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Impact of the New Abbott *m*PLUS Feature on Clinical Laboratory Efficiencies of Abbott RealTi*me* Assays for Detection of HIV-1, Hepatitis C Virus, Hepatitis B Virus, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae*

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Diagnostic laboratories are under increasing pressure to improve and expand their services. Greater flexibility in sample processing is a critical factor that can improve the time to results while reducing reagent waste, making laboratories more efficient and cost-effective. The introduction of the Abbott *m*PLUS feature, with the capacity for extended use of amplification reagents, significantly increases the flexibility of the *m*2000 platform and enables laboratories to customize their workflows based on sample arrival patterns. The flexibility in sample batch size offered by *m*PLUS enables significant reductions in processing times. For hepatitis B virus tests, a reduction in sample turnaround times of up to 30% (105 min) was observed for batches of 12 samples compared with those for batches of 24 samples; for *Chlamydia trachomatis*/*Neisseria gonorrhoeae* tests, the ability to run batches of 24 samples reduced the turnaround time by 83% (54 min) compared with that for batches of 48 samples. Excellent correlations between *m*PLUS and *m*2000 standard condition results were observed for all RealTi*me* viral load assays evaluated in this study, with correlation *r* values of 0.998 for all assays tested. For the qualitative RealTi*me C. trachomatis*/*N. gonorrhoeae* assay, the overall agreements between the two conditions tested were >98% for *C. trachomatis* and 100% for *N. gonorrhoeae*. Comparable precision results were observed for the two conditions tested for all RealTi*me* assays. The enhanced *m*PLUS capability provides clinical laboratories with increased efficiencies to meet increasingly stringent turnaround time requirements without increased costs associated with discarding partially used amplification reagents.

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 to expand their services while reducing costs and at the same time maintaining the highest levels of quality in their services (2). Many laboratories are challenged to maintain rapid turnaround time and to reduce costs while performing high-volume tests such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* tests as well as low-volume esoteric tests such as Epstein-Barr virus (EBV) and herpes simplex virus (HSV) tests. Greater flexibility in sample batch size and reagent storage time is a critical factor that can improve the time to results while reducing waste, making laboratories more efficient and cost-effective. The capabilities of molecular diagnostic instruments can have significant impacts on laboratory resource allocation and staffing (3). The two common platforms for HIV-1, hepatitis C virus (HCV), and hepatitis B virus (HBV) load testing are the Abbott *m*2000 and Roche COBAS AmpliPrep/COBAS TaqMan systems. Several comparative workflow analyses have been performed for these platforms (4–6). Those studies highlight platform daily maintenance, sample throughput, laboratory tube flexibility, the number of controls per batch, and the time to results.

The Abbott *m*2000 Plus (*m*PLUS) software feature allows laboratories to use the existing *m*2000 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 the tests remaining 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 patterns. This study evaluated process efficiencies and *m*2000 RealTi*me* assay performance with the new *m*PLUS capabilities.

MATERIALS AND METHODS

Currently, in order to optimize sample processing and reagent use, Abbott RealTi*me* HIV-1 and HCV assays are utilized in 24-, 48-, 72-, or 96-sample configurations, the HBV assay in 24- or 48-sample configurations, and the *C. trachomatis*/*N. gonorrhoeae* assay as 48 or 96 samples in a single run. RealTi*me* amplification reagents are stored at −10°C or below and are thawed at 2 to 8°C or 15 to 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 below or 2 to 8°C), capped, and protected from light for an assay-specific period before a second use. The internal control (IC) for all assays also may be used again within an assay-

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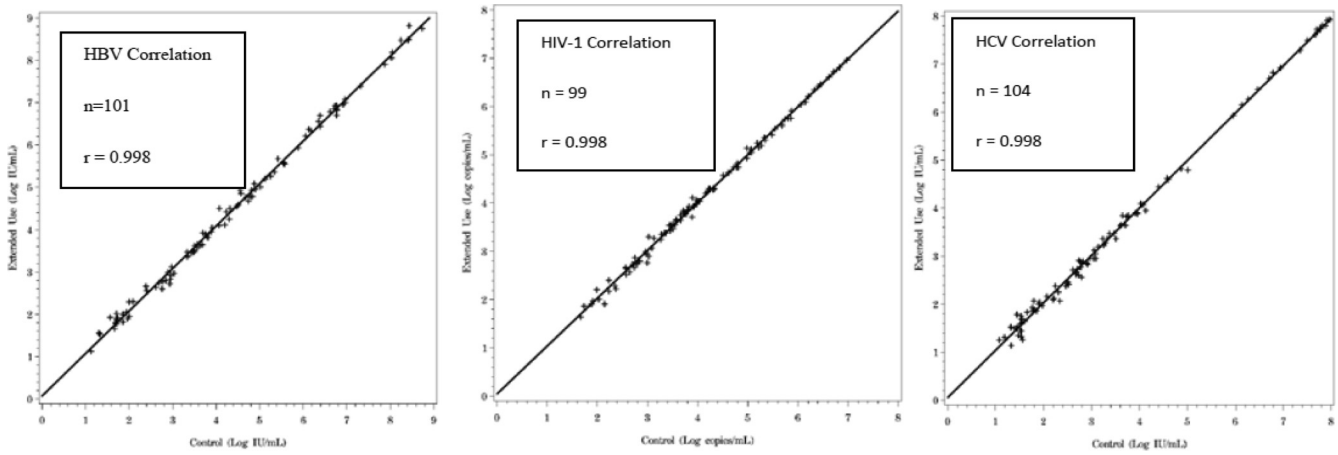

FIG 1 Correlation of Abbott RealTi*me* assay results obtained under *m*PLUS and *m*2000 conditions. Regression coefficients (*r*) of 0.998 were observed for all viral load assays.

specific period 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 min after removal from storage (−10°C or below or 2 to 8°C). The performance of Abbott RealTi*me* HIV-1, HCV, HBV, and *C. trachomatis*/*N. gonorrhoeae* assays under *m*PLUS conditions was evaluated by comparing precision, clinical correlation, and linearity with the results obtained under standard *m*2000 RealTi*me* assay conditions.

Studies were conducted using paired matched samples and reagents for the *m*PLUS and standard *m*2000 RealTi*me* assay conditions. HIV-1-, HCV-, and HBV-positive samples were obtained from either PromedDx (Norton, MA) or Northwest Biomedical (Everett, WA). Samples were tested on the same day with *m*2000 and *m*PLUS conditions, with storage at 2 to 8°C between runs. The *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 *C. trachomatis*/*N. gonorrhoeae*, percent agreement between *m*PLUS and *m*2000 RealTi*me* assay conditions was tested with 289 positive urine samples from male and female patients. For each quantitative viral load test evaluated, clinical specimens were identified or panels were created from spiked patient samples or armored RNA to cover the dynamic range of the test. Correlations between *m*PLUS and *m*2000 RealTi*me* assay conditions were evaluated with 107 HBV plasma samples (56 HBV-positive patient specimens and 51 HBV samples prepared by spiking normal human plasma with HBV-positive patient specimens), 124 HCV plasma samples (82 HCV-positive patient specimens and 42 samples prepared by spiking normal human plasma with HCV-positive patient specimens or HCV armored RNA [Asuragen, Inc., Austin TX]), and 108 HIV-1 plasma samples (70 HIV-1-positive specimens and 38 samples prepared by spiking normal plasma with HIV-1 armored RNA [Asuragen, Inc., Austin TX]).

*m*PLUS assay performance was evaluated for precision and linearity with panels 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, and 20.0 IU/ml for HBV, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, and 25.0 IU/ml for HCV, and 5, 10, 20, 30, 40, 50, 60, 75, and 100 cps/ml for HIV-1. Thirty-six replicates of panels were processed under *m*PLUS and *m*2000 RealTi*me* assay conditions, and sensitivities were established using probit analysis.

Instrument processing times to first results for the *m*PLUS feature using the Abbott RealTi*me* HIV-1, HCV, HBV, and *C. trachomatis*/*N. gonorrhoeae* assays with batch sizes of 8, 12, 24, 38, 48, 62, 72, 86, and 96 samples 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 correlations between *m*PLUS and *m*2000 conditions were observed for all RealTi*me* viral load assays evaluated in this study (Fig. 1). For the RealTi*me* *C. trachomatis*/*N. gonorrhoeae* assay, the overall agreement between the two conditions tested was >98% for *C. trachomatis* and 100% for *N. gonorrhoeae* (Table 1). For the RealTi*me* 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% confidence interval [CI], 0.99 to 1.01]), with a mean difference of 0.08 log IU/ml (95% CI, 0.06 to 0.11 log IU/ml). For the RealTi*me* 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 to 1.00]), with a mean difference of 0.00 log IU/ml (95% CI, −0.02 to 0.03 log IU/ml). For the RealTi*me* HIV-1 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 to 1.00]), with a mean difference of 0.01 log copies/ml (95% CI, −0.01 to 0.02 log copies/ml). All results observed were well within the expected ranges for precision and sensitivity, as observed in the literature and standard *m*2000 assay package inserts (Tables 2 and 3). All RealTi*me* viral load assays evaluated in this study were linear across their entire dynamic ranges under *m*PLUS and *m*2000 conditions. Also, evaluation of assay sensitivity under *m*PLUS and standard *m*2000 conditions showed that extended use of activated master mix (*m*PLUS

TABLE 1 Percent agreement of Abbott RealTi*me* *C. trachomatis*/*N. gonorrhoeae* assay results under *m*PLUS and *m*2000 conditions

Agreement	Results of tests for:					
	C. trachomatis			*N. gonorrhoeae*		
	Total no.	No. agreed	Agreement (%)	Total no.	No. agreed	Agreement (%)
Total assay	279	274	98.2	279	279	100
Negative	208	205	98.6	265	265	100
Positive	71	69	97.2	14	14	100

TABLE 2 Abbott RealTi*me* assay precision using *m*PLUS conditions

Results for RealTi*me* assay for[a]:								
HCV			HIV-1			HBV		
Viral load (log IU/ml)	*n*	Total SD (log IU/ml)	Viral load (log cps/ml)	*n*	Total SD (log cps/ml)	Viral load (log IU/ml)	*n*	Total SD (log IU/ml)
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

[a] SD, standard deviation; ND, not determined.

condition) did not have a negative impact on sensitivity for any of the assays (Table 3).

The flexibility in sample batch size offered by *m*PLUS enables significant reductions in processing time. For HBV tests, a reduction of up to 57% (154 min) in sample turnaround time was observed for batches of 8 samples, compared with batches of 48 samples; for *C. trachomatis*/*N. gonorrhoeae* tests, the ability to run a batch of 8 samples reduced the turnaround time by 38% (54 min), compared with batches of 48 samples (Fig. 2). The ability to store and then to use amplification reagents allows these time efficiencies to be achieved without significant increases in costs per reportable result due to wasted amplification reagents. The requirement to process only three assay controls for each instrument run regardless of the number of samples assayed provides *m*2000 and *m*PLUS a high degree of efficiency, enabling as many as 31 patient results to be generated per assay control processed (93 samples/3 assay controls yields 31 patient results per control). *m*PLUS offers significant advantages in costs per reportable result in comparison with the Roche COBAS AmpliPrep/COBAS TaqMan instrument, which can generate only 7 patient results per assay control (Fig. 3A and B).

DISCUSSION

In this study, the use of activated, stored reagents had no impact on Abbott RealTi*me* assay precision and the correlation of patient results for quantitative HIV-1, HBV, and HCV assays ($r = 0.998$). For qualitative *C. trachomatis*/*N. gonorrhoeae* tests, agreement between the two conditions was >98%.

Introduction of the new *m*PLUS feature for the Abbott *m*2000 system increases system flexibility by enabling laboratories to perform runs of any size and then store and reuse activated master

TABLE 3 Abbott RealTi*me* assay limit of detection, by probit analysis, under *m*PLUS conditions

Assay	Limit of detection (95% CI)
RealTi*me* HBV	5.3 IU/ml (3.6–9.1 IU/ml)
RealTi*me* HCV	4.0 IU/ml (3.1–6.1 IU/ml)
RealTi*me* HIV-1	40 cps/ml (33–51 cps/ml)

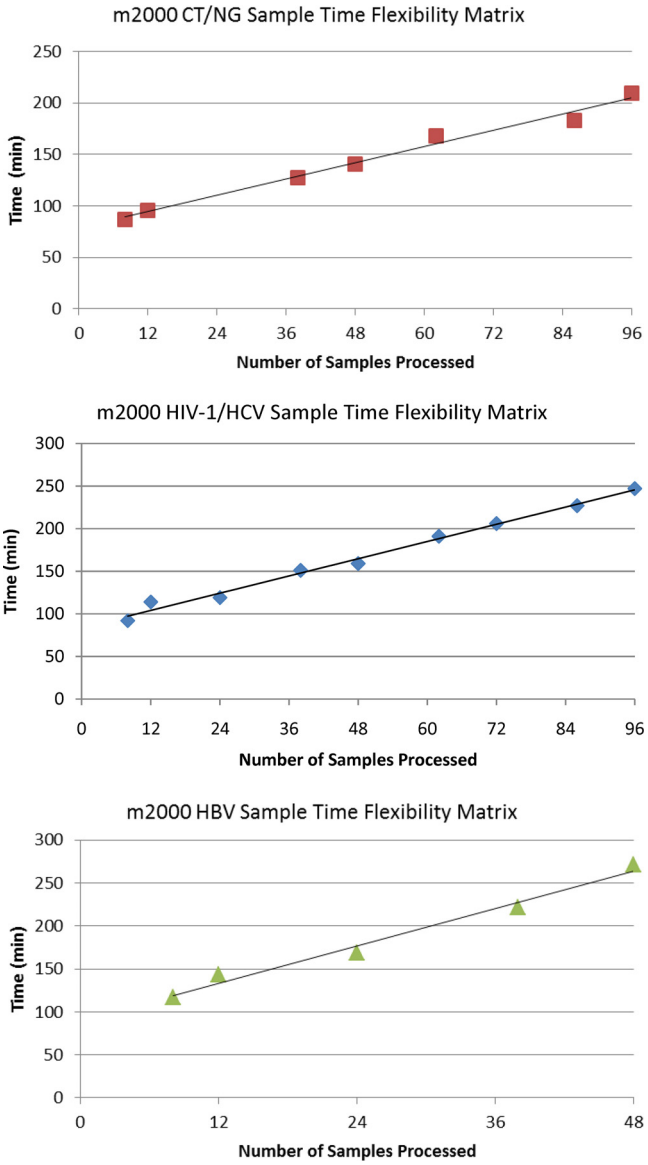

FIG 2 Instrument processing times to first result for the *m*PLUS feature using the Abbott RealTi*me* HIV-1, HCV, HBV, and *C. trachomatis*/*N. gonorrhoeae* (CT/NG) assays with batch sizes of 8, 12, 24, 38, 48, 62, 72, 86, and 96 samples.

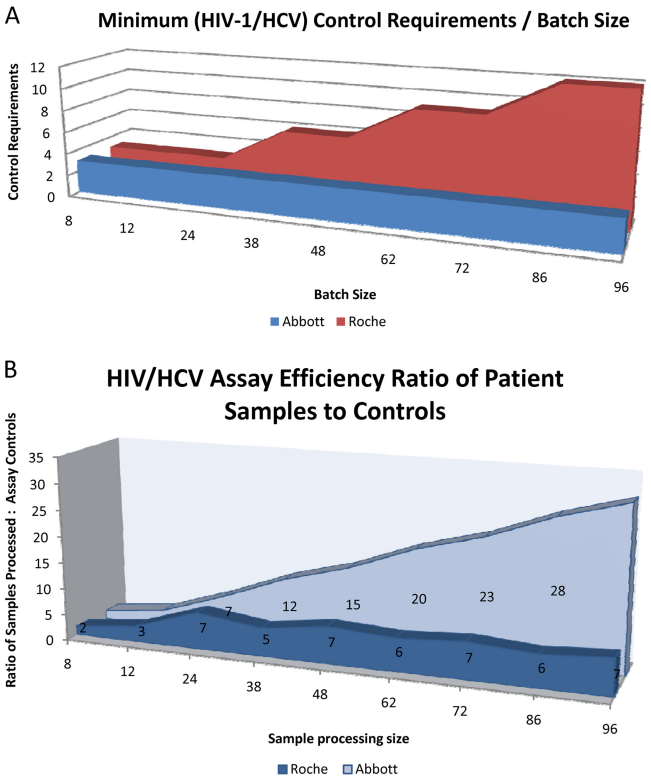

FIG 3 (A) Minimum control requirements with respect to batch size for the Abbott *m*2000 RealTi*m*e and Roche COBAS AmpliPrep/COBAS TaqMan systems. The Abbott *m*2000 RealTi*m*e system can process 96 samples in one run, while the Roche COBAS AmpliPrep/COBAS TaqMan system would need two runs to process the same number of specimens. (B) Comparison of process efficiencies (assay control versus patient result) for the Abbott *m*2000 RealTi*m*e and Roche COBAS AmpliPrep/COBAS TaqMan instruments. The Abbott *m*2000 RealTi*m*e system can yield up to 31 patient results per control, while the Roche COBAS AmpliPrep/COBAS TaqMan system can yield up to 7 patient results per control.

mix in a subsequent run. The enhanced *m*PLUS capability provides clinical microbiology and virology laboratories with increased efficiencies to meet increasingly stringent turnaround time requirements without increased costs associated with discarding partially used reagents. For small batches (<24 samples), processing times have been reduced by 25%, thus improving turnaround times while reducing costs associated with wasted amplification reagent. For larger runs with batch sizes that are not multiples of 24 (>24 samples but <96 samples), 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 and costs per reportable result were seen with the Abbott *m*PLUS system in comparison with the Roche COBAS AmpliPrep/COBAS TaqMan instrument, based on the minimum number of controls needed to run various sample sizes on each instrument. The maximum patient sample/assay control ratio possible with the Roche COBAS AmpliPrep/COBAS TaqMan is 7:1 for batches of 24 to 96 samples (7). In contrast, the ratios for the *m*2000 and *m*PLUS system range from 7:1 for batches of 24 samples up to 31:1 (93 samples/3 assay controls, yielding 31 patient results per control), representing significant reductions in costs per reportable result for laboratories running batches larger than 24 samples. This capability also translates into improved turnaround time, as the *m*2000 system is able to process more samples in a given time period than the Roche COBAS AmpliPrep/COBAS TaqMan system. Upon receipt of 100 samples into a laboratory, a single *m*2000 system is able to process 93% of the samples in a standard 8-h shift and 7% of the samples are carried over to the next day, while a single Roche COBAS AmpliPrep/COBAS TaqMan system is able to process 84% of the samples in a standard 8-h shift and 16% of the samples are carried over to the next day. The reduction in costs per reportable result and the improved turnaround time allow laboratories to expand their services and at the same time improve client satisfaction.

While preanalytical requirements have not been addressed in this study, a study by Vallefuoco et al. evaluated the resources needed to manage laboratory workflow from the bar-coded laboratory tubes to the final results (8). The Roche COBAS AmpliPrep instrument accepts input samples in sealed tubes (S-tubes), and each sample is manually transferred from the primary laboratory tube to the S-tube as part of the preanalytical sample-handling process. Vallefuoco et al. quantified this preanalytical hands-on time as approximately 2 h 40 min for 120 samples. This manual manipulation of samples introduces pipetting errors as well as risks associated with repetitive stress injuries. On the other hand, the Abbott *m*2000 platform is capable of accepting primary laboratory tubes with tube diameters ranging from 11.5 mm to 16 mm, thus reducing the need for sample aliquoting or an independent pipetting station. In addition, the *m*2000 system is capable of providing full automated sample traceability of primary laboratory tubes by utilizing the platform's primary tube bar-code scanner. This alone provides labor savings of approximately 1.3 min per sample processed. Daily maintenance of instruments is another area of interest to laboratories 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 system 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 the Abbott and Roche instrument manuals, which state that daily maintenance requires 12 to 16 min and 52 to 65 min, respectively (7–9). Following the publication of the study by Sloma et al., 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. These recommendations were to be used, in addition to the standard daily maintenance, in instances in which sample or internal control quantitation inhibition was observed (10). The results with *m*PLUS were comparable to the performance of the *m*2000 system using standard test packs; *m*PLUS provided cost savings from reuse of reagents and use of fewer controls and improved turnaround times by allowing tests to be performed on demand, using smaller batch sizes.

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