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Interpreting quantitative HBV, HCV and HIV-1 nucleic acid testing

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ABSTRACT

Monitoring of HBV, HCV and HIV-infected individuals which are on antiviral treatment, ideally requires periodic viral load measurements to track legitimate response to treatment. Quantification of viral load has greatly improved the monitoring of therapies for infected individuals. There are several commercially available assays for the quantitation of HBV, HCV and HIV-1 load, but still the viral load could not be presented accurately for the initiation of antiviral therapy and follow up therapy. The objective of this study is to provide information for interpretation of viral load data of an infected patient for selection of an appropriate molecular assay for initiation and follow up of antiviral therapy. Twenty nucleic acid positive plasma samples each of HBV, HCV, HIV-1 were used in the study. The same molecular assay in subsequent run showed up to 0.2-log₁₀ variations and different assays showed up to 0.4-log₁₀ viral load variations for the same set of samples. The large range of variations were observed in samples having higher viral loads (>one million copies/ml). Several times it has been observed that the viral load results of >100 copies/ml are more reliable in comparison to low viral load results. The molecular assays of different manufacturers showed sizeable differences in viral loads for samples having HBV genotype A in comparison to HBV genotype D. The factors like clinically relevant cut-off, natural variability of viral load, the inherent variability of viral load assays, inter and intra assay variations, precision, linear range of an assay should be normalised before determining the effectiveness of an antiviral therapy.

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KEYWORDS

Molecular assay;
Viral load;
NAT.

INTRODUCTION

Massive advances have been made in molecular diagnostic since its inception and these PCR-based technologies are being used in clinical diagnostic laboratories for the management of various viral infections. The real-time PCR has revolutionised overall performance

of these assays and viral load quantitation has become the major tool for disease prognosis and antiviral therapy management for HBV, HCV and HIV-1 infected patients. Viral load testing using polymerase chain reaction (PCR) is the preferred method for early detection of therapeutic failure^[1-3]. The commercialization of viral load assays has allowed widespread and routine moni-

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toring of viral nucleic acid levels in infected persons. In developing countries HBV, HCV and HIV-1 viral load assays are urgently needed in the context of expected universal access to prevention and antiviral treatment programs. The commercially available assays vary in their ability to accurately measure and detect significant changes in plasma viral load^[4]. A more precise assay can accurately distinguish true clinically significant changes in viral plasma load from background noise, or systematic variation. The precision of a viral load assay is critical to patient management and gives the clinician a clear picture of the patient's true virologic status that is attributable to infection or treatment as opposed to systematic variation in assays. With successful active antiviral therapy, viral load detection limits are observed to drop below 500 copies/ml^[5-7]. This degree of reduction in viral load detection limits and the general consensus that highly active antiretroviral therapy should aim to suppress viral replication as fully as possible, has prompted the need for even more sensitive viral load quantification assays. Therefore, a number of manufacturers have adapted their existing viral load assays to permit a lower limit of detection^[4]. The use of a viral load assay with a 50 copies/ml detection limit would provide more informative data for patient treatment.

In 2008, international AIDS Society reaffirmed the importance of accurate and sensitive viral load assessment and by necessity, access, to viral load assays^[8]. Viral load testing is considered essential when initiating anti-viral therapy, when monitoring anti-viral therapy response and when considering switching anti-viral therapy regimens. The demand for accurate, reproducible and cost effective quantitative molecular assays is therefore a global issue^[9-11]. The explanation based on our data, would help in understanding the results of viral load monitoring assays. Further, the interpretation based on this study will help the clinicians in deciding anti-viral therapy.

EXPERIMENTAL

Plasma samples

This study involved 60 plasma samples comprising of 20 HBV-DNA positive, 20 HCV-RNA positive and 20 HIV-1-RNA positive plasma samples. These

plasma samples have been fractionated from the blood bags found positive for HBV, HCV and HIV-1 respectively, during the screening of blood, donated to various blood banks. The reference materials used were (1) 3rd WHO international standard for Hepatitis B virus for nucleic acid amplification techniques (NIBSC code: 10/264), (2) 2nd HCV RNA Genotype panel for nucleic acid amplification techniques (NIBSC code: 08/264) and (3) HIV-1 Genotype panel (NIBSC code: 08/358).

Platforms and reagents used

1. Abbott Realtime HIV-1 amplification reagent kit, Abbott Realtime HCV amplification reagent kit and Abbott Realtime HBV amplification reagent kit on Abbott m2000rt platform.
2. artus HI Virus-1 RG-RT-PCR kit, artus HCV RG-RT-PCR kit and artus HBV RG PCR kit on rotorGene platform.
3. artus HI Virus-1 QS-RGQ kit, artus HCV QS-RGQ kit and artus HBV QS-RGQ kit on QIA Symphony SP & AS & Rotor Gene platform.
4. Cobas TaqMan HBV test, Cobas TaqMan HCV test v.2 and Cobas TaqMan HIV-1 test v.2 for high pure system on Cobas TaqMan 48 platform.
5. Cobas AmpliPrep/Cobas TaqMan HIV-1 test v.2, Cobas AmpliPrep/Cobas TaqMan HCV test and Cobas AmpliPrep/Cobas TaqMan HBV test v.2 on Cobas AmpliPrep/Cobas TaqMan platform.

RESULTS

Nucleic Acid testing laboratory at this Institute has been established for performance evaluation of molecular assays. During year 2013-14, the laboratory has evaluated 13 batches each of HBV, HCV and HIV-1 quantitative molecular assay test kits manufactured by M/s Abbott molecular, USA, M/s Roche Inc., USA and M/s QIAGEN GmbH, Germany. The panels of 20 HBV, 20 HCV and 20 HIV-1 positive plasma samples having a dynamic range of viral load were used for evaluation of respective test kits. The molecular assays of different manufacturers showed different viral load for the same set of samples. The inter-assay viral load differ-

ences ranged up to 0.4-log₁₀ for 80% of samples and in rest of the cases differences were above 0.5-log₁₀ but below 1-log₁₀. The variations in viral load were also shown by same assay for the same set of samples during subsequent runs and these variations ranged up to 0.2-log₁₀ for 95% of samples. Variations in viral load for HBV up to 0.08-log₁₀ IU/ml has been observed for the same sample when put in duplicate at the same time with the same reagent in the same run and by the same machine and same operator. The large range of variations were observed for samples having higher viral loads (> one million IU/ml). It has also been observed that the viral load results of >100 IU/ml were more reproducible in comparison to low viral load (<100 IU/ml) results. The viral load results for low copy number samples (<25 IU/ml) were unreliable. The samples showing viral load of <25 IU/ml by a molecular assay were not detected in subsequent or repeat run by the same assay and also were not detected by molecular assay of the other manufacturers. The molecular assays of different manufacturers showed significant differences in viral loads for samples having HBV genotype A. However viral load results were similar for HBV genotype D. But in case of HCV such differences were not observed for genotype 1 and 3.

DISCUSSION

During the performance evaluation variations in viral load results were observed using the same molecular assay and testing the same set of plasma samples. Overall, differences were up to 0.2-log₁₀ for 95% of samples, by same molecular assay and differences were up to 0.4-log₁₀ for 80% of samples, between molecular assays of different manufacturers. The CDC recommendations and reports of year 2001^[12], have also described variability among the results reported by laboratories using the same molecular assay and testing the same donor samples (i.e., the duplicate) within a survey or the same donor sample (i.e., the replicate) used in other survey. The variations reported were up to 1.8-log₁₀. However, the absolute values of HIV viral load measured in the same plasma sample by using two different assays can differ by ≥ 2 -fold^[12]. Similar results were reported by Katsoulidou et al., 2011 for 86% of the samples, between Abbott RealTime and

CobasTaqMan assay for HIV-1 plasma viral load on genetically diverse samples from Greece and none of the samples showed a deviation of more than 1.0 log₁₀^[13]. The CDC recommendations and reports of year 2001,^[12] mentions that HIV RNA levels can vary by approximately threefold (0.5-log₁₀) in either direction upon repeated measurements among clinically stable, HIV-infected persons. The Changes >0.5-log₁₀ usually cannot be explained by inherent biological of assay variability and likely reflect a biologically and clinically relevant change in the level of plasma HIV RNA. Therefore, the minimal change in viral load considered to be statistically significant is threefold, or a 0.5-log₁₀ copies/ml change and WHO guidelines state that a difference of <0.5-log₁₀ copies/ml is clinically not significant, and this variation has no clinical consequence^[14]. However, plasma HIV RNA assays vary greater towards the lower limits of sensitivity. Thus, difference between repeated measures of >0.5-log₁₀ might occur at low plasma HIV RNA values and might not reflect a substantive biological or clinical change^[12]. The differences in precision between assays are profound at low, near cut-off levels, but also occur throughout the dynamic range of the assays^[15]. Karasi et al., 2011 has compared Roche cobasAmpliPrep/cobasTaqMan HIV-1 v2.0 and AbbottRealTime HIV-1 assays for quantification of viral load in HIV-1 B and non-B subtypes, and showed high correlation. One subtype C was severely underquantified by TaqMan test v2.0 for which sequence analysis revealed multiple mismatches between the viral sequence and the primer/probe regions. For specimens under 200 copies/ml, the overall agreement was 90% at the cut-off of 50 copies/ml and 67% at assay's lower limit of detection of 20 and 40 copies/ml. The 309 samples were retested by the cobasmplicor HIV-1 MONITOR TEST, V.1.5 and disagreement among the three assays around their lower limit of quantification was revealed^[16]. Church et al., 2011 showed that none of the assays including the Abbott RealTime HIV-1 test, and Roche cobasAmpliPrep/cobasTaqMan HIV-1 48 test detected or accurately quantified diverse HIV subtype samples within the accepted clinical range required. Although RealTime HIV-1 test and cobasAmpliPrep/cobasTaqMan had low rates of false negatives, but these non-detected samples were different for each

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assay^[17]. The comparative study by Scott et al., 2009 with a 20-member subtype panel derived from HIV-1-infected blood donors in South Africa reported least variability in differences between Abbott RealTime HIV-1 test and Roche cobasAmpliPrep/cobasTaqMan HIV-1 test. According to them RealTime HIV-1 tended to generate higher values than the other assays and showed overall good performance and potential for use with samples from populations predominantly infected with HIV-1 subtype C^[18]. In India at NARI Khopkar et al., 2013 compared the performance of Standard Roche COBAS Amplicor HIV-1 Monitor Test, version 1.5 with Abbott HIV-1 RealTime assay using 125 specimens and observed 94.4% agreement. This group also reported that Abbott HIV-1 RealTime assay has higher analytical sensitivity compared to Amplicor HIV-1 Monitor Test^[19].

The different target regions in the viral genomes for different assays might be responsible for variations in viral loads because, the HIV-1 target region for Abbott RealTime HIV-1 test is highly conserved region within *pol* (*integrase*), for cobasTaqMan 48 HIV-1 is highly conserved region of the *gag* gene and for cobasAmpliPrep/cobasTaqMan assay is a conserved region of the *gag* p41 gene^[20]. It may also be said that different molecular assays target different subtypes/genotypes of a single virus differently i.e. the compatibility of their primers varies depending upon the presence of subtype/genotype in a sample and in some cases, it may be due to the degeneracy of primers, which have been used in order to target all subtypes/genotypes simultaneously^[18,20]. But due to this degeneracy the exact determination of viral load is compromised up to some extent. Therefore, for accurate determination of viral load, the subtype/genotype specific primer assay could be tried. The variations observed by us between different viral load platforms for HBV genotype A and D might be explained on similar lines in context to target regions in HBV genome. Performance significantly vary between HIV viral load platforms according to subtype, and highlighted that viral diversity in the population being tested must be assessed and considered in the selection of a viral load platform^[20,21]. This challenge although present in the developed world is even more important in the developing world where the major burden of viral diversity exists. Non-detection, under-

quantification, or even over quantification of plasma viremia, has the potential to cause serious error in patient care. Discordant samples should be re-tested using a second viral load assay that targets an alternative gene region (i.e., HIV-1 *gag* if *pol* based assay was initially used, and vice versa). Periodic evaluation and modification of these assays may be necessary to ensure reliable quantification of divergent subtypes. In case of RT-PCR/PCR based assays the other factor for variations might be loss of a few nucleic acid molecules during its extraction. Since, all RT-PCR/PCR based assays use ~40 PCR amplification cycles and a dsDNA or dscDNA molecule at the end of 40 amplification cycles gets amplified to $n \times 2^{40}$ molecules. Therefore, the loss of single nucleic acid molecule may lead to loss of 2^{40} dsDNA molecules from amplicons.

CONCLUSION

The viral load variations may sometimes mislead the reports of patients under antiviral drug therapy. It is sometimes suggested that during antiviral drug treatment the periodic viral load monitoring should be done with the same viral load molecular assay. Because the circulating recombinant forms and unique recombinant forms and genetic diversity have pressure on accuracy of quantification. The viral load results that are inconsistent with previous trends should be repeated and treatment decisions usually should be based on two or more similar values. The factors like clinically relevant cut-off, natural variability of viral load, the inherent variability of viral load assays, inter and intra assay variations, precision, linear range of an assay should be taken into account for interpretation of molecular assay results to reach any conclusion and to determine the effectiveness of drug in a patient under treatment.

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CONFLICT OF INTEREST

None declared.

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