VALIDATION OF QIAGILITY ROBOTIC WORKSTATION FOR QUANTIFILER HUMAN AND QUANTIFILER Y REACTION PLATE SETUP

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A validation study was designed for the QIAgility to determine its suitability for automated setup of RT-PCR reaction plates. This summary includes the methods, results, and conclusions obtained from studies performed using the Quantifiler Human and Quantifiler Y quantification kits with the above mentioned instrument.

The Human DNA Standard in the Quantifiler Human and Quantifiler Y kits was used to prepare the serial dilutions to serve as known values. The QIAgility was programmed to prepare the standards following the dilution scheme given in the Quantifiler Kit User's Manual. The master mixes were prepared using the QIAgility and two QAS run files created during the validation. The run files allow the user to define the number and type of samples needed for each plate. The QIAgility then distributes the master mix and DNA standards onto the plate. For these validation studies and in all casework operations at the JPRDL, all samples are added manually outside of the QIAgility.

Sensitivity and linearity were tested using a serial dilution of DNA standard. A reference sample from a known individual was extracted and purified and used to test for reproducibility and precision. Seven samples containing female saliva and male blood in varying ratios were prepared and purified to be used as mixture samples. Twenty previously extracted DNA samples were chosen for the concordance study and tested for comparison against the laboratory's current method. These included non-probative samples. The sources of the DNA were from blood stains, cigarette butts, cell phone swabs, gun swabs, baseball caps, t-shirts, and hair.

In conjunction with the concordance/non-probative and reproducibility studies, a cross contamination study was performed. This consisted of wells with samples alternating with blanks or wells without DNA in a checkerboard pattern. For the purposes of the cross-contamination study samples were either considered positive or negative (blanks). A DNA sample in the NIST 2372 kit was used for NIST traceability.

The QIAgility was found to prepare DNA standard dilutions resulting in R^2 values greater than 0.99 for both Quantifiler Human and Y. Based upon the results obtained from the studies performed it was determined that the QIAgility satisfactorily prepares DNA standards yielding results equal to or better than those obtained via manual pipetting, the QIAgility satisfactorily distributes 23 µL of Quantifiler Human and/or Quantifiler Y master mix into 96-well plates, and the QIAgility satisfactorily distributes 2 µL of DNA standards into 96-well plates.

When set up with the QIAgility and run on the ABI SDS 7000 the Quantifiler Human and Quantifiler Y kits satisfactorily determine the amount of human or male DNA present in the sample. The QIAgility and the ABI 7000 instruments yield satisfactory RT-PCR results using the Quantifiler Human and Quantifiler Y kits as observed by comparing all results against the current JPSO DNA Lab casework operations. ⌘