Background:
Our laboratory has been performing HIV-1 and HCV viraemia counts since many years with Roche Cobas Amplicor kits. As the company decided to stop commercializing these products we chose to switch our analyses to Cobas TaqMan format. Roche diagnostics recommends to process the plasma samples with a manual extraction Kit (High PureSystem Viral Nucleic Acid Kit) or with the COBAS®AmpliPrep Instrument. The manual extraction implies many steps, some specific laboratory equipment and requires a long hands-on time.

As our laboratory is equipped with a NucliSens® easyMag™ extractor we chose to use it for the processing of our plasma samples, and to compare the results with those previously obtained with Cobas®Amplicor HIV-1 Monitor® method.

Material and Methods:
Ninety-seven plasma samples from HIV and 37 plasma samples from HCV patients whose viraemia counts were previously obtained with Roche Cobas®Amplicor HIV-1 Monitor® Test v1.5 and Cobas®Amplicor HCV Monitor® Test v2.0 respectively, were tested with the new Roche Cobas® TaqMan® HIV-1 Test, v2.0 and Cobas® TaqMan® HCV Test, v2.0. Both of these tests are intended to be used with the High Pure System as a manual extraction method.

Five hundred microliters of each sample and plasma controls from the kit were extracted, using the specific B protocol, on the NucliSens®easyMag™ instrument. Before extraction 7µl of HIV-15QS (Quantification Standard) or 4.7µl of HCV-QS were added to each sample and plasma controls. The elution volume was 75µl, out of which 50 µl was used for amplification.

The two Master Mixes were prepared according to the manufacturer protocols. The amplification and detection steps were performed according to Roche’s instructions on a Cobas® TaqMan® 48 instrument. The results obtained with the two different platforms were then compared.

Results
Fifty-three out of the 97 HIV plasmas yielded viraemia counts lying within the linearity limits of both methods (HIV-1 Monitor: 50 copies/ml-100 000 copies/ml; TaqMan HIV-1: 34 copies×10^7 copies/ml). The linear regression analysis for these samples generated a R^2 (square of correlation coefficient) of 0.95, comparable with the R^2 (0.94) given by Roche for Cobas®Amplicor HIV-1 Monitor® Test v1.5 and Cobas®TaqMan® HIV-1 Test with manual High pure extraction.

Regarding the HCV tests, all 37 samples yielded results within linearity limits with both methods (HCV Monitor: 600 IU/ml-700 000 IU/ml; TaqMan HCV: 28 IU/ml-14×10^6 IU/ml). The R^2 obtained with the regression analysis was 0.97

Discussion and Conclusion:
From these results we conclude that an extraction of plasma samples with the NucliSens® easyMag™ instrument can be used with both the Cobas® TaqMan® HIV-1 Test and Cobas® TaqMan® HCV platforms instead of the High pure manual extraction.

This protocol allows us to avoid a cumbersome work and a long hands-on time without significant change in final results. The use of an automat is the guarantee of a better standardisation of the extractions than a manual method. With this procedure we have also been able to optimise the use of the automaton we already had in the laboratory, and to spare lab technician time and money.