians and laboratory scientific staff to understand the mechanisms of both true and apparent heparin resistance to ensure optimal care for patients.

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Method

A systematic review of English literature was undertaken using PubMed National Library of Medicine database. Search strategies included search terms "heparin resistance", "structure of heparin", and "pharmacology of heparin". Bibliographies of the included literature were also searched for additional references.

Structure and action of heparin

Heparin is a large linear polymer which is produced by the Golgi apparatus of mast cells and basophils and stored in the basophilic specific granules (Green 2020). It consists of repeating units of pyranosyluronic acid and glucosamine residues and has the highest negative charge density of any known biological macromolecule (Capila and Linhardt 2002). The most prevalent sequences within the heparin polymer are trisulphated disaccharide repeats of IdoA2-GlcN, as demonstrated in Figure 1. The antithrombin binding region, a specific pentasaccharide sequence consisting of GlcNAc6SO3-GlcAGlcNSO3(3,6SO3)-IdoA2SO3-GlcNSO36SO3, is scattered throughout the molecule (Wat *et al* 2018). Endogenous plasma heparin levels are present in concentrations ranging from 153 to 177 mIU/mL (Davids *et al* 2010). The effects of endogenous heparin are not currently completely understood, but are thought to include anticoagulant, anti-inflammatory and potentially anti-angiogenic effects as well as paradoxically, contributing to the initiation of the coagulation cascade (Hull *et al* 2021). Guilarte *et al* (2017) describe how mast cell heparin release due to tissue injury can provide the negatively-charged surface required for binding and activation of factor XII, thus initiating the clotting cascade.

The use of heparin as an anticoagulant began in 1935. Heparin in clinical use is isolated from bovine lungs and porcine intestines (Lee and Kong 2015). It is a mixture of polysaccharides of varying lengths, with an average size of approximately 45 saccharide units (Hull *et al* 2021). As an anticoagulant, unfractionated heparin (UFH) has multiple mechanisms of action which include heparin-cofactor II-dependent inhibition of thrombin, and inhibition of factor X activation by the intrinsic tenase complex. However, the predominant therapeutic action of UFH is the antithrombin-dependent inhibition of thrombin and anti-Xa (Buyue *et al* 2012).

Antithrombin (AT) is a 432-amino acid serine protease inhibitor, or serpin, which acts as the major physiological inhibitor of coagulation (Gandrille *et al* 1990; Mulder *et al* 2017). Antithrombin principally targets thrombin and factor Xa (Mulder *et al* 2017). Antithrombin exists in the plasma in two forms – an inactive "latent" form and an active monomer (Bauer 2021). Antithrombin is an unusual serpin in that it requires activation by a sulphated glycosaminoglycan – heparan sulphate or heparin – in order to inhibit its target proteases at a physiologically significant rate (Zhang *et al* 2005). The ability of heparin to increase the inhibition rate of thrombin and activated factor X by antithrombin is dependent on a specific pentasaccharide sequence in the polysaccharide chain. There are four key residues in AT which are key to heparin binding as highlighted in Figure 2: Arg47, Lys114, Lys125 and Arg129 (Schedin-Weiss *et al* 2002). Binding of the pentasaccharide sequence to antithrombin results in a substantial conformational change in the enzyme. The hinge region is ejected from β-sheet A, the reactive bond-loop is exposed and becomes more accessible as shown in Figure 3 (Johnson *et al* 2006). This conformational change results in a 1000-fold increase in AT activity (Bauer 2021).

Heparin acts in two key ways to activate antithrombin: as an allosteric activator of the inhibitor and as a bridging co-factor (Richard *et al* 2018). The inhibition of FXa by AT only requires the heparin-induced conformational change of AT. Thrombin inhibition by AT relies on a critical length of heparin to form a bridge between AT and thrombin, as demonstrated in Figure 4 (Wat *et al* 2018). This critical length of heparin has been found to be at least 18 polysaccharide units (Lehman and Frank 2009). The bridging mechanism greatly enhances AT reactivity with thrombin, leading to a three times more efficient inactivation of thrombin when compared to FXa inactivation (Green 2020).

Only approximately one-third of an administered UFH dose binds to AT, with the remaining two-thirds having minimal anticoagulant activity at therapeutic doses (Baluwala *et al* 2017). There is considerable non-selective binding of UFH to various proteins and cells within the body due to the dense negative charge surrounding larger molecular weight molecules (Lehman and Frank 2009). This includes binding to macrophages, endothelial cells and other plasma proteins (Finley and Greenbury 2013). Heparin is cleared in a biphasic fashion. In the initial phase, heparin binds to endothelial cell surface receptors and macrophages, is internalised and then depolymerised into smaller oligosaccharides (Weitz and Weitz 2010). This depolymerisation step is dependent on the dose of UFH administered (Lehman and Frank 2009), with higher doses able to saturate this mechanism of removal. The smaller oligosaccharides re-enter the circulation and are cleared by the kidneys (Weitz and Weitz 2010). Heparin is also metabolised by the liver to a minimal extent (Onishi *et al* 2016). Plasma heparin levels peak at two to four hours after intravenous administration (Hull *et al* 2021).

Monitoring of unfractionated heparin therapy

The most widely used test for monitoring of UFH therapy is the APTT (Smahi *et al* 2020). The APTT is performed on platelet-poor plasma from a peripheral blood sample collected into 0.9% sodium citrate anticoagulant. The plasma aliquot is warmed to 37° C, and the APTT reagent,

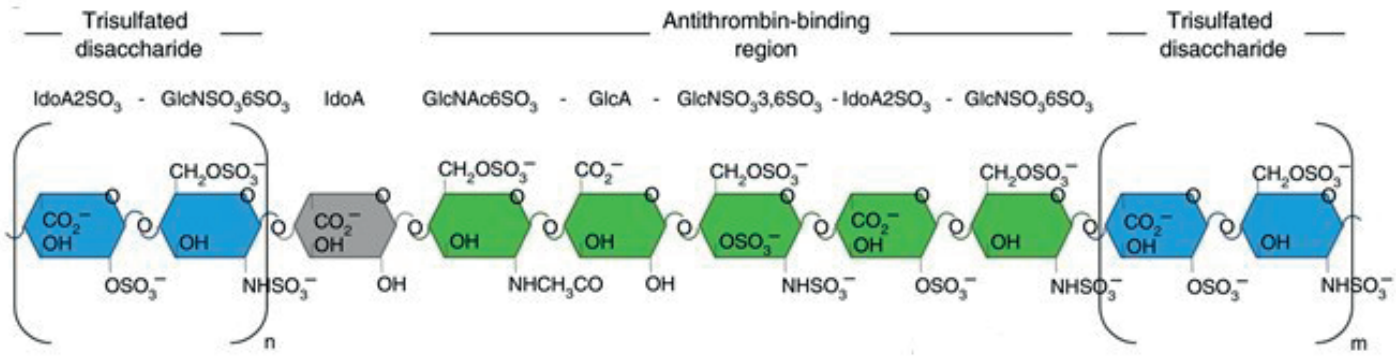

Figure 1. *Molecular structure of heparin. Heparin is an unbranched glycosaminoglycan consisting of iduronic acids (IdoAs), glucuronic acids (GlcAs) and glucosamines (GlcNs) with varying sulfation and acetylation patterns. Its anticoagulant properties depend on the antithrombin-binding region, a specific pentasaccharide sequence that serves as the binding site for the serine protease inhibitor antithrombin, which inhibits coagulation proteases such as factor Xa and thrombin. (Wat et al 2018, J Thromb Hemost 16: 1510–1522). Reproduced with permission.*

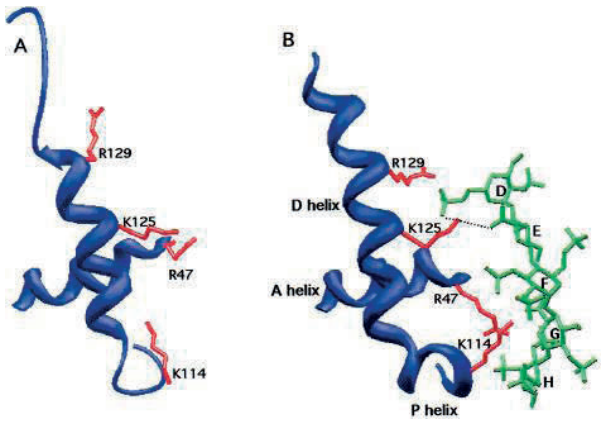

Figure 2. *Close up of the heparin binding site of antithrombin. (A) Antithrombin alone and (B) the antithrombin-pentasaccharide complex. The N-terminal end of the A helix, the D helix, and the P helix in the antithrombin-pentasaccharide complex and the corresponding regions in antithrombin alone are shown in blue. The side chains of the major pentasaccharide-binding residues (Arg47, Lys114, Lys125, and Arg129) are drawn in red. The pentasaccharide, DEFGH, with individual saccharide units denoted in alphabetical order from the nonreducing end, is represented in green. The interactions between Lys125 and the D and E units of the pentasaccharide are denoted with dotted lines. Drawn from PDB structures 2ant and 1azx. (Schedin-Weiss et 2002, 13, 14 Biochemistry 41: 4779–4788). Reproduced with permission.*

which contains a contact activator and phospholipid, is added and incubated for 3 – 5 mins to allow contact pathway activation. Calcium chloride is added to initiate the clotting process (Baluwala *et al* 2017). Figure 5 summarises the *in vitro* coagulation activation pathway leading to fibrin clot formation (Rasmussen *et al* 2020). The time in seconds from the addition of calcium to the formation of a fibrin clot is the APTT result (Baluwala *et al* 2017). Monitoring of UFH therapy using the APTT is relatively simple and cost effective (Smahi *et al* 2020). However, there are a number of factors that affect the APTT and its usefulness in heparin monitoring.

The APTT is highly dependent on preanalytical variables. The type of anticoagulant used, sampling tube composition and the timing of sample collection in relation to therapy can all affect the APTT result (Anderson and Saenko 2002). Patient factors such as elevated

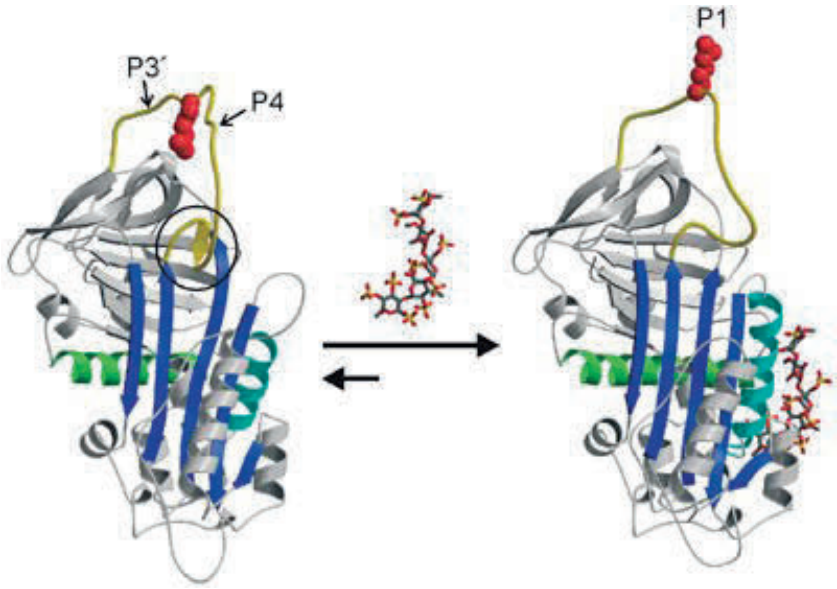

Figure 3. *The heparin-binding mechanism of AT. The structural features of native and pentasaccharide-activated AT are illustrated by ribbon diagrams. AT is shown in the classic orientation, with b-sheet A (blue) facing, helix A (green) in the back, the principal heparin-binding helix D (cyan) to the right, and the reactive centre loop (RCL) (yellow) at the top. RCL residues are numbered from the scissile P1–P10 bond towards the N- and C-termini, respectively, and the positions of theP4 and P30 residues are indicated. In native AT the N-terminal region of the RCL (hinge region, circle) is incorporated as strand 4 in b-sheet A, which constrains the RCL and the P1 Arg393 side chain (red space-filling). The heparin pentasaccharide (rods with gray C, red O, and yellow S) binds with contacts on helices A and D and induces local and global conformational changes in AT. Of particular relevance is the expulsion of the hinge region from b-sheet A, which predictably releases the constraints on the RCL and reorients the P1 side chain. (Johnson et al 2006, EMBO 25: 2029–2037). Reproduced with permission.*

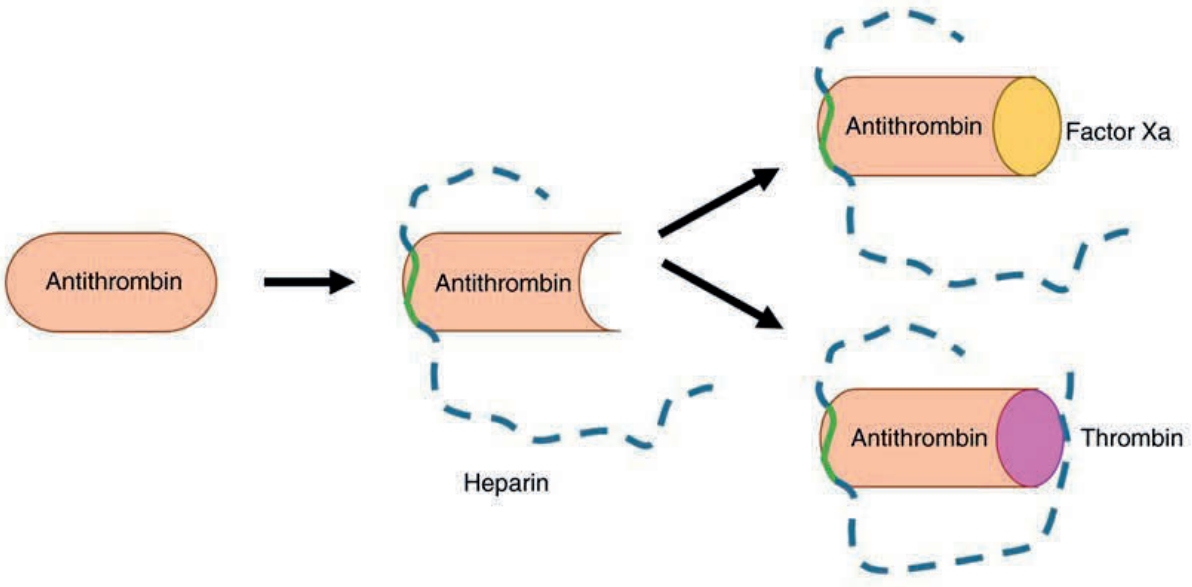

Figure 4. *Inhibition of FXa requires only the heparin-induced conformational change in antithrombin, inhibition of thrombin also depends on a certain length of heparin adjacent to the antithrombin-binding region to act as a bridge between the protease and antithrombin. (Wat et al 2018, J Thromb Hemost 16: 1510–1522). Reproduced with permission.*

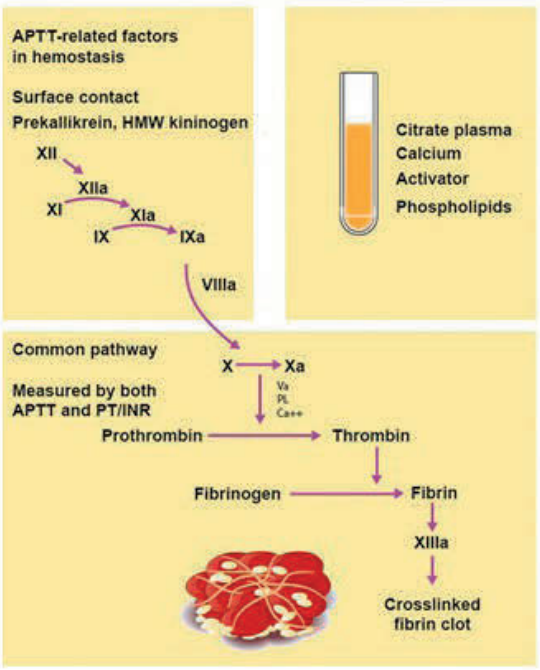

Figure 5. *Overview of the APTT related in vitro factor activation. Activation involves the coagulation factors of the intrinsic and the common pathway of the coagulation cascade. Initially, activation of contact factors (high-molecular-weight [HMW] kininogen, prekallikrein, factor XII, and factor XI) is achieved by addition of an activator, such as ellagic acid or silica. Deficiency of coagulation factors in the in vitro activation of the intrinsic and the common pathways leads to a prolongation of APTT. For an isolated prolonged APTT, the normal PT, however, indicates that the activation of the common pathway is intact. Prothrombin is factor II and thrombin is factor IIa. HMW, high molecular weight. (Rasmussen et al 2020 Eur J Haematol. 104: 519– 525). Reproduced with permission.*

haematocrit or anaemia can impact on the citrate-plasma ratio (McCraw *et al* 2010). Circadian variability of the APTT has been demonstrated by Budkowska *et al* (2019), with the most prolonged APTT values found at 8am, and the shortest APTT values found at 2pm. Other patient factors include the FVIII:c and fibrinogen levels of the patient, as well as increased levels of other acute phase proteins (Marlar *et al* 2017). Platelet activation during collection can cause release of platelet factor 4 (PF4), which results in heparin neutralisation *in vitro* and a risk of underestimation of heparin activity (Hardy *et al* 2020). Platelet activation can also occur with traumatic handling of the sample, such as vibration or agitation (McCraw *et al* 2010). PF4-mediated neutralisation of heparin can occur as rapidly as one hour post collection, meaning it is critical for the sample to be centrifuged and separated within this time if testing is to be delayed (Eikelboom and Hirsh 2006).

The APTT reagent used by the laboratory has a significant effect on monitoring of heparin therapy. APTT reagents will have different contact factor activators, phospholipid source and phospholipid concentration (Kershaw 2017). In particular, the relative proportion of phosphatidylserine in the phospholipid component of the APTT reagent is an important determinant of heparin sensitivity (Kitchen *et al* 1999). These variations lead to different sensitivities to UFH. In addition, different lots of APTT reagent will generally demonstrate different sensitivities to UFH. Each reagent lot number requires determination of the heparin therapeutic range to be used within that laboratory. Different clot detection methods (for example, optical versus mechanical clot detection) will also influence APTT values (Favaloro *et al* 2019). In patients with elevated levels of C-reactive protein (CRP) an acute phase reactant, APTT levels may be uninterpretable when using optical-based clot detection systems due to the formation of complexes between low-density lipoprotein and CRP in the presence of calcium (Hardy *et al* 2020).

The anti-Xa activity assay has been suggested as an alternative to the APTT for heparin monitoring. The principle of the anti-Xa activity is demonstrated in

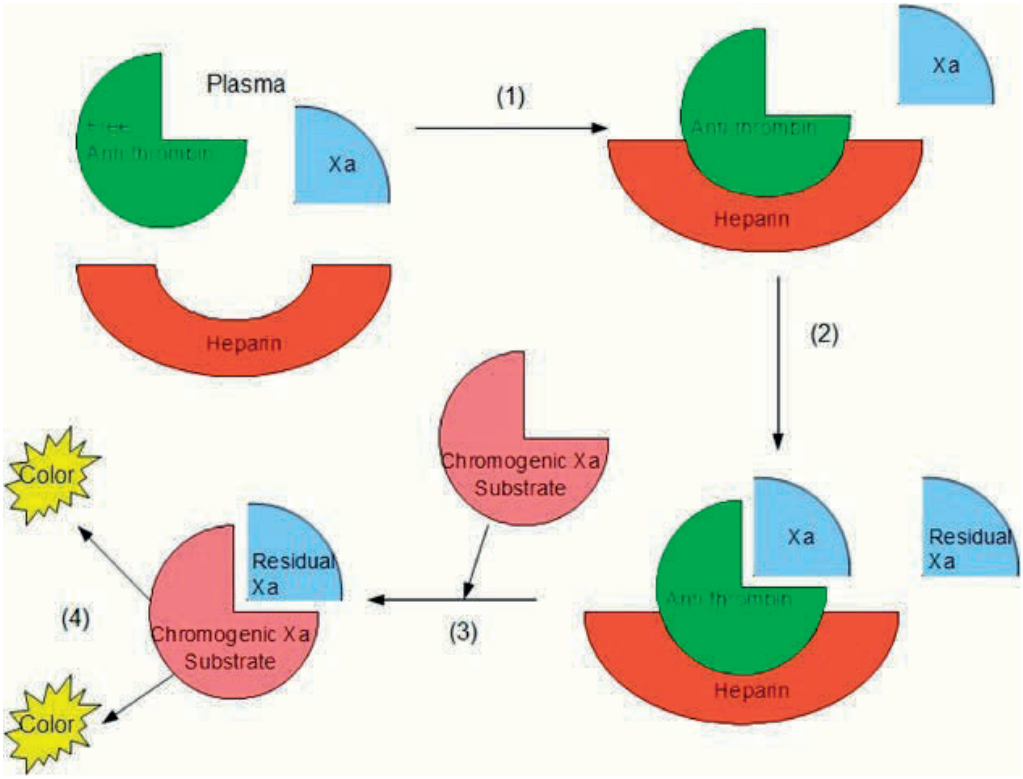

Figure 6. *Steps involved in the antifactor Xa assay for measuring the functional activity of heparin. (1) Plasma heparin binds to free antithrombin to form an antithrombin-heparin complex. Note that some antifactor Xa assays add an excess amount of free antithrombin. (2) Antithrombin-heparin complex binds to plasma factor Xa. (3) Chromogenic factor Xa substrate is added, which binds to residual Xa that remains unbound by antithrombin-heparin complex. (4) Binding of chromogenic Xa substrate to residual Xa produces color that can be measured optically. The quantity of the color produced is inversely proportional to the activity of heparin present. This is plotted against a standard curve, and results are expressed as units/ml of antifactor Xa activity and frequently quantitated as the heparin concentration. (Vandiver and Vandracek 2012, Pharmacotherapy 32: 546–558). Reproduced with permission.*

Figure 6. Briefly, citrate platelet-poor plasma is incubated with a known amount of Factor Xa and a chromogenic substrate specific for Factor Xa and the residual Factor Xa activity is measured. The residual Xa activity is inversely proportional to the concentration of heparin in the sample. For use in UFH monitoring, the anti-Xa activity assay must be calibrated using specific UFH/LMWH calibrators. The anti-Xa activity assay provides a direct measurement of heparin activity (Coons *et al* 2019). There is less interference from preanalytical factors, particularly PF4. Some anti-Xa activity reagents include dextran sulfate, which displaces heparin from its binding to non-specific proteins such as PF4 (Hardy *et al* 2020). One study by Toulon *et al* (2020) determined that the maximum delay for testing could be extended up to four hours when using the anti-Xa activity assay for heparin monitoring. The anti-Xa activity assay is also not affected by acute phase reactants or other clotting factors (Downie *et al* 2019). A therapeutic target range of 0.3 – 0.7 IU/mL of UFH has been recommended for the anti-Xa activity assay (Lehman and Frank 2009). The anti-Xa activity assay has also been associated with decreased time to achieve therapeutic anticoagulation, fewer dosage changes and reduced requirement for monitoring (Coons *et al* 2020). However there are some caveats to the use of the anti-Xa activity assay for UFH monitoring. Clinicians are less familiar with UFH dosing based on anti-Xa activity assays, the assay itself is less likely to be routinely available in hospital laboratories, and the cost of the assay is higher in comparison with an APTT assay (Hull *et al* 2021). The anti-Xa activity assay may also be affected by recent use of LMWH or direct oral anticoagulants and by interference from triglycerides or bilirubin, which should not impact APTT measurement by mechanical clot detection methods (McLaughlin *et al* 2019).

Heparin resistance

Heparin resistance is defined as a dose requirement of greater than 35000 units of heparin within a 24h period to reach the therapeutic range as determined by APTT measurement (Thota *et al* 2012). There are two recognised forms of heparin resistance: true heparin resistance, which reflects *in vivo* blunting of the anticoagulant effect of heparin, and apparent heparin resistance, where a subtherapeutic APTT persists despite therapeutic anticoagulation *in vivo* (Downie *et al* 2019).

Heparin resistance can be differentiated by comparing APTT and the anti-Xa assay on the same sample. In true heparin resistance, the APTT and anti-Xa results will both be lower than expected. In apparent heparin resistance, the anti-Xa assay will indicate results within the therapeutic range even though the APTT remains subtherapeutic (Downie *et al* 2019). Other assays which can assist in differentiating between true and apparent heparin resistance include AT, factor VIII and fibrinogen assays (Thota *et al* 2012). It is important to differentiate between true and apparent heparin resistance as this will guide clinical treatment. True heparin resistance can pose a significant risk for venous thromboembolism (VTE) (Fetters and Sirianni 2021). Apparent heparin resistance presents a peculiar dilemma as the *in vivo* UFH concentration is at sufficient anticoagulant levels but the monitoring is not appropriate. This may result in increased administration of UFH, leading to supratherapeutic heparin levels and subsequent possible bleeding complications (Durrani *et al* 2018).

There is some blurring of the lines between true and heparin resistance however, Uprichard *et al* (2010) describe how high factor VIII levels can cause a heparin blunting effect when thromboelastometry testing is performed. They also suggest that the absence of APTT prolongation in UFH treatment when factor VIII levels are high may in fact reflect genuine heparin resistance.

Predictors of heparin resistance include age 65 or above, AT activity less than or equal to 60%, increased factor VIII and fibrinogen levels and platelets greater than 300 x 109/L (Durrani *et al* 2018). It has been suggested that heparin resistance in older patients could be due to changes in heparin binding to AT with age, and the development of an age-related generalised hypercoagulable state (Grichnik *et al* 1999).

True heparin resistance

The most common cause of true heparin resistance is AT deficiency (Thota *et al* 2012) which can be congenital or acquired (Bauer *et al* 2021). Congenital AT deficiency is further divided into type I deficiency, which is a quantitative deficiency where the AT antigen level is equal to the activity level, and type II deficiency which is a qualitative deficiency characterised by an AT activity level lower than the AT antigen level (Richard *et al* 2018). Type II AT deficiency is then further subclassified into whether the heparin binding site of AT is affected, the reactive site of AT is affected or whether there are multiple effects on both heparin binding and progressive AT activity (Bauer 2021). Acquired causes of AT deficiency are more common than inherited causes (Gausmann and Marlar 2017).

Acquired AT deficiency is found in a number of clinical situations. The use of UFH can reduce AT levels by as much as 30% (Durrani *et al* 2018). Sepsis can lead to an acquired AT deficiency and this is thought to be secondary to a downregulation in AT production, which is then aggravated by an increased plasma protein turnover (Hage *et al* 2019). Disseminated intravascular coagulation and acute thrombosis can cause AT deficiency through AT consumption. Haemodialysis and extracorporeal membrane oxygenation (ECMO) have both been associated with acquired AT deficiency (Bauer 2021). One mechanism for acquired AT deficiency in ECMO therapy is the adsorption of AT to circuit tubing (Sorial *et al* 2020). L-asparaginase therapy, a chemotherapy agent commonly used in treatment of acute lymphoblastic leukaemia is also a cause of acquired AT deficiency (Bauer 2021). L-asparaginase causes depletion of L-asparagine, leading to a global impairment of plasma protein synthesis including AT (Fulcher and Carrier 2020). Other causes of acquired AT deficiency include oral contraceptive and oestrogen use, surgery, trauma and liver disease (Bauer 2021).

Congenital AT deficiency is inherited in an autosomal dominant pattern, with variable penetrance (Bauer 2021). The AT protein is encoded by the SERPIN1C gene, located on chromosome 1q23-25. The SERPIN1C gene contains seven exons and six introns (Mulder *et al* 2017). The most common mutations causing AT deficiency are insertions, deletions and missense mutations. Type I, or quantitative, AT deficiencies are typically associated with insertions or deletions in the SERPIN1C gene (Schedin-Weiss *et al* 2002). These mutations have only been described in the heterozygous form, as homozygous type I AT deficiency is not thought to be compatible with life (Muszbek *et al* 2010). There is parallel reduction of antigen levels and functional activity usually ranging from 40–60% of normal (Bock 2013). Most of the genetic mutations result in reduced synthesis of AT. Other changes include signal peptide changes, impaired post-translational processing, or reduced AT stability (Bauer 2021).

Type II, or qualitative, defects are most commonly caused by missense mutations. There are three subtypes of type II AT deficiency. Type II reactive site (RS) AT variants have mutations which occur in or around the reactive site of AT

(Schedin-Weiss *et al* 2002). There are two distinct clusters of mutations in type II RS: those at residues Ala382 and Ala384 in the AT hinge region and those involving residues 392, 393 and 394 in the reactive domain (Patnaik and Moll 2008). These mutations are generally located near the thrombin binding site (Bauer 2021). Type II heparin binding site (HBS) AT variants are the most common type of AT variants (Bauer 2021). Lys114, Lys125 and Arg129 have been demonstrated to be "hotspots" for heparin binding to AT and conformational activation. Mutations in any one of these specific amino acids result in massive losses in heparin pentasaccharide binding (Richard *et al* 2018). Type II HBS AT mutations are associated with a much lower risk of thrombosis when compared to other AT variants (Schedin-Weiss *et al* 2002). The third subtype of type II AT deficiency, Type II pleiotropic (PE), involves a mutation at the carboxyterminal end of the AT molecule. These mutations can cause conformational changes in the protein and can induce multiple effects including reduction in both heparin binding and progressive AT activity (Bauer 2021). One unique type II mutation, antithrombin Cambridge II, results from a change in amino acid 416 from alanine to serine (Mulder *et al* 2017). In the presence of UFH, this mutation results in AT Cambridge II acting as a substrate for factor Xa and thrombin, instead of an inhibitor. This imparts a slightly greater thrombophilic risk when compared to other type II HBS mutations (Mushunje *et al* 2003).

Diagnosis and subtyping of AT deficiency requires the use of specific AT assays. The recommended screening test is a functional assay as antigen levels will be normal in Type II AT deficiency. Functional assays may be either anti-IIa or anti-Xa based (Khor and Van Cott 2010). Anti-Xa based testing is preferred, as falsely elevated results are demonstrated in human thrombin-based assays due to heparin cofactor II activity (Bohner *et al* 1994). These activity assays use heparin and the results depend on both the RS and the HBS of AT. If the functional assay is decreased, an antigen assay may be used to aid in differentiating the type of AT deficiency. Type I AT deficiency will demonstrate both antigenic and functional deficiency, while Type II AT deficiency will in general demonstrate reduced function but normal antigen levels (Khor and van Cott 2010). Type II PE mutations may demonstrate both reduced functional and antigenic AT levels, but the functional level will be lower than the antigen level. A variation of the functional assays may be used to help differentiate type II AT deficiency. Exogenous heparin is removed and the incubation time of the assay is extended to 300 sec. This variant assay measures AT function independent of the HBS. Type II HBS mutations demonstrate progressive function, while type II RS mutations do not (Khor and van Cott 2010). There is however one intriguing exception as AT Cambridge II presents in an unusual way in AT assays. Heterozygous patients cannot be detected by either functional or antigenic assays. In homozygous patients however and in the presence of unfractionated heparin, the results from IIa-based antithrombin assays are reduced by approximately 15%, when compared to results from Xa-based antithrombin assays (Corral *et al* 2007). There are over 127 genetic mutations known to confer antithrombin deficiency (Khor and Van Cott 2010) and definitive diagnosis of antithrombin deficiency, particularly type II deficiencies, is often only achievable by genetic analysis (Bravo-Pérez *et al* 2019).

Other causes of true heparin resistance include increased non-specific protein binding and increased clearance of heparin (Downie *et al* 2019). UFH is highly negatively-charged and can bind through electrostatic interactions to positively-charged plasma proteins (Baluwala *et al* 2017). These proteins include apolipoprotein B, histidine-rich glycoprotein, complement factor H, fibronectin, fibrinogen and PF4 (Ignjatovic *et al* 2010). Non-specific binding can also occur between UFH, platelets and activated endothelial cells. Many of these proteins are acute phase reactants and will be elevated in acutely ill patients (Eikelboom and Hirsh 2006). In the hepatic acute phase response, in addition to production of acute phase proteins, there is alteration in the transport of ions and changes in many metabolic pathways which may also affect UFH clearance (Liu and Ahearn 2011). Another suggested mechanism of heparin resistance in acutely ill patients is increased heparanase activity (Streng *et al* 2020). Heparanase is expressed by activated white blood cells and platelets and cleaves heparan sulfate (Nadir and Brenner 2014). It also demonstrates affinity for UFH (Streng *et al* 2020). Heparanase activity appears to be increased during inflammatory disease and based on its ability to cleave UFH may be implicated in the aetiology of heparin resistance (Streng *et al* 2020).

Apparent heparin resistance

The mechanisms of apparent heparin resistance, where the APTT will remain subtherapeutic despite adequate anticoagulation demonstrated by an anti-Xa activity assay, are incompletely understood. One of the more common suggested causes of apparent heparin resistance is elevated factor VIII levels (Thota *et al* 2012). Factor VIII is an acute phase protein which can be induced in response to a number of physiological stresses, including burns, bacterial infections and trauma. Following tissue injury, a systemic alteration in normal biological set points is induced, known as the acute phase response. The immune system is activated, in particular monocytes and macrophages, and an assortment of cytokines, or soluble signalling molecules, are secreted. The three major mediators of the acute phase response are interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor α

(TNFα). These are transcription factors which act through signal transduction pathways to upregulate transcription of a number of proteins involved in processes such as blood clotting, tissue remodelling and wound healing (Begbie 2000). IL-6 is particularly important in promoting transcription of the FVIII gene. Levels of FVIII mRNA have been demonstrated to be increased six to nine-fold in liver cell lines after IL-6 stimulation (Bittar *et al* 2014). High FVIII levels, e.g. ≥ 150%, are a known risk factor for thrombosis (Mohren *et al* 2016). Elevated FVIII levels also have a significant effect on the APTT. Mitsuguro *et al* (2015) determined that high FVIII concentrations caused shortening of the APTT by up to 50% in the presence of 0.5 U/mL UFH.

Elevated fibrinogen levels are another common finding in the acute phase response. The production of fibrinogen is also mediated by IL-6 (Brock *et al* 2011). Elevated fibrinogen levels can also shorten the APTT, but the effect is not as significant as the effect of elevated factor VIII (Mitsuguro *et al* 2015). Other preanalytical and analytical factors affecting the APTT, as previously discussed, may also contribute to apparent heparin resistance.

Heparin resistance in SARS-COV-2 infections

A novel coronavirus, severe acute respiratory syndrome coronavirus 2, or SARS-Cov-2, was identified in late 2019. This coronavirus rapidly spread globally to reach pandemic proportions by early 2020 (Cuker and Peyvandi 2021). The World Health Organisation has designated the disease caused by SARS-Cov-2 as COVID-19 (McIntosh 2021).

Coronaviruses are large, enveloped, single-stranded RNA viruses. SARS-Cov-2 has a diameter ranging from 60nm to 140nm, and is covered in distinctive spike proteins, which range in size from 9nm to 12nm. It is these spike proteins which bind to the angiotensin-converting enzyme 2 (ACE2) receptor and allow SARS-Cov-2 to enter into nasal and bronchial epithelial cells and pneumocytes (Wiersinga *et al* 2020). The SARS-Cov-2 virus then causes downregulation of membrane ACE2 expression (Iwasaki *et al* 2021). The replication of SARS-Cov-2 and subsequent cellular destruction activates the innate immune response (Luo *et al* 2021). This inflammatory response activates monocytes and macrophages and induces the release of cytokines (Zeng *et al* 2020). At the present time, 81% of confirmed COVID-19 cases show mild disease, 14% of cases present with severe disease, and a further 5% of cases exhibit critical disease (McIntosh 2021). An excessive immune response, known as "cytokine storm", is a feature of severe COVID-19 disease (Luo *et al* 2021). Elevation of inflammatory markers such as IL-6, serum ferritin and CRP have been shown to be significantly associated with the development of severe COVID-19 (Zeng *et al* 2020) The substantial inflammatory state evident in severe COVID-19 leads to significant haemostatic changes (Luo *et al* 2021). These haemostatic changes help create an intensely hypercoagulable state in patients with COVID-19 (Cuker and Peyvandi 2021).

Understanding of the hypercoagulable state evident in COVID-19 patients is still evolving (Hardy *et al* 2020). Both platelets and coagulation factors are directly involved in modulation of the host immune response, and display proinflammatory functions independent of their haemostatic effects (Ponzetto *et al* 2021). The major complication in severe COVID-19 is acute respiratory distress syndrome (ARDS) (McIntosh 2021). ARDS is associated with a substantial thrombotic risk due to expression of tissue factor on mononuclear cells such as monocytes and macrophages, as well as the inhibition of fibrinolysis from cytokines such as IL-1, IL-6 and TNFα (Hardy *et al* 2020). Platelets are activated and release their granules which increases plasma PF4 levels (Streng *et al* 2020). Increased levels of IL-6 in cytokine storms also lead to upregulated production of both FVIII and fibrinogen (Hardy *et al* 2020). One published reference range for FVIII is 0.45 – 1.58 IU/mL (Laffan and Manning 2017) but the average FVIII level in severe COVID-19 is markedly increased at 2.97 IU/mL. Normal fibrinogen concentration ranges from 2.0 to 4.5 g/L (Levy *et al* 2014) but these levels are also significantly increased with an average level of 6.80 g/L (Cuker and Peyvandi 2021). Contributory factors to the hypercoagulable state include the severe acute inflammatory process leading to sustained release of plasminogen activator inhibitor-1 (PAI-1), thus inhibiting fibrinolysis (Hardy *et al* 2020). Other suggested causes of hypercoagulation in COVID-19 include neutrophil extracellular traps, circulating prothrombotic microparticles and hyperviscosity (Cuker and Peyvandi 2021).

There is a high incidence of thrombosis in patients with severe COVID-19, with one hospital reporting a cumulative incidence of arterial and venous thrombosis of 30%. Anticoagulation therapy is therefore critical to the management of severe COVID-19. However, the rates of heparin resistance are also high, with reports of heparin resistance in as many as 80% of cases (White *et al* 2020). This is most likely due to apparent heparin resistance caused by elevated levels of FVIII and fibrinogen (White *et al* 2020) however an element of true heparin resistance may also be a contributor. While predominantly normal AT levels are generally found in severe COVID-19, there are high levels of platelet activation and release of PF4 (Streng *et al* 2020). This can lead to neutralisation of UFH *in vivo* and increase dose requirements.

The use of APTT for monitoring heparin use in patients with COVID-19 is also problematic. Transient antiphospholipid antibodies are a well-known phenomenon in viral infections and this can prolong the baseline APTT and confound UFH monitoring (Hardy *et al* 2020). A study by Kostousov *et al* (2021) also demonstrated that increased concentrations of CRP, as seen in severe COVID-19 infections, is associated with a small, but statistically significant prolongation of the APTT. This effect is enhanced in heparinised plasma samples. High levels of CRP may also affect the heparin sensitivity of the APTT reagent used through CRP binding to phosphatidylcholine (Kostousov *et al* 2021). There may also be interference by CRP on measurement of the APTT through complex formation with low density lipoproteins (Hardy *et al* 2020).

UFH therapy may have an alternate role in treatment of COVID-19. Long heparin chains in particular have been reported to demonstrate anti-inflammatory activity, by binding and neutralising inflammatory cytokines and acute phase proteins and there may also be a protective effect on endothelial cells (Hardy *et al* 2020). Neutrophil recruitment into tissues may be impaired, and the activity of neutrophil proteases such as human leukocyte elastase and cathepsin G may be inhibited (Hardy *et al* 2020). One hypothesis also suggests that heparin may hamper the interaction between SARS-Cov-2 and the host cell, thus reducing the rate of infected cells. More research needs to be conducted into these effects to learn more precisely what extent heparin is clinically effective in COVID-19 (Hardy *et al* 2020).

Conclusion

UFH is the most common anticoagulant used in the hospital setting and it is considered safe for use in all patient populations, including neonates, children and in pregnancy. The narrow therapeutic window of UFH and high patient response variability necessitates careful laboratory monitoring to ensure optimal patient care.

The most common method of monitoring UFH therapy is the APTT, which, while simple to perform and readily available in most coagulation laboratories, is also subject to several complicating factors including the phenomenon of heparin resistance. Determining the type of heparin resistance, whether true or apparent, is important to guide clinical treatment and ongoing laboratory monitoring. It is important for both clinicians and laboratory scientific staff to understand the mechanisms of both true and apparent heparin resistance, to ensure optimal care for patients.

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Checklist for conformity evaluation of reviewing requirements for internal auditing: a quality compliance tool for International Standard ISO 15189:2012 accredited medical laboratories

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Abstract

The aim of this paper was to develop a compliance tool for internal auditors to determine the conformity status of reviewing requirements (RevReqs) in International Standard ISO 15189:2012 accredited medical laboratories. The objectives include the elicitation of RevReqs and the development of a checklist for conformity evaluation of RevReqs for internal auditing. The relevant RevReqs in Clauses 4 and 5 of ISO 15189:2012 were identified by content analysis. The content analysis results were used for distribution analysis in the ISO 15189:2012 process based quality management system model in the value chain model as well as used as specific audit criteria for the RevReqs checklist for conformity evaluation. A total of 26/26 (100 %) RevReqs was elicited in ISO 15189:2012. The 26/26 (100 %) RevReqs were found distributed among the four stages of ISO 15189:2012 process based quality management system model, ranged from 3/26 (12 %) RevReqs in the process control, design and planning stage to 11/26 (42 %) RevReqs in the process evaluation and improvement stage. When the stages were allocated in the value chain model, the primary activities and costs component contains 7/26 (27 %) RevReqs and the support activities and costs component contain 19/26 (73 %) RevReqs. The elicited RevReqs were then used to develop the RevReqs for conformity evaluation. The findings presented in this study contribute to existing knowledge of ISO 15189:2012 accreditation compliance management by providing internal auditors with a RevReqs checklist to determine the conformity status in the medical laboratory.

Keywords: accreditation, management audit, quality improvement

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Introduction

The pathology services industry plays a critical role in the support of healthcare by providing diagnostic information. This has been highlighted recently by the emergence of the 'Wuhan virus' (Yuwei and Keyue 2020), officially known as the 'SARSCoV2' by the International Union of Microbiological Societies, pressuring the medical laboratory to produce competent results with enhanced effectiveness and efficiency. To support the medical laboratory in fulfilling the expected specifications, a management system could be implemented (Pallas and Bartlett 2016), such as International Standard ISO 15189:2012 [International Organization for Standardization (ISO) 2014] prepared by the ISO (Behets *et al* 2021) which is a Type B international organisation according to the Union of International Associations. ISO 15189:2012 is an international consensus management system standard (MSS) which can be used by the medical laboratory to demonstrate its competence and quality by undergoing an accreditation. ISO 15189:2012 is classified as Type A MSS that has been defined as 'MSS providing requirements', as defined in Item SL.2.5 of Directives ISO/IEC DIR 1:2021 [ISO and International Electrotechnical Commission (IEC) 2021] prepared by the ISO and the IEC (Behets *et al* 2021), and it has been used by 74/104 (71 %) accreditation bodies in 89/249 (36 %) countries as International Laboratory Accreditation Cooperation (ILAC) mutual recognition arrangement signatories to ISO 15189:2012 (ILAC 2022).

ISO 15189:2012 is also characterised by having 1515 conformance requirements (CReqs) (Mok *et al* 2015) and 119 administrative requirements (Mok and Chowdhury 2016) that can be used for accreditation implementation purposes by medical laboratories. The medical laboratory determines whether the processes meet its quality management system specifications by implementing evaluation and internal audit processes, as specified in Subclause 4.14 (Evaluation and audits) of ISO 15189:2012 with the direct support of management review (Mok *et al* 2020b), as specified in Subclause 4.15 (Management review) of ISO 15189:2012, to ensure processes are subject to regular reviews. The term 'review' has been defined as 'determination of the suitability, adequacy or effectiveness of an object to achieve established objectives', as defined in Item 3.10.3 of International Standard ISO 22886:2020 (ISO 2020, p. 8) prepared by the ISO. Despite the checking measures available to support the ISO 15189:2012 implementation, no previous study has investigated the development of a quantitative analytical tool for internal auditors to evaluate the reviewing requirements (RevReqs) in Clause 4 (Management requirements) of ISO 15189:2012 and Clause 5 (Technical requirements) of ISO 15189:2012. The primary aim of this paper, to provide internal auditors with a quantitative approach to the comprehensive evaluation of all relevant review-associated CReqs in ISO 15189:2012, was achieved as follows. First, a content analysis was performed on Clauses 4 and 5 of ISO 15189:2012 to identify RevReqs. All relevant reviewing actions were considered as RevReqs. The results were required to support the RevReqs checklist. Second, a quantitative analysis was performed on the content analysis results to develop the RevReqs checklist. Third, a distribution analysis was performed on RevReqs in the ISO 15189:2012 process-based quality management system model in the value chain model of the medical laboratory (Porter 1985; Mok 2017; Mok *et al* 2020b). Finally, a RevReqs conformity status map was developed to support the final interpretation of the quantitative analysis.

The findings should make a practical contribution to the ISO 15189:2012 internal audit field by allowing internal auditors to evaluate the effectiveness of reviewing processes. This is the first study to focus specifically on auditing RevReqs in the medical laboratory, therefore conformity evaluations of the RevReqs in Clauses 4 and 5 of ISO 15189:2012 and using the developed RevReqs checklist should permit internal auditors to ensure reviewing processes of the medical laboratory are effectively implemented. In addition, the RevReqs checklist could provide another means to ensure Rev-Reqassociated CReqs are competently implemented and in alignment with the medical laboratory quality management system specifications.

Materials and methods

Elicitation of reviewing requirements in Clauses 4 (Management requirements) and 5 (Technical requirements) of ISO 15189:2012 for the conformity evaluation

Relevant Rev-Reqassociated CReqs that could be used as audit criteria for the conformity evaluation were identified by the technique of content analysis. The content analysis was performed using ISO 15189:2012 due to its suitability to quantify requirements in International Standards (Mok *et al* 2015; Mok *et al* 2017). The specific areas of interest were primarily in Clauses 4 and 5 of ISO 15189:2012. The content analysis was prepared by adapting the procedure previously used by Mok *et al* (2020b). Briefly, all CReqs that are associated with reviewing actions were identified as RevReqs and were quantified according to their locations in Clauses 4 and 5 of ISO 15189:2012.

Selection of graphical symbols for use in the development of conformity evaluation of reviewing requirement checklist

Relevant graphical symbols (n = 4) were selected from International Standard IEC 60417:2002 DB (IEC 2002), prepared by the IEC, to present information in the development of conformity evaluation of RevReqs checklist.

The following symbols were selected: IEC 604175662 (DB:200210), IEC 604175664 (DB:200210), IEC 604176334A (DB:201506) and IEC 604176334B (DB:201506).

Distribution analysis of reviewing requirements in the ISO 15189:2012 process-based quality management system model in the value chain model

The distribution analysis was performed using the content analysis results, especially the RevReq locations in Clauses 4 and 5 of ISO 15189:2012. The distribution analysis was prepared by adapting the procedure previously used by Mok *et al* (2020b). Briefly, the distribution is analysed in the ISO 15189:2012 process-based quality management system model in the value chain model of the medical laboratory.

Development of conformity evaluation of reviewing requirement checklist and conformity status map

The conformity evaluation checklist was developed using the content and distribution analysis results. The checklist was prepared such that the internal auditor could record results quantitatively for interpretation. This is supported by the conformity status map that was prepared by adapting the procedure previously used by Mok *et al* (2020b). The map could provide an interpretation of final results using three-colour-coded grading. The colour-coded grading was developed according to International Standard ISO 11429:1996 (ISO 1996) prepared by the ISO.

Results

Quantitation of reviewing requirements in Clauses 4 and 5 of ISO 15189:2012

Content analysis was used to elicit the RevReqs in Clauses 4 and 5 of ISO 15189:2012. Clauses 4 and 5 of ISO 15189:2012 was found to contain a total of 26/26 (100 %) RevReqs. Clause 4 of ISO 15189:2012 contains 16/26 (62 %) RevReqs. Clause 5 of ISO 15189:2012 was found to contain 10/26 (38 %) RevReqs. The overall range was 1/26 (4 %) RevReqs to 4/26 (15 %) RevReqs in Subclause 4.14 (Evaluation and audits) of ISO 15189:2012 and 4/26 (15 %) RevReqs in Subclause 5.5 (Examination processes) of ISO 15189:2012 (Figure 1).

The frequency of reviewing requirements in the ISO 15189:2012 process-based quality management system model

The ISO 15189:2012 process-based quality management system model comprises four stages. The strategic management stage was found to contain 5/26 (19 %) RevReqs (Mok and Chowdhury 2019), the process control, design and planning stage was found to contain 3/26 (12 %) RevReqs (Mok and Chowdhury 2020), the process evaluation and improvement stage was found to contain 11/26 (42 %) RevReqs (Mok *et al* 2020a), and the analytical processes stage was found to contain 7/26 (27 %) RevReqs (Figure 1).

The frequency of reviewing requirements in the value chain model

The analytical processes stage, containing 7/26 (27 %) RevReqs, operates within the primary activities and costs component of the value chain model; and, the strategic management stage, the process control, design and planning stage, and the process evaluation and improvement stage, containing 19/26 (73 %) RevReqs, operate within the support activities and costs component of the value chain model (Figure 1).

Conformity evaluation of reviewing requirement checklist

The RevReqs checklist was developed using the 26/26 (100 %) RevReqs in Clauses 4 and 5 of ISO 15189:2012 (Figure 2). The final score could be expressed quantitively and ranged from 1/26 (4 %) RevReqs to 26/26 (100 %) RevReqs (Figure 2).

Reviewing requirement conformity status map

The RevReqs conformity status map was prepared by adapting the management review input conformity status map, as previously developed by Mok et al (2020b) (Figure 2). The three-colour-coded grading was designed in accordance with the scheme for colours of visual signals, as specified in Subclause 5.3 (Scheme of visual signal colours) of ISO 11429:1996 (ISO 1996).

Discussion

The present study was designed to develop a practical tool for trained internal auditors to perform conformity evaluation of RevReqs in Clauses 4 and 5 of ISO 15189:2012 for medical laboratories claiming compliance to these standards. The developed conformity evaluation tool in the format of checklist has the potential to provide an additional support to the evaluation and internal audit processes (Kohl 2020), as specified in Subclause 4.14 of ISO 15189:2012, providing the checklist is used at planned intervals in conjunction with internal audit tools for assessment of ISO 15189:2012 CReqs and other requirements established by the medical laboratory. The conformity evaluation of RevReq results could also provide input to the laboratory management for management review purposes, as specified in Subclause 4.15.2 d) of ISO 15189:2012.

The use of the conformity evaluation of the RevReqs checklist has three technical considerations and implications that need to be considered by the medical laboratory. First, the medical laboratory should be using trained internal auditors for the conformity evaluation of RevReqs. There are no specific obligatory or regulatory requirements however governing how to train internal auditors. Current applicable guidance documents do not generally specify training requirements for medical

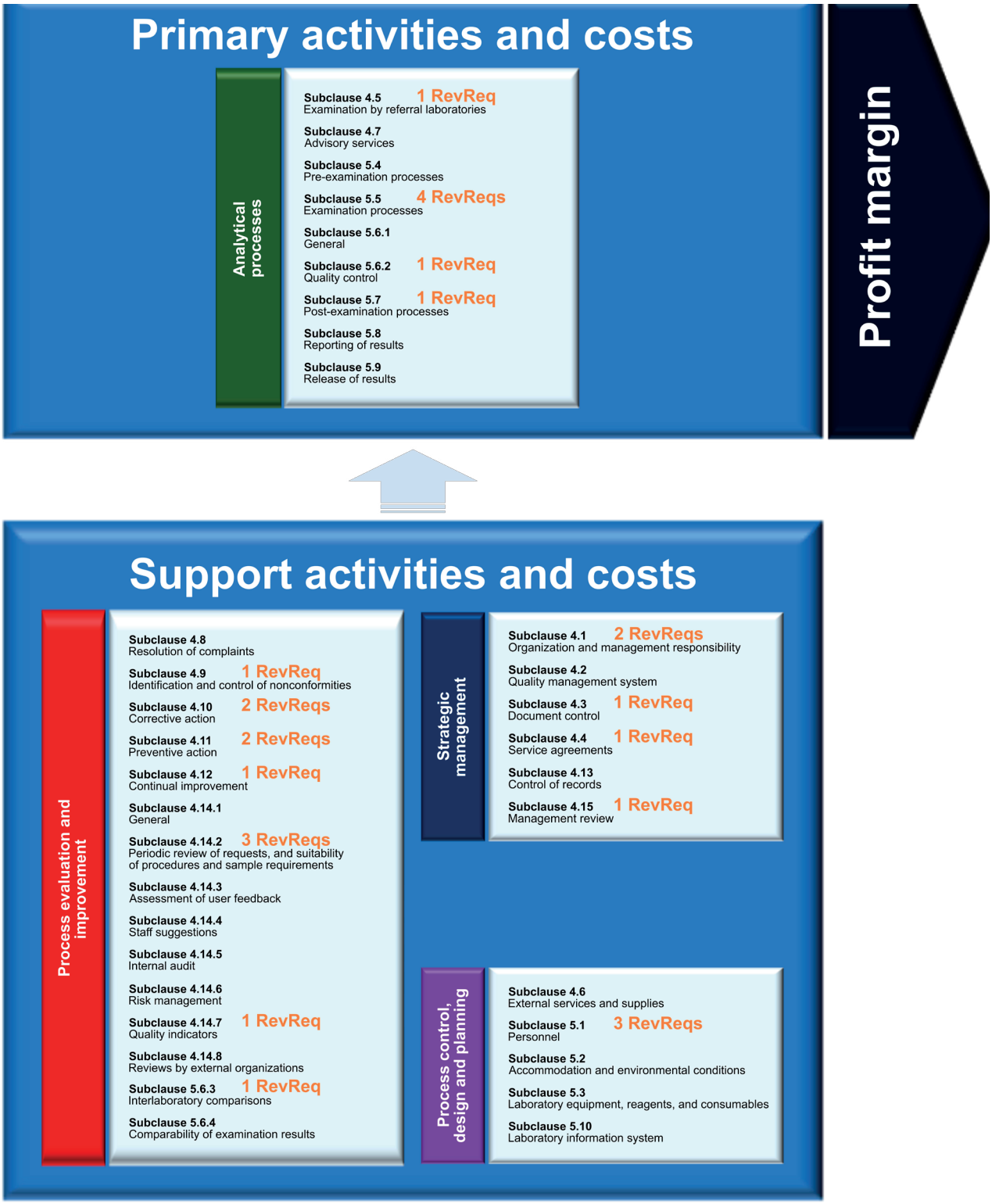

Figure 1. *Distribution of reviewing requirements among the four major stages of ISO 15189:2012 process based quality management system model in the value chain model.*

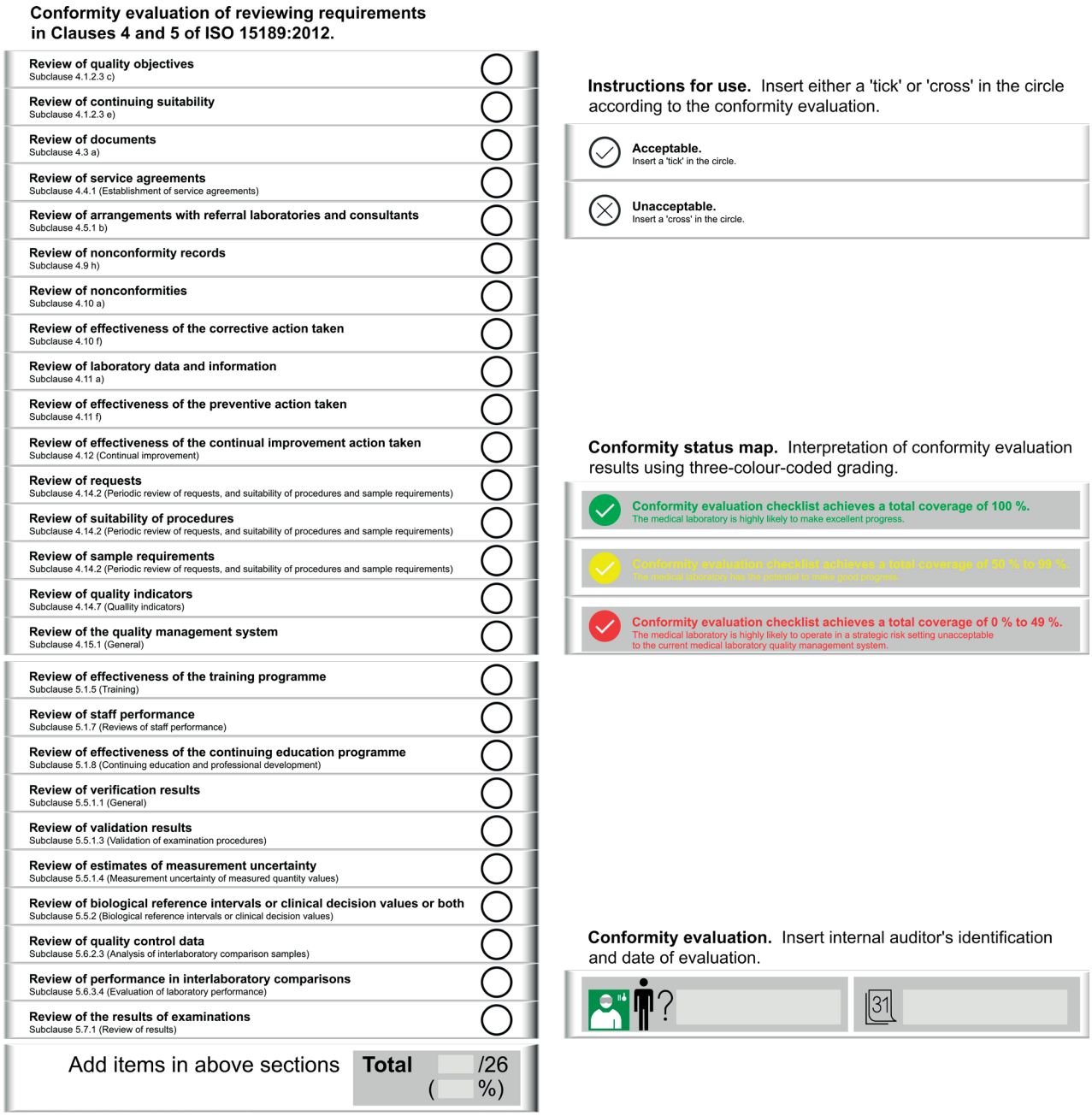

Figure 2. *Conformity evaluation of reviewing requirements in Clauses 4 and 5 of ISO 15189:2012 checklist. The first column entitled 'Conformity evaluation of reviewing requirements in Clauses 4 and 5 of ISO 15189:2012' requires the internal auditor to indicate the grade of either 'acceptable' or 'unacceptable'. The internal auditor then needs to sum up the number of acceptable grades for the percentage calculation. The second column entitled 'Instructions for use' requires the internal auditor to insert either a 'tick' [Symbol IEC 60417 6334A (DB:2015 06)] or 'cross' [Symbol IEC 60417 6334B (DB:2015 06)] in the first column. The second column entitled 'Conformity status map' supports the interpretation of conformity evaluation results by a three colour coded grading. Green highlights that the conformity evaluation checklist indicates a total coverage of 100 %. Yellow highlights that the conformity evaluation checklist indicates a total coverage of 50 % to 99 %. Red highlights that the conformity evaluation checklist indicates a total coverage of 0 % to 49 %. The second column entitled 'Conformity evaluation' requires the internal auditor to indicate: the internal auditor's 'Person identification' [Symbol IEC 60417-5664 (DB:2002 10)] and the 'Date' [Symbol IEC 60417 5662 (DB:2002 10)] that the evaluation was performed.*

laboratory internal auditors. Therefore it is important for the medical laboratory to establish internal guidelines and measures for the selection of trained internal auditors to conduct internal audits. The medical laboratory should carefully weigh up auditing competencies of internal auditors to make reasonably practicable decisions to ensure that all practices are compliant and that action is taken immediately if they are not. This point is quite important, particularly if the internal auditors are required to use procedures and tools that are assigned by the medical laboratory, as then the medical laboratory must provide relevant training to enable internal auditors to function with an appropriate level of scientific certainty, as specified in Subclause 5.1.5 b) of ISO 15189:2012. This however assumes that internal auditors are tasked to meet obligations relating to the medical laboratory established evaluation and internal audit processes, as specified in Subclause 4.14.1 (General) of ISO 15189:2012. The developed checklist should be used by internal auditors who are familiar with the processes, but it is important to note that this may restrict the impartiality and objectivity of the internal audit process. Nevertheless, the proposed structured approach should counterbalance the availability and suitability of ways to minimise the risk of producing unreliable auditing observations. From a utilitarian perspective, the selection of suitable internal auditors using the developed checklist should produce optimised auditing results that address the areas of vulnerability in the medical laboratory quality management system. An implication of this is the possibility of consulting with other international organisations to support the establishment of internal guidelines and measures for the selection of trained internal auditors to conduct internal audits. Although the International Standard ISO 19011:2018 produced by the ISO has detailed the competence requirements that auditors need to achieve, as specified in Subclause 7.2 (Determining auditor competence) of ISO 19011:2018 the '*one size fits all*' solution may not be adequate to address the issue. Another international organisation, the International Personnel Certification Association (IPC) has developed a management system auditor's certification scheme specifying the knowledge and skills for medical laboratory internal auditors (IPC 2020). This scheme should be consulted to support the development of internal guidelines and measures. Overall, the medical laboratory cannot adopt a shortsighted approach as it is clearly preferable to implement well considered programs that are in alignment with the overall quality policy deployment (Lee *et al* 2016).

Secondly, the most significant finding of the present study is that the majority of RevReqs (19/26 (73 %) were identified in the support activities and costs component of value chain. There were 3/26 (12 %) RevReqs in the support activities and costs component incorporated the process control, design and planning stage, 11/26 (42 %) in the process evaluation and improvement stage and 5/26 (19 %) found in the strategic management stage. It is important to note that 8/19 (42 %) RevReqs in Subclauses 4.3 a), 4.4.1, 4.9 h), 4.10 a), 4.10 f), 4.11 a), 4.11 f) and 5.6.3.4 of ISO 15189:2012 that are located in the support activities and costs component belong to CReqs for documented procedures, whereas the majority of RevReqs 11/19 (58 %) in Subclauses 4.1.2.3 c), 4.1.2.3 e), 4.12, 4.14.2, 4.14.7, 4.15.1, 5.1.5, 5.1.7 and 5.1.8 of ISO 15189:2012 do not belong to CReqs relating to documented procedures. Therefore, special attention is required by internal auditors to seek relevant audit evidence to classify the results as acceptable. The medical laboratory needs to ensure the support activities and costs component RevReqs are implemented properly, so that the primary activities and costs component that contains the analytical processes stage is supported competently. In the primary activities and costs component, the majority of RevReqs 6/7 (86 %) in Subclauses 5.5.1.2, 5.5.1.3, 5.5.1.4, 5.5.2, 5.6.2.3 and 5.7.1 of ISO 15189:2012 do not belong to CReqs relating to documented procedures. If the core component of the value chain is not supported properly, then it can be safely assumed that this is detrimental to the efficiency of producing optimised outputs (Martin 2019). Overall, the results of this study have established a structured approach that could be used by the medical laboratory to achieve optimisation of the quality management system (Du *et al* 2009).

Thirdly, the selection of a checklist format from among other tools has further considerations that need to be applied by the internal auditor (Dale *et al* 2016). It is important note that the use of documented information to support internal audits is highly recommended because results can more accurately reflect the effectiveness of the process being audited, as recommended in Subclause 6.3.4 (Preparing documented information for audit) of ISO 19011:2018 (ISO 2018). The checklist format is a practical tool for the provision of internal auditing for the medical laboratory (Mok *et al* 2018; Mok *et al* 2021); for example, it can produce quick quantitative results to determine compliance risk. The first potential implication for compliance is that the medical laboratory must conduct internal audits to determine whether all activities in the quality management system conform to specifications, as specified in Subclause 4.14.5 (Internal audit) of ISO 15189:2012. The referred activities must include the evaluation and internal audit processes, therefore the internal audit activities associated with the conformity evaluation of the RevReqs checklist must also be subject to internal audits. The second potential implication for compliance is that the conformity evaluation of the RevReqs checklist can be used conveniently as a record that can be treated as evidence of compliance (Minami *et al* 2017). In fact,

record keeping of all relevant internal audit information is a specific record requirement applicable to material evidence of compliance, as specified in Subclause 4.13 r) of ISO 15189:2012. The internal audit records can also be presented as review input to management review, as specified in Subclause 4.15.2 d) of ISO 15189:2012 as well as external organisations for audits or investigations, if required, as specified in Subclause 4.14.8 (Reviews by external organizations) of ISO 15189:2012. The third potential implication for compliance that relates to the use of the conformity evaluation of the RevReqs checklist, which has potential to be a negative implication for internal auditors is unfamiliarity with the collection of intangible evidence of compliance. The primary activities and costs component [6/7 (86 %) RevReqs] and the support activities and costs component [11/19 (58 %) RevReqs] contains RevReqs that do not belong to CReqs relating to documented procedures, therefore special attention is required for the selection of internal auditor who has suitable skills to obtain relevant evidence of compliance. Ideally, the internal auditor should have been trained in the application of different qualitative audit methods in order to achieve the audit objectives (Hignett and McDermott 2015), as specified in Annex A1 (Applying audit methods) of ISO 19011:2018 (ISO 2018).

The present study was designed to develop a tool for ISO 15189:2012 accredited medical laboratories to conduct internal audits to ensure all review associated practices are comprehensively evaluated to a reasonably practicable extent. The medical laboratory internal auditors should be able to use the conformity evaluation of the RevReqs checklist to conduct internal audits and provide relatively quick quantitative audit results and present reports competently to laboratory management and the medical laboratory at an expert level, providing relevant audit methods are used to obtain evidence of compliance (Nikolova 2019). This is the first study to focus on auditing RevReqs in the medical laboratory and it may enable internal auditors to enhance the reliability and validity of audit performance. Overall, the conformity evaluation of the RevReqs checklist has been developed to support internal auditors in ensuring the RevReqs relating to ISO 15189:2012 accredited medical laboratory practices are competently meeting the relevant specifications. Gaining an indepth understanding of the medical laboratory compliance status can limit its exposure to liability and nonconformity risks. A positive implication of this research is the possibility of developing more internal audit tools that are reasonably practicable for specific practices that are being implemented by the medical laboratory.

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CASE STUDY

Hypodysfibrinogenaemia: a diagnostic challenge

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Abstract

Congenital fibrinogen disorders are caused by genetic mutations in the fibrinogen molecule. These mutations result in a qualitative and/or quantitative range of disorders that can demonstrate diverse clinical outcomes for the patient. This is a case of a 41-year-old asymptomatic female who initially gave undetectable results for a routine coagulation screen. This could lead in some cases to a potential misdiagnosis. Additional testing in a central laboratory using a range of techniques including light transmittable and electromechanical methods was required to provide accurate fibrinogen determination. Immunological analysis of fibrinogen levels revealed a diagnosis of hypodysfibrinogenemia. This case highlights the issues faced in diagnosing patients, especially in regional laboratory settings where often only a single fibrinogen determination method is available. The role of a central laboratory to support and confirm initial diagnosis using multiple analysis methods is demonstrated here.

Keywords: hypodysfibrinogenemia, congenital fibrinogen disorders, diagnosis

Introduction

Fibrinogen disorders can be either acquired or congenital disorders. Acquired hypofibrinogenaemia is due to either reduced fibrinogen production or increased fibrinolysis. This can be caused by conditions such as disseminated intravascular coagulation, surgery, trauma, or asparaginase therapy. Acquired dysfibrinogenaemia results from a disparity between total fibrinogen and functional fibrinogen seen in liver disease, myeloma, and systemic lupus erythematosus (SLE) (Besser et MAcDonald 2016). Abnormalities in the fibrinogen molecule arise from a diverse range of genetic mutations in FGA, FGB and FGG genes on chromosome 4 and are causative for a range of congenital fibrinogen disorders. Their classification has been updated in recent years from recommendations of the Subcommittee on Factor XIII and Fibrinogen for ISTH (Casini *et al* 2018). This classification encompasses the following quantitative and qualitative fibrinogen disorders with correlating clinical outcomes (Table 1).

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In order to highlight some of the issues in diagnosing these conditions, a case of hypodysfibrinogenemia that required extensive testing by multiple methods across regional, central and reference laboratories to obtain the correct diagnosis is presented.

Case report

A 41-year-old female presented for routine coagulation testing and lupus anticoagulation testing at a regional centre following a telehealth consultation. The patient had presented with joint pain in hands and an elevated rheumatoid factor. The patient was not on any anticoagulants and had a normal platelet count. A coagulation screen consisting of prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and thrombin clotting time (TCT) were tested on a Sysmex CA-660 analyser, which utilises a photo-optical clot detection method. Each test reported a "No coagulation" instrument error, indicating a valid endpoint could not be determined in the allotted analysis read time.

Initial troubleshooting involved verifying pre-analytical patient and sample details including assessing for the presence of preanalytical specimen activation or clotting, lipaemia or haemolysis, confirming the collection time, assuring correct vial fill level and the correct specimen type;

Table 1. *Classification of qualitative and quantitative fibrinogen disorders*

Type 1 - Afibrinogenemia (functional fibrinogen levels <0.1g/L)
1A - Bleeding or Asymptomatic
1B -Thrombotic
Type 2 - Hypofibrinogenemia
2A - Severe (functional fibrinogen levels <0.5g/L)
2B - Moderate (functional fibrinogen levels 0.5-0.9g/L)
2C - Mild (functional fibrinogen levels 1.0 g/L - normal range lower limit)
2D - Fibrinogen storage disease (histologically proven hepatocyte fibrinogen accumulation)
Type 3 - Dysfibrinogenemia
3A - Bleeding/Thrombotic or Asymptomatic
3B - Thrombotic Related*
Type 4 - Hypodysfibrinogenemia
4A – Severe (antigenic fibrinogen levels <0.5g/L)
4B - Moderate (antigenic fibrinogen levels 0.5-0.9g/L)
4C – Mild (antigenic fibrinogen levels 1.0g/L - normal range lower limit)

**Specific Mutations of Fibrinogen Dusart, Fibrinogen Caracas V, Fibrinogen Ijmuiden, Fibrinogen New York I, Fibrinogen Nijmegen, Fibrinogen Naples (homozygous), Fibrinogen Melun OR a thrombotic episode with a first-degree familial thrombotic history*

all of which were acceptable. However, due to the grossly abnormal results and disparity with patient presentation, a second sample was collected to confirm results.

This sample was processed again for a coagulation screen and the same instrument errors occurred. The sample was double spun, aliquoted, frozen and transported to the central laboratory for further evaluation.

Analysis in the central laboratory was performed on a Sysmex CS5100 analyser, which also utilises a photo-optical clot detection method. All assays again resulted in "No coagulation" instrument errors. The fibrinogen assay was performed at multiple dilutions with no change in results. Reptilase time and lupus anticoagulant testing also did not generate valid results. An APTT mix (1:1) with normal plasma produced a result of 30sec. A D-dimer test was slightly elevated at 0.42µg/mL FEU (RR 0.19 - 0.40) As an investigative measure, a complete panel of clotting factors were analysed, and all results were within normal limits.

As part of the investigation, a basic manual test was performed in order to determine the presence of any fibrinogen. A suspension of thrombin (Dade Thrombin reagent-2.5 NHI units/ml) was prepared at ten times the standard strength used for routine TCT testing, and a TCT was performed in a 37°C water bath. This resulted in visual confirmation of a transparent, bulky clot indicating the presence of fibrinogen. The PT, APTT and fibrinogen tests were then processed on a STart MAX (Stago) instrument, which utilises electromechanical clot detection. The results obtained were PT 18s (RR 12-14s), APTT 39s (RR 24-38s) and Clauss fibrinogen level of 0.35g/L (RR 1.8-4.2g/L). The sample was then sent to a reference laboratory for immunological fibrinogen, which gave a level of 1.1 g/L. The rare diagnosis of hypodysfibrinogenemia was made on reduced levels of fibrinogen and the discrepancy between the functional level of 0.35g/L and the antigenic value of 1.1g/L.

The patient's other laboratory tests, medication, and clinical history were reviewed. The liver function tests were normal. The patient had previously delivered two children without complication and there were no indications of DIC. On this basis, it was concluded it was more likely to be congenital rather than an acquired disorder.

Discussion

This case identified the limitations of single platform testing in regional areas in enabling an accurate diagnosis for this patient. The ability to refer to a central laboratory for broader investigation allowed an accurate and timely diagnosis for the patient.

When diagnosing patients like this one, it is important to consider all potential causes of error in methods that use the function of fibrinogen to determine its total levels in plasma (eg. Clauss methods). These methods were used on different platforms in the regional and central laboratories, with varying methods of clot detection. The differences in various types of coagulation reagent and/or analyser used can affect results in qualitative fibrinogen disorders as described by Jennings *et al* (2017). Further examples can be seen in studies by Vasse *et al* (2020) where using bovine thrombin vs human thrombin reagents could be shown to dramatically alter results.

Two Sysmex series analysers with varying optical clot detection methods provided the initial workup for this patient. A regional CA series analyser utilised a Clauss fibrinogen assay with photo-optical clot detection methods, where light illuminates the sample and measures the changes in scattered light intensity as turbidity is increased at 660nm.

In contrast, the CS series analyser at the central laboratory operates with transmitted light detection methods from a source of 5 split light beams at varying wavelengths to detect clots. This allows for increased sensitivity for the influence of physiological interfering factors. Low fibrinogen levels in this setting can be measured at a variety of wavelengths. Despite these differences, the Clauss method on both platforms is known to have reduced sensitivity in dysfibrinogenemia and in situations of extremely reduced levels of fibrinogen (afibrinogenemia) as demonstrated in this case.

While the regional laboratory only had access to optical clot determination, which can have limitations in patients with dysfibrinogenaemia, the central laboratory provided both a confirmation method for the regional laboratory's optical determination and an alternative clot detection method using a Stago Start Max analyser, which utilises electromechanical clot determination for fibrinogen estimation.

In this case, while optical detection systems were unable to assess an endpoint due to the translucent nature of the clot (as demonstrated on manual testing), formation of a clot resulting in increased plasma viscosity was detected by the mechanical technique.

Results were therefore able to be generated for all coagulation screen tests. The functional fibrinogen level of 0.35g/L would be classified as a severe hypofibrinogenaemia (<0.5g/L). In comparison, the total fibrinogen result from the antigenic determination was 1.1g/L. The inconsistency in functional and total levels, indicates hypodysfibrinogenemia of a mild form (Type 4C). As the patient was asymptomatic and this was an incidental finding, further family studies including genetic if available, were recommended.

This rare presentation highlights variations in Clauss testing using various clot determination methodologies from light scatter/transmission to electromechanical, revealing a potential for misdiagnosis of fibrinogen disorders where only one method of determination is available. Therefore, in routine laboratories there must be alternative methodologies available, either on site or by a larger, central laboratory, in order to diagnose cases of hypodysfibrinogenemia. Confidence in robust systematic processes allowed regional laboratories to be supported by their central counterparts in making this rare diagnosis.

Acknowledgements

We extend our thanks for Robyn Coleman for assistance and testing of this patient study and for patiently teaching us and imparting her enthusiasm for coagulation.

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ORIGINAL ARTICLE

Who is educating our future Medical Laboratory Scientists? A perspective of AIMS accredited Medical Laboratory Science programs in Australia

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Abstract

The primary qualification to obtain employment as a Medical Laboratory Scientist is a university undergraduate degree in Medical Laboratory Science (MLS) or equivalent. University education for this degree is provided by academics who may not have a qualification in MLS or have experience as a Medical Laboratory Scientist. Due to the paucity of literature that describes the demographics of MLS academics, their qualifications, and previous experience as a Medical Laboratory Scientist, a descriptive cross-sectional study design was used to attempt to attain this information. Ten AIMS accredited academic programs participated in the study. Despite reporting experience in teaching over 100 MLS subjects, only a third of the academics had MLS qualifications and over half of the respondents had never worked in a pathology laboratory. Further research is needed to understand how this contributes to the employment outlook in the pathology industry and the graduate student outcomes.

Keywords: Medical Laboratory Science, pathology, tertiary education, academic, graduate

Introduction

The overarching requirement of a medical pathology service is to provide competent medical testing within a quality system that determines the cause and nature of diseases (Royal College of Pathologists of Australasia 2017). In Australia, Medical Laboratory Scientists contribute to this service in public and private pathology organisations. The number of people working as Medical Laboratory Scientists grew very strongly over 5 years from 16,200 in 2014 to 24,100 in 2019 and is likely to reach 28,400 by 2026 (Labour Market Insights 2022). The actual figures are an estimate as the number is extracted from data obtained when the ANSCO ID 2346 is applied, and this group can include other related industries. The primary university qualification is a three to four year undergraduate degree in Medical Laboratory Science (MLS), Medical Laboratory Science (Pathology), or Laboratory Medicine relevant to pathology which meets accreditation requirements by the Australian Institute of Medical and Clinical Scientists (AIMS). In Australia, at the end of 2021, there were 12 undergraduate and four postgraduate university programs that were accredited by AIMS (Australian Institute of Medical and Clinical Scientists 2021). The universities include: Central Queensland University, Charles Darwin University, Charles Sturt University, Curtin University, Griffith University, James Cook University, Murdoch University, Queensland University of Technology, RMIT University, University of South Australia, University of Tasmania, and the University of Southern Queensland.

University education is mostly provided by academics who teach general science and health subjects such as anatomy, physiology, and foundations in medical science. Some academics also specialise in MLS subjects such as clinical chemistry, haematology, transfusion science, medical microbiology, histopathology and have a research interest in MLS. However, not all academics that teach in MLS programs have a qualification in MLS or have experience as a Medical Laboratory Scientist in a pathology laboratory. Previous literature has reported the level of appointment and research track level of Australian MLS academics (Donkin *et al* 2020) however, there is a paucity of literature that describes the demographics of MLS academics, their qualifications, and previous experience as a Medical Laboratory Scientist.

A report by the Victorian Allied Health Workforce Research Program (Victorian Allied Health Workforce

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Research Program Medical Laboratory Science Workforce Report 2018) investigated the MLS workforce in Victoria where most Australian Medical Laboratory Scientists are employed (32% of all employed in Australia) (Labour Market Insights 2022). This study identified that the predominant qualification to practice as a Medical Laboratory Scientist was a directed MLS bachelor degree (64%, n=265) followed by 5% (n=19) entering the profession with a graduate entry MLS master's degree and the remaining having a range of other post-graduate qualifications including graduate certificates (n=21), graduate diplomas (n=51), masters degrees (management, research or other n=41), professional doctorates (n=8), and PhDs (n=24) (Victorian Allied Health Workforce Research Program Medical Laboratory Science Workforce Report 2018). This begs the question, who are the academics teaching MLS that cover all of these programs, and do they have a MLS qualification or relevant experience as a Medical Laboratory Scientist?

It has also been reported that graduate dissatisfaction with job environment and organisational structure needs to be addressed by academics who educate and train MLS students to prepare them for the workforce (Al-Enezi et al 2008) and there is no precise definition of the duties and responsibilities of the Australian Medical Laboratory Scientist (Badrick and St John 2012).

We anticipate knowledge attained from this study will inform the tertiary sector and AIMS on data of academics teaching in MLS programs in Australia and provide further data on student graduate outcomes and employability.

The specific research questions this study explored were:

1. What are the academic qualifications, teaching and MLS experience of MLS academic staff in Australia from 2019-2021?

2. What are the number of students enrolled and graduated in MLS programs in Australia from 2019-2021?

3. What are the employment outcomes for MLS graduates from 2019-2021?

Materials and Methods

A descriptive cross-sectional study design was used to recruit MLS academics and MLS program coordinators from AIMS accredited programs in Australia to collect quantitative and qualitative data through an online survey questionnaire. Participants were purposefully recruited through university AIMS accredited MLS program directories available on public websites. Written informed consent, built into the online survey, was obtained when participants completed the online survey questionnaire. Participation in this study was completely voluntary and participants could withdraw at any time. Human ethics was sought and approved for this study (University of the Sunshine Coast human ethics number A211550).

The survey collected academic characteristics such as the university academic program, level of appointment, previous education qualifications or previous work experience related to MLS, and years of experience, for example, the discipline area and years spent teaching or working as a Medical Laboratory Scientist in a pathology laboratory. Data on student graduate and employment outcomes were collated through the university MLS program coordinator where available.

Results

The survey was completed by 31 academic staff from 10 Australian AIMS accredited academic programs. The highest response rate was from Griffith University Bachelor of Medical Laboratory Science academic staff (n=9, 29%). The responders were employed as Program Directors or as academics from the level Lecturer to Professor. The number of years each academic had taught at university for their whole career (not just current employment) ranged from 1-20+ years with the most common years of teaching between 11-20 years (n=12, 39%).

Only nine (29%) academics had an undergraduate degree in MLS and not all were obtained from AIMS accredited academic programs. The universities and degrees obtained by the academics included RMIT, University of South Australia, Tasmanian College of Advanced Education (now known as the University of Tasmania), Queensland Institute of Technology (now known as Queensland University of Technology), University of Technology Sydney (Bachelor of Science Medical Science), and Curtin University (Bachelor of Applied Science in Medical Technology; Bachelor of Science Laboratory Medicine).

The highest level of education achieved by the academics was predominantly a PhD (n=28, 90%) followed by a Masters degree (n=3, 10%). However, over half of the respondents (n=17, 55%) had never worked as a Medical Laboratory Scientist or Technician in a private or public pathology laboratory. Of those academics that had worked in a pathology laboratory, nine (29%) had worked more than 10 years and five (16%) had worked 10 or fewer years in a pathology laboratory. For those that had experience in MLS, the field of work experience was widespread with most respondents reporting working across more than one discipline or multi-disciplinary including specimen reception. The most common disciplines of experience included Haematology (n=7, 15%), followed by Transfusion and Clinical Chemistry (both n=5, 11%), and then Clinical Microbiology (n=3, 6%). The least common areas of experience from the respondents were

in quality control (n=2, 4%) and Anatomical Pathology/ Cytology and Cytogenetics (n=1, 2%). No respondents had work experience in Molecular Pathology. At the time of completing the survey in 2021, only one academic was employed in an AIMS accredited program as an academic and was working as a Medical Laboratory Scientist, all other respondents were not currently employed as a Medical Laboratory Scientist.

Academic Teaching

Of the 31 academic respondents, accumulatively they taught into 100 university subjects. These included basic science subjects such as Biochemistry (n=9, 9%), Physiology (n=8, 8%), Pathophysiology (n=6, 6%), Genetics (n=4, 4%), Anatomy (n=4, 4%), Biosciences (n=3, 3%), Chemistry (n=1, 1%) and Biostatistics (n=1, 1%). Subjects specific to MLS programs included Pathology (n=5, 5%), Microbiology (n=4, 4%), Immunology (n=3, 3%), Haematology (n=7, 7%), Molecular Pathology (n=5, 5%), Histopathology (n=5, 5%), Work Integrated Learning (n=7, 7%), Transfusion Science (n=11, 11%), Biotechnology (n=3, 3%), Parasitology (n=2, 2%) and Cytology (n=1, 1%). Academics also taught into other subjects in non-MLS programs such as Medicine and Exercise Physiology.

Academics were asked to complete an open-ended question to reflect on factors that make it easy to teach MLS programs. The thematic analysis provided six themes: 1) Prior experience in the discipline or pathology industry; 2) Teaching team; 3) Practical based subjects with hands-on learning; 4) Relevancy of subjects to the profession and career pathway; 5) Engaged students; and 6) Accreditation standards. Academics were then asked to reflect on factors that make it difficult (harder) to teach MLS subjects. The thematic analysis provided 11 themes: 1) Lack of clinical pathology or industry experience; 2) Increased academic workload; 3) reduced teaching support; 4) Covid-19 pandemic; 5) Accreditation requirements; 6) Keeping current with new technologies and laboratory testing; 7) Lack of recognition for industry experience; 8) Finding and keeping experienced staff; 9) Teaching facilities; 10) Work-integrated learning opportunities; and 11) Undergraduate student entry-level requirements.

Nine of the academic staff had received teaching awards for their service in university teaching. These ranged from three academics with international Higher Education Academy awards (UK Professional Standards Framework for teaching and learning support in higher education) to national citations for the Australian Awards for University Teaching, Australian Government Office for Learning and Teaching Citation, Carrick Award for Australian University Teaching and UniJobs Lecturer of the Year Award top 10. One academic had received two local university awards for Excellence in Teaching.

MLS Graduate and Employment Outcomes

Of the 12 AIMS accredited academic programs invited to participate seven responded with student graduate enrolment and outcome measures. From the seven AIMS accredited academic programs in 2019, a total of 1,078 students were enrolled in MLS undergraduate degrees (UTAS only provided year 1 enrolment figures). In 2020 there was a meagre growth of 0.8% in enrolment to 1,087 students. In 2021 a growth in enrolment of 12.1% occurred with 1219 students enrolled in a MLS program which may have been attributed to a new program starting at Charles Sturt University Bachelor of Medical Laboratory Science (Pathology). Figure 1 details the number of students enrolled by program.

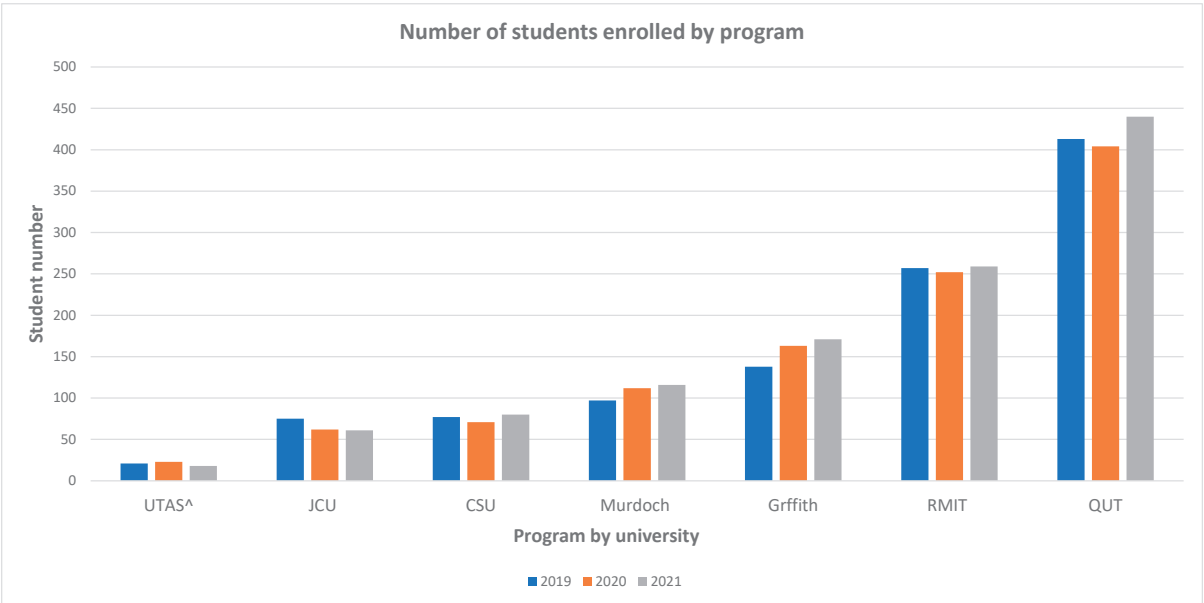

Figure 1. *Number of students enrolled by program across university affiliation (^UTAS year 1 data only)*

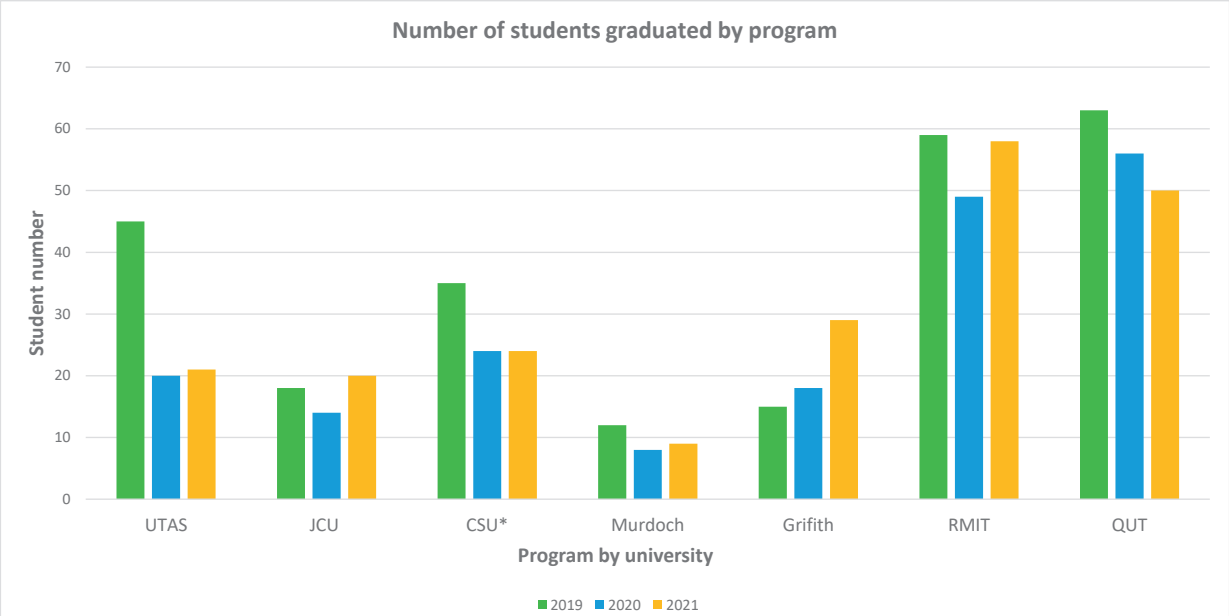

Figure 2. *Number of students graduated by program across university affiliation (*CSU new program in 2021)*

From the seven responding AIMS accredited academic programs in 2019 a total of 247 students graduated from MLS undergraduate degrees, while in 2020 (with the start of the Covid-19 pandemic in Australia) only 189 students graduated. Due to the timing of the survey at the end of 2021, not all universities had finalised their graduating numbers, the predicted 2021 student graduation number was 211. Figure 2 depicts the number of students graduated by program.

The number of graduates who had obtained full or part-time employment after graduating from an AIMS accredited academic program varied between each program. Comprehensive details of employers and examples of job specifications were incomplete. Where available Program Coordinators could identify examples of employment for 2019-2020 only. Examples included graduate positions with Pathology Queensland, multi-disciplinary Medical Laboratory Scientists and part-time roles in Covid-testing molecular laboratories. Employment was also reported in non-MLS industries such as fertility clinics or graduates enrolled in further research such as Honours and Higher Degree by Research Masters or PhDs after completing an undergraduate MLS program. Table 1 provides further details on each AIMS accredited academic program that responded to the survey.

Discussion

This study examined the academic characteristics of 31 staff from 10 Australian AIMS accredited academic programs. The academic qualifications in MLS and MLS experience reported by the respondents were low. Only nine (29%) academics had an undergraduate degree in MLS and over half of the respondents (n=17, 55%) had never worked as a Medical Laboratory Scientist or Technician in a private or public pathology laboratory. This highlights the importance of work-integrated learning in MLS programs to provide students with the specific bench skills required to perform pathology testing and the expertise provided by pathology Scientists and Technicians who provide this service on a daily basis.

This study also attempted to identify the number of students and graduates of MLS programs in Australia and the employment outcomes for these graduates. Of the 12 AIMS accredited academic programs invited to participate seven responded with student graduate enrolment and outcome measures. The total enrolled number of students from 2019-2021 ranged from 1078-1219 students, while the number of students graduating in 2019-2021 ranged from 189-247 students. The program durations were either 3.5 or 4 years full time and enrolment included both full-time and part-time data across all year levels in the program (except for UTAS who only supplied year 1 enrolment data).

Mindful that this study was conducted during the Covid-19 pandemic and educational and employment opportunities may have been disrupted, the enrolment data had modest growth in programs only at Murdoch and Griffith universities. The number of students graduating by program declined from 2019-2021 with the exception of the program at Griffith university and the limited data available from Charles Sturt University with the introduction of a new program. Identifying the reasons for the limited growth

Table 1. *Each AIMS accredited academic program that responded to the survey*

	Number of students enrolled	Number of students graduated	Full time employed MLS Scientist/technician	Part time employed	Examples of employment
	RMIT Bachelor of Biomedical Science (Laboratory Medicine) - 4 years full time				
2019	257	59	95%	n/a	n/a
2020	252	49	58%	21.00%	n/a
2021	259	58	n/a	n/a	n/a
	JCU Bachelor of Medical Laboratory Science (Honours) - 4 years full time				
2019	75	18	100%		Graduate Position PQ, Local SNP full time scientist
2020	62	14	100%		Graduate Position PQ, Local SNP full time scientist, full time HP3 PQ TSV
2021	61	20	n/a	n/a	8 students accepted to PQ Graduate Positions for 2022
	Murdoch University Bachelor of Science/Bachelor of Laboratory Medicine - 4 years full time				
2019	97	12	100%		Medical Scientist Special Biochemistry/Toxicology; Technical Assistant Diagnostic Genomics; Laboratory Assistant Histopathology; employment found in both private and public labs.
2020	112	8	100%		Medical Scientist Haematology (x3); Laboratory Technician Haematology; all employed in public sector
2021	116	9	n/a		Currently 7 are working part-time, mainly in COVID-testing molecular microbiology labs.
	Griffith Bachelor of Medical Laboratory Science - 4 years full time				
2019	138	15	100%		Majority in diagnostic labs including ADF and fertility clinics
2020	163	18	100%		2-3 enroled in a research by higher degree
2021	171	29	n/a		
	UTAS Bachelor of Laboratory Medicine - 3.5 years full time				
2019	21^	45	78%	n/a	About 20% Tasmanian Paths labs, remainder interstate or overseas
2020	23^	20	75%	n/a	About 20% Tasmanian Paths labs, remainder interstate or overseas
2021	18^	21	n/a	n/a	About 20% Tasmanian Paths labs, remainder interstate or overseas
	Charles Sturt University Bachelor of Biomedical Science (Honours) - 4 years full time				
2019	77	35	n/a	n/a	Many of our students are already employed usually as a Technical Officer or in Central Specimen Reception. Mostly
2020	71	24	n/a	n/a	distance students enrolled in our program so that they can upgrade to a Medical Scientist. A few progress into
2021	80	24	n/a	n/a	research degree. Most students get employed in science related jobs.
	Charles Sturt University Bachelor of Medical Laboratory Science (Pathology) - 3.5 years full time NEW PROGRAM				
2021	74	n/a	n/a	n/a	n/a
	QUT Bachelor of Medical Laboratory Science - 4 years full time				
2019	413	63	86%	n/a	Most students obtain positions primarily at SNP, QML, Pathology Queensland, Mater Pathology, Aquesta Pathology,
2020	404	56	71%	n/a	InfinityPATH, and IDEXX
2021	440	50	n/a	n/a	

^Year 1 only

in AIMS accredited MLS programs was beyond the scope of this study. However, a longitudinal study that captures accurate data from all Australian universities may provide this information. It could be postulated that students use the MLS programs as a springboard for other medical or allied health programs or research that prepares them in the field of health or science. Alternatively, students may enrol part-time in an MLS program or enrol with the view to obtain a high-grade point average (GPA) to apply for entrance into a medical program or assist in preparation for the Graduate Medical School Admissions Test (GAMSAT) (Mercer *et al* 2015).

A focussed plan to improve retention of MLS graduates and provide better support for the profession to improve graduate employability is required by both the university and AIMS. How this is to be achieved requires long term planning and was beyond the scope of this study. Graduates of AIMS accredited programs are classified as a Medical Laboratory Scientist ANZSCO 234611 however the onus is on the graduate to ensure that the university has complied with the AIMS accredited requirements (AIMS 2022). The AIMS website states "It is the responsibility of all prospective applicants who hold an AIMS accredited degree to ensure the subjects completed comply with the AIMS accreditation requirements in order to gain assessment as a medical scientist or Professional membership of AIMS. These requirements are stated in the AIMS accreditation report held by the University. Prospective applicants should contact the program co-ordinator at the University for advice on these requirements".

Findings from the Australian Institute of Medical and Clinical Scientists Annual Report (2020-2021) stated that there were 2,305 AIMS accredited members. There were 14 Bachelor degree programs and four Masters degrees by coursework in medical laboratory science conducted by universities in Australia and overseas that were accredited by AIMS in this period. It could be estimated that between 2,000-3,000 students are enrolled in these programs annually with approximately 500-600 graduates each year. This suggests a shortfall in graduates who become AIMS accredited members.

Limitations

Findings from this study should be interpreted with care as the data provided did not include all AIMS accredited programs and may not reflect all universities and their academic profiles. Of note there are differences amongst programs in relation to the duration and subject content however, all have met accreditation standards by AIMS. As the study was conducted partially during the Covid-19 pandemic data may have been influenced by external

circumstances and disruptions that is not a true reflection of the programs, academic profiles and student outcomes. Accurate data of graduates and their employability outcomes would provide meaningful information regarding the growth and future planning of MLS. Moreover, accurate data on the number of employed MLS Scientists and Technicians are hindered by Australia not formally regulating Medical Scientists and Technical Officers. The Australian Council for Certification of Medical Laboratory Scientific Workforce Limited (CMLS) has provided ongoing certification of Medical Scientists and Technical Officers and as of the Board meeting of Directors in February 2022, the number of certified Technicians, Scientists, and Clinical scientists was noted at 316 members (AIMS member update 2022). As this is a self-certification scheme and it is not compulsory for a graduate to be certified and it is difficult to record accurate data and maintain minimum standards for ongoing assessment of competency and continuing professional development in MLS.

Conclusion

Knowledge attained from this study contributes to providing national data of academic qualifications, MLS experience and teaching interest of MLS staff who contribute to educating MLS graduates to benchmark academic standards. This study also strengthens the understanding of most AIMS accredited MLS programs and the MLS student outcomes. Further research is needed to understand how this contributes to the employment outlook in the MLS industry and the graduate student outcomes as accurate records of AIMS employed graduates are not captured through university institutes and may better fall under a regulatory body such as CMLS or AIMS that could capture this data. Publicly publishing this information could focus the curriculum design of MLS programs that better prepares students for employment. Innovative and advanced testing in molecular pathology and personalised medicine requiring point-of-care testing which is rapidly changing, is not comprehensively taught at the tertiary level. At AIMS accredited academic programs included in this study, there appears to be a gap between academic characteristics and MLS experience to best advocate for AIMS accredited degrees and pathology employment. In this study cohort, the majority of academics with one or more specific MLS teaching focussed subjects did not necessarily have equivalent MLS laboratory experience in that discipline/subject. Prior experience as an MLS Scientist does not appear to influence the discipline that the academic teaches in, with over 100 different subjects taught from the 31 respondents. It is unclear whether an academic with or without MLS qualifications or experience better prepares students for employment in pathology. It is likely that academic knowledge and skills are reinforced through work-integrated learning when students are supervised by qualified pathology scientists and technicians however, further research in work-integrated learning is required to assess this.

Declaration of interest

This project was funded in part by the Australian Institute of Medical and Clinical Scientists (AIMS) Education and Leadership/Management Grants.

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Disclaimer

This article is the opinion of the authors and is not necessarily the opinion of the editors of the Australian Journal of Medical Science or of the Australian Institute of Medical and Clinical Scientists.

Australian Professional Acknowledgement of Continuing Education (APACE)

3 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly.
24 credits maximum per accreditation period claim.

Journal-based CPD No. 84
Page 1 of 1

Questions relating to the article *'Heparin resistance – a clinical and laboratory conundrum'* at page 39 of this issue.

1.	UFH is administered either intravenously or subcutaneously, as it is not readily absorbed by the gastrointestinal tract.	True/False
2.	UFH has a short half-life of approximately 60 mins when administered subcutaneously and 15 mins when administered intravenously.	True/False
3.	The APTT is a global coagulation test that reflects both the intrinsic and common coagulation pathways.	True/False
4.	Heparin resistance is not commonly observed in acutely ill patients, with relatively high rates of heparin resistance being observed in the current SARS-Cov-2 pandemic.	True/False
5.	Heparin is a large linear polymer which is produced by the Golgi apparatus of mast cells and neutrophils and stored in the basophilic specific granules.	True/False
6.	The most prevalent sequences within the heparin polymer are trisulphated disaccharide repeats of $IdoA2SO^3$-$GlcNSO^3$.	True/False
7.	The use of heparin as an anticoagulant began in 1905.	True/False
8.	Antithrombin (AT) is a 432-amino acid serine protease inhibitor, or serpin, which acts as the major physiological inhibitor of coagulation.	True/False
9.	Heparin acts in two key ways to activate antithrombin: as an allosteric activator of the inhibitor and as a bridging co-factor.	True/False
10.	APTT reagents will have different contact factor activators, phospholipid source and phospholipid concentration.	True/False

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Journal-based CPD No. 85
Page 1 of 1

Questions relating to the article '*Checklist for conformity evaluation of reviewing requirements for internal auditing: a quality compliance tool for International Standard ISO 15189:2012 accredited medical laboratories*' at page 52 of this issue.

1.	The pathology services industry plays a critical role in the support of healthcare by providing diagnostic information.	True/False
2.	A total of 86/26 (100 %) RevReqs was elicited in ISO 15189:2012.	True/False
3.	The most significant finding of the present study is that the majority of RevReqs (19/26 (73 %) were identified in the support activities and costs component of value chain.	True/False
4.	A distribution analysis was performed on RevReqs in the ISO 15189:2012 process based quality management system model in the value chain model of the medical laboratory.	True/False
5.	The specific areas of interest were primarily in Clauses 4 and 5 of ISO 15189:2012.	True/False
6.	The distribution analysis was performed using the content analysis results, especially the RevReq locations in Clauses 4 and 5 of ISO 15189:2012.	True/False
7.	The ISO 15189:2012 process based quality management system model comprises four stages.	True/False
8.	The RevReqs checklist was developed using the 29/29 (100 %) RevReqs in Clauses 4 and 5 of ISO 15189:2012.	True/False
9.	The use of the conformity evaluation of the RevReqs checklist has three technical considerations and implications that need to be considered by the medical laboratory.	True/False
10.	The most significant finding of the present study is that the majority of RevReqs (19/26 (73 %) were identified in the support activities and costs component of value chain.	True/False

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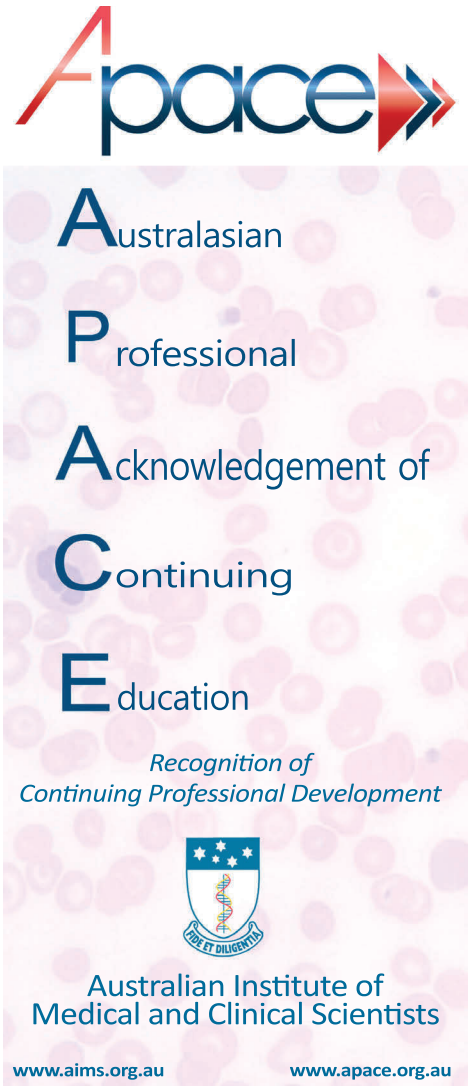

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Changes to APACE due to COVID-19 pandemic UPDATE

An APACE Certificate is usually awarded on attaining 100 CEU credits within a two year period.

As webinars and online conferences, meetings and workshops are all interactive, it was considered that this is the same as attending in person, therefore the same number of points will be awarded for attendance either virtually or face-to-face. This should enable more members to attend as no travelling time, costs and in some cases the online attendance with be without cost to the attendee.

Therefore, the extended time frame due to Covid-19 will no longer apply.

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BOOKS FOR REVIEW

Following is a list of books available for review by resource consultants and members of the Institute with particular expertise in the field. The reviewer is invited to retain the complimentary copy of the book once the review is received.

As per our agreement with the book publishing companies, complimentary books are submitted to the Institute provided that all reviews are published in the Australian Journal of Medical Science. These reviews must be of a high quality as buying decisions and the reputation of the book and author are important considerations.

Books not requested will be allocated at discretion of the Editors for the Australian Journal of Medical Science. Reviews should be 300 to 700 words depending on the volume of the book. Time limit for return of review is **six** weeks.

Please send your request to: Australian Institute of Medical Scientists and Clinical Scientists
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1. **Bifidobacteria: Genomics & Molecular Aspects** edited by B. Mayo, & D. Van Sinderen. Caister Academic Press. xii + 260 pages.

2. **Medicine and Sport Science Volume 55: Cytokines, Growth Mediators & Physical Activity in Children during Puberty** edited by J. Jurimae, A.P. Hills & T. Jurimae. Karger. viii+178 pages.

3. **Digestive Diseases The Keys to IBD 2010: Treatment, Diagnosis & Pathophysiology.** Edited by G. Rogler & W. Sandborn. Karger. 188 pages.

4. **Else Kröner-Fresenuis Symposia Volume 1: Molecular Mechanisms of Adult Stem Cell Aging** edited by K.L. Rudolph. Karger. xii+108 pages**.**

5. **Endocrine Development Volume 24: Hormone Resistance and Hypersensitivity** edited by M. Maghnie, S. Loche, M. Cappa, L. Ghizzoni & R. Lorini. Karger. viii + 160 pages.

6. **Frontiers of Hormone Research Volume 41: Endocrine Tumor Syndromes and Their Genetics** edited by C.A Stratakis. Karger. xii + 187 pages.

7. **Frontiers of Hormone Research Volume 39: Kallmann Syndrome & Hypogonadotropic Hypogonadism** edited by R. Quinton. Karger. x+174 pages.

8. **Generic: The Unbranding of Modern Medicine** by Jeremy A. Greene. John Hopkins University Press. 368 pages.

9. **Human Pathogenic Fungi: Molecular Biology and Pathogenic Mechanisms** edited by Derek J. Sullivan & Gary P. Moran, Caister Academic Press. x + 342 pages.

10. **Internal Medicine: A Doctor's Stories** by Terrence Holt. Black Inc. 273 pages.

11. **Intolerant Bodies: A Short History of Autoimmunity** by Warwick Anderson and & Ian R. Mackay. John Hopkins University Press. 250 pages

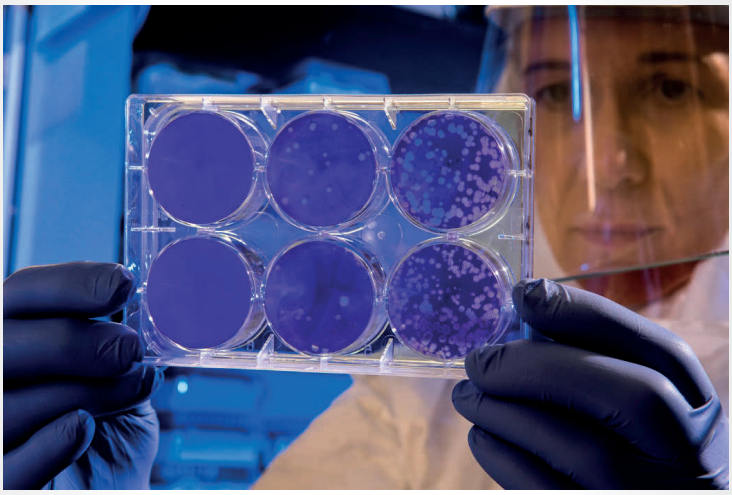
12. **Lyme disease and relapsing fever spirochetes** edited by Justin D. Radolf and D. Scott Samuels. Caiser Academic Press. 760 pages

13. **More Than Hot: A Short History of Fever** by Christopher Hamlin. John Hopkins University Press. 400 pages.

14. **Pediatric and Adolescent Medicine Volume 19: Metabolic Syndrome and Obsesity in Childhood and Adolescence** edited by W. Kiess, M. Wabitsch, C. Maffeis, A.M. Sharma. Karger. x + 202 pages.

15. **Phage Therapy - Current Research and Applications** edited by Jan Borysowski, Ryszard Miedzybrodzki & Andrzej Gorski. Caister Academic Press. 368 pages.

16. **Shigella: Molecular and Cellular Biology** edited by William D. Picking & Wendy L. Picking. Caister Academic Press. 280 pages.



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AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

Instructions to authors

The following instructions are based on the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", also known as the Declaration of Vancouver, and on the *Australian Government Style manual: for authors, editors and printers*, 6th edition, 2002. URLs were correct on September 29th, 2008.

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The *Australian Journal of Medical Science (AJMS)* will consider for publication any paper relevant to the field of Medical Science. Disciplines include blood banking, clinical biochemistry, haematology, histopathology, immunology, microbiology and molecular biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

Papers published in the *AJMS* are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the *AJMS* and those accepted become the property of the *AJMS*.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

Articles should be submitted in electronic format to programs@aims.org.au. If an article is too large to be submitted by email, it should be submitted on an or USB stick.

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

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The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g., William Smith, Susan Yeo, ..."). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.

Abstract & keywords

The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

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The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible, observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance. Do not include a separate section for conclusions, these should be given in the discussion.

Introduction

Clearly state the purpose of the article leading the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials & methods

Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

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Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

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Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However, avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:

(i) Research in this area (Jones 1999) …

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Examples of the correct form for references are given below:

Journal Reference:

Stein MK, Downing RW, Rickels K 1978. Self-estimates in anxious and depressed outpatients treated with pharmacotherapy. *Psychol Rep* 43: 487-492.

Personal Author(s) of a book:

Osler AG 1976. *Complement: mechanisms and functions*. Englewood Cliffs: Prentice-Hall.

Editor, Compiler, Chairman as Author:

Rhodes AJ, Van Rooyen CE, comps. 1968. *Textbook of virology: for students and practitioners of medicine and the other health sciences*. 5th ed. Baltimore: Williams and Wilkins.

Chapter in Book:

Weinstein L, Swartz MM 1974. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 457-472.

Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

Tables

Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence:

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In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a CD. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

In most instances, figures will be reduced to one column in width. All letters and numbers should be drawn to be at least 1.5 mm high after reduction, symbols at least 1.0 mm. Titles for illustrations belong in the legends for illustrations and not on the illustrations themselves.

Photomicrographs must have internal scale markers and the magnification must be stated. Symbols, arrows, or letters used in the photomicrographs should contrast with the background.

Cite each figure in the text in consecutive order, e.g., "Figure 1 illustrates …" or "… as shown (Figure 2)". If a figure has been published, acknowledge the original source and submit with the manuscript written permission from the copyright holder to reproduce the material. Permission is required, regardless of authorship or publisher, except for documents in the public domain.

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Abbreviations

Use only standard abbreviations (see list of commonly used abbreviations).

Avoid abbreviations in the title. The full term for which an abbreviation stands must precede its first use in the text unless it is a standard abbreviation for a unit of measurement.

Report measurements in the units in which the measurements were made. In most countries the International System of Units (SI) is standard.

Commonly used abbreviations

Abbreviation or Symbol	Standard Units of Measurement
g	gram
g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

O'Connor M, Woodford FP. *Writing scientific papers in English: an ELSE-Ciba Foundation guide for authors.* Amsterdam, Oxford, New York: Elsevier-Excerpta Medica, 1975.

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

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