Programs (RCPAQAP) offers quality assurance testing for APCR twice a year. Participants either used activated partial thromboplastin time (APTT) or Russell viper venom time (RVVT) to perform clotting assays, with the end point of identification or exclusion of APCR. We aimed to identify trends in APCR testing, which methods and reagent kits are better in the identification against those reagent kits at risk of reporting false positive and/or false negative APCR.

Data from 40 APCR samples 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, with participants reporting fewer incorrect interpretations in 2019 (3.0%) than in 2010 (16.8%). There was a move from APTT to RVVT based methods, with 59% users in 2019, compared to 48% in 2010. This 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.

DRUG-INDUCED AUTOIMMUNE HAEMOLYTIC ANAEMIA IN A PATIENT TREATED WITH ARTESUNATE FOR MALARIA INFECTION

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A 54-year-old woman was diagnosed with severe *Plasmodium falciparum* malaria following travel to Guinea. Her haemoglobin (Hb) at presentation was 138 g/L (ref range 115–160), with an initial parasite density of 5% and otherwise normal red cell morphology. The patient showed a good response to second line anti-malarial therapy with artesunate but re-presented 16 days post treatment initiation with symptomatic anaemia (Hb of 64 g/L), and displayed typical laboratory features consistent with haemolysis. The direct antiglobulin test (DAT) was positive (3/4) showing IgG and C3d specificity. The antibody screen was also positive, with grade 4+ reactions against all screening cells. Drug-induced autoimmune haemolytic anaemia (DIIHA) from artesunate therapy was suspected.

Prednisolone 1 mg/kg therapy was commenced, and transfusion was avoided due to the presence of the autoantibody and high risk of transfusion reaction. Treatment was successful with rapid resolution of haemolysis and Hb completely normalised on follow-up testing within 2 weeks. Response was sustained with tapering of prednisolone.

Post-artesunate delayed haemolysis (PADH) is estimated to occur in about 15–30% of patients treated with the drug.¹ Cases with a positive DAT are less common but are increasingly being reported and raise the possibility that artesunate may cause DIIHA.

Reference

1. Camprubi D, Pereira A, Rodriguez-Valero N, *et al.* Positive direct antiglobulin test in post-artesunate delayed haemolysis: more than a coincidence? *Malar J* 2019; 15: 123.

HIGHLIGHTING THE NEED FOR HARMONISATION OF G6PD REPORTING FOLLOWING THE INTRODUCTION OF NEW ANTIMALARIALS

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The recent introduction of new antimalarial drugs (e.g., tafenoquine)¹ comes with a recommendation to ascertain G6PD deficiency in order to avoid drug induced haemolysis.

A recent review of RCPAQAP G6PD survey results revealed a degree of variation in the interpretation of assay results in the range of 2.0–9.0 U/g Hb.

Aim: To assess the current status of G6PD testing and reporting with a view to providing additional information to laboratories and clinicians when assessing patients prior to prescribing anti-malarial drugs.

Method: An online survey was forwarded to laboratories participating in the 2019 RCPAQAP Haematology G6PD program seeking further information about their testing, interpretation and reporting of G6PD results. 76 of the 144 participating labs (53%) responded.

Results: The key findings were:

- A wide variation in reference intervals being reported (lower limit of normal ranging from 4.6 to 11 U/g Hb).
- 69% of respondents did not normally refer G6PD results for haematologist review.
- 93% of respondents do not specify a cut-off level of G6PD and/or interpretive comment below which it is not advisable to administer antimalarial drugs.

Conclusion: The key findings highlight the need to harmonise the reporting and interpretation of G6PD, particularly in light of new antimalarial treatments.

Reference

1. Tse EG, Korsik M, Todd MH. The past, present and future of anti-malarial medicines. *Malar J* 2019; 18: 93.

MICROSCOPY AND ICT VS LAMP ASSAY IN THE DIAGNOSIS OF MALARIA: A REAL-WORLD TIME AND COST-EFFECTIVE ANALYSIS

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Background: Malaria has traditionally been diagnosed using microscopy and immunochromatographic (ICT) assay. However, they are not without limitations including limited sensitivities.¹ Alethia Malaria is a DNA assay using LAMP (loop-mediated isothermal amplification) for the direct detection of *Plasmodium* spp. Studies have shown good sensitivity and specificity with a higher level of detection compared to microscopy.²

Aim: To determine a time and cost effective analysis of malaria diagnostic testing using microscopy and ICT vs LAMP assay in a community laboratory.

Methods: Malaria requests were identified at Australian Clinical Laboratories, Clayton, until 20 samples were obtained. All samples had microscopy, ICT and LAMP assay performed. The duration for the preparation of each test, microscopy review by

two scientists, and LAMP analyser time were recorded. Consumables, equipment and labour cost were calculated.

Results: The median time to result for microscopy and ICT was 49 min (including 14 min microscopy review). The cost per sample was AU\$19.45 or AU\$42.95 for 3 samples. The median time to result for the LAMP assay was 53 min (13 min sample preparation, 40 min analyser time) with a cost of AU\$21.50 per sample.

Conclusion: The LAMP assay is a time and cost effective malaria diagnostic screening tool and should be considered for implementation in the laboratory.

References

1. Mathison BA, Pritt BS. Update on malaria diagnostics and test utilization. *J Clin Microbiol* 2017; 55: 2009–17.
2. Rypien C, Chow B, Chan WW, *et al.* Detection of Plasmodium infection by the Illumigene Malaria Assay compared to reference microscopy and real-time PCR. *J Clin Microbiol* 2017; 55: 3037–45.

EVALUATION OF A T-CELL PROLYMPHOCYTIC LEUKAEMIA (T-PLL) EQA CASE STUDY

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Morphology continues to be a key diagnostic tool in identifying haematological disorders. The Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) offers an external proficiency program (EQA) in diagnostic morphology. The aim of the program is to assess the ability of participants to diagnose a case study through recognition of key morphological diagnostic features. Findings on a T-cell prolymphocytic leukaemia (T-PLL) case study were reviewed as a potential indicator of diagnostic capability among participating laboratories.

Method: The morphology program consists of 4 runs per year and three case studies per run. This case study featured a T-PLL. Participants were assessed using a predetermined scoring system for both the morphological and diagnostic component.

Results: The expected key morphological diagnostic features were a marked lymphocytosis with a predominance of small abnormal lymphoid cells containing prominent cytoplasmic blebbing. In addition, mature nuclear chromatin with a high magnification comment indicating highly convoluted nuclei with most containing a single small nucleolus.¹ Of the 499 participants, 76% (379) indicated the presence of the abnormal lymphocytes (T-cell prolymphocytes). However, only 45% (223) submitted a diagnosis of T-PLL.

Conclusion: This case study highlights an ongoing need for EQA programs to provide challenges to improve education and training in morphology.

Reference

1. Swerdlow SH, Campo E, Harris NL, *et al.* *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Revised 4th ed. Lyon: IARC, 2017; 346–8.

GUIDELINES FOR REPORTING BLOOD CELL MORPHOLOGY – ARE LABORATORIES FOLLOWING?

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In 2015, the International Council for Standardization in Haematology (ICSH) released a report outlining recommendations for the standardisation of nomenclature and grading of peripheral blood cell morphological features. Based on these, lymphoid cells with a suspected malignant or clonal aetiology should be classified as abnormal lymphocytes with an accompanying description of the cells.¹ We sought to evaluate if laboratories were following the ICSH reporting recommendations.

Method: Laboratories enrolled in the 2019 RCPAQAP Manual Differential program were provided with clinical notes, a stained blood film (which featured a T-cell prolymphocytic leukaemia case) and asked to categorise the different white blood cells. Assessment was based on robust statistical analysis and the clinical scenario.

Results: 79% (378/480) of labs reported the presence of abnormal lymphocytes, however, only 10% (41) provided a description of the abnormal cells. 23 participants included the abnormal cells as part of the lymphocyte count, while 15 participants counted the abnormal cells as ‘others’ without a description.

Discussion and conclusion: The main morphological features that were expected to be reported were the presence of abnormal lymphocytes and an associated description (T-cell prolymphocytes). Based on the results for this survey, either laboratories have a low uptake on the ICSH guidelines or they are missing important morphological features indicating the need for standardisation in blood film morphology reporting.

Reference

1. Palmer L, Briggs C, McFadden S, *et al.* ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Haematol* 2015; 37: 287–303.

DROPLET DIGITAL PCR FOR FETAL HPA-1A TYPING – NIPT PROOF OF PRINCIPLE STUDY

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Background: There is a clinical need for a non-invasive prenatal test (NIPT) that accurately assesses fetal HPA-1a status in fetoneonatal alloimmune thrombocytopenia (FNAIT) investigations. This is relevant for HPA-1bb women who have developed HPA-1a antibodies.

Aims: To establish proof of principle that droplet digital (dd) PCR is a suitable platform for NIPT, to detect fetal HPA-1 status.

Methods: Maternal blood samples were collected from four subjects, gestation ages ranges from 12⁺₄, 13, 22 and 39 weeks. Cell-free fetal (cff)DNA was extracted from maternal plasma for ddPCR. Cultured amniocytes or infant cord serology were used to confirm the findings.

Results: Optimisation demonstrated that the ddPCR assay could be performed using the same reaction and cycling conditions as NIPT assays developed for fetal red cell antigen predictions. Fetal specific signals were detected for HPA1a sequences for three of the four cases: only the 13-week case lacked signals, predicting that the baby was negative. Genotyping outcomes correlated with cultured amniocyte genotype.