EQA monitoring of BCR-ABL1 levels in CML using the International Scale (IS) to determine a molecular response

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Background

Leukaemia is the 8th leading cause of death from cancer in Australia. Chronic myeloid leukaemia (CML) is a blood cancer affecting more than 300 people in Australia annually[1]. CML is caused by the genetic formation of a fusion oncogene *BCR-ABL1*, through a translocation between chromosomes 9 and 22 (t(9;22)) resulting in the expression of a pathogenic chimeric *BCR-ABL1* tyrosine kinase[2]. *BCR-ABL1* is primarily found in CML patients but may also be detected in patients with acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML). Tyrosine kinase inhibitors (TKI) are effective for inhibiting the *BCR-ABL1* oncogenic transcript[3, 4]. A patient's major molecular response (MMR) to TKI therapies is monitored by measuring *BCR-ABL1* transcript levels. Using a conversion factor, laboratories can convert their *BCR-ABL1* transcript levels to the IS5. This allows for the effectiveness of TKI therapy to be monitored and is additionally used for proficiency testing for identifying consistency between multiple BCR-ABL1 clinical testing laboratories.

Method

In 2010, the WHO *BCR-ABL1* International Genetic Reference Panel was developed for BCR-ABL1 quantification on the IS. As these samples were limited, laboratories became involved in a sample exchange with a laboratory in Adelaide for IS calibration. Due to a need for these standards, a commercial *BCR-ABL1* secondary reference panel, adapted from the WHO panel was developed[6, 7]. In 2017, the RCPAQAP sent out the *BCR-ABL1* secondary reference panel, containing five lyophilised samples, to 45 laboratories across 14 countries. Each sample was prepared from a mixture of cell lines. However, the relative level of *ABL* and *BCR* control genes in these mixtures are not fully representative of physiological levels. Thus, the values assigned to these samples may differ according to the control gene used for quantitative-PCR of the *BCR-ABL1* transcript. The assigned values for these samples were based on analyses using *ABL* as the control gene and were therefore not applicable to laboratories using *BCR* as their control gene. For those laboratories using *BCR* as a control gene, values were assigned based on the average *BCR* value for the samples tested over 2 years. Graphical analysis was performed using the Bland-Altman plot to determine mean difference (bias) and limits of agreement8, 9 between the assigned standard values and the laboratories values. In addition, laboratories were asked to provide a clinical interpretation on their IS result. Laboratories were also assessed on their ability to detect the lowest level of *BCR-ABL1* transcript levels, which is a strategy used to determine assay sensitivity.

Results

For this EQA, laboratories were asked to report on the *BCR-ABL1* transcript type and to convert their results from the *BCR-ABL1* measurement to a recommended IS value5. This ensures that comparable results between laboratories are provided and can be monitored. Thirty-three laboratories reported using *ABL*, four laboratories used *BCR*, and two laboratories used both *ABL* and *BCR* as the control gene. The common e14a2 *BCR-ABL1* transcript was present in all five samples (Table I). Table II defines the molecular response of *BCR-ABL1* levels on the IS.

Table I. Target result for each sample

Sample ID.	Translocation	IS (%)		Acceptable Clinical Interpretation
		ABL	BCR	
Sample A	e14a2	0.0032	0.010	$DMR^{4.5}$ or DMR^{4}
Sample B	e14a2	0.01	0.023	DMR^{4} or DMMR
Sample C	e14a2	0.10	0.20	DMMR or NMMR
Sample D	e14a2	1.0	2.6	NMMR
Sample E	e14a2	10	36	NMMR

Table II. Legend for clinical interpretation

Code	Clinical Interpretation
NMMR	The *BCR-ABL1* value is not within the range for a MMR
DMMR	Detectable *BCR-ABL1* indicating a MMR
DMR4	Detectable *BCR-ABL1* indicating MR^{4}
DMR4.5	Detectable *BCR-ABL1* indicating $MR^{4.5}$
UMMR	Undetectable *BCR-ABL1* indicating that at least a MMR is achieved
UMR4	Undetectable *BCR-ABL1* indicating MR^{4}
UMR4.5	Undetectable *BCR-ABL1* indicating $MR^{4.5}$

Figure 1. Overall fold bias plot against the assigned value. The red solid lines represent the range +/- 2-fold of the average BCR-ABL1 IS values. The blue solid line represents the mean bias (0) between the assigned value and laboratories results.

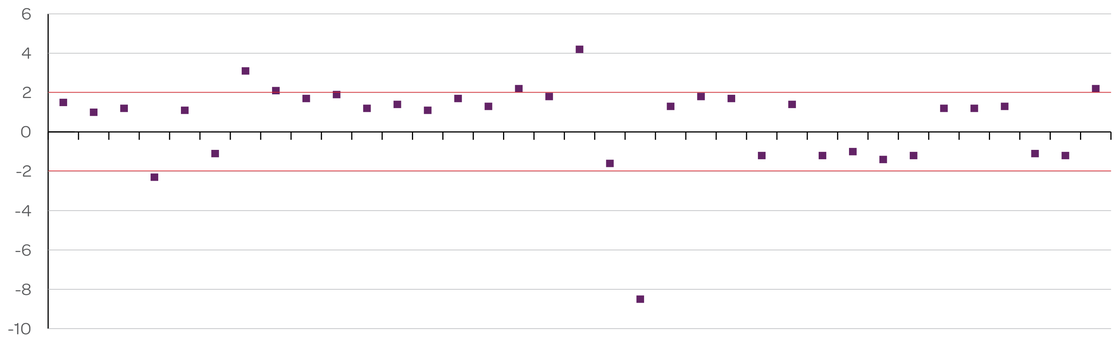
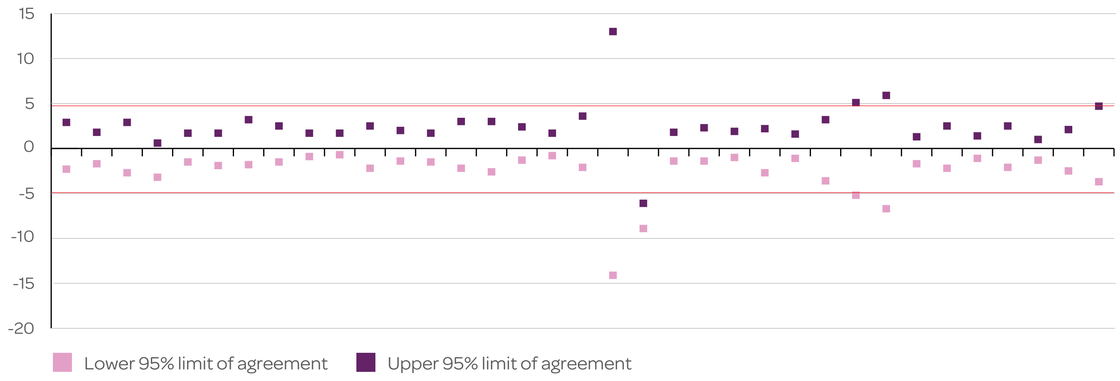


Figure 2. Overall limits of agreement (+/– 5-fold)



Discussion & Conclusion

- Specific codes for the interpretation of the molecular response were incorporated into the assessment (Table II).
- Laboratories that did not supply an interpretative comment were not assessed. Interpreting the molecular response in regards to treatment is essential for clinicians in the development of appropriate patient management.
- 36 laboratories performed quantitative *BCR-ABL1* testing for all samples.
- Samples A and B, three laboratories did not return a *BCR-ABL1* result and samples C, D and E, one laboratory did not return a *BCR-ABL1* result. These laboratories were not assessed.
- For all samples, ~six laboratories that reported a *BCR-ABL1* result failed to provide any clinical interpretation of their data and were therefore not assessed.
- ~86% laboratories correctly detected the lowest *BCR-ABL1* level (0.0032%IS) at MR4.5 and MR4.
- Data from all laboratories were compared against the assigned values. Below are the overall performance for all samples:
  - Sample A: ~58% concordance between laboratories in providing the correct molecular response for the IS value reported.
  - Sample B: ~69% concordance between laboratories in providing the correct molecular response for the IS value reported.
  - Sample C: ~75% concordance between laboratories in providing the correct molecular response for the IS value reported.
  - Sample D: ~80% concordance between laboratories in providing the correct molecular response for the IS value reported.
  - Sample E: ~78% concordance between laboratories in providing the correct molecular response for the IS value reported.
- The bias between the standard assigned value and the laboratories reported values for all five samples were calculated for values reported on the IS and for laboratories where four or five IS values were reported as positive. These results are reported as the bias, displayed in Figure 1. Since the analysis only includes a small number of samples for each laboratory, the bias was considered acceptable if it was within +/- 2-fold. ~75% of laboratories were within +/- 2-fold.
- The 95% limits of agreement include a comparison of the assigned value and laboratories reported values[8, 9]. The limit of agreement measures the total error between the two values. ~90% of laboratories were within +/- 5-fold of the assigned values, shown in Figure 2.

References

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