Hygiena™ Prep Xpress Liquid Handling Automation Platform Internal Validation

Introduction

The Prep Xpress automation instrument is designed for high-throughput liquid handling. These protocols have been optimized for high throughput utilizing the 8-well multi-channel pipette head. This instrument is complimentary to BAX® System sample preparation protocols for filling cluster tubes with lysis solutions or BHI media, transferring from a sample or BHI cluster tube into lysis solution cluster tubes, or transferring from a prepared lysate cluster tubes into PCR tubes. This instrument does not perform onboard heating steps, therefore the use of a manual or automated heating block is required to complete portions of the BAX System sample preparation protocols.

High-throughput laboratories require skilled technicians to perform long hours of repetitive tasks that ultimately determine food safety risk from results. However, as sample inputs increase, training and retaining labor becomes more difficult in today's environment. The purpose of this study was to verify that the utilization of the Prep Xpress automation platform performs equivalently to manual processes throughout BAX System PCR methods to provide a reliable tool for high-throughput laboratories.

Required Protocols and Labware

Additional details regarding the protocols and labware (Table 1) listed below can be found in the Hygiena Prep Xpress Protocol Installation and User's Manual at www.hygiena.com.

Protocols:
Prep-Lysis_Solution_(6_racks)
Prep-BHI_Solution_(5_Racks)
Transfer-5_uL_Cluster_Tube_to_Cluster_Tube
Transfer-20_uL_Cluster_Tube_to_Cluster_Tube
Hydrate-30_uL-Lysate_to_PCR
Hydrate-50_uL-Lysate_to_PCR
Hydrate-30_uL-Lysate_to_PCR_SINGLE
Hydrate-50_uL-Lysate_to_PCR_SINGLE

Table 1. Required Labware for Prep Xpress Protocols

Product Code	Labware Type	Description
MIS2103	Plate	Hygiena Cluster Tube Rack w Adapter 96 Green 1.1 mL
MIS2104	Reservoir	Vblock 250 mL Open Reservoir, V-bottom, Clear PP
MIS2111	Reservoir	Seahorse-Agilent, Reservoir, 1 cavity, 8 Row, 290ml, Clear PP
MIS2107	Tips	50 µL Framed Conductive Filtered
MIS2082	Tips	300 µL Framed Conductive Filtered
MIS2108	Tips	1000 µL Framed Conductive Filtered
MIS2109	Rack	Eppendorf PCR Cooler, 200 µl, Strip-Well, PCR, White Well
MCH2075	Instrument	Prep Xpress Automation Instrument

Validation Methodology

The BAX System sample preparation steps were integrated into the Prep Xpress by executing modular protocols being Bulk Lysis Fill, Sample to Lysis (5 or 20 µL), and PCR Hydration, which can be utilized individually or in combination. Verification was performed in triplicate, across three instruments, to verify pipetting accuracy, assay performance, and cross-contamination for each protocol individually and in combination in comparison to manual processes. Each protocol was validated in triplicate across three different instruments to ensure user and instrument variability were considered.

Pipetting accuracy was determined by liquid weight with standard margin of error less than 20% established by the instrument manufacturer (Hamilton® Robotics). Assay performance and sensitivity claims were verified by utilizing post-enrichment inoculated samples to create lysates and PCR tablet hydration.

Cross-contamination was determined using a checkerboard pattern with 8.0 Log CFU/mL of bacteria and un-spiked media to perform all BAX System sample preparation to PCR steps (Figure 1). For any cross-contamination experiments, pure cultures of *Salmonella* Typhimurium DD13557 and *E. coli* O157:H7 DD916 were grown overnight in Brain Heart Infusion (BHI) broth at 35 °C. Each culture was then serially diluted 1:10 in additional BHI broth and plated in triplicate onto BHI agar for enumeration.

Full process systematic testing was performed utilizing beef enrichment samples inoculated with *Salmonella* and *E. coli* O157:H7 at low (6.0 Log CFU/mL) and high (8.0 Log CFU/mL) concentrations and uninoculated controls manually transferred into cluster tubes, automated transfer into prepared lysis solution tubes, heat steps performed utilizing thermal blocks outside of instrument, and automated hydration to PCR with the Prep Xpress automation instrument.

All Real-Time PCR tablets were hydrated with 30 µL of the appropriate lysate, sealed with flat optical caps and held for 10 minutes on a chilled (4 °C) PCR cooling block if required. Standard PCR tablets were hydrated with 50 µL of the appropriate lysate and sealed with flat optical caps. All PCR tablets were then loaded into the Q7 instrument, and a full process was run according to the instructions in the BAX System User Guide.

Results

The Hygiena Prep Xpress automation instrument performed equivalently compared to manual pipetting across all BAX System PCR method steps. All assay performance criteria and sensitivity claims were met with no cross-contamination during utilizing the Prep Xpress for any protocol individually or in combination (Figure 2). The variability for pipetting accuracy was <1% for the bulk lysis fill, 20 µL transfer and PCR tablet hydration. A 6.6% variability was observed for the 5 µL transfer protocols, which is equivalent to a ± 0.33 µL (Figure 3).

Figure 1: Checkerboard cross-contamination BAX Q7 software rack set-up for testing BAX System Real-Time PCR Assays for *Salmonella* and *E. coli* O157:H7 Exact.

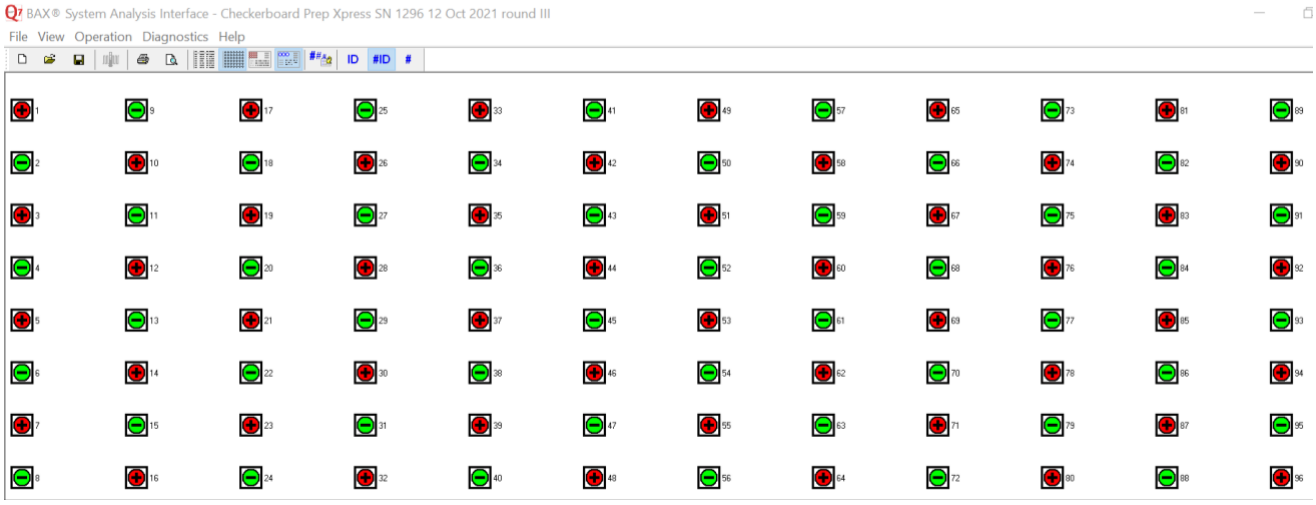


Figure 2: Checkerboard cross-contamination experiments across three instruments utilizing the Hydrate-30_uL-Lysate_to_PCR protocol with the BAX System Real-Time PCR Assays for *Salmonella*.

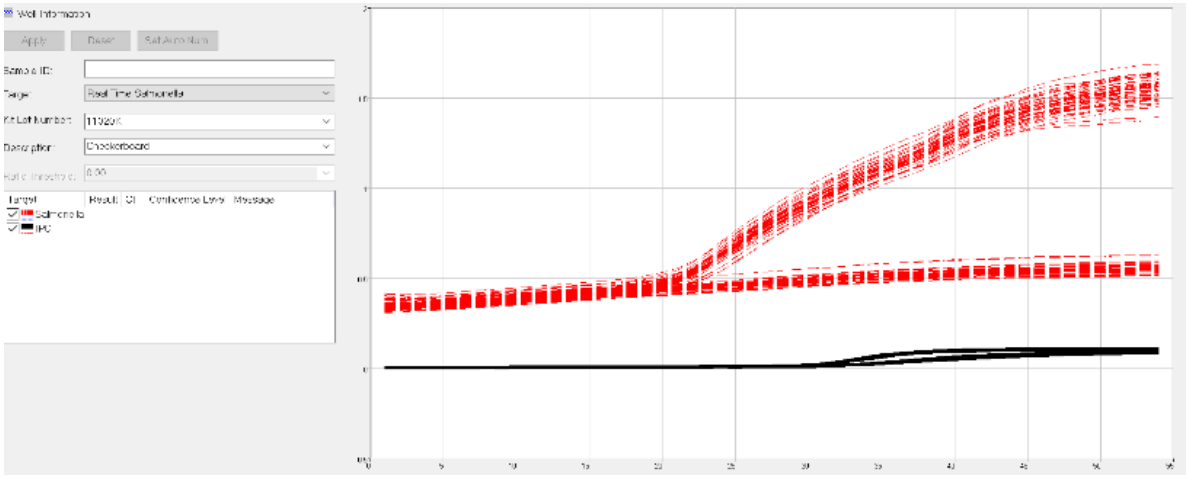
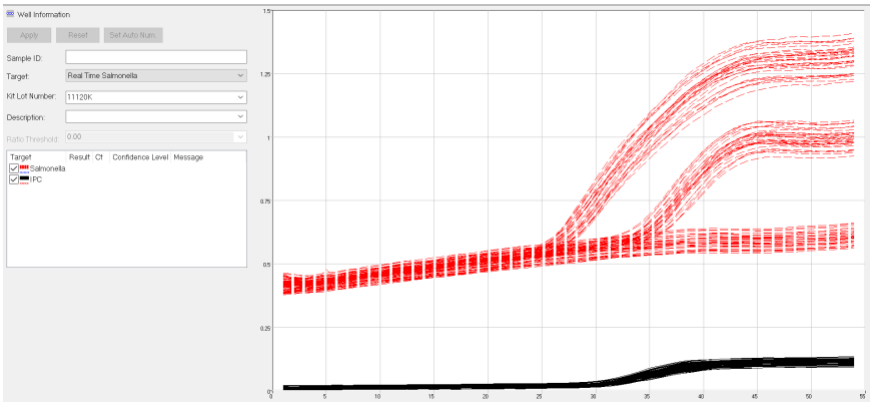
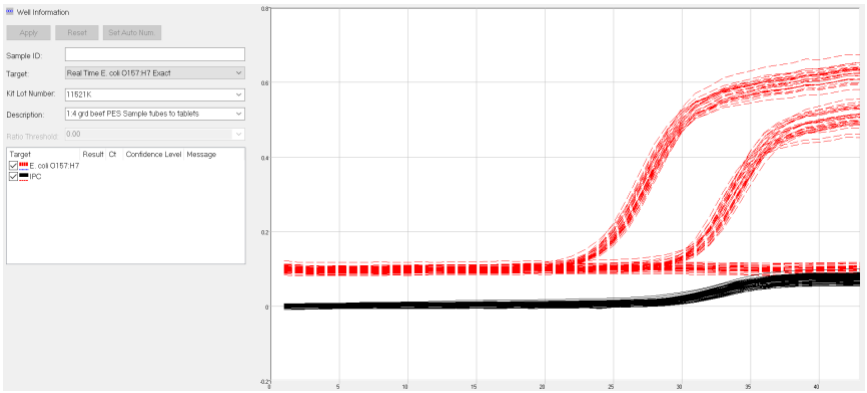


Figure 3: Full process systematic testing utilizing beef enrichment samples inoculated with *Salmonella* and *E. coli* O157:H7 in cluster tubes to lysis solution, heat steps utilizing thermal blocks outside of instrument, and hydration to PCR with the Prep Xpress automation instrument.

Salmonella



***E. coli* O157:H7**



Conclusions

Integration of automation solutions that modulate laboratory procedures can increase lab efficiencies by reducing the burden on technicians from compounding repetitive processes, like pipetting, that require long-term, high levels of accuracy and precision.