Evaluation of the INTEGRA Biosciences D-ONE Single Channel Pipetting Module on the ASSIST PLUS Robot, Paired with the MINI 96 Portable Electronic Pipette for Semi-Automated Set-Up of the CDC Influenza/SARS CoV-2 rRT-PCR Assay

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Introduction

INTEGRA Biosciences launched the D-ONE single channel pipetting module for use on the ASSIST PLUS automated pipetting platform in February 2022. Used in conjunction with the INTEGRA Biosciences MINI 96, a 96 channel portable electronic pipette, the D-ONE on the ASSIST PLUS allows for automated master mix preparation, automated PCR plate setup followed by single step addition of 96 specimens/controls to a PCR reaction plate. This functionality was evaluated for use with the Centers for Disease Control and Prevention (CDC) Influenza/SARS CoV-2 rRT-PCR Assay (Flu/SC2).

Method

Serial dilutions of five previously tested SARS CoV-2 positive specimens were prepared, extracted, and pooled. Ct values were evaluated and displayed linearity from 19-34. Programming of the ASSIST PLUS was accomplished using VIALAB Software (INTEGRA Biosciences). Master mix was prepared and plated using the D-ONE on the ASSIST PLUS. Samples were added using the MINI 96. Five replicates of each sample were tested over 5 days for a total of 125 tests per method. SC2 results were compared to results obtained from manual master mix preparation, master mix plating and sample plating with an electronic repeating handheld pipette. Results were evaluated for accuracy, precision, and analyst hands on time. Influenza test results were not included in this evaluation.
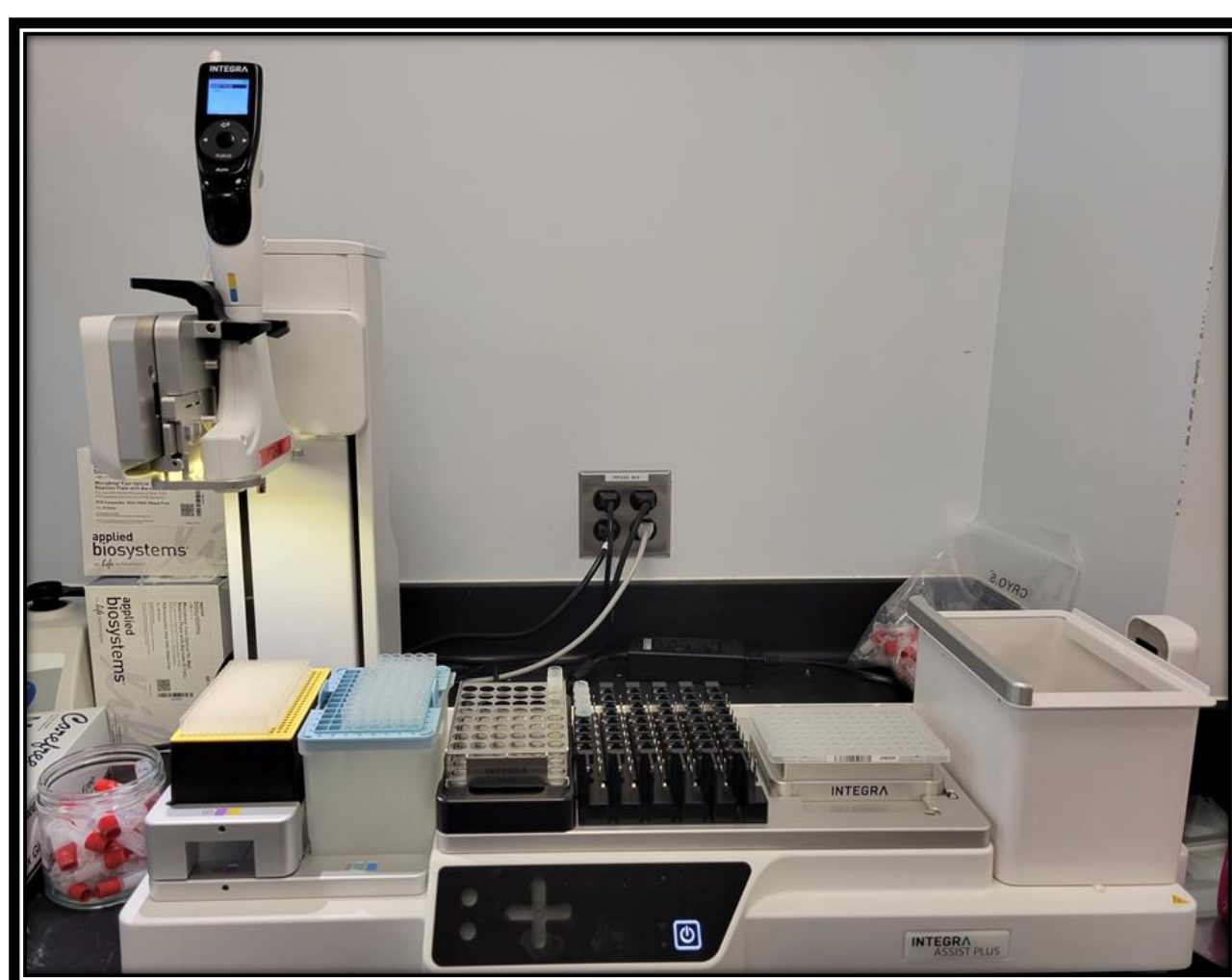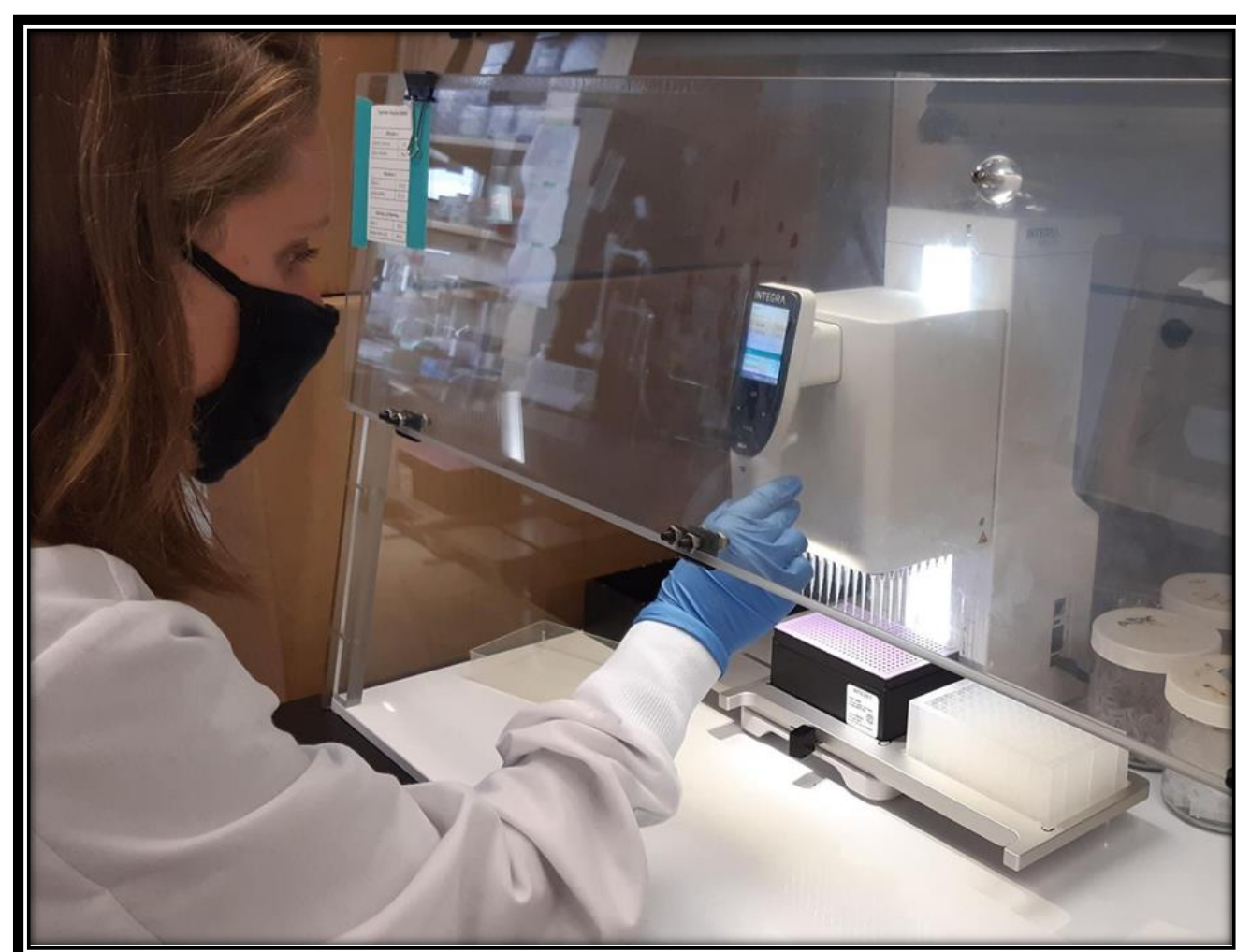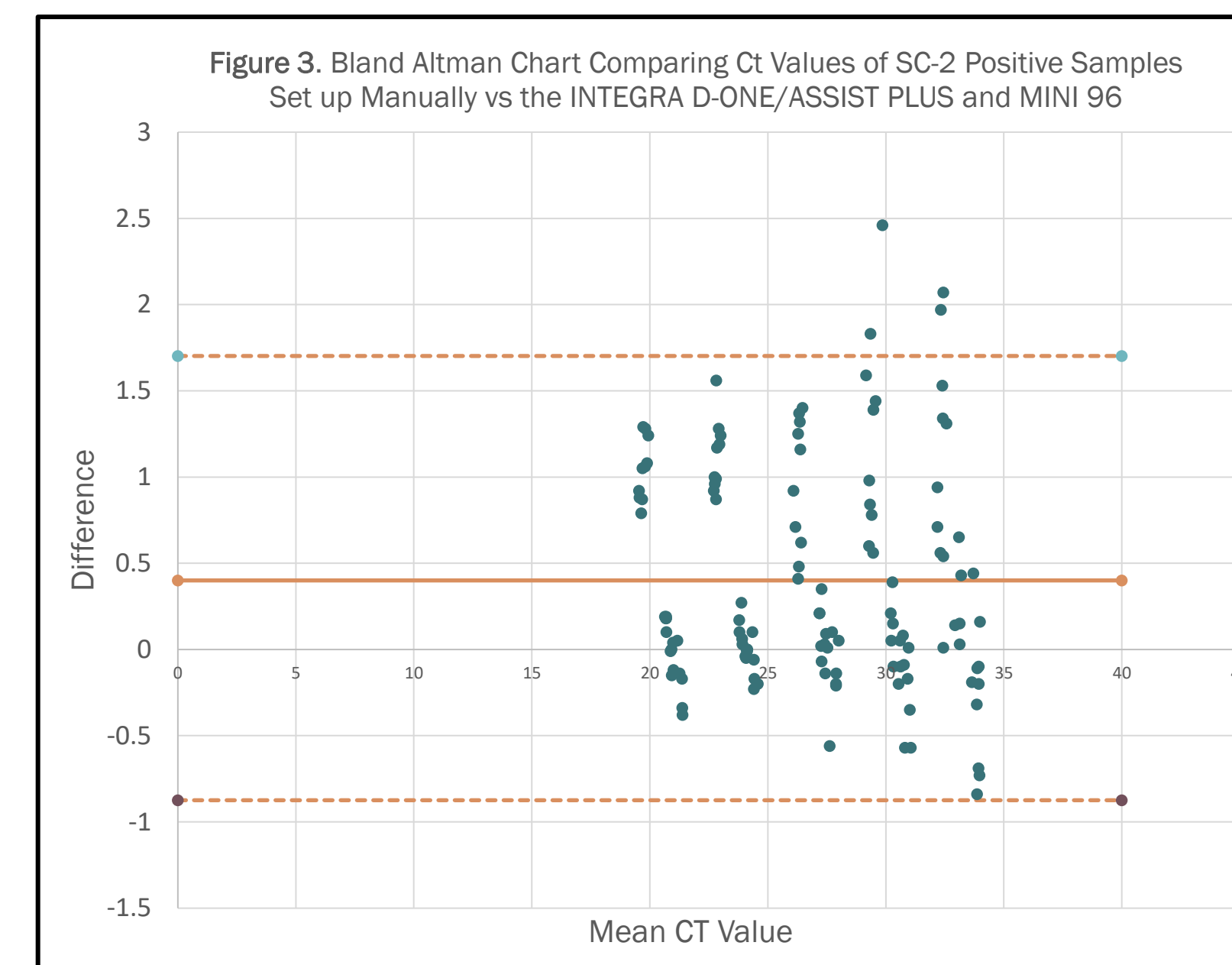
Results

Accuracy

125 samples were tested in this evaluation. As compared to manual plate preparation, automated PCR plate set-up by the D-ONE/ASSIST PLUS and MINI 96 demonstrated 100% accuracy. Ct values from the automated plate preparation averaged 0.43 cycles higher compared to manual plate preparation. Bland-Altman analysis shows higher variability in Ct values as viral load decreases

Precision

Precision was evaluated by calculating the coefficient of variation (CV) of Ct values of all samples tested. Automated PCR plate preparation displayed a CV of 0.39%, while manual plate preparation displayed a CV of 0.52%.

Hands-On Time

Hands-on time when using the D-ONE/ASSIST PLUS with MINI 96 was reduced by 27% as compared to the time required for manual plate preparation.

Figure 3. Bland Altman Chart Comparing Ct Values of SC-2 Positive Samples Set up Manually vs the INTEGRA D-ONE/ASSIST PLUS and MINI 96

Discussion

Surges in SARS CoV-2 testing and high laboratory staff turnover pose a challenge to Public Health Laboratories across the country. One strategy to address this challenge is to use more automation in the laboratory. The ASSIST PLUS (INTEGRA Biosciences) is an open platform pipetting robot, easily programmed using INTEGRA VIALAB software. Until recently, a limitation of the ASSIST PLUS was that it could only be used with a multi-channel pipette, resulting in limited functionality for tasks requiring single tube access (e.g., small volume reagent prep, hit-picking, normalizations). The release of the D-ONE single channel pipetting module provides this capability. We evaluated this capability for SC-2 detection. This evaluation showed that the D-ONE/ASSIST PLUS/MINI 96 preparation of Flu/SC-2 PCR plates was highly accurate and precise for SC-2. While Bland-Altman analysis showed samples with lower viral load exhibit a larger variation in Ct values between tests prepared manually and with automation, the distribution is within the expected normal range.

When paired with the MINI 96, a 96 channel electronic pipette that can deliver up to 96 extracted nucleic acid samples in one pipetting step, the D-ONE on the ASSIST PLUS provided reliable, robust pipetting capability for automated preparation and plating of Flu/SC2 master mix, reduced the opportunity for pipetting errors and freed up technicians to perform other tasks. The ability to control the robot from a laptop enables easy modification of the number of master mix reactions to be prepared as the workload varies, as well as post-run reports for quality assurance. Priced at under $25,000 for the ASSIST PLUS base unit and the D-ONE pipetting module, and $12,500 for the MINI 96, this system supports laboratory capacity for SC2 detection and surveillance at a competitive price.

Figure 1. The ASSIST PLUS with D-ONE Single Channel Pipetting Module set up to perform Master Mix Preparation and Plating

Figure 2. The MINI 96 Portable Electronic Pipette, set up to transfer 96 specimens in one transfer step.