

# Validation of an automated combined extraction of RNA and DNA to detect Hepatitis C and Parvovirus by PCR in blood plasma pools

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## Abstract

In order to fulfill international requirements for the safety of blood plasma pools, an automated protocol was developed to analyze a large number of samples for HCV RNA and Parvovirus DNA. We validated the extraction of viral nucleic acids from plasma pools using the Biorobot 9604 (Qiagen) in conjunction with the QIamp virus protocol. To detect HCV-RNA we used the Amplicor HCV Test version 2.0 (Roche) giving a qualitative result. Additionally we validated the extraction system for DNA using a quantitative real-time PCR (LightCycler, Roche) for the measuring of Parvovirus B19 DNA content.

Our results show a high sensitivity and a good reproducibility. Quantitative results are precise and accurate only in the range above 610geq/ml. The validation results comply with international guidelines.

Keywords: automated RNA/DNA extraction, HCV, Parvovirus B19, PCR, validation, blood plasma pools

## Characterization of the HCV detection

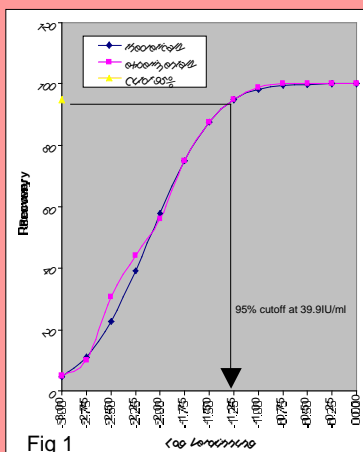


Fig 1

## Characterization of the Parvovirus B19 determination

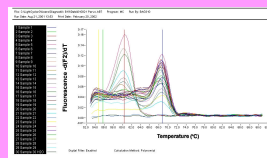


Fig 2

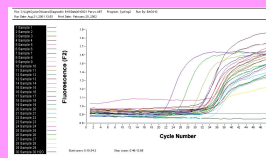


Fig 3

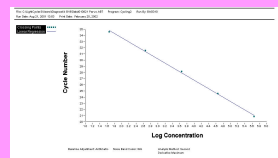


Fig 4

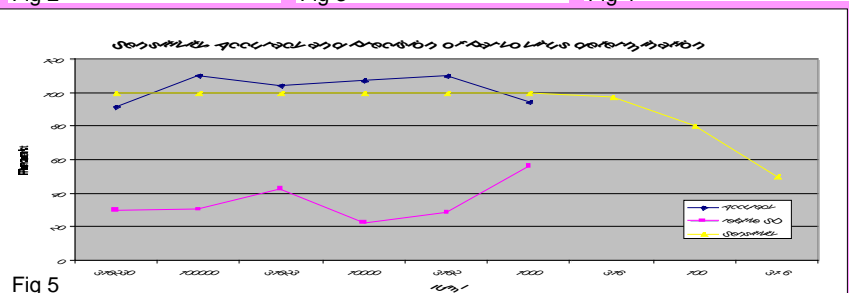


Fig 5

## Materials and Methods

### Extraction, amplification and detection

In order to extract viral RNA and DNA we applied the QIAamp 96 Virus Biorobot Kit in combination with the Biorobot 9604. The internal control of the Amplicor HCV extraction kit and an in house DNA extraction control for Parvovirus B19 were added to the lysis buffer before extraction of the samples (200ml). After extraction of the nucleic acids in duplicate, the two elutes were pooled. To amplify, 50ul for the HCV test (Amplicor Version 2.0) and 6ul for the Parvovirus B19 test were used for the PCR-assay.

The amplification of parvovirus B19 DNA was done by using an in house realtime PCR on the LightCycler (hybridization probes, Fig 2). The internal control of the Parvovirus B19 gave a signal with a 10°C lower melting point which is clear distinguishable from the wt-signal (Fig 2). Linearity of the PCR amplification was excellent over 5 orders of magnitude (Fig 4)

### Sequence of the primers and probes for Parvovirus detection

Primers B19459U19: cag tat cag cag cag tgg t B19rev: agg tgt gta gaa ggc ttc tt

Probes B193\*Fl: cgc gct cta gta cgc cca tcc cc B195\*LC: acc agt tca gga aga att att tgt cgg aa

### Cycling program:

10min 95°C 50x(95°C 20sec; 62°C-57°C 0.2°C/step 5sec single; 72°C 10sec 2°C ramp)

### Standards

Well characterized and widely used HCV and Parvovirus B19 viral standards were used from the National Institute for Biological Standards and Control (NIBSC, Blanche Lane, South Mimms Potters Bar, Hertfordshire EN 6 3Q, United Kingdom)

### Qualitymanagement

The complete analysis was designed to be run in the accredited scope (ISO 17025). Therefore complete process documentation was important and achieved. The individual run was validated positive using minimal requirements of the signals from the internal standards and external extraction controls.

### Positive and negative run controls

A run control must ensure detection of a systematic low performance of the assay. Seven samples of the NIBSC HCV and Parvovirus working standard diluted from 1:10 to 1:320 in negative sera served as positive run-controls. The nucleic acids of these samples were extracted and the quantitative results served as validation criteria of the individual run including all factors influencing the sensitivity, accuracy and precision of the

## Discussion

HCV: The data presented here show that the extraction and amplification assay used here, produces highly reproducible results also when extracting sera containing titer below 100IU/ml HCV-RNA. The achieved sensitivity at 95% cutoff was at 35IU/ml which fulfills the requirement (Fig 1). The sensitivity was similar to the one achieved when using the QIamp viral RNA kit which does not contain a Proteinase K step and extracts RNA only.

Parvovirus B19:

The sensitivity for Parvovirus B19 was below 312IU/ml (Fig 5). This gives approximately 15 geq per amplification reaction which is close to the theoretical lowest limit possible regarding the PCR-reaction (6geq). For high titer samples a relative variation of about 20% is achieved which is common in nucleic acid determination using real-time-PCR (Fig 5). The precision of the assay decreases and gets an increased relative variation of 60% when the virus titer becomes lower than 610geq/ml. Therefore quantitative results can only be obtained in an elevated range above 610geq/ml with satisfactory interval of confidence in a single measurement. Otherwise the sample has to be assayed multiple times and the results have to be averaged. However this method is practicable and fulfills the requirements in a fast and economic way.

The coextraction of RNA and DNA from bloodplasma is possible and very convenient. It opens the possibility to address many nucleic acid based quality relevant questions using a single extraction method.