Challenges in Immunophenotyping EQA

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ACS Hobart

RCPAQAP

The Royal College of Pathologists of Australasia
Quality Assurance Programs

I pay respect to the traditional and original owners of this land the muwinina people, to pay respect to those that have passed before us and to acknowledge today's Tasmanian Aboriginal people who are the custodians of this land.

For the next 20 minutes

- A little bit about the QAP
- The QAP Immunophenotyping program
  - Collection
  - Processing and distribution
  - Report review and assessment
  - Neat vs stabilised dot plots
  - The assessment process
- Troubleshooting

The RCPAQAP – who are we and what do we do?

The Royal College of Pathologists of Australasia Quality Assurance Programs

- Its in our name!
- We are an independent self-sustaining organisation
- We offer a comprehensive range of EQA for all disciplines of pathology. Our programs are offered in Australia and internationally in over 100 countries. RCPAQAP is committed to providing an efficient and customer-focused service to participants
- Our mission statement: To provide resources, products, services, data and insights for assessing the diagnostic and technical proficiency of laboratories in each discipline of Pathology and for the purposes of supporting patient safety, continuing medical education and research
- Our work is more than just EQA

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What is the role of EQA

Participants are provided with frequent challenges, peer-reviewed assessments, and educational activities to monitor the quality of their laboratory services and ensure they meet accreditation requirements. Our strength lies in the expertise and support provided by an extensive advisory network consisting of pathologists and scientific staff from Australia and internationally.

How does this help you?

The ability to compare the quality of laboratory performance with others on a national/international scale

To provide assurance to consumers that their laboratory results are of good standard

To encourage good IQC processes

To identify errors and implement corrective actions

To encourage standardised procedures and good quality reagents

To educate laboratories involved in the program

To maintain best practice if/when standards/guidelines change

Immunophenotyping (IP) programs

Immunology immunophenotyping – lymphocyte subsets

Haematology immunophenotyping – Primarily LPD panel

Haematology immunophenotyping – Acute panel if we get lucky

Haematology immunophenotyping – PNH

Haematology IP Program design

- The program is designed to test your testing protocols from the moment a sample is collected i.e we are testing the whole process
- Real patient
- We provide the clinical/presentation history
- FBC results provided
  - We will provide details of which panel to test, sometimes there is enough information in the details provided for you to proceed without a prompt from QAP
- Blood film digital scan of the actual sample
- Actual flow results reported in the survey report

Where do we get our samples from?

- For both Immunology and Haematology the samples are single donor patient samples
- Samples are sourced by our collections scientist (Elysse Dean) – based at RPA
  - Ethics agreement with NSWHP
  - Collection based on surplus to pathology samples or
  - Disease specific
  - Donors are de-identified
  - All samples are safety tested
- Immunology – utilise Haemochromatosis patient donors
  - Venesected blood bag/tubes – consented
  - These patients have regular clinic appointments and IM can mostly adhere to a schedule
  - Collection based around a schedule

Where do we get our samples from?

- Haematology IP
  - LPD patients – better able to collect as they present regularly to clinic
  - But still need the donor consent at time of collection
  - Collection dependant on donor availability
- Usually – less than a week's notice for a potential collection
- But, until the donor is at the clinic, has consented and goes through with the collection the donation is not confirmed
- Most of the time, we know only on the day of collection that the samples are coming
- Acute collections are unicorns!

Before samples arrive at QAP

Any recent results, clinical history are sent to the Senior Scientist to assess if results/history are fit for purpose

Samples collected, Lithium Heparin and EDTA if possible, plus safety testing

Meanwhile at QAP – we get ready for processing and dispatch

Customer service creates the packing lists and slips

Logistics creates the connotes and books dispatch dates

Samples should be ready by 12 pm on day of the dispatch

Haem Team starts the paperwork, informs collaborators of impending pretesting samples, and vials are labelled and prepared for aliquoting. Slide stainer prepared, VM scanning informed of urgent scan requirement. Survey dispatch scheduled email sent to CAT team for approval

Samples arrive at QAP. Run an FBC. We assess volume requirements. Stabilise and aliquot based on the volume of sample received including vials for homogeneity and pretesting

Run homogeneity, send neat and stabilised samples for pretesting flow (gives the baseline results)

Blood film made and stained, slide is coverslipped and dried overnight

Samples are packed and ready for dispatch the next day

The next day,

Safety testing should be back – we can now send the samples

VM scanning starts

Labware/myQAP/IT prepared for survey open

CAT approved emails can be sent to participants of survey open and close dates

Complete labware/myQAP requirements

Enter patient clinical details and testing requirements

Assess suitability of VM scan for inclusion in survey instructions

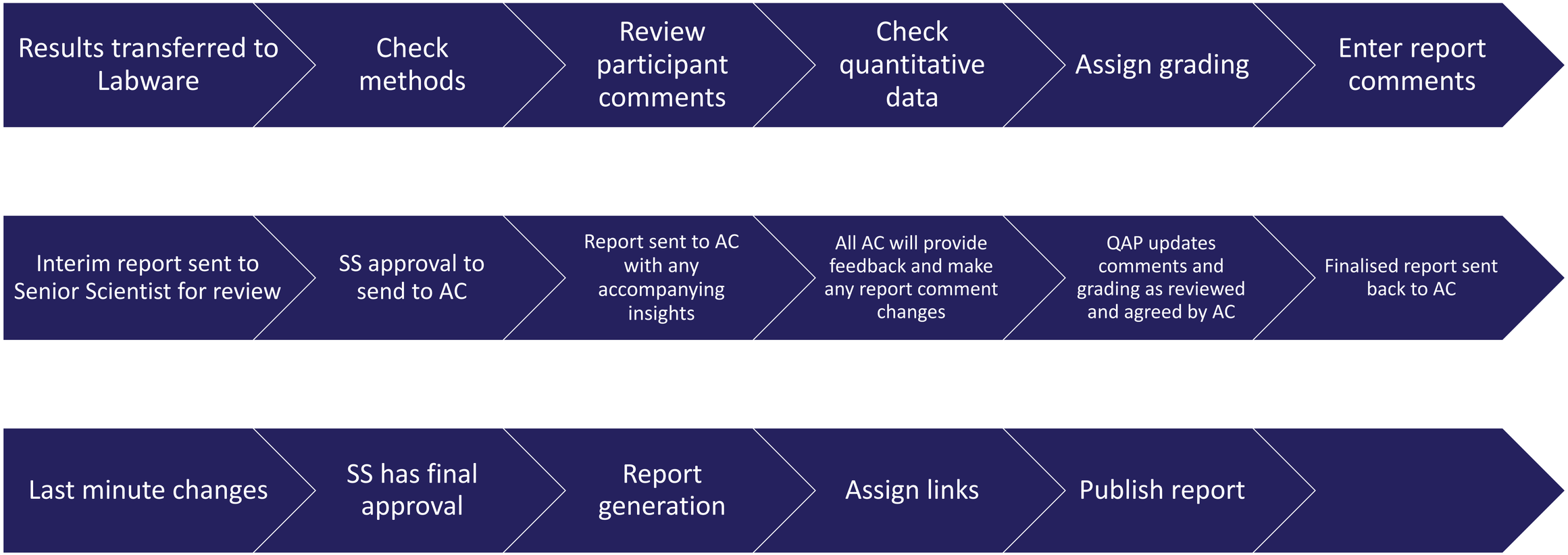
Complete all paperwork, check all details ready for survey open

Survey opens

While the survey is open

- Check pretesting results
- Check requests
- Interim data load
- Start report commentary

After survey close



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Survey report and assessment

Assessments based on bioanalytical method

Assessment of measurands (usually) falls into 1 of 4 categories

Quantitative	**Uses calibration standard to determine the absolute quantitative values for unknown samples. The reference material is well defined and fully representative of the endogenous analyte.**
Relative quantitative	**Uses a calibration standard to estimate the absolute quantitative values for unknown samples. The reference material is not fully representative of the endogenous analyte**
Quasi-quantitative	**Does not use calibration standard, but has a continuous response. Numeric data is reported.**
Qualitative	**Lacks proportionality to the amount of analyte. Categorical data is reported.**

Flow cytometric methods largely fall in the two latter categories and are essentially quasi-quantitative or qualitative.

Reference: Wood et al. Cytometry Part B (Clinical Cytometry) 84B:315–323 (2013)

IP program assessment

- The nature of quasi-quantitative reporting means we cannot apply the usual APS (assessment by median) criteria for assessment
- Multi level assessment is provided using
  - Z-score for quantitative data
  - Concordance assessment on the qualitative data
  - Concordance assessment on diagnostic interpretation
- Comprehensive report commentary provided

IP program assessment

Data analysis and assessment criteria: https://dataanalysis.rcpaqap.com.au/

A core set of antigens are selected based on a consensus phenotype. Antigens are assessed individually, based on their result distribution profile i.e. where results form a cluster. A mean and 2SDs will be applied and results are assessed based on the z-score that is calculated. Z-scores that are greater than 2.0 and less than 3.0 are highlighted for review in ‘amber’, if 3.0 or greater, highlighted in red. Quantitative responses are not assessed if the results of a marker show variation greater than 50%.

The report provides participants with a summary of performance, result review pages, comprehensive discussion on the case study and cumulative performance for the survey year. Results use the standardised assessment grades used across disciplines of Concordant, Minor discordant, Differential Diagnosis or Discordant

Survey reports

Summary of Performance

Target Source = Specific Target (Assessment is based on the z-score) z-score = 2.0 – 3.0 (requires review) z-score = >3.0 (requires action)

Performance Assessment

	Sample: HA-IP-22-03				
Test	**Your Result**	**Mean/Expected Result**	**Review**	**Z-score**	**nPart**
CD3%	1	3.2		-0.2	74
CD3 - Interpretation	Negative	Negative	Concordant		75
CD23%	7	2.9		0.4	72
CD23 - Interpretation	Negative	Negative	Concordant		72
CD25%	1	2.8		-0.2	72
CD25 - Interpretation	Negative	Positive	Discordant		71
CD38%	1	5.1		-0.3	49
CD38 - Interpretation	Negative	Negative	Concordant		48
CD45%	100	94.4		0.3	55
CD45 - Interpretation	Positive	Positive	Concordant		52
CD103%	10	3.5		0.6	76
CD103 - Interpretation	Partial	Negative	Minor Discordance		76
CD123%	4	0.4		7.1	50
CD123 - Interpretation	Negative	Negative	Concordant		50
CD200%	10	2.6		1.0	70
CD200 - Interpretation	Negative	Negative	Concordant		70
KAPPA%	2	1.4		0.2	86
KAPPA - Interpretation	Negative	Negative	Concordant		86
LAMBDA%	97	83.3		0.5	89
LAMBDA - Interpretation	Positive	Positive	Concordant		87
FMC-7%		46			42
FMC-7 - Interpretation		Positive	Not Assessed		42
Diagnostic Interpretation	Splenic B cell lymphoma/leukaemia, unclassifiable	Hairy cell leukaemia variant	Minor Discordance		92

Overall Performance

Please review results returned for:

Sample HA-IP-22-03 | CD103 - Interpretation, Diagnostic Interpretation

Summary of performance illustrating the assessment criteria. The table represents the markers reported by the participant, illustrating the result returned, the expected result & calculated mean value, illustrating the assessment. The overall performance provides all measurands that require review for further action.

Troubleshooting

Stabilisation and transit

Sample type	Advantages	Disadvantages
Fresh	As close as possible to commutable. Allows for real patient scenarios	Cells start deteriorating within hours of collection
Cryopreserved	Rare cases	Cost, loss of cells during thawing, artifact

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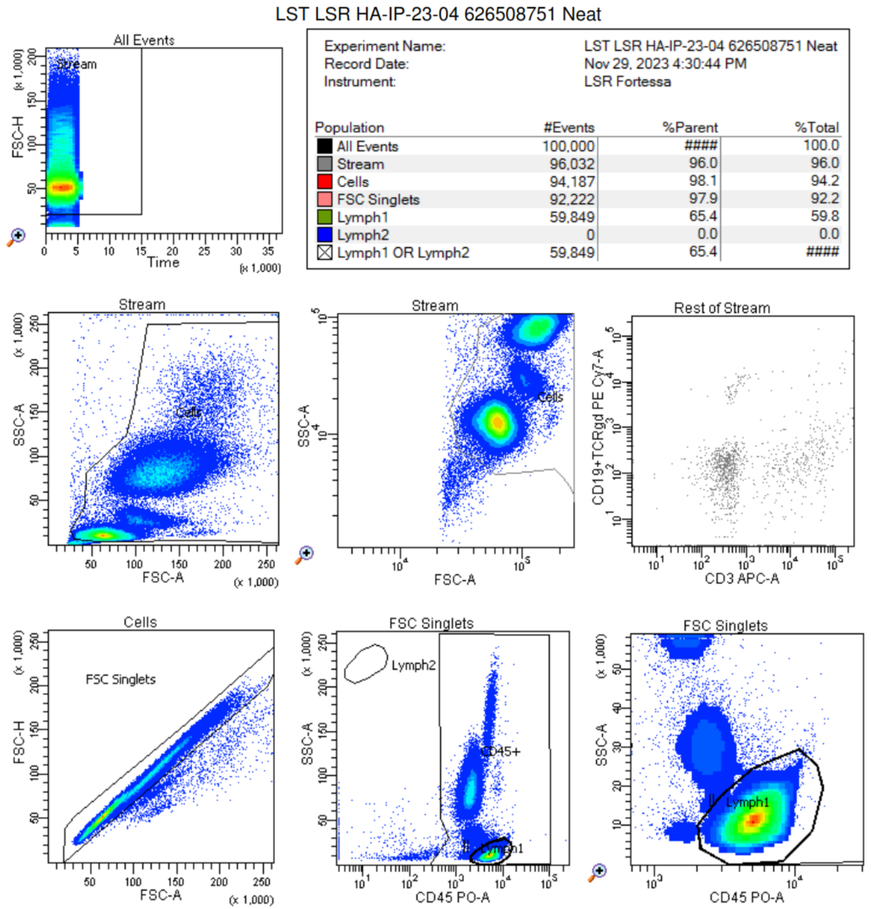
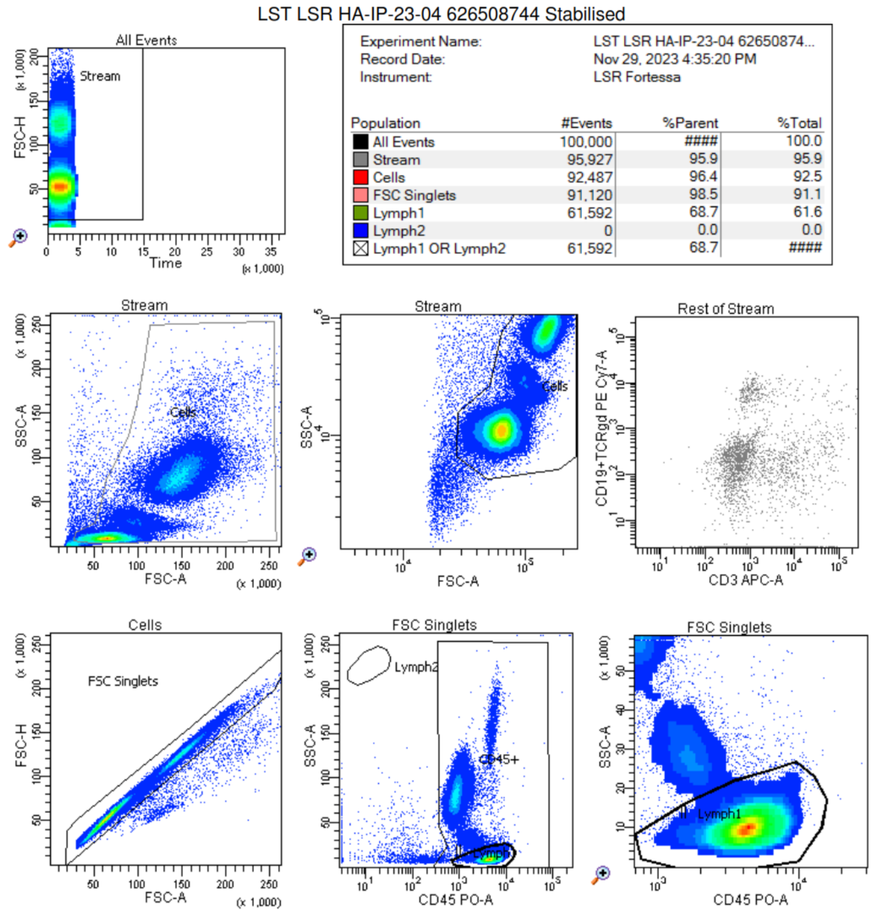
Stabilised vs Neat sample dot plots

HA-IP-23-04 Neat (SBLPN)(HCLv)



HA-IP-23-04 Stabilised



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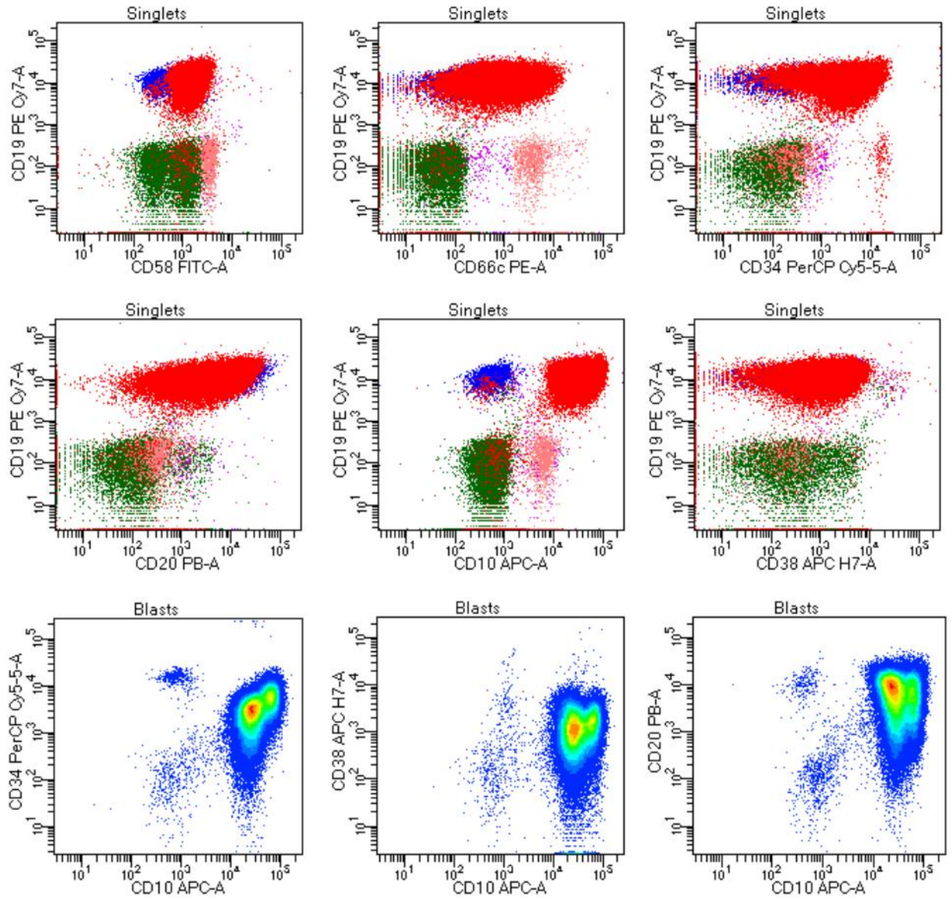
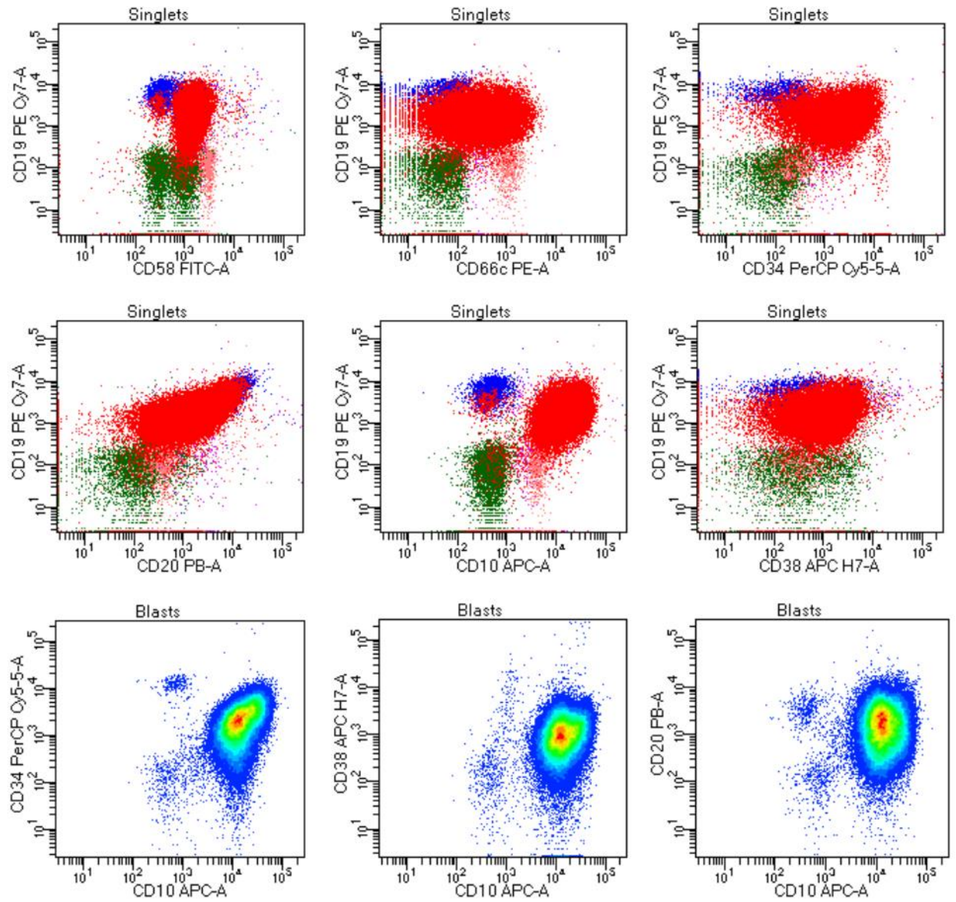
Stabilised vs Neat sample dot plots

HA-IP-24-01 Neat B-ALL, NOS



HA-IP-24-01 Stabilised



Troubleshooting tips

Issue	Potential Causes	Potential Solution
Poor sample quality (increased debris)	Transit-related; temperature or delay resulting in suboptimal survey material for testing	Request replacement sample ASAP by logging a request through myQAP or calling (02) 9045 6040
Insufficient lysing of sample	Stabiliser – fixation of EQA samples may cause insufficient lysing action	1. Check the temperature of the laboratory is within the range specified by the manufacturer of the lysis buffer 2. Additional volume of lysis buffer or additional lysis time may be required
Reduced forward scatter – due to increased debris or unlysed red cells	Interferences from fixation of EQA sample	Increase the gain of the forward scatter parameter
Cell clumping	Insufficient mixing	Invert sample at least 25 times prior to analysis as per product insert
Poor staining	Reduced antibody binding can occur if the sample is not brought to room temperature	Samples should be brought to room temperature (20-25°C) before analysis

Other considerations

- EQA samples may be handled by the most experienced operator in the lab (or the person in charge)
- Sometimes assigned to only 1 individual to test (operator biases)
- May not be randomly loaded in the run
- Interpretation of result a “group effort”
- Collusion with other labs
- More rigorous checking procedures
- Doing further testing (especially on a negative sample)
  - (no we are not trying to trick you)

2025 updates

- PNH program
  - Changes to result entry and survey report
  - to align with ICCS/ESCCA Consensus Guidelines

- IP program result entry and report refresh

- Viable CD34 will be available to AU participants only

- Current CD34 Program
  - Change in supplier
  - Samples will look different
  - Unable to assign different levels – no Precision and Accuracy

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Thank you

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