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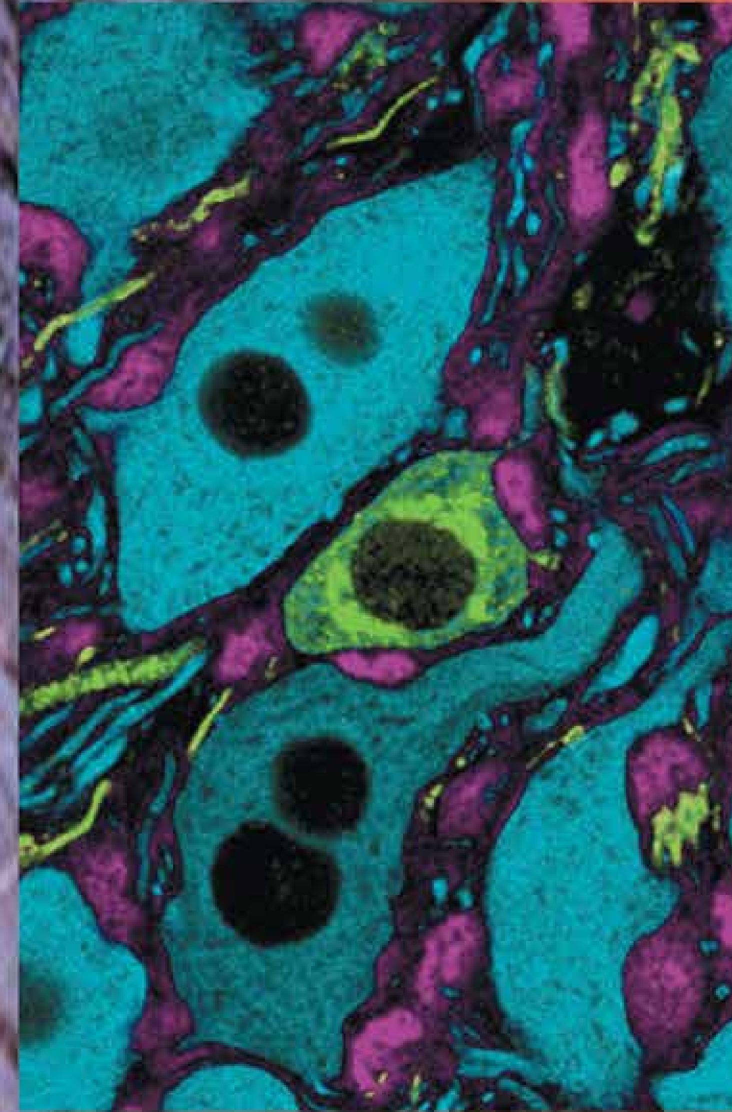

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ORIGINAL ARTICLE

Cobalamin deficiency in companion animals: a review

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Abstract

Serum cobalamin levels are often analysed in the serum of companion animals during the investigation of suspected coblamine deficiency. Robust testing methods and reference ranges are therefore required to detect abnormal levels. There are currently no recommended guidelines describing standardized methods to be used to determine levels or appropriate reference ranges to be used when interpreting serum cobalamin levels in dogs and cats.

This review focuses on cobalamin detection methods used in published studies available on multiple international literature databases. The literature searched was published between January 1970 and December 2020. In the search for available published papers based on cobalamin related keywords, a total of 11,906 studies were identified. Based on the selection criteria used for this study requiring reference to original data and a direct relationship to canines and cobalamin, felines and cobalamin or cobalamin and reference intervals or reference interval validation, 39 papers were found to be relevant to this review. It subsequently identified the need for standardised procedures for developing serum cobalamin reference ranges and testing protocols. One method for developing standardised reference ranges is a protocol developed by the Clinical Laboratory and Standards Institute (CLSI) to test human samples, which has since been adopted and adapted for testing animal samples by the American Society for Veterinary Clinical Pathology.

In developing standard guidelines for testing serum cobalamin in dogs and cats, it is essential to check the validity of the reference range for a specific laboratory as various laboratories may have different procedures, reagents, and patient populations. The lower reference intervals used for canines and felines ranged between 186-251 pmol/L and 213-261 pmol/L, respectively, while the upper reference range was between 670-908 pmol/L 500-1451 pmol/L respectively. Eleven of the 50 papers did not describe how the reference ranges had been validated, referring to the laboratories pre-existing reference interval for their research.

This review is the first Australian comprehensive review of studies reporting cobalamin detection in serum for canines and felines to the best of the author's knowledge. Based upon this review it is clear more studies leading to the development of better guidelines in relation to cobalamin detection and interpretation of cobalamin deficiency in cats and dogs are required.

Keywords: ***Cobalamin, Vitamin B12, canine, feline***

Introduction

Cobalamin, otherwise known as Vitamin B12 (VB12), is a sizeable water-soluble analyte found in most animal products. With a molecular weight of 1355 it is incapable of crossing the cell membrane without being broken down and transported by the R-protein which is synthesized by dogs and cats in the stomach and salivary gland (McLeish *et al* 2019; Ruaux 2013). Once cobalamin within food is ingested, the intestinal microbiota breaks down the food releasing cobalamin in the presence of cobalt, pepsin, and hydrochloric acid (Cook *et al* 2011). The newly freed cobalamin intrinsically binds to the R-protein (glycoprotein). The cobalamin-glycoprotein complex then binds to intrinsic factor (IF) excreted by the exocrine pancreas in the duodenum. R-protein cobalamin is then transported to the tissue through the bloodstream when bound to transcobalamin II (McLeish *et al* 2019; Weisberg and Rhodin 1970).

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Cobalamin disorders can be caused by inherited mechanisms or can be induced by lack of absorption from food into the bloodstream. Examples include inherited defects of cobalamin metabolism and absorption as well as cobalimin deficiency secondary to other disorders (Kook 2013; Kook *et al* 2014). Juvenile hypocobalaminaemia manifests in breeds such as Beagles and Border Collies with an autosomal recessive genetic mutation on the Cubam (CUBN) gene on chromosome 10 (Kather *et al* 2020; Kook *et al* 2014; Maunder *et al* 2012). Other genetically predisposed canines include the Australian Shepherd, and Giant Schnauzer breeds with an amnionless (AMN) gene mutation on chromosome 14, and canines with Imerslund-Gräsbeck syndrome (IGS) can exhibit malabsorption, proteinuria, megaloblastic anaemia and typical hypocobalminaemia at an early age. Megaloblastic anaemia can be caused by hypocobalaminaemia in felines but is more common in canines (Battersby *et al* 2005; Gräsbeck 2006; He *et al* 2005; Suchodolski and Steiner 2003).

Cobalamin deficiencies secondary to other disorders in canine or felines include gastro-intestinal diseases, chronic inflammatory enteropathies, and disturbances to gut microbiota which can decrease available receptors in the small intestines and minimise cobalamin absorption (Kather *et al* 2020). The literature suggests that up to 73% of canines and 61% of felines with chronic intestinal disease are hypocobalaminaemic, as the cobalamin half-life decreases from 11-14 days to 4.5-5.5 days. Healthy animals receive enough cobalamin supplementation through commercial pet food bought in Australia. Therefore, diet alone is an unlikely source of deficiency in hypocobalaminaemic animals and further supplementation can be necessary (Dossin 2011; Wakefield *et al* 2006). Hypocobalaminaemia can also occur due to increased physiological demand due to various conditions including pregnancy leading to pregnancy-related anaemia (Hunt and Jugan 2021; Nivy *et al* 2019; Simpson *et al* 2001). As felines age from 3 to 9 years, hypocobalaminaemia occurs more frequently as a natural but pathological process (Hill *et al* 2018; Ruaux *et al* 2009; Salas *et al* 2014).

Cobalamin reference interval requirements are well documented in humans where the CLSI guidelines are well established as the gold standard technique for identifying reference ranges. Conversely, very little has been reported in the literature regarding a standard method when measuring cobalamin levels and developing guidelines for reference intervals in animals, including dogs and cats (Friedrichs *et al* 2012; Jiang *et al* 2020; Ventrella *et al* 2016).

The American Society of Clinical Pathology has adopted the human-based CLSI guidelines in the veterinary pathology field and there is currently no standard method for identifying abnormal cobalamin levels in Australia's veterinary pathology. There is also no standardised approach to reference range verification/validation in veterinary diagnostic laboratories at present. Individual laboratories set up their ranges based on their method, scientific background and available population data.

The aims of this review were to:

- identify peer-reviewed studies describing reference intervals for serum cobalamin levels in felines and canines
- identify the analytical methods used for determining cobalamin in serum from felines and canines.

Method

A systematic review was carried out according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) as described below. Figure 1 provides insight into the search and review process undertaken (Page *et al* 2020).

Literature Search strategy

Relevant papers were identified in electronic databases, including Google Scholar, PubMed Central, Scopus, Elsevier Science Direct, and Primo search. Each database was searched using the keywords associated with feline and canine cobalamin (Table 1).

Literature review process

Criteria used to select papers for this review

The process of selecting papers used in the review was multifaceted. The first step was to identify useful keywords and a date range relevant to cobalamin testing The dates searched were between January 1970 and December 2021.

Papers that did not report data on either cobalamin reference ranges or testing methods were not included in the review.

Data extraction and analysis

Papers identified as relevant based upon keywords used (Table 1) were downloaded into EndNote™ and duplicate papers were deleted after databases were searched. These papers were then tabulated with information on cobalamin detection into an Excel spreadsheet. A total of 39 papers identified suited a systematic review more so than a meta-analysis. All papers were assessed to ensure that the studies reported statistically significant data (P-value was ≤ 0.05).

Results

The review identified a range of parameters that could identify at-risk canines and felines from healthy individuals and normal cobalamin results for the respective instrument types used in the studies.

The most common type of instrument used for determining cobalamin in feline and canine serum was the Siemens Immulite platform (Table 2). The accuracy of the clinical or

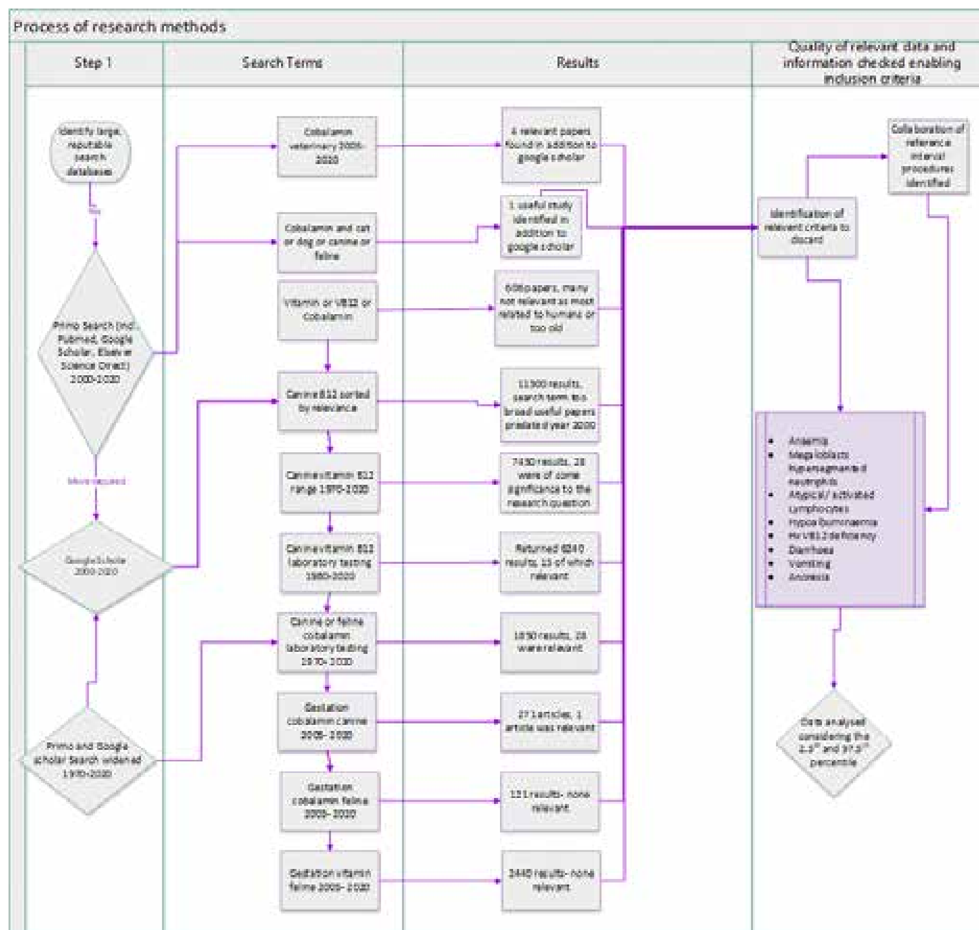

Figure 1. *Process of selecting papers to be included in the review. A search of the literature was conducted with a view to selecting papers for this review. Most papers identified using relevant search terms could not be included as they did not fit with the in Flow diagram showing the search process of current literature and subsequent selection of papers for this review.*

Table 1. *Results of the literature search performed on databases Pubmed, Google Scholar, Elsevier Science Direct using various search terms.*

Search terms	Number of relevant articles retrieved
Cobalamin + veterinary (2005-2020)	4
Cobalamin + cat or dog or feline + canine	5
Canine B12 range (1970-2020)	28
Canine + vitamin B 12 + laboratory testing	15
Canine or feline cobalamin laboratory testing	28
Gestation + cobalamin + canine	1

tissue correlation in these studies was somewhat hampered as serum cobalamin measurement is limited in that levels are detected only in serum. Therefore, the serum reference intervals can only indicate the approximate intracellular cobalamin levels available within the mitochondria and cytoplasm as cellular levels cannot be measured directly and sometimes do not reflect intracellular levels (Kather *et al* 2020).

In the initial search from 1970-2020 a total of 11,906 studies were identified using search terms: vitamin, VB12, cobalamin, trypsin-like immunoreactivity (TLI) (which aids in the identification of exocrine pancreatic insufficiency), and methylmalonic acid (MMA) (which is increased in the absence of enough VB12). Many of these papers were not relevant to canine and feline reference intervals as the search terms were too broad or were published prior to 2000.

Due to the lack of pertinent recent articles, the combinations of search terms - canine, feline, cobalamin, B12, gestation, laboratory and vitamin were extended to include the years 1970-2021 and 81 of 23,823 papers were identified as potentially useful (Table 1). Subsequently eleven reports covering reference interval information were identified as relevant to this review (Table 2).

Current knowledge and practice of detecting cobalamin abnormalities

A summary of the cobalamin reference ranges reported by the papers reviewed here is shown in Table 2 and Figures 2 and 3. Of the nine studies reporting reference ranges (MMA and cobalamin), only one study Berghoff *et al* (2012) used their current data to determine the reference ranges for

Table 2. *Literature reporting cobalamin reference ranges in dogs and cats.*

Source of samples	Platform (instrument)	Lower RI pmol/L	Upper RI pmol/L	Mean RI pmol/L	Reference
Feline	Immulite 2000	213	1106	659.5	Geesman *et al* 2016
	Immulite 2000	225	1451	838	Kempf *et al* 2018
	Multiple platforms	221	1106	663.5	Jugan and August 2017
	Roche platform	261	1169	715	Hill *et al* 2018
	Tosoh Bioscience	220	500	360	McLeish *et al* 2019
Canine					
	Immulite 2000	186	670	428	Cook *et al* 2011
	Immulite 2000	192	739	465.5	Idexx internal distribution (personal communication, Idexx laboratory)
	Immulite 2000	185	670	523	Kather *et al* 2020
	Immulite 2000	251	908	579.5	Berghoff *et al* 2012
	Immulite 2000	251	908	579.5	Grutzner *et al* 2012
	Tosoh Bioscience	200	400	300	McLeish *et al* 2019

MMA against previously tested cobalamin ranges.

In another study, investigators compared their findings against reference ranges reported by another laboratory (McLeish *et al* 2019). There appeared to be little correlation between instrument and assay methodologies from different diagnostic companies. This further supports the importance for a laboratory to develop reference ranges for their local population. For the other seven studies, investigators used the laboratories pre-existing reference intervals with no indication of the methods used for determining this.

Methods of determining cobalamin concentration in canine and feline serum

Seven of the nine papers used a chemiluminescent assay (Immulite) (Siemens Healthcare Diagnostics, Tarrytown, New York) (Table 2). However methods used were not fully

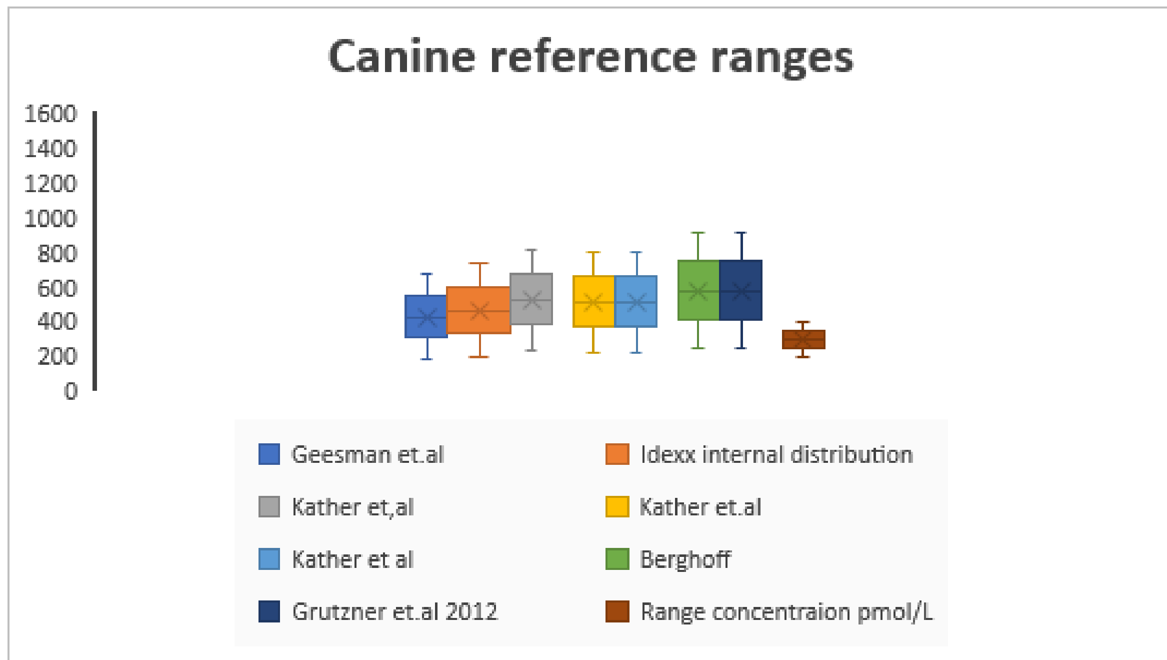

Figure 2. *Canine reference range companion pmol/L*

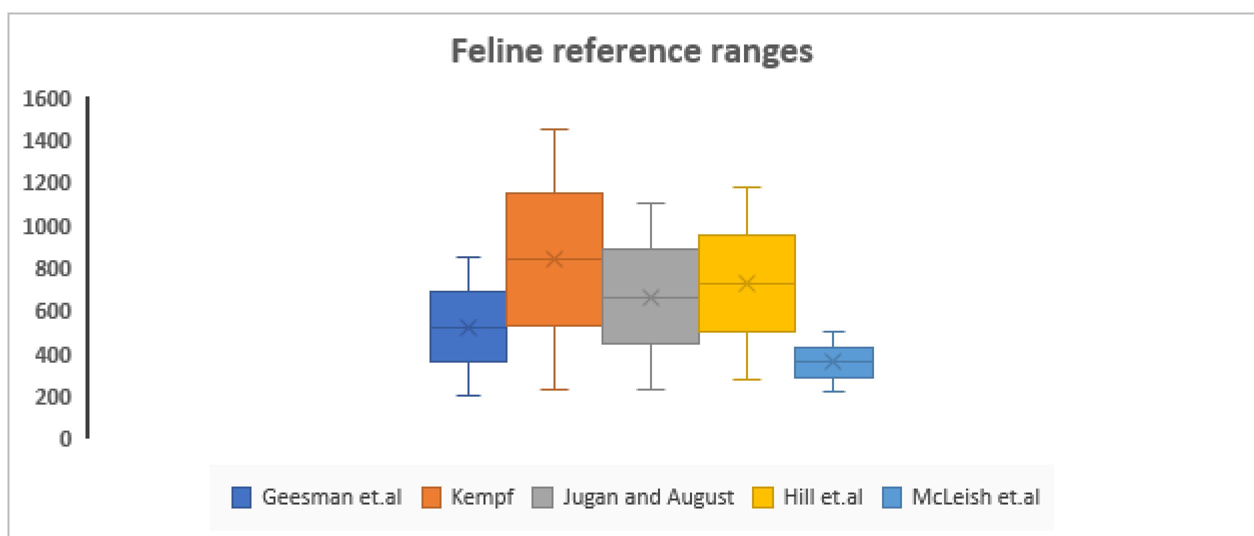

Figure 3. *Feline reference range companion pmol/L*

disclosed for many of the studies reviewed, and information such as pre-treatment procedures used in the analytical phase of testing would have assisted in the comparison of reference intervals. McLeish *et al* (2019) compared the Immulite assay with a 2-site immunoenzymometric method (Tosoh Bioscience instrument). They also discussed a pre-treatment procedure for the immunoenzymometric method; they found that the two methods were not comparable indicating a requirement for assay dependent reference ranges.

In another study by Hill *et al* (2018) the investigators described the electrochemiluminescent assay available from Roche. The reference interval obtained using this method did not differ significantly from other platforms (Table 2).

The Immulite chemiluminescent assay is the most commonly used method for determining cobalamin levels in serum in the veterinary laboratory based on the published literature.

References ranges available for serum cobalamin in canines

In the five published studies on cobalamin levels in canines reviewed (Table 2), the authors utilised their laboratory reference intervals, which did not appear to be standardised when identifying cobalamin status. The exception is the paper by McLeish *et al* (2019) in which the authors used a different method and retrospectively analysed the data to determine the appropriate cut-offs for adequate cobalamine levels and to develop the reference range for their lab (Table 2).

There were disparities in the lower and upper cut-off values in the reference ranges used in these studies to determine cobalamin levels in canine serum. The lower range cut-off value used to identify hypocobalaminaemia ranged between 186-251 pmol/L. The upper range cut-off value used to identify hypercobalaminaemia ranged between 670-908 pmol/L (Berghoff *et al* 2012; Cook *et al* 2011; Grützner *et al* 2012; Kather *et al* 2014; McLeaish *et al* 2019). This indicates that there may be issues in obtaining reliable diagnoses of these conditions based on current testing methods.

References ranges available for serum cobalamin in felines

Jugan and August (2017) used data from 207 cats for their study comparing values reported from multiple instruments. Hill *et al* (2018) used data from 65 felines, broken down into three age categories. McLeish *et al* (2019) used samples from 68 clinical cases, a mixture of felines and canines. The inter-instrument correlation was shown using Bland-Altman analysis to have poor agreement between the Immulite method and the immunoenzymometric method using the Tosoh Bioscience instrument. This was due to using different technologies and reagent ingredients. McLeish *et al* (2019) concluded the reference interval could not be calculated or correlated from their study due to the significant difference in results of normal patients obtained by the different instruments.

The cut-off value to identify hypocobalaminaemia ranged between 213-261 pmol/L, and for hypercobalaminaemia between 1106 and 1451 pmol/L (Geesaman *et al* 2016; Hill *et al* 2018; Jugan and August 2017; Kempf *et al* 2018; McLeish *et al* 2019) with the outlier being the McLeish study quoting a high range of 500 pmol/L (McLeish *et al* 2019).

Discussion

During this review it became apparent that many more articles are available on the detection of cobalamin in humans compared to felines and canines combined. Due to the variability in study populations in the low number of studies included in this review, it was deemed appropriate to undertake a systematic review (Higgins *et al* 2019). A meta-analysis would not have yielded meaningful data as it would not have been possible to combine data from multiple studies.

Assay methods for determining cobalamin concentration in canine and feline serum

Cobalamin assays often require multiple manual steps and temperature changes. The lack of harmonization of the different assays used between laboratories means using reference ranges developed by another group should be avoided. In an example of reference interval verification method published in the veterinary field, an iron reference interval study used the 2.5th and 97.5th percentile and is more in line with practices in some laboratories (Ventrella *et al* 2016).

The Siemens Immulite is the most documented instrument and the Immulite method was the most commonly reported method for reporting cobalamin in canine and feline serum. This is most likely due to Siemens instruments being widely used in high throughput veterinary pathology laboratories. This enables a reference range to be validated, and remain stable unless the normal population changes or the reference calibration stock solution is changed by the manufacturer (Ferraro and Panteghini 2019). It should be noted however that there are no standardized calibration materials recommended for laboratories to use for calibration in relation to cobalamin testing in felines and canines. This is an important first step in the harmonization process allowing for inter assay laboratory comparisons (Ferraro and Panteghini 2019).

For two of the studies reviewed, the investigators reported a variation from the standard Immulite method. One study used the Immulite as the gold standard to compare the 2-site immunoenzymometric method (McLeish, Burt and

Papasouliotis 2019). This paper concluded that correlation was not possible between the Immulite (Siemens) and the Tosoh Bioscience instruments. The second paper used the electrochemiluminescent assay method (Hill *et al* 2018). Despite the differing methodologies between the enzymatic and electrochemiluminescent assay methods, the cut off value for identifying hypocobalaminaemia was not significantly affected whereas it altered the cut off for identifying hypercobalaminaemia.

Considering the impact of pre-analytical variation

The large variability in the lower range cut off values (Figs 2 and 3) used to identify hypocobalaminaemia could be due to the impact of pre-analytical variation. In several studies reviewed, it was demonstrated that a reference range for cobalamin may be impacted by animal age, location and breed (Berghoff *et al* 2012). Differences in genetics (various breeds), diet, climate, and differences in testing procedures may all impact the development of reference ranges and therefore it is clear that each laboratory will need to develop a standardised approach to creating locally applicable reference ranges.

Consideration must also be given for the large variety of breeds and ages. The biological variation amongst feline and canine breeds means reference intervals used by diagnostic laboratories need to reflect the local breeds as much as possible (Berghoff *et al* 2012). It is also possible that reference ranges for specific breeds need to be developed. Researchers not considering breeds used in reference range verification used by the laboratory potentially can hinder the studies.

Standardised approaches to reference range setting in veterinary diagnostics

The importance of a relevant reference interval is integral to any laboratory diagnostic test (Cray 2012; Friedrichs *et al* 2012). The development of universal reference ranges requires a standardised approach particularly for use in the in the veterinary field (Friedrichs *et al* 2012). Many laboratory methods used in the veterinary field are first validated for use in human diagnostic testing, which the veterinary laboratory then needs to validate for veterinary use using available samples from healthy animals. Other papers attempted to correlate physical changes observed macroscopically or microscopically through histopathology and ultrasound interpretation (Jugan and August 2017). Additionally, serological changes such as methylmalonic acid and homocysteine concentration (Rossi *et al* 2013), cobalamin concentration post collection in serum, the connection between hyperthyroidism, exocrine pancreatic insufficiency, the correlation between TLI and cobalamin concentration (Grützner *et al* 2012) and hypocobalaminaemia (Cook *et al* 2011) were all investigated leading to slightly different suggestions for reference range cut off. These caveats can easily lead to differences in reference range verification methods depending on the amount of available data and sample or test and cost constrictions (Cray 2012). Currently, recording of conclusive results linking MMA, pancreatic insufficiency, and histopathology interpretations to cobalamin deficiency can only provide limited benefit in supporting a diagnosis of hypocobalaminaemia in dogs and cats.

Typical processes discussed when identifying methods for setting reference intervals included identifying healthy cats and dogs at the veterinary surgery and asking the owners for permission to draw blood. However this may not be practical as most people do not take a healthy animal to the vet unless they are concerned about a predisposition and require pathology testing. The number of available healthy animal samples is minimal when compared to real time data collection when running the assay for an extended period. When working up a de novo study, sample numbers tend to be minimal, impacting the validity of the reference interval. Reference intervals are more reliable when checked against large sample numbers over some time (Friedrichs *et al* 2012; Ventrella *et al* 2016). This indicates that it is essential to regularly check the specificity and sensitivity of the assay, as well as compare existing assays with new assays as they are developed, and reference intervals should be suitable for the testing population.

The second option is to use reference ranges from published papers with a population and testing method comparable to the assay in use in your laboratory. Most reports however gave insufficient information to ensure laboratories replicating the study use the same techniques and equipment. The third option is to verify reference intervals with retrospective data, similar to methods undertaken in human medicine (Jiang *et al* 2020). One approach to this may be using retrospective laboratory data to verify previously published reference interval suitability.

Most of the studies reviewed used the pre-set reference ranges set by their own laboratory with no indication of how the author of the study verified these ranges for their species, age, or study type. It would have been useful to know how or if the verification of the reference range was checked against the study population as this has a potential impact on the findings. Further investigation is also required to identify if there is a correlation between total cobalamin levels and intracellular concentration as currently animals at risk of intracellular hypocobalaminaemia cannot be easily identified. Only one veterinary cobalamin study (Cook *et al* 2011) utilised CLSI guidelines as the guiding principle when identifying reference intervals.

Another interesting finding from the data was that cats required a higher reference range than dogs and this was

contrary to ranges reported from other similar studies. Recently, unpublished data obtained from a Sydney laboratory (personal communication, Idexx) collected over three years (2019-2021) from 113 healthy felines and 1067 canines (a much longer period than than that reported by other published studies) resulted in a cobalamin reference range for dogs of 218-845 pmol/L and 208-867 pmol/L for felines.

The data from this laboratory also highlighted the stability and robustness of the Immulite assay used by the laboratory. They reported a between run CV of 15%, 14% and 13% across the concentrations 240 pmol/L, 390 pmol/L and 570 pmol/L respectively over a 12 month period. Notably the sigma range is 0.82, 0.97, and 1.01. These data are important for developing recommendations to identify cobalamin deficicinecy in dogs and cats similar to guidelines available for testing in humans (RCPA manual).

To the best of the author's knowledge, this is the first attempt to develop a reference range for serum cobalamin in dogs and cats in the Australian context, with over 1000 canine and 100 feline samples tested and validated utilising CLSI guidelines. When searching more broadly for reference interval procedures accepted by veterinary groups, backed by clinical laboratory groups, the author found no papers for cobalamin reference intervals utilising the CLSI recommendations for veterinary companion animals.

However the American Society of Veterinary Pathology has adopted CLSI guidelines as the gold standard when identifying reference intervals and de novo assays (Friedrichs *et al* 2012; Cray 2012). It would be useful to have an international consensus on gold standard practice for identifying and verifying reference intervals for veterinary assays, including cobalamin. An agreement in the veterinary scientific community would allow for a more robust reference interval and greater participation in multi-centre trials.

Conclusion

The reference interval for serum cobalamin measurement in dogs and cats differs across the literature, dependent on the assay technique used for identification and measurement of serum cobalamin concentration. The differences in the ranges reported could be due to a combination of pre analytical factors including genetic variation amongst various breeds, sample size, and variability in the methods used to determine cobalamin levels and to calculate the reference interval. It would be useful to have a reliable gold standard method identified in Australia and agreed upon by the veterinary laboratory communities to develop robust reference intervals for determining cobalamin abnormalities.

Additionally, as new breeds evolve (cross-breeding) reference intervals need to be updated accordingly. Therefore multi-centre studies looking at more data are required to develop robust reference ranges for cobalamin. Future studies should ideally involve data collected over an extended period e.g five to ten years to thoroughly evaluate the method used and enable comprehensive testing of all breeds of dogs and cats.

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Conflict of interest

The authors have no conflict of interest to declare.

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ORIGINAL ARTICLE

Professional practice evaluation of medical laboratory scientists in support of patient safety: a compliance tool for International Standard ISO 15189:2012 accredited medical laboratories

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Abstract

The aim of this paper is to develop a practical tool for International Standard ISO 15189:2012 accredited medical laboratories to determine the conformity status of patient safety. The objectives include identifying relevant patient safety requirements (PSReqs) in the position paper entitled 'IFBLS position paper on patient safety' and conformance requirements (CReqs) in ISO 15189:2012, and developing a tool to evaluate the conformity status of patient safety. Content analysis was used to identify PSReqs in the position paper and CReqs in ISO 15189:2012. The elicited PSReqs and CReqs were subject to crosswalk analysis according to their interoperability in contributing appropriate levels to patient safety. The CReqs distribution was analysed across the ISO 15189:2012 process-based quality management system model. The correlated PSReqs and CReqs were used to develop a compliance tool for patient safety evaluation. Content analysis identified 37 PSReqs in the position paper and 1515 CReqs in ISO 15189:2012. Crosswalk analysis identified that 259/1515 (17%) CReqs were associated with 37 PSReqs. Distribution analysis found that 259/1515 (17%) CReqs were distributed across the four stages of the process based quality management system model. Finally, the 37 PSReqs and 259/1515 (17%) referred CReqs were used to develop conformity rating scales for the evaluating laboratory personnel responsibilities. This study has developed a reasonably practical tool to determine the conformity status of patient safety for accredited medical laboratories.

Keywords: ***Accreditation, Patient safety, Quality improvement, Quality management, Total quality management***

Introduction

The pathology services industry plays an integral role in delivering technically valid results to support medical decision making in patient management. Medical laboratory operations are characterised by deploying the most advanced range of medical laboratory capabilities to accomplish investigative tasks. Medical laboratories implement relevant standardised processes to support their practices. A common approach to demonstrate the ability to provide technically competent results is to obtain accreditation with a relevant accreditation body that is a signatory to the International Laboratory Accreditation Cooperation (ILAC) (Behets *et al* 2021, p. 1839) mutual recognition arrangement.

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The ILAC mutual recognition arrangement is between accrediting bodies that include the accreditation of medical laboratories to the ISO 15189:2012 standard [International Organization for Standardization (ISO) 2014]. Currently there are 107 accrediting bodies registered with ILAC, of which 78 accredit medical laboratories to the ISO 15189:2012 standard (ILAC 2022).

The ISO (Behets *et al* 2021, p. 1896; Romaniuk 2022, p. 896) has been producing relevant guidance documents for medical laboratories to consult with, from ISO Guide

25 (ISO 1978) to ISO 15189:2012, and works with other international organisations, such as the Institute of Electrical and Electronics Engineers (Behets *et al* 2021, p. 1481) and the International Electrotechnical Commission (Behets *et a*l 2021, p. 1741; Romaniuk 2022, p. 1195), to produce relevant guidance documents. Although implementing ISO 15189:2012 remains a formidable project for any medical laboratory (Mok *et al* 2013; Mok and Ang 2016), it is equally challenging to maintain currency (Mok and Chowdhury 2019; Mok and Chowdhury 2020; Mok *et al* 2020). ISO 15189:2012 accreditation requires the medical laboratory to fulfil specific conformance requirements (CReqs) in Clauses 4 (Management requirements) and 5 (Technical requirements) of ISO 15189:2012 (ISO 2014, pp. 6-39) and the conformity status can be classified according to the process-based quality management system model of ISO 15189:2012 (Mok 2017). Overall, the compliance status reflects on the managerial and technical capabilities of the medical laboratory.

Recently, there has been increased interest in patient safety and many models have been established (Kingston-Riechers *et al* 2010; National Academies of Sciences 2015; WHO Regional Office for the Eastern Mediterranean 2016). Despite the importance of the relationship between medical laboratory personnel professional practices and patient safety, limited relevant literature has been available. The International Federation of Biomedical Laboratory Science (IFBLS) (Behets *et al* 2021, p. 1760) has recently released a position paper entitled 'IFBLS position paper on patient safety' to address the issue (IFBLS 2020). No previous study has assessed the relationship between laboratory personnel practices and patient safety, and no known tools are available for medical laboratories to perform empirical investigations.

The aim of this paper is to develop a reasonably practical tool for ISO 15189:2012 accredited medical laboratories to determine the conformity status of patient safety. The tool was developed in five steps.

First, relevant patient safety requirements (PSReqs) in the IFBLS's position paper were identified. The relevant PSReqs were established from a published quantitative analysis, as previously described (Mok *et al* 2015).

Second, relevant CReqs in Clauses 4 and 5 of ISO 15189:2012 that could be performed against the conformity evaluation of PSReqs were identified.

Third, elicited PSReqs were correlated with elicited CReqs according to their interoperability and their linkages were charted for crosswalk analysis.

Fourth, the distribution of CReqs were analysed across Clauses 4 and 5 of ISO 15189:2012 as well as across the process-based quality management system model of ISO 15189:2012.

Fifth, a compliance tool to address the conformity status of patient safety was developed based on the PSReqs and referred CReqs. The compliance tool has been designed so laboratory personnel may evaluate practices directly related to patient safety in ISO 15189:2012 accredited medical laboratories.

Method

Quantitative analysis of patient safety requirements for the conformity evaluation of patient safety

The technique of content analysis was used for the quantitative analysis of the position paper entitled 'IFBLS position paper on patient safety' prepared by the IFBLS (IFBLS 2020). Content analysis was selected because of it is a reliable and suitable approach to elicit relevant CReqs in the analysis of International Standards (Mok *et al* 2015; Mok *et al* 2017; Mok *et al* 2020).

Briefly, two types of content analysis were used during the analysis. First, textual analysis was used for the quantitation of specific words in the text, such as the term 'shall'. The term 'shall' in ISO 15189:2012 implies at least one CReq is to be implemented in medical laboratory practice. Second, conceptual analysis was used to quantify the existence and frequency of CReqs. Relevant PSReqs relating to the conformity evaluation were elicited, as previously described (Mok *et al* 2015).

The position paper's requirements were either objects (nouns) or actions (verbs) for implementation purposes. Relevant objects and actions were tagged and numbered.

Quantitative analysis of conformance requirements of ISO 15189:2012 for the crosswalk analysis

Relevant CReqs that could be performed against the conformity evaluation of PSReqs were identified and selected in ISO 15189:2012 by content analysis (Mok *et al* 2015). Relevant CReqs relating to the conformity evaluation were elicited from ISO 15189:2012, as previously described (Mok *et al* 2015).

Crosswalk analysis of patienty safety requirments and conformance requirements of ISO 15189:2012

The elicited PSReqs that contained verifiable certainties were compared with elicited CReqs according to their interoperability in contributing at an appropriate level to patient safety. Specific CReqs that showed related informational contents were connected to specific PSReqs to enhance comparability and their linkages were charted for crosswalk analysis. RAWGraphs (Version 2.0) designed by DensityDesign, Milan, Italy, was used to compose an

alluvial diagram demonstrating the contrast between CReqs and PSReqs.

Distribution analysis of patienty safety requirments in the process-based quality management system model of ISO 15189:2012

The distribution of CReqs can be represented effectively across the process-based quality management system model of ISO 15189:2012 (Mok 2017). The distribution of CReqs that could be used for auditing purposes was represented using this model to show the extent of involvement at each stage. The CReqs distribution among the four stages was analysed: the strategic management stage had six subclauses and was represented in blue; the process, control, design and planning stage had five subclauses and was represented in purple; the analytical processes stage had nine subclauses and was represented in green; and the process evaluation and improvement stage had eight subclauses and was represented in orange.

Limitation of the crosswalk analysis

The crosswalk analysis has two limitations relating to the content analysis. First, the CReqs elicited in ISO 15189:2012 contain administrative requirements, such as management review, that are highly likely to contain degrees of variability in the implementation required by different accreditation bodies; accreditation bodies may contain supplementary accreditation requirements for implementation.

Second, it is possible that medical laboratories may wish to include more CReqs in ISO 15189:2012 to support the PSReqs because established processes could provide further parameters for indications. This situation can arise when the medical laboratory has the maturity to operate at an optimised level of performance.

Results

Quantitation of patient safety requirements for the conformity evaluation of patient safety

Content analysis was used to identify the relevant PSReqs from the position paper of IFBLS, as previously described (Mok *et al* 2015). A total of 37 PSReqs was identified (Figure 1) and used for the conformity evaluation. The laboratory personnel responsibilities for ensuring the safety of each step of the laboratory testing process contained 13/37 (35%) PSReqs. The laboratory personnel responsibilities for the effectiveness of laboratory testing contained 5/37 (14%) PSReqs. The laboratory personnel responsibilities for improving the efficiency of laboratory testing in all settings contained 7/37 (19%) PSReqs. The laboratory personnel responsibilities for ensuring the timeliness of laboratory testing in all settings contained 5/37 (14%) PSReqs. The laboratory personnel responsibilities for equity in the delivery of laboratory testing services contained 2/37 (5%) PSReqs. The laboratory personnel responsibilities for providing laboratory testing services that are patient-centred contained a total of 5/37 (14%) PSReqs.

The number of PSReqs identified in each aspect ranged from 2/37 (5%) PSReqs in the equity aspect to 13/37 (35%) PSReqs in the safety aspect.

Correlation of patient safety requirements and conformance requirements of ISO 15189:2012

Content analysis was used to identify the relevant CReqs in ISO 15189:2012, as previously described (Mok *et al* 2015). A total of 1515 CReqs was identified in Clauses 4 and 5 of ISO 15189:2012. The total number was in agreement with previous analysis results (Mok *et al* 2015). A total of 259/1515 (17%) CReqs were found to show correlation, in terms of interoperability (not correlation coefficient analysis), with 37 PSReqs (Figure 2).

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

The CReqs are distributed across the four-stage process-based quality management system model of ISO 15189:2012 and 259/1515 (17%) CReqs were found to associate with 37 PSReqs (Figure 2). The strategic management stage has six subclauses containing 399/1515 (26%) CReqs and 25/399 (6%) CReqs were associated with PSReqs.

The process control, design and planning stage has eight subclauses containing 477/1515 (31%) CReqs and 8/477 (2%) CReqs were associated with PSReqs. The analytical processes stage has nine subclauses containing 387/1515 (26%) CReqs and 84/387 (22%) CReqs were associated with PSReqs. The process evaluation and improvement stage has eight subclauses containing 252/1515 (17%) CReqs and 142/252 (56%) CReqs were associated with PSReqs (Figure 3).

Conformity rating scale for the conformity evaluation for patient safety requirements by using conformance requirements of ISO 15189:2012

The conformity rating scale was developed for interpretation of results using colour-coded grading in four colours (Figure 4 to Figure 9). The conformity rating scale can be used to display the level of conformity achieved. Red indicates a total coverage of 0%. Orange indicates a total coverage of 50%. Green indicates a total coverage of 75%. Blue indicates a total coverage of 100%.

Discussion

The present study aimed to develop a reasonably practical tool for ISO 15189:2012 accredited medical laboratories to determine the conformity status of patient safety. This was

Biomedical Laboratory Scientists are responsible for ensuring the safety of each step of the laboratory testing process by:

- Adhering to current high standards of practice [1] and the application of quality assured protocols [2] and governance [3];
- Focusing upon preventing errors [4] in the entire laboratory testing process;
- Ensuring that laboratories conform with national [5] and international [6] standards of accreditation;
- Maintaining high standards for qualifications [7] and continuing professional development [8];
- Including patient safety concepts [9] and competencies [10] into academic [11] and continuing professional development [12] requirements for Biomedical Laboratory Scientists;
- Improving laboratory testing services applying continuous quality improvement principles [13].

Biomedical Laboratory Scientists are responsible for the effectiveness of laboratory testing by:

- Incorporating current scientific evidence of appropriate use of laboratory testing [14];
- Ensuring optimal use of laboratory testing services as an aide to diagnosis [15], and monitoring treatment of disease [16].
- Improving appropriate utilization of existing [17] and new technology [18] to optimize the benefits of laboratory investigations.

Biomedical Laboratory Scientists are responsible for improving the efficiency of laboratory testing in all settings by:

- Using cost effective methods for all processes without compromising standards [19];
- Eliminating defects in the total laboratory testing process through audit [20] and quality assurance [21].
- Improving workforce productivity with technology [22], informatics [23] and continuous review [24] and evaluation of processes [25].

Biomedical Laboratory Scientists are responsible for ensuring the timeliness of laboratory testing in all settings by:

- Employing analytical test methods that use optimal procedures [26] and technology [27].
- Using new technology and informatics to optimize delivery of laboratory results [28] and interpretative information [29] to ensure maximum benefit to the patient [30].

Biomedical Laboratory Scientists are responsible for equity in the delivery of laboratory testing services by:

- Ensuring access to laboratory testing services for all individuals based on clinical need [31];
- Evaluating access to laboratory services with a monitoring system [32].

Biomedical Laboratory Scientists are responsible for providing laboratory testing services that are patient-centered by:

- Maintaining patient confidentiality throughout all phases of laboratory testing [33];
- Providing patients with information about laboratory testing prior to specimen collection in order to give informed consent [34];
- Providing test results [35] and interpretation [36] to inform the diagnosis;
- Focusing on improving patient outcomes using new technologies [37], such as personalized medicine by contributing our professional expertise.

***Figure 1**. **Identification of requirements in the position paper entitled 'IFBLS position paper on patient safety' prepared by the International Federation of Biomedical Laboratory Science**.*

A requirement can either be an object or an action for implementation purposes. Relevant objects (nouns) and actions (verbs) in the position paper were tagged in purple and numbered from 1 to 37.

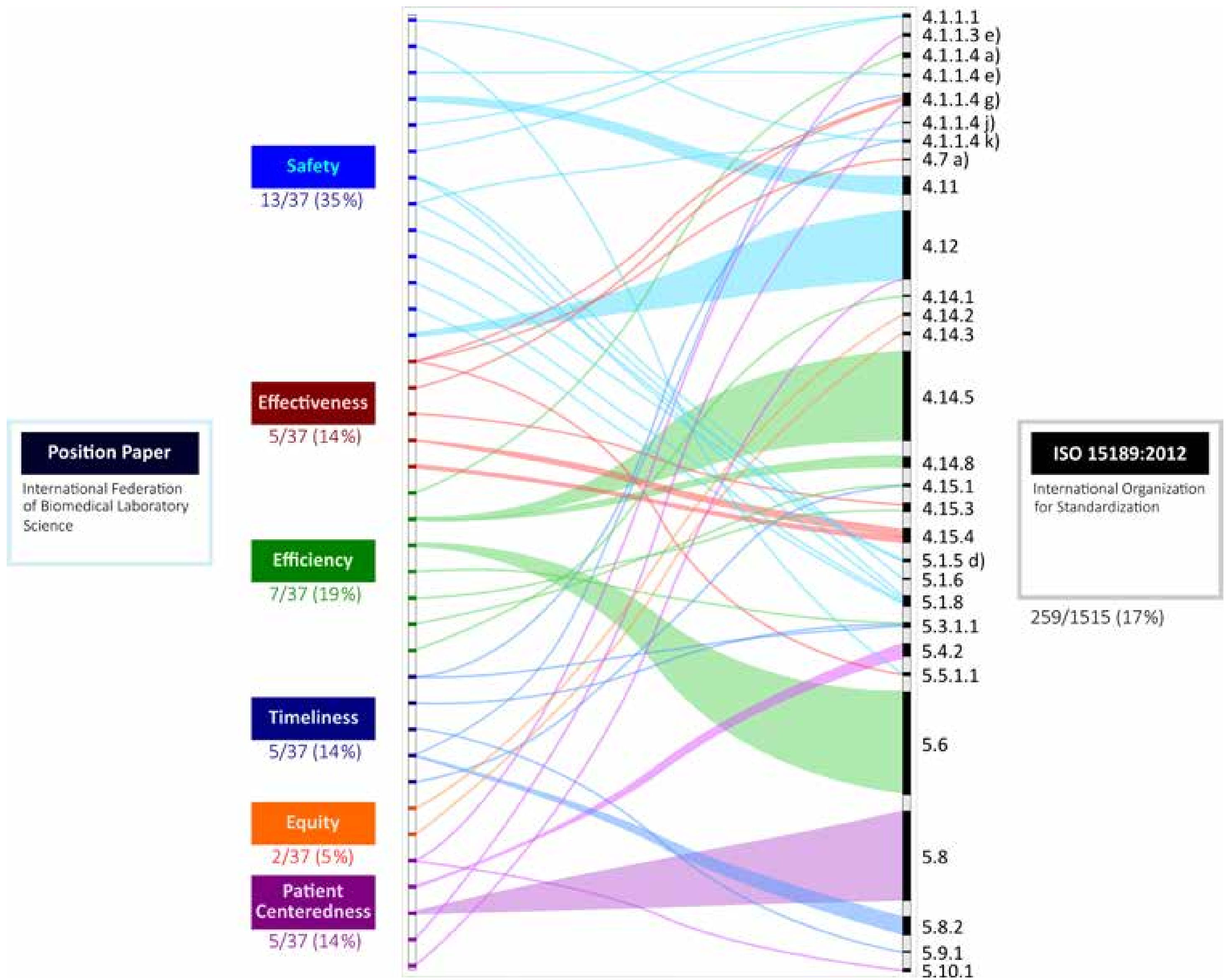

***Figure 2**. **Crosswalk analysis of patient safety requirements**.*

Crosswalk analysis of patient safety requirements of the International Federation of Biomedical Laboratory Science's recommended responsibilities relating to patient safety and conformance requirements of ISO 15189:2012. The left column represents the 37 patient safety requirements of the International Federation of Biomedical Laboratory Science's position paper. The right column represents 259/1515 (17%) conformance requirements of ISO 15189:2012.

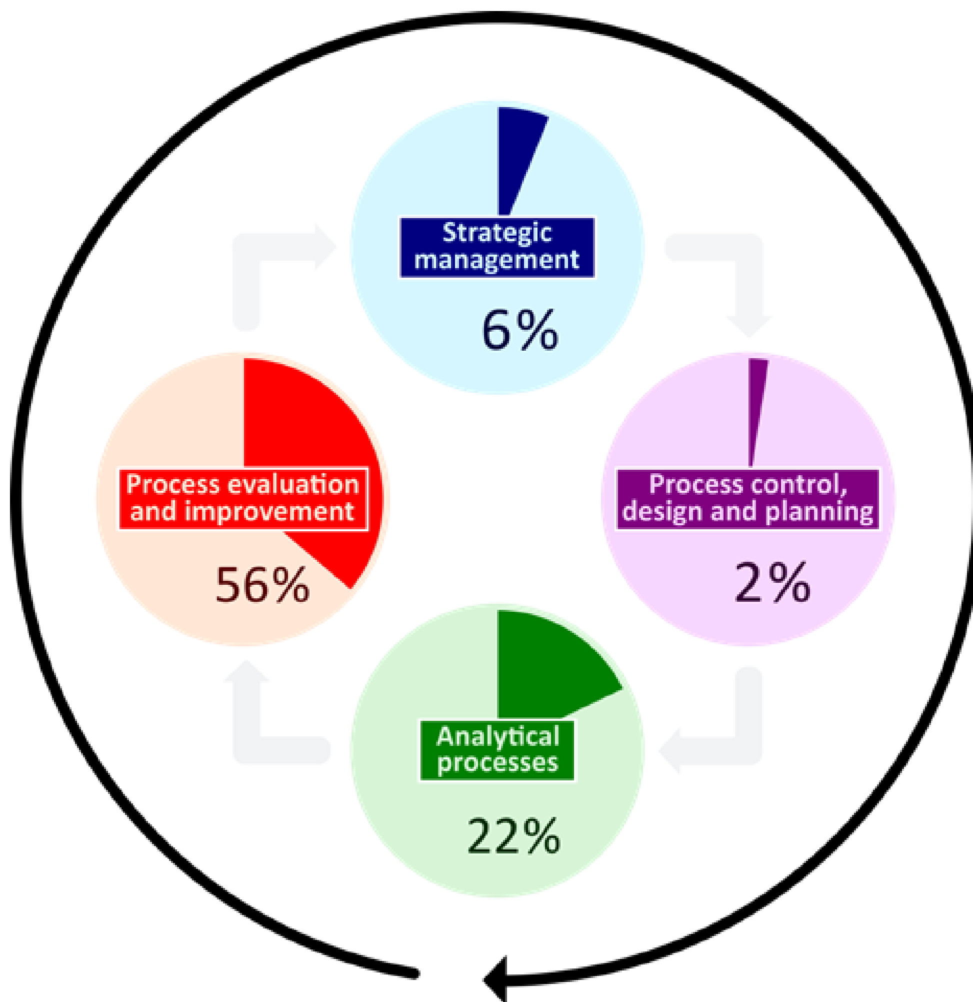

***Figure 3**. **Distribution analysis of patient safety model requirements**.*

Distribution analysis of patient safety model requirements prepared by the International Federation of Biomedical Laboratory Science across the four stages of the ISO 15189:2012 process based quality management system model. The distribution of conformance requirements across Clauses 4 (Management requirements) and 5 (Technical requirements) of ISO 15189:2012 is presented in four stages. The strategic management stage, represented by a blue circle, has six subclauses containing 399/1515 (26%) conformance requirements, and 25/399 (6%) conformance requirements were found to be associated with patient safety requirements.

The process control, design and planning stage, represented by a purple circle, has eight subclauses containing 477/1515 (31%) conformance requirements, and 8/477 (2%) conformance requirements were found to be associated with patient safety requirements. The analytical processes stage, represented by a green circle, has nine subclauses containing 387/1515 (26%) conformance requirements, and 84/387 (22%) conformance requirements were found to be associated with patient safety requirements. The process evaluation and improvement stage, represented by an orange circle, has eight subclauses containing 252/1515 (17%) conformance requirements, and 142/252 (56%) conformance requirements were found to be associated with patient safety requirements.

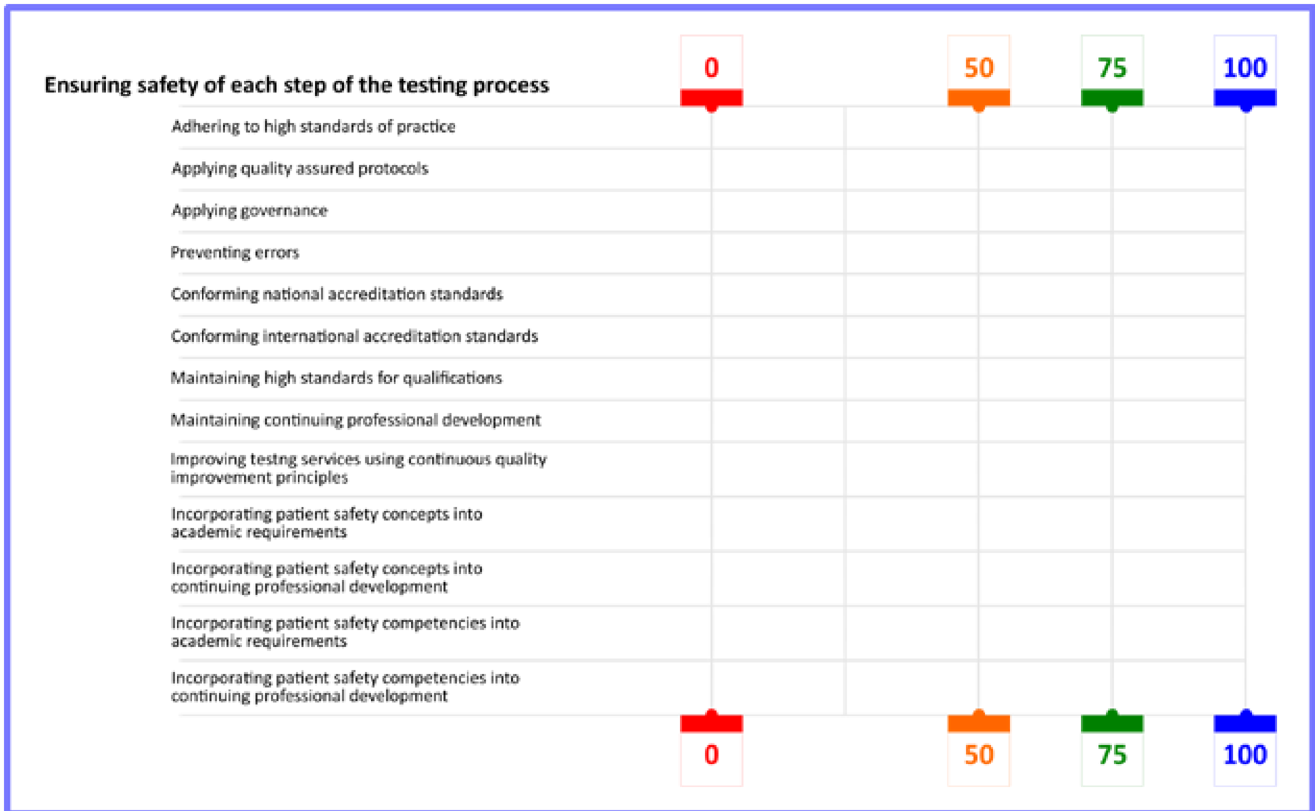

***Figure 4*. *Conformity rating scale on safety*.**

Conformity rating scale for the evaluation of laboratory personnel responsibilities for ensuring the safety of each step of the laboratory testing process. The 13/37 (35%) patient safety requirements conformity status should be evaluated against the referred 56/259 (22%) conformance requirements conformity status in Subclauses 4.1.1.1 (General), 4.1.1.4 e), 4.1.1.4 j), 4.1.1.4 k), 4.11 (Preventive action), 4.12 (Continual improvement), 5.5.1.1 (General), 5.1.6 (Competence assessment) and 5.1.8 (Continuing education and professional development) in ISO 15189:2012.

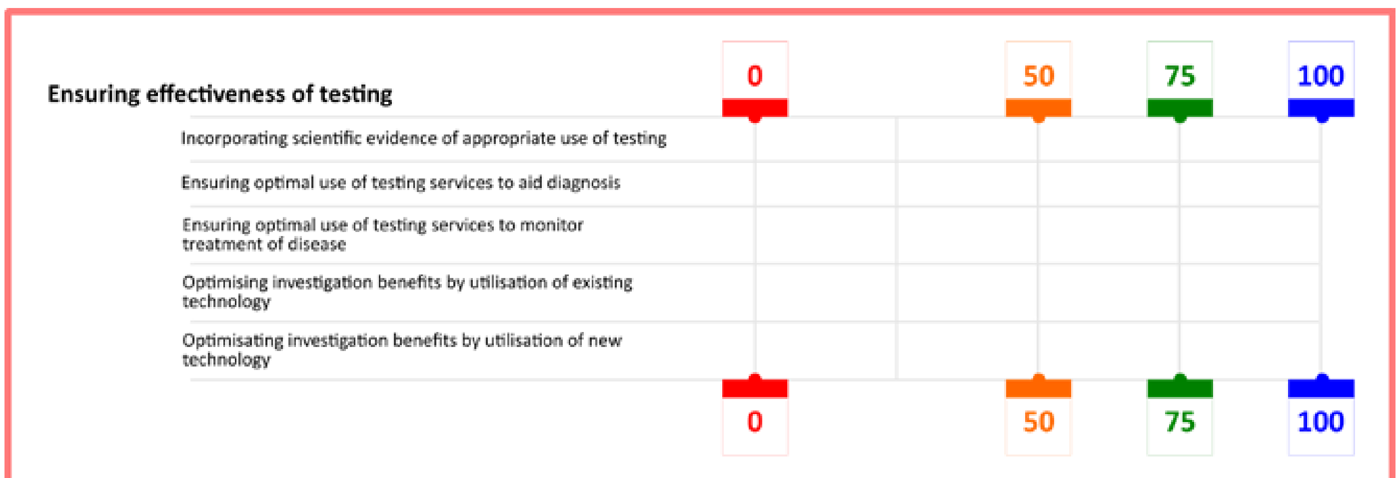

***Figure 5*. *Conformity rating scale on effectiveness*.**

Conformity rating scale for the evaluation of laboratory personnel responsibilities for the effectiveness of laboratory testing. The 5/37 (14%) patient safety requirements conformity status should be evaluated against the referred 11/259 (4%) conformance requirements conformity status in Subclauses 4.1.1.4 g), 4.7 a), 4.15.3 (Review activities), 4.15.4 (Review output) and 5.5.1.1 (General) in ISO 15189:2012.

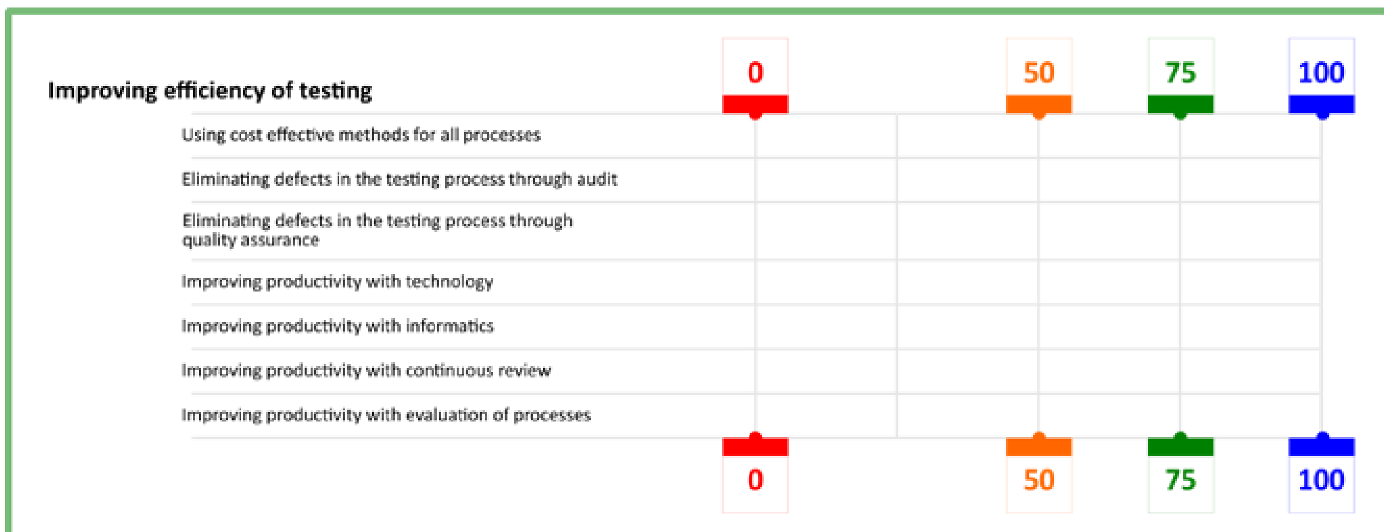

***Figure 6*. *Conformity rating scale on effeciency*.**
Conformity rating scale for the evaluation of laboratory personnel responsibilities for improving the efficiency of laboratory testing in all settings. The 7/37 (19%) patient safety requirements conformity status should be evaluated against the referred 116/259 (45%) conformance requirements conformity status in Subclauses 4.1.1.4 a), 4.14.5 (Internal audit), 4.14.8 (Reviews by external organizations), 4.15.1 (General), 4.15.3 (Review activities) and 5.6 (Ensuring quality of examination results) in ISO 15189:2012.

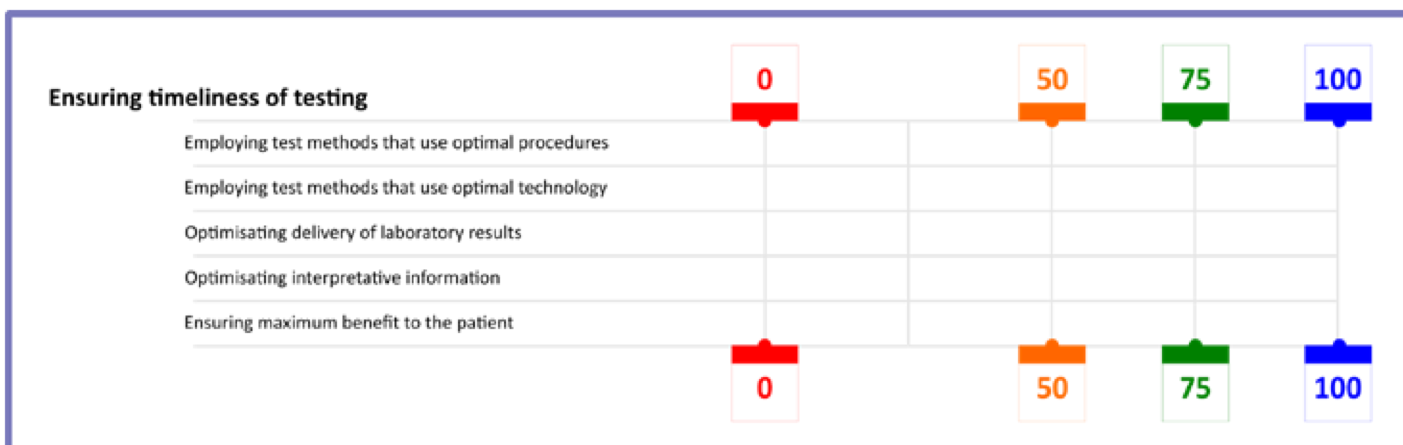

***Figure 7*. *Conformity rating scale on timeliness*.**
Conformity rating scale for the evaluation of laboratory personnel responsibilities for ensuring the timeliness of laboratory testing in all settings. The 5/37 (14%) patient safety requirements conformity status should be evaluated against the referred 12/259 (5%) conformance requirements conformity status in Subclauses 4.1.1.4 g), 4.1.1.4 k), 4.15.1 (General), 5.3.1.1 (General), 5.5.1.1 (General), 5.8.2 (Report attributes) and 5.9.1 (General) in ISO 15189:2012.

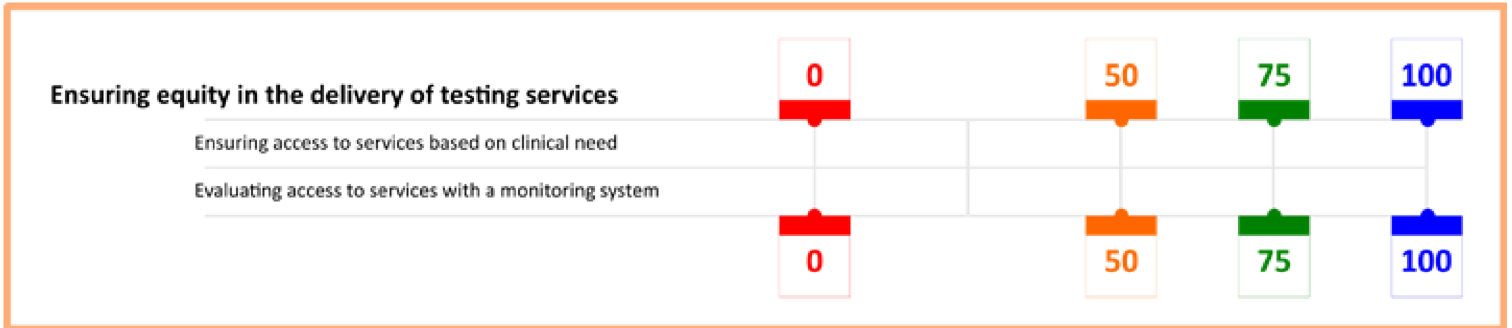

***Figure 8*. Conformity rating scale on equity in the delivery of laboratory testing services**.
Conformity rating scale for the evaluation of equity in the delivery of laboratory testing services. The 2/37 (5%) patient safety requirement conformity status should be evaluated against the referred 12/259 (5%) conformance requirements conformity status in Subclauses 4.14.2 (Periodic review of requests, and suitability of procedures and sample requirements) and 4.14.3 (Assessment of user feedback) in ISO 15189:2012.

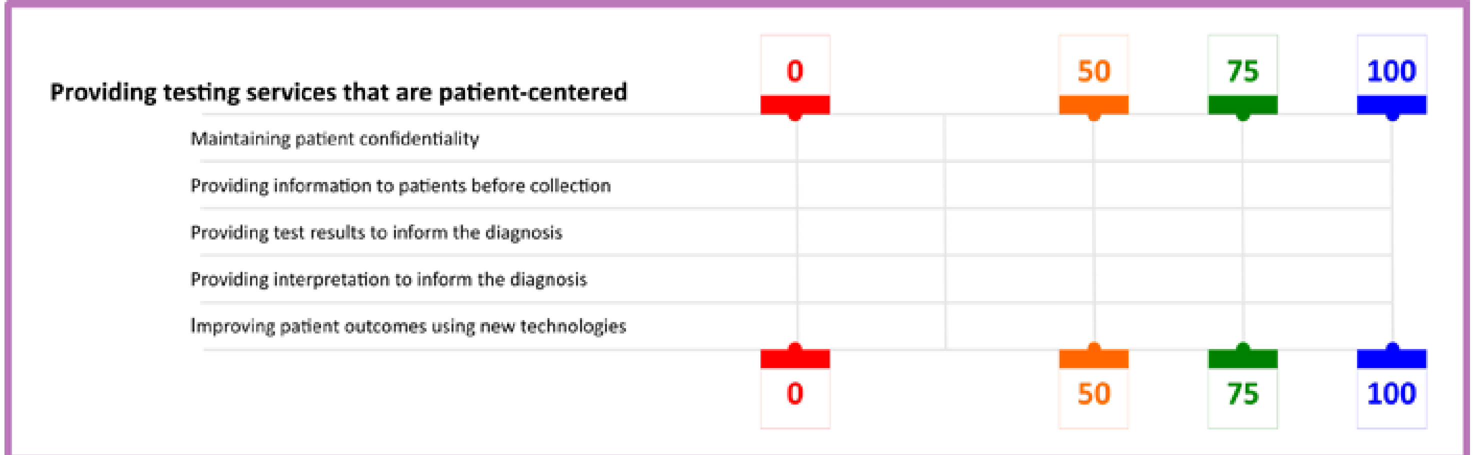

***Figure 9*. Conformity rating scale on testing services that are patient-centred**.
Conformity rating scale for the evaluation of laboratory personnel responsibilities for providing laboratory testing services that are patient centred. The 5/37 (14%) patient safety requirements conformity status should be evaluated against the referred 60/259 (23%) conformance requirements conformity status in Subclauses 4.1.1.3 e), 4.1.1.4 g), 4.12 (Continual improvement), 5.4.2 (Information for patients and users), Subclause 5.8 (Reporting of results) and 5.10.1 (General) in ISO 15189:2012.

achieved by the development of conformity rating scales based on the content analysis results of a recently released position paper entitled 'IFBLS position paper on patient safety' and ISO 15189:2012. The current study identified that the position paper contained 37 PSReqs and could be evaluated against 259/1515 (17%) referred CReqs in Subclauses 4.1.1.1 (General) in ISO 15189:2012 (ISO 2014, p. 6) to 5.10.1 (General) in ISO 15189:2012 (ISO 2014, p. 38). Overall, the current study found that the tool can provide useful information to support ISO 15189:2012 accredited medical laboratories.

The use of conformity rating scales to determine the conformity status of patient safety could add value to the medical laboratory quality management system by providing quantifiable information at the strategic management level. The results are also highly likely to give meaningful feedback to laboratory personnel who are associated with the examination processes. Despite the potential advantages of using the tool to provide useful information, the findings may be somewhat limited by seven potential areas of concern, if not first clarified properly. These limitations may offer unique challenges which require laboratory personnel to implement measures to reduce the susceptibility to produce further uncertainties. These potential obstacles are highly likely to cause variation in determining the compliance status of specific CReqs in ISO 15189:2012 which in turn will influence the rating of PSReqs. While it is impossible to implement 'one-size-fits-all' countermeasures to such obstacles, laboratory personnel should implement reasonably practical solutions using appropriate, relevant resources to perform in the most economical and efficient manner. The potential areas for laboratory personnel to clarify and consider prior to using the tool to determine the conformity status of patient safety are discussed and partial solutions are suggested below.

The first potential area is the clarification of what 'high standards of practice' are, because the IFBLS has not yet provided exact specifications. High standards could range from having relevant certifications to full registration of laboratory personnel to practise medical laboratory science. The variation may cause uncertainties in certain areas of practice; therefore, the term 'high standards of practice' must be explicitly defined by medical laboratories, so that laboratory personnel can align their practices to achieve complete compliance. It is possible that the high standards of practice are supported by relevant good practices. It is important to note that the term 'good practice', defined as a 'method that has been proven to work well and produce good results, and is therefore recommended as a model' in Subclause 3.1.3 of ISO 14055-1:2017 (ISO 2017, p. 2), differs from the term 'best practice' (Boon 2016), defined as a 'method that has been proven to work well and produce the best results, and is therefore recommended as a model' in Subclause 3.5 of ISO 10014:2021 (ISO 2021, p. 2). Laboratory personnel could also identify specific practices for implementation by translating proficient performance into competitive good practices, particularly for high-risk activities. Laboratory personnel may wish to select relevant good practices that could maximise operational effectiveness while complying with accreditation requirements; for example, if levels of applicable theoretical and practical requirements are measured by using the local registration process, then the laboratory personnel must hold valid registration to practise in an accredited medical laboratory, where it is operationally feasible (Emmerling 2015). Employing special exemptions in registrations to practise in order to manage legal necessities while ignoring quality of performance is highly likely to open a window of vulnerability into the quality management system whereby technically valid results are not delivered, which contradicts the intent of accreditation.

The second potential area for clarification is preventing errors in the examination processes. The requirement to have a documented procedure for preventive action is highly unlikely to be included in the fourth edition of ISO 15189 (Mok and Ang 2016, p. 49), and it is not a requirement in the implementation of ISO 9001:2015 (ISO 2015). In case of this scenario, the medical laboratory may wish to invest more resources into risk management (Holt 2015) with the specific support of ISO 22367:2020 (ISO 2020) to support their prevention-related actions. In addition, IEC 31010:2019 (IEC 2019) has detailed relevant risk assessment techniques for medical laboratories to apply. However, the routine practice of preventive maintenance for laboratory equipment should remain an important measure to prevent errors. Preventive maintenance plays a vital role in ensuring the examination processes produce credible information to support diagnosis, and laboratory personnel could contribute by performing the maintenance tasks competently or ensuring the maintenance programme is both effective and efficient in taking reasonable steps to minimise errors.

The third potential area for clarification is the format of continuing professional development (CPD). The term CPD has been defined as 'activities undertaken by a person after initial education or training to maintain, improve or increase his/her knowledge and skills related to his/her professional activities' in Subclause 2.27 of ISO/IEC TS 17027:2014 (ISO and IEC 2014, p. 4). It is important to note that the definition of CPD is highly likely to differ between accreditation bodies, and at the same time the accreditation requirements to implement CPD-related activities are likely to vary. Nevertheless, laboratory personnel must engage in CPD to a sufficient level, as specified in Subclause 5.1.8 (Continuing education and professional development) of ISO 15189:2012 (ISO 2014, p. 21). It is important to note that laboratory personnel who make professional judgments with

reference to examinations must have relevant competencies to fulfil the role, as specified in Subclause 5.1.2 (Personnel qualifications) of ISO 15189:2012 (ISO 2014, p. 19) and this should be fully supported by relevant CPD, as specified in Subclause 5.1.8 of ISO 15189:2012.

The fourth potential area for clarification is the technical difficulties laboratory personnel sometimes face in providing relevant clinical advice relating to the choice of examinations and use of services directly to the users of medical laboratories. This may be related to the clinical governance structure that assigns accountability and responsibility for supervision. It is possible to address this area by obtaining higher qualifications, such as clinical scientist qualifications obtained through fellowship qualifications of the Royal College of Pathologists, the Royal College of Pathologists of Australasia (Behets *et al* 2021, p. 2468), and the Australian Institute of Medical and Clinical Scientists, which require laboratory personnel to complete an extensive specialist training programme. By enhancing qualifications, the laboratory personnel can increase capabilities that reinforce patient safety.

The fifth potential area for clarification is the selection of cost effective methods for testings without compromising standards. While cost effectiveness remains a continuous discussion, it is important for laboratory personnel to evaluate the medical laboratory's capabilities, competitiveness and resources comprehensively to gain situational awareness (Thompson Jr *et al* 2021, pp. 78-114). Once costs and effectiveness are determined, the medical laboratory can competitively strengthen its quality management system by aligning with key performance indicators of relevant requirements (Gandellini *et al* 2013, pp. 101-107). This delicate balancing act may require skills that are beyond laboratory personnel, therefore the support of financial management professionals to perform the analysis may be required.

The sixth potential area for clarification is the mechanisms involved in using audits to eliminate defects. The effective use of internal audits throughout the medical laboratory is highly likely to identify strengths and weaknesses of examination processes. However, it remains difficult for laboratory personnel to obtain relevant internal auditing qualifications to perform such audits. Internal audits must be conducted by trained laboratory personnel, as specified in Subclause 4.14.5 (Internal audit) of ISO 15189:2012 (ISO 2014, p. 17). The current acceptable practice is to use laboratory personnel who have completed training courses that have been certified by the International Personnel Certification Association (Behets *et al* 2021, pp. 1906-1907). Internal auditing training courses in ISO 9001:2015 remain easily accessible; however, this is not the case for ISO 15189:2012. Laboratory personnel should complete training courses from organisations certified to ISO/IEC 17024:2012 (ISO and IEC 2012) before conducting internal audits for the medical laboratory, such as Exemplar Global and PECB.

The seventh potential area for clarification is the degree of stringency required to maintain patient confidentiality throughout all phases of testing. It remains a routine responsibility for laboratory personnel to take steps to protect personal information from misuse and loss and from unauthorised access, modification and disclosure; this is often supported by a certification of ISO/IEC 27001:2013 (ISO and IEC 2013). However, laboratory personnel must also remain firm under undue influences and pressures, even from internal upper management (Åkerström 2014). Laboratory personnel must take all reasonable steps to maintain patient confidentiality at all times.

The present study was undertaken to develop a practical tool for ISO 15189:2012 accredited medical laboratories to determine the conformity status of patient safety. Six areas of responsibility containing PSReqs were used to identify referred CReqs in ISO 15189:2012 to develop conformity rating scales for evaluation. The main advantage of the tool is to provide a quantitative and structured approach to medical laboratories to perform gap analysis for continual improvement purposes, if they see fit. Overall, this study has developed a reasonably practicable tool to determine the conformity status of patient safety for ISO 15189:2012 accredited medical laboratories.

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NSW Health Pathology Research Forum 2022

"Together Towards Tomorrow Today"

Oral Presentations and Posters – Meeting Abstracts

Background

The NSW Health Pathology Research Forum 2022 "Together Towards Tomorrow Today" is the second annual Research Forum from NSW Health Pathology.

The first such forum, held over two days in November, and was entitled "Collaboration for the Future: Pathology Research Towards 2025".

The 2022 Research Forum provides a similar forum, with the additional daily themes of 'COVID-19 and beyond' and 'Research Initiatives and Beyond'. This was a free online event open for anyone to attend virtually. The forum included a variety of talks, including international and national invited speakers in several Plenary sessions, and additional concurrent sessions highlighting research and innovation activities of different clinical streams and NSW Health Pathology services. Separate sessions included "rapid fire presentations" by early career researchers. Here are the speakers and abstracts for the meeting.

Speakers and presentation titles

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The dynamic nature and public health relevance of SARS-CoV-2 within-host diversity

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A longitudinal genomic survey of invasive pneumococcal serotype 3 disease in Australia

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Single-cell analysis of CD8+T-cells in newly diagnosed multiple myeloma patients

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Genomic surveillance of invasive Haemophilus influenzae in New South Wales

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When COVID-19 changes the rules: Understanding the rapid implementation of a pathology result notification service – A case study

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Quality Control monitoring in a Forensic Biology Laboratory: A Success Story

Hirani R 112
Seroprevalence of SARS-CoV-2 antibodies among Australian blood donors following the arrival of the Omicron variant

Hitchcock C 112
Validation of the Precision ID™ mtDNA whole genome panel and the Ion GeneStudio™ S5 Plus Sequencer for mitochondrial DNA casework

Johnson-Mackinnon J 113
Using Respiratory Viral Oligo Panel to identify co-infections in SARS-CoV-2 positive patients

Kot C 113
Small scale assessment of false positive rapid antigen tests causation

Sam M 113
Development and Evaluation of an LC-MS method for therapeutic monitoring of Infliximab drug levels for use in the routine clinical laboratory

Sim E 114
Genomics aided surveillance of Shiga-toxin producing Escherichia coli in New South Wales

Walker G 114
Rapid evaluation of clinical immunotherapeutics against emerging SARS-CoV-2 variants of concern

Zhu Y 115
Implementation of the Genomic Annotation Interpretation Application (GAIA) Platform in a diagnostic Massively Parallel Sequencing service

Poster Presentations

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Birth of a healthy baby using autologous sperm cryostored for over 25 years

Domazetovska A 115
Culture-free phylogenetic analysis of Legionella pneumophila using targeted CRISPR/Cas9 Next Generation Sequencing

Elton J 116
Fridge temperature monitoring and logging Solution

Favaloro E 116
Harmonisation of haemostasis assays within NSW Health Pathology

Green E 117
Whole-genome sequencing analysis of invasive Streptococcus pyogenes isolates from patients within the Hunter Region, New South Wales, Australia

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Halving the Way: Reduced Volume Quantifiler™ Trio Processing using an Automated Workflow

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Degrading DNA Profiles to a specific level using UV exposure

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Abstracts

Oral Presentations

The dynamic nature and public health relevance of SARS-CoV-2 within-host diversity

Jessica Agius[1,2], Jessica Johnson-Mackinnon[2], Winkie Fong[2], Kerri Basile[2], Vitali Sintchenko[2], Rebecca Rockett[2]

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Introduction: During the COVID-19 pandemic, SARS-CoV-2 genomic surveillance has facilitated real-time monitoring of the virus to track its spread and identify emerging variants. However, SARS-CoV-2 research, and surveillance has predominantly focused on mutations observed in the consensus genome, which represent the dominant variants.

This study delves beneath the consensus to discover within-host variants (iSNVs). Within-host viral diversity can provide early indications of diagnostic dropouts, identify transmission events, and inform public health surveillance.

Methods: We quantified iSNVs among mild and severe clinical cases and longitudinally within in vitro culture. Respiratory samples were collected in NSW between March 2020-August 2021, and captured with the Respiratory Viral Oligo Panel and sequenced.

Results: Within-host variants were detected in mild (83%), severe (100%), and culture (100%) specimens. Patterns and frequencies of iSNVs were dynamic and increased longitudinally. In culture, iSNVs were consistent across sampling days and dilutions. Within-host variants detected in some SARS-CoV-2 lineages were shared in clusters of community transmission, highlighting transmission events between household members.

Conclusion: We have demonstrated the significant heterogeneity and dynamic frequency of within-host variants

of SARS-CoV-2. Therefore, monitoring of sub-consensus mutations is needed for SARS-CoV-2 surveillance. This can assist with determining the effectiveness of immunisation and therapeutics during the pandemic.

A longitudinal genomic survey of invasive pneumococcal serotype 3 disease in Australia

Shona Chandra[1], Rebecca Rockett[2,3], Bianca Crowder[1,4], Shahin Oftadeh[4], Vitali Sintchenko[2,3]

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Introduction: Prior to the introduction of the pneumococcal conjugate vaccines (PCV7 and PCV13), invasive pneumococcal disease (IPD) was associated with high rates of morbidity and mortality in children and the elderly. However, serotype 3 infections characteristically result in severe clinical presentations. Despite the inclusion of serotype 3 in the PCV13 vaccine formulation, it continues to evade antibody-mediated clearance and remains a significant cause of IPD in Australia.

Methods: This study aimed to understand the evolution and genomic characteristics of IPD caused by Streptococcus pneumoniae serotype 3. Historical isolates of S. pneumoniae recovered from IPD cases diagnosed in New South Wales between January 1999 and December 2020 (n=134) were subjected to serotyping, whole genome sequencing (WGS) and bioinformatics analysis.

Results: The population structure of IPD isolates were determined. The S. pneumoniae genomes of serotype 3 isolates were predominately classified as sequence type (ST)-180 (86.4%, 108/125).

However, the incidence of a more divergent and virulent lineage within ST-180 (Clade II, 15.2%, 19/125) emerged and increased after 2005. Internationally, Clade II has been associated with increased virulence, genetic recombination, and antimicrobial resistance to first-line agents, notably due to the acquisition of the Tn916-like conjugative transposon.

Conclusions: These findings indicate a genetic divergence in S. pneumoniae serotype 3 genomes, which is postulated to be driving the increased incidence and persistence of serotype 3 IPD in Australia.

Solving Crime Through Family Ties

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Introduction: Familial Searching is a forensic tool that takes advantage of the sharing of DNA between closely related individuals to help identify candidates that may be biologically related to an unknown profile. In cases where direct DNA database searching has failed to lead to identification of an unknown profile, a familial search can be used to identify potential closely related individuals. The familial search predominately identifies parent/child and sibling relationships. Case examples of familial searching contributions to investigative outcomes will be showcased.

Methods: The familial searching workflow is an evolving process, with the refinements aimed at maximising the familial candidates captured by the process while minimising the resources outlaid.

Results: Potential persons of interest have been identified through familial searching. The associated upgrading of DNA reference profiles to the current autosomal DNA typing kit in addition to male DNA profiling has also resulted in investigative links.

Conclusion: The intelligence obtained through familial searching has proved to be a useful investigative tool for NSW Police, providing new leads in unsolved cold and contemporary cases.

Dope Ropes: Exploration of analysis techniques for Cannabinoids found in edibles

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Introduction: In recent years, there has been an increase in the prevalence and diversity of commercially produced cannabinoid infused edibles. These commercially produced edibles pose a major health risk to the general population as the product is easily mistaken for popular confectionary, particularly to youths and inexperienced users.

The rise in high-quality edibles, particularly commercially manufactured edibles, present two major challenges; the isolation of cannabinoids from the complex matrix and obtaining a representative sample. Due to the unique composition of these edibles, a uniform extraction

approach is not conducive to producing reproducible results. Across all brands and subcategories of edibles, cannabinoid composition, concentration and uniformity vary considerably, creating the need for a more thorough investigation into extraction practices.

Methods: An exploratory study was conducted on chemical extraction and homogenisation techniques. Exploitation of solubilities, ratios, soaking and sonication times, pH dependences as well as sampling aids, such as liquid nitrogen and grinding, were utilised to address the current limitations surrounding isolation and identification of cannabinoids from edibles.

Results: The results of the study were evaluated and categorised by extraction efficiencies, cost benefits and improvements to workflow.

Conclusion: Conclusions made aided in the recommendations for optimising techniques within high-throughput illicit drug laboratories, which has meant the implementation of a new procedure in the laboratory.

Moving from epi-curves to epi-fishplots: visualising the evolution of genomic clusters

Jenny Draper[1,2], Alicia Arnott[1], Elena Martinez[1,2], Mailie Gall[1,2], Andrew Ginn[1,2], Grace Blackwell[1], Rebecca Rockett[1,2], Qinning Wang[1], Jen Kok[1], Dominic Dwyer[1,2], Vitali Sintchenko[1,2]

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Introduction: The rise and fall of disease outbreaks has traditionally been illustrated with an “epidemiological curve”, typically a histogram or stacked bar chart illustrating how incidence changes over time in response to events or public health measures. However, traditional “epi-curves” fail to adequately represent outbreaks involving multiple strains which are introduced, evolve, or are eliminated over time. Here we introduce a new visualisation tool, the “epidemiological fishplot” (epi-fishplot) to communicate complex genomic data to public health professionals and illustrate the emergence, spread, and evolution of genomic clusters over time. Initially developed to communicate the real-time evolution of the SARS-CoV-2 epidemic in New South Wales, the epi-fishplot can be used for different types of genomic epidemiological surveillance data.

Methods: The “fishplot” is a variant of a “stream graph”, originally developed to illustrate the evolution of cell lineages in cancer tumours. In a fishplot, lineages can arise independently or evolve into sublineages, clearly illustrating and quantifying the relative emergence and evolution of genomic lineages in a microbial population over time. We developed an R package “epifish” that adapts fishplots to handle genomic epidemiological data.

Results: We demonstrate how the epi-fishplot documents the course of the COVID-19 epidemic in New South Wales, as well as effect of COVID-19 pandemic public health measures (lockdowns) on the circulating genomic lineages of the sexually transmitted pathogen Shigella sonnei and antimicrobial resistance (AMR) plasmids.

Assessment of COVID-19 vaccine-induced thrombotic thrombocytopenia in Australia

Emmanuel J Favaloro[1,2,3], Joanne Clifford[4], Emma Leitinger[4], Michael Parker[4], Pauline Sung[4], Sanjeev Chunilal[4], Huyen Tran[5], Geoffrey Kershaw[6], Suki Fu[6], Freda Passam[6], Monica Ahuja[7], Shir Jing Ho[7], Elizabeth Duncan[8], Olivia Yacoub[8], Chee Wee Tan[8,9], Lisa Kaminskis[10], Natasha Modica[10], Dominic Pepperell[10], Leanne Ballard[11], Lisa Clarke[12,13], Christine SM Lee[14], Elizabeth E Gardiner[15], Philip Young-Ill Choi[15,16], Ibrahim Tohidi-Esfahani[14], Robert Bird[17], Timothy Brighton[18], Vivien Chen[12,14]

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Introduction: COVID-19 vaccination aims to prevent and minimise COVID-19 and related pathophysiology. A rare complication of COVID-19 vaccination is vaccine-induced thrombotic thrombocytopenia (VITT), which in early reports were fatal in upwards of 40% of VITT cases. We aim to describe patterns of testing for VITT in a large Australian cohort.

Methods: In this multicentre study, VITT ELISA ('immunological') testing was performed in 1284 suspected VITT patients over a period of 12 months, supplemented in select cohorts by additional tests, including functional platelet activation assays.

Results: Overall, 20.7% ELISA results were classified as positive, with similar positivity rates seen in six participating centres. Functional VITT assays showed associations with immunological test results, but we also identified functional positivity in up to a third of ELISA negative samples, suggesting false negative VITT by ELISA in these cases.

Conclusion: To our knowledge, this is the largest ever multicentre evaluation of ELISA testing for investigation of VITT. Discrepancies in test results in some patients highlighted limitations in relying on single methods for identification of VITT, and also highlights the variability in phenotypic presentation of VITT. Our co-ordinated nationwide testing approach led to rapid diagnosis or exclusion of VITT, early intervention and treatment, and a low mortality rate in the Australian VITT cohort (<5%).

Single-cell analysis of CD8+T-cells in newly diagnosed multiple myeloma patients

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Introduction: CD8+T-cells have a well-established role in multiple myeloma (MM) control. We assessed the impact of MM on CD8+T-cells in the bone marrow (BM) and peripheral blood (PB) of newly diagnosed (ND)MM patients.

Methods: CD8+T-cells from paired BM and PB samples from NDMM patients were subjected to scRNA-seq, inclusive of paired TCR-seq. Samples from NDMM, MGUS and age-matched controls were assessed by high-dimensional flow and mass cytometry. Analysis was performed using custom bioinformatics pipelines and FlowJo.

Results: Differences between BM and PB were apparent by cluster restricted expression of GZMB and GZMK, with the latter chiefly evident within the BM and largely co-expressing CD69, reflecting activation and/or BM-retention. CD69 expression was restricted to the BM and delineated two independent CD8+T-cell subsets. BM-restricted CD8+CD69+T-cells appeared largely unperturbed by disease, while marked differences were observable in the CD69 negative subset, which reflected infiltrating T-cells, inclusive of previously described, Vβ-restricted clonal CD8+T-cell expansions [1]. Clonal CD8+T-cells, evident in BM and PB, potentially specific for common peptides previously reported over-expressed in MM [2], were observable across multiple individuals.

Conclusion: While BM-CD8+CD69-T-cells cells appear phenotypically diverse and contain clonally expanded CD8+T-cells in NDMM, BM-CD8+CD69+ T-cells are conserved and do not show clonal expansion, suggesting they may be preserved from disease influence.

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Genomic surveillance of invasive Haemophilus influenzae in New South Wales

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Background: Haemophilus influenzae (HI) is a recognised cause of severe invasive infections which is characterised by the presence or absence of the polysaccharide capsule into serotypes A-F (encapsulated) and NTHi (non-typeable). The disease caused by HI serotype B is a vaccine-preventable and notifiable disease.

However, despite high rates of vaccination, HI cases have been reported in Australia and globally. This study aimed to characterise the strains of HI circulating in New South Wales.

Methods: A total of 70 cases of invasive HI infections (n=71 isolates) were referred to the ICPMR-NSW Health Pathology for typing between January 2017 and September 2021. The isolates were characterised using slide agglutination with antisera and whole genome sequencing (WGS) to determine serotype. Strains were further investigated for sequence type via MLST and antimicrobial resistance markers.

Results: The median age of cases associated with invasive HI infections was 50.25 years, ranging between 0 to 97 years of age. HI isolates were classified into five serotypes – A (n=2), B (n=39), E (n=3), F (n=2) and NTHi (n=25). Eleven HI isolates were resistant to β-lactams as they encode a β-lactamase. In conjunction with epidemiological data, the study demonstrated the current capabilities of clustering isolates based on core-genome, SNP, and serotyping analysis.

Conclusion: These findings demonstrated that in silico serotyping based on WGS data carries the capability to replace slide agglutination serotyping. This study was able to characterise the HI in NSW and was able to determine potential links between cases.

When COVID-19 changes the rules: Understanding the rapid implementation of a pathology result notification service – A case study

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Introduction: During the COVID-19 pandemic, high rates of testing helped identify positive patients, monitor outbreaks and limit spread of infection. Without the traditional clinician referral pathways, the surge in COVID-19 testing made it difficult to rapidly notify the large volume of patient awaiting results. This occurred manually by frontline health staff and took up to ten days to contact patients. The rapid roll-out of the NSWHP COVID-19 SMS Result Service alleviated pressure on frontline health staff and supported greater focus on patients with COVID-19 detected results including clinical management and contact tracing. However, it was unclear what contextual factors were critical to its adoption by LHD partners.

Methods: A mixed method approach was used involving a semi-structured survey issued to 239 staff from a representative sample of four LHDs (metropolitan, regional and rural) and supplemented by NSWHP enterprise data related to COVID-19 testing and Result Service uptake rates.

Results: Within a month of implementation of the Results service, LHD registration rates increased sharply from 10% to 75%. Despite the low response rate 13% (31), several themes emerged from the survey. Most participants agreed that the Result Service benefitted the people of NSW and their LHD. The highest ranked factors that influenced the implementation were demand on frontline staff (4.57/5) and patient demand (4.48/5).

Conclusions: The study identified the contextual factors that influenced LHD adoption of the Result Service including increasing demands on front-line staff, unfunded resource impacts and pressure from patients.

Quality Control monitoring in a Forensic Biology Laboratory: A Success Story

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Introduction: Good quality management is critical in forensic laboratories as it demonstrates laboratory's ability to provide reliable, high-quality results to stakeholders in the criminal and coronial justice system. An important aspect of quality management is the ongoing monitoring of quality controls.

Methods: One example of ongoing quality control monitoring in the Forensic Biology/DNA Laboratory at FASS, relates to the assay used for DNA quantification. The results from this assay provide an estimate of total human DNA and male DNA quantities that informs downstream processing. Ongoing monitoring allows for the early identification of any non-conforming results which could affect the quality of DNA profiles generated by the laboratory.

The monitoring system in place relies on a strict pass/fail criteria based on validation, casework data and plotting of quality control data used for trend analysis.

Results: We present a recent example where trend analysis of quality control data for the DNA quantification assay was critical in swiftly identifying and resolving a performance issue. Staff identified a deviation in assay performance compared to previous quality control data, which indicated a potential manufacturing issue. The quality control data was valuable in demonstrating the issue to the manufacturer and helped with a prompt resolution.

Conclusion: Undertaking trend analysis of quantification quality control data has enabled FASS staff to notice and resolve performance issues promptly. This contributes to a strong quality management system to ensure we deliver a quality service to the NSW community.

Seroprevalence of SARS-CoV-2 antibodies among Australian blood donors following the arrival of the Omicron variant

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Introduction: Until the emergence of the Omicron SARS-CoV-2 variants, Australia had relatively low case numbers. Following detection of Omicron, cumulative infection notifications rose dramatically to over 2.1 million by 31/01/2022. Since the spread of Omicron put considerable strain on routine testing and contact tracing, serosurveillance of blood donors provides estimations of the potential spread of this variant in the community.

Methods: We have conducted 2 of 4 planned serosurveys among blood donors (aged 18+ years) to estimate SARS-CoV-2 seroprevalence. Residual samples were randomly selected from Australian Red Cross Lifeblood blood donors. Analysis was conducted on the Roche Diagnostics Elecsys® Anti-SARS-CoV-2 immunoassay to detect anti-spike (S) and anti-nucleocapsid (N) antibodies.

Detection of anti-S indicates response from natural infection or vaccination and anti-N informs a response from recent (past 3-6 months) natural exposure only.

Results: In total, 10000 specimens donated between 23/02/2022–03/03/2022 and between 09-18/06/2022, were analysed. Crude anti-S seroprevalence was high across Australia (99.0%; 98.7–99.2) which was constant nationally and in all age groups. Anti-N seroprevalence was 17.0% (16.0–18.0) in round 1 and increased to 46.2% (44.8-47.6) in round 2 and was highest among donors aged 18-29 years at 62%.

Conclusions: Preliminary results suggest that anti-S seroprevalence was high among Australian blood donors. Anti-N seroprevalence was consistent with age-specific patterns based on cumulative case notifications. The next sample collection will be conducted in early September.

Validation of the Precision ID™ mtDNA whole genome panel and the Ion GeneStudio™ S5 Plus Sequencer for mitochondrial DNA casework

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Introduction: FASS has provided a NATA accredited mitochondrial DNA (mtDNA) analysis service since 2015, which has assisted with major criminal and coronial cases where samples were compromised or nuclear DNA analysis was not possible.

Until recently, mtDNA analysis has been performed using two hypervariable sites of the mitochondrial genome (mtGenome) with traditional Sanger sequencing but the discrimination power of this method can be limited when a common mtDNA profile is recovered. A large number of variants, however, are located outside the hypervariable regions and sequencing the whole genome captures all the variation present significantly increasing the power of discrimination between individuals.

Methods: FASS acquired the Ion Chef and Ion GeneStudio S5 Plus Sequencer to undertake massively parallel sequencing (MPS) as it enables the rapid sequencing of multiple samples simultaneously. The validation of the Precision ID mtDNA Whole Genome Panel on this equipment involved evaluating: the performance of the panel amplicons, library input amount, library quantification, template input amount, repeatability, cross-contamination studies and analysis thresholds.

Results: Testing demonstrated the MPS system could successfully sequence person samples for mtDNA analysis and that 60-85% of the variants were detected outside the hypervariable regions. Samples sharing a common mtDNA haplotype using Sanger sequencing were able to be

differentiated using whole mtDNA sequencing.

Conclusion: The MPS system is fit for purpose for processing casework person samples and has been implemented into the Forensic DNA laboratory.

Using Respiratory Viral Oligo Panel to identify co-infections in SARS-CoV-2 positive patients

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Introduction: The ability to detect co-infections in SARS-CoV-2 patients is often limited due to an over-reliance on SARS-CoV-2 specific RT-PCR assays and SARS-CoV-2 amplification-based sequencing protocols. However, as public health containment strategies ease it is expected that there will be an increase in high-burden respiratory pathogens for which capacity to conduct genomic surveillance remains limited.

Methods: We utilised a probe-based method, the Illumina Respiratory Viral Oligo Panel (RVOP) that can hybridise the genomes of 42 distinct respiratory pathogens. In this study we spiked positive SARS-CoV-2 respiratory samples with human influenza A virus, respiratory syncytial virus (RSV) A, human adenovirus and rhinoviruses to simulate mixed viral respiratory infections. Here we (i) demonstrate the sensitivity of the RVOP method to capture viral co-infections, and (ii) present a case study using RVOP to uncover multiple viral pathogens infecting a SARS-CoV-2 positive patient.

Results: RVOP had high sensitivity and was able to generate consensus genomes from simulated viral co-infections where each pathogen had a viral load of approximately 2512 copies. In the case study of a child PCR positive for SARS-CoV-2 and HIA the RVOP, method uncovered complete genomes to SARS-CoV-2 and 229E CoV along with sequencing reads identifying regions of Human Influenza A and KI polyomavirus.

Conclusions: These findings highlight the high sensitivity and capacity of RVOP to identify respiratory viruses of clinical and public health relevance that are not routinely tested for.

Small scale assessment of false positive rapid antigen tests causation

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Introduction: Currently the gold standard testing strategy used globally for SARS-CoV-2 is RT-PCR. Rapid Antigen Tests (RATs) screening were incorporated into everyday testing methods as a quicker alternative approach to RT-PCR. An evaluation was conducted to outline the shortcomings of newly introduced RATs. It has been globally reported that various chemicals and food-based items have triggered false positives within RATs.

Method: This evaluation utilised 8 different RAT products with 7 varying solutions in the attempt to produce false positive results. Each solution was tested in Vitro with: 100% test solution, 50% test solution 50% buffer and 100% buffer as a control and in Vivo consumption: 0 minutes after, 30 minutes after and 60 minutes after.

Results: All RATs were subject to a pH curve between 1 and 7.1. Major results found that acidic solutions resulted in false positive results for RATs with saliva RATs recording the highest results of false positives when compared to nasal RATs. When subject to the pH curve, a pH of ≤ 3.9 resulted in false positives in all RATs.

Conclusion: The sudden mass introduction of RATs into standard SARS-CoV-2 testing comes with an alarming shortcoming. The rate of false positives, as well as the ease to generate false positives in RATs generated by this study, highlights the limitations of RATs. Additional information to mitigate the risk of false positive results should be circulated if RATs are going to be a staple of society.

Development and Evaluation of an LC-MS method for therapeutic monitoring of Infliximab drug levels for use in the routine clinical laboratory.

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Introduction: Therapeutic drug monitoring (TDM) of anti- tumour necrosis factor-alpha drugs infliximab (IFX) is increasingly used in patient management protocols in several autoimmune diseases. Traditionally both drug and anti-drug antibody (ADA) levels are measured using immunoassays, yet there can be significant variation in drug

and antibody concentrations obtained by different assay methods. Recently, assays for the measurement of IFX drug levels have been developed using LC-MS techniques.

The aims of this study are to develop and validate LCMS assays for measuring IFX drug levels for routine use.

Methods: We spiked IFX into buffer at concentrations of 0.5-100 mg/L to produce a calibration curve. Samples were digested using an optimised trypsin digestion protocol. Replicates were analysed to determine intra- and inter-assay precision (%CV). IFX spiked into serum was isolated using ZEBA© and Melon Gel© columns.

Results: A standard curve across the range of 0.5-100 mg/ml was developed and results show good precision ($R2 = 0.99$, Inter-assay %CV< 6.5, Intra-assay %CV = 6.2). We developed a method to isolate IFX from serum which is dose responsive and determined the recovery rate compared to ELISA (Mean 86% n=12).

Conclusions: We have developed an LC-MS/MS assay for the measurement of total IFX in serum, which has a good recovery rate, precision and a broader measurement range compared to ELISA (0.5-100 mg/L vs 0.63-35.99 mg/L). This assay can be used to measure total IFX in patients with suspected ADA development. A free IFX assay to replace ELISAs in the clinical laboratory is in progress.

Genomics aided surveillance of Shiga-toxin producing Escherichia coli in New South Wales

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Introduction: Shiga-toxin producing Escherichia coli (STEC) is a group of bacteria spanning across multiple serotypes that can cause gastroenteritis and sometimes lead to haemolytic uremic syndrome (HUS). Two immunologically distinct Shiga toxins (Stx) produced by STEC, Stx1 and Stx2 can be further subtyped as different subtypes have different risks of clinical disease. Internationally, whole genome sequencing of STEC has been utilised to great effect for genetic epidemiology.

In this report we developed a genomics workflow and describe our experience in providing genomics guided surveillance for STEC in NSW.

Methods: STEC isolated from faeces were sequenced and put through an in-house genomics workflow where each genome will be assigned a virulence barcode and a risk category. In addition, a two-tiered approach consisting of whole genome multi-locus sequence typing (wgMLST) and core single nucleotide polymorphisms (SNP) clustering were performed for genomic clustering.

Results: This genomic workflow allowed pertinent STEC information consisting of serotype, MLST, and Shiga-toxin subtype, and the potential risk of severity of illness to be captured. Utilisation of both wgMLST and SNP clustering allowed for the inference of genomic diversity and the detection of isolates likely to be epidemiologically linked.

Conclusion: Genomic surveillance of STEC is an important part of public health response and ongoing interrogations could unlock additional insights for public health monitoring of this group of pathogens.

Rapid evaluation of clinical immunotherapeutics against emerging SARS-CoV-2 variants of concern

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Introduction: The rapid evolution of SARS-CoV-2 is an ongoing challenge to the development and clinical use of immunotherapeutics to treat COVID-19. Timely evaluation of clinically important monoclonal antibodies (mAbs) against new SARS-CoV-2 variants is needed to inform on their optimal use in treating and preventing infection.

Methods: We have built a pipeline that enables the rapid identification, culture, and characterisation of new variants within weeks of their emerging in Australia. This pipeline was used to assess in-vitro neutralisation of Beta, Delta, and Omicron (BA1, BA2, and BA4/5) SARS-CoV-2 variants by TGA-approved monoclonal antibodies.

Results: Both sotrovimab (Xevudy, GSK) and tixagevimab/cilgavimab (Evusheld, AstraZeneca) potently neutralised non-Omicron variants. Sotrovimab activity was retained against BA1 (805 ng/mL, 6-fold), but was significantly reduced against BA2 (non-neutralising) and BA5 (3826 ng/mL, 29-fold), relative to neutralisation of the reference strain (133 ng/mL).

Tixagevimab/cilgavimab weakly neutralised BA1 (1910 ng/mL, 70-fold), but retained activity against BA2 (71 ng/mL, 4-fold) and BA5 (454 ng/mL, 29-fold), relative to neutralisation of the reference strain (16 ng/mL).

Conclusions: The in-vitro neutralisation potency of therapeutic monoclonal antibodies is reduced by, and differs across, Omicron lineages. Our findings have been pivotal in updating COVID-19 treatment guidelines and demonstrate

the continued importance of SARS-CoV-2 molecular surveillance.

Ongoing evaluation of immunotherapeutics will be required as new SARS-CoV-2 variants inevitably emerge.

Implementation of the Genomic Annotation Interpretation Application (GAIA) Platform in a diagnostic Massively Parallel Sequencing service

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Background: The clinical interpretation of Massively Parallel Sequencing (MPS) data has become an important methodology in molecular diagnostics. Commercial bioinformatic applications may not meet individual laboratory needs and conversely open-source bioinformatics tools usually target a specific analysis type and require tailoring by bioinformaticians.

Aim: To implement an accredited clinical diagnostic pipeline designed for clinicians and scientists requiring no bioinformatics expertise for use, including variant prioritization, storage and quality control of data from panels/exomes/genomes.

Method: For single nucleotide variants (SNV), variant filtering is based on inheritance pattern, zygosity, amalgamated population frequency databases, variant impact severity and best practice in silico pathogenicity scores.

For Copy Number Variants (CNV), variants are identified by four CNV callers, then filtered according to morbid gene content relevant to the referral phenotype and the number of callers identifying a potential CNV.

Results: GAIA has achieved NATA-accreditation for diagnostic services and has demonstrated educational capacity in training workshops to perform rapid queries across all types of clinical referrals. GAIA has facilitated the identification of numerous novel genes in neurocognitive and other Mendelian disorders.

Discussion: GAIA is a comprehensive web-based annotation, filtration and visualization platform. It allows straight-forward laboratory workflows without regular bioinformatic support. It is well-maintained and extensively adjustable for future requirements from both clinicians and scientists.

Poster Presentations

Birth of a healthy baby using autologous sperm cryostored for over 25 years

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Introduction: Autologous sperm cryostorage provides fertility insurance for men facing gonadotoxic cancer or other treatments that risk temporary or permanent infertility. Our sperm cryostorage program started in 1978 (Andrology Unit, RPAH) relocating to the Clinical Andrology Lab, Concord Hospital in 1999 and is the largest and longest running single-centre sperm bank in the world. Cryostored sperm are considered to retain fertilising capacity indefinitely; however, long-term fertilising ability is not often proven.

Method: In 1996, a 15-year-old schoolboy with Hodgkin's disease and extensive thoracic mass was referred for sperm cryostorage prior to 6 months of MOPP/ABV chemotherapy. Three semen samples were collected and cryostored in liquid nitrogen. During follow-up the patient remained azoospermic on multiple samples. In 2021-2022, he and his 35-year-old wife underwent one IVF procedure in Brisbane using the cryostored sperm.

Results: The 3 cryostored samples had between 34-78.7 million sperm per ejaculate, 54 frozen sperm straws and post thaw motility of 17%-34%. In the IVF cycle, 27 oocytes were harvested with 21 fertilising normally with the cryostored sperm resulting in 10 embryos. A single transfer of 5 fertilised embryos was carried out and the remaining 5 embryos were frozen.

The transfer resulted in the live birth of healthy boy on the 17/2/2022 (4.3 kg and 57 cm long), more than 25 years after the sperm were cryostored.

Conclusion: We report possibly the longest case of a healthy birth from autologous sperm stored for over 25 years confirming the preservation of fertilising ability of cryostored human sperm.

Culture-free phylogenetic analysis of Legionella pneumophila using targeted CRISPR/Cas9 Next Generation Sequencing

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Introduction: Currently available methods for the laboratory

investigation of Legionella pneumophila outbreaks require organism culture. The ability to sequence L. pneumophila directly from clinical samples would significantly reduce delays. Here, we develop a method for targeted next generation sequencing (NGS) of selected L. pneumophila genes utilising a CRISPR/Cas9 based target enrichment system. We determine the method's utility by typing cultured L. pneumophila isolates, and subsequently apply the method directly to patient samples.

Methods: We sequenced 10 L. pneumophila isolates by 2 methods: (i) whole genome sequencing (WGS) and (ii) targeted (CRISPR/Cas9-based) NGS (FLASH-NGS) – sequencing 57 selected genes. We subsequently performed targeted NGS of L. pneumophila directly on patient respiratory samples.

Results: The targeted NGS of 57 genes was more efficient than WGS, and phylogenetic analysis of the 57 genes yielded the same classification of the L. pneumophila isolates as that based on analysis of whole genome data. Targeted NGS of L. pneumophila performed directly on patient respiratory samples correctly classified the patients according to their corresponding cultured isolates.

Conclusion: We provide proof of concept that targeted NGS can be used to sequence L. pneumophila directly from patient samples. Studies on a larger number of patient samples will help validate this method further. Nonetheless, CRISPR/Cas9 targeted NGS methods have the potential to be widely applicable to microbial outbreak investigations in the future, particularly in the context of difficult and slow growing organisms.

Fridge temperature monitoring and logging Solution

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[1]Point of Care Testing, NSW Health Pathology

Introduction: It is difficult to put a monetary value on a nurses' time but if we can free them of unnecessary tasks, we can not only improve their working conditions, but also make a significant saving for NSW Health.

An automated fridge monitor will free nurses from the task of manual data entry of a Fridges temperature and allow for quicker responses to cold chain breaches.

Method: A Raspberry Pi 3 Model B+ and a Ruuvitag Temperature sensor have been used to automatically log temperatures and alert users to temperature breaches. The Ruuvitag sensor transmits its temperature and battery life every ten minutes to the Raspberry Pi which is then passed to the Internet of Things Gateway, temperatures are then stored in a database.

Results: A prototype temperature sensor and logging system has been created. Recorded temperatures are stored in a database, and a monthly report is generated for review, showing daily maximum and minimums. This report can be accessed by computer, phone, or tablet. Out of bounds alerts can be sent to users by email.

Conclusion: This solution aims to automatically log temperatures of fridges, store that information in one easily accessible place, graph and display the data, alert staff if a fridge is out of bounds and make strong backup copies of the data. It has the potential to save NSW Health time and money by automating a tedious and time-consuming task.

Harmonisation of haemostasis assays within NSW Health Pathology

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Introduction: Haemostasis reflects an in vivo homeostasis mechanism to help prevent bleeding or thrombosis. Within the general discipline of haematology, all 60 NSW Health Pathology (NSWHP) laboratories perform some haemostasis assays, generally routine coagulation tests, with some major sites also performing specialised (diagnostic) haemostasis assays. In the past, NSWHP laboratories performed testing according to local network protocols.

Methods: Evaluation and advisory NSWHP teams were formed to harmonise all haemostasis assays across NSWHP. These co-ordinated the evaluation of coagulation methods for use on new ACL TOP instrumentation, and evaluations of other haemostasis test methods performed on other instruments. Consensus for harmonisation of all methods was achieved by discussion and agreement.

Results: The evaluation team has successfully evaluated and harmonised all routine coagulation tests to be performed on ACL TOP analysers in all 60 laboratories. Separate evaluations for specialised (diagnostic) haemostasis assays has also permitted harmonisation of several other assays to date, with more in progress. To date, the group have published five papers describing our approach in the scientific literature.

Conclusion: To our knowledge, this is the largest ever harmonisation drive for haemostasis testing. All 60 NSWHP sites have harmonised routine coagulation tests, and the larger laboratories are in the process of harmonising other haemostasis assays.

Whole-genome sequencing analysis of invasive Streptococcus pyogenes isolates from patients within the Hunter Region, New South Wales, Australia

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[1]NSW Health Pathology, Microbiology, John Hunter Hospital

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Background: Streptococcus pyogenes [or Group A Streptococcus (GAS)] is a significant cause of invasive infections. Epidemiological evidence suggests invasive GAS infections (iGAS) cases are increasing within Hunter New England Local Health District, from an average of 3.77 cases per 100,000 population per year for 2008-2013 to 5.24 cases per 100,000 population per year for 2014-2019.

The purpose of this study was to understand the molecular epidemiology of iGAS isolates by whole genome sequencing (WGS), and to identify antimicrobial resistance (AMR) determinants. We sequenced 182 iGAS isolates collected between 2007 and 2017 from patients located within the Hunter Region, NSW, Australia.

Methods: S. pyogenes strains were frozen after isolation and susceptibility testing. Isolates were sub-cultured on blood agar, genomic DNA was extracted, and sequencing libraries were prepared. 2×300bp sequencing was performed on an Illumina MiSeq. Bioinformatic analyses were performed for quality control checks, de-novo genome assembly, multi-locus sequence typing, in-silico emm-typing, and AMR screening.

Results: Molecular typing revealed at least 53 different S. pyogenes strain types. The six most prevalent strains were emm1-ST28, emm28-ST52, emm89-ST14, emm3.1-ST1, emm12-ST36, and emm4-ST39. Tetracycline resistance was found in 9/182 isolates, and all nine contained tetM genes.

Halving the Way: Reduced Volume Quantifiler™ Trio Processing using an Automated Workflow

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Background: The Quantifiler™ Trio DNA quantification assay has been widely adopted in forensic laboratories for the quantification of total and male DNA from casework samples. The Forensic DNA Laboratory implemented this assay into automated casework processing in 2017. Since implementation the quantification assay has been a valuable addition to the workflow, providing enhanced information for casework samples to improve downstream DNA amplification strategies, particularly in the sexual assault workflow.

Methods: To improve operational efficiency, the performance of the DNA quantification method was evaluated using a half reaction volume. A previous validation study for another DNA amplification chemistry demonstrated similar or improved amplification efficiency when using a reduced reaction volume.

Results: The evaluation of this assay included a detailed assessment and testing of the half reaction volume compared to a full reaction volume in relation to sensitivity, cross-contamination, performance with male:female mixtures and other challenging casework samples. Several changes were made to the robotic script to accommodate the reduced volume, including adjustments to the pipetting parameters to ensure the accurate delivery of 1 µL of sample to the final reaction plate.

Conclusion: The implementation of the half reaction volume resulted in a significant cost saving for the laboratory which has enabled investment in further operational enhancements.

Degrading DNA Profiles to a specific level using UV exposure

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Introduction: The production of degraded DNA samples can be very useful for forensic DNA research as this can mimic the condition of crime samples and therefore the expected results. Methods which use quick and cost-effective Ultraviolet light (UV) exposure are not well documented, hence this study aimed to establish a procedure and define the parameters required to generate DNA profiles at different degradation levels.

Methods: UV exposure times between 0-60 seconds were tested using genomic DNA. Samples were quantified with Quantifiler Trio pre- and post-UV exposure, and select samples were profiled using PowerPlex 21. The quantification data and DNA profiles were analysed to identify metrics which indicated the degradation severity.

Results: As expected, samples were observed to be degraded by UV exposure and this was evidenced by several established DNA degradation markers. No specific metric was identified to determine the severity of sample degradation, but it was noted that UV exposure between 5-10 seconds produced mild degradation, between 15-25 seconds produced moderate degradation, and between 45-60 seconds produced severe degradation. This study indicated that quantification before and after UV exposure may be useful to estimate the rate of change in one of the quantification targets, estimating the degree of degradation.

Conclusion: A method for genomic DNA degradation via UV exposure was established with treatment times identified to produce mild, moderate, and severe degradation. It was recommended that samples be quantified prior to and following treatment to estimate degradation severity.

Rapid clinical testing for SARS-CoV-2 using the Roche Liate

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Introduction: With the onset of SARS-CoV-2 in Australia, many collection centres and laboratories had to pivot to respond to the growing outbreak. Collection and PCR testing can often take 24-48 hours to accurately diagnose an infection. Front-line healthcare workers and patients determined high-risk, vulnerable, or low compliance were required to be quickly screened for SARS-CoV-2 to better protect the community and individuals. Time delays can be problematic when implementing elimination and suppression strategies.

Method: In response to the Delta and Omicron wave, NSW Health Pathology and the Point of Care Team (PoCT) implemented a robust mobile Rapid Testing Clinic (RTC). Each RTC was equipped with rapid Point of Care PCR devices (Roche Liats). The RTC concept established a 30-minute benchmark from collection to a formal result. Here we examine one RTC based at the University of Newcastle campus, which utilised up to 6 Roche Liats. During its operation from August 2021-Feburary 2022, the Ministry of Health directed over 8000 high-risk patients to the RTC. At the RTC, patients who returned positive to SARS-CoV-2 were immediately consulted on-site by skilled nurses and directed to quarantine at dedicated facilities or at home to reduce the risk of further community spread.

Result/Conclusion: The work carried out by the RTC alleviated the pressure from regular testing centres and laboratories which were often overwhelmed by surged testing. This approach proved to be a valuable resource for the Ministry of Health during the SARS-CoV-2 Delta and Omicron outbreak in Newcastle, Australia.

High-sensitive troponin point of care assessment of the Siemens Healthineers VTLi

William Hamilton, Huy Tran, Amanda Caswell, Doug Chesher, Andrea Rita Horvath, Margaret Janu, Christopher John Farrell, Don Clausen, Frank Alvaro, Jason Chung, Brian Heffernan, Terry Grissell, Brittany Thorn, John Paul Smiles, Michael Zhang, Daniel Thompson, Christopher Kot, Andrew Sargeant

[1]Point of Care Testing (PoCT), NSW Health Pathology

Introduction: Between 2020-2021, 2.9% of the Australian adult population reported symptoms of coronary heart disease. The prevalence of which increases rapidly with age, affecting 11% of adults 75 and over. A key biomarker which has been use, d to indicate the presence of acute coronary symptoms is cardiac troponin I.

Currently, there is a gap in the healthcare setting as laboratory's use high-sensitivity troponin assays, while point of care assays do not meet this standard.

Method: This clinical trial and verification evaluated the Siemens Healthineers Atellica VTLi as a point of care device that aims to address this issue. The VTLi was directly compared to a current NSW Health Pathology approved cardiac troponin I high-sensitivity assay, which is run on the Abbott Architect. Patients enrolled into the trial at John

Hunter Hospital were tested on the Architect from a standard Lithium Heparin Vacuette collection, as well as an additional Lithium Heparin Vacuette collected simultaneously, to be run in parallel on the VTLi. Key analysis of the VTLi included determining limits of detection, quantitation and blank, utilising clinical adjudication for discordant results, and incorporating standard statistical analyses as per NSW Health Pathology requirements.

Results/Conclusion: The interim findings have demonstrated that the VTLi shows a promising avenue to explore. With additional correspondence and improvement with Siemens Healthineers, the VTLi can act as an acceptable response to the current clinical requirements that are present within emergency departments.

Illuminating the mechanism: A novel approach to detecting cross-contamination on automated platforms

Catherine Hitchcock, Felicity Poulsen

[1]NSW Health Pathology, Forensic & Analytical Science Service (FASS), Forensic Biology/DNA Unit

Introduction: The implementation of automated platforms in forensic laboratories has dramatically improved sample processing by streamlining sample analysis, standardising results and reducing DNA processing turn-around times. The use of automated platforms has also significantly reduced the risk of cross-contamination events that previously occurred through manual processing methods. Nevertheless, automated platforms require extensive cross-contamination testing prior to implementation and ongoing assessment to ensure sample integrity. If contamination has been detected, the identification of the mechanism can be extremely challenging particularly when the issue is intermittent.

Methods: Typically, cross-contamination studies involve processing high DNA content samples alternating with samples containing no DNA in specific patterns. However, frequently this method does not reveal the mechanism of contamination. In the FASS Forensic DNA Laboratory, we used a novel fluorescein technique to visualise the contamination mechanism of an intermittent cross-contamination issue.

Results: The novel testing method was able to reveal the root cause of the contamination events under investigation. This resulted in the identification of a faulty labware item and enabled FASS to notify the manufacturer to take measures to rectify the issue.

Conclusion: This fluorescein method was found to be effective for troubleshooting cross-contamination incidents on automated platforms. In addition, the method is cheap, easy and quick to perform.

Using microflow liquid chromatography and high-resolution mass spectrometry to identify a commonly occurring interferent in a high sensitivity oestradiol assay

Chris Hodgkins, Simon Thompson, Renee Sahertian, Kevin Mantik, Andrea Rita Horvath

[1]NSW Health Pathology Randwick, Sydney, NSW

Introduction: Mass-spectrometry (MS) assays are recommended for samples expected to have low oestradiol (E2) concentrations. Whilst MS assays usually avoid cross-reactivity with structurally similar analytes, interferences from isobaric compounds remain possible. We previously reported on a common interfering substance in our MS E2 assay which we were able to mitigate by an extended chromatographic separation. The aim of this study was to identify the substance using high-resolution MS technology.

Methods: The interference was present in samples from a 76-year-old female patient. Serum extracts were separated by microflow chromatography and analysed by a combination of data-dependent (DDA) and data-independent-acquisition (DIA) on a Q-TOF tandem MS, with data processing by manual review and library searching of MS/MS spectra.

Results: DIA analysis revealed the interfering compound precursor m/z (271.1) to be an in-source fragment of an unknown precursor with m/z 408.1. Cross-referencing the high-resolution MS/MS spectrum of the 408.1 m/z precursor with a library of drugs and metabolites returned a library match score of 98.4 (out of 100) for ezetimibe, a lipid-lowering medication.

We reviewed the clinical information from 20 other patients in which the interference was present. Medication history, where available (n=13), showed ezetimibe use.

Conclusion: Using high-resolution MS measurements on a sample that returned an elevated result, we confirmed a commonly prescribed drug interferes with our sensitive E2 assay. This knowledge has helped us optimise our routine method while still reducing total analysis time.

High-throughput liquid chromatography-tandem mass spectrometry: same separation, shorter run-time

Chris Hodgkins, Renee Sahertian, Laura Buckton, Kevin Mantik, Andrea Rita Horvath

[1]NSW Health Pathology Randwick, Sydney, NSW

Introduction: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become indispensable in the chemical pathology laboratory due to its incomparable

selectivity. However, the relatively low throughput of LC-MS/MS limits its use to a subset of the laboratory test repertoire.

While the mass spectrometer operates on a time scale of seconds, the requirement for the liquid chromatograph (LC) to separate sample components in time makes the duration of analysis per sample in the order of minutes.

Methods: We adapted techniques demonstrating a four-fold increase in LC-MS/MS throughput for a range of analytical targets. The analytes included a panel of three steroids already assayed by a validated ~9-minute LC-MS/MS run, a drug used to treat COVID-19 patients (baricitinib), a thyroid hormone currently tested with radioimmunoassay (rT3) and a panel of virus-derived peptides.

Results: Across all high-throughput methods, inter-analyte resolution was fit-for-purpose. Where LC performance could be directly compared, resolution remained or was reduced by <20% despite a 3x increase in throughput.

Similarly, sensitivity of the methods remained within the clinically relevant ranges. Trueness assessments against lower-throughput LC-MS/MS assays showed <1% bias for three commonly measured steroids.

Conclusion: The throughput of modern LC-MS/MS systems can be significantly improved while maintaining the accuracy, precision and trueness of results. Higher throughput methods will provide improved turn-around times, better utilisation of expensive LC-MS/MS instruments and a faster return on investment.

Weathering of Water-based and Oil-based Architectural Paints in the Australian Climate

Fiona Jackson[1,2], Claude Roux[2], Joanna Bunford[3]

[1]Forensic & Analytical Science Service, NSW Health Pathology

[2]Centre for Forensic Science, University of Technology Sydney, NSW

[3]Australian Federal Police, Canberra, ACT

This research explored three separate routes for data collection to assist with the interpretation of the evidential value of vehicle and architectural paint. This was achieved first, by a survey that was conducted on the common colours of vehicles seen on the roads and a motorway within Western Sydney.

A separate survey looked at the most common apparent vehicle paint colours found on stationary objects, such as on the pillars and walls, in five different carparks around Western Sydney. The next part of the research focused on the effects of weathering on clear coats and other layers of selected OEM vehicle paints.

The final part of the research was focused on the weathering of architectural paint. This was conducted on eight different paint samples using either oil or water-based formulations. These samples were exposed to the natural weathering climate in Sydney, Australia for up to 3 years. Weathering was found to affect the physical and chemical profiles of a number of the paint samples.

There were unexpected and significant changes seen across all of the paint samples after only 12-months of weathering, most notably within the water-based samples. These changes continued to occur after the first 12-months of weathering and were seen right up to the 3 years of exposure.

Overall, the findings from this research study have provided supporting information that will assist in improving the evaluation of the evidential value of paint.

Rapid antigen test school feasibility trial

Christopher Kot, Catherine Pitman, Hemalatha Varadhan, William Hamilton, Brittany Thorn, Andrew Sargeant

[1]Point of Care Testing, NSW Health Pathology

[2]NSW Health Pathology, Microbiology, John Hunter Hospital

Introduction: SARS-CoV-2 minimised the movement of people causing significant global, social, and economic impact. This feasibility trial assessed the effectiveness of at-home, self-collected rapid antigen tests (RATs) and the general experience of self-collecting.

Methods: Students and staff at Newcastle Grammar School were given the option to evaluate at-home collection methods for both RATs and PCR. Saliva, Nasal and RhinoSwab collection methods were tested in parallel. Participants provided feedback about the collection by survey.

Results: During the trial, 382 data points were collected from RATs. 6 were recorded as invalid (1.57%) and 3 were false positive (0.78%). There were 495 samples collected for PCR with a 94% collection success rate. Participants generally tolerated all 3 swab types with Saliva 1st, RhinoSwab 2nd, and Nasal 3rd. This ranking does not consider the analytical ranking based on swab type.

Conclusion: These findings highlight that a decentralised approach to SARS-CoV-2 screening is feasible and was well tolerated by the general community represented in this assessment. This approach would dramatically decrease waiting times associated with pathology collections and could potentially be considered for other respiratory

conditions. Home collection of RATs has been demonstrated to be feasible with participants wanting schools to remain open with frequent RAT screening. This feasibility assessment did not assess the analytical performance of the RATs used in the trial, only which collection methods could be implemented at home to reduce the overall burden and costs to the healthcare system.

Fantastic yeasts and where to find them: Investigating possible microbial origins of anomalous DNA sequence data generated from stored bone using MPS

Felicity Poulsen, Catherine Hitchcock

[1]NSW Health Pathology, Forensic & Analytical Science Service (FASS), Forensic Biology/DNA Unit

Introduction: Microbial contamination is a common challenge impacting DNA sequencing success from compromised skeletal remains. In cases where microbial overgrowth is suspected as the cause of failed DNA sequence analysis, targeted Sanger sequencing of 16S ribosomal RNA has previously been used to confirm the presence and origin of microbial DNA in a sample.

Massively parallel sequencing (MPS) technology generates an enormous amount of sequence information and the bioinformatic pipelines used for MPS data analysis enable greater scrutiny of sequencing results.

These MPS features can simplify investigations of possible instances of microbial contamination.

Methods: We present a case study of two stored femur samples which failed analysis using Ion Torrent™ MPS technology for targeted SNP genotyping. Non-specific amplification during library preparation was suspected as the cause of failed analysis, with the observation of >300,000 unaligned sequence reads. Select sequence strings were searched using NCBI's nucleotide BLAST to investigate the possible origin of non-human DNA in the samples.

Results: Several sequence strings were identified with homology to the genomes of various Proteobacteria and Fungi species, supporting the idea that non-specification amplification of microbial DNA contamination had occurred and contributed to the failed analysis.

Conclusion: Further research is required to develop methods to improve the success of forensic DNA sequencing applications with skeletal remains contaminated with microbial DNA. MPS methods utilising allele hybridisation strategies for human DNA enrichment appear promising.

A verification study to reduce the sample incubation time of the ABAcard® p30 test for the forensic identification of semen

Kadir Sarun, Felicity Poulsen, Rebecca Douglas, Catherine Hitchcock

[1]NSW Health Pathology, Forensic & Analytical Science Service (FASS), Forensic Biology/DNA Unit

Introduction: Prostate-specific antigen, also known as p30, is a protein synthesised by the prostate gland. It is abundantly secreted into the seminal plasma hence making it an ideal marker for detecting semen in forensic casework.

The Forensic Biology/DNA Unit currently uses the ABAcard® p30 Kit as a confirmatory test for the presence of human semen in sexual assault cases. This study aimed to improve the test efficiency by reducing the current sample incubation time of two hours without compromising assay performance.

Methods: Semen samples were prepared in a range of dilutions to test for sensitivity. Preliminary time point testing was conducted to determine the shortest incubation time where assay sensitivity was maintained. To confirm suitability, the reduced incubation time was directly compared to the current method across the dilution range. Substrate specificity testing was conducted by seeding semen on a range of common substrates. Fluid specificity was also tested with other human biological fluids to test for cross-reactivity.

Results: Sensitivity testing showed that an incubation time of 10 minutes-maintained assay sensitivity compared to the current method. Also, the substrate and fluid specificity tests were not negatively affected by the reduced incubation time.

Conclusion: Reducing the sample incubation time from two hours to 10 minutes did not impact the sensitivity or specificity of the ABAcard® p30 test for semen. Reducing the sample incubation time has significantly improved the efficiency of the p30 testing protocol.

Validation of Sperm DNA Fragmentation Testing in a Clinical Andrology Laboratory

Sue Sleiman, Fey Bacha

[1]Clinical Andrology Laboratory, Concord Repatriation General Hospital

Introduction: Sperm DNA fragmentation (SDF) contributes to impaired natural and IVF fertility. Elevated SDF has been associated with delayed conception, poor embryo quality,

implantation failure and miscarriage. We evaluated the performance of the Halosperm G2 kit for SDF testing, prior to introducing it into routine laboratory workflow.

Method/Results: In replicate fresh and frozen semen samples (n=19) by Bland-Altman (BA) deviance analysis, intra-class correlation (ICC) and Spearman correlation (rs) results displayed a high degree of within-observer reproducibility for fresh (BA -4.1 [+9.6, -17.7], ICC>0.988) and frozen samples (BA -5.1 [7.6, -17.7], ICC >0.993) with no bias between fresh and frozen samples (BA -0.1 [11.9, -12.1]).

However, significant between-observer bias was present but eliminated in a second set of samples (n=13) following reconciliation of scoring (BA -1,2 [6.2, -8.7], ICC>0.997). The test showed excellent agreement with another SDF test (SCSA) in another laboratory (BA 3.4 [24.1, -17.3]). SDF was correlated with abstinence interval (rs0.65, 0.01), sperm motility (rs -0.58, 0.02) and vitality (rs -0.57, 0.05) but not sperm output or morphology and by ANOVA was significantly influenced by technician (p<0.001) but not replicates or sample condition (p>0.31).

Conclusion: We conclude that excellent reproducibility and validity of the Halosperm G2 kit was achieved after eliminating between-observer bias.

When implemented as a rapid and cost-effective test without needing complex training or new equipment, it has resulted in enhanced quality of service, high and growing test demand and non-Medicare revenue.

PoCT Bench Design

Emily Whelan, Phoebe Hollott, Callum Davies, Simranjit Virk

[1]Point of Care Tests (PoCT), NSW Health Pathology

[2]University of Newcastle

Currently Point of Care Testing (PoCT) occurs at fixed stations which limits the reach of the testing devices. A mobile PoCT bench would enable the testing devices (i-Stat Alinity, Coagucheck, Liat, Hemascreen, Gem Premier) to be moved around within and outside of NSW hospitals. This will reduce the stigma and remove any difficulty in visiting a hospital. The many constraints of this project are how it can be easily maneuvered (in hospital and in/out van for transport), vibration dampening and device mounting.

Features of the bench include:

- Mounting and powering PoCT devices
- An iPad mount
- Transportable in a van
- A handwashing sink
- Infection control
- Securing points and vibration dampening for device protection
- Storage spaces for extra equipment

Current results are limited with future results to be visualized in models and drawings. As the PoCT bench project progresses, more fabricated results, designed and constructed by our team, will become available.

Quality review of a rapid FISH service

Catherine Wren[1], Elizabeth Tegg[1,2]

[1]Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology

[2]University of Sydney

Introduction: This is a retrospective review of the rapid FISH testing undertaken at ICPMR-NSWHP.

Methods: The total number of rapid FISH performed were audited and the following determined:

1. Probe type
2. Signal quality
3. Abnormality rate and clone size
4. Where possible, information was also gathered on the clinical benefit to patients in having a rapid FISH result available.

Results: A total of 169 rapid FISH tests were performed from August 2018 until May 2022. The most requested probes for rapid testing were BCR/ABL1 and PML/RARA. The hybridization quality was shown to be excellent with most hybridizations graded 4+. The overall abnormality rate was 43%. This method was able to detect patients with multiple and small clones.

Conclusions: This rapid FISH method gave a high-quality accurate FISH result which allowed patients rapid access to targeted therapies and clinical trials. The most frequently requested probes for rapid testing were BCR/ABL1 and PML/RARA. The hybridizations were of high quality (score 4+) and all results concurred with external findings. No adverse outcomes were identified. Even negative results (BCR/ABL1) were important for patient access to clinical trials.

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Journal-based CPD No. 86
Page 1 of 1

Questions relating to the article *'Challenges faced by veterinary laboratories in diagnosing cobalamin deficiency in companion animals: a review'* at page 83 of this issue.

1.	The Clinical Laboratory and Standards Institute (CLSI) have developed a protocol for standardising reference ranges for animals.	True/False
2.	The most common type of instrument used for determining cobalamin in feline and canine serum was the Abbott platform.	True/False
3.	Hypocobalaminaemia can also occur due to increased physiological demand due to various conditions including pregnancy.	True/False
4.	The dates searched for useful keywords initially were between January 1950 and December 2020.	True/False
5.	The transportation protein, the R-protein, is synthesized by dogs and cats in the stomach and salivary glans.	True/False
6.	The literature suggests that up to 73% of canines and 61% of felines with chronic intestinal disease are hypocobalaminaemic.	True/False
7.	Cobalamin is otherwise known as Vitamin B10.	True/False
8.	Reference ranges for specific breeds of dogs have been developed.	True/False
9.	The lower range cut-off value used to identify hypocobalaminaemia in cats ranged between 186-251 pmol/l.	True/False
10.	Megaloblastic anaemia can be caused by hypocobalaminaemia in felines but is more common in canines.	True/False

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Journal-based CPD No. 87
Page 1 of 1

Questions relating to the article '*Professional practice evaluation of medical laboratory scientists in support of patient safety: a compliance tool for International Standard ISO 15189:2012 accredited medical laboratories*' at page 93 of this issue.

1.	ISO works with other international organisations, such as the Institute of Electrical and Electronics Engineers and the International Electrotechnical Commission to produce relevant guidance documents.	True/False
2.	Even though the relationship between medical laboratory personnel professional practices and patient safety is extremely important, limited relevant literature has been available.	True/False
3.	The accreditation of medical laboratories is to the ISO 14069:2020 standard.	True/False
4.	According to the IFBLS position paper, biomedical lab scientists are responsible for the safety of procedures by adhering to the current standards of practice.	True/False
5.	A total of 1845 CReqs was identified in Clauses 4 and 5 of ISO 15189:2012.	True/False
6.	The term 'shall' in ISO 15189:2012 implies at least one CReq is to be implemented in medical laboratory practice.	True/False
7.	The current study identified 57 PSReqs in the IFBLS position paper that could be evaluated against 259/1515 referred CReqs in ISO 15189:2012.	True/False
8.	'Best practice' is defined by ISO as a 'method that has been proven to work well and produce good results'.	True/False
9.	The term CPD has been defined by ISO as 'activities undertaken by a person after initial education or training to maintain, improve or increase his/her knowledge and skills related to his/her professional activities.	True/False
10.	IFBLS released a position paper on patient safety in 2020.	True/False

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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.

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AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

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Arrange the article in the following sequence:

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

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

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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.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus (see http://www.nlm.nih.gov/mesh/). Keywords should be given below the Abstract.

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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.

Results

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

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

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(ii) It has been successfully demonstrated that (Smith and Brown 1981; Auteur 1995; Scienziato *et al* 2007).

(iii) Following further investigation, Wetenschapper (2002 highlighted the difficulties inherent in…

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

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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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