Detecting BCR-ABL1^{IS} and scoring MR: Results from a CE-IVD kit run on non-IVD PCR instruments: a comparison with data from a EUTOS validated lab.

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INTRODUCTION

Detection of the BCR-ABL1 fusion transcript in CML patients using RT-qPCR allows sensitive monitoring of disease levels (minimal residual disease, MRD), which is important for determining prognosis and making treatment decisions. To interpret MRD results correctly in respect to data from clinical trials, it is necessary report data as IS (International Scale), which is a standardized unit for reporting the level of BCR-ABL1 transcripts. In order to report on the IS scale, a conversion factor is required, which currently is calculated by comparing MRD data from numerous samples exchanged with a reference laboratory; an exhausting process. Alternatively, commercial BCR-ABL1 kits can allow reporting on the IS scale, however IVD kits may be validated on specific equipment not available in the current laboratory setting, which may rise concerns about the performance under these conditions. Here we report data from a commercial CE-IVD BCR-ABL1 detection kit run on non-IVD instruments, compared to results obtained from our own EUTOS validated lab.

OBJECTIVE(S)

The use of IVD kits can be very helpful for maintaining a stable analysis in your laboratory, however often the material requirements specified by the manufacturer of the kit may not be available at your specific location, and/or quite costly to acquire. Here we aim to confirm whether the use of non-IVD validated materials and equipment will affect the kits ability to provide results equal to those obtained using our EUTOS validated method.

METHOD(S)

A EUTOS validated assay following EAC and EUTOS guidelines for detecting and scoring BCR-ABL1 was applied as described in guideline papers[1, 2], to detect the presence of BCR-ABL1 in 56 RNA samples. More specifically, RNA was purified from peripheral blood collected and frozen in PAX-gene tubes, and the concentration measured on a Nanodrop. cDNA synthesis was performed using the Superscript Vilo enzyme (Invitrogen) with 1.5ug of RNA in each reaction. The qPCR reaction was run using Brilliant III qPCR mastermix (Agilent) with primers from the EAC guidelines, and 50 cycles on a Stratagene Mx3005 qPCR machine. GUSB was used as reference gene together with 6 replicates of BCR-ABL1 for increased sensitivity. GUSB and BCR-ABL1 copy numbers were calculated from a standard curve using the ERM-AD623 plasmids.

A commercial CE-IVD kit, QuatideX qPCR BCR-ABL IS KIT (Asuragen), was also used to detect the presence of BCR-ABL1 in the same 56 RNA samples, following the manufactures recommendations, with the following alterations: RNA was from blood collected in PAX-gene instead of EDTA-tubes, a Stratagene Mx3005 instrument was used for qPCR instead of the ABI 7500 Fast Dx Real-Time PCR instrument or Roche cobas z 480 Analyzer, and %IS and MR was calculated on a spreadsheet instead of through the Asuragen QuantideX Reporter Software.

RESULT(S)

Initially, 17 RNA samples were tested with the commercial CE-IVD kit: QuantideX qPCR BCR-ABL IS KIT (Asuragen) in parallel on two different qPCR machines; the ABI7500-Fast as recommended for the IVD kit, as well as the Stratagene Mx3005, which we use for the majority of our routine analyses of clinical samples. We observed good correlation between the data from the two machines (Figure 1), indicating that valid results with the kit could be obtained on the non-IVD machine.

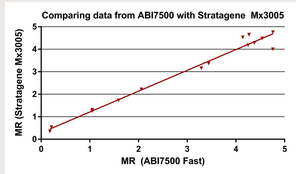

Figure 1:
MR values for 17 RNA samples were obtained using the Asuragen CE-IVD kit in parallel on two different qPCR machines, plotted against each other and a regression line calculated, resulting in an equation of Y=0.92x+0.28, with an R^2 of 0.9783.

We then proceeded to test 56 RNA samples on the same instrument (Stratagene Mx3005), using two different methods: our inhouse EAC-based protocol, and the Asuragen QuantideX kit, in order to detect any differences in the reported MR/IS. Figure 2 shows the data from all samples, depicted as MR vrs MR.

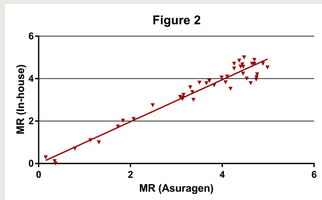

Figure 2:
MR values for 56 RNA samples were obtained using the Asuragen CE-IVD kit as well as the in-house EAC method in parallel on the Stratagene Mx 3005 qPCR instrument, plotted against each other. The regression line was calculated, resulting in an equation of Y=0.99x-0.007, with an R^2 of 0.9528.

The % IS values from both methods were calculated for all samples and are shown in the figures below. Due to the large range difference in the BCR-ABL1 levels, the samples have been divided up into Figure 3, 4 and 5 according to the number of transcripts detected in the in-house assay, for a better overview of the data from the two methods.

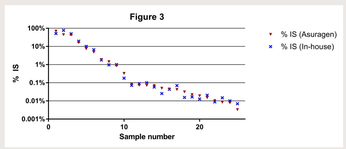

Figure 3:
IS values from samples containing BCR-ABL1 transcripts detected in all 6 wells in the In-house assay (i.e. those samples with least stochastic variation). The reported IS between the two methods are similar, with an average fold change of 1.2 between the assays (ranging from 0.4 to 2.1).

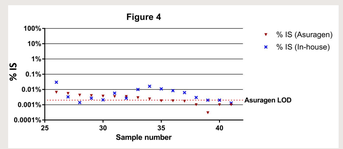

Figure 4:
IS values from samples with detectable low-level disease in the In-house assay where one or more BCR-ABL1 wells contain under 3 transcripts. Two samples (40-41) with detectable disease with the In-house assay were not detectable with the commercial assay, these were also below the LOD.

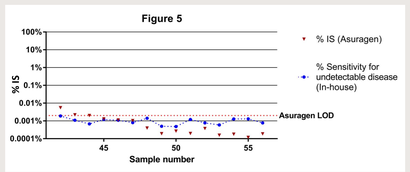

Figure 5:
IS values from samples with undetectable disease in the In-house assay. The first 4 samples (42-45) show detectable disease when using the commercial kit. The remaining samples (46-56) have undetectable disease with both methods.

CONCLUSION(S)

In our hands, data obtained from the Asuragen kit was essentially equal to our in-house assay, conforming to EAC/EUTOS standards, even when run on equipment as yet not IVD approved by Asuragen. The largest variations were seen for samples close to the limit of detection (MR=4.7 or IS= 0.002%), where our In-house assay had several BCR-ABL1 wells containing less than 3 transcripts.

The recent EUTOS guidelines recommend using 3 as the lowest number when scoring a positive well, and this may in some cases result in IS overestimation. At least, in our experiment, the largest differences in %IS between the two methods was observed for samples where our in-house method had several wells containing only 1 transcript (sample number 33-39). Transcript number using the commercial kit was much larger due to the single-well multiplex setup, thereby reducing the effect of stochastic variation in the samples close to the LOD.

In the 6 samples where there was discrepancy between the methods, in regards to detectable/non-detectable disease, the level of BCR-ABL1 was so low it is down to random chance whether or not the few transcripts present manage to be pipetted into the PCR well.

ACKNOWLEDGEMENTS

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REFERENCES

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2) **Gabert J et al.**, Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. *Leukemia (2003) 17, 2318-2357*

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