Local plasma separation results in more frequent HIV RNA undetectability when using the Roche TaqMan v2.0 assay

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Background

The issue of raised HIV viral load results in patients taking antiretroviral therapy (ART) currently affects many HIV centres[1,2]. The aetiology of this is as yet fully understood, but appears to be more complex than due solely to poor adherence.

We noted that HIV viral load results for patients on ART from a peripheral centre were more frequently raised than those results from an HIV centre on the same site as the virology laboratory.

On reviewing results from the peripheral centre it became apparent that samples were often processed at >=24hours.

The manufacturing guidelines from Roche regarding the TaqMan v2.0 assay state 24 hours as a suggested time from sampling to centrifugation and plasma separation - a change from the v1.0 guidelines which state 6 hours.

A service evaluation[3] showed that if samples were centrifuged locally with the plasma separated at under 6 hours, prior to transportation to the virology lab, results were more likely to achieve undetectability.

The clinic then switched to local centrifugation and separation of plasma in all samples for HIV-1 PCR testing.

Methods

We retrieved all HIV-1 PCR results for patients stable on ART for the 6 months prior to and following the change in sample processing described above. The assay used was Roche TaqMan v 2.0.

Inclusion criteria

Samples from patients commencing ART >= 6 months prior to the test.

Exclusion criteria

Samples from patients where there was concern regarding adherence.

Results

There were 135 samples (A) fulfilling the criteria from the 6 months prior to the change to local centrifugation and 160 (B) in the following 6 months.

Results from 42 samples obtained during the service evaluation were also included. The median HIV-1 PCR result for samples A was 151.

The median result for samples B was 0.

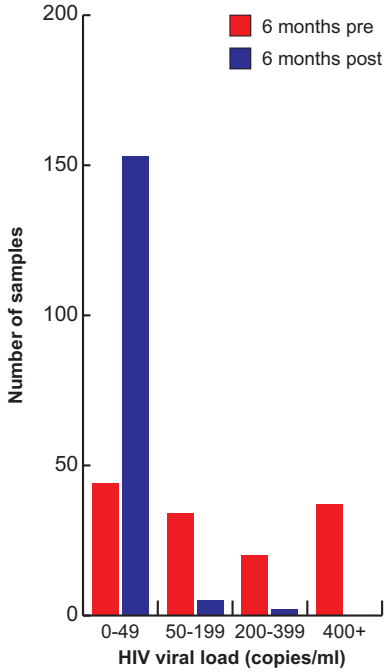
This gives a highly significant result ($p<0.001$) using the Mann-Whitney U non-parametric test.

The range of values for samples A was 0 to 8000, for samples B it was 0 to 330.

	Transported as whole blood	Local centrifugation and plasma separation <6h
Number of samples	135	160
Median HIV-1 PCR result	151*	0*
Range of HIV-1 PCR results	0-8000	0-330

** p<0.001 Mann-Whitney U test*

HIV viral load results prior to and following the change to local plasma separation



Discussion

We have shown that a delay in processing of whole blood samples is associated with inaccurate HIV-1 PCR results in patients stable on ART. We suspect that this may be due to natural cell lysis occurring over time, allowing more sensitive HIV assays to amplify proviral DNA. This data is of significance to other centres utilising the TaqMan v2.0 assay. This issue has also been highlighted to the writers of the BHIVA monitoring guidelines[4] as they suggest that leaving EDTA samples as whole blood for 2-3 days is acceptable.

Research looking at the reproducibility of HIV-1 RNA results over time in patients on ART is lacking. Previous data[5,6] relates to patients not taking effective ART. Concern in these studies is mainly regarding a reduction in VL over time, rather than an increase. However, a small increase in HIV VL in patients on ART has far wider reaching implications than a small reduction in VL in patients not on treatment.

More work is needed to clarify the optimal time from sampling to centrifugation & plasma separation in patients on ART. Until then, when a sample is taken as part of a research trial, or during pregnancy, we suggest that the timing of plasma separation is crucial. Our data suggests that the sooner this occurs, the more accurate the result for HIV-1 PCR using the TaqMan v2.0 assay.

Key Points

- **In patients taking ART, a delay in centrifugation and plasma separation of >= 24 hours, in samples taken for HIV-1 PCR testing, is associated with more frequently detectable results using the TaqMan v2.0 assay**
- **Research is needed into the reproducibility of HIV-1 PCR results over time for patients on ART when the EDTA sample remains as whole blood**

References

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