Comparison of the QuantideX® qPCR BCR-ABL IS Kit CE and the modified EAC protocol for the monitoring of major molecular response in CML patients.

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SUMMARY

- **The rapid evolution of CML treatment, has sparked interest in the possibility of treatment free remission, leading to increased demands of molecular monitoring and standardised reporting of very low levels of disease[1].**
- **The QuantideX® qPCR BCR-ABL IS monitoring assay incorporates both reverse transcription and RQ-PCR, has a proven limit of detection of MR4.7 (0.002% IS) and allows direct reporting on the International Scale, without the need for conversion factor calculations[4].**
- **Results from the QuantideX® kit are comparable to our in house method, with no statistically significant difference identified in our final cohort (P=0.559).**
- **Advantages of the kit include elimination of lengthy conversion factor validation, faster turnaround of results, and superior reverse transcription , when compared with our in house method.**
- **Disadvantages to consider are the cost implications when compared with our in house method.**

AIMS

- To evaluate performance of the QuantideX® qPCR BCR-ABL IS kit, in the monitoring of prospective patient samples.
- To carry out a cost burden analysis, and determine economic viability of introducing the kit into routine practice.

INTRODUCTION

The treatment of CML was revolutionised by the use of the tyrosine kinase inhibitor (TKI) Imatinib Mesylate against the BCR-ABL1 fusion gene. The goal of successful treatment is the achievement of major molecular response (MMR); a 3 log reduction in transcript levels, equivalent to a BCR-ABL:ABL ratio of 0.1% on the international scale (IS), which greatly increases progression free survival[1].

Many patients will achieve MMR in the first 6-12 months of TKI therapy and a large number of these will continue to have further reduction in transcript levels, leading to a deeper molecular response and possibly undetectable disease[1].

This has sparked interest in the possibility of treatment free remission (TFR) for those patients achieving a long term deep molecular response, and therefore increases the need for the laboratory to produce high quality RNA, accurately assess and monitor low level BCR-ABL1 transcripts, and to report them on the International Scale (IS)[1].

We have evaluated the QuantideX® qPCR BCR-ABL IS kit as an alternative to our in-house method for BCR-ABL:ABL quantification.

The QuantideX® qPCR BCR-ABL IS Kit reports ABL1 copy numbers, MR values, and %IS values traceable to the WHO primary reference materials, allowing direct reporting on the International Scale, without the need for sample exchange or conversion factor calculations[2].

It has been proven to generate precise results with high sensitivity sufficient for studies in deep molecular responses (>MR4.5) [2].

Previous validation studies have demonstrated reduced hands-on time, allowing for 49 samples, plus 11 standards and controls to be run within 4 hours when used at full capacity, leading to improved workflow (fig. 1)[2].

1

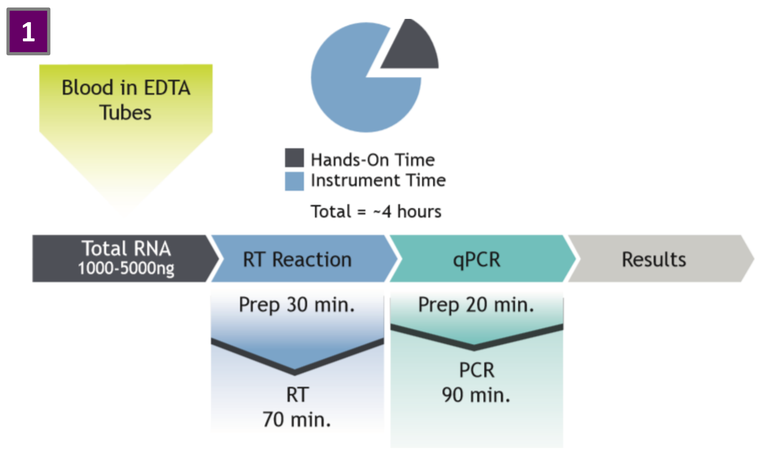

Figure 1: Assay Workflow of the QuantideX® Kit: Previous studies have demonstrated that using 1000-5000 ng in the RT (from 100-500 ng/µL of RNA) facilitates accurate measurement of BCR-ABL1 and ABL1, with total hands-on-time of ~1 hour and on-board instrument time of ≤4 hours[2].

With the rapid evolution of CML therapy, and the introduction of second generation TKIs[1], the QuantideX® qPCR BCR-ABL IS kit, offers a potential solution to meet the increased demands of molecular monitoring, and standardised reporting of low levels of disease, without the need for conversion factor calculations.

ABBREVIATIONS

MR – *Molecular response*
MMR – *Major Molecular Response*
EAC – *Europe Against Cancer*
UK IS CF – *UK International Standard Conversion Factor*
CE IVD – *European CE marking for in vitro diagnostic devices*
L Con – *Asuragen Low Control*
H Con – *Asuragen High Control*

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METHODS

To evaluate the performance of the kit, we carried out a direct comparison with our in house method (modified EAC protocol)[5], on live patient samples.

Our comparison was split into three phases: a 'learning phase' to trial the kit and establish a protocol, an 'initial testing phase,' on 20 patient samples, and a 'final testing phase,' using the kit at full capacity on 49 live CML monitoring samples.

Initial Testing Phase

RNA extraction was performed on 32 live patient samples using the Maxwell RSC instrument, eluting into 50µl of RNase free water. Following Nanodrop quantification, 20 prospective samples were selected from a wide range of concentrations (including 2 NEQAS EQA samples).

RNA was split into 2 aliquots, with one aliquot following our in-house method, and the other following the QuantideX® kit, allowing a direct comparison of results.

Final Testing Phase

RNA extraction was performed on 60 live patient samples using the Maxwell RSC instrument eluting into 50µl of RNase free water. These samples were frozen and stored as RNA for 24 hours, to test performance on frozen RNA. Following Nanodrop quantification, 49 prospective samples were selected from a wide range of concentrations, as well as the Asuragen low and high controls.

RNA was split into 2 aliquots, with one aliquot following our in-house method, and the other following the QuantideX® kit, allowing a direct comparison of results.

The QuantideX® qPCR BCR-ABL IS Kit

The Major breakpoint monitoring assay uses Armored RNA Quant® (ARQ) technology to deliver a nuclease-resistant 4-point standard curve containing both BCR-ABL1 and ABL1 transcripts. The assay contains three controls (high, low, and negative), as well as calibrators which are traceable to the WHO Primary BCR-ABL1 reference materials[2]. The QuantideX® kit comprises both reverse transcription and RQ reagents, with BCR-ABL and ABL1 multiplexed into one well. Samples were tested on the ABI 7500 for both RT and qPCR step.

RESULTS

Initial Testing Phase

From our run with the Asuragen QuantideX® qPCR BCR-ABL IS Kit, raw data from the 7500 was exported into the QuantideX® qPCR BCR-ABL IS Kit analysis software to produce BCR-ABL1 %IS. This was compared to our in-house data using our current conversion factor of 0.58 (generated from the UK IS CF project). In the initial testing phase, we identified a statistically significant difference between the two data sets (P=0.023 by Wilcoxon Signed Rank Test), although this is possibly due to the small cohort included.

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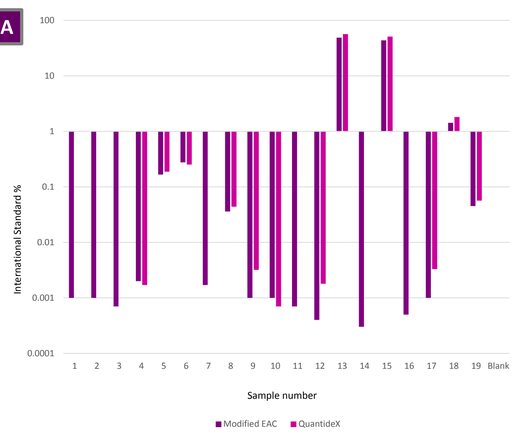

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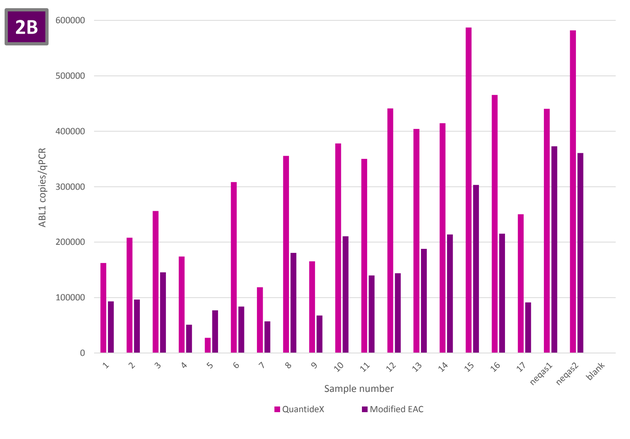

Figure 2A: Initial comparison of %IS values for our modified EAC protocol and the QuantideX® kit. The results appear generally comparable, however a statistically significant difference was identified between the two data sets. This may be due to the small sample size. A larger cohort was included in the final testing phase.

Figure 2B: Initial comparison of ABL1 copy numbers for our modified EAC protocol and the QuantideX® kit. The QuantideX® kit appears to be superior in generating higher ABL 1 copy numbers, than our in house method, in all samples except patient 5.

(*Note: to allow a comparison between the 2 methods we had to split patient samples and therefore the ABL1 results generated in house have allowed a comparison of the 2 methods, but do not reflect our usual ABL1 transcript levels).

Final Testing Phase

In the final testing phase, which included a larger cohort of 49 samples, plus the Asuragen High and Low controls, there was no statistically significant difference between the two data sets (P=0.559 by Wilcoxon Signed Rank Test).

3A

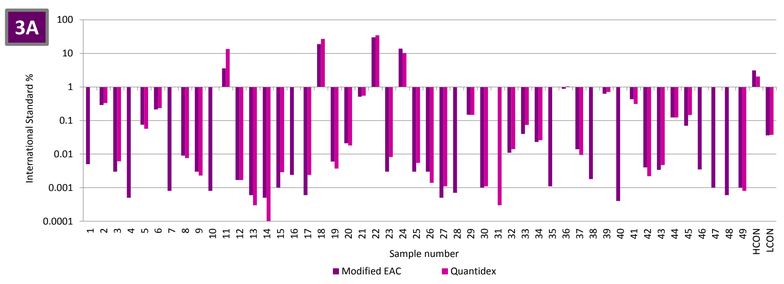

Figure 3A: Final comparison of %IS values for our modified EAC protocol and the QuantideX® kit. Results are comparable, and there was no statistically significant difference identified between the two data sets (p=0.559).

3B

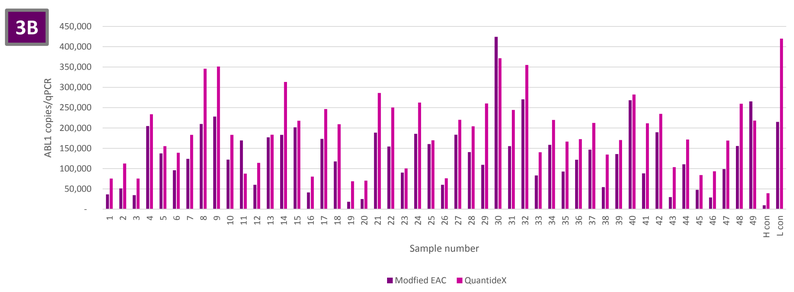

Figure 3B: Final comparison of ABL1 copy numbers for our modified EAC protocol and the QuantideX® kit. The QuantideX® kit appears to be superior in generating higher ABL1 copy numbers, than our in house method, in all but 3 samples (patients 11, 30 and 49).*

To interpret the data, we looked at the level of molecular response, as scored using the EUTOS working definitions for scoring deep molecular response following treatment of CML (fig. 4)[3].

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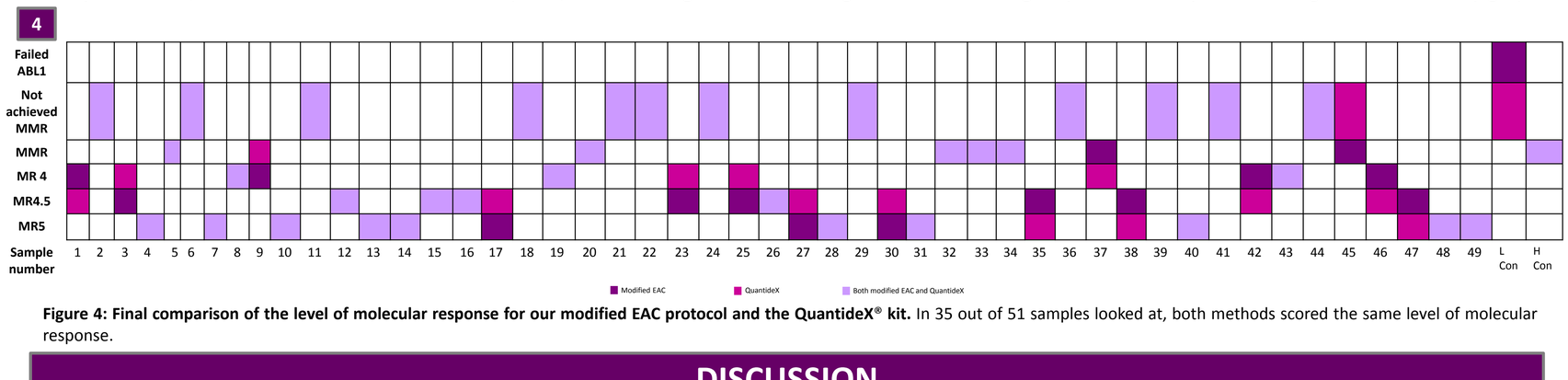

Figure 4: Final comparison of the level of molecular response for our modified EAC protocol and the QuantideX® kit. In 35 out of 51 samples looked at, both methods scored the same level of molecular response.

DISCUSSION

The QuantideX® reverse transcription method proved superior in generating higher ABL1 transcripts in 18/19 samples tested in the initial phase, and 50/51 samples in the final phase, when compared to our in house method. Additionally all 51 samples in the final phase achieved an ABL1 copy number of at least 32,000 (required to score deep molecular response at MR4.5) , compared to our in house method, with only 90% achieving 32,000 ABL1 transcripts.

Discrepancy in the scoring of molecular response between the 2 methods was seen in 16/51 patients. The majority of these discrepancies were seen in the scoring of deep molecular response[1]. Higher ABL1 transcripts using the kit allowed a deeper molecular response to be scored in 4/51 patients.

Both methods identified the same patients at risk of treatment failure and sub-optimal response[3] by not achieving MMR. There was, however a single borderline result which failed to achieve MMR with the kit, but was scored as MMR by our in-house method (0.1457% IS kit vs 0.07% EAC).

In-house methods found 6 patients with no detectable disease, but BCR-ABL1 transcripts were detected with the kit. Likewise, a single patient with detectable disease in-house had no BCR-ABL1 transcripts with the kit. This is possibly due to differences in assay optimisation[1], but the false positive and negative rate requires further assessment.

ADVANTAGES

1) No requirement to undertake lengthy conversion factor re-validation.
Any changes to in-house methodologies can invalidate a conversion factor. We currently have 2 real-time PCR machines, but use only one for CML monitoring, as the other has not been validated using conversion factor reagents. This kit would enable use of both machines.

2) The kit appears superior at reverse transcribing RNA when compared to our in-house method.

3) The kit is much quicker at generating results.
Although hands on time was not evaluated, the kit was significantly quicker than our current method. Samples were reverse transcribed in a 96 well plate and then multi-channelled into a second 96 well plate for RQ-PCR. Since the assay is multiplexed in a single well, this significantly reduced pipetting time and the number of plates from 4 to 1 for the RQ PCR step.

4) The kit has undertaken comprehensive validation using patient material.
A limit of detection has been established using patient samples.

DISADVANTAGES

For all their advantages CE IVD developed tests are generally more expensive than in-house laboratory developed tests (LDT) and as such there will be a significant cost increase per sample. Whether the increased sensitivity will allow more patients to cease TKI therapy and thus achieve monetary saving on medication remains to be seen.

Future work:

We will be undertaking a 6 month validation of the kit from November 2019, and running it alongside or current method to determine whether we can take the kit forward into routine use.

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