LOW VIRAL LOAD HIV-1 GENOTYPIC RESISTANCE ASSAY USING VIROSEQ KIT

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Introduction

Current antiretroviral therapy (ART) regimes are responsible for turning HIV-1 into a chronic manageable disease, however, drug resistance remains a problem. When patients are administered HIV therapy, the goal is to achieve viral suppression below the limit of clinical significance. To assist in achieving this goal, HIV-1 genotypic resistance assay (GRA) is routinely performed to identify point mutations which are known to be associated with HIV-1 drug resistance. The Abbott Viroseq HIV-1 Genotyping System requires a minimum HIV-1 viral load of 2000 copies/mL in order to perform the assay. Therefore, clinicians suspecting drug resistance in their patients must wait for the HIV-1 viral load to increase to 2000 copies/mL before a GRA can be attempted at Pathology Queensland.

By implementing an alternative extraction protocol (QIAGEN QIAamp Viral RNA Mini kit) instead of the extraction method used in the Abbott Viroseq HIV-1 Genotyping System, a purer extract can be eluted. This allows the sensitivity of the Abbott Viroseq assay to be increased well below the recommended cut off of 2000 copies/mL.

By increasing the sensitivity of the HIV-1 GRA assay to below 2000 copies/mL, clinicians may be able to pick up potential drug failures/drug resistances earlier in patients, therefore resulting in improved patient management and care.

Method

- HIV-1 viral load was determined using the Abbott RealTime HIV-1 m2000 platform
- 1mL of plasma was microcentrifuged at 24000 x g for 1 hour
- Supernatant was carefully removed (without touching/disturbing the viral pellet) leaving behind 140uL of plasma
- The QIAGEN QIAamp Viral RNA Mini kit extraction protocol was followed
- RT-PCR / PCR and Sequencing was performed on the QIAGEN extract using the Abbott Viroseq HIV-1 Genotyping Systems protocols
- Sequences were performed on the ABI 3730XL
- Sixteen (16) HIV-1 samples of various known viral loads below 2000 copies/mL with previous HIV-1 genotypic resistance profile were extracted using the QIAamp Viral RNA Mini kit and amplified/sequenced using the Viroseq Kit
- Five (5) HIV-1 samples of various known viral loads below 2000 copies/mL (with no previous HIV-1 genotypic resistance profile) were extracted using the QIAamp Viral RNA Mini kit and amplified/sequenced using the Viroseq Kit
- Quality of sequences were observed and comparisons were made with previous HIV-1 genotypic resistance profiles (where available)

Discussion

The quality of the sequences for all patients tested were good, obtaining an average QV of >= 40. Samples which had a previous HIV-1 genotypic resistance profile were compared. All samples showed highly concordant results producing the same subtype, and expected “other mutations”. All samples that had a significant mutation in the previous sample were still detected. There was no evidence that the modification to the Viroseq method reduced the effectiveness of the assay to detect significant mutations.

Five (out of sixteen) patients that had a previous GRA profile, demonstrated a significant mutation (causing resistance) that was not previously detected. All five patients were on antiretroviral therapy and had a persistently low but detectable HIV-1 viral load. This indicates that the patient developed a significant mutation whilst on therapy, which may explain why the HIV-1 viral load was not full suppressed.

Furthermore, significant mutations (causing resistance) were detected in two (out of five) patients with no previous GRA data.

With less RNA being analysed, random errors due to the processes of PCR must also be considered. It is possible that resistant mutations are present in a minority population of the virus species cannot be detected; however, our finding suggests that significant mutations can still be detected early in low viral load patients. This provides the treating clinician with the opportunity to perform GRA’s on patients with persistently low but detectable HIV-1 viral loads where ART failure is suspected. By detecting potential development of drug resistance earlier in the course of treatment, without needing to wait for the HIV-1 viral load to increase above 2000 copies/mL, clinicians will be able to change the patient’s therapy earlier, thus resulting in better patient care.