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UK NEQAS *BCR-ABL1* Quantitation Programme: Impact of UK Guidelines for *BCR-ABL1* Transcripts Quantitation in Chronic Myeloid Leukaemia

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INTRODUCTION

Real time Quantitative Polymerase Chain Reaction (RQ-PCR) is an established technique in the monitoring of Philadelphia (Ph) positive CML patients receiving Tyrosine Kinase Inhibitor (TKI) treatment. Recently, published UK recommendations for the quantification of *BCR-ABL1* have highlighted the need to amplify/detect greater than 10,000 *ABL1* copies in order to obtain the required sensitivity and resolution needed to confidently report *BCR-ABL1* levels at Major Molecular Response (MMR) level.

The aim of this project was to determine if UK Guidelines for *BCR-ABL1* transcript quantitation are impacted by the different RNA extraction and/or cDNA synthesis methods in use and how these affect *ABL1* copy numbers.

METHODS

- Stable lyophilised samples were produced by mixing the *BCR-ABL1* positive cell line K562 with the *BCR-ABL1* negative cell line HL-60.
- Prior to sample issue, local batch testing ensured *ABL1* copies were greater than 10,000 in both sample 112 and 113.
- Participating laboratories were asked to assay the samples using their local methodology.
- Results were submitted to UK NEQAS LI along with details of RNA extraction, cDNA synthesis and RQ-PCR methodologies.
- The consensus %*BCR-ABL1* was calculated from all quantitative results submitted.

RESULTS

- Ninety one laboratories submitted %*BCR-ABL1/ABL1* with a median *ABL1* copy number of 25930 molecules (inter quartile range (IQR): 45882) for sample 112 and 24130 molecules (IQR: 38717) for sample 113.
- Sixteen (18%) and 24 (26%) of laboratories reported *ABL1* levels below the recommend 10000 copy number for samples 112 and 113 respectively.
- No significant difference was found in *ABL1* copy number between the RNA extraction methods phenol-chloroform and silica binding (sample 112 p=0.2488, sample 113 p=0.8840) (Fig 1), or whether the RNA extraction was automated or manually processed (sample 112 p=0.9810, sample 113 p=0.6794).
- When comparing the reverse transcriptase step, no significant difference was found in *ABL1* levels when comparing in-house versus commercial cDNA synthesis protocols and reagents (sample 112 p=0.4797, sample 113 p=0.5636) (Fig 2), In addition no significant difference was seen when comparing Superscript versus Moloney Murine Leukaemia Virus (MMLV) reverse transcriptase (sample 112 p=0.2523, sample 113 p=0.3185) (Fig 3).

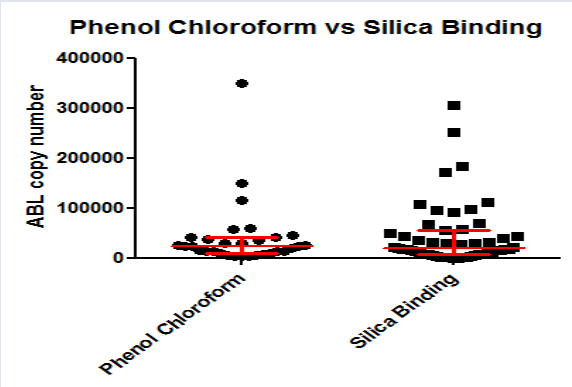

Figure 1: *ABL1* copy number categorised by RNA extraction method.

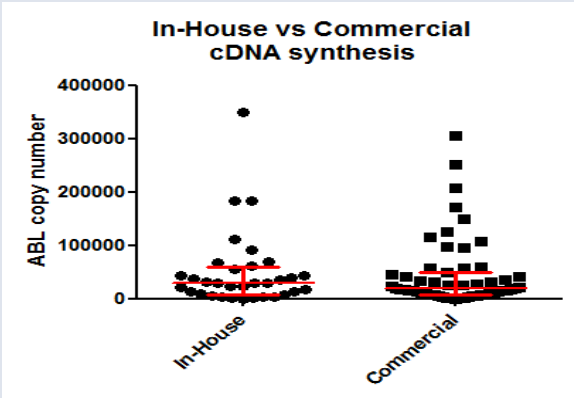

Figure 2: *ABL1* copy number categorised by, cDNA method.

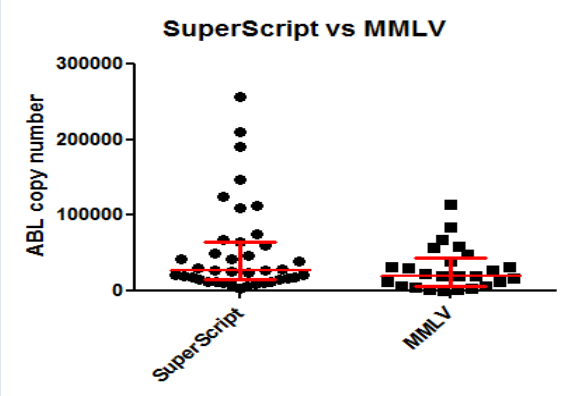

Figure 3: *ABL1* copy number categorised by reverse transcriptase enzyme

CONCLUSION

- Our data shows that the variability in results observed for low *ABL1* copy numbers is independent of RNA extraction or cDNA synthesis process and suggests that to date, despite the availability of UK guidelines, significant inter-laboratory variability still persists.