Use and Interpretation of Quantitative HIV-1 RNA Test Results

Testing and Reporting Guidance for Laboratories

This document outlines the importance of accurately reporting results from quantitative HIV-1 RNA assays.

Nucleic acid tests (NATs), including qualitative and quantitative HIV-1 RNA assays, can detect HIV-1 RNA approximately 10-12 days after exposure.[1,2] In comparison, currently available HIV-1/2 antigen/antibody (Ag/Ab) immunoassays have a window period of approximately 15-24 days after exposure, making NATs essential in detecting acute HIV infections.[1]

While qualitative HIV-1 RNA assays are used primarily for diagnostic purposes, quantitative HIV-1 RNA assays are typically used to help monitor disease progression and treatment efficacy. Currently two quantitative HIV-1 RNA assays, the Aptima® HIV-1 Quant Dx Assay and the Alinity m HIV-1 Assay, have a dual claim when plasma (not serum) is tested, that allows them to be used for HIV-1 diagnosis and viral load monitoring.

Quantitative NATs have both a limit of detection and a lower limit of quantification. Patient samples that have HIV-1 RNA concentrations below the lower limit of quantitation (LLoQ) and above the limit of detection (LoD) of the assay may be either incorrectly reported by laboratories, incorrectly interpreted by clinicians or both. Incorrect reporting or interpretation of these quantitative HIV-1 RNA results has significant clinical and public health implications and can cause patients undue stress.

Virologic Marker of Response to Antiretroviral Treatment

In the United States, antiretroviral therapy (ART) is recommended for all persons with HIV and should be initiated as soon as possible after an HIV diagnosis.[3] Quantitative HIV-1 RNA assays, commonly referred to as viral load assays, measure the amount of viral RNA in the blood, which is used as a surrogate marker to monitor the effectiveness of ART and disease progression. An initial quantitative HIV-1 RNA assay is performed prior to ART initiation to establish a “baseline” viral load. While on ART, quantitative HIV-1 RNA assays are performed at regular intervals to measure the viral load. This provides essential information to achieve and maintain viral suppression with the prescribed therapy.

Limit of detection (LoD) is the lowest measured concentration of HIV-1 RNA (analyte) that is possible to detect in the test sample with acceptable certainty, typically ≥95% under routine laboratory conditions.

Lower limit of quantitation (LLoQ) is the lowest measured concentration of analyte (HIV-1 RNA) that can be quantified within the specified degree of accuracy and precision.

Upper limit of quantitation (ULoQ) is the greatest measured concentration that the analyte/HIV-1 RNA can be quantified within the specified degree of accuracy and precision.

HIV Viral Suppression is defined by the CDC as an HIV-1 NAT result of <200 copies per mL.

Undetectable means that the amount of HIV-1 RNA in a patient sample is below the LoD of the assay used.

It is important to consider the distinction between viral suppression and an "undetectable" HIV-1 NAT result, especially as patients focus on the message that "Undetectable equals Untransmittable" and as HIV-1 NATs have become more sensitive. The US Centers for Disease Control and Prevention (CDC) uses a confirmed HIV-1 NAT result of <200 copies/mL as the definition of HIV-1 viral suppression,[4] whereas the World Health Organization defines three categories based upon HIV-1 viral load: unsuppressed (>1000 copies/mL), suppressed (detected but ≤1000 copies/mL) and undetectable (viral load not detected by test used).[5] The Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV acknowledges that an undetectable HIV-1 NAT indicates optimal viral suppression but defines virologic failure as "as the inability to achieve or maintain suppression of viral replication to HIV RNA level <200 copies/mL."[3] These definitions account for the fact that when HIV-1 is suppressed to < 200 copies/mL there is no risk of sexual transmission of HIV-1 and the risk for emergence of drug resistance is low.[3-5]

Regardless, quantitative HIV-1 RNA results must be evaluated in the context of HIV serological results, ART including PrEP, and viral load history and other clinical markers to ensure that the determination of HIV status, ART effectiveness and disease progression is accurate. Accordingly, accurate reporting of these results is essential to preventing transmission of the virus, supporting clinical management and conducting accurate disease surveillance. There are several assays approved by the US Food and Drug Administration (FDA) for quantitation of HIV-1 RNA in human plasma on automated or semi-automated systems (**Table 1**) including the two with dual intended use claims named above. Each quantitative HIV-1 RNA assay has specific performance characteristics and specifications including LoD, LLoQ and ULoQ which are included in the instructions for use (**Table 1**) and depicted visually in **Figure 1**. For all FDA approved HIV-1 quantitative assays, the LoD and LLoQ are below 200 viral copies/mL. While these thresholds are standardized for a particular HIV-1 RNA assay, there may be variability between different assays (usually less than 0.5 log) which laboratories and clinicians should be aware of, particularly for monitoring response to therapy.

LoD vs LLoQ—What do you need to know?

- **The LLoQ can either be equal to or greater than the concentration of the LoD.**
  When the LLoQ is a greater concentration than the LoD, the challenge of result interpretation and reporting arises. If a sample's concentration falls between the LoD and LLoQ, a quantitative HIV-1 RNA assay is able to detect HIV, but cannot quantify the concentration (**Figure 1**: Yellow Bar).
- **The LLoQ cannot be less than the concentration of the LoD.**

Table 1. Reporting Language for Currently Available FDA-approved Quantitative HIV-1 RNA Tests[a, b]

Assay and Manufacturer (PMA#[c])	HIV-1 RNA concentration below LoD	HIV-1 RNA concentration above LoD and below LLoQ	HIV-1 RNA concentration at or above LLoQ and within Linear Range
	Result, Interpretation		
Abbott RealTime HIV-1[d] Abbott (BP060002)	**Not Detected,** Target not detected	**< 1.60 Log_{10} or < 40 cp/mL,**[e] Detected	**1.6-7.0 Log_{10} 40-10,000,000 cp/mL**
Alinity m HIV-1[f, g] Abbott (BP200455)	**Not Detected,** Target not detected	**<LLoQ,** Detected <LLoQ	**20 Copies/mL to ≤ULoQ,** Detected and quantified (20-10,000,000 cp/mL)
Aptima HIV-1 Quant Dx Assay[e] Hologic (BP150318)	**Not Detected,** HIV-1 RNA not detected	**<1.47 Log10 or < 30 cp/mL detected,** HIV-1 RNA is detected but at a level below the LLoQ	**1.47-7.0 Log10 30-10,000,000 cp/mL,** HIV Concentration is within the linear range of 30 to 10,000,000 cp/mL
cobas HIV-1(6800/8800) Roche (BP150262)	**Target Not Detected,** HIV-1 not detected	**< Titer Min,**[h] HIV-1 detected, less than (Titer Min)	**Titer,** (Titer) of HIV-1 Detected (20-10,000,000 cp/mL)
COBAS Ampliprep/COBAS Taqman HIV-1 v.2.0 Roche (BP050069)	**Target Not Detected,** HIV-1 RNA not detected	**< 2.00E+01 cp/mL (20 cp/mL),** HIV-1 RNA detected, less than 20 HIV-1 RNA cp/mL	**≥ 2.00E+01 cp/mL and ≤1.00E+07 cp/mL** (20-10,000,000 cp/mL)

a Package inserts (also known as "instructions for use" (IFU)) are provided for reference use only. For the most updated version please either login to your account with the manufac- turer, review the package insert with the assay that was included with the version that is currently being run, or contact a sales representative.

b Reporting Language provided in the table are based on the IFU for neat or undiluted specimens with regards to the LoD, LLoQ and ULoQ. Please refer to the IFU for the specific assay if using diluted specimens or for further clarification on reporting for each assay.

c PMA: Pre-market Approval (per FDA website where the acronym comes from)

d The LoD and LLoQ for the Abbott RealTime HIV-1 Assay varies depending on the sample volume. Reporting Language is for sample volume of 1 mL.

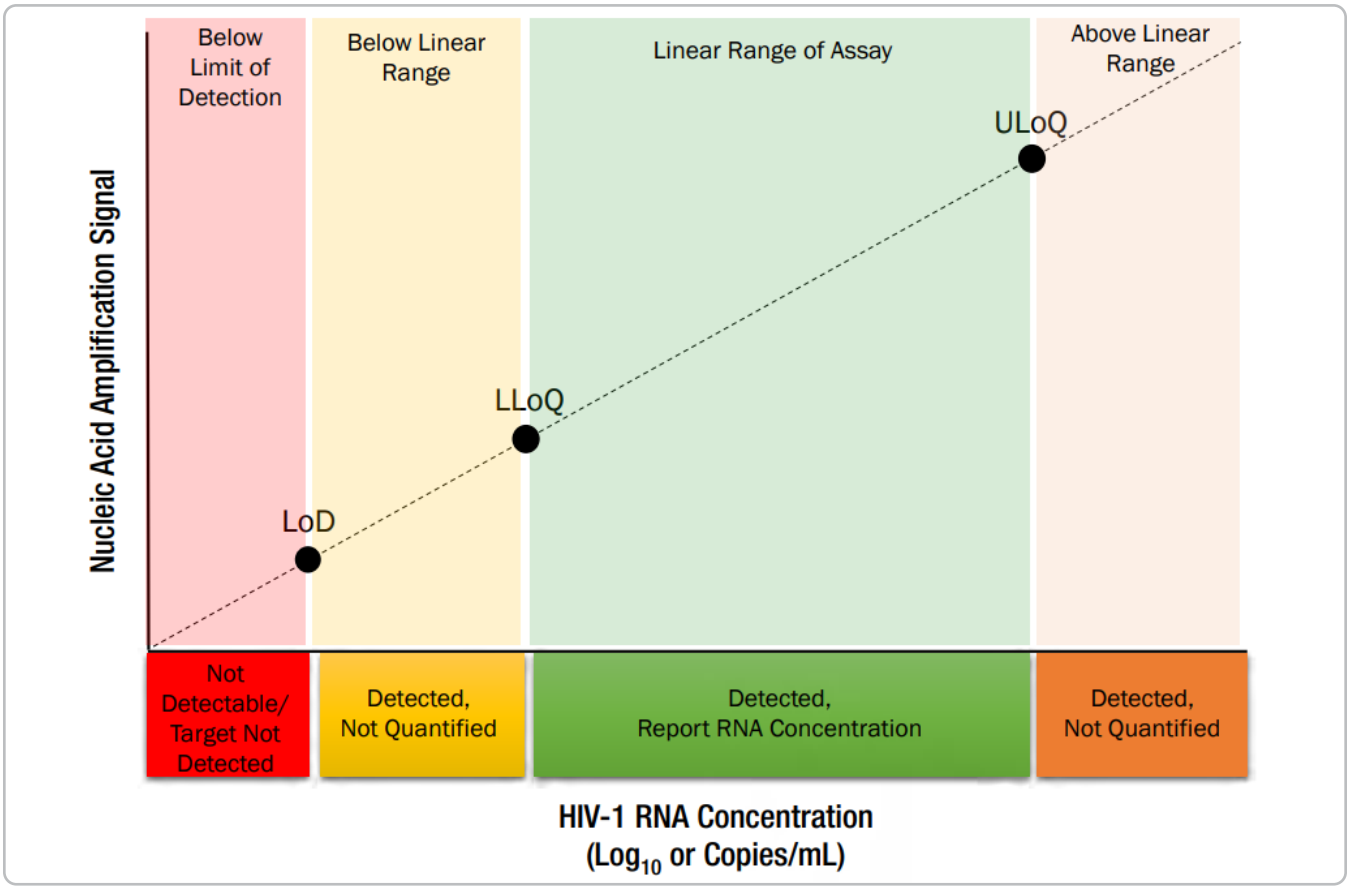
e cp/mL: RNA copies per milliliter

f The LoD and LLoQ for the Alinity m HIV-1 assay is 20 cp/mL (1.3 log cp/mL) for specimens tested without dilution (neat). The ULoQ is 10,000,000 cp/mL. Diluted specimens (1:25 or 1:50) may be used but if a diluted specimen has an analyte concentration <LoD a message code appears and they should be retested with a new neat specimen, they cannot be interpreted as "target not detected."

g The Alinity m HIV-1 and the Aptima HIV-1 Quant Dx have dual approved intended uses—one for diagnosis and one for viral load monitoring. This table only includes information for the intended use of monitoring/viral load or quantitation of HIV-1 RNA using plasma, but the assay has an approved intended use as a qualitative assay for the aid in the diagnosis of HIV-1 infection and confirmation of HIV-1 infection. It is intended for use as an aid in the diagnosis of HIV-1 infection, as a confirmation of HIV-1 infection, and as an aid in clinical management of patients infected with HIV-1. See the Instructions for Use for more details.

h The cobas HIV-1 (6800/8800) uses the term "Titer Min" for LLoQ. Actual Titer Min for the assay is 20 cp/mL for a 500 µL specimen and 50 cp/mL for a 200 µL sample.

Figure 1. Representation of Quantitative HIV-1 RNA Assay Results and Interpretations Between LoD, LLoQ and ULoQ[i]



Reporting Results from Quantitative HIV-1 RNA Assays

Reporting results from quantitative HIV-1 RNA assays is straightforward when HIV-1 RNA is not detected or is detected within (or above) the quantitation range of the assay. When HIV-1 RNA is not detected (either because the RNA concentration is below the LoD or not present), the reporting language may include the terms "not detected," "target not detected" or "HIV-1 RNA not detected." When HIV-1 RNA is detected and the concentration is within the linear range of the assay, the specific RNA concentration is reported, alone (Log10 cp/mL or cp/mL) or with terms such as: "target detected," "HIV-1 RNA Detected," "HIV-1 Detected" (See Table 1, Column 4).

However, reporting and interpreting results from quantitative HIV-1 RNA assays can be confusing if the HIV-1 RNA concentration is greater than the LoD but less than the LLoQ. Samples with an HIV-1 RNA concentration that falls within this zone (Figure 1, yellow bar) are colloquially referred to as "detected, not quantified" or "detectable, not quantifiable" because the assay detects the presence of HIV-1 RNA, but the RNA concentration is lower than the linear range of the assay and, therefore, cannot be quantified reliably. Many manufacturers include a suggested interpretation that is intended to provide more clarity. Specifically for this result, most manufacturers suggest inclusion of interpretations that include the word "detected" (Table 1, Column 3). However, based on reports from US HIV Surveillance Programs, this may not always be reported by the laboratory, may not be consumed properly by the electronic health record (EHR) or may be reported in such a way that the result and result interpretation are not clearly associated with each other.

i Abbreviations: **LoD** – Limit of detection; **LLoQ** – Lower limit of quantitation; **ULoQ** – Upper limit of quantitation

It is inaccurate and clinically misleading to report or interpret a result of detected, not quantified as HIV-1 RNA negative (target not detected, not detected or undetected). Every effort should be made to ensure that when data are transmitted electronically, details that might clarify the result (i.e., interpretations and comments that accompany the result) are not lost, stripped or otherwise excluded from the version that a healthcare provider reviews to make clinical decisions, as this can lead to misinformed clinical decisions.

Therefore, APHL recommends that laboratories reporting results from quantitative HIV-1 RNA assays for samples that have an HIV-1 RNA concentration above the LoD but below the LLoQ should make it clear in reporting the result that HIV-1 RNA was detected by including specific language in the result report that includes the numerical value of the LLoQ (i.e., HIV-1 RNA detected, <# copies/mL or for dual claim assays, HIV-1 RNA Positive/target detected, <#copies/mL) and an interpretation (i.e., HIV-1 RNA Detected, Below the Limit of Quantitation for the Assay). Additional language may also be needed to inform providers what additional steps need to be taken, keeping in mind that viral suppression is achieved when the viral load is less than 200 copies/mL. It may be useful to note that the Infectious Diseases Society of America defines viral suppression as a viral load persistently below the level of quantification of an assay, but also acknowledges that "the threshold for prevention of HIV sexual transmission is considered to be < 200 copies/ml."[6]

Table 2: Three examples to illustrate how results and interpretations can vary between different quantitative HIV-1 RNA Assays.[j] Note that all examples illustrate virologic suppression with a result < 200 copies/mL.

	HIV-1 VL Assay A	HIV-1 VL Assay B
LoD	10 copies/mL	10 copies/mL
LLoQ	20 copies/mL	15 copies/mL
Example 1: Sample A has an HIV-1 RNA concentration of 8 copies/mL which is lower than the LoD of both assays and therefore is unlikely to be detected by either assay. It would fall in the range of the red bar in Figure 1.		
Sample A Result	Not Detected	Not Detected
Sample A Interpretation	HIV-1 RNA Not Detected	HIV-1 RNA Not Detected
Example 2: Sample B has an HIV-1 RNA concentration of 15 copies/mL which is greater than the LoD of both assays, lower than the LLoQ for Assay A and equivalent to the LLoQ for Assay B. It should be detected by both assays but will not be able to be quantified by Assay A since it is below the LLoQ. For Assay A it would fall in the range of the yellow bar and with Assay B it would fall in the range of the green bar in Figure 1.		
Sample B Result	<20 copies/mL	15 copies/mL
Sample B Interpretation	HIV-1 RNA Detected at a level below the LLoQ	HIV-1 RNA Detected
Example 3: Sample C has an HIV-1 RNA concentration of 50 copies/mL which is greater than the LoD and the LLoQ for both assays and should be detected and quantified by both assays. This sample would fall within the range of the green bar in Figure 1.		
Sample C Result	50 copies/mL	50 copies/mL
Sample C Interpretation	HIV-1 RNA Detected	HIV-1 RNA Detected

j Terminology and format for reporting the result and interpretation should follow the manufacturers IFU.

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