Abschlussbericht Teilprojekt 2.2

Projekttitel: Qualitätskontrolle für virologisches Tests

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I. Short description

Aims of the project

The major aim of the project is the standardization of the virologic tests, trials, and assessment of new diagnostic procedures and reagents.

Background

One important task of the Hep-Net is to maintain and improve the already existing high standards of viral diagnostics in participating laboratories and institutions. This requires that all centers involved in the network take part, at least once per two years, in external quality assessment schemes provided by INSTAND, the Virology Quality Control program, and the Quality Control Concerted Action. The members of the Hep-Net project could also participate in standardization of the HCV genotyping techniques in collaboration with the Central Serum and DNA Bank, Essen using the HCV genotyping panel. The developed panel could serve as a useful cross-platform quality control standard for diagnostic and clinical laboratories employing different nucleic acid testing technologies. The reagents for this panel should be replenished on a regular basis to answer the demands.

Cooperation with other participants of the Hep-Net

The project is meant to serve all participants of the Hep-Net. The prepared diagnostic reagents, including HCV genotyping panel and plasmids, containing genome fragments from different HCV types, are available upon a request to all members of the network

II. Detailed description

Results

According to the plan, the first step of the project comprised the preparation of working standards for HCV diagnostics. Reagents for the HCV genotyping panel developed by the National Reference Centre for Hepatitis C (Essen) were replenished to answer a possible demand. At the moment, the HCV genotyping

panel includes 9 serum samples containing major HCV genotypes and subtypes (Table 1) and can be send on a request to any Hep-Net participant. By now this panel was sent to a more than 100 laboratories in Germany and abroad. Recently, the HCV genotype 6 positive blood donors were identified and this sample will be included into the panel. Thus, this panel will includes all major HCV genotypes and subtypes.

Table 1. The genotyping panel

Probe	**PCR**	**Quantification**		**Genotyping**				
	Amplicor	Cobas Amplicor HCV Monitor 2.0 (IU/ml))	VERSANT bDNA 3.0 (IU/ml)	Inno-Lipa HCV 2	GEN-ETI-K DEIA	PCR with type-specific primers Widell [1]	Ohno [2]	Sequencing [3]
1a	positive	$6{,}2 \times 10^4$	$4{,}4 \times 10^4$	1a/b	1°	1a	1a	1a
1b	positive	$1{,}2 \times 10^5$	$8{,}0 \times 10^4$	1b	1b	1b	n. t.	1b
2a	positive	$3{,}9 \times 10^5$	$5{,}0 \times 10^5$	2a/c	2a	2a	2a	2a
2b	positive	$1{,}5 \times 10^5$	$7{,}7 \times 10^4$	2b	2b	2b	2b	2b
2c	positive	$3{,}0 \times 10^5$	$3{,}1 \times 10^5$	2a/c	2c	n. t.	n. t.	2c
2i	positive	$1{,}2 \times 10^5$	$7{,}6 \times 10^4$	2a/c	2	n. t.	n. t.	2i
3a	positive	$3{,}2 \times 10^5$	$3{,}3 \times 10^5$	3a	3a	3a	3a	3a
4	positive	$2{,}3 \times 10^5$	$5{,}6 \times 10^5$	4c/d	4	n. t.	4	4
5a	positive	$1{,}1 \times 10^5$	$5{,}9 \times 10^4$	5a	5	n. t.	5	5a

1 Widell et al.: J. Med. Virol. 44: 272 - 279 (1994), [2] Ohno et al.: J. Clin. Microbiol. 35: 201 - 207 (1997), n. t.: not typable, [3] With primers from HCV core region (Viazov et al.: J. Med. Virol 53: 36 - 40 [1997]).

This panel can be used not only for standardization of the HCV genotyping techniques but, after calibration in WHO units, could also serve as a cross-platform quality control standard for laboratories using different nucleic acid detection technologies. At the moment, work is in progress on the preparation of a set of plasmids encoding 5'-UTR and core regions derived from major HCV types and subtypes, including 1b, 1a, 2, 3, and 4. In 2005, the preparation of plasmids containing the genome fragments of HCV type 5 and 6 is planned. These plasmids could be used for in vitro preparation of RNA transcripts. Such RNA transcripts are necessary for the assessment of the sensitivity of the in-house and commercial HCV diagnostic tests and kits for the detection of different HCV genotypes.

In the frame of this project, the staff of the National Reference Center for HCV upon the requests provided an expertise on diagnostic procedures and reagents. It also

took part in assessment of new diagnostic reagents. Thus, performance characteristics of the third generation branched DNA-based signal amplification assay for HCV RNA quantification was studied. This assay showed an analytical specificity of 98%. Mean intra- and between-run imprecisions were 6.8 and 11.2%, respectively. The assay was linear over its entire dynamic range. Quantitation appeared to be unaffected by genotype. A comparison of bDNA 3.0 with the second-generation bDNA assay calibrated against WHO HCV RNA standard, and the PCR-based Cobas Amplicor HCV Monitor 2.0 revealed a fairly good correlation among the assays. Twenty-nine and 11% of the paired quantitative results differed by more than log (10) 0,5 (i.e. three-fold). All three assays after calibration against WHO standard also yielded clinically comparable results with regard to the tailoring of interferon/ribavirin treatment duration in patients infected with HCV genotypes 1, 4, and 5.

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III. Plans for the future

In general, the whole study was run in full accordance with the program and the plan envisaged in the initial project proposals.

The activities during the second funding period will be directed at the standardization of the virological tests, trials and assessment of new diagnostic procedures and reagents. Special attention will be attributed to the standardization of HCV genotyping procedures; the major aim is the achievement of an adequate level of standardization on the European level.

Establishment of collaboration with other institutions involved in the standardization of virological tests. Thus, attempts are being made to include a number of samples, identified during the first funding period, into the different panels, distributed by Instand (Berlin) and Quality Control for Molecular Diagnostic (QCMD, Glasgow) in the framework of the external quality control assessment

schemes conducted at European level. This work should result in the establishment of a higher levels of standardization of HCV genotyping methods.

The activities during the second funding period will be also directed at standardization of the preanalytical phase of HCV RNA detection procedures. It is well recognized that the final outcome of all test for HCV RNA to a significant extent depends on a way the starting clinical materials are kept and processed. The relative instability of HCV particles means possible viral RNA degradation during processing and storage of clinical materials, especially during the shipment of these samples from clinical institutions to diagnostic laboratories. Recently, a number of new reagents for stabilization of RNA in tissues were developed. In collaboration with several institutions and serum bank we plan to perform a series of special trials of the influence of such reagents on stabilization of HCV RNA in different clinical materials used for routine laboratory diagnostic, including the whole blood, plasma and serum. The obtained results should lead to development of the optimal schemes of HCV RNA preservation and higher level of standardization of diagnostic procedures. In collaboration with the industry we also plan to participate in trials and assessments of new diagnostic procedures and novel modifications of the existing ones.