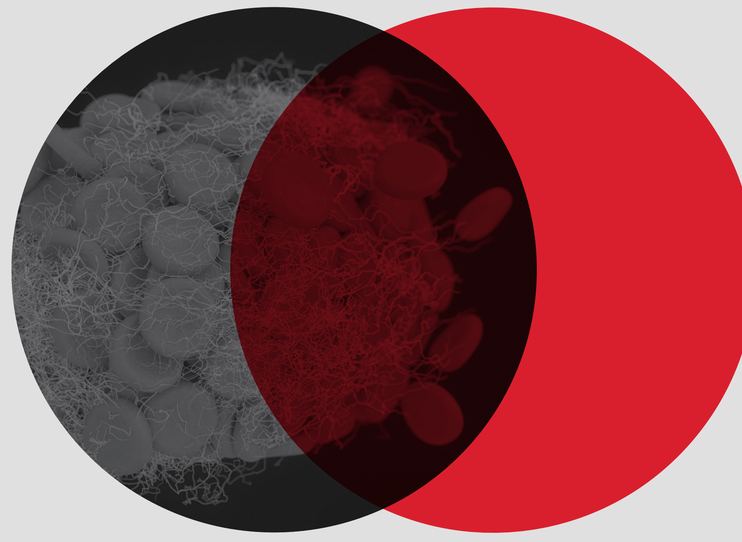
The Changing Face of Activated Protein C Resistance Testing – A 10 Year Review



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Introduction

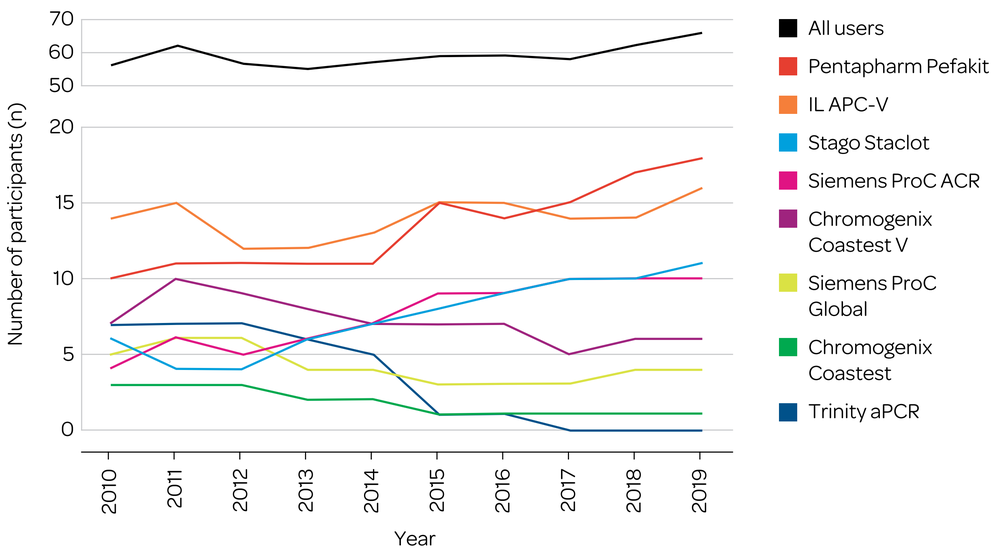
Activated protein C resistance (APCR) is a hypercoagulable condition that increases the risk of venous thrombosis. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) offers external quality assessment testing for APCR twice a year. Participants either used activated partial thromboplastin time (APTT) or Russell viper venom time (RVVT) based methods to perform clotting assays. We aimed to identify which methods and reagent kits perform better in the identification of APCR.

Method

The RCPAQAP distributes four APCR samples per year to 66 current participating laboratories. Data for this report was collected from the past 10 years, representing a total of 40 samples. APCR enrolment numbers, ratio results, interpretations, reagents, and methods were analysed. False positive and false negative rates were calculated per sample, and for each reagent method/kit. Here, 'false positive' interpretations are defined as those where a negative APCR sample was reported as positive, while a 'false negative' was reported as negative on an APCR positive sample.

Results

Figure 1. Total enrolment numbers of the APCR program and individual kits



The number of participants in the APCR program increased overall by 18% from 2010 to 2019. Individual kit uptake varied throughout this period. Eight different commercial APCR reagent kits were recorded, with some growing in popularity; Pentapharm's Pefakit saw an 80% increase and Stago's Staclot an 83% increase. In comparison, other kits have diminished in use, with Trinity's aPCR reagent and Chromogenix's Coatest decreasing to 1 and 0 users. The enrolment numbers for the other reagents have remained fairly stable (Figure 1).

RVVT based methods have come into favour over APTT based methods. From 2010 to 2012, APTT based APCR assays were used by the majority of participants. RVVT based assays became the preferred method in 2013, and have continued to be so since. In 2019, 39 of 66 (59%) responses came from participants using RVVT based assays, 11% more than in 2010 (Figure 2).

Figure 2. Enrolment of APTT vs RVVT based APCR methods

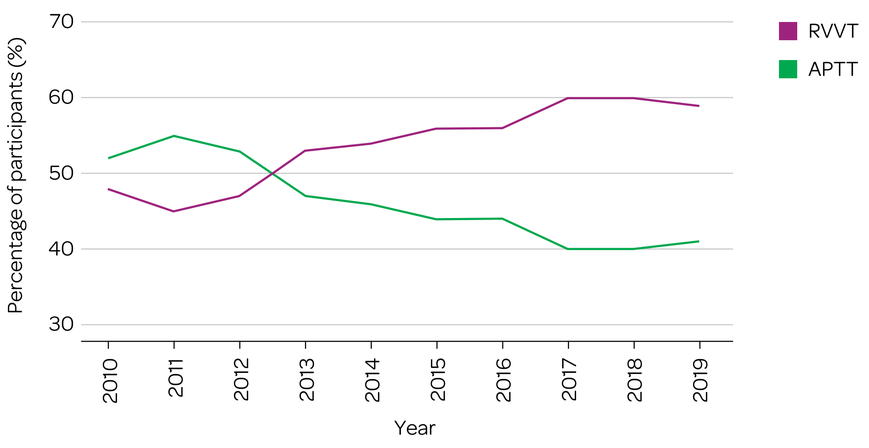
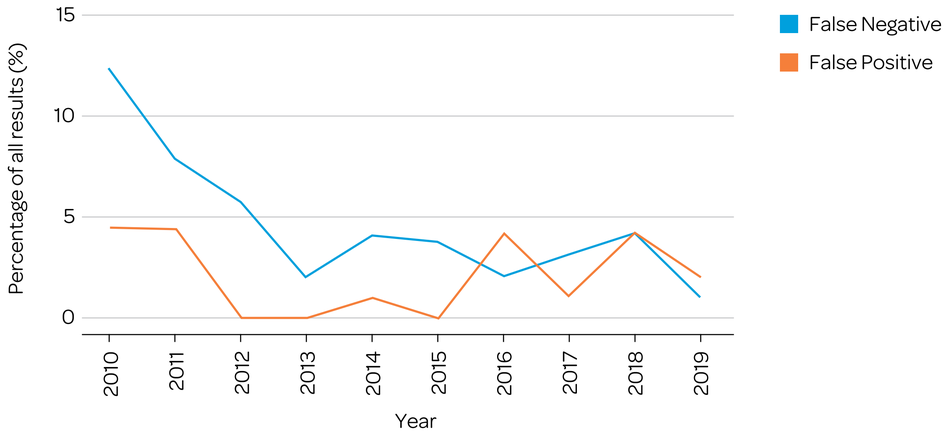


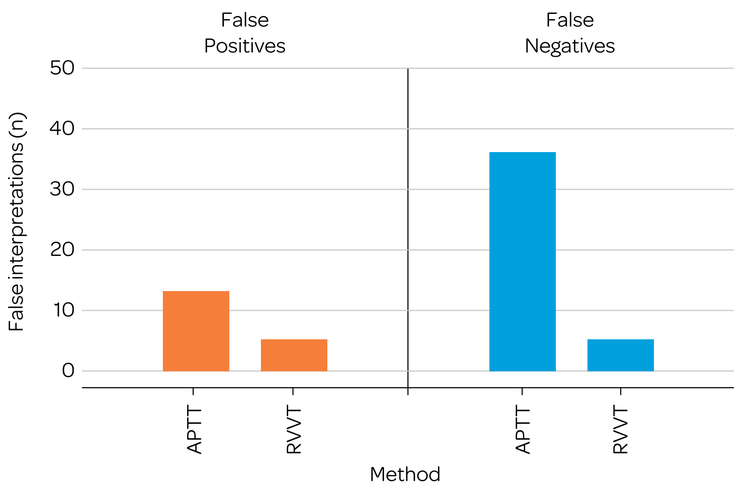
Figure 3. Overall percentage of APCR incorrect interpretations



False negative interpretations, including both APTT and RVVT based methods, have dropped from 12.3% to 1.0% over the 10-year period, while false positive interpretations peaked above 4% in 2010, 2011, 2016 & 2018, and were at or below 1% in 2012–2015 (Figure 3).

The kits based on APTT (IL-APC, Coatest, and ProC Global) accounted for 72.2% (13/18) of false positives and 87.8% (36/41) of false negatives. In contrast, the RVVT based methods (Pefakit, ProC ACR, Staclot and Trinity aPCR) accounted for 27.8% (5/18) of false positives and 12.2% (5/41) false negatives (Figure 4).

Figure 4. Incorrect interpretations – APTT based vs RVVT based methods



Discussion

Over the 10-year period of analysis, we saw an overall increase APCR participants (Figure 1). By 2010, APCR testing had been in laboratory use for nearly 20 years. Nevertheless, not all haemostasis laboratories would have taken it up during this time. As the assay has become more mainstream, we naturally saw an overall increase in enrolments.

Pefakit is now the most widely used APCR kit by RCPAQAP participants. Its popularity is likely due to the reagent's robustness to interference by anticoagulants, which otherwise affect RVVT-based testing[1,2]. Explanation of the diminished number of users for Trinity's aPCR reagent and Chromogenix's Coatest probably lies in the trend of laboratories moving away from APTT based to RVVT based assays (Figure 2). Indeed, 100% (10/10) of participants in this evaluation who moved away from Trinity aPCR and Coatest have since adopted an RVVT based assay.

This trend away from APTT based, to RVVT based APCR assays is likely due to the findings published in many studies that RVVT based methods are more sensitive than APTT based methods to the presence of Factor V Leiden, the major cause of APCR[3,4]. Users ability to interpret APCR ratios as positive or negative for APCR has improved over the period of analysis. The percentage of incorrect interpretations dropped most notably for false negatives, while false positives followed a less consistent pattern (Figure 3). This improvement is likely due to the move away from APTT towards RVVT based APCR assays, which appear to be more robust in terms of true detection of APCR as seen in Figure 4.

Conclusion

Overall, we have seen an increase in APCR users, and participants reporting fewer incorrect interpretations over the past 10-years. APTT based methods resulted in more false negatives and false positives than RVVT based methods. We believe this is a major reason why users are moving from APTT based to RVVT based APCR assays. Further, active participation and review of results in the RCPAQAP APCR program has likely been a contributing factor to better performance in identifying APCR.

References

1. Favaloro EJ. Diagnostic issues in thrombophilia: a laboratory scientist's view. Semin Thromb Hemost. 2005 Feb;31(1):11-6.
2. Bonar R, Favaloro EJ, Mohammed S, Ahuja M, Pasalic L, Sioufi J, Marsden K. The effect of the direct factor Xa inhibitors apixaban and rivaroxaban on haemostasis tests: a comprehensive assessment using in vitro and ex vivo samples. Pathology, 2016 Jan;48(1):60-71.
3. Mohammed S, Favaloro EJ. Laboratory Testing for Activated Protein C Resistance (APCR). Methods Mol Biol. 2017;1646:137-143.
4. Favaloro EJ, Mirochnik O, & McDonald D. Functional Activated Protein C Resistance Assays: correlation with Factor V DNA analysis is better with RVVT- than APTT- based assays. British Journal of Biomedical Science, 1999; 56: 23-33.

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