50 Years as World Leaders in EQA 1969–2019

UK NEQAS International Quality Expertise

Reticulocyte Haemoglobin Content: UK NEQAS External Quality Assessment

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Introduction

Reticulocyte Haemoglobin Content (RHC) is considered a reliable, early biomarker of iron deficiency, which also demonstrates a response to iron therapy and is recommended for the diagnosis of iron deficiency in chronic kidney disease. RHC parameters are offered on all major automated haematology platforms, although they vary in their methodologies. There is no external quality assessment (EQA) for RHC in the UK, restricting the wider clinical application of this parameter. The UK National External Quality Assessment Scheme (UK NEQAS) for Haematology has completed a pilot study for RHC EQA. The purpose of the initial studies was to demonstrate that the assay material used gave clinically acceptable and comparable results between different instrument types and was stable for a sufficiently long period of time and in transit to be suitable for use in EQA.

Table 1. Retic Hb parameters offered by different analyser platforms

Manufacturer	Instrument	Parameter
Abbott	Alinity hq	MCHr
Beckman Coulter	DxH 900	RSF and LHD%
HORIBA	Yumizen H2500	RHCc
Siemens	ADVIA 2120 and 2120i ADVIA 120 and 120i	CHr
Sysmex	XN-10, XN-1000 and XN-20	Ret-HE

Key:
MCHr – Cellular Hb concentration for retics (pg)
RSF – RBC size factor (fL)
LHD% - Low Hb Density
RHCc – Retic Hb Cellular content (pg)
CHr – Mean Hb content for retics (pg)
Ret-HE – Hb content of retics (pg)

Note:
Beckman Coulter instruments were not included in these studies because insufficient instruments were available to provide an effective assessment of the survey material.

Methods

Blood was collected with informed consent from two JAK2-positive, polycythaemia vera (PV) patients undergoing regular venesection, into acid-citrate-dextrose (ACD), and stabilised within 48h using glutaraldehyde, formaldehyde and broad-spectrum antibiotics (specimens RH01 and RH02). Blood from a volunteer donor, collected into citrate-phosphate-dextrose (CPD), was treated in the same way (specimen RH03). Aliquots of each donation were distributed to Abbott, Horiba, Siemens and Sysmex analyser users for RHC analysis on days 1, 3, 5 and 7 post-distribution.

The JAK-2 Positive Donors
Both donors were *JAK2 V617F mutation*-positive. They were venesected as part of their treatment regime and the blood used with the patient's consent, rather than being discarded as clinical waste.

RH01 was an 82-year-old female patient with borderline microcytosis (Hb 157 g/L, RBC 5.7x10^{12}/L, MCV 84 fL, MCH 27.5 pg, RDW 15%).

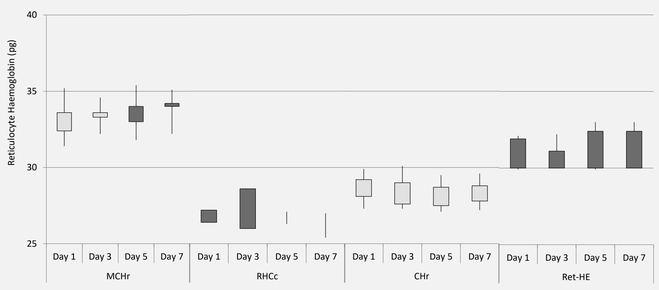
RH02 was a 76-year-old, male patient with marked microcytosis (Hb 152 g/L, RBC 6.9x10^{12}/L, MCV 68 fL, MCH 22.2 pg, RDW 19%).

Results

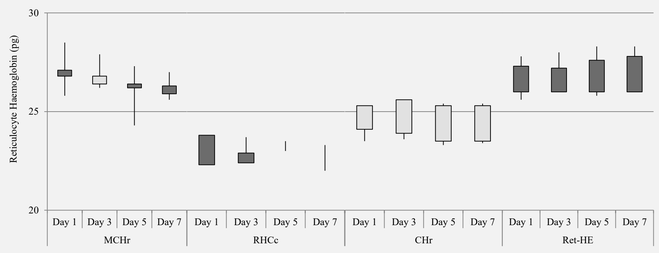
Results were received from 20 laboratories using 4 different platforms. There was no significant within platform difference ($p>0.5$) for mean and standard deviation (SD) between testing days, although there was a statistically significant difference ($p< 0.001$) between different platforms (see Fig. 1A-1C). The greatest differences were seen between Abbott MCHr and Horiba RhCc parameters (-6.14+/-1.25pg), Abbott MCHr and Siemens CHr (-4.69+/-1.25pg), and Sysmex Ret-HE and Horiba RhCc (3.95+/-1.35pg). The Horiba RhCc gave the lowest RHC and the Abbott MCHr the highest. The inter-platform differences did not preclude the clinically appropriate interpretation of results regardless of platform (see Fig. 1D-1F) using British Society of Haematology and National Institute for Health and Care Excellence (NICE) published guidelines, which recommend a RHC cut-off of 29 pg as indicative of iron restriction.

Figure 1. Results returned for specimens RH01, RH02 and RH03
Figures 1A, 1B and 1C show the daily medians and distribution of results for testing days 1, 3, 5 and 7, by platform. Figures 1D, 1E and 1F show box and whisker plots of all results received from all platforms, by specimen.

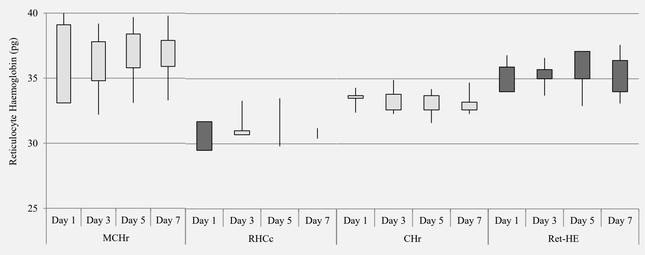
1A. RH01



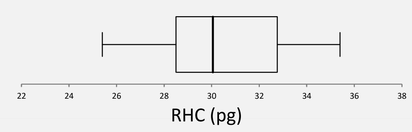
1B. RH02



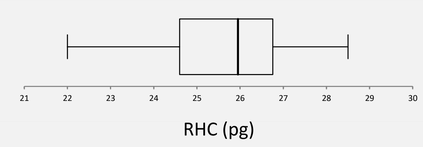
1C. RH03



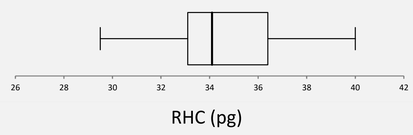
1D. RH01
The median of all results received for RH01 was 30 pg (range 24-36 pg). This distribution is consistent with a borderline iron deficient status.



1E. RH02
The median of all results received for RH02 was 26 pg (range 22-29 pg). This distribution is consistent with an iron restricted status.



1F. RH03
The median of all results received for RH03 was 34 pg (range 30-40 pg). This distribution is consistent with a normal iron status.



Conclusions

RHC is an advanced, readily available, automated cell counting parameter, which can deliver extra information with increasingly evidence-based clinical utility in the investigation of iron deficiency.

The final hurdle in converting research only parameters, such as the RHC, into established parameters in for diagnosis or monitoring is the provision of an EQA programme, the need for which is indicated by the statistically significant inter-platform RHC differences.

UK NEQAS Haematology has provided a survey material with the potential to support an EQA programme. Two further pilot distributions will be distributed in 2023 and the programme will be offered on a pilot basis in 2024, with the development of performance assessment and online operation.