Received: 23 June 2023 | Accepted: 28 October 2023

DOI: 10.1002/jmv.29223

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Performance evaluation of fully automated cobas® 6800 CMV PCR for the detection and quantification of cytomegalovirus DNA in neonatal urine and saliva, and adult urine, saliva, and vaginal secretion

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Funding information
Merck Sharp & Dohme,
Grant/Award Number: MISP58414

Abstract

Laboratory testing for cytomegalovirus (CMV) in bodily fluids is essential to manage congenital and prenatal CMV infection. The rapid and fully automated cobas® CMV PCR is approved only for the testing of plasma in transplant patients. To evaluate the performance of the cobas® CMV to detect and quantify CMV DNA in neonatal and adult female urine, saliva, and vaginal secretion, the limit of detection (LoD), limit of quantification (LoQ), imprecision, linearity, PCR efficiency, bias, analytical specificity, cross-reactivity, and cross-contamination of the cobas® CMV for urine, saliva, and vaginal secretion was determined. The performance of the assay was evaluated prospectively with two laboratory-developed PCR assays using neonatal and adult urine, saliva swabs, and vaginal swabs. The LoD and LoQ were 31 and 100 IU/mL, respectively, for urine, and 81 and 100 IU/mL, respectively, for vaginal secretion. The LoD and LoQ for saliva were the same (200 IU/mL). The cobas® CMV was precise (coefficient of variation ≤10%), linear ($R^2 \geq 0.995$), and efficient (1.07 and 1.09) between 100 and 250,000 IU/mL for the sample types. The bias and analytical specificity was <±0.30 $\log_{10}$ IU/mL and 100%, respectively. Cross-reactivity with non-CMV pathogens was not detected. Cross-contamination rate was 0.28%. The diagnostic accuracy, sensitivity, and specificity of the cobas® CMV for neonatal urine and saliva were ≥95.0%, ≥93.3%, and ≥90.4%, respectively. The overall percent agreement for adult urine, saliva, and vaginal secretion was 86.6%, 94.5%, and 89.4%, respectively. Taken together, the cobas® CMV demonstrated acceptable analytical and diagnostic performance, and is suitable for routine diagnostic laboratory investigation of CMV infection in neonates and adults.

KEYWORDS
cytomegalovirus, PCR, saliva, urine, vaginal secretion

Cassie F. Pope and David Carrington are co-senior authors and contributed equally to this study.

This work was presented in part at the 33rd European Congress of Clinical Microbiology and Infectious Diseases, Copenhagen, 15–18 April 2023.

1 | INTRODUCTION

Human cytomegalovirus (CMV) is the most common congenital viral infection and a leading cause of sensorineural hearing loss and neurodevelopmental disability in children.[1] Worldwide, congenital CMV (cCMV) causes not only long-term suffering in affected families, but also imposes a substantial economic burden on health and social care systems.[2] In pregnant women, CMV shedding in bodily fluids is associated with miscarriages and vertical transmission.[3–7]

Rapid laboratory testing for CMV in neonatal urine and saliva is recommended to diagnose cCMV and guide clinical management.[8] In newborns, rapid detection of CMV in urine and saliva may also facilitate a large-scale screening for cCMV.[9–11] The need to improve CMV diagnostics to support timely patient management and universal screening has been reported.[12,13] In pregnant women, testing for CMV shedding in urine, saliva, and vaginal secretion not only allows the detection of maternal infection and prediction of the risks of vertical transmission, but may also support early discussion for further investigation and potential medical referral.[5,6,14]

Until very recently, a rapid, fully automated, and high throughput CMV test to support clinicians' decision-making and patient management for congenital and prenatal CMV was not available. As a result, a myriad of manual and semiautomated extraction methods, amplification platforms, and laboratory-developed or modified commercial assays were used by laboratories to detect and quantify CMV DNA in urine, saliva, and vaginal secretion.[3–7,9–11] The methodological variations partially have limited the portability and comparison of PCR results.[14–16] In addition, the inefficient laboratory processes and long test turnaround time have contributed to suboptimal patient care.[12] For laboratories that do not have access to a locally validated test (such as ours), samples were referred to reference laboratories for testing which resulted in further delays.

The cobas® 6800 is a rapid and fully automated sample-to-result high throughput PCR system that was implemented in our laboratory in 2016 for the detection and quantification of viral load.[17] The cobas® 6800 and assays were developed to improve laboratory efficiency, test turnaround times, and analytical and clinical performance.[18] One of the implemented tests was cobas® CMV. However, the assay is FDA-approved and IVDR-certified only for the detection and quantification of CMV DNAemia in transplant patients, which impedes its use for nonplasma samples to support a timely management of congenital and prenatal CMV infection unless its fitness for intended purpose has been validated and evaluated.[19,20] Therefore, the aim of this study was to validate and evaluate the diagnostic performance of the cobas® CMV assay for neonatal urine and saliva, and adult female urine, saliva, and vaginal secretion, to facilitate a rapid, accurate and efficient laboratory detection and quantification of CMV DNA.

2 | MATERIALS AND METHODS

2.1 | CMV PCR assays

cobas® CMV PCR was performed on the cobas® 6800 instrument in line with the manufacturer's instructions (Roche Diagnostics). Briefly, samples (urine and vaginal swabs preserved in cobas® PCR media [CPM] [Roche Diagnostics], and saliva swabs preserved in eNAT® PCR media [eNAT] [Copan Italia SpA, Italy]) were loaded directly onto the instrument following homogenization and removal of swabs. Further manual intervention of the testing process was not required. The instrument performs fully automated sample extraction, PCR amplification, and result analysis using proprietary algorithms. To evaluate the performance of the cobas® CMV with ISO 15189-accredited PCR assays, samples were tested also by reference laboratory M (within 20 h of receipt, urine and vaginal swabs) or reference laboratory R (within 5 days of receipt, saliva swabs). Samples were stored at 2–8°C at the respective reference laboratory before testing. The reference laboratories were blinded to the cobas® CMV results. The PCR protocol, workflow, and turnaround time for the cobas® and reference laboratory assays are summarized in Supporting Information: Table S1.

2.2 | Collection of samples

For the validation of the cobas® CMV, nonmenstrual and CMV PCR negative urine and vaginal swabs were collected from five nonpregnant female volunteers as described previously.[21] Urine preserved in CPM was prepared by adding 5 mL urine to 4.3 mL CPM. CMV PCR negative saliva fluids and swabs were collected and prepared from three of the five aforementioned volunteers as described previously.[21] For the cross-reactivity experiment, self-collected saliva swabs were obtained from four of the five volunteers. All samples were homogenized before being tested or pooled for spiking experiments.

2.3 | Limit of detection (LoD)

Eight CMV titers (0–90 IU/mL, urine and vaginal secretion preserved in CPM; 0–250 IU/mL, saliva preserved in eNAT) were prepared using the 1st WHO standard for CMV.[22] Samples were tested in line with the Clinical and Laboratory Standards Institute (CLSI) EP17-A2 guidance.[23] The procedural variations are described in Supporting Information: Tables S2–S4.

2.4 | Bias, imprecision, limit of quantification (LoQ), linearity, and PCR efficiency

Eight CMV titers (20–250,000 IU/mL) in urine, saliva, and vaginal secretion preserved in PCR media were prepared using the 1st WHO

standard for CMV.[22] Samples were tested in line with the CLSI EP17-A2, EP15-A3, and EP05-A3 guidance.[23–25] The procedural variations are described in Supporting Information: Tables S5–S7.

2.5 | Analytical specificity

CMV DNA negative urine, saliva, and vaginal secretion preserved in PCR media was tested with eight cobas® CMV reagent lots.

2.6 | Cross-reactivity

Cross-reactivity was determined in line with CLSI MM03 guidance by testing 32 viruses, 11 bacteria, *Trichomonas vaginalis*, and *Pneumocystis jirovecii* (Supporting Information: Tables S8–S10) with CMV DNA positive (at 1950 IU/mL) and CMV DNA negative urine, saliva, and vaginal secretion preserved PCR media.[26]

2.7 | Cross-contamination

CMV DNA negative cobas® Diluent and urine, saliva, and vaginal secretion preserved in PCR media was tested whenever the cobas® CMV PCR was performed. The negative samples were placed randomly among high titers CMV samples.

2.8 | Performance evaluation of the cobas® CMV for neonatal urine and saliva

The evaluation was performed prospectively by testing neonatal urine and/or saliva swabs submitted to the diagnostic laboratory between 2020 and 2023 for CMV PCR as part of the clinical investigation of, or follow-up for, cCMV infection from three acute tertiary referral hospitals in London (Croydon University Hospital, Kingston Hospital [KH], and St George's Hospital [SGH]). Seven neonatal saliva swabs submitted between April and July 2021 for the investigation of a possible cCMV infection as part of the *Cytomegalovirus Shedding Characteristics in Pregnant Women (cCHIPS)* study (NCT04021628) were also included.[27]

2.9 | Performance evaluation of the cobas® CMV for adult urine, saliva, and vaginal secretion

The evaluation was performed prospectively by testing pregnant and nonpregnant adult female urine, saliva swabs, and/or vaginal swabs collected between 2019 and 2023 for the diagnostic investigation of, or follow-up for, maternal CMV infection from two hospitals (KH and SGH), cobas® CMV assay validation, and cCHIPS study.

2.10 | Data analysis

CMV DNA titer was $\log_{10}$-transformed before analysis. Data were summarized as mean ± SD, or median, and the minimum and maximum range, as appropriate. LoD was estimated using probit regression at 95% probability.[23] For the determination of bias, the difference between the measured and nominal CMV titers was determined, using a goal of ≤±0.30 $\log_{10}$ IU/mL acceptable error.[24] Imprecision was determined for the repeatability and intermediate precision conditions of measurement and expressed as coefficient of variation (CV).[25] The LoQ was the lowest titer with a hit rate of 100%, CV ≤ 10%, and ≤0.50 $\log_{10}$ IU/mL total analytical error (TAE).[23] Linearity was assessed by regression and Kolmogorov–Smirnov-CUSUM test, using a goal of ≤0.50 $\log_{10}$ IU/mL allowable difference.[28] PCR efficiency was determined with the formula $E = [10\text{^}(-1/\text{slope})]-1$.[29] Analytical specificity and cross-contamination rate with 95% confidence interval (CI) was determined. For the performance evaluation of the cobas® CMV for neonatal urine and saliva, the diagnostic accuracy, sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio (LR+), and negative likelihood ratio (LR-) with 95% CI of the cobas® CMV was determined.[30] For adult samples, because currently there is neither a consensus definition of CMV "shedding", nor an established reference assay for the detection and interpretation of CMV DNA in urine, saliva, and vaginal secretion, the performance of the cobas® CMV, using reference laboratory M and R assays as comparators, was reported as overall percent agreement, positive percent agreement (PPA), and negative percent agreement (NPA) with 95% CI.[30] Data analysis was performed using Analyse-it software (Analyse-it Software Ltd). p values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | LoD

In total, 15 replicate was tested for each CMV titer (Supporting Information: Tables S2–S4). The LoD was 30.87 (95% CI 19.86–47.98), 200.0 (95% CI 172.5–237.0), and 80.58 (95% CI 50.04–129.80) IU/mL for urine, saliva, and vaginal secretion, respectively (Table 1).

3.2 | Bias, imprecision, LoQ, linearity, and PCR efficiency

Between 100 and 250,000 IU/mL, the detection and quantification of CMV DNA in urine, saliva, and vaginal secretion was accurate. At these titers, the bias between the measured and nominal titers was <±0.30 $\log_{10}$ IU/mL (Table 1). The detection and quantification of CMV DNA was less reliable at low titers (≤50 IU/mL) (Supporting Information: Tables S5–S7). There was a satisfactory imprecision for all sample types; for CMV titers ≥1000 IU/mL, the CVs for repeatability and intermediate precision were ≤5%, and for titers <1000 IU/mL, the CVs for repeatability

TABLE 1 Analytical performance characteristics of the cobas® CMV for urine, saliva, and vaginal secretion.

Characteristic	Matrix			Supporting Information
	Urine	Saliva	Vaginal secretion	
LoD (95% CI) (IU/mL)	30.87 (19.86–47.98)	200.0 (172.50–237.0)	80.58 (50.04–129.80)	Tables S2–S4
Bias[a] (Log_{10} IU/mL)	−0.10 to 0.06	−0.19 to 0.03	−0.27 to −0.11	Tables S5–S7
Imprecision	For titers ≥1000 IU/mL, the CVs for repeatability and intermediate precision were ≤5%. For titers <1000 IU/mL, the CVs for repeatability and intermediate precision (if quantifiable) were ≤10%.			Tables S5–S7
LoQ (IU/mL) (TAE, Log_{10} IU/mL)	100 (0.25)	200 (0.27)	100 (0.34)	Tables S5–S7
Linear range (IU/mL)	100–250,000	200–250,000	100–250,000	Figure S1
Linearity				
R^2 (KS-CUSUM's p)	0.998 ($p = 0.61$)	0.996 ($p = 0.61$)	0.995 ($p = 0.91$)	Figure S1
Y-intercept (95% CI)	0.07 (−0.03 to 0.16)	0.06 (−0.08 to 0.19)	−0.05 (−0.21 to 0.11)	
Slope (95% CI)	0.97 (0.94–0.99)	0.95 (0.92–0.99)	0.96 (0.92–1.00)	
PCR efficiency	1.07	1.09	1.07	
Analytical specificity (%) (95% CI)	100 (94.8–100)	100 (94.8–100)	100 (94.8–100)	
Cross-reactivity[b]	Not detected	Not detected	Not detected	Tables S8–S10
Cross-contamination (%) (95% CI)	0.28 (0.01–1.57)			

Abbreviations: CI, confidence interval; CV, coefficient of variation; KS-CUSUM's *p*, *p* value determined using Kolmogorov-Smirnov-CUSUM test; LoD, limit of detection; LoQ, limit of quantification; TAE, total analytical error.

[a]Bias for CMV titers between 100 and 250,000 IU/mL.

[b]Cross-reactivity with non-CMV pathogens include 32 viruses, 11 bacteria, *Trichomonas vaginalis*, and *Pneumocystis jirovecii*.

and intermediate precision (if quantifiable) were ≤10% (Table 1). Using the criteria of a 100% hit rate, CV ≤10% and ≤0.50 log_{10} IU/mL TAE, the LoQ was designated as 100 IU/mL for urine and vaginal secretion. For saliva, as the LoD was 200 IU/mL, the LoQ was designated as 200 IU/mL. The cobas® CMV was found to be linear and efficient between 100 and 250,000 IU/mL for all sample types. At these titers, the coefficient of determination and PCR efficiency were 0.998 and 1.07, respectively, for urine, 0.996 and 1.09 for saliva, and 0.995 and 1.07 for vaginal secretion (Table 1, Supporting Information: Figure S1).

3.3 | Analytical specificity

Sixty-nine vials of each sample type were tested with eight cobas® CMV reagent lots. All samples tested negative for CMV DNA. The analytical specificity of the cobas® CMV for each sample type was 100% (95% CI 94.8–100.0).

3.4 | Cross-reactivity

A molecular interference of the cobas® CMV with non-CMV pathogens (Supporting Information: Tables S8–S10) was not detected (Table 1). The mean CMV titer recovered from the theoretical input of 3.29 log_{10} IU/mL by cobas® CMV was 3.13 (range 2.96–3.25), 3.00 (range 2.76–3.11), and 3.03 (range 2.87–3.12) log_{10} IU/mL for urine, saliva, and vaginal secretion, respectively (Supporting Information: Table S10).

3.5 | Cross-contamination

CMV DNA negative cobas® Diluent ($n = 146$) and urine, saliva, and vaginal secretion ($n = 207$) was tested between 2019 and 2023. All but one (cobas® Diluent, <34.5 IU/mL) were CMV DNA negative, resulting in a cross-contamination rate of 0.28% (95% CI 0.01–1.57).

3.6 | Performance evaluation of the cobas® CMV for neonatal urine and saliva

One hundred and forty-eight urine and 100 saliva swabs from 25 and 12 neonates, respectively, were tested by the cobas® CMV in parallel with reference laboratory PCR assays (Supporting Information: Table S11). As the cobas® CMV was not validated for the testing of urine and saliva swabs, only the reference laboratory PCR results were reported to clinicians for patient management. The most common indications for a CMV PCR request were "congenital

CMV", "intrauterine growth restriction", "suspected sepsis" (urine and saliva), "premature baby" (urine), and "failed newborn hearing test" (saliva). Overall, the performance of the cobas® CMV was excellent (Table 2). The diagnostic accuracy, sensitivity, and specificity were 96.0% (95% CI 91.4–98.1), 99.0% (95% CI 94.3–99.8) and 90.4% (95% CI 79.4–95.8), respectively, for urine, and 95.0% (95% CI 88.8–97.9), 93.3% (95% CI 85.3–97.1) and 100.0% (95% CI 86.7–100.0), respectively, for saliva swabs. The LR+ for urine (10.29, 95% CI 4.80–23.69) indicated that the cobas® CMV may be useful to identify a CMV infection when the result is detected, and the LR- (0.01, 95% CI 0.00–0.06, urine; 0.07, 95% CI 0.03–0.15, saliva swabs) may be sufficiently small to exclude an infection when the result is not detected. Reference laboratory M and R assays detected CMV DNA in 1 urine and 5 saliva swabs, respectively, that were not detected by the cobas® assay, whilst the cobas® assay detected CMV DNA in 5 urine samples that were not detected by the reference laboratory M assay. All 11 samples had low CMV titers (Table 2).

TABLE 2 Performance of the cobas® CMV for neonatal urine and saliva.

	LDT PCR (Laboratory M) Urine		LDT PCR (Laboratory R) Saliva	
	Detected	Not detected	Detected	Not detected
cobas® CMV				
Detected	95[a]	5[d]	70[b]	0
Not detected	1[c]	47	5[e]	25
Diag. accuracy (95% CI)	96.0 (91.4–98.1)		95.0 (88.8–97.9)	
Diag. sensitivity (95% CI)	99.0 (94.3–99.8)		93.3 (85.3–97.1)	
Diag. specificity (95% CI)	90.4 (79.4–95.8)		100.0 (86.7–100.0)	
PPV (95% CI)	95.0 (89.2–97.8)		100.0 (94.9–100.0)	
NPV (95% CI)	97.9 (87.0–99.7)		83.3 (68.2–92.1)	
LR+ (95% CI)	10.29 (4.80–23.69)		–	
LR- (95% CI)	0.01 (0.00–0.06)		0.07 (0.03–0.15)	

Abbreviations: CI, confidence interval; LDT, laboratory-developed test; LR-, likelihood ratio negative; LR+, likelihood ratio positive; NPV, negative predictive value; PPV, positive predictive value.

[a]Consists of 28 low positive (10^1 to 10^2 copies/mL), 43 medium positive (10^3 to 10^4 copies/mL), and 24 high positive (≥10^5 copies/mL) samples quantified by Laboratory M assay.

[b]Consists of 24 samples with cycle threshold (Ct) ≥32.0 (median 34.4, range 32.3–36.2), 25 samples with Ct ≥ 28.0 but <32.0 (median 30.1, range 28.2–31.9), and 21 samples with Ct < 28.0 (median 25.4, range 21.0–27.9) detected by Laboratory R assay.

[c]CMV DNA 62 copies/mL (LDT PCR, Lab M).

[d]CMV DNA < 34.5 IU/mL (same value for all five samples; cobas® CMV).

[e]Median Ct 37.6, range 35.2–38.8 (LDT PCR, Lab R).

3.7 | Performance evaluation of the cobas® CMV for adult urine, saliva, and vaginal secretion

One hundred and forty-nine urine, 145 saliva swabs, and 161 vaginal swabs from 36, 60, and 43 adult females, respectively, were tested by the cobas® CMV in parallel with reference laboratory PCR assays (Supporting Information: Tables S12 and S13). Of these samples, 8 urine, 5 saliva swabs, and 5 vaginal swabs were collected from four patients for the diagnostic investigation of, or follow up for, CMV infection in pregnancy. As the detection of CMV in these samples to support patient consultation was not part of a routine clinical practice, CMV PCR results were reported with qualification to requesting clinicians following an informed consent of the non-approved use of the test. Overall, the cobas® CMV demonstrated satisfactory agreement with reference laboratory assays (Table 3). The PPA was 98.9% (95% CI 94.0–99.8), 91.4% (95% CI 83.9–95.6), and 88.8% (95% CI 81.4–93.5) for urine, saliva swabs, and vaginal swabs, respectively. The cobas® assay demonstrated a lower NPA with the reference laboratory M assay for urine samples (67.8% [95% CI 55.1–78.3]) due to the detection of 19 (32%) low titer CMV samples by the cobas® assay that were missed by the reference laboratory assay. The cobas® assay also detected 5 (9%) low titer vaginal swabs that would otherwise be missed. However, the cobas® assay did not detect low titer CMV in 1 (1%) urine, 8 (9%) saliva swabs, and 12 (11%) vaginal swabs (Table 3).

4 | DISCUSSION

Laboratory testing for CMV infection in neonatal urine and saliva is essential to diagnose and manage cCMV.[8] In pregnant women, as CMV shedding in bodily fluids is correlated with miscarriages and vertical transmission, laboratory testing for CMV may be utilized to support patient consultation.[3–7,14] We found the cobas® CMV PCR demonstrated the speed, accuracy, and efficiency required for diagnostic laboratory detection and quantification of CMV DNA in urine, saliva, and vaginal secretion. If implemented, the assay may reduce the delay in initiating antiviral therapy for cCMV, and support a timely management of CMV infection in neonates and adults.[8,12,13]

In the context of diagnosing cCMV in neonates, a sensitive and specific test is required. The cobas® assay was found to be fit for this purpose. The discordant samples at low CMV titers were detected only by the cobas® or reference assay, and could be due to a laboratory contamination and/or differences in assay sensitivity. The discordant results may also be due to PCR stochastic variations or subsampling error inherent to low template concentration.[31] Due to a limited sample volume and requirement for a higher CMV titer for sequencing (≥10^4 IU/mL), the samples were not retested to resolve the discrepancy. It is worth noting that the practice of discrepant resolution, that is, revising original data following a repeat testing of discordant samples, has recently been questioned.[32] The significance of the discordant results is uncertain, but is unlikely to be a major concern as CMV shedding in urine and saliva is often at high levels in cCMV, and the cobas® CMV is unlikely

TABLE 3 Performance of the cobas® CMV for adult urine, saliva, and vaginal secretion.

	LDT PCR (Laboratory M)				LDT PCR (Laboratory R)	
	Urine		Vaginal secretion[a]		Saliva	
	Detected	Not detected	Detected	Not detected	Detected	Not detected
cobas® CMV						
Detected	89[b]	19[f]	95[c]	5[h]	85[d]	0
Not detected	1[e]	40	12[g]	49	8[i]	52
OPA (95% CI)	86.6 (80.2–91.1)		89.4 (83.7–93.3)		94.5 (89.5–97.2)	
PPA (95% CI)	98.9 (94.0–99.8)		88.8 (81.4–93.5)		91.4 (83.9–95.6)	
NPA (95% CI)	67.8 (55.1–78.3)		90.7 (80.1–96.0)		100.0 (93.1–100.0)	

Abbreviations: CI, confidence interval; LDT, laboratory-developed test; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement.

[a]Two samples excluded from analysis due to a run control failure on the cobas® 6800.

[b]Consists of 31 low positive (10^1–10^2 copies/mL), 50 medium positive (10^3–10^4 copies/mL), and 8 high positive (≥10^5 copies/mL) samples quantified by Laboratory M assay.

[c]Consists of 68 samples with a cycle threshold (Ct) ≥32.0 (median 35.9, range 32.4–40.9), 16 samples with Ct ≥ 28.0 but <32.0 (median 29.0, range 28.5–31.9), and 11 samples with Ct < 28.0 (median 26.6, range 25.9–27.7) detected by Laboratory M assay.

[d]Consists of 46 samples with Ct ≥ 32.0 (median 34.8, range 32.6–39.1), 20 samples with Ct ≥ 28.0 but <32.0 (median 30.8, range 28.0–31.5), and 19 samples with Ct < 28.0 (median 26.9, range 25.4–27.9) detected by Laboratory R assay.

[e]CMV DNA 296 copies/mL (LDT PCR, Lab M).

[f]Median CMV DNA <34.5 IU/mL, range <34.5–367 IU/mL (cobas® CMV).

[g]Median Ct 38.5, range 34.8–40.8 (LDT PCR, Lab M).

[h]Median CMV DNA 150 IU/mL, range 93–174 IU/mL (cobas® CMV).

[i]Median Ct 36.5, range 35.4–38.1 (LDT PCR, Lab R).

to miss a detection due its high sensitivity.[33,34] Nevertheless, as low-level shedding is possible, clinicians should repeat sampling to confirm positive results or if results are inconsistent with clinical signs and/or history.[8,33–36] To avoid confusion with low-level false positives, saliva swabs should be taken ≥1 h after breastfeeding.[36,37] The importance of optimal swab sampling and using validated sample collection devices and transport media to reduce the variability of results and the risk of false negatives and PCR inhibition cannot be overemphasized.[9,21,38–40] Diagnostic laboratories should also optimize preanalytical handling of samples to avoid false negatives due to CMV DNA degradation.[21,38,39]

For the detection of CMV shedding in adult females, the cobas® assay also has demonstrated satisfactory concordance with comparator assays for urine, saliva, and vaginal secretion. As aforementioned, a discrepant resolution was not performed partially due to limited sample availability but also to avoid analysis bias.[32] Although detecting CMV shedding is not part of routine practice, clinicians and/or pregnant women do request CMV PCR to support patient discussions about current or future pregnancies. However, as the significance of CMV shedding in pregnancy remains unclear, a cautious interpretation of results is warranted.[3–7,14,41] In addition, as CMV shedding is often at low levels, it is imperative to utilize a sensitive and fully-validated assay that can ensure the validity and reliability of results.[14,15,42–44] A fully-validated assay is also a prerequisite for the comparison of CMV shedding prevalence.[15,42–44]

In agreement with analytical performance data and the manufacturer's cross-reactivity results, a significant molecular interference with non-CMV pathogens or sample matrices (in CPM or eNAT transport media) was not detected.[19] The largest difference between the theoretical and the quantified CMV DNA load was 0.53 $\log_{10}$ IU/mL for CMV-BK virus in saliva sample (an equivalent of 1.77 cycle threshold shift), which is not considered to be a significant interference or competitive inhibition.[45] Consistent with our previous studies, a PCR inhibition caused by biological samples was not observed in the current study, presumably due to the use of validated swab types and transport media that permit an optimal stabilization and recovery of CMV DNA.[21,39] Therefore, the lower NPA between the cobas® CMV and reference laboratory M assays for adult urine samples was unlikely to be due to CMV DNA degradation or PCR inhibition, and more likely to be due to differences in extraction efficiency between the cobas® 6800 and Kingfisher Flex extractors as reported.[21] Nevertheless, we cannot rule out the possibility of the differences in detection capability between the assays as a more concentrated eluate and a higher ratio of PCR input were used in the cobas® CMV assay. The possibility of detecting small amplicons or fragmented CMV DNA by the cobas® assay also cannot be excluded, though unlikely as a similar rate of disagreement was not observed for saliva and vaginal secretion.[46,47]

To our knowledge, this is the first study that investigated the clinical utility of a rapid and fully automated CMV PCR to support

timely decision-making and management of congenital and prenatal CMV infection. Although Roh and colleagues also investigated the utility of the cobas® CMV assay to detect and quantify CMV DNA in urine samples, the group did not assess saliva and vaginal secretion samples.[48] In addition, differences in methodology and the population investigated prevent a direct comparison with our results. Nonetheless, both studies have demonstrated the potential utility of the cobas® CMV assay for non-plasma samples to inform patient care. Compared with reference laboratory M and R, the cobas® CMV PCR test demonstrates several significant improvements in laboratory workflow and efficiency. These include (1) a shorter turnaround time due to the removal of the need to refer samples to, and a delay in receiving results from, reference laboratories, (2) a reduction in the number of user's intervention and risk of errors as samples are pipetted directly from primary containers and processed by fully automated sample-to-result processes, and (3) the removal of the need to remove reagents for storage which reduces hands-on time and prevents contamination (Supporting Information: Table S1). These improvements will support a timely management of patients and universal screening for cCMV.[12,13] However, the benefits are contingent on a laboratory access to the cobas® CMV assay and a minimum of 350 µL sample volume, which may not be realistic for some laboratories or patients such as low birth weight infants. In addition, as with all automated solutions, loss of clinical samples is possible due to instrument failure. Fortunately, the failure rate of the cobas® instrument or assay is very low in our laboratory.

The present study has limitations. First, our laboratory method evaluation study cannot be regarded as a true diagnostic accuracy study. The diagnostic performance of the cobas® CMV needs to be verified in clinical studies with predefined eligibility and inclusion criteria that fulfill the requirements of the *Standards for Reporting Diagnostic Accuracy Studies* (STARD).[49] However, before such studies can be performed, a consensus definition of CMV shedding will first need to be established. Similarly, the analytical performance data also need to be confirmed in larger studies. Notwithstanding the limitations, we believe our results may inform future clinical and laboratory studies as currently there is a paucity of data on the validation and evaluation of the cobas® CMV in neonatal and adult women cohorts. Second, amniotic fluid has not been assessed in the current study due to a limited availability of clinical samples and the administrative and financial constraints to source amniotic fluid. As the detection of CMV DNA in amniotic fluid is vital for the diagnosis of cCMV in pregnancy, validation of this sample type on a rapid and accurate assay such as the cobas® CMV is warranted.[8] Third, the detection of potentially small amplicon and/or fragmented CMV DNA in urine, saliva, and vaginal secretion also have not been assessed, which may have significant clinical and technical implications such as the interpretation and comparison of CMV shedding results.[14–16,42–44] Finally, our performance data were based on the use of the described sample collection devices and assays, and cannot be generalized to other sampling and PCR methods. Laboratories that employ different sample collection devices and/or nucleic acid amplification technologies will need to conduct their own performance validation and evaluation before implementation into routine use.[20]

In conclusion, we have demonstrated the analytical and diagnostic performance of the cobas® CMV for the detection and quantification of CMV DNA in urine, saliva, and vaginal secretion. The assay demonstrated excellent performance and is suitable for a rapid, accurate, and efficient laboratory investigation of CMV infection in neonates and adults. Further clinical and laboratory studies to confirm the clinical utility of the cobas® CMV assay are warranted.

AUTHOR CONTRIBUTIONS

Ngee Keong Tan: Conceptualization; methodology; investigation; data curation; validation; formal analysis; visualization; writing—original draft; writing—review and editing. **Cassie Francesca Pope:** Methodology; writing—review and editing; supervision. **David Carrington:** Conceptualization; methodology; writing—review and editing; supervision; funding acquisition. All authors reviewed and approved the final draft of the manuscript.

ACKNOWLEDGMENTS

We thank volunteers, patients, and study participants for providing urine, saliva, and/or vaginal secretion. We gratefully acknowledge the technical assistance of Sima Patel, Hiral Patel, Ismahan Hassan Ali, John Maskell, Foziya Malik, Auet Asfaha, Kirstie Thompson, Nathalie Lenders, Ankita Jaiprakash, and Lowra Thelikada Hallinage in the Microbiology laboratory at South West London Pathology, and Robin Parsons, Dr Shari Sapuan and Vanessa Greening for the collection of samples from the study participants. We also thank Georgios Vlachos and Dr Murali Ponniah at Roche Diagnostics UK for constructive discussions and their support in this study. This work was supported in part by Professor Paul T. Heath and Dr Shari Sapuan (St George's, University of London) as part of the *Cytomegalovirus Shedding Characteristics in Pregnant Women* (cCHIPS) study funded by Merck Sharp & Dohme via the Merck Investigator Studies Program (MISP 58414). The funder has no role in the study design, data collection, result interpretation, writing of the manuscript, and the decision to submit the article for publication.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Ethical approval was not required as the study was defined as service evaluation/improvement/development project according to the UK Policy Framework for Health and Social Care Research. Diagnostic samples submitted by clinicians and patients for CMV PCR which was not part of a routine clinical practice were tested following an informed consent of the non-approved use of the tests. The cCHIPS study (NCT04021628) was approved by the NHS Health Research Authority, London-Brent Research Ethics Committee (19/LO/0161).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Tan NK, Pope CF, Carrington D. Performance evaluation of fully automated cobas® 6800 CMV PCR for the detection and quantification of cytomegalovirus DNA in neonatal urine and saliva, and adult urine, saliva, and vaginal secretion. *J Med Virol*. 2023;95:e29223. doi:10.1002/jmv.29223