Novel approaches to ensuring quality in laboratory diagnostics and external quality assessment

Quality in laboratory testing is a prerequisite to ensuring accuracy and precision of test results, and thus ensures its contribution to appropriate patient clinical care (1-3). Quality in laboratory testing is ensured by application of various 'quality' processes. Central to this quality of laboratory testing in ongoing application of internal quality control (IQC) and external quality assessment (EQA).

IQC is a process that utilises control samples, which in essence comprise a similar matrix to typical patient test samples, be it blood, plasma, serum (etc.), and which are tested in a given laboratory assay on a regular basis over a period of time. It is normal in IQC processes to assess at least two levels of an analyte, typically within a normal reference range (NRR), as well as outside that NRR, within a relevant 'abnormal' or 'pathological' range. This 'pathological' region of interest may be below or above the NRR, depending on the test, and associated pathology. The period of testing depends on the analyte. For continuous tests, for example, testing may be defined by the number of tests or by a length of time (e.g., after every 50 tests are performed, or after every 4 hours of testing). For batch testing, IQC are imbedded in the test run, for example at the start, middle, and/or end of a test run. The results of the IQC tests are collected and analysed, with the main aim being to assess test reproducibility, or precision. The aim is to have a test that has high reproducibility or high precision, or low imprecision. The assay variation if often measured by use of statistical metrics such as standard deviation (SD) or coefficient of variation (CV).

EQA represents a different approach, where test samples are provided by a third party (an External Quality Assessment Scheme or provider). These samples are provided and then tested by the laboratory on a periodic basis, with the frequency dependent on the test and the capacity of the EQA provider. For example, a frequently performed test, such as a prothrombin time (PT) or activated partial thromboplastin time (APTT) would be tested at a frequency of 4–6 times per year, whereas a less frequently offered or performed test would be tested at a frequency of 2–3 times per year. After testing, test results are returned to the EQA provider, which then analyses test results with comparison to those of other laboratories. In this way, a laboratory's test results can be compared with those of 'peer' laboratories and/or methods. The overall results undergo statistical analysis, and often the median or mean value of all methods, or that of a particular method, becomes the target value, by which all laboratories can then be assessed. Thus, a particular laboratory's result can be assessed against the target to determine its variation from this target. The EQC process thus acts primarily as a measure of accuracy, although overall data can also provide information on a method's precision. The assessments can be for individual analytes, groups of analytes, and also over the long term, which also enables assessment of assay bias.

This preface relates to a series of papers that have been collated in this journal to examine how quality in laboratory testing can be ensured. This contemporary collection should be of value to all laboratories involved in diagnostic testing, although the main focus is on hematology and hemostasis testing.

First, there are two papers by the Guest Editor of this series that explore IQC and EQA for two important test procedures in hemostasis, and which otherwise prove to be challenging for most laboratories and QA providers (4,5), namely von Willebrand disease (VWD) and platelet function testing.

VWD is recognised as the most common congenital bleeding disorder, and an acquired form named von Willebrand syndrome (AVWS) may arise in a variety of disease states (6). Thus, laboratory testing for VWD and AVWS represent key diagnostic activities with hematology facilities performing advanced hemostasis diagnostics (7). VWD and AVWS arise due to deficiency and/or defect(s) in the adhesive plasma protein called von Willebrand factor (VWF). VWF represents a key element of primary hemostasis, and also contributes to secondary hemostasis. Thus, deficiency and/or defects in VWF, either congenital or acquired, leads to a bleeding diathesis in affected individuals. A wide variety of laboratory tests may be performed to investigate, diagnose or exclude VWD/AVWS, including screening assays, more complex functional assays, and also molecular analysis (7). The overall diagnostic process is even more complicated since both individual tests and the pattern of overall test results from all tests is evaluated before a diagnosis of VWD can be made or excluded. That is, VWD testing incorporates a variety of processes, and potential methodologies. Although IQC and EQA is critical to ensure the quality of

laboratory testing in VWD, it is made difficult because of the nature and variety of tests available, as well as the heterogeneity of VWD/AVWS and their phenotypic presentation (8,9). The current review (4) looks at laboratory testing for VWD/AVWS from the perspective of IQC and EQA, and considers some novel approaches to ensure the accuracy and quality of this test activity in the future diagnostics landscape.

Platelet function testing is also a key diagnostic activity within hematology laboratories associated with advanced hemostasis diagnostics (10). Platelets represent a key component of primary hemostasis, and deficiency and/or defects, either congenital or acquired, also lead to bleeding diathesis in affected individuals. There are various levels of platelet function tests, from simple screens, to complex functional assays, as well as molecular analysis (11-13). Platelet function testing has evolved to now incorporate various processes, such as whole blood aggregometry, light transmission aggregometry, testing by platelet function analyser (PFA)-100 (or -200), flow cytometry, and many other methodologies. Despite some of these tests being available now for decades, and/or continuously evolving, IQC and EQA is limited, made difficult by the nature of the tests and test material, the latter typically representing functional cellular material (i.e., platelets) (14). The current review (5) looks at platelet function testing from the perspective of diagnostic screening, and highlights current limitations, as well as potential solutions that will enable more effective and accurate testing in the future. The primary focus of the review, however, is on IQC and EQA for the PFA-100/-200.

Coagulation factors are also representative of important analytes for hemostasis laboratories. In particular, factors VIII and IX are those most regularly tested by haemostasis laboratories, primarily because Hemophilia A (FVIII deficiency) and Hemophilia B (FIX deficiency) represent the most common of the coagulation factor bleeding disorders (15,16). Moreover, plasma levels of these coagulation factors can be associated with a given patient's bleeding risk. The Royal College of Pathologists Australasia Quality Assurance Programs (RCPAQAP) is an international EQ provider with over 100 laboratories enrolled in the Coagulation Factors VIII and IX Program. The aim of the reported work presented here (17) is to evaluate the assessment criteria for FVIII and FIX over a six-year period [2013–2018] and identify areas of benefit and weakness, in part resulting from a recent [2016] change in criteria (from assessment against factor deficient plasma reagent to plasma calibrator, as this was felt to be more relevant for determination of factor level). The authors identified an ongoing improvement [reduction in numbers of laboratories outside the RCPAQAP Analytical Performance Specifications (APS)] for both FVIII and FIX over this period of change in assessment criteria. FVIII outliers almost halved from 43 to 23 laboratories, and FIX outliers reduced by over 60% from 36 to 14 laboratories. It is further hypothesised that the change in assessment criteria contributed to this improvement in Coagulation Factors program performance by laboratories, although other elements may have also contributed.

Two other papers from the RCPAQAP are also presented in this series, with the focus turning from bleeding disorders (as above) to thrombotic risk (18,19). The first paper is on Activated Protein C Resistance (APCR), which is a hypercoagulable condition, mostly caused by genetic 'mutations' in clotting factor V, including factor V Leiden, which increases the risk of venous thrombosis (20). The RCPAQAP offers QA testing for APCR twice a year. Participants either used APTT or Russell viper venom time (RVVT) based methods to perform clotting assays, with these being variously sensitive factor V Leiden, or other causes of APCR, with the end point of analysis being identification or exclusion of APCR (according to overall targets based on participant responses). The authors also aimed to identify trends in APCR testing, which methods and reagent kits are better in the identification of APCR *vs.* those reagent kits with increased risk of reporting false positive and/or false negative APCR. Data from 40 APCR test samples (both positives and negatives for APCR) over the past 10 years was analysed. False positive and false negative rates were calculated per sample, and reagent kits. There was an 18% increase in participants performing APCR testing over the 10 years, with participants reporting fewer incorrect interpretations in 2019 (3.0%) than in 2010 (16.8%). This was coincident with a move away from APTT to RVVT based methods, with 59% users of RVVT methods in 2019, compared to 48% in 2010. This change likely contributed to lower percentages of incorrect results, as it was found that participants using APTT based assays reported more false negatives (87.8% of total) and false positives (72.2% of total) than those using RVVT based assays. This study thus informs on the accuracy of test methods for identification of APCR, and highlights the general superiority of RVVT methods for detection of APCR (21).

A second paper reports on data from the RCPAQAP in relation to antiphospholipid antibodies (aPL), which are associated with both thrombosis and pregnancy related morbidity and mortality (19). The most commonly tested aPL are anticardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies. These aPL represent important markers for the diagnosis of the

antiphospholipid syndrome (APS) (22). Previous studies have shown significant variability in results obtained from different kits and manufacturers for these antibodies. In response to this lack of homogeneity, there have been numerous international initiatives aimed at improving the reproducibility and standardization of these assays, and spanning the last two decades (23-30). To assess if these standardization initiatives have led to improved consistency in routine diagnostic laboratory reporting of these antibodies, the authors retrospectively reviewed 10 years of data from the RCPAQAP (19). Data submitted by laboratories participating in the aPL program over a ten-year period [2009–2018] for IgG and IgM aCL and IgG aβ2GPI antibodies were reviewed. Changes in assay methodologies, consensus of results against the target set by RCPAQAP, and the number of laboratories reporting semi-quantitative results were assessed. Methodologies used for the detection of aCL and aβ2GPI antibodies were found to have changed considerably since 2009, with a steady trend from ELISA towards non-ELISA based methodologies, such as chemiluminescence, fluorescence immunoassay and Luminex based techniques. Consensus in resulting (defined as ≥80% concordance in reporting "negative" or "positive" results for a sample) did not significantly change across the 10-year period for any test. There was a significant decrease in the proportion of laboratories reporting semi-qualitative results (i.e., low/medium/high positive) for IgG aCL (P=0.0036) and IgG aβ2GPI antibodies (P=0.007). No significant change was noted for IgM aCL antibodies (P>0.999). The authors conclude that despite concerted efforts by a number of international groups to improve the standardization of aCL and aβ2GPI antibodies assays, a review of data obtained over a 10-year period of EQA testing in diagnostic laboratories demonstrated that there was no evidence to support that these efforts have translated into improvements in the consistency of IgG/IgM aCL and IgG aβ2GPI antibody results.

The authors of the remaining pieces in this series of the journal move away from specific areas of IQC and EQA testing to general considerations in EQA. Firstly, Olson and colleagues provide an update on the EQATH (External Quality Assurance in Thrombosis and Hemostasis) group and arising initiatives (31). EQATH was formed in 2005, when several EQA providers met and formed the group, although a formal Charter was not enacted until 2016. The goals of EQATH are to: identify the organizations involved with EQA in thrombosis and hemostasis; determine the functions and scope of these EQA programs; share information with the goal of improving the quality of existing programs and to seek methods that may standardize some activities; develop EQA samples that can be shared among EQA programs to determine the variation that may exist is various regions of the world; inform laboratories participating in EQA programs of the identified problems in laboratory testing; work with existing International Society on Thrombosis and Hemostasis (ISTH) Scientific Standardisation Committees (SSCs) and Working Groups, providing information regarding clinical laboratory needs for standards and to help with the validation and value setting of standards; work with other organizations in developing recommendations and guidelines for EQA program activities; collaborate with other organizations and societies with interests in the quality of diagnostic coagulation testing. Since its beginning, the EQATH group has met regularly, completed two international projects (32,33), collaborated in the completion of four others and developed a guidance document for developing an EQA program in thrombosis and hemostasis. The group is now engaged in two additional projects that are underway.

Related to the above paper, and as noted therein, the guidance document for developing an EQA program in thrombosis and hemostasis is described in two additional papers in this series (34,35). In the first, Malar and colleagues provide an Editorial to, and in the second provide the guidance document itself. As noted before, accurate results in clinical testing (e.g., for thrombosis and hemostasis analytes) is critical, but can be a difficult goal. Importantly, accurate testing relies on a good EQA scheme (EQAS). Having access to a reliable program may not be readily available to all laboratories that perform thrombosis and hemostasis testing. As well, accreditation organizations and government regulations may require participation in an EQAS program. If an EQAS program does not exist or is not available to the laboratories in a region or country, then one may need to be developed. This article therefore provides the basic guidance for the development and implementation of such a program.

Finally, for this series, Gosselin and colleagues provide a review and critique the current EQA options in the USA for specialized hemostasis laboratories (36). Their aim is to assess whether (I) these options address all three phases of testing, and whether (II) the testing platforms are providing optimal assessment of hemostasis testing. Much of the history can be traced back to 1999, when the National Committee for Clinical Laboratory Standards (NCCLS, now known as Clinical & Laboratory Standards Institute) introduced the concepts of quality practice for clinical laboratories, with the notion to improve the pre-analytical, analytical and post-analytical phases of laboratory testing. Hemostasis testing is particularly is known to be sensitive to pre-analytical issues (37), but most QA measures are specific for the analytical phase of hemostasis

testing. The USA EQA programs are typically a biannual assessment using blinded samples that are intended to improve laboratory practice. The authors reflect that EQA practice in the USA is not ideal. The authors propose that EQA programs develop partnerships and provide improved services for esoteric tests. The authors also identify some barriers for international EQA in the USA, such as custom importing issues and the need for "local" sponsorship according to current regulations. Advantages to international partnerships, aside from potentially better EQA material, is the potential to harmonize EQA reporting and assessing leading to better clinical practice (38-40).

As the Guest Editor for this series, I therefore hope that the readership of *AOB* enjoys the compilation, and that these initiatives lead to improvements in quality of testing, thereby improving clinical support of patients undergoing laboratory testing.

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Emmanuel J. Favaloro

Emmanuel J. Favaloro[1,2,3]
[1]*Department of Haematology, Institute of Clinical Pathology and Medical Research, NSW Health Pathology, Westmead Hospital, Westmead, NSW Australia;*
[2]*Sydney Centres for Thrombosis and Haemostasis, Westmead, NSW Australia;*
[3]*School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, New South Wales, Australia.* (*Email: emmanuel.favaloro@health.nsw.gov.au*)
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