# Impact of HIV-1 RNA Assay Selection on Low-Level Viremia and Clinical Trial Endpoint Interpretations

Anna Maria Geretti, Kehui Wang, Bliss Leverette, Kai Hove, Richard Grove, Jerry L Jeffrey, Miriam Jones, Riya Moodley, Sherene Min, Rebecca A Borsi, Kimberley Brown, Emilio Letang, Jean van Wyk, Parul Patel, Bryn Jones

*Clinical Infectious Diseases*

**MAJOR ARTICLE**

# Impact of HIV-1 RNA Assay Selection on Low-Level Viremia and Clinical Trial Endpoint Interpretations

**Anna Maria Geretti,[1,2] Kehui Wang,[3] Bliss Leverette,[4] Kai Hove,[5] Richard Grove,[6] Jerry L. Jeffrey,[4] Miriam Jones,[5] Riya Moodley,[5] Sherene Min,[4] Rebecca A. Borsi,[4] Kimberley Brown,[4] Emilio Letang,[7] Jean van Wyk,[5] Parul Patel,[4] Bryn Jones[5]**

[1]Department of Medicine of Systems, University of Rome Tor Vergata, Rome, Italy; [2]Department of HIV Medicine, Royal Free London NHS Foundation Trust (NorthMid), London, UK; [3]GSK, Durham, NC, USA; [4]ViiV Healthcare, Durham, NC, USA; [5]ViiV Healthcare, London, UK; [6]GSK, London, UK; [7]ViiV Healthcare, Tres Cantos, Spain

**Background:** Accurate plasma HIV-1 viral load quantification is essential for monitoring responses to antiretroviral therapy (ART) and defining efficacy in clinical trials. Differences in assay performance characteristics may result in discordant results and affect clinical interpretation. We evaluated concordance between the Roche cobas 6800 and Abbott RealTi*m*e HIV-1 RNA assays in 2 ongoing, multicenter, phase 3b trials.

**Methods:** Stored paired plasma aliquots from adults with HIV-1 who were virologically suppressed or initiating ART (271 and 348 paired samples, respectively) were batch re-tested using the RealTi*m*e assay; sample selection included all specimens from pre-specified key study visits and was enriched for selected centrally reported cobas 6800 results ≥50 copies/mL. Concordance was evaluated at clinically relevant categorical thresholds (50 and 200 copies/mL).

**Results:** Overall, among samples with cobas 6800 HIV-1 RNA results ≥50 copies/mL, 92% (91/99) of samples from participants who were virologically suppressed and 68% (159/233) from

**Correspondence and reprint requests to:** Bliss Leverette, 410 Blackwell St, Durham, NC 27701, USA, Phone: 908-331-6986, Email: elizabeth.b.leverette@viivhealthcare.com





DOI: 10.1093/cid/ciag435

participants initiating ART were <50 copies/mL by RealTi*m*e. Among samples with cobas 6800 HIV-1 RNA results ≥200 copies/mL, 78% (14/18) and 83% (44/53), respectively, were <200 copies/mL by RealTi*m*e. Most samples classified as <50 copies/mL by cobas 6800 were <50 copies/mL by RealTi*m*e. All samples classified as <200 copies/mL by cobas 6800 were <200 copies/mL by RealTi*m*e. In both studies, per-protocol case review identified no clinical contributors in most cases of viremia.

**Conclusions:** Marked discordance between assays was observed near clinically relevant low HIV-1 RNA thresholds. These findings highlight the importance of assay-specific interpretation of low-level viremia in trials and routine care, particularly near decision-making thresholds.

**Keywords:** Abbott RealTi*m*e; clinical trial; concordance; HIV-1 RNA; Roche cobas 6800

## INTRODUCTION

Accurate quantification of plasma HIV-1 RNA is essential for monitoring responses to antiretroviral therapy (ART) in routine care and defining virologic endpoints in clinical trials. Current treatment guidelines typically define optimal virologic suppression as a plasma HIV-1 RNA level <50 copies/mL [1-4], or, in some cases, as the lower limit of quantification (LLOQ) of contemporary commercial viral load (VL) assays (e.g., <20 copies/mL) [5]. A higher threshold of 200 copies/mL is commonly used to define actionable virologic failure, which distinguishes it from low-level viremia, and to accommodate assay variability [1-3,5]. In clinical trials, efficacy is often determined using the last available VL measurement within one or more pre-specified time windows according to regulatory algorithms such as the US Food and Drug Administration Snapshot approach, with repeat testing of any elevated VL result within a specified window to confirm virologic failure [6]. As a consequence, assay-dependent variation near clinically relevant thresholds may significantly affect the classification of virologic responses [7].

Although commercially available HIV-1 RNA assays show high overall concordance, differences in assay characteristics, including nucleic acid extraction methods, gene targets, and analytic sensitivity and precision, can lead to discordant results, particularly at VL levels near the 50 copies/mL threshold [8-16]. Furthermore, pre-analytic factors, such as sample handling and the timing and procedures for plasma separation, may influence VL results near lower quantification thresholds [17-21]. Real-world evidence illustrates the potential clinical significance of assay discordance at low VL levels [16,22,23]. For example, if individuals with a previously suppressed VL are identified as having low-level viremia after switching to a different VL assay, this can create confusion in clinical interpretation, affecting both patients and clinicians [16,23]. Clinical laboratories and specialist panels have issued guidance and recommendations on sample handling, VL testing, and reporting and interpretation of low-level HIV-1 RNA results to support consistent clinical decision-making [1-3,5,21,23].

Registrational trials of dolutegravir/lamivudine (DTG/3TC) used the Abbott RealTi*m*e HIV-1 assay (Abbott Molecular Diagnostics, Des Plaines, IL) [24-26]. In contrast, 2 ongoing, multicenter, phase 3b studies in participants who were virologically suppressed or initiating ART used the Roche cobas 6800 HIV-1 assay (Roche Diagnostics, Indianapolis, IN) for centralized VL testing. During study conduct, differences were noted between centrally and locally performed VL measurements at some sites, with centrally reported results showing low-level viremia while locally performed testing indicated suppression. In most cases, no clear clinical factors were identified that could explain these discordant findings. The frequency of centrally reported low-level viremia was also overall higher than that observed in prior phase 3 DTG/3TC studies conducted with the RealTi*m*e assay [24-26]. These observations prompted a retrospective re-analysis of stored plasma samples using the RealTi*m*e assay. To our knowledge, this represents the first evaluation of the comparative performance of the cobas 6800 and RealTi*m*e assays in a clinical trial setting. Here, we report categorical concordance between the 2 assays and discuss implications for interpreting virologic outcomes in clinical trials and routine HIV care.

## METHODS

### Study description and selection of participant samples

Comparative assay results were evaluated using stored plasma samples collected in the EYEWITNESS (NCT05911360) and VOLITION (NCT05917509) clinical trials. Trial designs are described elsewhere [27,28]. Briefly, EYEWITNESS is evaluating the efficacy, safety, and tolerability of switching to once-daily DTG/3TC from bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) in people with HIV-1 aged ≥50 years who are virologically suppressed (HIV-1 RNA <50 copies/mL) [27]. VOLITION is evaluating the efficacy, safety, and tolerability of DTG/3TC in people initiating ART who, after achieving virologic suppression (HIV-1 RNA <50 copies/mL), are offered the option to switch to long-acting cabotegravir + rilpivirine at the Day of Choice (DoC) visit (occurring any time between 4-16 weeks after DTG/3TC initiation) [28,29]. In both trials, HIV-1 RNA was measured at a central laboratory using the cobas 6800 assay, which represented a change of assay relative to previous DTG/3TC studies [24-26]. The RealTi*m*e assay was not routinely available at the central laboratory used in EYEWITNESS and VOLITION and was made available for sample re-testing upon special request.

In participants who were virologically suppressed (Group A, EYEWITNESS), 2 sets of samples were selected for re-testing with the RealTi*m*e assay: (i) samples with centrally reported cobas 6800 HIV-1 RNA ≥50 copies/mL at any non–Week 48 visit, together with samples from unscheduled or withdrawal visits regardless of cobas 6800 VL value (n=82); and (ii) all samples within the Week 48 Snapshot window, regardless of cobas 6800 VL value (n=189). Combined, these sample sets comprised 271 paired cobas 6800/RealTi*m*e samples.

In participants newly initiating ART (Group B, VOLITION), 2 sets of samples were selected for re-testing with the RealTi*m*e assay: (i) samples with centrally reported cobas 6800 HIV-1 RNA 50 to 1000 copies/mL at any visit before initial virologic suppression (excluding baseline), together with samples from unscheduled or withdrawal visits regardless of cobas 6800 VL value (n=188); and (ii) all samples from the DoC visit, regardless of cobas 6800 VL value (n=160). Combined, these sample sets comprised 348 paired cobas 6800/RealTi*m*e samples.

Paired measurements were derived from plasma aliquots obtained from the same blood draw and time point. The re-testing strategy intentionally enriched the analysis for observations near the clinically relevant 50 copies/mL threshold rather than representing a fully unselected cohort.

**Plasma HIV-1 RNA assays**

At each study site, plasma was separated by centrifugation of whole blood collected in $K_2$EDTA tubes. Plasma aliquots were stored at −20°C or below at the site until next-day shipment on dry ice to the region-specific central laboratory in the United States (PPD GCL US, Highland Heights, KY) or Europe (PPD GCL EU, Zaventem, Belgium), where samples were stored at −70°C until analysis.

Initial testing was performed using the cobas HIV-1 quantitative assay on the automated cobas 6800 platform [30]. The assay targets the HIV-1 *gag* gene and the long-terminal repeat region. Using a sample processing volume of 0.5 mL, the linear range is 20 to 10,000,000 copies/mL, with an LLOQ of 20 copies/mL and a lower limit of detection of 13.2 copies/mL.

Retrospective batch re-testing of selected stored paired plasma aliquots was performed using the RealTi*m*e assay on the automated *m*2000 platform [31]. The assay targets the HIV-1 *pol integrase* region. Using a sample processing volume of 0.6 mL, the linear range is 40 to 10,000,000 copies/mL, with an LLOQ of 40 copies/mL; HIV-1 RNA detection below this threshold is reported qualitatively but not quantified.

Both assays use automated magnetic particle-based nucleic acid extraction followed by real-time reverse transcription polymerase chain reaction amplification. Manufacturer-recommended guidance for storage conditions, nucleic acid extraction, reagent stability, assay calibration, and permissible freeze-thaw cycles was followed for both assays.

**Statistical analysis**

Comparative assay performance characteristics were analyzed separately for each study (Group A and B) given different VL kinetics between individuals stably suppressed and those newly initiating ART. Concordance between assays was evaluated at clinically relevant categorical thresholds (50 and 200 copies/mL), with concordant results indicating classification into the same threshold category by both assays. Results were summarized using descriptive counts and

proportions of concordant and discordant paired measurements and displayed visually with scatterplots.

Bland-Altman analyses were used to assess assay agreement and were restricted to pre-specified key study visits to reduce the influence of enrichment-driven sampling and ensure a comparable measurement context. For participants who were virologically suppressed (Group A), analyses were limited to Week 48, the regulatory efficacy endpoint. For participants initiating ART (Group B), analyses were performed at the DoC visit after initial virologic suppression. For samples with VL values below the assays' LLOQ, values were imputed as 19 copies/mL for cobas 6800 and 39 copies/mL for RealTi*m*e. Because the sampling strategy enriched observations near the 50 copies/mL threshold, and many paired samples were <LLOQ (and therefore influenced by the imputing method), the analyses were interpreted descriptively (presented in Supplementary Material).

Viral load variability, defined as fluctuation in HIV-1 RNA measurements across the 50 copies/mL threshold, was further assessed across individual participants enrolled in each trial using side-by-side comparisons of VL trajectories for cobas 6800 and RealTi*m*e re-test results; if RealTi*m*e re-test results were not available, only cobas 6800 results were used.

## RESULTS

### Concordance analysis of plasma HIV-1 RNA results by cobas 6800 and realti*m*e assays

#### *Group A: participants who were virologically suppressed (EYEWITNESS)*

Viral load levels were consistently higher when measured with the cobas 6800 assay compared with the RealTi*m*e assay (Figure 1A). Categorical discordance was evident at the 50 copies/mL threshold (Table 1). Among samples classified as ≥50 copies/mL by cobas 6800, 74/79 (94%) at non–Week 48 visits and 17/20 (85%) at Week 48 were <50 copies/mL by RealTi*m*e; overall, 91/99 (92%) samples ≥50 copies/mL by cobas 6800 were <50 copies/mL by RealTi*m*e. Among 172 samples <50 copies/mL by cobas 6800, 170 (99%) were also <50 copies/mL by RealTi*m*e; only 2/172 (1%) samples <50 copies/mL by cobas were ≥50 copies/mL by RealTi*m*e.

In addition to the above, among 13 participants with cobas 6800 HIV-1 RNA ≥50 copies/mL at baseline (i.e., while receiving BIC/FTC/TAF and before switching to DTG/3TC), 11 had a paired RealTi*m*e result: 9/11 (82%) were <50 copies/mL and 2/11 (18%) were ≥50 copies/mL.

Categorical discordance was also observed at the ≥200 copies/mL threshold, although sample numbers were small. Among 18 samples classified as ≥200 copies/mL by cobas 6800 (including 15 from non–Week 48 and 3 from Week 48 visits), 14/18 (78%) were <200 copies/mL by RealTi*m*e (Table 1). In contrast, concordance was complete for samples classified as <200 copies/mL by cobas 6800, with all 253 samples also <200 copies/mL by RealTi*m*e. There were no

instances of RealTi*m*e reporting results ≥200 copies/mL when cobas 6800 results were <200 copies/mL.

Differences in HIV-1 RNA measurements of ≥0.5 $\log_{10}$ copies/mL between assays were observed for 29/271 (11%) paired samples overall, including 25/82 (30%) from non–Week 48 visits and 4/189 (2%) from Week 48 visits.

***Group B: participants initiating ART (VOLITION)***

Among participants initiating ART, VL values were consistently higher when measured with the cobas 6800 assay (Figure 1B), with categorical discordance observed at the 50 and 200 copies/mL thresholds (Table 2). At non-DoC visits, 115/181 (64%) samples ≥50 copies/mL by cobas 6800 were <50 copies/mL by RealTi*m*e. At the DoC visit, 44/52 (85%) samples ≥50 copies/mL by cobas 6800 were <50 copies/mL by RealTi*m*e. Overall, 159/233 (68%) samples ≥50 copies/mL by cobas 6800 were <50 copies/mL by RealTi*m*e. Among 115 samples <50 copies/mL by cobas 6800, 113 (98%) were also <50 copies/mL by RealTi*m*e; only 2/115 (2%) samples <50 copies/mL by cobas 6800 were ≥50 copies/mL by RealTi*m*e.

At the ≥200 copies/mL threshold, among 53 samples classified as ≥200 copies/mL by cobas 6800 (including 43 from non-DoC and 10 from DoC visits), 44/53 (83%) were <200 copies/mL by RealTi*m*e (Table 2). Of the 9 samples that were ≥200 copies/mL in both assays, only one occurred at the DoC visit. Complete concordance was observed for samples classified as <200 copies/mL by cobas 6800, with all 295 samples also <200 copies/mL by RealTi*m*e. There were no instances of RealTi*m*e reporting results ≥200 copies/mL when cobas 6800 results were <200 copies/mL.

Differences in HIV-1 RNA measurements of ≥0.5 $\log_{10}$ copies/mL between assays were observed for 60/348 (17%) paired samples overall, including 49/188 (26%) from non–DoC visits and 11/160 (7%) from DoC visits.

In the Bland-Altman analysis, the mean of VL differences between assays (cobas 6800 minus RealTi*m*e) was −0.32 $\log_{10}$ copies/mL for Group A (EYEWITNESS) and −0.08 $\log_{10}$ copies/mL for Group B (VOLITION; Supplementary Figure 1). However, these values were strongly influenced by imputation of measurements below the assays' LLOQ.

**Individual VL variability by assay over time**

Variability in HIV-1 RNA measurements over time was examined at the individual participant level in each trial (EYEWITNESS, N=205; VOLITION, N=171; Figure 2). These longitudinal displays reflect measurements obtained at visits with available data and therefore do not represent uniform paired sampling across all visits or participants. Moreover, some time points contain only cobas 6800 results as paired RealTi*m*e re-test results were not available. Variability (i.e., fluctuation across the <50 copies/mL threshold) was most apparent in the 50 to 200 copies/mL range for cobas 6800 results and less evident for RealTi*m*e re-test results. Specifically, among

participants who were virologically suppressed (Group A, EYEWITNESS), most RealTi*m*e values remained <50 copies/mL across study visits, whereas cobas 6800 values more frequently crossed the 50 copies/mL threshold. A similar pattern was observed in participants initiating ART (Group B, VOLITION) following virologic suppression.

In both studies, per-protocol review was conducted for all instances of viremia; in most cases, no clear clinical contributors were identified, including suboptimal adherence, intercurrent illness, vaccinations, or drug–drug interactions with concomitant medications or supplements.

## DISCUSSION

In this analysis of paired samples from 2 ongoing phase 3b clinical trials, marked discordance was observed between the Roche cobas 6800 and Abbott RealTi*m*e HIV-1 RNA assays at clinically relevant low VL thresholds. Across paired samples from participants who were virologically suppressed (Group A, EYEWITNESS) or initiating ART (Group B, VOLITION), a high proportion of samples categorized as ≥50 copies/mL by cobas 6800 were <50 copies/mL when re-tested by RealTi*m*e. Similarly, a high proportion of samples categorized as ≥200 copies/mL by cobas 6800 were <200 copies/mL by RealTi*m*e, although sample numbers at this threshold were smaller. In contrast, most samples classified as <50 copies/mL and all classified as <200 copies/mL by cobas 6800 were also classified as <50 and <200 copies/mL, respectively, by RealTi*m*e. Collectively, these findings indicate that assay discordance is most frequently observed when results fall near clinically relevant decision-making thresholds, where small differences in quantification can affect categorical classification. Although the mean inter-assay differences were small (<0.5 $\log_{10}$ copies/mL), modest analytic variation may still have clinical relevance at low VL levels used to define virologic suppression or low-level viremia. The unexpectedly high frequency of low-level viremia with the cobas 6800 assay is in marked contrast to the infrequent incidence of low-level viremia in prior DTG/3TC studies that used the RealTi*m*e assay [24-26]. This finding has important implications for participant virologic management and endpoint interpretation in clinical trials.

Compared with historical studies conducted using the RealTi*m*e assay [24-26], adoption of the cobas 6800 assay for the 2 ongoing trials was associated with a higher frequency of HIV-1 RNA ≥50 copies/mL. This difference, together with repeated and ongoing investigator-reported discrepancies between central and local VL testing during the studies, prompted a formal evaluation of inter-assay agreement using stored paired plasma samples. Importantly, in most cases, no clinical factors commonly associated with viremia [32,33] were identified through case review. Results of repeat testing using the RealTi*m*e assay showing maintenance of virologic suppression were consistent with clinician assessments of individual participants and with virologic outcomes observed in prior DTG/3TC studies [24-26].

Detection of residual HIV-1 RNA below conventional assay LLOQ thresholds (generally ≤11 copies/mL) is well-documented in individuals on durably suppressive ART [34-36]. Moreover, both transient ("blips") and confirmed (low-level viremia) HIV-1 RNA elevations up to approximately 200 copies/mL have been reported relatively frequently during stable ART. "Blips" (i.e., isolated HIV-1 RNA values between 50 and 200 copies/mL preceded and followed by values <50 copies/mL) are not considered clinically significant [2,3,5]. Although low-level viremia is associated with an increased risk of higher VL rebound in a subset of individuals [34,37-39], in most cases, it is not considered indicative of ongoing viral replication [36,40]. Thus, interpretation of VL results between 50 and 200 copies/mL requires careful consideration of assay performance characteristics, sample handling and processing integrity, and guideline-based definitions [1-3,5].

In this analysis, VL variability in the 50 to 200 copies/mL range was more evident with the cobas 6800 assay and less apparent when re-tested with the RealTi*m*e assay. While the data suggest greater analytic variability near the LLOQ with cobas 6800, variability at low VL levels, particularly in the 50 to 200 copies/mL range, is a recognized feature of HIV-1 RNA quantification. Our results are consistent with multiple published studies showing that HIV-1 RNA assays across multiple platforms differ in performance characteristics and may exhibit increased analytic variability at low VL levels [8-16]. Previous comparative studies have reported that Roche HIV-1 RNA assay platforms may report higher VL values than RealTi*m*e at low VL levels, with greater discordance at the 50 copies/mL threshold and improved concordance at higher thresholds [10,11,15]. Among these, a large multicenter evaluation comparing the cobas 6800 and RealTi*m*e assays showed good overall agreement between platforms but identified systematic differences in quantification [15]. The cobas 6800 assay tended to yield slightly higher HIV-1 RNA values (by an average of 0.3 $\log_{10}$ copies/mL) and classified approximately 10% to 12% more samples above the 50 copies/mL threshold [15]. These findings agree with our observations, together highlighting that assay-specific analytic characteristics can influence detectability and classification around clinically relevant thresholds.

Multiple mechanisms may contribute to assay discordances at low VL levels. Although the overall design of the cobas 6800 and RealTi*m*e assays is similar, differences in target regions, amplification chemistry, analytic sensitivity, and susceptibility to amplification of residual cell-associated HIV nucleic acids may contribute to variation in rates of low-level detectable viremia [10,15,30,31]. Some studies have suggested that certain methodologies, including some cobas assays, may be more susceptible to detecting HIV DNA released into plasma during cell lysis in cases of delays in sample processing time [15,17-19]. These findings do not imply inferior performance of any assay; rather, they highlight how technical and methodological differences can influence reported results near decision-making thresholds. Pre-analytic conditions, including sample handling and plasma separation procedures and timing, may affect sample integrity and VL measurements. In this analysis, no concerns were identified regarding assay reagents or assay calibration process. After the identification of assay discordance, reinforcement of required sample

handling procedures was implemented at sites during study conduct; however, the impact of these measures on assay agreement was not evaluated and cannot be inferred from the available data set.

These findings have implications for both clinical trials and routine HIV care. In clinical trials that rely on protocol-defined virologic management and withdrawal criteria to define efficacy outcomes, assay-dependent variability near low thresholds, particularly around 50 copies/mL, may influence endpoint classification and interpretation [7]. In clinical practice, "blips" are generally considered benign and distinguished from low-level viremia, which may prompt closer monitoring and further clinical evaluation for adherence, drug–drug interactions, or emerging resistance [1-3,5]. Assay-related variability near clinically relevant thresholds may increase the frequency of low-level viremia, potentially leading to patient and clinician concern and unnecessary regimen changes [16,22,23]. These findings may also have implications for patient counseling, as they may generate uncertainty regarding virologic suppression and the "Undetectable = Untransmissible" (U=U) messaging [23]. Awareness of assay-specific performance characteristics is therefore essential to appropriately contextualize low-level VL results and avoid unnecessary interventions or patient anxiety.

This analysis has several limitations. First, sample selection was enriched for observations at the clinically relevant threshold of 50 copies/mL and does not represent a fully unselected population. This focused re-testing strategy was pragmatic, as differences between assays at higher VLs were not expected to be large or to change participant management or endpoint adjudication. Second, re-testing was performed retrospectively on stored plasma aliquots rather than contemporaneously; however, paired aliquots were derived from the same blood draw and were not subjected to multiple freeze-thaw cycles. Although a small amount of sample loss during a single freeze-thaw cycle could contribute to assay discordance, assay manufacturers indicate that a limited number of freeze-thaw cycles does not affect assay performance [30,31]. Third, pre-analytic conditions, including sample handling and the timing of plasma separation procedures, could not be fully evaluated, and some sites may not have followed procedures exactly. Fourth, most samples were from participants receiving DTG/3TC, although a similar discordance was observed in the small number of paired baseline samples included from participants receiving BIC/FTC/TAF. Finally, the central laboratory performing HIV-1 RNA testing of samples on cobas 6800 and RealTi*m*e in EYEWITNESS and VOLITION trials differed from the central laboratory performing testing of RealTi*m*e samples in previous DTG/3TC trials [24-26]. Overall, the use of paired samples and consistent findings across multiple study sites with 2 distinct populations and different ART regimens strengthen the validity of the observations.

In conclusion, marked discordance between the cobas 6800 and RealTi*m*e HIV-1 assays was observed at low VL thresholds used to define virologic suppression and failure, indicating the need for HIV clinicians and researchers to be aware of assay-specific performance characteristics and technical factors that may influence VL results near lower quantification thresholds. Viral load measurements, particularly those indicating low-level viremia, should be interpreted in the context of an individual's clinical history, treatment adherence, and clinical markers.

Overall, these findings highlight the importance of anticipating assay-dependent effects when switching HIV-1 RNA testing platforms. In clinical trials with fixed virologic failure definitions, such assay-dependent differences may lead to apparent misclassifications that cannot be reconciled once a study is underway. Parallel testing during platform transitions may mitigate these challenges but is unlikely to be feasible in all settings, particularly in low-resource environments and large, multinational trials. Therefore, additional strategies will be needed to contextualize low-level VL measurements and support accurate interpretation of virologic outcomes in both HIV clinical trials and routine care.

## DISCLOSURES

***Author contributions:*** KW, KH, RG, MJ, RM, SM, KB, JvW, and BJ contributed to the conception and/or design of the study. AMG, KW, BL, KH, RG, JLJ, MJ, RM, SM, KB, EL, JvW, PP, and BJ contributed to the analysis and/or interpretation of the data. All authors contributed to drafting the manuscript and/or critically revising the manuscript for important intellectual content. All authors had full access to all the data in the study, had final responsibility for the decision to submit for publication, and approved the final version for submission.

***Potential conflicts of interest:*** AMG has received personal fees from Abbott, Gilead, GSK, MSD, Roche, and ViiV Healthcare; and institutional funding from Gilead, Roche, and ViiV Healthcare. KW, BL, KH, RG, JLJ, MJ, RM, SM, RAB, KB, EL, JvW, PP, and BJ are employees of GSK or ViiV Healthcare and own stock in GSK.

***Funding:*** This analysis and the EYEWITNESS and VOLITION studies were funded by ViiV Healthcare.

***Acknowledgments:*** Editorial assistance was provided under the direction of the authors by Jenny Johnson, PhD, ELS, CMPP, and Jennifer Rossi, MA, ELS, CMPP, Fingerpaint Medical, and funded by ViiV Healthcare.

***Data availability:*** Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

## REFERENCES

1. European AIDS Clinical Society. EACS guidelines for the clinical management of HIV and other infections in Europe. Version 13.0. 2025. Available at: https://www.eacsociety.org/guidelines/eacs-guidelines. Accessed 20 March 2026.
2. British HIV Association. BHIVA guidelines on antiretroviral treatment for adults living with HIV-1 2022 (2025 interim update). 2025. Available at: https://bhiva.org/wp-content/uploads/2025/10/BHIVA-guidelines-on-antiretroviral-treatment-for-adults-living-with-HIV-1-2025-interim-update.pdf. Accessed 20 March 2026.

3. Gandhi RT, Landovitz RJ, Sax PE, et al. Antiretroviral drugs for treatment and prevention of HIV in adults: 2024 recommendations of the International Antiviral Society–USA Panel. JAMA, **2025 ;** 333: 609-628.
4. World Health Organization. WHO updated recommendations on HIV clinical management: recommendations for a public health approach. 2025. Available at: https://www.who.int/publications/i/item/9789240119468. Accessed 20 March 2026.
5. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. Department of Health and Human Services. 2025. Available at: https://clinicalinfo.hiv.gov/en/guidelines/hiv-clinical-guidelines-adult-and-adolescent-arv/whats-new. Accessed 20 March 2026.
6. Center for Drug Evaluation and Research. Human immunodeficiency virus-1 infection: developing antiretroviral drugs for treatment. Guidance for industry. 2015. US Food and Drug Administration. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-immunodeficiency-virus-1-infection-developing-antiretroviral-drugs-treatment. Accessed 20 March 2026.
7. White K, Garner W, Wei L, et al. Repeat testing of low-level HIV-1 RNA: assay performance and implementation in clinical trials. AIDS, **2018 ;** 32: 1053-1057.
8. Association of Public Health Laboratories. Use and interpretation of quantitative HIV-1 RNA test results: testing and reporting guidance for laboratories. 2025. Available at: https://www.aphl.org/docs/default-source/technical/ID_HIV-1_RNA_Test_Reporting_Guide.pdf. Accessed 20 March 2026.
9. Ruelle J, Debaisieux L, Vancutsem E, et al. HIV-1 low-level viraemia assessed with 3 commercial real-time PCR assays show high variability. BMC Infect Dis, **2012 ;** 12: 100.
10. Swenson LC, Cobb B, Geretti AM, et al. Comparative performances of HIV-1 RNA load assays at low viral load levels: results of an international collaboration. J Clin Microbiol, **2014 ;** 52: 517-523.
11. Amendola A, Marsella P, Bloisi M, Forbici F, Angeletti C, Capobianchi MR. Ability of two commercially available assays (Abbott RealTime HIV-1 and Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 Version 2.0) to quantify low HIV-1 RNA levels (<1,000 copies/milliliter): comparison with clinical samples and NIBSC working reagent for nucleic acid testing assays. J Clin Microbiol, **2014 ;** 52: 2019-2026.
12. Garner W, White K, Szwarcberg J, McCallister S, Zhong L, Wulfsohn M. Concordance of HIV-1 RNA values by amplicor and TaqMan 2.0 in patients with confirmed suppression in clinical trials. Clin Infect Dis, **2016 ;** 62: 929-934.
13. Lalama CM, Jennings C, Johnson VA, et al. Comparison of three different FDA-approved plasma HIV-1 RNA assay platforms confirms the virologic failure endpoint of 200 copies per milliliter despite improved assay sensitivity. J Clin Microbiol, **2015 ;** 53: 2659-2666.
14. Hopkins M, Hau S, Tiernan C, et al. Comparative performance of the new Aptima HIV-1 Quant Dx assay with three commercial PCR-based HIV-1 RNA quantitation assays. J Clin Virol, **2015 ;** 69: 56-62.
15. Wiesmann F, Ehret R, Naeth G, et al. Multicenter evaluation of two next-generation HIV-1 quantitation assays, Aptima Quant Dx and Cobas 6800, in comparison to the RealTi*m*e HIV-1 reference assay. J Clin Microbiol, **2018 ;** 56: e00292-18.

16. Wirden M, Palich R, Abdi B, et al. More HIV-1 RNA detected and quantified with the Cobas 6800 system in patients on antiretroviral therapy. J Antimicrob Chemother, **2022 ;** 77: 2251-2256.
17. Wan H, Seth A, Rainen L, Fernandes H. Coamplification of HIV-1 proviral DNA and viral RNA in assays used for quantification of HIV-1 RNA. J Clin Microbiol, **2010 ;** 48: 2186-2190.
18. Portman MD, Lacey CJ. Apparent low-level HIV RNA viraemia related to sample processing time. HIV Med, **2012 ;** 13: 578-579.
19. Mortier V, Vancoillie L, Dauwe K, et al. Meticulous plasma isolation is essential to avoid false low-level viraemia in Roche Cobas HIV-1 viral load assays. Antivir Ther, **2018 ;** 23: 277-281.
20. Gutema G, Hailu H, W/semeyat B, et al. Effect of sample management on quantitative HIV-1 viral load measurement at Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. PLoS One, **2022 ;** 17: e0269943.
21. Public Health Ontario. HIV-1 RNA viral load. Updated 2025. Available at: https://www.publichealthontario.ca/en/laboratory-services/test-information-index/hiv-viral-load. Accessed 20 March 2026.
22. Leierer G, Grabmeier-Pfistershammer K, Steuer A, et al. Factors associated with low-level viraemia and virological failure: results from the Austrian HIV cohort study. PLoS One, **2015 ;** 10: e0142923.
23. Rodriguez MG, Syros A, Rodriguez AE, Serota DP. The perils of overly sensitive viral load testing for persons with human immunodeficiency virus. Open Forum Infect Dis, **2023 ;** 10: ofad494.
24. De Wit S, Bonnet F, Osiyemi O, et al. Durable efficacy of switching from a 3-or 4-drug tenofovir alafenamide–based regimen to the 2-drug regimen dolutegravir/lamivudine in the TANGO study through week 196. J Acquir Immune Defic Syndr, **2024 ;** 96: 156-160.
25. Cahn P, Madero JS, Arribas JR, et al. Three-year durable efficacy of dolutegravir plus lamivudine in antiretroviral therapy–naive adults with HIV-1 infection. AIDS, **2022 ;** 36: 39-48.
26. Llibre JM, Brites C, Cheng C-Y, et al. Efficacy and safety of switching to the 2-drug regimen dolutegravir/lamivudine versus continuing a 3-or 4-drug regimen for maintaining virologic suppression in adults living with human immunodeficiency virus 1 (HIV-1): week 48 results from the phase 3, noninferiority SALSA randomized trial. Clin Infect Dis, **2023 ;** 76: 720-729.
27. National Library of Medicine. A study to evaluate efficacy, safety and tolerability in antiretroviral therapy (ART)-experienced participants of at least 50 years of age living with human immunodeficiency virus (HIV) with virologic suppression who switch to DTG/3TC FDC from BIC/FTC/TAF (EYEWITNESS). NCT05911360. Updated 2025. Available at: https://clinicaltrials.gov/study/NCT05911360. Accessed 20 March 2026.
28. National Library of Medicine. A study to evaluate the efficacy, safety, participant choice and preference of an oral once-daily regimen or a long-acting injectable regimen every two months for treatment of human immunodeficiency virus (HIV-1) in adults who have not previously taken antiretroviral therapy (VOLITION). NCT05917509. Updated 2026. Available at: https://clinicaltrials.gov/study/NCT05917509. Accessed 20 March 2026.
29. Córdova E, Hove K, Hocqueloux L, et al. Rapid virologic suppression with DTG/3TC facilitates early switch to CAB+RPV LA for treatment-naive people living with HIV: suppression phase outcomes from the phase 3b VOLITION study. Presented at: 13th IAS Conference on HIV Science; July 13-17, 2025; Kigali, Rwanda; Poster WEPEB033.

30. Roche Molecular Systems. cobas HIV-1 quantitative nucleic acid test for use on the cobas 5800/6800/8800 systems [package insert]. 2025. Available at: https://elabdoc-prod.roche.com/eLD/api/downloads/b06079cc-f36e-f011-3191-005056a71a5d?countryIsoCode=XG. Accessed 20 March 2026.
31. Abbott Molecular Inc. Abbott RealTi*m*e HIV-1 [package insert]. 2011. Available at: https://www.molecular.abbott/content/dam/add/molecular/products/pdf-/realtime-hiv-1-package-insert.pdf. Accessed 20 March 2026.
32. Ekwaru JP, Campbell J, Malamba S, Moore DM, Were W, Mermin J. The effect of opportunistic illness on HIV RNA viral load and CD4+ T cell count among HIV-positive adults taking antiretroviral therapy. J Int AIDS Soc, **2013 ;** 16: 17355.
33. Calmy A, Bel M, Nguyen A, et al. Strong serological responses and HIV RNA increase following AS 03-adjuvanted pandemic immunization in HIV-infected patients. HIV Med, **2012 ;** 13: 207-218.
34. Doyle T, Geretti AM. Low-level viraemia on HAART: significance and management. Curr Opin Infect Dis, **2012 ;** 25: 17-25.
35. Ruggiero A, Cozzi-Lepri A, Beloukas A, et al. Factors associated with persistence of plasma HIV-1 RNA during long-term continuously suppressive firstline antiretroviral therapy. Open Forum Infect Dis, **2018 ;** 5: ofy032.
36. Wu F, Simonetti FR. Learning from persistent viremia: mechanisms and implications for clinical care and HIV-1 cure. Curr HIV/AIDS Rep, **2023 ;** 20: 428-439.
37. Elvstam O, Malmborn K, Elen S, et al. Virologic failure following low-level viremia and viral blips during antiretroviral therapy: results from a European multicenter cohort. Clin Infect Dis, **2023 ;** 76: 25-31.
38. Bernal E, Martinez-Rodriguez R, Gomez JM, et al. Low-level viremia linked to virological failure but not clinical events. AIDS, 2025 ; **39:** 1545-1557.
39. Lanz C, Meier J, Stockle M, et al. HIV-1 low-level viremia predicts viral failure in participants on antiretroviral therapy in the Swiss HIV cohort study. Clin Infect Dis, **2025 ;** 81: 57-66.
40. White JA, Wu F, Yasin S, et al. Clonally expanded HIV-1 proviruses with 5'-leader defects can give rise to nonsuppressible residual viremia. J Clin Invest, **2023 ;** 133: e165245.

## TABLES

**Table 1.** Categorical Concordance Between Roche cobas 6800 and Abbott RealTi*m*e Assays by Clinically Relevant Thresholds (50 and 200 copies/mL) in Participants Who Were Virologically Suppressed (Group A; EYEWITNESS)[a]

| | Roche cobas 6800 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Week 48 | | | Non–Week 48 | | | Overall | | |
| | <50 copies/mL | ≥50 copies/mL | Total | <50 copies/mL | ≥50 copies/mL | Total | <50 copies/mL | ≥50 copies/mL | Total |
| **Abbott RealTi*m*e** | | | | | | | | | |
| Total, N[b] | 169 | 20 | 189 | 3 | 79 | 82 | 172 | 99 | 271 |
| <50 copies/mL, n (%) | 167 (99) | 17 (85) | 184 (97) | 3 (100) | 74 (94) | 77 (94) | 170 (99) | 91 (92) | 261 (96) |
| ≥50 copies/mL, n (%) | 2 (1) | 3 (15) | 5 (3) | 0 | 5 (6) | 5 (6) | 2 (1) | 8 (8) | 10 (4) |
| Discordant results, n (%) | 2 (1) | 17 (85) | — | 0 | 74 (94) | — | 2 (1) | 91 (92) | — |
| | <200 copies/mL | ≥200 copies/mL | Total | <200 copies/mL | ≥200 copies/mL | Total | <200 copies/mL | ≥200 copies/mL | Total |
| **Abbott RealTi*m*e** | | | | | | | | | |
| Total, N[b] | 186 | 3 | 189 | 67 | 15 | 82 | 253 | 18 | 271 |
| <200 copies/mL, n (%) | 186 (100) | 2 (67) | 188 (99) | 67 (100) | 12 (80) | 79 (96) | 253 (100) | 14 (78) | 267 (99) |
| ≥200 copies/mL, n (%) | 0 | 1 (33) | 1 (1) | 0 | 3 (20) | 3 (4) | 0 | 4 (22) | 4 (1) |
| Discordant results, n (%) | 0 | 2 (67) | — | 0 | 12 (80) | — | 0 | 14 (78) | — |

[a]Paired samples were derived from the same blood draw and time point; sample selection was enriched for observations near clinically relevant viral load thresholds. [b]Percentages are based on column totals.

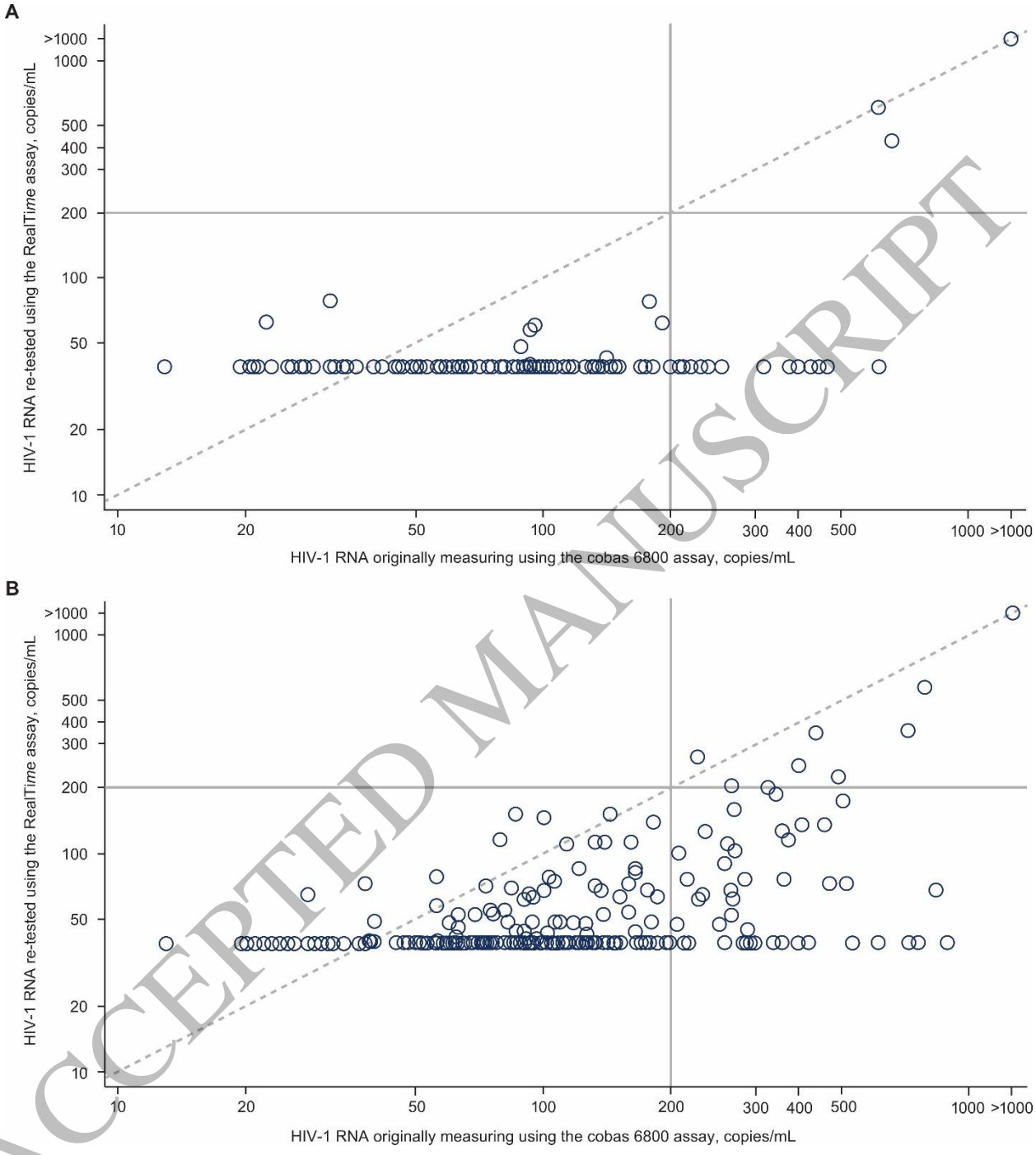
**Table 2.** Categorical Concordance Between Roche cobas 6800 and Abbott RealTi*m*e Assays by Clinically Relevant Thresholds (50 and 200 copies/mL) in Participants Initiating ART (Group B; VOLITION)[a,b]

| | Roche cobas 6800 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DoC visit | | | Non-DoC visit | | | Overall | | |
| | <50 copies/mL | ≥50 copies/mL | Total | <50 copies/mL | ≥50 copies/mL | Total | <50 copies/mL | ≥50 copies/mL | Total |
| **Abbott RealTi*m*e** | | | | | | | | | |
| Total, N[c] | 108 | 52 | 160 | 7 | 181 | 188 | 115 | 233 | 348 |
| <50 copies/mL, n (%) | 107 (99) | 44 (85) | 151 (94) | 6 (86) | 115 (64) | 121 (64) | 113 (98) | 159 (68) | 272 (78) |
| ≥50 copies/mL, n (%) | 1 (1) | 8 (15) | 9 (6) | 1 (14) | 66 (36) | 67 (36) | 2 (2) | 74 (32) | 76 (22) |
| Discordant results, n (%) | 1 (1) | 44 (85) | — | 1 (14) | 115 (64) | — | 2 (2) | 159 (68) | — |
| | <200 copies/mL | ≥200 copies/mL | Total | <200 copies/mL | ≥200 copies/mL | Total | <200 copies/mL | ≥200 copies/mL | Total |
| **Abbott RealTi*m*e** | | | | | | | | | |
| Total, N[c] | 150 | 10 | 160 | 145 | 43 | 188 | 295 | 53 | 348 |
| <200 copies/mL, n (%) | 150 (100) | 9 (90) | 159 (99) | 145 (100) | 35 (81) | 180 (96) | 295 (100) | 44 (83) | 339 (97) |
| ≥200 copies/mL, n (%) | 0 | 1 (10) | 1 (1) | 0 | 8 (19) | 8 (4) | 0 | 9 (17) | 9 (3) |
| Discordant results, n (%) | 0 | 9 (90) | — | 0 | 35 (81) | — | 0 | 44 (83) | — |

ART, antiretroviral therapy; DoC, Day of Choice. [a]Paired samples were derived from the same blood draw and time point; sample selection was enriched for observations near clinically relevant viral load thresholds. [b]In participants initiating ART (VOLITION), samples from any one or multiple scheduled visits with detectable viremia post-baseline from Weeks 4-16 were classified as non-DoC visits, whereas the sample from the DoC visit represented the first visit with HIV-1 RNA <50 copies/mL occurring any time within the same interval. [c]Percentages are based on column totals.

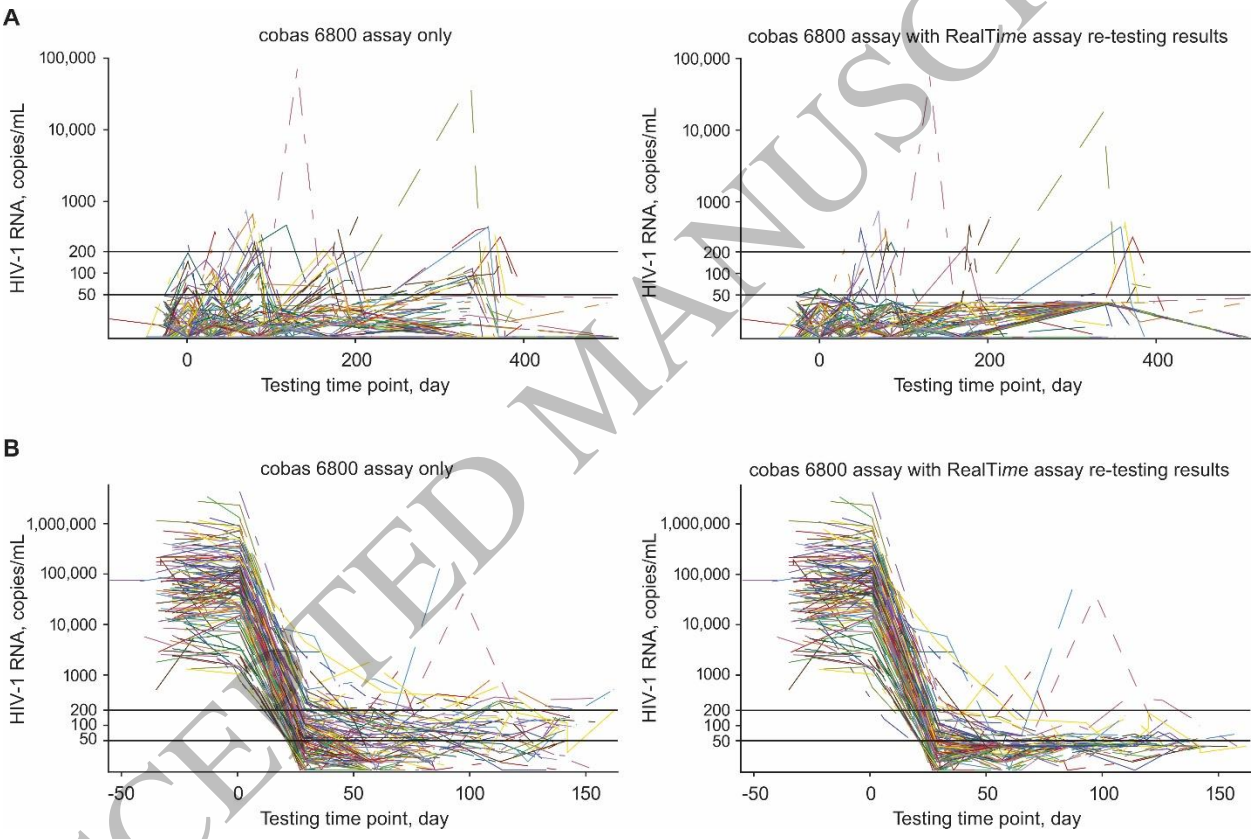
## FIGURE LEGENDS

**Figure 1.** Comparison of paired plasma HIV-1 RNA measurements obtained using Roche cobas 6800 and Abbott RealTi*m*e assays in **(A)** participants who were virologically suppressed (Group A; EYEWITNESS [n=271[a] paired samples]) and **(B)** participants initiating ART (Group B; VOLITION [n=348 paired samples]). Each point represents a paired sample derived from the same blood draw and time point; outlier data not shown in panel A for 2 participants with paired HIV-1 RNA results (46,400 vs 17,632 copies/mL and 71,400 vs 53,181 copies/mL for cobas 6800 vs RealTi*m*e, respectively). The solid grey line separates HIV-1 RNA above and below the 200 copies/mL threshold. The dashed line is the line of equality, where cobas 6800 values are equal to RealTi*m*e values; samples with RealTi*m*e values lower than cobas 6800 values appear below the line, and samples with RealTi*m*e values higher than cobas 6800 values appear above the line. Viral load values below the assays' LLOQ were manually imputed as 19 copies/mL for cobas 6800 (LLOQ, 20 copies/mL) and 39 copies/mL for RealTi*m*e (LLOQ, 40 copies/mL). ART, antiretroviral therapy; LLOQ, lower limit of quantification. [a]One additional paired sample from a screening visit was inadvertently selected for re-testing as this was a manual process. This data point was not used in the EYEWITNESS analysis but is included in the scatterplot.




**Alt-text:** Two scatterplots comparing HIV-1 RNA measurements by Roche cobas 6800 and Abbott RealTi*m*e. Panel A shows samples from participants who were virologically suppressed, and Panel B shows samples from participants initiating ART.

**Figure 2.** Individual HIV-1 RNA measurements over time by assay among **(A)** unique participants who were virologically suppressed (Group A; EYEWITNESS [N=205]) and **(B)** unique participants initiating ART (Group B; VOLITION [N=171]). The left graphs show Roche cobas 6800 results. The right graphs show Abbott RealTi*m*e re-test results when available; otherwise, cobas 6800 results are shown. In the left graphs (cobas 6800–only results), greater variability is observed in the low viral load range (approximately 50-200 copies/mL). In contrast, the right graphs show reduced variability in this range, largely remaining below 50 copies/mL. Viral load values below the assays' LLOQ were manually imputed as 19 copies/mL for cobas 6800 (LLOQ, 20 copies/mL) and 39 copies/mL for RealTi*m*e (LLOQ, 40 copies/mL). ART, antiretroviral therapy; LLOQ, lower limit of quantification.




**Alt-text:** Four line graphs arranged in 2 panels comparing HIV-1 RNA measurements over time. Panel A shows results for participants who were virologically suppressed, and Panel B displays results for participants initiating ART. Each panel includes a left graph showing Roche cobas 6800 results and a right graph showing Roche cobas 6800 with Abbott RealTi*m*e re-testing results.

Rukobia▼ (fostemsavir)


# Managing multidrug-resistant HIV-1 presents unique challenges[1]

**Rukobia, in combination with other antiretrovirals, is indicated for the treatment of adults with multidrug resistant HIV-1 infection for whom it is otherwise not possible to construct a suppressive antiviral regimen**

Rukobia▼ (fostemsavir) provides an option to be added in or used as a replacement ARV in people who are experiencing difficulty achieving viral suppression on their current ART. Here, you'll learn how Rukobia works differently from other ARVs and about its durable efficacy and robust CD4+ T-cell outcomes from the BRIGHTE trial over 5 years. Are you ready to see how Rukobia could help you meet the needs of eligible people living with MDR HIV-1?

## Discover what's possible with RUKOBIA:

Leads to a promotional ViiV website

- **Durable virologic suppression, demonstrated over 5 years of follow-up in the BRIGHTE Study.[3]**
- **Robust CD4+ T-cell recovery, as per the BRIGHTE study.[3]**
- **Low potential for clinically significant DDIs, as RUKOBIA can be co-administered with most common treatments for HIV and frequently prescribed medicines for comorbidities[2]**
- **Consistent safety and tolerability profile, with 5 years of follow-up data, as per the BRIGHTE study[2,3]**

**Adverse events should be reported. Reporting forms and information can be found at https://yellowcard.mhra.gov.uk/ or search for MHRA Yellowcard in the Google Play or Apple App store. Adverse events should also be reported to GSK via the GSK Reporting Tool or on 0800 221441.**
**Prescribing Information**

**ART,** antiretroviral therapy; **ARV,** antiretroviral; **CD4,** cluster of differentiation 4; **DDI,** drug–drug interaction; **HIV-1,** human immunodeficiency virus type 1; **MDR,** multidrug-resistant.

**References**

1. Ackerman P, Thompson M, Molina JM, et al. Long-term efficacy and safety of fostemsavir among subgroups of heavily treatment-experienced adults with HIV-1. AIDS. 2021;35(7):1061-1072.
2. RUKOBIA Summary of Product Characteristics.
3. Aberg J et al. Infect Dis Ther 2023; 12:2321-2335.

PM-GB-FST-ADVR-250001 Date of preparation: November 2025

created and funded by ViiV Healthcare