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Validation of an automated RNA extraction and RT-PCR analysis to detect Hepatitis C virus of blood plasma pools

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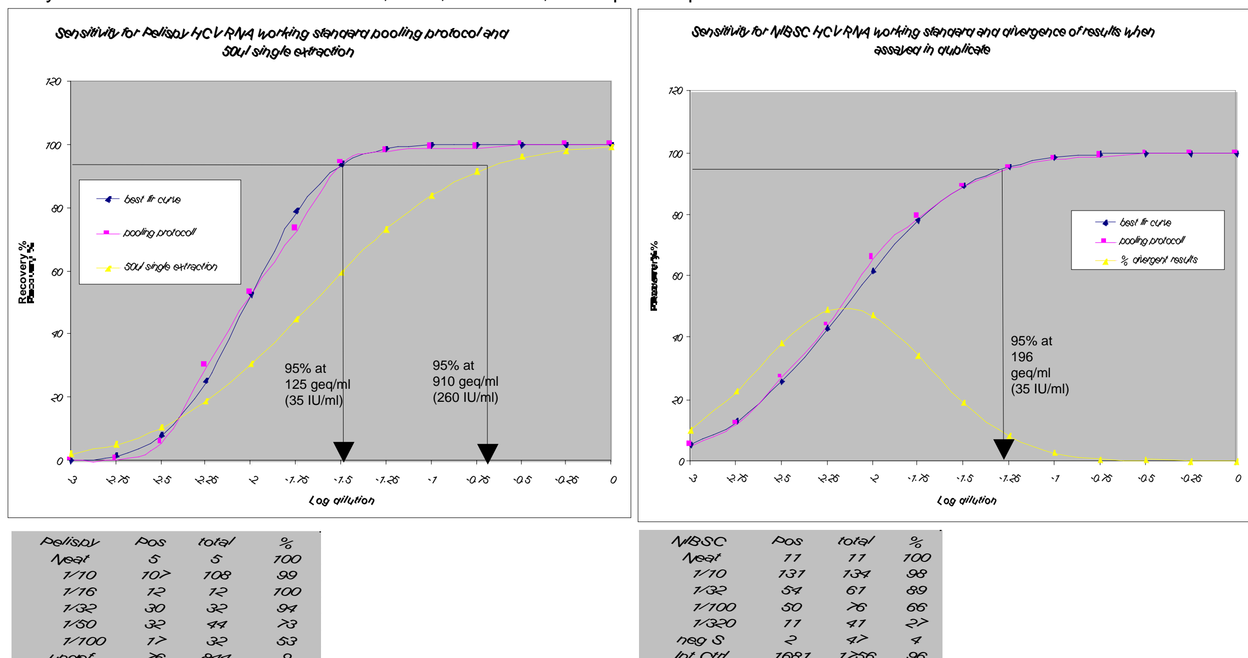
Abstract

To increase the safety margin of blood products plasma pools are tested for the absence of hepatitis C virus (HCV) RNA. In order to fulfil international requirements for sensitivity 100 international units per ml have to be detected with a rate of recovery of 95%. To analyze a large number of samples only an automated protocol can be implemented. Therefore we validated extraction of viral RNA from plasma pools using the Biorobot 9604 (Qiagen) followed by amplification and detection using the Amplicor HCV Test version 2.0 (Roche).

Our results show a high sensitivity and a good reproducibility only when samples are extracted twice and pooled. The validation results comply with international guidelines.

Keywords: automated RNA extraction , PCR, validation, blood plasma pools



Pelispy	Pos	total	%
Neat	5	5	100
1/10	107	108	99
1/16	12	12	100
1/32	30	32	94
1/50	32	44	73
1/100	17	32	53
undef	76	844	9

NIBSC	Pos	total	%
Neat	11	11	100
1/10	131	134	98
1/32	54	61	89
1/100	50	76	66
1/320	11	41	27
neg S	2	47	4
Int Ctrl	1681	1756	96

Materials and Methods

Extraction, amplification and detection

In order to extract viral RNA we applied the QIAamp 96 viral RNA test Kit in combination with the Biorobot 9604. The internal control of the Amplicor HCV extraction kit was added to the lysis buffer before extraction of the samples (200µl). After extraction (with 50µl of Amplicor Diluent) of the RNA in duplicate, the two elutes were pooled. For amplification two protocols were applied and compared.

The 50µl single extraction protocol:

50µl of single extracted samples were mixed with 50µl Mastermix of Amplicor HCV Test Version 2.0. Amplification products were detected using the Amplicor HCV Detection Kit (Art. No. 07 5758 6 /US 83280, Roche Diagnostics) and Amplicor Internal control detection Kit (Art. No. 07 6330 6/US 83324, Roche Diagnostics. This protocol was applied with Pelispy standard only.

The pooling protocol:

The double extracted sample was pooled and 50µl were mixed with 50µl Mastermix of Amplicor HCV Test Version 2.0 (HCV Amplification Kit Version 2.0, Art. No. 07 5930 9/US 83318, Roche Diagnostics) according to the manufacturer's protocol. When analyzing samples in a low range, divergent results are produced for statistical reasons (see right graphic, maxima at 50% recovery). Pooling of two extractions avoid this problem of interpretation.

Standards

Well characterized and widely used HCV viral standards are supplied from Paul Ehrlich Institute (PEI, Germany), the National Institute for Biological Standards and Control (NIBSC, Blanche Lane, South Mimms Potters Bar, Hertfordshire EN 6 3Q, United Kingdom) and the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Pelispy, PO Box 9091, 1006 AD Amsterdam the Netherlands) . We decided to use Pelispy (3600geq/ml,1000IU/ml genotype 1, PS 010398)) because data of Pelispy tested with the Amplicor Version 1.0 were available. Additionally we used NIBSC working standard (4000geq/ml, 710IU/ml, 96/586) because it is a genotype 3 virus which is known to be amplified with lower efficiency.

Positive and negative run controls

A run control must ensure detection of a systematic low performance of the assay. As positive control we included in every assay four samples of the NIBSC working standard diluted 1:10 in negative sera and three samples of Pelispy standard diluted 1:10 in negative sera. Negative controls are also included in every assay to ensure that positive results are specific and to trace crosscontaminations. One sample contains the negative sera in which the positive samples were diluted.

Dilutions to evaluate the detection limit. The positive standards are diluted in negative sera. The dilutions were neat (1/1), 1/4, 1/10, 1/16, 1/32, 1/50 and 1/100.

Discussion

The data presented here show that the extraction and amplification assay used here, produces highly reproducible results also when extracting sera containing low titer of 100IU/ml HCV-RNA (Pelispy and NIBSC working standard, 1/10 each). It does not discriminate between genotype 1 and genotype 3. Earlier data showed a discrimination of the genotype 3 followed by a underestimation of the viral load.

Pooling of double extractions before amplification increased the sensitivity from unsatisfactory 260IU/ml to satisfactory 35IU/ml, implying that extraction failure is a main reason for assay failure. If the sample is assayed in duplicate divergent results are produced at the detection limit (see yellow right graph). This leads to uncertainty. Double extraction and single amplification of the pooled extract overcomes this problem.

Only a single episode of cross contamination was observed during the whole validation period (1 Year). Column clotting is rare and depends strongly on the product to be analyzed.

During the Validation period the interpretation modes where changed. When applying the Amplicore Kit 1.0 mode of interpretation, 15.6% of the results had to be reanalyzed (10% undefined results of the sample 2% extraction failure and 3.6% positive negative control sera). This unsatisfactory finding obviously was the reason for changing the mode of interpretation with the new Amplicor Version 2.0 Kit. A single cutoff at 0.3OD was set. This reduces the amount of undefined results to 2% (failure of the internal control) which is satisfactory.

The presented procedure complies with international validation requirements. The method is sensitive, fast, convenient and cost effective.