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# Evaluation of the new Abbott *m*PLUS feature: Impact on clinical laboratory efficiencies with Abbott RealTi*m*e HIV-1, HCV, HBV and CT/NG assays

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**Abstract**

Diagnostic laboratories are under increasing pressure to improve and expand their services. Greater flexibility in sample processing is a critical factor which can improve time to results while reducing reagent waste, making laboratories more efficient and cost effective. The introduction of the Abbott *m*PLUS system with the capability for extended use of the amplification reagents, significantly increases the flexibility of the *m*2000 platform, enables laboratories to customize their workflow based on sample arrival pattern. The flexibility in sample batch size offered by *m*PLUS enables significant reductions in processing time. For HBV up to 30% (105 min) reduction in sample turnaround time was observed for batches of 12 when compared to batches of 24 while for CT/NG the ability to run a batch of 24 reduced the turnaround time by 83% or 54 min compared to a batch of 48 samples. Excellent correlation was observed between *m*PLUS and *m*2000 standard condition with all RealTi*m*e viral load assays evaluated in this study with a correlation r value of 0.998 for all assays tested. For the qualitative RealTi*m*e CT/NG assay the overall agreement between both conditions tested was >98% for CT and 100% for NG. Comparable precision was observed between the two conditions tested with all RealTi*m*e assays. The enhanced *m*PLUS capability provides the clinical laboratories with increased efficiencies to meet the increasingly stringent turnaround time requirements without increased costs associated with discarding partially used amplification reagents.

## Introduction

Molecular assays have become increasingly important for the detection of bacteria and viruses in clinical laboratories. Several criteria including the number of different tests performed and the diagnostic focus of the laboratory influence the choice of instrumentation used. Automation of nucleic acid extraction is an integral component of platform selection as it decreases the hands-on time per sample and improves assay performance including precision[1]. Diagnostic laboratories are under increasing pressure to improve and expand their services while reducing cost, and at the same time maintain the highest levels of quality in their service[2]. Many laboratories are challenged to maintain rapid turnaround time and to reduce costs while running high volume tests such as *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) as well as low volume esoteric tests such as Epstein Barr Virus (EBV) or Herpes Simplex Virus (HSV). Greater flexibility in sample batch size and reagent storage time is critical factors which can improve time to results while reducing waste, making laboratories more efficient and cost effective. The capabilities of molecular diagnostic instruments can have significant impact on laboratory resource allocation and staffing[3]. The two common platforms for HIV-1, Hepatitis C and B viral load testing are Abbott *m*2000 and Roche COBAS AmpliPrep/COBAS TaqMan. Several comparative workflow analyses have been performed for both platforms [4-6]. These studies highlight platform daily maintenance, sample throughput, laboratory tube flexibility, number of controls per batch and time to result.

The Abbott *m*2000 Plus (*m*PLUS) software feature allows laboratories to use the existing m2000 platform with the added benefit of extended use of the amplification reagents. The new software feature tracks the number of tests used as well as remaining tests within an amplification reagent pack. The introduction of *m*PLUS significantly increases the flexibility of the *m*2000 system enabling laboratories to adapt their workflow to actual sample arrival pattern.

This study evaluated process efficiencies and *m*2000 RealTi*m*e assay performance with the new *m*PLUS capabilities.

**Methods**

Currently, in order to optimize sample processing and reagent use, Abbott RealTi*m*e HIV-1 and HCV assays are utilized in 24, 48, 72 or 96 sample configurations, HBV in 24 or 48 sample configuration and the CT/NG assay is utilized as 48 or 96 samples in a single run. RealTi*m*e amplification reagents are stored at ≤ -10°C and are thawed at 2-8°C or 15-30°C prior to use. *m*PLUS allows amplification reagent packs containing prepared master mix to be stored at an assay-specific temperature (≤ -10°C or 2-8°C), capped and protected from light, for an assay-specific duration before a second use. The Internal Control (IC) for all assays may also be used again within an assay-specific duration if the vial remains capped at an assay-specific temperature until the second use. Amplification reagent packs and IC can be used a total of 2 times. *m*PLUS amplification reagents were used within 25 minutes after removal from storage (≤ -10°C or 2-8°C). Abbott RealTi*m*e HIV-1, HCV, HBV and CT/NG assays' performance under *m*PLUS conditions were evaluated by comparing precision, clinical correlation and linearity to the results obtained under standard *m*2000 RealTi*m*e assay conditions.

Studies were conducted using paired matched samples and reagents for both *m*PLUS and standard *m*2000 RealTi*m*e assay conditions. HIV-1, HCV and HBV positive samples were obtained from either PromedDx (Norton, Ma) or Northwest Biomedical (Everett, WA). Samples were tested the same day by m2000 and *m*PLUS with 2-8 C storage between runs. *m*2000 and *m*PLUS comparative studies were performed using the same instruments. Precision studies were performed across 3 instruments, 5 days and multiple operators. For CTNG, percent agreement between *m*PLUS and *m*2000 RealTi*m*e assay conditions was tested in 289 positive urine samples from males and females. For each quantitative viral load test evaluated, clinical specimens were identified or panels created from spiked patient samples or armored RNA to cover the dynamic range of the test. Correlation between *m*PLUS and *m*2000 RealTi*m*e assay conditions was evaluated in 107 HBV plasma samples (56 HBV positive patient specimens and 51 HBV samples prepared by spiking normal human plasma with HBV positive patient specimen), 124 HCV plasma samples (82 HCV positive patient specimens and 42 samples were prepared by spiking normal human plasma with HCV positive patient specimen or HCV armored RNA (Asuragen Inc., Austin TX)) and 108 HIV-1 plasma samples (70 HIV-1 positive specimens and 38 samples were prepared by spiking HIV-1 armored RNA (Asuragen Inc., Austin TX) into normal plasma).

*m*PLUS assay performance was evaluated for precision and linearity with panel members made from virus or armored RNA. Sensitivity was evaluated by creating high volume panels targeting viral concentrations of 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, 20.0 IU/mL for HBV, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0 IU/mL for HCV and 5, 10, 20, 30, 40, 50, 60, 75, 100 cps/mL for HIV-1. Thirty six replicates of panels were processed under *m*PLUS and *m*2000 RealTi*m*e assay conditions and sensitivity was established using probit analysis.

Instrument processing times to first result for the *m*PLUS system using the Abbott RealTi*m*e HIV-1, HCV, HBV and CT/NG assays with batch sizes of 8, 12, 24, 38, 48, 62, 72, 86 and 96 were measured by direct observation. Additional instrument process efficiencies were evaluated by measuring the minimum number of assay controls required for each sample processing size evaluated in this study and comparing this ratio (patient result: assay control) to those of the Roche COBAS AmpliPrep/ COBAS TaqMan instrument[7].

**Results**

Excellent correlation was observed between *m*PLUS and *m*2000 conditions with all RealTi*m*e viral load assays evaluated in this study (Figure 1). For the RealTi*m*e CT/NG assay, the overall agreement between both conditions tested was >98% for CT and 100% for NG (Table 1). For the RealTi*m*e HBV viral load assay, the correlation relationship between the *m*PLUS and *m*2000 conditions had a slope of 1.00 (r=0.998; 95% CI 0.99, 1.01) with a mean difference 0.08 log IU/ml (95% CI 0.06, 0.11). For the RealTi*m*e HCV viral load assay, the correlation relationship between the *m*PLUS and *m*2000 conditions had a slope of 0.99 (r=0.998; 95% CI 0.98, 1.00) with a mean difference of 0.00 log IU/ml (95% CI -0.02, 0.03). For the RealTi*m*e HIV-1 viral load assay the correlation relationship between the *m*PLUS and *m*2000 conditions had a slope 0.99 (r= 0.998; 95% CI 0.98,1.00) with a mean of differences is 0.01 log copies/ml (95% CI -0.01, 0.02). All results observed were well within the expected ranges for precision and sensativity as observed in literature and standard *m*2000 assay package inserts (Table 2 & 3). All RealTi*m*e viral load assays evaluated in this study were linear across their entire dynamic ranges under *m*PLUS and *m*2000 conditions. Also, evalution of assay sensitivity under *m*PLUS and standard *m*2000 conditions showed that extended use of activated mastermix (*m*PLUS condition) does not have a negative impact on sensitivity of any of the assays sensitivity equivalence (Table 3).

The flexibility in sample batch size offered by *m*PLUS enables significant reductions in processing time. For HBV up to 57% (154 min) reduction in sample turnaround time was observed for batches of 8 when compared to batches of 48 while for CT/NG the ability to run a batch of 8 reduced the turnaround time by 38% (54 min) compared to a batch of 48 samples (Figure 2). The ability to store and use amplification

reagents allows these time efficiencies to be achieved without significantly increasing cost per reportable result due to wasted amplification reagents. The requirement to only process three assay controls on each instrument run regardless of number of sample run size provides *m*2000 and *m*PLUS a high degree of efficiencey enabling as many as 31 patient results to be generated per assay control processed (93 samples/3 assay controls results in 31 patient results per control). *m*PLUS offers significant advantages in cost per reportable result comapred to the Roche COBAS AmpliPrep/COBAS TaqMan instrument which can only generate 7 patient results per assay control (Figure 3a-b).

**Discussion**

In this study the use of activated, stored reagents had no impact on Abbott RealTi*m*e precision and correlation of patient results for quantitative HIV-1, HBV and HCV assays (r=0.998). For the qualitative CTNG test the percent agreement between the two conditions were greater than 98%.

Introduction of the new *m*PLUS feature for the Abbott *m*2000 increases system flexibility by enabling laboratories to perform runs of any size, then store and reuse activated master mix in a subsequent run. The enhanced *m*PLUS capability provides the clinical microbiology and virology laboratories with the increased efficiencies to meet the increasingly stringent turnaround time requirements without increased costs associated with discarding partially used reagents. For small batches (<24) processing times have been reduced by 25%, thus improving turnaround time while reducing cost associated with wasted amplification reagent. For larger runs with batch sizes that are not multiples of 24 (>24 but <96), the *m*PLUS feature enables laboratories to process samples as they arrive, avoiding the need to carry over samples to the next day.

In addition to the use of activated reagents, other significant advantages in efficiency/costs per patient reportable result were seen with the Abbott *m*PLUS system when compared to the Roche COBAS AmpliPrep/COBAS TaqMan instrument based on the minimum number of controls needed to run various samples sizes on each instrument. The maximum ratio of patient sample to assay control possible with the Roche COBAS AmpliPrep/COBAS TaqMan is 7:1 for batches of 24 to 96 samples (7). In contrast, the ratio for the *m*2000 and *m*PLUS system ranges from 7:1 for batches of 24 samples, up to 31:1 (93 samples/3 assay controls results in 31 patient results per control) representing significant reductions in cost per reportable result for laboratories running batches greater than 24. This capability also translates into improved turnaround time as *m*2000 is able to process more samples in a given time period than the Roche COBAS AmpliPrep/COBAS TaqMan. Upon receipt of 100 samples into a laboratory, a single *m*2000 is able to process 93% of the samples in a standard 8hr shift and 7% of the samples would be

carried over to the next day while a single Roche COBAS AmpliPrep/COBAS TaqMan is able to process 84% of the samples in a standard 8hr shift and 16% of the samples would be carried over to the next day The reduction in cost per reportable result as well as improved turnaround time allows laboratories to expand their services and at the same time improve client satisfaction.

While pre-analytical requirements have not been addressed in this study, a study by Vallefuoco evaluated resources needed to manage laboratory workflow from bar coded laboratory tube to final result[8]. Roche COBAS AmpliPrep instrument accepts input samples in sealed tubes (S-tubes) and as such each sample is manually transferred from the primary laboratory tube to the S-tube as part of the pre-analytical sample handling process. Vallefuoco quantified this pre-analytical hands-on time to be approximately 2h 40min for 120 samples. This manual manipulation of samples introduces pipetting errors as well as risk associated with repetitive stress injury. On the other hand, the Abbott *m*2000 platform is capable of accepting primary laboratory tubes with tube diameter ranging from 11.5mm to 16mm and as such reducing the need for sample aliquoting or an independent pipetting station. In addition, the *m*2000 is capable of providing full and automated sample traceability of primary laboratory tubes by utilizing the platform's primary tube barcode scanner. This alone provides labor savings of approximately 1.3 minutes per sample processed. Instrument daily maintenance is another area of interest to laboratories when attempting to reduce hands-on time in order to improve staff and laboratory efficiency. A workflow study by Sloma et al, which included daily maintenance, concluded that the daily maintenance procedures for the Abbott *m*2000 required 8 min to complete, while a substantial portion of the hands-on time required to perform the initial Roche COBAS AmpliPrep/COBAS TaqMan run (30 of 46 min) was spent performing daily maintenance procedures[6]. These data are supported by Abbott and Roche instrument manuals which state that daily maintenance is 12-16min and 52-65min, respectively[7-9]. Following the publication of the Sloma et al. study, which described higher than expected levels of amplicon contamination with the docked COBAS AmpliPrep/COBAS TaqMan 96 systems, Roche issued supplemental "best practices" recommendations for periodic maintenance and cleaning of the instrument. This recommendation was, in addition to the standard daily maintenance, to be used in instances where sample or internal control quantitation inhibition was observed[10].

The results with *m*PLUS were comparable to the performance of the *m*2000 using standard test packs and provided cost savings from reuse of reagents, use of fewer controls and improved turnaround time by allowing tests to be performed on demand using smaller batch sizes.

**Aknowledgments:**

Authors are employees and shareholders of Abbott Laboratories; All authors reviewed and approved the final manuscript.

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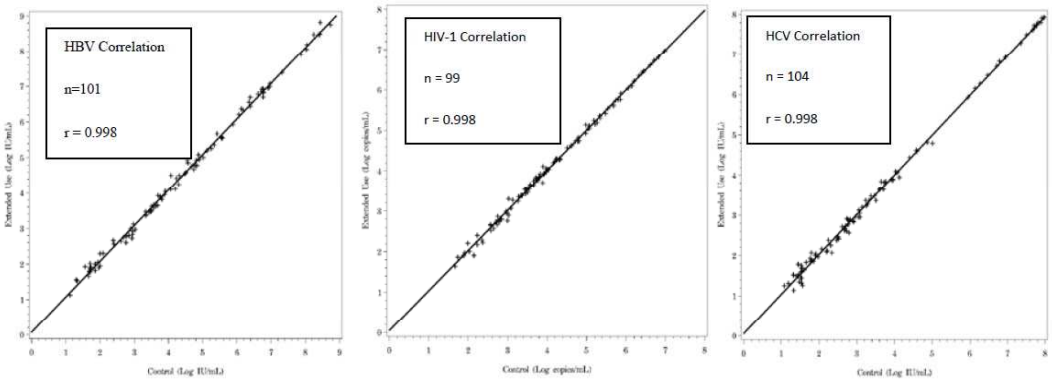
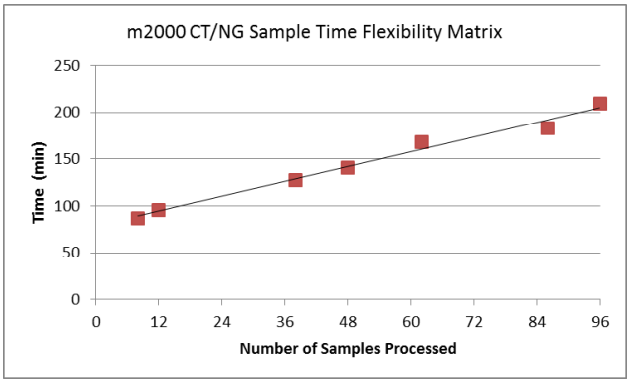
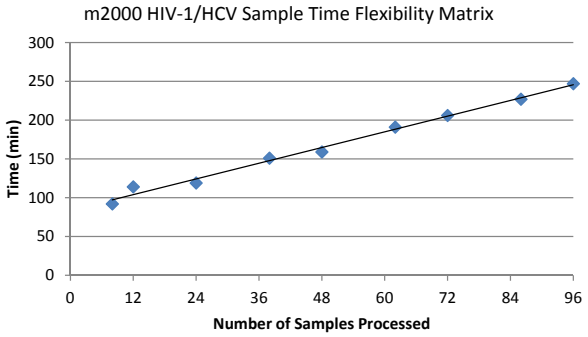



**Figure 1:** Correlation of Abbott RealTi*m*e assays between *m*PLUS and *m*2000. Regression coefficient (r) of 0.998 was observed among all viral load assays.





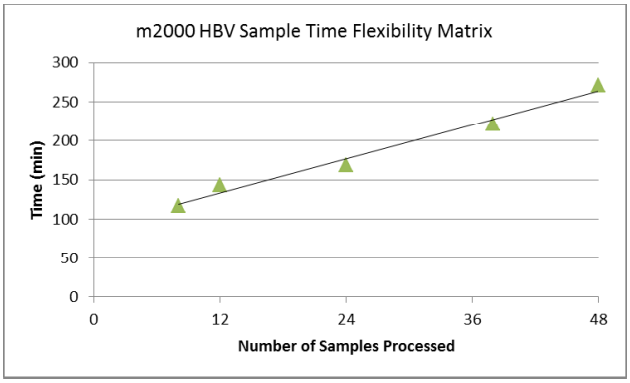



**Figure 2:** Instrument processing times to first result for the *m*PLUS system using the Abbott RealTi*m*e HIV-1, HCV, HBV and CT/NG assays with batch sizes of 8, 12, 24, 38, 48, 62, 72, 86 and 96.

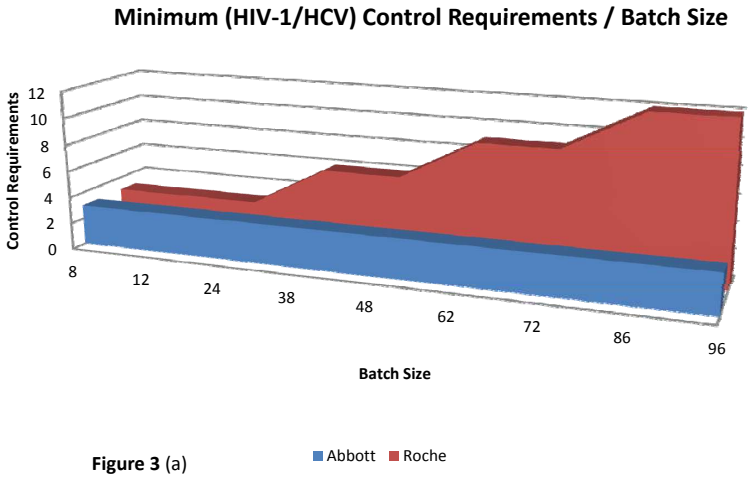



**Figure 3** (a)

**Figure 3** (a) Minimum control requirement per batch size between Abbott *m*2000 RealTi*m*e and Roche COBAS AmpliPrep/COBAS TaqMan. Note Abbott *m*2000 RealTi*m*e can process 96 samples in one run while Roche COBAS AmpliPrep/COBAS TaqMan would need to process two runs for the same number of specimens.

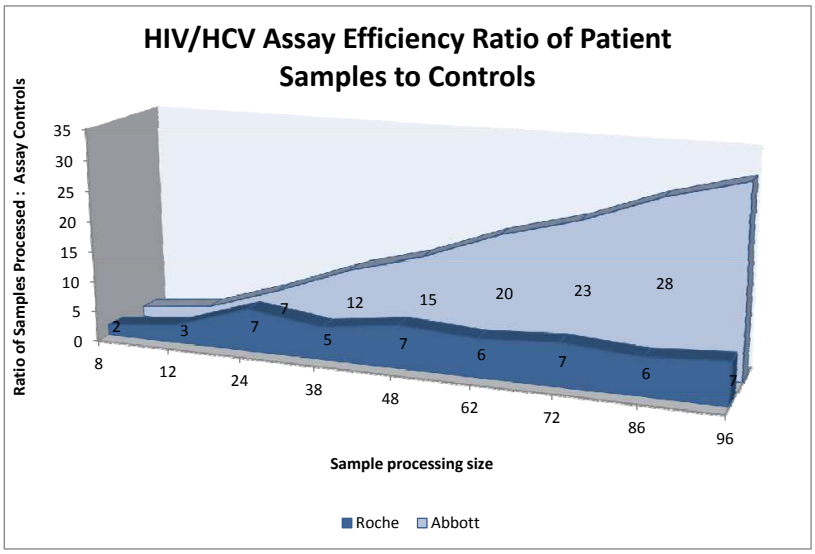



**Figure 3b**

**Figure 3** (b) Comparison of process efficiencies (Assay Control vs Patient Result) between Abbott *m*2000 RealTi*m*e and Roche COBAS AmpliPrep/COBAS TaqMan instruments. Note: Abbott *m*2000 RealTi*m*e can result up to 31 patient results per control, while Roche COBAS AmpliPrep/COBAS TaqMan can result up to 7 patient results per control.

| | Number of Tests CT | | | Number of Tests NG | | |
|---|---|---|---|---|---|---|
| | Total | Agreed | Agreement (%) | Total | Agreed | Agreement (%) |
| Assay Agreement | 279 | 274 | 98.2 | 279 | 279 | 100 |
| Negative Agreement | 208 | 205 | 98.6 | 265 | 265 | 100 |
| Positive Agreement | 71 | 69 | 97.2 | 14 | 14 | 100 |

**Table 1:** Percent Agreement of Abbott RealTi*m*e CT/NG Assay results between *m*PLUS and *m*2000.

| RealTime HCV Viral Load Precision | | | RealTime HIV-1 Viral Load Precision | | | RealTime HBV Viral Load Precision | | |
|---|---|---|---|---|---|---|---|---|
| Viral Load (log IU/mL) | n | Total SD | Viral Load (log cps/mL) | n | Total SD | Viral Load (log IU/mL) | n | Total SD |
| 1.10 | 57 | 0.16 | 1.45 | 27 | 0.16 | 1.37 | 60 | 0.35 |
| 2.05 | 57 | 0.09 | 2.11 | 56 | 0.18 | 2.15 | 60 | 0.10 |
| 2.98 | 57 | 0.05 | 3.07 | 56 | 0.08 | 3.31 | 57 | 0.08 |
| 3.96 | 57 | 0.05 | 4.05 | 56 | 0.06 | 4.33 | 60 | 0.07 |
| 2.12 | 57 | 0.09 | 5.07 | 55 | 0.05 | 5.32 | 60 | 0.07 |
| 3.03 | 57 | 0.06 | 6.09 | 56 | 0.04 | 6.40 | 60 | 0.07 |
| 4.00 | 57 | 0.06 | 7.59 | 56 | 0.06 | 7.40 | 60 | 0.07 |
| 5.01 | 56 | 0.06 | 2.10 | 54 | 0.13 | 8.51 | 60 | 0.06 |
| 6.04 | 54 | 0.05 | 3.04 | 56 | 0.08 | ND | ND | ND |
| 7.01 | 54 | 0.06 | 3.96 | 56 | 0.06 | ND | ND | ND |
| 8.18 | 45 | 0.05 | ND | ND | ND | ND | ND | ND |

**Table 2:** Abbott RealTime Assay Precision using *m*PLUS.

| | Sensitivity (95% CI) |
|---|---|
| Assay | Extended Use |
| RealTi*m*e HBV | 5.3 IU/mL (3.6 – 9.1 IU/mL) |
| RealTi*m*e HCV | 4.0 IU/mL (3.1 – 6.1 IU/mL) |
| RealTi*m*e HIV-1 | 40 cps/mL (33 – 51cps/mL) |

**Table 3:** Abbott RealTi*m*e assay limit of detection by probit analysis under *m*PLUS conditions.