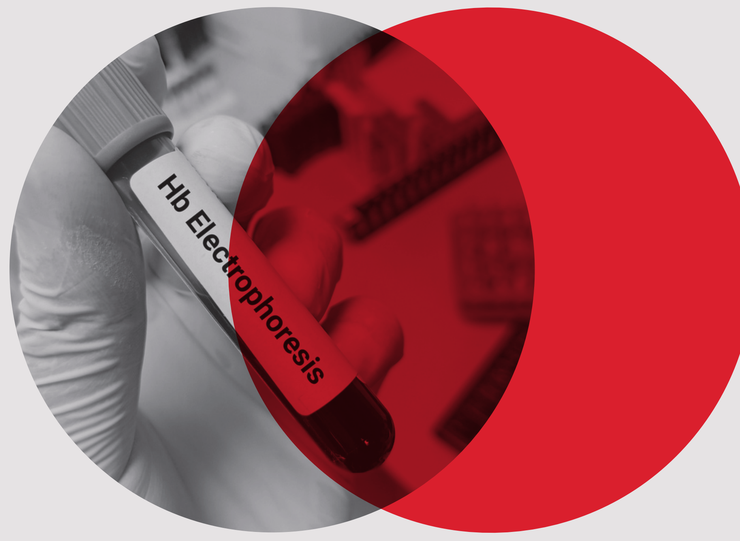
Analytical principle vs measurement system to assess Haemoglobinopathy EQA results



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Introduction

RCPAQAP regularly review the assessment criteria for their EQA programs to ensure that participant results flag appropriately.

Assessment category options include comparison with peers using the same instrument, reagent or analytical principle. A minimum of 6 participants are required for within category assessment.

The introduction of new HPLC instruments and their associated columns prompted a review of the assessment criteria (analytical principle vs measurement system) for the Haemoglobin A_2, Haemoglobin F and variant haemoglobin results submitted by participants in the RCPAQAP Hemoglobinopathy program.

Method

A retrospective analysis of five years' data representing twenty data sets of Hb A_2, Hb F and Hb variant (if present in the sample) were categorised and assessed according to the analytical principles i.e. high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) as well as the measurement systems (instruments). The mean, standard deviations (SD) and coefficients of variation (CV) were compared as a measure for determining the preferred category to evaluate results, and hence the performance of the laboratories enrolled in our program.

Data from samples containing haemoglobin variants from 2017 to 2021 (Table 1) were specifically reviewed to determine any potential impacts on the measurement of Hb A_2 and Hb F levels. Values of "0.0%" for the Hb A_2, and a "Hb A_2 + variant" (which is how we ask participants to report the presence of HbA_2 interference) were used as one of the indicators of a co-elution with HbA_2.

Table 1. Haemoglobin variant samples from 2017–2021 with potential to co-elute

Survey Sample	Target Diagnosis
May 2017	Heterozygous Haemoglobin E
March 2018	No haemoglobin variant present (No samples with variants were offered in 2018).
March 2019	Heterozygous Haemoglobin E/alpha thalassaemia
May 2020	Heterozygous Haemoglobin S
June 2021	Alpha chain variant

Results and Discussion

The differences between the mean, median, SD and CVs for Hb A_2 and Hb F for analytical principles vs measurement system when no haemoglobin variant was present, weren't deemed to be clinically significant (Figure 1, Table 2). Heterozygous E variants interfered with Hb A_2 reporting for HPLC methods. The variants in the 2020 and 2021 programs (Heterozygous Hb S and alpha chain respectively) did not interfere with either Hb A_2 or Hb F for HPLC or capillary methods, as expected (Figure 1). Table 2 does show that analytical principle CVs may be masking differences between instruments, and supports assessing by instrument. There were at least 6 users in each instrument group to facilitate the use of the within instrument review category.

Conclusion

The emergence of new HPLC instrumentation has improved the ability of determining HbA_2 where there is a variant haemoglobin co-eluting in the same position, such as Heterozygous Hb E. The decision to change the assessment criteria from analytical principle to instrument peer group now provides a more accurate assessment of performance in the RCPAQAP Haemoglobinopathy Program.

Table 2. Comparison of the CV% of Hb A_2 and Hb F where no haemoglobin variant was present (2017–2021)

	Bio-Rad Variant 11	Bio-Rad D-10	Sebia Capillarys/ Minicap	HPLC	CE
CV% – Hb A_2	5.6	10.0	3.6	7.8	3.9
CV% – Hb F	7.2	11.9	5.3	8.5	9.0

Note: Only method groups where n≥6 were included., and results > 3SD were excluded.

Figure 1. Comparison of medians for HbA_2 by instrument and method for the 2017–2021 period noting a 0% HbA_2 indicates variant interference.