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Effect of hemolysis on HIV-1 protease and reverse transcriptase genotyping and phenotyping success: a 2-year analysis

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Purpose of the study

Interference of clinical laboratory assays by endogenous and exogenous substances in the blood is well known. To date, there are no clear guidelines on the testing of hemolyzed plasma in clinical laboratories. A conservative approach of alerting the physician with re-sampling is advised. This study focuses on the effect of processed (2006/2007), hemolyzed (in vivo/in vitro) plasma (visual inspection) on genotyping (virco®TYPE HIV 1) and phenotyping (Antivirogram®) success at Virco.

Methods

The viral RNA extraction kits (QiaAmp Virus MDx kit: 965652 or Easymag Nuclisens: 280130–280135) are highly sensitive in deriving intact, good quality RNA from hemolyzed or non-hemolyzed plasma, leading to successful amplification of PR-RT genes. The validity of the obtained result was confirmed by comparing the results from previous or subsequent visits/services from the same patient, where available.

Summary of results

In 2006, 315 hemolyzed samples arrived at Virco. From the 263 genotype requests, 246 were successfully genotyped (positive) and for 17 no genotype was obtained (negative, four samples with VL <1000 cp/ml, eight unknown VL, and three with VL >1000 cp/ml). From the 78 phenotype requests, 72 were positive and six negative (three with VL unknown and three with VL >1000 cp/ml). In 2007, 277 hemolyzed samples arrived at Virco. From the 210 genotype requests, 197 were positive and 13 negative (one with VL <1000 cp/ml, seven unknown VL, and five with VL >1000 cp/ml). From the 49 phenotype requests, 43 were positive and six negative (three with VL unknown and three with VL >1000 cp/ml). No limitations were observed for the different Clades.

Conclusion

The success rate for hemolyzed samples with VL >1000 cp/ml was >97% for both years and is similar to the success rate for non-hemolyzed samples with VL >1000 cp/ml (>98% for 2006 and >97% for 2007), indicating that hemolyzed samples can be processed for resistance testing under circumstances where re-sampling is difficult. The sensitivity to phenotyping clearly demonstrates the integrity of the amplified product that can be used to generate viable recombinant virus stocks. Further investigation is required on the severity of hemolysis and detection of well-defined mixtures within hemolyzed samples.