RCPAQAP

The Royal College of Pathologists of Australasia
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RCPAQAP Molecular Genetics

Quality use of Pathology Program

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Title

The development of quality assurance programs for DNA extraction and for disease biomarkers associated with cancer, non-invasive prenatal testing, and leukaemia.

Final Report

A project funded under the Australian Government's Quality Use of Pathology Program

Contents

1. Executive Summary

Genetic disease diagnostic testing requires sequencing of patient DNA so that alterations in the DNA sequence can be identified and attributed to a genetic disorder. The reproducibility of DNA sequence information that accurately reflects the original clinical sample is therefore essential for all downstream clinical diagnostic interpretations. With the advancement of sequencing technology, DNA data from whole genome (entire human DNA) and whole exome (gene coding DNA regions only) sequencing are now proving that an excessive number of DNA sequence alterations exist in many human genetic diseases. As such, multiple DNA variants are starting to be recognised as clinically relevant biomarkers. In addition, the recent discovery of small DNA fragments found to be freely circulating in human blood is now providing a new liquid biopsy testing strategy for additional biomarker identification. It is therefore essential that external quality assurance (EQA) programs be devised to meet the clinical needs that arise following the discoveries of new biomarkers associated with genetic disease. As such, Commonwealth funding allows the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) to devise and develop novel EQA initiatives alongside current pathological findings so that clinical and patient needs can be met and continually addressed. Importantly, before newly identified DNA biomarkers can be added to the Medicare Benefits Schedule (MBS) for diagnostic testing, an EQA proficiency testing program first needs to be available.

The purpose of this Commonwealth funded study was to develop proficiency testing for laboratories performing diagnostic testing on (i) genomic DNA extracted from patient material (i.e., blood, skin, saliva, liver, pre-natal and tumour tissue), (ii) on circulating free DNA isolated from blood, and (iii) on patients with different types of leukaemia. The underlying principles for these pilot studies were to identify in the short-term, key problem areas associated with all methods used, and in monitoring laboratory reporting of biomarker data. Proficiency testing was performed for each participating laboratory and levels of concordance derived. The data from these pilot studies were then used to formulate medium- to long-term strategies for the development of fully accredited EQA programs.

The following represents an overview of the EQA results for each of the QUPP funded programs:

1.1 DNA Extraction programs
The DNA extraction program was divided into two separate sub-programs consisting of whole genomic DNA extraction and formalin-fixed paraffin-embedded (FFPE) DNA extraction.

1.1.1 Whole genomic DNA extraction

For the whole genomic DNA extraction program, laboratories were required to extract DNA from tissues that were of clinical interest and forward up to five of these samples to the RCPAQAP for quality testing and DNA analysis. In 2017, 11 laboratories submitted a total of 55 DNA samples, in 2018, 23 laboratories submitted a total of 115 DNA samples, and in 2019, 25 laboratories submitted a total of 125 DNA samples. Overall, 295 laboratory extracted DNA samples were submitted to the RCPAQAP for quality assessment from 2017 to 2019. Of the 295 samples submitted, 98% (54/55) in 2017, 99% (114/115) in 2018, and 99% (114/115, 10 samples were non-human and therefore not assessed) in 2019 were concordant and therefore considered suitable for laboratory genetic DNA diagnostic analysis.

1.1.2 Formalin-fixed paraffin-embedded (FFPE) DNA extraction

For the FFPE DNA extraction program, laboratories were required to extract DNA from clinical FFPE tissue and forward up to five of these samples to the RCPAQAP for quality testing and DNA analysis. In 2018, 9 laboratories submitted 45 FFPE DNA samples, and in 2019, 16 laboratories submitted 78 FFPE DNA samples. A total of 123 extracted FFPE DNA samples were therefore submitted to the RCPAQAP for assessment. Of these, 98% (44/45) of samples in 2018 and 96% (75/78) of samples in 2019 were concordant for genetic DNA diagnostic analysis.

1.2 Circulating fragmented DNA programs

The circulating fragmented DNA programs were divided in to two separate sub-programs consisting of circulating free DNA (cfDNA) analysis, and non-invasive prenatal testing (NIPT) analysis.

1.2.1 Circulating free DNA (cfDNA)

For the cfDNA program, seven laboratories in 2018 and six laboratories in 2019 participated for cfDNA proficiency testing. Laboratories were sent eight samples in 2018 (designed to contain nine gene variants in five genes with differing allelic frequencies), and five samples in 2019 (designed to contain six gene variants in four genes with differing allelic frequencies). Laboratories were required to test for variants that were of clinical interest to them and were therefore not required to test for all cfDNA variants in all samples. For the 2018 program, one laboratory did not provide any mutational identification data and were not assessed. Of the remining six laboratories, 33% (2/6) were overall concordant for detecting the cfDNA variants tested for. For the 2019 program, 33% (2/6) were overall

concordant for detecting the cfDNA variants tested for. However, four of the six laboratories could not be assessed due to the cfDNA variants tested for being below the limit of detection for the testing platforms used.

1.2.2 Non-invasive prenatal testing (NIPT)

The NIPT program was offered in 2019 as a pilot and five laboratories participated. Five reference testing samples were distributed to each laboratory for analysis. Laboratories were required to identify the chromosome abnormality and to report on the level of foetal fraction present in each test sample. Two of the five laboratories were concordant (100%) for all five samples tested. One laboratory was concordant for four of the five (80%) samples, one laboratory was concordant for two of the five (40%) samples, and one laboratory could not detect any abnormality due to incompatibility of the testing material with their testing platform.

Circulating free DNA analysis is a new area for diagnostic testing and more laboratories are likely to adopt testing of this material given that it can be non-invasively acquired (i.e., a simple blood sample) from the patient. This type of testing is therefore likely to replace the invasive strategy of tissue samples acquired by biopsy/excision processes.

1.3 Leukaemia programs

The leukaemia programs were divided in to three separate sub-programs consisting of chromosome 17p deletion detection for the diagnosis of chronic lymphocytic leukaemia (CLL), isocitrate dehydrogenase 1 and 2 (*IDH1*, *IDH2*) gene variant testing for the diagnosis of acute myeloid leukaemia (AML), and next generation sequencing of multiple genes for improved diagnosis of AML.

1.3.1 Chromosome 17p deletion detection

For the chromosome 17p deletion detection program, 13 laboratories in 2018 and 13 laboratories in 2019 participated for proficiency testing. Three reference testing Case samples were distributed in each year. For the 2018 program, 92% (12/13) of laboratories were concordant for reference testing Case 1, all laboratories (100%) were concordant for Case 2, and 85% (11/13) of laboratories were concordant for Case 3. For the 2019 program, 77% (10/13) of laboratories were concordant for Case

1, all laboratories (100%) were concordant for Case 2, and 92% (12/13) of laboratories were concordant for Case 3.

1.3.2 Isocitrate dehydrogenase 1 and 2 (*IDH1*, *IDH2*) program

For the *IDH1* and *IDH2* gene variant testing program, 12 laboratories in 2017, 15 laboratories in 2018, and 17 laboratories in 2019 participated for proficiency testing. Three reference testing Case samples were distributed in each year. For the 2017 program, 92% (11/12) of laboratories for Case A, and 100% (12/12) of laboratories for Cases B and C, were concordant. For 2018, 93% (14/15) of laboratories for Cases A and B, and 100% (15/15) of laboratories for Case C, were concordant. For 2019, 100% (16/16) of laboratories were concordant for all three cases tested for.

1.3.3 Next generation sequencing program

A next generation DNA sequencing EQA program was devised to proficiency test laboratories performing diagnostic analysis on multiple genes associated with acute myeloid leukaemia. A total of eight laboratories in 2018 and nine laboratories in 2019 participated in the EQA program. Ten samples in 2018 and two samples in 2019 were distributed for proficiency testing respectively. A total of 15 genes in 2018 were identified as representing a consensus core for diagnostic analysis. However, for 2019, this reduced to eight genes for clinical testing. All laboratories testing for the consensus core gene variants were concordant for each sample tested.

1.4 Conclusion

The 2017-2019 Commonwealth funded project has allowed the RCPAQAP to develop two new DNA extraction programs and five new DNA biomarker programs for proficiency testing human genetic disease. In the short-term, the availability of pilot EQA programs that reflect new developing areas of disease understanding and genetic diagnostics are critical for laboratories since diagnostic shortcomings can be identified and resolved. In the mid-term, these EQA programs will allow larger cohorts of laboratories to enrol for proficiency testing and benchmarking. In the long-term, these EQA programs will continue to ensure that extracted patient DNA and biomarker analysis is of the highest quality, particularly for laboratories adopting to new diagnostic strategies of liquid biopsy cfDNA testing and whole genome next generation sequencing. This will be key for diagnosing the complex diseases of cancer (i.e., breast, lung, prostate, gastrointestinal, leukaemia), neurological disorders (i.e., Alzheimer's disease, Parkinson's disease, Huntington's disease, epilepsy), and age-related disease (i.e., cardiovascular disease, type 2 diabetes, arthritis, osteoporosis). As such,

proficiency testing new diagnostic strategies and disease understandings will benefit pathology services and allow clinicians to make better informed decisions with respect to pharmaceutical intervention and patient management.

2. Aims of the Study

(*What was the aim/purpose of the project?*)

There were three key aims to be addressed in this study. The first aim was to determine the ability of laboratories to extract total DNA from patient blood and clinical tissue. Critically, there are no EQA programs available to monitor Australasian laboratories for their ability to extract total DNA from multiple different tissues. Importantly, the data generated from laboratory genetic tests are directly reflective of the initial DNA quality. Extraction of high-quality DNA is therefore essential for accurate downstream clinical interrogation and interpretation of gene mutations found in genetic disease. The DNA extraction programs therefore allows laboratories to monitor the quality of their DNA extracts for all genetic tests. The second aim was to assess laboratories performing novel diagnostics for the testing of circulating fragmented DNA found in cancer patients (for identifying tumour resistance) (Malapelle et al., 2016), and in maternal blood (for identifying abnormal foetal DNA) (El Khattabi et al., 2016). These strategies are generating great levels of interest from clinicians and diagnostic testing laboratories since circulating free DNA analysis is non-invasive and surgical tissue-extraction does not need to be performed. The circulating free DNA EQA programs therefore allows laboratories to identify and resolve any shortcomings in this new area of genetic diagnostics. The third aim was to assess laboratories performing different leukaemia diagnostics for either detecting chromosome 17p genetic rearrangements in chronic lymphocytic leukaemia, for identifying isocitrate dehydrogenase 1 and 2 (*IDH1, IDH2*) gene mutations in acute myeloid leukaemia, or for performing next generation sequencing on DNA isolated from acute myeloid leukaemia patients. Importantly, the development of next generation pharmaceutical compounds in the treatment of leukaemia is growing (Mondesir et al., 2016; O'Brien et al., 2016) and it is essential that EQA programs be developed and offered to meet this growing level of treatment. As such, the data generated from the new EQA programs developed here will help with future clinical diagnostic testing and aid in understanding the leukaemia disease process.

3. Background

(*What is the overview of the project and its importance for disease diagnostics?*)

Identifying abnormal cellular function in response to genetic DNA variation, microbial infection, or as a consequence of another underlying disease pathology is key to understanding the human disease process. Inherited disease, complex disease, and age-related diseases all require assessment of extracted patient DNA so that DNA sequence alterations in key genes can be identified and a disease-causing status assigned. However, assigning a specific gene variant as disease-causing can be difficult since the human genome is estimated to contain approximately 21,000 protein encoding genes (Willyard, 2018), and data from the 1000 genomes project has identified that 1000s of DNA variants exist in these genes in normal healthy individuals (1000 genomes project consortium, 2015). Importantly, some of these same DNA variants are also present in disease pathologies which makes interpreting specific DNA gene mutations as disease-causing difficult to clarify. An additional complexity is that gene mutations previously characterised as disease-causing have also been found in centenarians who are disease-free (Freudenberg-Hua et al., 2014; Zhang et al., 2019). Given this level of complexity, key organisations are now aiming to sequence the entire genome in disease individuals to fully identify multiple DNA gene variants associated with specific genetic disease. In particular, Genomics England are currently performing a 100,000 genomes study designed to map the entire human genome in 85,000 individuals (and their family members) with rare disease and in individuals with cancer (https://www.genomicsengland.co.uk/about-genomics-england/the-100000-genomes-project/). Completion of the 100,000 genomes project is expected to enhance our genetic understanding of rare disease which will provide a foundation for future developments of diagnostic, therapeutic, and preventive medical applications in other complex and age-related diseases. Clinical adoption of whole genome next generation sequencing is likely to occur in the near future as sequencing costs reduce. It is therefore important that DNA quality be of the highest level when performing this level of DNA sequencing.

The outcomes of this project were to confirm that (i) laboratories are extracting high quality DNA that is amenable for all downstream genetic diagnostic analyses, and (ii) that disease-associated DNA biomarkers can be correctly identified and reported. Current genetic testing strategies are highly varied with 64,874 genetic diagnostic tests being performed globally on 18,686 genes across 9858 different genetic conditions (https://www.ncbi.nlm.nih.gov/gtr/). Most of the 64,874 diagnostic tests are designed to target single DNA variants in small DNA regions of a known gene sequence.

However, whole genome next generation sequencing has the potential to reduce diagnostic analysis of all genetic diseases to a single assay. The EQA programs developed by the RCPAQAP are therefore important for providing laboratories access to these key quality testing resources and for promoting new disease understandings arising from data derived from highly sensitive sequencing technology.

As new disease diagnostics are developed, it is important that these are followed up with appropriate EQA programs. For example, liquid biopsy samples are emerging as key material for early diagnostic evaluations of disease. The liquid biopsy material comprises of (but not limited to) blood plasma, urine, saliva, and cerebrospinal fluid. However, blood plasma remains the most common, containing circulating cell-free DNA (cfDNA) that are representative of small DNA fragments ranging approximately 160–200 base pairs in length. These specific cfDNA fragments are released into the blood circulation by both normal healthy cells and abnormal tumour cells through cell growth and cell turnover processes (Kamat et al., 2006; Warton and Samimi, 2015). However, in cancer, treatment resistance tumour-specific cfDNA variants have been detected in various genes and can therefore be differentiated from normal cellular wild-type cfDNA. A primary clinical focus of cfDNA testing is to therefore identify and monitor specific variants that are associated with pharmaceutical treatment resistance in cancer (Vendrell et al., 2017; Thierry et al., 2017; Iwama et al., 2018). For example, the *EGFR* c.2369C>T (p.Thr790Met) variant is commonly found in the tumours of non-small cell lung cancer patients where resistance to tyrosine kinase inhibitor therapy is detected (Bordi et al., 2015; Del Re et al., 2017). Monitoring the onset of resistance through observing an increase in cfDNA *EGFR* c.2369C>T copy number allows for earlier intervention and the application of next generation therapeutic inhibitors. Analyses of plasma-derived cfDNA has therefore been proposed as an alternative test for cancer diagnostics [Vendrell et al., 2017; Eposito et al., 2017; Aggarwal et al., 2018). The key advantage of blood plasma cfDNA testing is that it is minimally invasive making it a very attractive technique over the invasiveness of surgery and of the risks associated with this. CfDNA analysis of maternal blood is particularly advantageous for the prenatal diagnosis of foetal DNA since existing prenatal diagnostic strategies are invasive and may induce miscarriage (Tabor and Alfirevic, 2010). The blood liquid biopsy genetic testing approach therefore represents a significant advancement for disease diagnostics and is likely to rapidly grow on a global scale. It is therefore essential that quality assurance programs be developed to allow for cross-laboratory proficiency testing to ensure that clinical diagnostics are maintained at a high standard.

Identifying underlying DNA variation in genes of known function is a key process for the development of pharmaceutical compounds that are actionable for alleviating disease symptoms or progression. In the treatment of leukaemia, new specific compounds have been designed to inhibit the gain-of-function mutations found in *IDH1* and *IDH2* genes (Mondesir et al., 2016), and for treating patients where resistance to traditional treating compounds has occurred (O'Brien et al., 2016). Furthermore, clinical application of next generation sequencing is continually identifying new potentially actionable leukaemia-associated gene variations (Wang et al., 2018). This information is essential for the development of new pharmaceutical medicines. However, quality assurance programs need to be developed to keep pace with advances in medical improvements. In Australia, this is key since Medicare rebates are unavailable for any new gene variant targeting compound in the absence of an accredited EQA program. The RCPAQAP therefore aim to continually develop new EQA programs that are clinically relevant for current diagnostic needs.

This project was therefore designed to quality assess seven key diagnosing areas comprising of whole genomic DNA extraction, tumour FFPE DNA extraction, cfDNA analysis, NIPT analysis, leukaemia chromosome 17p deletion, leukaemia *IDH1* and *IDH2* gene mutation testing, and leukaemia next generation sequencing. The technical ability of laboratories to accurately detect and report on gene targets is key for all genetic diagnostics. The development of new EQA programs in new key developing areas is essential as this allows laboratories to enrol for proficiency testing so that performance can be monitored, and improvements made where necessary.

4. Addressing essential needs

(*What need/s does this project address?*)

The final step in DNA testing involves clinical interpretation of the data so that appropriate decisions on patient management can be derived. There is therefore a critical requirement to monitor clinical interpretations of DNA gene variations in line with current DNA extraction practises, in the application of new understandings of disease processes, and in the adoption of new technologies used for disease diagnostics. This is of key concern especially if laboratories inadvertently miscall genetic variants due to poor quality DNA and therefore provide false negative/positive reports to the referring clinician. In addition, there is an unmet need in post market surveillance for monitoring tumour DNA sequence variation associated with treatment resistance in response to pharmaceutical intervention. This critical information will aid the clinical pathologist in making key management decisions for patient treatment. It is therefore important that EQA programs be consistently developed to match the needs of clinical testing laboratories so that diagnostic testing problems can be identified and rectified to maintain high levels of patient care.

4.1 EQA design process

The RCPAQAP continually strive to keep up to date with current technology and clinical testing needs. As such, advice from our Molecular Genetics Advisory Committee is key for understanding current diagnostic areas requiring appropriate EQA programs. Based on advice from our Advisory Committee, each EQA program was initially developed to address a current clinical need. However, the data produced, and feedback received from each participating laboratory further allows us to refine each EQA program so that they better fit the real-world testing requirements of each diagnostic laboratory. This EQA refining approach therefore allows the RCPAQAP to develop high quality EQA programs that are reflective of true patient testing and diagnostic practices.

5. Benefits

(*What benefit will the project be to consumers of pathology services?*)

A key benefit of this project is that it will ensure the availability of quality assurance programs to all participating genetic diagnostic testing laboratories in the Australasia region. In particular, monitoring for DNA extraction quality and in genetic testing using new technology and biomarker strategies for cancer, NIPT and leukaemia diagnostics will enable laboratories to apply for National Association of Testing Authorities (NATA) accreditation so that they can offer these new efficient diagnostic developments for routine diagnostics. Furthermore, laboratories performing these tests will be able to operate at a higher level of proficiency due to the availability of proficiency testing quality assurance programs.

Genetic tests are totally dependent on the quality of extracted DNA. All downstream diagnostic genetic assays and clinical interpretation will be reflective of the initial DNA isolate. Quality assuring DNA isolation will raise confidence in data generation and in clinical interpretation of any DNA variation. This is key as it will directly impact on appropriate clinical management of the patient. For example, data produced from high quality DNA whole genome sequencing is helping raise the clinical diagnosis of rare genetic disorders (Taylor et al., 2015). This has a direct impact on health as the referring clinician will be better informed for the diagnosis and genetic counselling of patients and their families. In addition, new therapeutic compounds targeting key gene mutations can only be offered if a quality assurance program is available for the quality monitoring of those DNA alterations. The need for quality assurance is therefore crucial and of great benefit to the continuing healthcare of patients and their families.

The identification of circulating free DNA in plasma is starting to cause a shift in thinking away from invasive biopsy techniques to now focus instead on plasma DNA for diagnostic characterisation. The non-invasive nature of the test makes it a very attractive technique over the invasiveness of surgery and of the risks associated with this. The ability to monitor the levels of circulating tumour DNA mutations in response to ongoing pharmaceutical intervention will help address the clinical significance between pharmaceutical treatment and molecular tumour DNA response. Additionally, the rapid determination of genetic abnormalities found in foetal DNA will greatly aid the clinician for informed decision making of a putative underlying progressive disease.

The benefit of quality monitoring of DNA extraction and genotyping results will ultimately allow clinicians to make better informed decisions with respect to pharmaceutical intervention and patient management. These EQA programs will therefore be of benefit to pathology communities comprising scientists, clinical geneticists, genetics pathologists and oncologists.

6. Reference samples

(*What were the samples used in the project*)

Reference testing samples were either commercially obtained or were sourced from participating laboratories. The number of samples required for each EQA program from 2017 to 2019 are provided in Table 1. An overview of the reference testing samples, including the genes required to be tested, are provided below for each EQA program.

Table 1. Total number of samples assayed for each Commonwealth QUPP funded EQA program.

PROGRAM	2017	2018	2019	TOTAL
Whole genomic DNA extraction	55	115	125	295
FFPE DNA extraction	-	45	78	123
cfDNA	-	56	35	91
NIPT	-	-	25	25
Leukaemia 17p deletion	-	39	39	78
Leukaemia *IDH1, IDH2*	36	45	51	132
Leukaemia next generation sequencing	-	80	18	98

6.1 Whole genomic DNA Extraction

No specific reference testing sample was used for the whole genomic DNA extraction program. Instead, laboratories were requested to submit to the RCPAQAP up to five extracted DNA samples that were of clinical interest. As such, laboratories could submit samples from multiple different tissue types for quality analysis. This program was designed to be specifically advantageous for tissue biobanks since these facilities have vast amounts of stored DNA and current EQA providers are unable to offer quality assurance on any stored DNA sample. The needs of tissue biobanks are therefore not addressed. The newly devised RCPAQAP program addresses this need for stored DNA.

6.2 Fixed formalin paraffin embedded (FFPE) DNA extraction

Reference testing FFPE samples were obtained from the National Centre for Clinical Laboratories (NCCL), Beijing, China, for the 2019 FFPE DNA extraction program. Laboratories were requested to submit to the RCPAQAP up to five extracted FFPE DNA samples (including the reference control sample). FFPE is a process that fixes thinly sliced tumour material on to a glass slide. This allows tumour tissue to be visualised under a microscope where areas of tumour growth can be identified. The DNA from these identified tumour areas can be extracted and sequenced to identify key gene mutations that may be responsible for promoting tumour progression. However, the FFPE fixation process severely damages DNA causing high levels of fragmentation. Current EQA programs for stored FFPE DNA material not exist. The newly devised RCPAQAP program therefore addresses this need for quality assessing stored FFPE DNA.

6.3 Circulating free DNA (cfDNA)

The cfDNA reference testing material was synthetically derived and was obtained from the NCCL (Beijing, China). The cfDNA material was designed to contain clinically relevant cfDNA variant genotypes in five common cancer associated genes (*TP53*, *EGFR, KRAS, NRAS,* and *BRAF*). The DNA variants in these genes ranged from 0.1% to 10% in comparison to the normal DNA sequence. Laboratories were requested to identify the gene variants that were of clinical interest to them and report on their mutational percentage level.

6.4 Non-invasive prenatal testing (NIPT)

The NIPT reference testing material was obtained from the NCCL (Beijing, China) and was designed to reflect common chromosomal abnormalities found in foetal DNA that is circulating in the mother's blood. Specifically, the EQA program was devised to proficiency test the detection of additional chromosomes (known as a trisomy) that are clinically relevant for either Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), or Patau syndrome (trisomy 13). Laboratories were requested to identify the trisomy abnormalities and report their findings.

6.5 Leukaemia Chromosome 17p deletion

The chromosome 17p deletion samples were obtained from a collaboration with the Australian Society of Diagnostic Genomics (ASDG). The reference testing material were reflective of chronic lymphocytic leukaemia (CLL) and samples were derived through the tissue culture of bone marrow cells prior to fixation onto a glass slide. The specific bone marrow cells contained differing sizes of

TP53 gene region deletions in chromosome 17p. Laboratories were requested to identify and report on the percentage of cells containing the chromosome 17p deletion.

6.6 Leukaemia isocitrate dehydrogenase 1 and 2 gene testing gene testing

Samples containing isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) gene variants were obtained from the Royal Prince Alfred Hospital. The *IDH1* and *IDH2* gene variants in these reference samples are associated with acute myeloid leukaemia. Laboratories were requested to identify and report on each gene variant in each sample tested.

6.7 Leukaemia next generation sequencing

The reference testing samples used for the next generation sequencing program were obtained from the Royal North Shore Hospital and were isolated from patients with acute myeloid leukaemia. These samples contained multiple gene DNA variants. Laboratories were requested to sequence and identify each gene variant routinely tested for in their facility.

7. Methods

(*Technical background of the study*)

7.1 Laboratories

All laboratories that participated in the Commonwealth funded EQA programs are accredited for clinical diagnostic genetic testing. The number of laboratories participating in each EQA program are provided in Table 2. Laboratories from nine different countries participated in these programs. The geographic location of all laboratories is provided in Figure 1. The participation of overseas laboratories is a consequence of these new EQA programs being unavailable elsewhere. This is also important for the RCPAQAP since this raises the profile of the company for developing and offering new EQA programs of clinical need.

Table 2. Total number of laboratories participating in each QUPP funded EQA program.

PROGRAM	2017	2018	2019
Whole genomic DNA extraction	11	23	25
FFPE DNA extraction	-	9	16
cfDNA	-	7	6
NIPT	-	-	5
Leukaemia 17p deletion	-	13	13
Leukaemia *IDH1, IDH2*	12	15	16
Leukaemia next generation sequencing	-	8	9
TOTAL	23	75	90
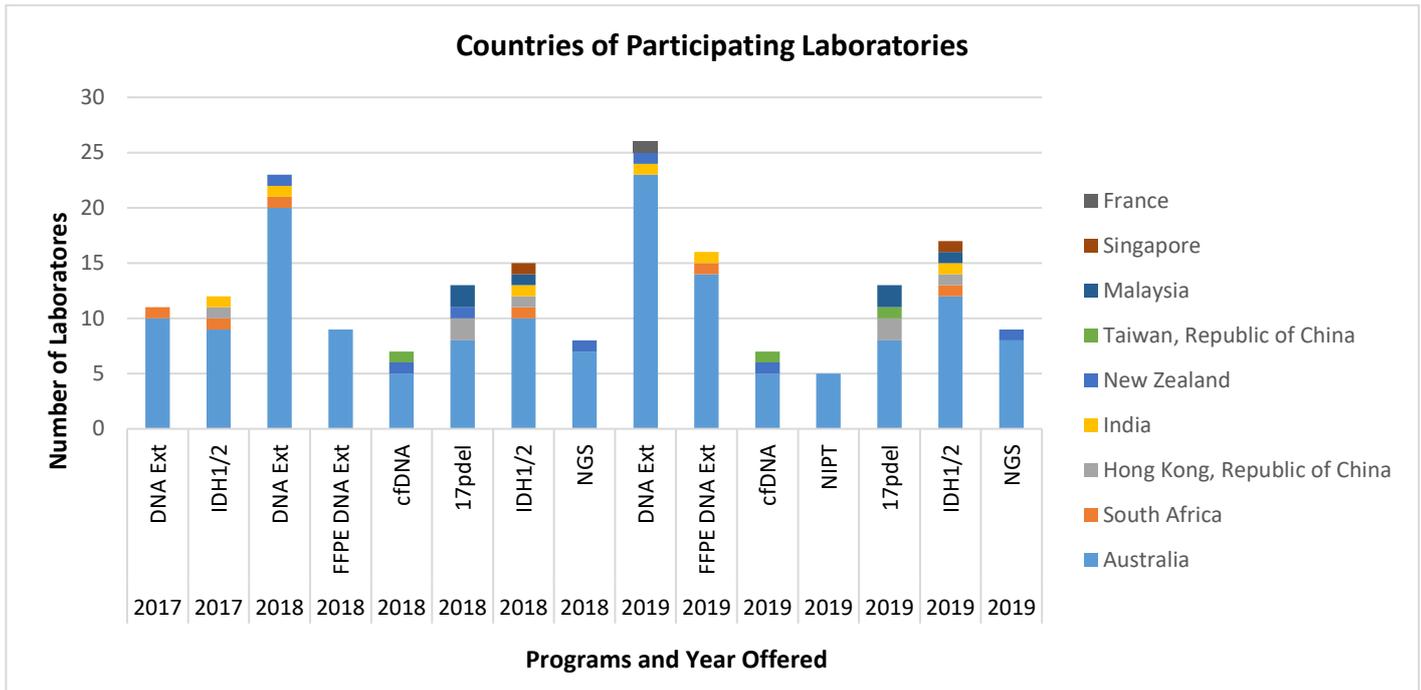
Figure 1. The geographic location of all participating laboratories during the 2017 – 2019 Commonwealth funded study.

7.2 Proficiency testing

7.2.1 Whole genomic DNA Extraction

The objective of this program was to assess the quality of laboratory extracted genomic DNA and to validate the suitability of each DNA extract for downstream genetic testing. Five DNA extracts were submitted by each participant for assessment. The RCPAQAP evaluated each DNA extract using three different measuring strategies; (i) total DNA integrity analysis using a DNA TapeStation 4200; (ii) analysis of DNA using multiplex-PCR; and (iii) analysis of DNA by real-time PCR. For DNA integrity analysis, quality assessment was performed to determine the level of DNA recovery and degradation in relation to the extraction process. For multiplex PCR and real-time PCR analyses, the amplification of 16 gene different genes in total were used to determine the applicability of each DNA extract for downstream applications (Table 3).

7.2.1.1 DNA Integrity

The integrity of each DNA extract was initially measured on the DNA Tape Station 4200 (Agilent Technologies, Santa Clara, USA). The TapeStation is a micro fluidic platform which assesses the

quantity and integrity of genomic DNA in the sizing range of 200 to >60000 base pairs. A software algorithm produces a virtual gel image and a DNA Integrity Number (DIN) that are representative of whole genomic DNA integrity. The DIN ranges from 0 (highly degraded DNA) to 10 (highly intact DNA). DNA extracts were diluted to a working concentration of 50ng/µl. Extracts that measured less than 50ng/µl were analysed undiluted. All assays were performed according to the manufacturer's instructions.

7.2.1.2 Multiplex-PCR (mPCR)

Amplification of five gene loci (Table 3) was performed in a single end-point PCR reaction using T100 thermal cycler (Bio-Rad, California, USA). Essentially, PCR amplification of DNA was performed in 25µL volumes consisting of a final concentration of 1X MyTaq reaction buffer (Bioline), 3µM primers (forward and reverse), 1 unit MyTaq HS DNA polymerase (Bioline), and 125ng DNA.

PCR cycling parameters were:

95°C for 3 mins
95°C x 30 secs ⎤
58°C x 30 secs ⎬ 35 cycles
68°C x 2 mins ⎦
68°C x 10 mins

Primer sequences (reported by van Dongen et al., 2003):

AFF1-600F	GGAGCAGCATTCCATCCAGC
AFF1-600R	CATCCATGGGCCGGACATAA
AFF1-400F	CCGCAGCAAGCAACGAACC
AFF1-400R	GCTTTCCTCTGGCGGCTCC
ZBTB16-300F	TGCGATGTGGTCATCATGGTG
ZBTB16-300R	CGTGTCATTGTCGTCTGAGGC
RAG1-200F	TGTTGACTCGATCCACCCCA
RAG1-200R	TGAGCTGCAAGTTTGGCTGAA
TBXAS1-100F	GCCCGACATTCTGCAAGTCC
TBXAS1-100R	GGTGTTGCCGGGAAGGGTT

All PCR products were visualised on the 4200 TapeStation. Five amplification products were expected to be amplified from each single PCR reaction.

7.2.1.3 Real-time PCR (RT-PCR)

RT-PCR was performed using the Quant Studio 3 System (Thermo Fisher Scientific, Massachusetts, USA). TaqMan assays (Thermo Fisher Scientific) representing 11 gene loci (Table 3) were used to amplify DNA extracted from each tissue type. TaqMan assays were performed in duplicate using 96-well plates. Briefly, a total volume of 17.4µl volumes consisting of a final concentration of 1X PCR master mix (Thermo Fisher Scientific), 1X TaqMan primer/probe mix (Thermo Fisher Scientific, Massachusetts, USA) and 10ng of DNA were used in accordance with the manufacturers protocols (Thermo Fisher Scientific).

PCR cycling parameters were:

96°C for 10 mins
98°C x 30 secs   ⎤
60°C x 2 mins   ⎦   39 cycles
60°C x 2 mins

Analyses of gene loci cycle threshold (Ct) values for all plates were determined using the Data Assist program (Thermo Fisher Scientific). The RT-PCR assays were expected to generate a Ct value for each of the 11 genes to confirm DNA amplification. Ct values less than 34 were accepted as successful amplification. Ct values 35 and above were rejected and considered as failed amplification.

7.2.1.4 Overall Quality Assessment

The number of gene regions amplified by mPCR and RT-PCR were compared against the total number of gene regions expected to be amplified to derive a ratio value. For example, four products observed from mPCR and 10 products observed from RT-PCR provide a total of 14 PCR products. A total of 16 PCR products are expected to be amplified. The PCR ratio is therefore $14/16 = 0.875$. The PCR ratio values were then used for a z-score calculation [Z = (PCR ratio – population mean PCR ratio) / standard deviation of the population PCR ratio]. Z-scores within ± 2 standard deviations from the mean were considered concordant. In contrast, z-scores greater than ± 2 standard deviations from the mean were considered discordant.

Table 3. Gene regions used for RT-PCR (RT) and mPCR (M) assessment of extracted DNA. The catalogue number refers to Thermo Fisher Scientific TaqMan assays.

Gene	Chromosome	RefSeq	Description	Dye	Size (bp)	Assay	Catalogue No./Reference
LRP1B	2	NG_051023.1	LDL receptor related protein 1B	FAM	114	RT	Hs02501162_cn
ROBO2	3	NG_027734.1	roundabout guidance receptor 2	FAM	77	RT	Hs03227040_cn
TERT	5	NG_009265.1	telomerase reverse transcriptase	VIC	88	RT	4403315
PDE4D	5	NG_027957.1	phosphodiesterase 4D	FAM	110	RT	Hs04290984_cn
EYS	6	NG_023443.2	eyes shut homolog (Drosophila)	FAM	110	RT	Hs04321318_cn
CNTNAP2	7	NG_007092.2	contactin associated protein like 2	FAM	107	RT	Hs05018255_cn
ASTN2	9	NG_021409.1	astrotactin 2	FAM	102	RT	Hs06843013_cn
PRKG1	10	NG_029982.1	protein kinase, cGMP-dependent, type I	FAM	82	RT	Hs03731145_cn
CNTN5	11	NG_047156.1	contactin 5	FAM	105	RT	Hs05228401_cn
RBFOX1	16	NG_011881.1	RNA binding fox-1 homolog 1	FAM	110	RT	Hs03953793_cn
DMD	X	NG_012232.1	dystrophin	FAM	105	RT	Hs00129944_cn
AFF1 (AF4)	4	NC_018915.2	AF4/FMR2 family member 1	None	600	M	van Dongen et al., (2003)
AFF1 (AF4)	4	NC_018915.2	AF4/FMR2 family member 1	None	400	M	van Dongen et al., (2003)
ZBTB16 (PLZF)	11	NG_012140.2	zinc finger and BTB domain containing 16	None	300	M	van Dongen et al., (2003)
RAG1	11	NG_007528.1	Recombination activating gene 1	None	200	M	van Dongen et al., (2003)
TBXAS1	7	NG_008422.2	Thromboxane A synthase 1	None	100	M	van Dongen et al., (2003)

7.2.2 Fixed formalin paraffin embedded (FFPE) DNA extraction

The objective of this program was to assess the quality of laboratory extracted FFPE DNA and to validate the suitability of each DNA extract for downstream genetic testing. Five FFPE DNA extracts were submitted by each participant for assessment. The RCPAQAP evaluated each DNA extract using multiplex-PCR and real-time PCR as described in the whole genomic DNA extraction section above.

7.2.3 Circulating free DNA (cfDNA)

This program was designed to assess laboratory performance in the detection of gene variant allelic frequency in circulating free DNA. The cfDNA liquid biopsy reference testing standards were generated as previously reported (Zhang et al., 2017). The derived reference testing material was designed to reflect commonly reported cfDNA variants identified in various cancers including adenocarcinoma (colon), non-small cell lung cancer, and melanoma (Mehrotra et al., 2017; Auliac et al., 2018; Tian et al., 2018; Byeon et al., 2019). This allowed laboratories to test for variants found in specific cancers that were of clinical interest to them. Laboratories were therefore not required to test for all variants in all cancer types.

Validation of each predicted cfDNA variant allelic frequency was performed using next generation sequencing (NGS) and digital PCR (dPCR) as previously reported (Zhang et al., 2017; Chai et al., 2019; Peng et al., 2019). The RCPAQAP also performed additional confirmation of the genotypes using digital PCR according to Zhang et al., (2017). Stability testing was performed by shipping samples at room temperature to a Singapore laboratory and having the samples returned to the RCPAQAP for repeat digital PCR analyses.

For 2018, laboratories were sent eight testing samples designed to contain nine clinically relevant cfDNA variant genotypes in the *EGFR, KRAS, NRAS, TP53,* and *BRAF* genes with predicted allelic frequencies ranging from 0.1% to 10%. Each sample consisted of 25µL of Tris EDTA (TE) buffer (pH8) containing 125ng of cfDNA. Laboratories were not required to extract the cfDNA prior to analysis. Any potential issues relating to laboratory-specific cfDNA extraction processes were therefore removed. For 2019, laboratories were sent five lyophilized testing samples designed to contain six clinically relevant cfDNA variant genotypes in the *EGFR, KRAS, TP53,* and *BRAF* genes with predicted allelic frequencies ranging from 0.1% to 5%. Laboratories were required to resuspend each sample in 1mL of water prior to analysis. All reference testing standards were distributed to

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each laboratory with accompanying instructions for storage and assay procedures. Genomic DNA extracted from healthy blood cells were also distributed to participants for use as a mutation negative control (for all variants tested for). Participating laboratories were requested to provide key information relating to the specific cfDNA detection methodology used including assay kits and limit of detection (LoD) of their testing platforms.

For proficiency testing, the RCPAQAP combined data from all laboratories in China and Australia who participated in the RCPAQAP/NCCL collaborative EQA program. Z-score calculations using the participant consensus mean for each gene allelic frequency were used to determine laboratory performance. Z scores (z = (individual laboratory data – laboratory mean) / standard deviation) between -2.0 to 2.0 were considered to be acceptable and therefore concordant. In contrast, z scores less than -2.0 or greater than 2.0 were considered discordant. Participants who did not test for specific genotypes, or where the LoD of their testing platform was above the allelic frequency range being tested for, where not assessed or penalized.

7.2.4 Non-invasive prenatal testing (NIPT)

The NIPT program was designed to assess laboratory performance in the detection of foetal DNA abnormalities in reference samples containing both foetal and maternal DNA. The derived reference testing material was synthetically designed to reflect the three commonly reported chromosomal abnormalities of trisomy 13, trisomy18, and trisomy 21. A trisomy is representative of an extra chromosome in the developing foetus that can be identified in pregnancy through DNA analysis. As such, laboratories were required to test for these three chromosomal abnormalities.

Validation of chromosomal abnormalities and DNA stability testing were performed and confirmed by NCCL. Five reference testing samples were distributed for to five laboratories for testing. Each sample consisted of 40-50ng of lyophilized DNA that needed to be resuspended prior to analysis. Laboratories were requested to resuspend DNA and perform analysis according to their standard operating procedure and to return all data to the RCPAQAP.

For proficiency testing, the overall laboratory generated data was used to derive a consensus. Concordance was awarded to laboratories providing data that matched the consensus data.

7.2.5 Leukaemia Chromosome 17p deletion

The objective of this program was to assess laboratory performance in the detection of a chromosome 17p deletion that contains the *TP53* gene. The derived reference testing material was designed to reflect *TP53* gene deletions found in chronic lymphocytic leukaemia. Validation of chromosomal abnormalities were confirmed by ASDG and a reference testing laboratory. Stability testing was performed by ASDG. Laboratories were requested to perform analysis according to their standard operating procedure and return data to the RCPAQAP. Three reference testing slide samples were distributed for analysis. One sample was a normal control and two samples consisted of chromosome 17p deletions. Laboratories were requested to perform analysis according to their standard operating procedure and return data to the RCPAQAP.

For proficiency testing, laboratories were assessed against the relevant Australian guidelines including ISO15189, The National Pathology Accreditation Advisory Council (NPAAC) Requirements for Cytogenetic Testing (Third Edition, 2013), and An International System for Human Cytogenomic Nomenclature (ISCN) (2016). Assessment was made of the quality and technical proficiency of the hybridisations through examination of the supplied raw images. Each submitted report was assessed for accuracy and completeness according to the relevant guidelines. Laboratories were also requested to report the percentage of cells containing a deletion for each slide analysed. Z-scores were then derived from the reported data and concordance determined.

7.2.6 Leukaemia isocitrate dehydrogenase 1 and 2 gene testing

The objective of this program was to assess laboratory performance in the detection of *IDH1* and *IDH2* gene variants that are associated with acute myeloid leukaemia. The reference testing samples were confirmed to contain gene variants by the Royal Prince Alfred Hospital. Variants were validated by a reference testing laboratory. Stability testing was performed by the RCPAQAP. Three reference testing samples were distributed for analysis. Each sample consisted of 1ug of DNA in 20uL volumes of TE buffer. Laboratories were requested to perform analysis according to their standard operating procedure and return data to the RCPAQAP.

For proficiency testing, laboratories were assessed directly against the reference target for detection or non-detection of each gene variant.

7.2.7 Leukaemia next generation sequencing

The objective of this program was to assess laboratory performance using next generation sequencing in the detection of multiple gene variants that are associated with acute myeloid leukaemia. The reference testing samples were obtained from the Royal North Shore Hospital and were confirmed to contain DNA variants in different genes. For the 2018 program, ten reference testing samples were distributed to each laboratory for analysis. For the 2019 program, two samples were distributed for analysis. Each sample consisted of 1ug of DNA in 20uL volumes of TE buffer. Laboratories were requested to perform analysis according to their standard operating procedure for acute myeloid leukaemia diagnoses and return data to the RCPAQAP.

For proficiency testing, laboratories were assessed directly against the consensus data for detection or non-detection of each gene variant.

8. Results and Discussion

(*Results and understandings from the study*)

8.1 Whole genomic DNA Extraction

The three measuring platforms used in this program provide key information relating to the quality of each DNA extract. The combination of the three testing platforms is important for providing a comprehensive analysis of laboratory extracted DNA. The DNA TapeStation is useful for providing an initial quality assessment that is independent of any contaminants that may be present in the DNA sample. However, low DNA TapeStation scores (i.e., high levels of DNA fragmentation) do not necessarily indicate that the DNA sample is not fit for purpose. The mPCR and RT-PCR assays are therefore key for verifying amplification and confirming that the DNA sample can be used for DNA diagnostic testing.

For 2017, 11 laboratories submitted a total of 55 samples for analysis. The DNA integrity, mPCR and RT-PCR data are represented in Table 4. Laboratory 2 did not provide adequate DNA for complete analysis of Samples A, C, and D. For these samples, concordance was awarded since the DNA integrity number is high which is representative of good quality DNA (Table 4). For Laboratory 7, Sample A was discordant since the DNA integrity number was very low (poor quality DNA) and both mPCR and RT-PCR assays failed. These data indicate that Sample A (blood DNA) is either too fragmented for usage, or that very little or no DNA was successfully extracted by Laboratory 7. Of the 55 samples analysed, 98% (54/55) were concordant. DNA was extracted from 15 different tissue types (Figure 2).

For 2018, 23 laboratories submitted a total of 115 samples for analysis. The DNA integrity, mPCR and RT-PCR data are represented in Table 5. For Laboratory 15, Sample B (product of conception) was discordant since a DNA integrity number could not be generated (indicating poor quality DNA) and both mPCR and RT-PCR assays failed. This is indicative of very poor DNA quality or absence of DNA. Of the 115 samples analysed, 99% (114/115) were concordant. DNA was extracted from 16 different tissue types (Figure 3).

For 2019, 25 laboratories submitted a total of 125 samples for analysis. The DNA integrity, mPCR and RT-PCR data are represented in Table 6. For Laboratory 9, Sample E (Bone) was discordant since a DNA integrity number could not be generated (indicating poor quality DNA) and both mPCR

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and RT-PCR assays failed to amplify. Laboratories 16 and 23 were not assessed due to the samples submitted being non-human. Although DIN values could be produced (Table 6), the mPCR and RT-PCR assays were not performed given that all PCR primers were designed to amplify human DNA only and not DNA from other species. Of the 125 samples submitted, 115 could be tested and 99% (114/115) were concordant. DNA was extracted from 18 different tissue types (Figure 4).

The RCPAQAP whole genomic DNA extraction program is different from other EQA providers in that analysis of DNA extracts is directly performed by the RCPAQAP. Each individual DNA sample can therefore be easily identified as being amenable for genetic diagnostic testing. The proficiency assessment data is key for laboratories for identifying DNA extracts (including archived DNA) that are of good quality for diagnostic usage. In addition, the identification of poor quality DNA allows laboratories to address the underlying issues leading to the generation poor DNA extraction quality. As such, laboratory interest in the whole genomic DNA extraction EQA program is growing and participation has more than doubled from an initial 11 laboratories in 2017 to 25 laboratories in 2019.
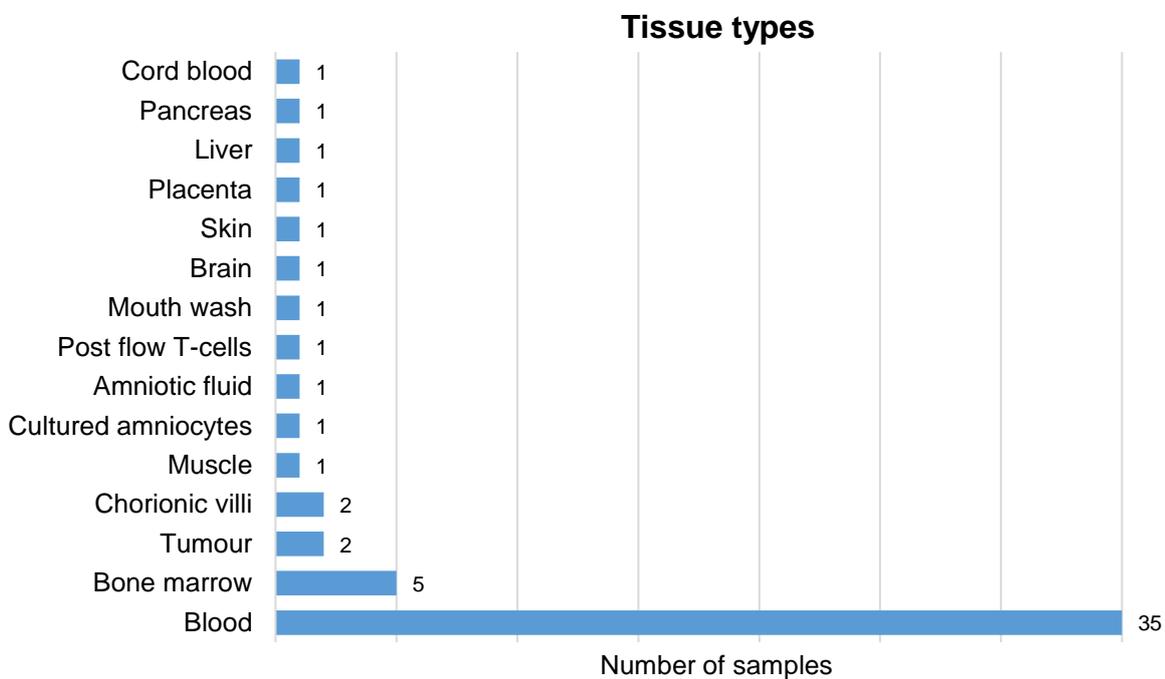
Figure 2. The total number of DNA extracts isolated from different tissue types for the 2017 whole genomic DNA extraction program.
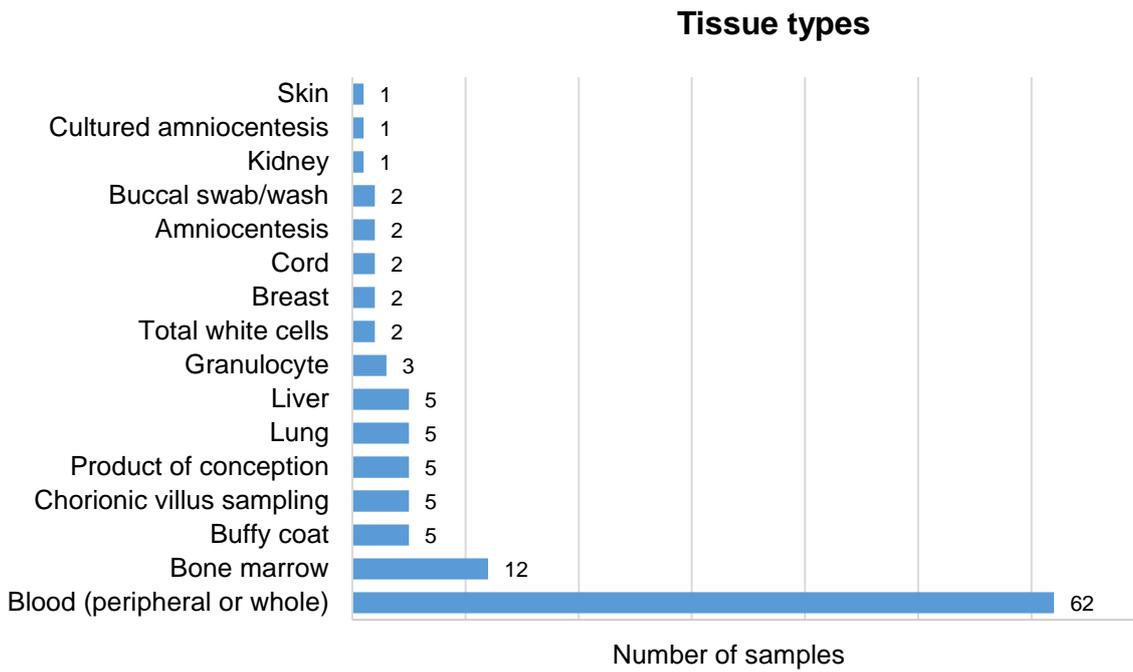
Tissue types

Figure 3. The total number of DNA extracts isolated from different tissue types for the 2018 whole genomic DNA extraction program.
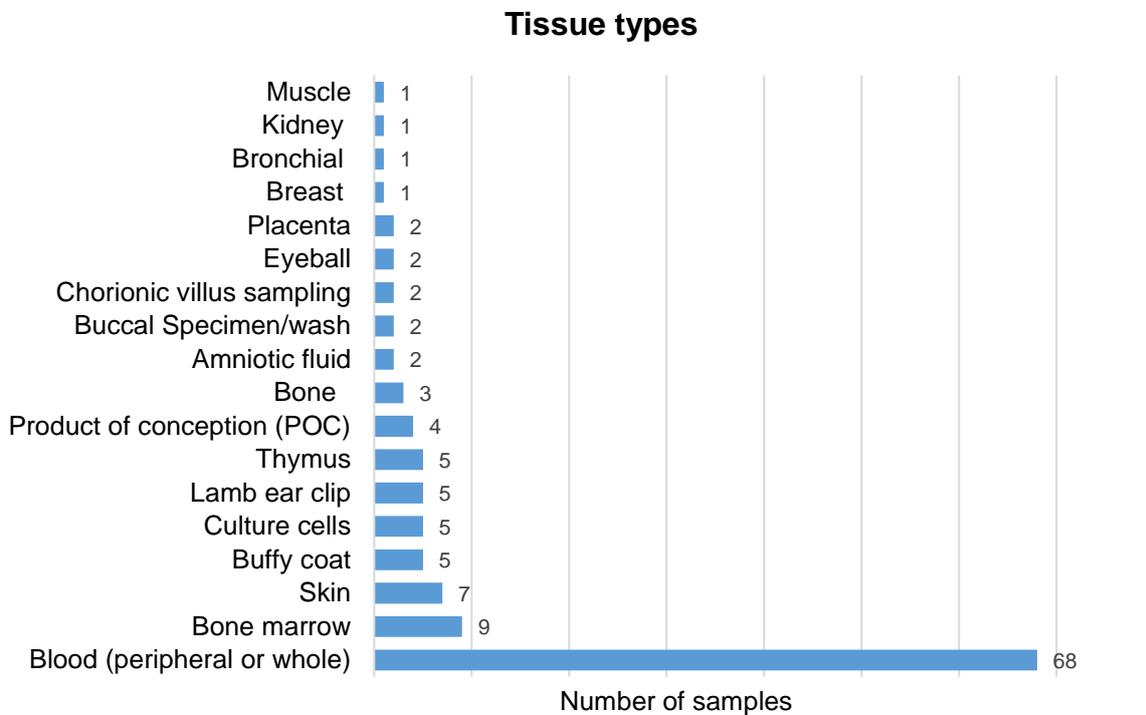
Tissue types

Figure 4. The total number of DNA extracts isolated from different tissue types for the 2019 whole genomic DNA extraction program.

Table 4. Assessment of tissues submitted for the 2017 DNA Extraction EQA program.

Laboratory	Sample	Tissue	Integrity (DIN)	mPCR products	RT- PCR products	PCR Ratio	Z-score	Overall Assessment
1	A	Tumour	7.2	5	11	1	0.14	Concordant
1	B	Blood	9.3	5	11	1	0.14	Concordant
1	C	Bone marrow	9.2	5	11	1	0.14	Concordant
1	D	Blood	7.4	5	11	1	0.14	Concordant
1	E	Blood	8.1	5	11	1	0.14	Concordant
2	A	Blood	8.8	DNA exhausted	DNA exhausted	-	Not assessed	Concordant
2	B	Blood	9.0	5	11	1	0.14	Concordant
2	C	Blood	8.4	DNA exhausted	DNA exhausted	-	Not assessed	Concordant
2	D	Blood	8.3	DNA exhausted	DNA exhausted	-	Not assessed	Concordant
2	E	Blood	8.0	5	11	1	0.14	Concordant
3	A	Blood	8.6	5	11	1	0.14	Concordant
3	B	Blood	8.0	5	11	1	0.14	Concordant
3	C	Cultured amniocytes	7.4	5	11	1	0.14	Concordant
3	D	Tumour	9.7	5	11	1	0.14	Concordant
3	E	Chorionic villi	7.6	5	11	1	0.14	Concordant
4	A	Bone marrow	8.3	5	11	1	0.14	Concordant
4	B	Blood	9.6	5	11	1	0.14	Concordant
4	C	Blood	9.3	5	11	1	0.14	Concordant
4	D	Post flow T-cells	5.9	5	11	1	0.14	Concordant
4	E	Mouth wash	1.4	5	11	1	0.14	Concordant
5	A	Blood	7.1	5	11	1	0.14	Concordant
5	B	Blood	6.2	5	11	1	0.14	Concordant
5	C	Blood	7.0	5	11	1	0.14	Concordant
5	D	Blood	5.0	5	11	1	0.14	Concordant
5	E	Blood	7.6	5	11	1	0.14	Concordant
6	A	Blood	8.5	5	11	1	0.14	Concordant
6	B	Blood	8.3	5	11	1	0.14	Concordant
6	C	Blood	8.2	5	11	1	0.14	Concordant
6	D	Blood	8.4	5	11	1	0.14	Concordant
6	E	Blood	8.8	5	11	1	0.14	Concordant
7	A	Blood	1.1	0	0	0	-7.07	Discordant
7	B	Blood	9.4	5	11	1	0.14	Concordant
7	C	Bone marrow	9.3	5	11	1	0.14	Concordant
7	D	Bone marrow	9.3	5	11	1	0.14	Concordant
7	E	Bone marrow	9.3	5	11	1	0.14	Concordant
8	A	Blood	8.0	5	11	1	0.14	Concordant
8	B	Blood	6.4	5	11	1	0.14	Concordant
8	C	Blood	6.3	5	11	1	0.14	Concordant

8	D	Blood	7.8	5	11	1	0.14	Concordant
8	E	Blood	6.3	5	11	1	0.14	Concordant
9	A	Brain	8.6	5	11	1	0.14	Concordant
9	B	Skin	7.4	5	11	1	0.14	Concordant
9	C	Placenta	6.5	5	11	1	0.14	Concordant
9	D	Liver	7.8	5	11	1	0.14	Concordant
9	E	Pancreas	6.6	5	11	1	0.14	Concordant
10	A	Blood	6.6	5	11	1	0.14	Concordant
10	B	Blood	7.9	5	11	1	0.14	Concordant
10	C	Blood	7.6	5	11	1	0.14	Concordant
10	D	Blood	8.0	5	11	1	0.14	Concordant
10	E	Blood	7.8	5	11	1	0.14	Concordant
11	A	Chorionic villi	8.1	5	11	1	0.14	Concordant
11	B	Amniotic fluid	5.7	5	11	1	0.14	Concordant
11	C	Muscle	4.2	5	11	1	0.14	Concordant
11	D	Cord blood	9.3	5	11	1	0.14	Concordant
11	E	Blood	6.4	5	11	1	0.14	Concordant

Table 5. Assessment of tissues submitted for the 2018 DNA Extraction EQA program.

Laboratory	Sample	Tissue	Integrity (DIN)	mPCR products	RT- PCR products	PCR Ratio	Z-score	Overall Assessment
1	A	Buffy coat	7.6	5	11	1	0.11	Concordant
1	B	Buffy coat	6.4	5	11	1	0.11	Concordant
1	C	Buffy coat	6.6	5	11	1	0.11	Concordant
1	D	Buffy coat	7.6	5	11	1	0.11	Concordant
1	E	Buffy coat	7.1	5	11	1	0.11	Concordant
2	A	Peripheral blood	6.4	5	11	1	0.11	Concordant
2	B	Bone marrow	8.3	5	11	1	0.11	Concordant
2	C	Granulocyte	6.6	5	11	1	0.11	Concordant
2	D	Peripheral blood	7.0	5	11	1	0.11	Concordant
2	E	Bone marrow	8.5	5	11	1	0.11	Concordant
3	A	EDTA whole blood	9.5	5	11	1	0.11	Concordant
3	B	EDTA whole bone marrow	7.6	5	11	1	0.11	Concordant
3	C	EDTA whole blood	8.7	5	11	1	0.11	Concordant
3	D	EDTA whole blood	8.7	5	11	1	0.11	Concordant
3	E	EDTA whole blood	8.9	5	11	1	0.11	Concordant
4	A	Peripheral blood	9.7	5	11	1	0.11	Concordant
4	B	Peripheral blood	9.5	5	11	1	0.11	Concordant
4	C	Peripheral blood	6.2	5	11	1	0.11	Concordant
4	D	CVS	9.8	5	11	1	0.11	Concordant
4	E	CVS	9.8	5	11	1	0.11	Concordant
5	A	Peripheral blood total white cells	8.0	5	11	1	0.11	Concordant
5	B	Peripheral blood granulocytes	8.9	5	11	1	0.11	Concordant
5	C	Blood total white cells	7.2	5	11	1	0.11	Concordant
5	D	Bone marrow	7.6	5	11	1	0.11	Concordant
5	E	Peripheral blood granulocytes	7.7	5	11	1	0.11	Concordant
6	A	EDTA blood	9.6	5	11	1	0.11	Concordant
6	B	EDTA blood	9.7	5	11	1	0.11	Concordant
6	C	EDTA blood	9.6	5	11	1	0.11	Concordant
6	D	EDTA blood	9.4	5	11	1	0.11	Concordant
6	E	EDTA blood	9.4	5	11	1	0.11	Concordant
7	A	Breast tissue	8.4	5	11	1	0.11	Concordant
7	B	Bone marrow	6.7	5	11	1	0.11	Concordant
7	C	Breast tissue	8.4	5	11	1	0.11	Concordant
7	D	Peripheral blood	7.4	5	11	1	0.11	Concordant
7	E	Kidney tissue	8.7	5	11	1	0.11	Concordant
8	A	Lung	7.3	5	11	1	0.11	Concordant
8	B	Lung	1.1	4	11	0.94	-0.58	Concordant
8	C	Villi	5.9	5	11	1	0.11	Concordant
8	D	Lung	9.1	5	11	1	0.11	Concordant
8	E	Lung	1.8	5	11	1	0.11	Concordant

9	A	Bone marrow	9.7	5	11	1	0.11	Concordant
9	B	Bone marrow	9.6	5	11	1	0.11	Concordant
9	C	Bone marrow	9.8	5	11	1	0.11	Concordant
9	D	Bone marrow	9.5	5	11	1	0.11	Concordant
9	E	Bone marrow	9.6	5	11	1	0.11	Concordant
10	A	Blood	8.6	5	11	1	0.11	Concordant
10	B	Blood	9.4	5	11	1	0.11	Concordant
10	C	Blood	9.0	5	11	1	0.11	Concordant
10	D	Blood	8.7	5	11	1	0.11	Concordant
10	E	Blood	8.8	5	11	1	0.11	Concordant
11	A	Whole blood	8.2	5	11	1	0.11	Concordant
11	B	Whole blood	8.1	5	11	1	0.11	Concordant
11	C	Whole blood	9.1	5	11	1	0.11	Concordant
11	D	Whole blood	9.3	5	11	1	0.11	Concordant
11	E	Whole blood	9.0	5	11	1	0.11	Concordant
12	A	Blood	8.7	5	11	1	0.11	Concordant
12	B	Blood	8.0	5	11	1	0.11	Concordant
12	C	Blood	9.9	5	11	1	0.11	Concordant
12	D	Cord	9.9	5	11	1	0.11	Concordant
12	E	Cord	9.9	5	11	1	0.11	Concordant
13	A	Cultured Amniocentesis	9.0	5	11	1	0.11	Concordant
13	B	Amniocentesis	6.3	5	11	1	0.11	Concordant
13	C	Placental villi	8.0	5	11	1	0.11	Concordant
13	D	Liver	6.7	5	11	1	0.11	Concordant
13	E	Blood	7.4	5	11	1	0.11	Concordant
14	A	Buccal wash	6.5	5	11	1	0.11	Concordant
14	B	EDTA blood	9.8	5	11	1	0.11	Concordant
14	C	EDTA blood	9.3	5	11	1	0.11	Concordant
14	D	EDTA blood	7.8	5	11	1	0.11	Concordant
14	E	EDTA blood	7.7	5	11	1	0.11	Concordant
15	A	Product of conception	9.7	5	11	1	0.11	Concordant
15	B	Product of conception	-	0	0	0	-11.00	Discordant
15	C	Product of conception	6.7	5	11	1	0.11	Concordant
15	D	Product of conception	-	5	11	1	0.11	Concordant
15	E	Product of conception	-	5	9	0.88	-1.28	Concordant
16	A	EDTA blood	7.7	5	11	1	0.11	Concordant
16	B	EDTA blood	9.1	5	11	1	0.11	Concordant
16	C	EDTA blood	9.1	5	11	1	0.11	Concordant
16	D	EDTA blood	8.5	5	11	1	0.11	Concordant
16	E	EDTA blood	8.0	5	11	1	0.11	Concordant
17	A	Blood	9.6	5	11	1	0.11	Concordant
17	B	Blood	9.3	5	10	0.94	-0.58	Concordant
17	C	Bone marrow	9.7	5	11	1	0.11	Concordant
17	D	Bone marrow	9.6	5	11	1	0.11	Concordant

17	E	Bone marrow	9.4	5	11	1	0.11	Concordant
18	A	Blood	7.6	5	11	1	0.11	Concordant
18	B	Blood	7.7	5	11	1	0.11	Concordant
18	C	Blood	7.8	5	11	1	0.11	Concordant
18	D	Blood	7.9	5	11	1	0.11	Concordant
18	E	Blood	7.8	5	11	1	0.11	Concordant
19	A	Liver	6.8	5	11	1	0.11	Concordant
19	B	Liver	6.6	5	10	0.94	-0.58	Concordant
19	C	Liver	7.3	5	11	1	0.11	Concordant
19	D	Lung	1.8	5	11	1	0.11	Concordant
19	E	Liver	1.0	4	11	0.94	-0.58	Concordant
20	A	Blood	9.3	5	11	1	0.11	Concordant
20	B	Blood	8.6	5	11	1	0.11	Concordant
20	C	Blood	8.9	5	11	1	0.11	Concordant
20	D	Blood	8.8	5	11	1	0.11	Concordant
20	E	Blood	9.0	5	11	1	0.11	Concordant
21	A	Blood	7.6	5	11	1	0.11	Concordant
21	B	Blood	8.2	5	11	1	0.11	Concordant
21	C	Blood	7.4	5	11	1	0.11	Concordant
21	D	Blood	7.7	5	11	1	0.11	Concordant
21	E	Blood	7.9	5	11	1	0.11	Concordant
22	A	Blood	8.4	5	11	1	0.11	Concordant
22	B	Blood	8.3	5	11	1	0.11	Concordant
22	C	Blood	8.5	5	11	1	0.11	Concordant
22	D	Blood	5.8	5	11	1	0.11	Concordant
22	E	Blood	6.7	5	11	1	0.11	Concordant
23	A	CVS	7.5	5	11	1	0.11	Concordant
23	B	Amnio	1.9	5	11	1	0.11	Concordant
23	C	Skin	8.0	5	11	1	0.11	Concordant
23	D	Buccal swab	7.8	5	11	1	0.11	Concordant
23	E	Blood	8.6	5	11	1	0.11	Concordant

Table 6. Assessment of tissues submitted for the 2019 DNA Extraction EQA program.

Laboratory	Sample	Tissue	Integrity (DIN)	mPCR products	RT- PCR products	PCR Ratio	Z-score	Overall Assessment
1	A	Buffy coat	8.3	5	6	1	0.35	Concordant
1	B	Buffy coat	8.7	5	6	1	0.35	Concordant
1	C	Buffy coat	8.3	5	6	1	0.35	Concordant
1	D	Buffy coat	7.3	5	6	1	0.35	Concordant
1	E	Buffy coat	8.2	5	6	1	0.35	Concordant
2	A	Bone Marrow	8.4	5	6	1	0.35	Concordant
2	B	Bone Marrow	8.7	5	6	1	0.35	Concordant
2	C	Bone Marrow	9	5	6	1	0.35	Concordant
2	D	Peripheral Blood	8.4	5	6	1	0.35	Concordant
2	E	Peripheral Blood	9.1	5	6	1	0.35	Concordant
3	A	Bone marrow aspirate	9	5	6	1	0.35	Concordant
3	B	EDTA Whole blood	8.5	5	6	1	0.35	Concordant
3	C	EDTA Whole blood	9.4	5	6	1	0.35	Concordant
3	D	EDTA Whole blood	9.3	5	6	1	0.35	Concordant
3	E	EDTA Whole blood	9	5	6	1	0.35	Concordant
4	A	CVS Tissue	9.1	5	6	1	0.35	Concordant
4	B	CVS Tissue	9.5	5	6	1	0.35	Concordant
4	C	Blood	9.6	5	6	1	0.35	Concordant
4	D	Blood	9.2	5	6	1	0.35	Concordant
4	E	Blood	6.5	5	6	1	0.35	Concordant
5	A	Peripheral Blood Total White Cells	8.3	5	6	1	0.35	Concordant
5	B	Peripheral Blood Granulocytes	7.5	5	6	1	0.35	Concordant
5	C	Bone Marrow	6.8	5	6	1	0.35	Concordant
5	D	Peripheral Blood Granulocytes	7.9	5	6	1	0.35	Concordant
5	E	Peripheral Blood Total White Cells	8.3	5	6	1	0.35	Concordant
6	A	Blood	8.4	5	6	1	0.35	Concordant
6	B	Blood	9.5	5	6	1	0.35	Concordant
6	C	Blood	8.9	5	6	1	0.35	Concordant
6	D	Blood	9.6	5	6	1	0.35	Concordant
6	E	Blood	9.4	5	6	1	0.35	Concordant
7	A	Blood	9.4	5	6	1	0.35	Concordant
7	B	Bone Marrow	1.4	5	4	0.81	-0.48	Concordant
7	C	Breast Tissue	8.3	5	6	1	0.35	Concordant
7	D	Bronchial Tissue	6.3	5	6	1	0.35	Concordant
7	E	Kidney Tissue	8.4	5	6	1	0.35	Concordant
8	A	Placenta	7	5	6	1	0.35	Concordant
8	B	Peripheral Blood	9.1	5	6	1	0.35	Concordant
8	C	Peripheral Blood	9.8	5	6	1	0.35	Concordant
8	D	Peripheral Blood	9.8	5	6	1	0.35	Concordant
8	E	Placenta	7.8	5	6	1	0.35	Concordant
9	A	Eyeball	1.3	4	6	0.9	-0.09	Concordant

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9	B	Bone	7.7	5	6	1	0.35	Concordant
9	C	Eyeball	7.2	5	6	1	0.35	Concordant
9	D	Bone	8.3	5	6	1	0.35	Concordant
9	E	Bone	-	0	0	0	-4.00	Discordant
10	A	Blood	7.1	5	6	1	0.35	Concordant
10	B	Blood	7.3	5	6	1	0.35	Concordant
10	C	Blood	7.4	5	6	1	0.35	Concordant
10	D	Blood	7.2	5	6	1	0.35	Concordant
10	E	Blood	7	5	6	1	0.35	Concordant
11	A	whole blood	8.7	5	6	1	0.35	Concordant
11	B	bone marrow	8.9	5	6	1	0.35	Concordant
11	C	whole blood	8.7	5	6	1	0.35	Concordant
11	D	whole blood	8.7	5	6	1	0.35	Concordant
11	E	whole blood	9.1	5	6	1	0.35	Concordant
12	A	culture cells	9.4	5	6	1	0.35	Concordant
12	B	culture cells	9	5	6	1	0.35	Concordant
12	C	culture cells	8.2	5	6	1	0.35	Concordant
12	D	culture cells	9.3	5	6	1	0.35	Concordant
12	E	culture cells	8.7	5	6	1	0.35	Concordant
13	A	Blood	9	5	6	1	0.35	Concordant
13	B	Blood	8.7	5	6	1	0.35	Concordant
13	C	Blood	8	5	6	1	0.35	Concordant
13	D	Bone marrow	8.1	3	6	0.81	-0.48	Concordant
13	E	Product of conception (POC)	8.1	5	6	1	0.35	Concordant
14	A	whole blood	8.4	5	6	1	0.35	Concordant
14	B	whole blood	8.5	5	6	1	0.35	Concordant
14	C	whole blood	7.4	5	6	1	0.35	Concordant
14	D	whole blood	8	5	6	1	0.35	Concordant
14	E	whole blood	5.8	5	6	1	0.35	Concordant
15	A	Blood	8.6	5	6	1	0.35	Concordant
15	B	Blood	7.1	5	6	1	0.35	Concordant
15	C	Blood	7.3	5	6	1	0.35	Concordant
15	D	Blood	8.6	5	6	1	0.35	Concordant
15	E	Mouth Wash	7.8	5	6	1	0.35	Concordant
16	A	Mouse Skin	6.5	-	-	-	-	Not assessed
16	B	Mouse Skin	7.5	-	-	-	-	Not assessed
16	C	Mouse Skin	6.4	-	-	-	-	Not assessed
16	D	Mouse Skin	5.6	-	-	-	-	Not assessed
16	E	Mouse Skin	5.2	-	-	-	-	Not assessed
17	A	Blood	6.9	5	6	1	0.35	Concordant
17	B	Blood	9.3	5	6	1	0.35	Concordant
17	C	Products of Conception - Villi	8.6	5	6	1	0.35	Concordant
17	D	Products of Conception - Villi	6.8	5	6	1	0.35	Concordant
17	E	Products of Conception - Rib	8.6	5	6	1	0.35	Concordant

18	A	EDTA Blood	7.2	5	6	1	0.35	Concordant
18	B	EDTA Blood	9.2	5	6	1	0.35	Concordant
18	C	EDTA Blood	8.8	5	6	1	0.35	Concordant
18	D	EDTA Blood	9.6	5	6	1	0.35	Concordant
18	E	EDTA Blood	8.8	5	6	1	0.35	Concordant
19	A	Bone Marrow	9.4	5	6	1	0.35	Concordant
19	B	Peripheral Blood	9.4	5	6	1	0.35	Concordant
19	C	Peripheral Blood	9.6	5	6	1	0.35	Concordant
19	D	Bone Marrow	7.5	5	6	1	0.35	Concordant
19	E	Bone Marrow	9.2	5	6	1	0.35	Concordant
20	A	Blood	7.9	5	6	1	0.35	Concordant
20	B	Blood	8.2	5	6	1	0.35	Concordant
20	C	Blood	8.3	5	6	1	0.35	Concordant
20	D	Blood	7.7	5	6	1	0.35	Concordant
20	E	Blood	8.2	5	6	1	0.35	Concordant
21	A	Whole Blood EDTA	8.1	5	6	1	0.35	Concordant
21	B	Whole Blood EDTA	7.3	5	6	1	0.35	Concordant
21	C	Whole Blood EDTA	9.2	5	6	1	0.35	Concordant
21	D	Whole Blood EDTA	8.8	5	5	0.9	-0.09	Concordant
21	E	Whole Blood EDTA	8	5	6	1	0.35	Concordant
22	A	Thymus	1.2	5	6	1	0.35	Concordant
22	B	Thymus	4.1	5	6	1	0.35	Concordant
22	C	Thymus	1.8	5	6	1	0.35	Concordant
22	D	Thymus	7	5	6	1	0.35	Concordant
22	E	Thymus	3.5	5	6	1	0.35	Concordant
23	A	Lamb Ear clip	7.9	-	-	-	-	Not assessed
23	B	Lamb Ear clip	8.9	-	-	-	-	Not assessed
23	C	Lamb Ear clip	8.3	-	-	-	-	Not assessed
23	D	Lamb Ear clip	8.7	-	-	-	-	Not assessed
23	E	Lamb Ear clip	8.7	-	-	-	-	Not assessed
24	A	Blood	8.4	5	6	1	0.35	Concordant
24	B	Blood	9.4	5	6	1	0.35	Concordant
24	C	Blood	9.3	5	6	1	0.35	Concordant
24	D	Skin	7	5	6	1	0.35	Concordant
24	E	Skin	8	5	6	1	0.35	Concordant
25	A	Muscle	5.7	5	6	1	0.35	Concordant
25	B	Uncultured Chorionic Villus	7.5	5	6	1	0.35	Concordant
25	C	Uncultured Amniotic Fluid	5.8	5	6	1	0.35	Concordant
25	D	Buccal Specimen	6.9	5	6	1	0.35	Concordant
25	E	Peripheral blood	9.1	5	6	1	0.35	Concordant

8.2 Formalin-fixed paraffin-embedded DNA Extraction

The two measuring platforms of mPCR and RT-PCR were used in this program to provide key information relating to the quality of each FFPE DNA extract. Given that FFPE DNA is fragmented, the combination of the mPCR and RT-PCR testing platforms are important for providing information on the amplification capacity of laboratory extracted FFPE DNA. The mPCR and RT-PCR data are therefore key for verifying amplification and confirming that the DNA sample can be used for DNA diagnostic testing.

For 2018, nine laboratories submitted a total of 45 FFPE DNA samples for analysis. The mPCR and RT-PCR data are represented in Table 7. Laboratory 2 did not provide adequate DNA for complete RT-PCR analysis of Samples A, B, C, D and E. PCR ratio values were therefore derived using the observed products produced for each sample divided by the expected value of 13 for Samples A and B, 11 for Sample C, 9 for Sample D, and 12 for Sample E (Table 7). For Laboratory 6, Sample D (right hemicolectomy) was discordant since only eight PCR products could be amplified from a total of 16 which is reflected in a z-score of -3.5. Therefore, Sample D appears to be too fragmented for diagnostic usage. Of the 45 samples analysed, 98% (44/45) were concordant. DNA was extracted from 14 different FFPE tissue types (Figure 5).

For 2019, 16 laboratories submitted a total of 78 FFPE DNA samples for analysis with 16 of these representing a RCPAQAP reference sample. The mPCR and RT-PCR data are represented in Table 8. Due to limited availability of DNA samples, the total number of genes amplified in the RT-PCR assay were reduced to six. For Laboratory 9, Samples B, D, and E were discordant since the none of the six RT-PCR genes could be amplified. These samples therefore appear to be too fragmented for diagnostic usage. Of the 78 samples analysed, 96% (75/78) were concordant. DNA was extracted from 19 different FFPE tissue types (Figure 6).

The RCPAQAP FFPE DNA extraction program is particularly useful for tissue biobanks who have archived FFPE tissue samples. The RCPAQAP are therefore the first to offer quality assurance for archived tissue. Each submitted FFPE DNA sample can be tested for quality and for being amenable for genetic diagnostic testing. The proficiency assessment data is key for laboratories for identifying DNA extracts that are of good quality for diagnostic usage. In addition, the identification of poor quality DNA allows laboratories to address the underlying issues leading to the generation poor DNA extraction quality or for monitoring the degradation of archived DNA over time. As such, laboratory

interest in the FFPE DNA extraction EQA program is growing and participation has almost doubled from an initial 9 laboratories in 2018 to 16 laboratories in 2019.
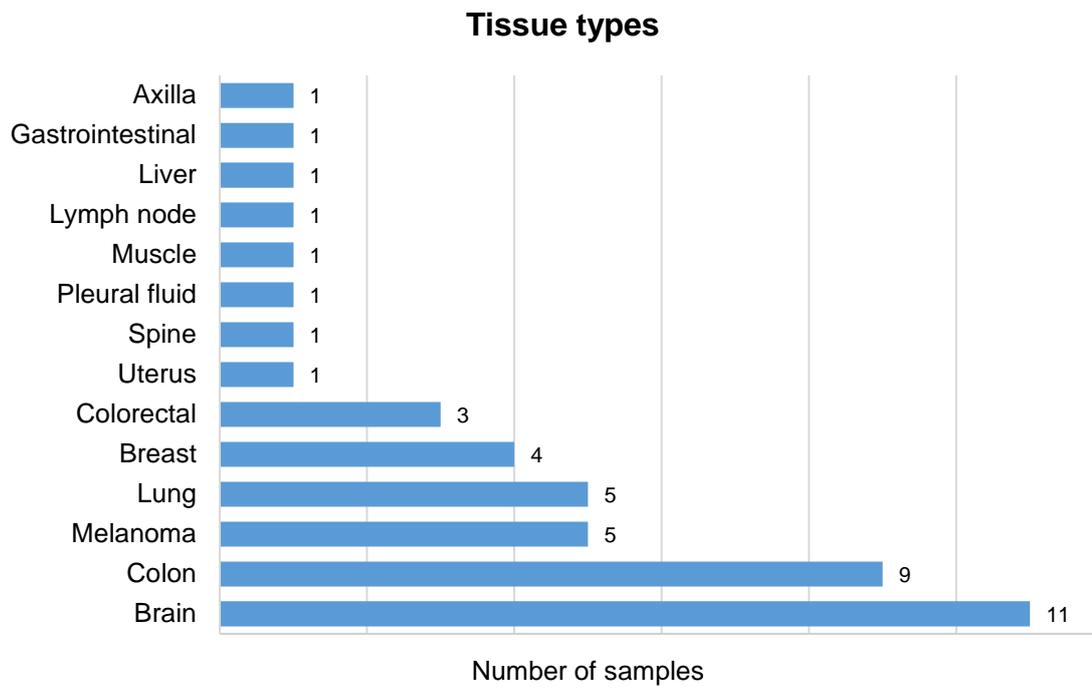
**Figure 5**. The total number of DNA extracts isolated from different tissue types for the 2018 FFPE DNA extraction program.
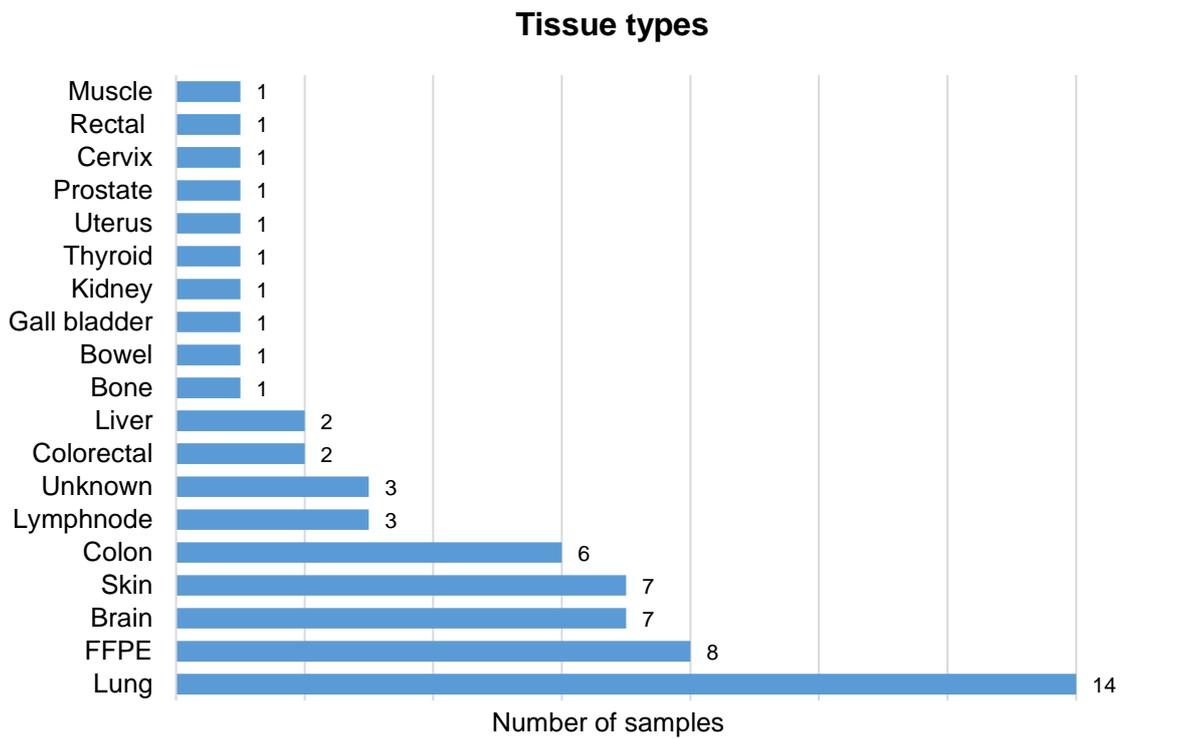
Tissue types

Figure 6. The total number of DNA extracts isolated from different tissue types for the 2019 FFPE DNA extraction program.

Table 7. Assessment of tissues submitted for the 2018 FFPE DNA Extraction EQA program.

Laboratory	Sample	Tissue	mPCR products	RT- PCR products	PCR Ratio	Z-score	Overall Assessment
1	A	Uterine carcinoma	4	10	0.88	-0.38	Concordant
1	B	Malignant melanoma	4	10	0.88	-0.38	Concordant
1	C	Malignant melanoma	5	10	0.94	0.15	Concordant
1	D	Malignant melanoma	5	11	1	0.67	Concordant
1	E	Breast	5	11	1	0.67	Concordant
2	A	Brain Tumour	4	8	0.92*	0.00	Concordant
2	B	Brain Tumour	5	8	1*	0.67	Concordant
2	C	Brain Tumour	4	6	0.90*	-0.17	Concordant
2	D	Brain Tumour	5	4	1*	0.67	Concordant
2	E	Brain Tumour	3	7	0.83*	-0.75	Concordant
3	A	Colon	4	10	0.88	-0.38	Concordant
3	B	Melanoma	4	11	0.94	0.15	Concordant
3	C	Colon	4	11	0.94	0.15	Concordant
3	D	Brain	5	11	1	0.67	Concordant
3	E	Brain	4	11	0.94	0.15	Concordant
4	A	Breast Tissue	4	11	0.94	0.15	Concordant
4	B	Breast Tissue	5	11	1	0.67	Concordant
4	C	T3 Vertebral Metastisis	5	11	1	0.67	Concordant
4	D	Breast Tissue	5	11	1	0.67	Concordant
4	E	Axilla	5	10	0.94	0.15	Concordant
5	A	Colon	5	11	1	0.67	Concordant
5	B	Colon	5	11	1	0.67	Concordant
5	C	Colon	5	11	1	0.67	Concordant
5	D	Brain	5	11	1	0.67	Concordant
5	E	Brain	4	11	0.94	0.15	Concordant
6	A	Right upper lobe wedge	5	11	1	0.67	Concordant
6	B	Splenic flexure	4	11	0.94	0.15	Concordant
6	C	Lymph node	3	11	0.88	-0.38	Concordant
6	D	right hemicolectomy	3	5	0.50	-3.50	Discordant
6	E	lower back tissue	5	11	1	0.67	Concordant
7	A	Lung	5	11	1	0.67	Concordant
7	B	Lung	4	11	0.94	0.15	Concordant
7	C	Brain	5	11	1	0.67	Concordant
7	D	Pleural fluid	5	11	1	0.67	Concordant
7	E	Colon	4	11	0.94	0.15	Concordant
8	A	Colorectal	5	11	1	0.67	Concordant
8	B	Melanoma	5	11	1	0.67	Concordant
8	C	Gastrointestinal	5	11	1	0.67	Concordant
8	D	Colorectal	5	11	1	0.67	Concordant
8	E	Colorectal	5	11	1	0.67	Concordant
9	A	Colon	4	11	0.94	0.15	Concordant

9	B	Lung	4	11	0.94	0.15	Concordant
9	C	Liver	5	11	1	0.67	Concordant
9	D	Brain	4	11	0.94	0.15	Concordant
9	E	Lung	4	11	0.94	0.15	Concordant

*Laboratory 2 did not provide adequate DNA for complete RT-PCR analysis of Samples A, B, C, D and E. PCR ratio values were therefore derived using the observed products produced from mPCR and RT-PCR for each sample and dividing this by the expected value of 13 for Samples A and B, 11 for Sample C, 9 for Sample D, and 12 for Sample E.

Table 8. Assessment of tissues submitted for the 2019 FFPE DNA Extraction EQA program.

Laboratory	Sample	Tissue	mPCR products	RT- PCR products	PCR Ratio	Z-score	Overall Assessment
1	A	QAP sample	4	6	0.91	0.05	Concordant
1	B	Skin	5	6	1	0.56	Concordant
1	C	Colon	5	6	1	0.56	Concordant
1	D	Skin	5	6	1	0.56	Concordant
2	A	QAP sample	4	6	0.91	0.05	Concordant
2	B	FFPE	4	6	0.91	0.05	Concordant
2	C	FFPE	4	6	0.91	0.05	Concordant
2	D	FFPE	5	6	1	0.56	Concordant
2	E	FFPE	5	6	1	0.56	Concordant
3	A	QAP sample	0	6	0.55	-1.97	Concordant
3	B	FFPE Brain tumour tissue	0	6	0.55	-1.97	Concordant
3	C	FFPE Brain tumour tissue	4	6	0.91	0.05	Concordant
3	D	FFPE Brain tumour tissue	4	6	0.91	0.05	Concordant
3	E	FFPE Brain tumour tissue	5	6	1	0.56	Concordant
4	A	QAP sample	4	6	0.91	0.05	Concordant
4	B	Unknown	3	6	0.82	-0.45	Concordant
4	C	Unknown	4	6	0.91	0.05	Concordant
4	D	Unknown	4	6	0.91	0.05	Concordant
5	A	QAP sample	4	6	0.91	0.05	Concordant
5	B	Retrocaval Lymphnode	4	6	0.91	0.05	Concordant
5	C	Right Hemicolectomy	5	6	1	0.56	Concordant
5	D	Pleural Biopsy	5	6	1	0.56	Concordant
5	E	Right Supraclavic ular Lymphnode	5	6	1	0.56	Concordant
6	A	QAP sample	4	6	0.91	0.05	Concordant
6	B	Liver tissue	5	6	1	0.56	Concordant
6	C	Prostate tissue	5	6	1	0.56	Concordant
6	D	Brain (Frontal Lesion)	5	6	1	0.56	Concordant
6	E	Bowel (Colectomy)	5	6	1	0.56	Concordant
7	A	QAP sample	4	6	0.91	0.05	Concordant
7	B	Bowel	4	6	0.91	0.05	Concordant
7	C	Lung	4	6	0.91	0.05	Concordant
7	D	Rectal polyp	4	6	0.91	0.05	Concordant
7	E	Uterus	4	6	0.91	0.05	Concordant
8	A	QAP sample	4	6	0.91	0.05	Concordant
8	B	FFPE tissue	5	6	1	0.56	Concordant
8	C	FFPE tissue	4	6	0.91	0.05	Concordant
8	D	FFPE tissue	5	6	1	0.56	Concordant
8	E	FFPE tissue	4	6	0.91	0.05	Concordant
9	A	QAP sample	4	6	0.91	0.05	Concordant
9	B	Cervical lymph node	2	0	0.18	-3.99	Discordant
9	C	Gallbladder	2	6	0.73	-0.96	Concordant

9	D	Cervix	1	0	0.09	-4.49	Discordant
9	E	Muscle	2	0	0.18	-3.99	Discordant
10	A	QAP sample	4	6	0.91	0.05	Concordant
10	B	Right Hemicolectomy	5	6	1	0.56	Concordant
10	C	Right Hemicolectomy	4	6	0.91	0.05	Concordant
10	D	Left lateral liver	4	6	0.91	0.05	Concordant
10	E	Lung-right lower lobe	5	6	1	0.56	Concordant
11	A	QAP sample	4	6	0.91	0.05	Concordant
11	B	Lung	4	6	0.91	0.05	Concordant
11	C	Lung	4	6	0.91	0.05	Concordant
11	D	Lung	5	6	1	0.56	Concordant
11	E	Lung	5	6	1	0.56	Concordant
12	A	QAP sample	4	6	0.91	0.05	Concordant
12	B	Melanoma	5	6	1	0.56	Concordant
12	C	Lung	5	6	1	0.56	Concordant
12	D	Colorectal	5	6	1	0.56	Concordant
12	E	Thyroid	5	6	1	0.56	Concordant
13	A	QAP sample	4	6	0.91	0.05	Concordant
13	B	Recto-sigmoid	4	6	0.91	0.05	Concordant
13	C	Colon	4	6	0.91	0.05	Concordant
13	D	Melanoma (left temple)	0	6	0.55	-1.97	Concordant
13	E	Lung	4	6	0.91	0.05	Concordant
14	A	QAP sample	4	6	0.91	0.05	Concordant
14	B	Brain	5	6	1	0.56	Concordant
14	C	Lung	3	6	0.82	-0.45	Concordant
14	D	Lung	4	6	0.91	0.05	Concordant
14	E	Colorectal	4	6	0.91	0.05	Concordant
15	A	QAP sample	4	4	0.73	-0.96	Concordant
15	B	FFPE Rib (Bone)	5	6	1	0.56	Concordant
15	C	FFPE Lung	5	6	1	0.56	Concordant
15	D	FFPE Lung	5	6	1	0.56	Concordant
15	E	FFPE Kidney	5	6	1	0.56	Concordant
16	A	QAP sample	4	6	0.91	0.05	Concordant
16	B	Lacrimal gland	5	6	1	0.56	Concordant
16	C	Mediastinal mass	5	6	1	0.56	Concordant
16	D	Skin	5	6	1	0.56	Concordant
16	E	Skin	5	6	1	0.56	Concordant

8.3 Circulating free DNA (cfDNA)

For the cfDNA program, collaborative data from Australian and Chinese participating laboratories were combined. This allowed for more accurate proficiency assessment of laboratory performance. Only the performance of the RCPAQAP enrolled laboratories are reported here.

For 2018, eight samples were distributed to seven laboratories for analysis. The reference testing samples contained a total of nine gene mutations in five genes at 18 different percentage allelic frequencies (Table 9). Importantly, the average laboratory consensus allelic frequency data closely matched that of the reference testing laboratory (Table 9 and Figure 7). For proficiency assessment, the data from seven Australian enrolled laboratories were combined with 83 laboratories participating in China. A total of 90 laboratories were therefore involved with this collaborative RCPAQAP/NCCL EQA program. Z-scores were derived from the consensus allelic frequency data and the performance of the Australian laboratories are provided in Table 10 (the discordant z-scores are highlighted red). Laboratory 1 did not provide any mutational allelic frequency data and were not assessed. Laboratories 3, 4 and 7 each reported DNA variant allelic frequencies that were higher than the consensus data and are reflected in discordant z-scores (Table 10). Laboratory 5 failed to detect the *EGFR* c.2310_2311ins variant that was within their platform's limit of detection (LoD) and were awarded discordance (Table 10). The LoD for each laboratory are provided in Table 11. Laboratories were not assessed for variants that were below the LoD for the testing platform used.

For 2019, five samples were distributed to seven laboratories for analysis with six laboratories returning results. The reference testing samples contained a total of six gene mutations in four genes at seven different percentage allelic frequencies (Table 12). The average laboratory consensus allelic frequency data closely matched that of the reference testing laboratory (Table 12 and Figure 8). For proficiency assessment, the data from the six Australian enrolled laboratories were combined with the 125 laboratories participating in China. A total of 131 laboratories were therefore involved with this collaborative RCPAQAP/NCCL EQA program. Z-scores were derived from all data and the performance of the Australian laboratories are provided in Table 13 (the discordant variants are highlighted red). Laboratory 1 has a LoD of 0.1% but failed to detect the *EGFR* variants tested for in Sample C (Table 13). However, the consensus allelic frequencies of 0.13% (for c.2573T>G) and 0.15% (for c.2235_2249del) are borderline for the LoD of the Laboratory 1 testing platform and this may help explain the negative finding. Laboratory 2 and Laboratory 4 each identified false variants with *EGFR* c.2369C>T being reported in Samples A and C (Laboratory 2), and TP53 c.553A>C

being reported in Samples A and B (Laboratory 4) (Table 14). The LoD for each laboratory are provided in Table 15. Six of the seven consensus allelic frequency variants are below 1% (Table 12). This value is primarily below the LoD of the platforms used by the Australian participating laboratories (Table 15). As such, Laboratories were not assessed for variants that were below the LoD for each testing platform used (Tables 13 and 15).

The RCPAQAP cfDNA program is important for laboratories performing non-invasive liquid biopsy testing of cancer material. Liquid biopsy analysis is rapidly growing owing to the non-invasive nature of the technique. The RCPAQAP reference testing material contained multiple DNA variants associated with different cancers which allows for a more challenging test for participating laboratories. It is important for laboratories to report the LoD of a testing platform to avoid being recorded as discordant for variants below the platform's LoD. This information also allows the RCPAQAP to identify areas for improvement for those variants that are miscalled or where the allelic frequency data are incorrect. For example, in 2018, Laboratories 3, 4 and 7 each reported higher than expected allelic frequency values (discordant variants in Table 10). A clinical evaluation of these cfDNA data would suggest that the patient's tumour is becoming resistant to chemotherapeutic treatment which is an incorrect finding. Such reported findings could have a detrimental impact for the ongoing clinical management of a patient. These data highlight the importance of participating in an EQA so that areas of error can be identified.

Table 9. Gene variants used for the 2018 EQA program. The reference and consensus allelic frequencies (AF) are similar. LRG represents the Locus Reference Genomic sequence for each gene.

| Sample	Gene	Expected Genotypes	Reference AF	Consensus AF
A	TP53	LRG_321t1:c.742C>G	10%	14.75%
	EGFR	LRG_304t1:c.2235_2249delGGAATTAAGAGAAGC	5%	7.37%
B	TP53	LRG_321t1:c.742C>G	5%	6.34%
	EGFR	LRG_304t1:c.2235_2249delGGAATTAAGAGAAGC	1%	0.69%
		LRG_304t1:c.2369C>T	0.5%	0.5%
C	TP53	LRG_321t1:c.742C>G	1%	0.83%
	EGFR	LRG_304t1:c.2235_2249delGGAATTAAGAGAAGC	0.1%	0.15%
		LRG_304t1:c.2369C>T	0.1%	0.29%
		LRG_304t1:c.2390G>C	0.25%	0.08%
D	EGFR	LRG_304t1:c.2235_2249delGGAATTAAGAGAAGC	2.5%	3.72%
E	KRAS	NM_004985.4:c.35G>A	0.5%	0.8%
F	-	No gene variants	0%	0%
	-	No gene variants	0%	0%
G	BRAF	LRG_299t1:c.1801A>G	0.05%	0%
	NRAS	LRG_92t1:c.181C>G	2.5%	4.29%
H	EGFR	LRG_304t1:c.2310_2311insGGT	1%	0.5%
	BRAF	LRG_299t1:c.1799T>A	1%	0.39%
	NRAS	LRG_92t1:c.181C>G	0.5%	0.54%
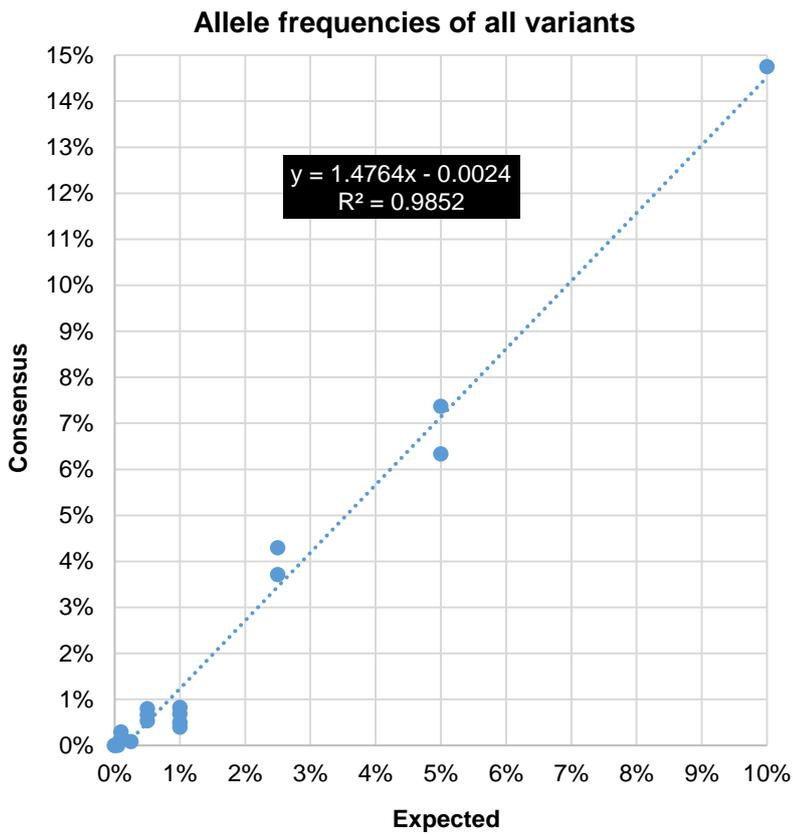
Figure 7. Correlation between all the expected reference allelic frequencies and the produced laboratory consensus allelic frequencies.

Table 10. Performance of Australian enrolled laboratories in the 2018 cfDNA program. AF (allelic frequency), N/A (not assessed), NT (not tested), LoD (limit of detection), FN (false negative), ND (not detected). Highlighted z-scores are discordant.

Sample	Gene/Variant	Consensus AF	Lab 1 AF	Z-score	Lab 2 AF	Z-score	Lab 3 AF	Z-score	Lab 4 AF	Z-score	Lab 5 AF	Z-score	Lab 6 AF	Z-score	Lab 7 AF	Z-score
A	TP53 c.742C>G	14.75%	N/A	N/A	NT	NT	NT	NT	NT	NT	11.0%	-0.79	NT	NT	NT	NT
	EGFR c.2235_2249del	7.37%	N/A	N/A	8.50%	0.33	17.39%	2.91	24%	4.83	6.20%	-0.34	10.52%	0.92	15%	2.22
B	TP53 c.742C>G	6.34%	N/A	N/A	NT	NT	NT	NT	NT	NT	3.2%	-1.28	NT	NT	NT	NT
	EGFR c.2235_2249del	0.69%	N/A	N/A	0.70%	0.01	13.64%	8.93	LoD	N/A	0.80%	0.08	1.05%	0.25	LoD	N/A
	EGFR c.2369C>T	0.5%	N/A	N/A	0.80%	0.13	9.58%	8.91	LoD	N/A	0.50%	-0.17	0.95%	0.28	LoD	N/A
C	TP53 c.742C>G	0.83%	N/A	N/A	NT	NT	NT	NT	NT	NT	0.8%	-0.09	NT	NT	NT	NT
	EGFR c.2235_2249del	0.15%	N/A	N/A	LoD	N/A	LoD	N/A	LoD	N/A	LoD	N/A	LoD	N/A	LoD	N/A
	EGFR c.2369C>T	0.29%	N/A	N/A	0.09%	-0.86	LoD	N/A	LoD	N/A	LoD	N/A	LoD	N/A	LoD	N/A
	EGFR c.2390G>C	0.08%	N/A	N/A	NT	NT	LoD	N/A	LoD	N/A	LoD	N/A	0.51%	1.57	LoD	N/A
D	EGFR c.2235_2249del	3.72%	N/A	N/A	4.80%	0.47	16.41%	5.47	15%	4.86	1.90%	-0.78	5.54%	0.78	9%	2.28
E	KRAS c.35G>A	0.8%	N/A	N/A	NT	NT	NT	NT	NT	NT	LoD	N/A	NT	NT	NT	NT
F	EGFR wildtype	0%	N/A	N/A	0%	0.00	0%	0.00	0%	0.00	0%	0.00	0%	0.00	0%	0.00
	BRAF wildtype	0%	N/A	N/A	0%	0.00	NT	NT	0%	0.00	0%	0.00	0%	0.00	0%	0.00
G	BRAF c.1801A>G	0%	N/A	N/A	NT	NT	NT	NT	LoD	N/A	LoD	N/A	LoD	N/A	LoD	N/A
	NRAS c.181C>G	4.29%	N/A	N/A	NT	NT	NT	NT	2%	-1.51	3.90%	-0.26	NT	NT	6%	2.43
H	EGFR c.2310_2311ins	0.5%	N/A	N/A	NT	NT	4.08%	6.68	NT	NT	FN	ND	NT	NT	LoD	N/A
	BRAF c.1799T>A	0.39%	N/A	N/A	0.40%	-0.43	NT	NT	LoD	N/A	0.40%	-0.43	0.56%	0.26	LoD	N/A
	NRAS c.181C>G	0.54%	N/A	N/A	NT	NT	NT	NT	LoD	N/A	LoD	N/A	NT	NT	LoD	N/A

Table 11. Platform limit of detection (LoD) for participating laboratories in the 2018 cfDNA program.

Lab	LoD (%)	Comments on submitted data
1	1	Allelic frequency not reported
2	0.1	Allelic frequency of 0.1% or less not detected
3	Not Reported	Allelic frequency of 1% or less not detected. Large over estimation of other variants
4	1	Allelic frequency of 1% or less not detected. Large over estimation of other variants
5	0.5	Allelic frequency of 0.5% or less and EGFR ins D770 (1%) not detected
6	0.5	Allelic frequency of 0.1% or less not detected.
7	5	Allelic frequency of 1% or less not detected. Over estimation of other variants

Table 12. Gene variants and expected allelic frequency (AF) for the 2019 EQA program. LRG represents the Locus Reference Genomic sequence for each gene.

| Sample	Gene	Expected Genotypes	Expected AF	Consensus AF
A	-	No gene variants	0%	0%
B	*TP53*	LRG_321t1:c.742C>G	0.1%	0.15%
	KRAS	NM_004985.4:c.35G>A	0.5%	0.21%
C	*EGFR*	LRG_304t1:c.2573T>G	0.1%	0.13%
	EGFR	LRG_304t1:c.2235_2249delGGAATTAAGAGAAGC	0.25%	0.15%
D	*TP53*	LRG_321t1:c.742C>G	5%	9.18%
	BRAF	LRG_299t1:c.1799T>A	0.25%	0.45%
E	*BRAF*	LRG_299t1:c.1801A>G	1%	0.76%
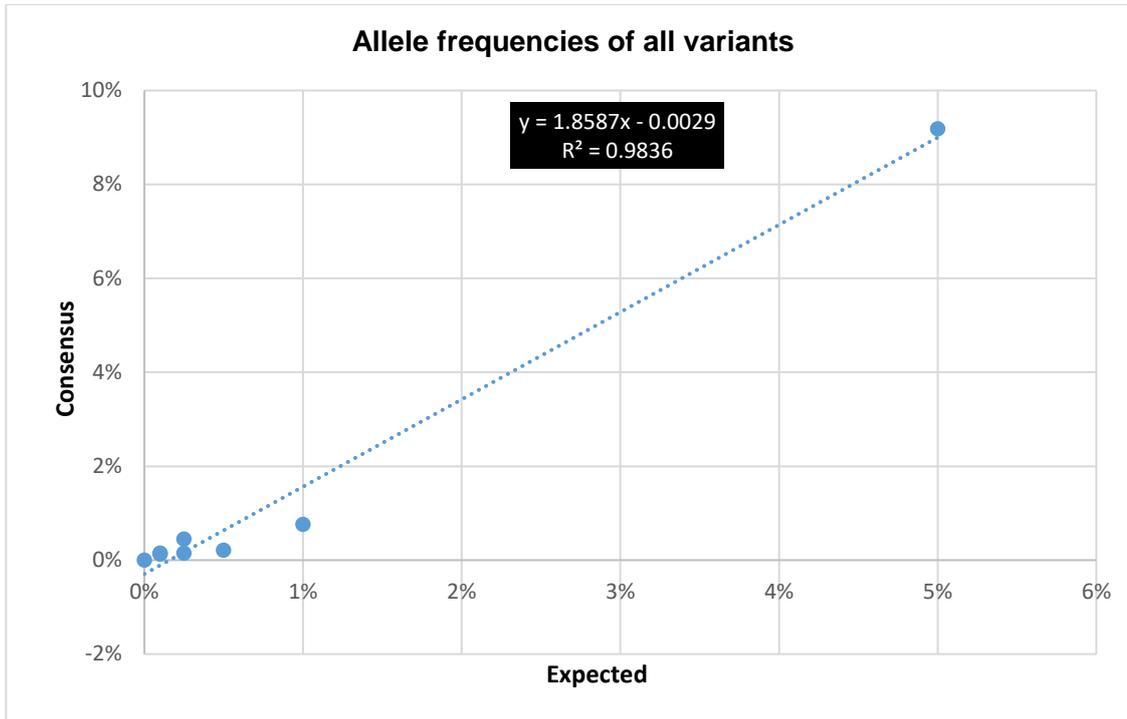
Figure 8. Correlation between all the expected reference allelic frequencies and the produced laboratory consensus allelic frequencies.

Table 13. Performance of Australian enrolled laboratories in the 2019 cfDNA program. AF (allelic frequency), N/A (not assessed), NT (not tested), LoD (limit of detection), FN (false negative), FP (false positive), ND (not detected). Highlighted are discordant.

Sample	Gene/Variant	Consensus AF	Lab 1 AF	Z-score	Lab 2 AF	Z-score	Lab 3 AF	Z-score	Lab 4 AF	Z-score	Lab 5 AF	Z-score	Lab 6 AF	Z-score
A	Wildtype no gene variants	0%	0%	0	FP	-	0%	0	FP	-	0%	0	0%	0
B	TP53 c.742C>G	0.15%	NT	N/A	NT	N/A	NT	N/A	LoD	N/A	NT	N/A	NT	N/A
	KRAS c.35G>A	0.21%	NT	N/A	LoD	N/A	NT	N/A	LoD	N/A	NT	N/A	LoD	N/A
C	EGFR c.2573T>G	0.13%	FN	ND	LoD	N/A	LoD	N/A	LoD	N/A	0.18	0.83	LoD	N/A
	EGFR c.2235_2249del	0.15%	FN	ND	LoD	N/A	NT	N/A	0.16%	0.14	LoD	N/A	LoD	N/A
D	TP53 c.742C>G	9.18%	NT	N/A	NT	N/A	NT	N/A	9.6	0.15	NT	N/A	NT	N/A
	BRAF c.1799T>A	0.45%	NT	N/A	LoD	N/A	NT	N/A	0.82	1.68	0.35	-0.45	LoD	N/A
E	BRAF c.1801A>G	0.76%	NT	N/A	LoD	N/A	NT	N/A	1.24	1.45	1.04	0.85	LoD	N/A

Table 14. False positive variants reported in the 2019 program.

Sample	Lab 2	Lab 4
A	EGFR c.2369C>T	TP53 c.553A>C
B		TP53 c.553A>C
C	EGFR c.2369C>T	

Table 15. Platform limit of detection (LoD) for participating laboratories in the 2019 cfDNA program.

Lab	LoD (%)	Comments on submitted data
1	0.1	Allelic frequency of 0.1% not detected (variants are borderline for the LoD)
2	1	Allelic frequency of 1% or less not detected
3	1	Allelic frequency of 1% or less not detected.
4	0.5	Allelic frequency of 0.5% or less not detected. However, EGFR del (in Sample C), and BRAF (in Sample D) detected at less than 1%
5	0.5	Allelic frequency of 0.5% or less not detected. However, (EGFR in Sample C) and BRAF (in Sample D) detected at less than 1%
6	5	Allelic frequency of 5% or less not detected.

8.4 Non-invasive prenatal testing (NIPT)

NIPT was performed in 2019. Five samples were distributed to five testing laboratories for analysis with all laboratories returning results. The reference testing samples contained different chromosomal trisomies with different foetal fractions (Table 16). For proficiency assessment, the laboratory data were compared directly against the laboratory consensus data (Table 17).

Next generation sequencing was used as the testing strategy by four of the five participating laboratories (laboratories 1, 3, 4, and 5). Laboratory 2 used the Harmony Prenatal Test (Roche) using the technique of digital analysis of selected regions prior to DNA sequencing. Laboratories 1 and 4 were concordant for all five samples tested (Table 17). Laboratory 2 were unable to generate any data. This indicates that the testing strategy used by laboratory 2 may be incompatible with the current reference testing material. As such, laboratory 2 were not assessed (Table 17). Laboratory 3 informed the RCPAQAP that they did not adhere to the sample storage instructions. This appeared to lead to sample degradation since three of the five samples tested could not be detected. These three samples were not assessed due to the samples being compromised (Table 17). Laboratory five reported that their internal quality control of sample NIPT 19E failed and as such did not proceed with analysis of the DNA. Laboratory 5 were therefore not assessed for this sample (Table 17).

The RCPAQAP NIPT program is important for offering proficiency testing to laboratories performing non-invasive liquid biopsy testing of DNA material isolated from pregnant mothers. Liquid biopsy assays relating to NIPT are rapidly growing owing to the non-invasive nature of the technique. The RCPAQAP therefore need to develop new programs that reflect the expectation and needs of clinically testing laboratories and to keep pace with current testing strategies. As such, the reference testing material used in this program was designed to test for foetal chromosome abnormalities in the three common foetal genetic conditions of Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), and Down syndrome (trisomy 21).

Table 16. NIPT expected data and the laboratory consensus data. FF represents the percent of the foetal fraction in each sample. The laboratory consensus FF values are based on fragment length instead of overall coverage statistics. The expected (reference) FF values represent the coverage statistics.

Sample	Expected Result	Expected FF (%)	Consensus Result	Consensus FF (%)
NIPT 19A	Trisomy 18	20	Trisomy 18	7
NIPT 19B	Negative	10	Negative	5
NIPT 19C	Trisomy 18	15	Trisomy 18	7
NIPT 19D	Trisomy 21	10	Trisomy 21	5
NIPT 19E	Trisomy 13	10	Trisomy 13	6

Table 17. NIPT data from each participating laboratory. Assessments were based on the consensus data. ND (not detected).

Sample	Lab 1	FF (%)	Assessment	Lab 2	FF (%)	Assessment	Lab 3	FF (%)	Assessment	Lab 4	FF (%)	Assessment	Lab 5	FF (%)	Assessment
NIPT 19A	T18	5	Concordant	ND	ND	Not assessed	ND	ND	Not assessed	T18	6	Concordant	T18	8.9	Concordant
NIPT 19B	Neg	6	Concordant	ND	ND	Not assessed	ND	ND	Not assessed	Neg	5	Concordant	Neg	4	Concordant
NIPT 19C	T18	4	Concordant	ND	ND	Not assessed	T18	10	Concordant	T18	6	Concordant	T18	6.9	Concordant
NIPT 19D	T21	2	Concordant	ND	ND	Not assessed	T21	10	Concordant	T21	3	Concordant	T21	6.5	Concordant
NIPT 19E	T13	5	Concordant	ND	ND	Not assessed	ND	ND	Not assessed	T13	7	Concordant	ND	ND	Not assessed

8.5 Leukaemia chromosome 17p deletion

All laboratories used the technique of fluorescence in situ hybridization (FISH) to detect chromosome 17p deletions in the reference testing samples. Laboratories were requested to analyse each slide and report on the percentage of cells containing the deletion.

For 2018, three reference case samples were distributed to 13 laboratories for analysis. The RCPAQAP reference testing laboratory identified a chromosome 17p deletion at 80% for Case 1, 0% for Case 2, and 36% for Case 3. However, for z-score analysis, the average consensus data produced by all laboratories were used for a more accurate proficiency assessment. The consensus deletion data for Case 1 was reported at 74%, the consensus deletion data for Case was reported at 0%, and the consensus deletion data for Case 3 was reported at 35% (Table 18). These data closely match the reference laboratory data (Table 18). All testing laboratories were concordant for each case except for Laboratory 2 and Laboratory 10. Laboratory 2 were discordant for Case 1 (reporting a low deletion level) and Case 3 (failure to detect a deletion), whilst Laboratory 10 were discordant for Case 3 (reporting a low deletion level) (Table 18). The probe detection sets used by each laboratory are provided in Table 19.

For 2019, three reference case samples were distributed to 13 laboratories for analysis. The RCPAQAP reference testing laboratory identified a chromosome 17p deletion at 18% for Case 1, 0% for Case 2, and 75% for Case 3. The consensus deletion data for Case 1 was reported at 17%, the consensus deletion data for Case 2 was reported at 0%, and the consensus deletion data for Case 3 was reported at 77% (Table 20). These data closely match the reference laboratory data (Table 20). Laboratories 2, 3 and 13 were discordant for Case 1 (failing to detect a deletion). Laboratory 7 was discordant for Case 3 (reporting a higher level of deletion containing cells) (Table 20). The probe detection sets used by each laboratory are provided in Table 20.

The RCPAQAP chromosome 17p deletion program is important for laboratories performing large deletion analysis of leukaemia cancer material. The RCPAQAP reference testing material contained deletions at differing percentage levels allowing for a more challenging test for participating laboratories. It is important for laboratories to identify and report on the correct percentage of cells containing a deletion. This information allows the RCPAQAP to identify if a laboratory FISH technique needs to be improved. For example, Laboratory 2 in 2018, failed to detect the deletion in Case 3 (Table 18), and Laboratories 2, 3 and 13 in 2019, failed to detect the deletion in Case 1 (Table

20). Clinical evaluations of these results would suggest that the patient's tumour is free of any disease-causing deletion mutation, which is an incorrect finding. Such reported findings could have a detrimental impact for the ongoing clinical management of a patient. In addition, the underestimation of tumour percentage (Laboratory 2 for Case 1 and Laboratory 10 for Case 3 in 2018) or the over estimation of tumour percentage (Laboratory 7 for Case 3 in 2019) indicate that the FISH technique used by these laboratories needs to be further optimised. These data highlight the importance of participating in an EQA program so that sources of error can be identified.

Table 18. Assessment of laboratories performing FISH analysis for chromosome 17p deletions in 2018. The average consensus data for detecting the deletion in Case 1 was 74%, for Case 2 was 0%, and for Case 3 was 35%. Discordant values are highlighted red.

Laboratory	Case 1 (Ave 74%)	Z-score	Case 2 (Ave 0%)	Z-score	Case 3 (Ave 35%)	Z-score
Reference	80%	0.85	0%	0	36	0.16
Lab 1	67%	-0.97	0%	0	41	0.93
Lab 2	58%	-2.24	0%	0	0	-5.33
Lab 3	77%	0.43	0%	0	27	-1.21
Lab 4	72%	-0.27	0%	0	34	-0.14
Lab 5	78%	0.57	0%	0	34	-0.14
Lab 6	75%	0.15	0%	0	40	0.78
Lab 7	84%	1.41	0%	0	39	0.62
Lab 8	79%	0.71	0%	0	46	1.69
Lab 9	67%	-0.97	0%	0	32	-0.45
Lab 10	67%	-0.97	0%	0	21	-2.13
Lab 11	74%	0.01	0%	0	33	-0.29
Lab 12	75%	0.15	0%	0	31	-0.60
Lab 13	82%	1.13	0%	0	40	0.78

Table 19. Deletion detecting FISH probe sets used by each participating laboratory in the 2018 chromosome 17p deletion program.

Laboratory	FISH probe set
Lab 1	Cytocell TP53/D17Z1 Probe Set
Lab 2	Cytocell P53 (TP53) deletion Probe Set
Lab 3	MetaSystems XL ATM/TP53 Deletion Probe Set
Lab 4	MetaSystems XL ATM/TP53 Probe Set
Lab 5	Cytocell Aquarius P53/ATM Probe Set
Lab 6	Cytocell TP53 deletion Probe Set
Lab 7	MetaSystems CLL Panel - ATM and TP53 Probe Set
Lab 8	MetaSystems XL ATM/TP53 Probe Set
Lab 9	Abbott Vysis CLL Panel ATM/TP53 Probe Set
Lab 10	Abbott Vysis LSI TP53/ATM Probe Set
Lab 11	Cytocell P53 Deletion Probe Set
Lab 12	Cytocell P53(TP53)/ATM Probe Set
Lab 13	Abbott Vysis CLL Panel ATM/TP53 Probe Set

Table 20. Assessment of laboratories performing FISH analysis for chromosome 17p deletions in 2019. The average consensus data for detecting the deletion was 17% for Case 1, 0% for Case 2, and 77% for Case 3. Discordant values are highlighted red.

Laboratory	Case 1 (Ave 17%)	Z-score	Case 2 (Ave 0%)	Z-score	Case 3 (Ave 77%)	Z-score
Reference	18	0.20	0%	0	75	-0.40
Lab 1	15	-0.40	0%	0	79	0.29
Lab 2	0	-3.99	0%	0	85	1.22
Lab 3	0	-3.99	0%	0	81	0.60
Lab 4	12	-1.12	0%	0	82	0.76
Lab 5	19	0.56	0%	0	69	-1.26
Lab 6	21	1.03	0%	0	75	-0.33
Lab 7	17	0.08	0%	0	92	2.31
Lab 8	12	-1.12	0%	0	76	-0.17
Lab 9	10	-1.60	0%	0	72	-0.79
Lab 10	16	-0.16	0%	0	71	-0.95
Lab 11	23	1.51	0%	0	72	-0.79
Lab 12	21	1.03	0%	0	71	-0.95
Lab 13	0	-3.99	0%	0	80	0.45

Table 21. Deletion detecting FISH probe sets used by each participating laboratory in the 2019 chromosome 17p deletion program.

Laboratory	FISH probe set
Lab 1	MetaSystems TP53 Probe Set
Lab 2	Cytocell TP53 Deletion Probe Set
Lab 3	ZytoVision TP53 Deletion Probe Set
Lab 4	MetaSystems ATM / TP53 Probe Set
Lab 5	Cytocell TP53 Deletion Probe Set
Lab 6	MetaSystems ATM / TP53 Probe Set
Lab 7	MetaSystems ATM / TP53 Probe Set
Lab 8	Abbott Vysis TP53 Deletion Probe Set
Lab 9	MetaSystems ATM / TP53 Probe Set
Lab 10	MetaSystems ATM / TP53 Probe Set
Lab 11	MetaSystems ATM / TP53 Probe Set
Lab 12	Cytocell TP53 Deletion Probe Set
Lab 13	Abbott Vysis TP53 Deletion Probe Set

8.6 Leukaemia isocitrate dehydrogenase 1 and 2 gene testing

All enrolled clinical testing laboratories were requested to identify *IDH1* and *IDH2* gene variants in acute myeloid leukaemia associated RCPAQAP reference testing samples and to report on the DNA gene variants detected.

For 2017, three reference samples were distributed to 12 laboratories for analysis. The RCPAQAP reference testing laboratory identified an *IDH1* c.395G>A variant in Sample A, no gene variants were detected in Sample B, and an *IDH2* c.515G>A variant in Sample C. Concordance was awarded for correct identification of each variant for each sample. For Sample A, 92% (11/12) of laboratories were concordant, and for Samples B and C, 100% (12/12) of laboratories were concordant (Table 22). Laboratory 7 were discordant for failing to detect and report on the *IDH1* c.395G>A gene variant in Sample A (Table 22).

For 2018, three reference samples were distributed to 15 laboratories for analysis. The RCPAQAP reference testing laboratory identified an *IDH2* c.419G>A variant in Sample A, an *IDH1* c.395G>A variant in Sample B, and an *IDH2* c.515G>A variant in Sample C. Concordance was awarded for

correct identification of each variant for each sample. For Samples A and B, 93% (14/15) of laboratories were concordant, and for Sample C, 100% (15/15) of laboratories were concordant (Table 23). Laboratory 4 were discordant for failing to detect and report on the *IDH2* c.419G>A gene variant in Sample A, and Laboratory 8 were discordant for failing to detect and report on the *IDH1* c.395G>A gene variant in Sample B (Table 23).

For 2019, three reference samples were distributed to 17 laboratories for analysis with 16 laboratories returning results. The RCPAQAP reference testing laboratory identified no gene variants in Sample A, an *IDH2* c.419G>A variant in Sample B, and no gene variants in Sample C. Concordance was awarded for correct identification of each variant for each sample. For Samples A, B, and C, all laboratories (16/16) were concordant (Table 24).

The RCPAQAP leukaemia *IDH1*/*IDH2* gene testing program is designed for laboratories performing acute myeloid leukaemia DNA mutation analysis on the two key target genes of *IDH1* and *IDH2*. The RCPAQAP reference testing material consisted of different variants in the two genes. It is critical for laboratories to identify and report on all variants detected. This information allows the RCPAQAP to identify underperforming laboratories and to advise on improvement. For example, in 2017, Laboratory 7 failed to detect the *IDH1* gene variant in Sample A (Table 22). Clinical evaluation of this result would suggest that the patient's DNA is free of any disease-associated variant which is an incorrect finding. Such reported findings could have a detrimental impact for the ongoing clinical management of a patient. These data highlight the importance of participating in an EQA so that sources of error can be identified.

Table 22. Assessment of laboratories performing *IDH1* and *IDH2* gene variant testing in the 2017 EQA program.

Laboratory	Sample A Variant	Assessment	Sample B Variant	Assessment	Sample C Variant	Assessment
Reference	IDH1 c.395G>A	-	No variants detected	-	IDH2 c.515G>A	-
Lab 1	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 2	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 3	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 4	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 5	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 6	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 7	Not detected	Discordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 8	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 9	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 10	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 11	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant
Lab 12	IDH1 c.395G>A	Concordant	No variants detected	Concordant	IDH2 c.515G>A	Concordant

Table 23. Assessment of laboratories performing *IDH1* and *IDH2* gene variant testing in the 2018 EQA program.

Laboratory	Sample A Variant	Assessment	Sample B Variant	Assessment	Sample C Variant	Assessment
Reference	IDH2 c.419G>A	-	IDH1 c.395G>A	-	IDH2 c.515G>A	-
Lab 1	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 2	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 3	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 4	Not Detected	Discordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 5	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 6	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 7	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 8	IDH2 c.419G>A	Concordant	Not Detected	Discordant	IDH2 c.515G>A	Concordant
Lab 9	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 10	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 11	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 12	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 13	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 14	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant
Lab 15	IDH2 c.419G>A	Concordant	IDH1 c.395G>A	Concordant	IDH2 c.515G>A	Concordant

Table 24. Assessment of laboratories performing *IDH1* and *IDH2* gene variant testing in the 2019 EQA program.

Laboratory	Sample A Variant	Assessment	Sample B Variant	Assessment	Sample C Variant	Assessment
Reference	No variants detected	-	IDH2 c.419G>A	-	No variants detected	-
Lab 1	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 2	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 3	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 4	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 5	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 6	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 7	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 8	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 9	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 10	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 11	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 12	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 13	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 14	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 15	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant
Lab 16	No variants detected	Concordant	IDH2 c.419G>A	Concordant	No variants detected	Concordant

8.7 Leukaemia next generation sequencing

Laboratories were requested to perform next generation sequencing (NGS) to identify multiple gene variants associated with acute myeloid leukaemia (AML). The RCPAQAP reference testing samples were confirmed to contain DNA variants in different genes. However, for proficiency testing, laboratory consensus data were derived for all tested gene variants and laboratories were assessed for the detection or non-detection of each consensus variant. A consensus gene variant was accepted if at least three testing laboratories detected and reported on the same variant. Concordance was awarded for gene variant detection that matched the consensus data.

For 2018, 10 reference DNA samples were distributed to eight clinical testing laboratories for NGS DNA analysis. A total of 15 genes and 27 DNA variants were identified (Table 25). Not all laboratories tested for all variants. Laboratories that did not test for gene variants were not penalised. All laboratories were concordant for detecting their specific gene variant of interest (Table 26).

For 2019, two reference DNA samples were distributed to nine clinical testing laboratories for analysis. A total of eight genes and were identified as containing consensus variants (Table 27). Not all laboratories tested for all variants. All laboratories were concordant for detecting their specific gene variant of interest (Table 28)

NGS testing is growing in clinical diagnostics but the identification of clinically relevant genes is challenging. The RCPAQAP leukaemia next generation sequencing program was therefore designed to initially identify a core set of consensus genes that are of clinical interest. The 2018 data identified 15 genes that can potentially serve as a core set of testing genes for the clinical diagnosis of AML (Table 25). However, for 2019, the number of consensus genes tested reduced to eight. This program is therefore adaptable, and can proficiency test any number of genes that are considered clinically relevant. The data also suggest that the NGS technology is sensitive for AML diagnostic testing.

Table 25. Consensus genes and DNA variants identified in the 2018 AML program.

Gene	Consensus DNA variant and predicted protein impact
BCOR	c.3052-2A>G
DNMT3A	c.1502A>G; p.Asn501Ser
	c.1628G>C; p.Gly543Ala
	c.2371delG; p.Ala791Profs*11
	c.1648G>A; p.Gly550Arg
	c.2645G>A; p.Arg882His
FLT3	Internal tandem duplication detected
	c.2503G>T; p.Asp835Tyr
IDH1	c.395G>A; p.Arg132His
IDH2	c.419G>A; p.Arg140Gln
KIT	c.2447A>T; p.Asp816Val
KRAS	c.35G>A; p.Gly12Asp
	c.38G>A; p.Gly13Asp
NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12
NRAS	c.182A>G; p.Gln61Arg
	c.35G>A; p.Gly12Asp
	c.38G>A; p.Gly13Asp
PTPN11	c.181G>C; p.Asp61His
	c.1504T>G; p.Ser502Ala
RAD21	c.100G>T; p.Glu34*
	c.58G>C; p.Ala20Pro
SF3B1	c.2098A>G; p.Lys700Glu
SRSF2	c.284C>G; p.Pro95Arg
TET2	c.2911G>T; p.Glu971*
	c.5618T>C; p.Ile1873Thr
WT1	c.1151delA; p.Glu384Glyfs*65
	*c.1107del; Arg370fs

Table 26. Assessment of laboratories performing next generation sequencing for the 2018 AML program.

Sample	Consensus genes	Mutation	Laboratory Detection	Concordance	Variant Not Tested
1	DNMT3A	c.1502A>G; p.Asn501Ser	1,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	FLT3	Internal tandem duplication detected	All labs	100% (8/8)	-
2	KRAS	c.35G>A; p.Gly12Asp	2,3,4,5,7,8	75% (6/8)	25% (2/8)
	KRAS	c.38G>A; p.Gly13Asp	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	NRAS	c.182A>G; p.Gln61Arg	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	SRSF2	c.284C>G; p.Pro95Arg	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	TET2	c.2911G>T; p.Glu971*	2,3,4,5,7,8	75% (6/8)	25% (2/8)
3	FLT3	Internal tandem duplication detected	1,2,4,5,6,7	75% (6/8)	25% (2/8)
	NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12	All labs	100% (8/8)	-
	WT1	c.1151delA; p.Glu384Glyfs*65	2,3,4,5,7,8	75% (6/8)	25% (2/8)
4	DNMT3A	c.1628G>C; p.Gly543Ala	1,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	FLT3	Internal tandem duplication detected	1,2,4,5,6,7	75% (6/8)	25% (2/8)
	NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12	All labs	100% (8/8)	-
	NRAS	c.35G>A; p.Gly12Asp	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	RAD21	c.100G>T; p.Glu34*	3,6,8	37.5% (3/8)	62.5% (5/8)
	TET2	c.5618T>C; p.Ile1873Thr	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
5	DNMT3A	c.2371delG; p.Ala791Profs*11	1,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	IDH1	c.395G>A; p.Arg132His	All labs	100% (8/8)	-
	NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12	All labs	100% (8/8)	-
	SF3B1	c.2098A>G; p.Lys700Glu	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
6	DNMT3A	c.1648G>A; p.Gly550Arg	1,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	NPM1	c.863_864insCATG; p.Trp288Cysfs*12	All labs	100% (8/8)	-
	PTPN11	c.181G>C; p.Asp61His	3,5,6,7,8	62.5% (5/8)	37.5% (3/8)
7	BCOR	c.3052-2A>G	3,6,7	37.5% (3/8)	62.5% (5/8)
	PTPN11	c.1504T>G; p.Ser502Ala	3,5,6,7,8	62.5% (5/8)	37.5% (3/8)
	WT1	*c.1107del; Arg370fs	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
8	IDH1	c.395G>A; p.Arg132His	All labs	100% (8/8)	-
	NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12	All labs	100% (8/8)	-
9	KIT	c.2447A>T; p.Asp816Val	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
	NRAS	c.38G>A; p.Gly13Asp	2,3,4,5,6,7,8	87.5% (7/8)	12.5% (1/8)
10	DNMT3A	c.2645G>A; p.Arg882His	All labs	100% (8/8)	-
	FLT3	Internal tandem duplication detected	1,2,4,6,7	62.5% (5/8)	37.5% (3/8)
	FLT3	c.2503G>T; p.Asp835Tyr	All labs	100% (8/8)	-
	IDH2	c.419G>A; p.Arg140Gln	All labs	100% (8/8)	-
	NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12	All labs	100% (8/8)	-
	RAD21	c.58G>C; p.Ala20Pro	3,6,8	37.5% (3/8)	62.5% (5/8)

Table 27. Consensus genes and DNA variants identified in the 2019 AML program.

Gene	Consensus DNA variant and predicted protein impact
DNMT3A	c.2644C>T; p.Arg882Cys
FLT3	Internal tandem duplication detected
IDH1	c.395G>A; p.Arg132His
NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12
NRAS	c.35G>A; p.Gly12Asp
PTPN11	c.214G>A; p.Ala72Thr
TET2	c.3866G>A; p.Cys1289Tyr
WT1	Structural (deletion/insertion) variant at position c.1149

Table 28. Assessment of laboratories performing next generation sequencing for the 2019 AML program.

Sample	Consensus genes	Mutation	Laboratory Detection	Concordance	Variant Not Tested
1	*WT1*	Structural variant at c.1149	1,3,4,8,9	55.6% (5/9)	44.4% (4/9)
	FLT3	Internal tandem duplication detected	1,2,3,4,5,6,8,9	89% (8/9)	11% (1/9)
2	*IDH1*	c.395G>A; p.Arg132His	All laboratories	100% (9/9)	-
	NPM1	c.860_863dupTCTG; p.Trp288Cysfs*12	1,2,3,4,5,6,8,9	89% (8/9)	11% (1/9)
	NRAS	c.35G>A; p.Gly12Asp	1,2,3,4,5,6,8,9	89% (8/9)	11% (1/9)
	PTPN11	c.214G>A; p.Ala72Thr	1,2,6	33% (3/9)	66% (6/9)
	TET2	c.3866G>A; p.Cys1289Tyr	1,3,4,5,6,7,8,9	89% (8/9)	11% (1/9)
	DNMT3A	c.2644C>T; p.Arg882Cys	All laboratories	100% (9/9)	-

9. Project Findings

9.1 Objectives and results

The 2017 – 2019 QUPP Commonwealth funding allowed the RCPAQAP to develop seven novel diagnostically important EQA programs. The seven EQA programs required large numbers of samples for assessing the proficiency of each clinical testing laboratory. These programs were designed to be challenging to fully test each participating laboratory. In total, 832 individual samples/assays were analysed with the majority being for the whole genomic DNA extraction program (35%, 295/832). The remaining samples/assays were analysed for leukaemia *IDH1* and *IDH2* gene variant testing (16%, 132/832), FFPE DNA extraction, (15%, 123/832), leukaemia next generation sequencing, (12%, 98/832), cfDNA testing, (10%, 81/832), leukaemia chromosome 17p deletion, (9%, 78/832), and NIPT (3%, 25/832). The data from each of the three key aims/objectives (as outlined on page 9) were as follows:

9.1.1 Aim 1 – DNA Extraction

To determine the ability of laboratories to extract total DNA from patient blood and clinical tissue.

Overall DNA extraction data

A total of 418 DNA extracts (295 from the genomic DNA extraction program and 123 from the FFPE DNA extraction program) were assessed for diagnostic testing quality. Assessments were based on two RCPAQAP in-house testing techniques comprising of (i) DNA Tapestation analysis for the initial identification of overall DNA quality, and (ii) using classical DNA polymerase chain reaction (PCR) analysis to determine whether the DNA can be amplified from different DNA regions. However, if the DNA could be amplified, then this was deemed as the most important indication for overall DNA quality and samples were assessed as concordant. Essentially, most of the received DNA extracts were at a quality amenable for clinical diagnostic testing. Very few samples were assessed as being discordant (see below). These data indicate that, although many different samples were extracted using a variety of DNA extraction techniques, they are nonetheless functioning at a clinical acceptable level for DNA disease diagnostics. This was an important finding since it confirmed that different laboratories using different techniques/kits/platforms (from different commercial companies) are operating at equivalent and acceptable levels for DNA extraction.

9.1.2 Aim 2 – Circulating free DNA

To assess laboratories performing novel diagnostics for the testing of circulating fragmented DNA found in cancer patients (for identifying tumour resistance) and in maternal blood (for identifying abnormal foetal DNA).

Overall circulating free DNA data

In combination, a total of 106 circulating free DNA assays (81 from the cancer program, and 25 from the NIPT program) were assessed for the mutational testing of cfDNA variants. Assessments were based on the correct identification of each variant as reported from the reference testing facility. Overall, most laboratories were concordant for identifying the correct DNA mutation using their standard operating procedure. However, some cfDNA mutations were below the limits of detection sensitivity for certain testing platforms used. Laboratories failing to identify these variants were not penalised due to this limitation. These data provide evidence that cfDNA testing facilities are capable of diagnosing cfDNA variants at acceptable clinical levels. However, the limits of detection of some testing platforms needs to be addressed to increase sensitivity for the future testing of this new diagnostic technique.

9.1.3 Aim 3 – Leukaemia

To assess laboratories performing different leukaemia diagnostics for either detecting chromosome 17p genetic rearrangements in chronic lymphocytic leukaemia, for identifying isocitrate dehydrogenase 1 and 2 (*IDH1, IDH2*) gene mutations in acute myeloid leukaemia, or for performing next generation sequencing on DNA isolated from acute myeloid leukaemia patients.

Overall leukaemia data

A total of 308 DNA assays (132 from the *IDH1* and *IDH2* program, 98 from the next generation sequencing program, and 78 from the chromosome 17p deletion program) were assessed for mutational testing of leukaemia associated DNA variants. Assessments were based on the correct identification of each variant as reported from each of the reference testing facilities. Overall, most laboratories were concordant for identifying the correct DNA mutation using their standard operating procedure. Very few assays were discordant (see below). These data indicate that current leukaemic diagnostic testing facilities are operating at acceptable clinical testing levels.

9.2 Identification of discordant samples

Discordant samples were identified in the EQA programs of whole genomic and FFPE DNA extraction, cfDNA, chromosome 17p deletion, and in *IDH1* and *IDH2* gene testing. A summary of overall levels of discordance for each EQA program are provided in Table 29.

Table 29. Identification of all discordant samples in each EQA program.

Program Designation	Specific EQA Program	Total Assays Assessed	Total Discordant samples
DNA Extraction	Whole genomic DNA extraction	295	3 (1%)
	FFPE DNA extraction	123	4 (3%)
Circulating free DNA	Cancer	81	13 (16%)
	NIPT	25	0 (0%)
Leukaemia	Chromosome 17p deletion	78	7 (9%)
	IDH1 and IDH2	132	3 (2%)
	Next generation sequencing	98	0 (0%)
TOTAL		**832**	**30 (4%)**

From a total of 832 assays assessed by the RCPAQAP across seven different EQA programs, only 30 (4%) were found to be discordant. These data therefore indicate that diagnostic testing laboratories are performing at high acceptable levels for extracting DNA, and for clinical genetic disease diagnostic characterisation. However, the identification of discordance, although low, is still nonetheless a key finding since this can signify problems associated with a diagnostic testing strategy. Identifying sources of error is therefore a primary role for the EQA provider so that testing issues can be highlighted, and where appropriate, improvements can be recommended to the participating laboratory. Any recommended improvements or worsening of performance can be monitored over time and relayed back to the appropriate laboratory for their own internal performance monitoring. The data generated from these EQA programs demonstrate the importance of laboratories enrolling on external proficiency testing programs so that comparisons with their peers can be made and improvements made where necessary.

This QUPP funded study therefore achieved its aim in developing new diagnostic testing EQA programs and in three key areas of diagnostics (as outlined in the aims above).

10. National Association of Testing Authorities (NATA) Accreditation

The Commonwealth QUPP funding has allowed the RCPAQAP the successfully apply to NATA for accreditation of the cfDNA testing program and the leukaemia *IDH1* and *IDH2* gene testing program. The leukaemia chromosome 17p deletion program has been accredited previously for the ASDG. These programs are compliance with ISO/IEC 17043:2010 (Accreditation Number: 14863) and will be offered by the RCPAQAP as a full EQA proficiency testing programs. The remining DNA extraction, FFPE DNA extraction, NIPT, and next generation sequencing EQA programs will be assessed for accreditation in 2020/21 once we have obtained sufficient data to satisfy the NATA requirements.

11. Problems Encountered

Two key problems were encountered in this study and these relate to the cfDNA and NIPT programs. The first issue was in receiving the reference testing material in an appropriate time-frame from our collaborator in Beijing, China. This issue relates to our collaborator having to apply and obtain a license from the Chinese authority for shipping materials to Australia. This caused delays in distributing material to participating laboratories. The second issue relates to the cfDNA material being different in 2019 from what was ordered and expected. The consequence of this was that participating laboratories were required to perform extra steps for the 2019 cfDNA analysis in comparison to the 2018 cfDNA analysis and this impacted on the results generated. However, these encountered issues were precisely what this project was aimed at discovering given that these are new areas for human diagnostics and key issues need to be identified and resolved.

12. Future diagnostics

(*Do the projects complement other similar services, activities and resources?*)

The data from this project directly complements two previously funded QUPP projects. In 2017, Commonwealth QUPP funding (Agreement id: 4-4Z3AWAN) was awarded for developing a mass spectrometry EQA proficiency testing program to identify human disease-associated biomarker proteins. The identification of protein biomarkers (that are potentially circulating in the blood) are therefore directly complementary to the cfDNA and NIPT programs. For example, future protein diagnostics may be used with DNA analyses to identify circulating levels of both proteins and DNA that are associated with different human diseases (Cohen et al 2017). Such analyses would be for more informative for the clinician and may help identify disease development early so that appropriate clinical management can be initiated.

In 2019, Commonwealth QUPP funding was awarded (Agreement id: 4-ALD5HVH) for developing a cardiovascular disease EQA program to proficiency test laboratories performing DNA mutational analysis of multiple gene variants. This program complements the DNA extraction program since isolated DNA for clinical testing needs to be of high quality for mutational analysis as laboratory reporting of incorrect results (due to poor quality DNA) can have severe consequences for the clinical management of patients.

The development of new EQA programs also complements our existing programs. For example, we have molecular quality assurance programs in the areas of inherited haematological disorders (i.e., haemochromatosis, thrombosis, and thalassaemia) and in the qualitative and quantitative detection of nucleic acids from infectious micro-organisms including viruses and bacteria. It is also critical to develop new programs that are of immediate clinical demand, as is the case for the cfDNA program, which was designed to reflect the diagnostic need in the new era of non-invasive cancer diagnostics.

13. Long term outcomes

(*Do the projects provide value for money including: Economy, Efficiency and Effectiveness - delivering a better service or getting a better return for the same amount of expense, time or effort?*)

The rapid growth in molecular testing is now allowing the detection of multiple DNA sequence variations associated with either inherited (genetic) or acquired (cancer) diseases. Diagnostic testing laboratories do however require good quality DNA so that confidence in the molecular results can be attained. In addition, the new technology of next generation sequencing (NGS) can now detect DNA variation in the entire human genome in a single assay. All other genetic techniques/technologies are restricted to analyses of very small single gene regions only (i.e., can largely only detect a single DNA variation at a time, as is the case for the *IDH1* and *IDH2* EQA program). Nonetheless, the NGS technology has not yet been fully adopted for clinical use due to cost and the complex nature of the substantial data produced. As such, EQA programs for single gene analysis are still very important. Although the cost of NGS is high, it is nevertheless reducing, which is allowing laboratories to perform a more intensive interrogation of the disease genome. The complex data is also becoming easier to characterise due to new developments of sequencing software programs for data analysis. These new developments however require new EQA programs. There is therefore a constant need for EQA providers to keep pace with the advancement and clinical adoption of new technology and analysis systems so that proficiency testing can be offered to ensure that diagnostic testing laboratories are performing at clinically acceptable levels. Therefore, the EQA programs developed from this Commonwealth funding were devised to reflect the need and demands of diagnostic testing laboratories and to keep pace with current and emerging technology.

13.1 Economy

Proficiency assessments of existing and new technologies allows laboratories to benchmark their overall performance, and to lower their assay development costs by reducing the time required to optimise a key diagnostic test. The availability of an EQA for assessments of clinical diagnostics will ensure that all clinicians are working to the same standard for diagnoses. The cost of healthcare and treatment plans can therefore be significantly reduced and implemented earlier. This will increase patient care by allowing earlier diagnoses which will benefit patient management strategies and thus increase cost effectiveness.

13.2 Efficiency

Diagnostic applications incorporating new strategies for isolating patient diagnosable material, and in using emerging technology (i.e., NGS) is rapidly growing. As such, new understandings of disease processes combined with new technology allows for increased levels of diagnostic sensitivity, high throughput capacity, reduced patient invasiveness and increased efficiency in terms of time and cost. Diagnostic laboratories now have the capacity to sequence the whole genome in a single assay. This increases the detection rate of DNA variation in a much shorter time-period with high accuracy and sensitivity. EQA proficiency testing allows for aberrant testing issues to be identified and solved so that improvements in a testing technology can be continually recommended.

13.3 Effectiveness

The development of new EQA programs will additionally allow the establishment of cross functional RCPAQAP inter-discipline collaboration. For example, the RCPAQAP Anatomical Pathology, Biosecurity, Chemical Pathology, Cytopathology, Haematology, Immunology, Serology and Microbiology disciplines will benefit from new understandings of disease-associated DNA biomarkers since new disease processes can be linked in to other disciplines for improved proficiency testing and clinical interpretation of data. This combined discipline approach will result in the production of highly-developed quality assurance programs for future efficiency testing and cost-effective analyses across the RCPAQAP.

14. Project Sustainability

Given that molecular genetic disease diagnostics is growing, it is anticipated that multiple laboratories will want to enrol for participation in new EQA programs that are of clinical relevance. As such, an increase in laboratory participation will make these EQA programs sustainable in the future. Importantly, the RCPAQAP collaboration with the European Molecular genetics Quality Network (EMQN) will further allow these novel EQA programs to be offered to the global market making sustainability more likely.

15. Publications arising from QUPP funding

1. DNA Extraction EQA program

Horan MP, Chai SY, Pillay N, Tay KH, Bennetts B, Badrick T (2018) Proficiency testing and pre-diagnostic characterization of extracted DNA for external quality assurance. Int. J. Inf. Res. Rev. 5:5790-5796.

2. Circulating free DNA EQA program

Chai SY, Peng R, Zhang R, Zhou L, Pillay N, Tay KH, Badrick T, Li J, Horan MP (2020) External Quality Assurance of Current Technology for the Testing of Cancer-Associated Circulating Free DNA Variants. Pathol. Oncol. Res. 26:1595-1603.

Peng R, Zhang R, Horan MP, Zhou L, Chai SY, Pillay N, Tay KH, Badrick T, Li J (2020) From Somatic Variants Toward Precision Oncology: An Investigation of Reporting Practice for Next-Generation Sequencing-Based Circulating Tumor DNA Analysis. Oncologist. 25:218-228.

3. Leukaemia EQA program

Corboy G, Greg, Othman J, Lee L, Wei A, Blomberry P, Fong C, Brown A, Grove C, Enjeti A, Iland H, Bohlander S, Horan M, Stevenson W (2020) Laboratory quality assessment of candidate gene panel testing for Acute Myeloid Leukaemia: A joint ALLG / RCPA QAP initiative. Pathology (submitted for publication)

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