Study design: For each NAT technology employed in this study, NAP™ HCV RNA panels were tested in multiple runs on different days, with multiple kit lots. Results were analyzed using the data reduction protocols specific to each technology. The proprietary units from each assay were compared to WHO International Units (IU).

Results: Evaluation of the NAP™ HCV RNA in a variety of NAT methodologies demonstrated linearity across the entire range of target concentrations (0, 50, 500, 5,000, 50,000, 200,000, 500,000 and 2,000,000 IU/mL). Each panel member fell within 0.5 log of the expected value. As expected, the correspondence of assay specific proprietary units to IU differed depending upon the technology utilized.

Conclusions: NAP™ HCV RNA provides a consistent, standardized method for comparing results across patients, laboratories and technologies, and is useful in ensuring the quality of NAT testing for HCV RNA independent of the methodology used.

Session Pathogens

Quantification of Viral Load: Clinical Relevance for Human Immunodeficiency Virus, Hepatitis B Virus, and Hepatitis C Virus Infection

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In the last five years considerable scientific and financial efforts have been invested in the development of quantitative nucleic acid detection technology.

Quantitative culture of human immunodeficiency virus (HIV) is time consuming, cumbersome and requires appropriate laboratory safety equipment. Determination of p24 antigen with enzyme immunoassay (EIA) is of limited value due to its relatively poor sensitivity. Therefore, quantitative determination of viral load using nucleic acid amplification techniques represents the most accurate prognostic marker for HIV-1 infection, independently of CD4+ cell count.

For hepatitis B virus (HBV), which is not cultivable in vitro, serological assays permit an accurate diagnosis and follow-up of acute or chronic infection. While serology permits an accurate follow-up of HBV infection, HBV DNA quantification is used for monitoring of antiviral therapy, determination of infectivity, and in combination with serological markers for the resolution of unusual profiles, i. e., isolated anti-HBc reactivity.

Hepatitis C virus (HCV) can only be detected by molecular based assays, because there is no cell culture system, which would permit a reliable isolation of clinical specimens. Furthermore, early diagnosis and follow-up of infection cannot be reliably achieved with serology. The prognostic relevance of HCV RNA determination is of limited value for the long-term prognosis of chronic hepatitis C, however, viral load may predict the outcome of antiviral therapy.

Performance Characteristics of HIV and HCV PCR Quantification Assays in Blood

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The quantification of HIV and HCV is a useful test to give information about prognosis and monitoring of patients receiving antiviral therapy. For HIV PCR, different methods as conventional PCR from Roche Diagnostics, branched DNA from Chiron without amplification of nucleic acids, and NASBA technique with isothermal amplification from Organon Teknika exist. In addition, different processing methods give a higher sensitivity.

For routine purposes, commercially available tests are used (Cobas Amplicor HCV version 2.0 and HIV-1 version 1.5, Roche Diagnostics). The coefficient of variance (CV) for HIV-1 ranges from 19% to 42% and for HCV from 15% to 35%. For HCV, Gutekunst (1997) and Yen-Lieberman (1997) reported a CV of 35%, for HIV Schockmel (1997) a CV of 30% and Lin (1998) a CV between 16 and 88%. For HCV infections with genotype 3, the amplification rates are lower than for HCV genotype 1b. In comparison to genotype 1, the sensitivities of HCV primers have been shown 11% for genotype 2 and 8% for genotype 3 (Berger 1998).

Although these results are mostly in agreement with the clinical situation of the patients, especially for low copy numbers the reproducibility is not good enough. As a conclusion it is to be assumed that quantification of the HIV and HCV RNA should be improved with regard to international standardisation and reproducibility.

Borderline and/or Discordant Amplicor HCV Test Version 2.0 Results: Clinical Significance

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Background and Approach: The Amplicor HCV Test Version 2.0 (Roche Diagnostics) allows highly sensitive detection of hepatitis C virus (HCV) RNA in patient samples (100 copies/ml with 100% reproducibility) and generally yields reproducible, unambiguous results that correlate well with relevant serological and clinical parameters. Occasionally, however, results are borderline (defined by Roche as O.D. 0.15 - 1.0) and/or discordant upon repetition. Such results are difficult to interpret clinically. Do they represent false positives or reflect a very low level viremia (i.e., <100 copies/ml)? In an attempt to clarify the situation, we (1) tested serial dilutions of 4 HCV standards or quantified patient sera, and (2) correlated ambiguous results from 19 patients with available clinical and laboratory data.

Results: (1) All dilutions containing >100 copies/ml yielded O.D. values of >1.40 (average 2.35) and 100% concordant replicates. All dilutions containing <100 copies/ml, however, yielded O.D. values of 0.01 - 2.70 (average 0.97, 2 values 0.93 and 1.29) and <100% concordant rates. (2) All 19 patients had either a confirmed HCV infection (n=12, seropositive, most of them undergoing interferon therapy) or