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Quantification and genotyping in management of virus infection

P-101
Quantification of cytomegalovirus load by fret-based real time PCR with the lightcycler instrument
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We developed and evaluated a LightCycler-based quantitative Cytomegalovirus (CMV) PCR applying the fluorescence resonance energy transfer (FRET) technology. The primers and both hybridization probes were located in the CMV glycoprotein B gene. The noise band crossing points and the log template concentration were correlated ($r = -0.99, P,0.0001, 95\% \text{ CI} -0.999 -- 0.990$) in a range between $10^2$ and $10^8$ copies per milliliter plasmid standard, the end point detection limit was < 100 copies per milliliter standard. Human specimens ($n = 200$) were tested with the LightCycler PCR and compared to the Cobas Aplicor CMV Monitor assay and an inhouse PCR assay. Samples determined reactive in two assays were classified as CMV DNA positive ($n = 95, 47.5\%$), samples determined non-reactive in two assays as CMV DNA negative ($n = 105, 52.5\%$). CMV DNA was detected in 91 of the 95 CMV DNA positive human samples (sensitivity 95.8\%, 95\% CI 89.6--98.8) and in 1 of the CMV DNA negative human samples (specificity 99\%, 95\% CI 94.8--99.8) by the LightCycler test. The CMV load quantification by both quantitative test systems was correlated ($r = 0.73, P < 0.0001, 95\% \text{ CI} 0.61--0.81$), but undiluted samples with high CMV loads were quantified with LightCycler test more precisely than with the Cobas Amplicor CMV Monitor test. The level of sensitivity, specificity, and quantification accuracy makes the LightCycler technology a favorable test platform for the quantification of cytomegalovirus load.

P-102
Real-time quantitative PCR for the measurement of GBV-B in tamarin serum using the taqman detection system
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Objective: The aim of this study was to develop a new assay for rapid and accurate measurement of GBV-B viral load in Tamarins, based on real-time quantitative PCR with the TaqMan® system. The GBV-B Tamarin system has been developed as a surrogate model of Hepatitis C infection in humans.

Methods: The TaqMan® EZ single-step RT-PCR kit (PE Biosystems) was used with a set of oligonucleotide primers and a fluorophore-labelled probe specific for a highly conserved core region of
GBV-B. Assay optimization was carried out using in vitro transcribed RNA corresponding to a 407 bp core region of GBV-B. Assay validation was achieved by extracting RNA from 50 μl of GBV-B-infected Tamarin serum that tested positive in standard RT-PCR. Relative quantification of GBV-B RNA genomes was achieved by comparison to a standard curve generated from serial 10-fold dilutions of the in vitro transcribed RNA. To assess assay variability, pooled GBV-B-infected serum was divided in to four and tested in separate experiments.

**Results:** The detection limit of the TaqMan assay using in vitro transcribed RNA was 100 copies. Using 50 μl of GBV B infected Tamarin serum the limit of detection reached was $2.5 \times 10^4$ copies/ml. Only 2 fold variability was observed over four replicate assays in determining the GBV B genome copy number in the same Tamarin serum sample.

**Conclusions:** This single step assay is capable of detecting and quantifying GBV B RNA in small volume Tamarin serum samples. Additionally the reproducibility of the assay means that variations of 2 fold in GBV B viral load can be detected.

P-103

**CMV glycoprotein B and H genotypes in urine isolates from HIV patients**
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The envelope glycoproteins B and H of cytomegalovirus appear to be involved in both attachment and entry of the virus into host cells and are likely to play an important role in viral pathogenesis. Restriction enzyme analysis has shown that clinical isolates of cytomegalovirus (CMV) can be classified into one of four categories based on gB genotype and into one of two categories based on gH genotype. In the present study, we determined the gB and gH genotypes present in CMV isolates from urine specimens of a group of HIV positive patients with CD4 + T cell counts below 50 cells/mm³.

DNA was extracted by the guanidium isothicyanate method and subjected to nested PCR for amplification of gB and single round PCR for amplification of gH gene sequences. The glycoprotein subtypes were determined by restriction enzyme analysis.

A total of 35 samples were tested. Analysis of gB genotype showed: 11% type 1, 54% type 2, 29% type 3, 3% type 4 and 3% mixed genotype. Analysis of gH genotype showed: 34% type 1 and 66% type 2. Multiple samples collected over periods ranging from 3 to 7 months were available for 7 of these patients. The gB and gH types remained the same throughout the collection period for 5/7. For 2 patients however the genotype changed: in the case of one the gB type changed from 1 to 2 and the gH type fluctuated between 1 and 2. For the other patient the gB type remained type 3 throughout, but the gH type changed from 1 to 2.

These data, although gathered from few patients, suggest the CMV genotype can alter during the course of infection and may explain the prevalence of the more pathogenic genotypes at the advanced stage of HIV disease.

P-104

**Serological and molecular diagnosis of measles during the 1998 Argentine outbreak**
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Global eradication of measles (MV) will require continued commitment to increase vaccination coverage levels. The laboratory will play a critical role in monitoring the success of measles control strategies by developing more sensitive diagnostic reagents.

A total of 500 serum and saliva paired samples taken from clinical cases of MV were tested for specific MV IgM antibodies using a capture ELISA assay. Among these, 100 samples were confirmed positive by an RT-PCR method using primers designed to amplify 605 bp corresponding to the variable 3' region of the nucleoprotein (N) gene.

Results obtained by these methods showed that in 95% of the samples were detected high levels of IgM in both serum and saliva taken from each patient. IgM titers in these samples were positively correlated ($r = 0.95$, $P < 0.001$, Pearson Coefficient). Accordingly, amplification products of 605 bp were detected in all samples screened by RT-PCR. Similar results were obtained with the Edmonston strain present in available vaccines. Confirmed (IgM positives), 68% of cases belong to infants (<6 months, 25%) and small children (<13 months, 43%) demonstrating lack of maternal antibody protection.

These results demonstrate that a single saliva sample can be used either for confirming MV clinical disease or genotyping of circulating wild type strains.

**P-105**

**Evaluation of the cobas ampiclicor CMV monitor (CA CMM) test for quantitative detection of viral DNA in amniotic fluid specimens**

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**Background:** PCR amplification of human cytomegalovirus (HCMV) DNA in amniotic fluid is an important method for diagnosis of intrauterine infection but the test is not standardized and has variable sensitivity.

**Objective:** To evaluate a commercial, quantitative PCR assay (Cobas Amplicor CMV Monitor Test; Roche Diagnostic Systems, Inc) for the detection and quantitation of HCMV DNA in amniotic fluid specimens.

**Design:** Nine CNM positive and 29 negative amniotic fluid specimens, were included in the study. Intrauterine infection was confirmed in 7 of 9 positive specimens by viral isolation, in one by virus culture from the urine of a day old newborn and one by histopathological findings of viral inclusion bodies in an aborted foetus. CMV negative samples were all culture negative and were obtained from asymptomatic CMV IgM-negative mothers. In house semi-nested (sn) PCR and CA CMM were performed on all 38 specimens.

**Results:** CA CMM was positive in all 9 CMV positive specimens with viral load range of 6,670-8,090,000 copies/ml. SnPCR was positive in 8 of 9 samples (89%). The sample which was CA MMC-positive and snPCR-negative had viral load of 6,670 copies/ml and was culture negative. Viral load measurements were reproducible (in at least 3 consecutive experiments) and were not affected by 3 cycles of freezing and thawing of the amniotic fluid specimens. Non infected samples were all negative by both methods.

**Conclusion:** CA CMM test is sensitive, specific and responsible for detecting HCMV in amniotic fluid and can be standardized for the clinical laboratory. The use of a quantitative assay may shed new light on the pathogenesis of intrauterine CMV infection.
Abstract

P-106

**Typing of cytomegalovirus strains causing congenital infection by molecular analysis of UL55 GENE**

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The glycoprotein B of cytomegalovirus is a constituent of the viral envelope that is primarily involved in viral infectivity and in immunological recognition. Molecular analysis of the gene allows the classification of 5 genotypes. In order to determine the relative proportion of the genotypes and to investigate the relationship between genotype and virulence in an homogeneous group of cases of primary infection we analysed the viral DNA extracted from samples collected from 98 congenitally infected babies (41 symptomatic at birth). CMV DNA was extracted from dried blood spots on Guthrie cards (Barbi et al, 1996) and from urine/saliva samples collected in the first days of life, without any passage in cell culture. The products of a n-PCR amplifying the sequence corresponding to the cleavage site of gB protein were digested with Mae III enzyme (Shepp et al, 1996). Electrophoresis patterns corresponding to genotypes 1–4 were identified. The same genotyping results were obtained in urine and blood samples of the same baby. The most prevalent genotype was gB1 (42%) followed by gB3 (26%), gB2 (19%) and gB4 (13%). The same relative proportion was found in the 41 cases with congenital disease. No statistically significant relationship was found between genotypes and presence of neurological signs at birth.

Our results confirm the genotype prevalence reported by others both in bone marrow transplant patients and in infected babies and children. The presence of symptoms at birth, in particular of the neurological ones, seems not to be correlated to the infecting genotype.

P-107

**Serum epstein-barr virus DNA as a prognostic marker for naso-paryngeal carcinoma patient with distant relapse**

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Primary treatment of NPC is by radiotherapy. Advance disease is associated with a poor treatment outcome and an increased frequency of recurrent disease of up to 70%. We undertook a retrospective longitudinal study of EBV DNA in serum of NPC by in-house nested PCR for up to 5 years after radiotherapy.

By this method, detectable EBV DNA was observed in 31/97 NPC sera before treatment and high positive rate was associated in advance disease. Follow up of 40 NPC patients, 10 who remained remission up to 5 years, was consistently found undetectable EBV DNA in serum, while 2/10 of locoregional relapse were found positive in one serum of the follow-up samples. However, 8/10 (80%) in patients with distant metastatic disease was found DNA positives in single or multiple sera samples. Detectable serum EBV DNA can be observed as early as 12 months before patients who developed distant metastasis. Significant elevation in serum EBV DNA was also observed in 6 patients after distant relapse.

On the contrary, 3/6 was found decreased in antibody level to VCA and EA after relapse and dead finally.

We conclude these results that serum EBV DNA may be a reliable marker for early detection of distant relapse. Combination of serum EBV DNA and serology may be of prognostic value for relapse of NPC.
P-108

**HBV DNA levels in healthy antenatal carriers of hepatitis B**  
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**Objective:** To study the spectrum of HBV DNA levels in healthy carriers of hepatitis B virus (HBV) and study the variation over time in the absence of antiviral therapy. To assess infectivity in terms of HBV DNA concentration by examining perinatal transmission events.

**Methods:** Three commercial HBV DNA assays, Digene Hybrid Capture, Chiron Quantiplex and Roche Monitor were used to study the HBV DNA levels in over 100 antenatal patients.

**Results:** About 40% of HBeAg negative carriers were negative for HBV DNA (<400 copies/ml). A biphasic distribution of HBV DNA levels was observed with HBeAg positive and a few HBeAg negative patients having levels >10^7 copies/ml, the majority of HBeAg negative samples had levels below 10^5 copies/ml. Few samples fell within the range 10^5 and 10^7 copies/ml although this was the range over which the two most sensitive assays (Chiron and Roche) overlapped. Perinatal transmission from HBeAg negative mothers was noted to occur at relatively modest levels of HBV DNA. HBV DNA levels in single patients can vary widely over time, while others show a more stable pattern. The highest HBV DNA levels in HBeAg negative subjects were associated with both codon 1 and codon 28 variants.

**Conclusions:** HBV DNA levels are not stable over time even in HBeAg negative carriers. Transmission events are not always associated with the highest HBV DNA levels.

P-109

**Monitoring of EBV viral load in peripheral blood mononuclear cells of immunocompromised patients: an helpful tool for lymphoproliferative diseases**  
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Post-transplant lymphoproliferative disease (PTLD) is a severe complication of organ and bone marrow transplantation and more than 90% of PTLDs are positive for EBV. Different therapeutic approaches have been: reduction in immunosuppressive drugs, surgery for localized disease, chemotherapy and antiviral treatment and, more recently, infusion of donor derived EBV specific cytotoxic T lymphocytes and administration of anti CD20 monoclonal antibody. For the diagnosis and the monitoring of EBV related PTLDs, the quantification of EBV in the blood of transplant recipients seems to be an important tool.

We have developed a new quantitative competitive polymerase chain reaction assay (QC PCR) with a plasmide as internal standard (IS) derived from the qualitative EBV PCR primers which amplified a 121bp fragment located in BamH1C. A standard curve was generated after QC PCR of serial dilution of Namalwa cells and a constant number of IS. The amplified products were analyzed by differential hybridization in a microtiter plate. A wide linear range (10 40 000 copies) was established.

We used this assay to follow the fluctuation of EBV DNA load in peripheral blood mononuclear cells (PBMC’s) of 18 immunosuppressed patients, included solid organ and bone marrow recipients. The 6 patients who developed an EBV proliferation had a viral load higher than 500 copies/10^5 PBMC’s; in addition, the efficiency of the treatment has been monitored by the evolution of the viral load.

We concluded that quantitation of EBV DNA in the blood of immunosuppressed patients is a usefull tool that may be essential for the diagnostic and the monitoring of EBV induced PTLDs.
P-110

Detection and quantification of HHV 8, EBV, and HSV 2 DNA in cervical secretions from Swedish women
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Epstein Barr virus (EBV) and human herpesvirus 8 (HHV 8) are two related herpesviruses that both have been suggested to, at least in part, be transmitted sexually. We have developed real time PCR systems for detection and quantification of DNA from these viruses, and also herpes simplex virus 2 (HSV 2). These methods were used for examination of cervical secretions from 112 Swedish women. HHV 8 PCR on peripheral blood mononuclear cells (PBMC) and HHV 8 EBV, and HSV 2 serology was also performed on samples from all subjects. Antibodies to HHV 8 latent antigen were found in 3/112 (2.3%) and to HHV 8 lytic antigen in 27/112 (24%). No cervical secretion sample or PBMC contained detectable HHV 8 DNA.

EBV DNA was found in 10/112 (9%) of cervical secretion samples with a median number of 5700 (range 990–3 000 000) genomes/ml. Antibodies to HSV-2 were detected in serum from 13/112 (12%) individuals and of those 2/13 (15%) had detectable HSV 2 DNA in cervical secretions with 200 and 900 genomes/ml respectively. This study gives no further support to a sexual route of transmission for HHV8, but indicates that EBV could be transmitted through sexual contact. These new real-time PCR systems could be valuable in future studies of relations between virus load and disease.

P-111

Viral subtype and discrepancies between HIV load assays in 34 antiretroviral naive individuals
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Background: Discrepant results between different HIV load assays, or unexpectedly low RNA levels, have been observed in a significant proportion of HIV-1 infected individuals in southeast London.

Objective: To compare the results of three different HIV RNA quantification assays and determine the viral subtype in antiretroviral naive patients with discrepant virus load results.

Methods: Eligible patients were antiretroviral naive and had either plasma HIV load of &lt; / = 1500 copies/ml in at least one of three assays (NASBA, Chiron quantiplex version 3.0, and Roche amplicor version 1.5) or a clear discrepancy between results of different assays. Samples were serotyped by V3 loop peptide ELISA.

Results: The 34 eligible patients comprised 22 Africans, 8 Caucasians and 4 Caribbeans. Twenty-four (71%) had non-B subtype infections, including 19 Africans, 4 Caucasians, and one Caribbean. Of these 24 patients, 8 had an HIV load &lt; / = 1500 copies per ml in all three assays. The median CD4 count was 481 cells/mm3 (range 74–679). The remainder of the study group comprised 4 Caucasians, 3 Africans and 3 Caribbeans, of which 6 had an HIV load &lt; / = 1500 copies per ml in all three assays and a median CD4 count of 383 cells/mm3 (range 264 688).

Conclusions: Although a proportion of this cohort may be long-term non-progressors, this study has identified a subset of patients infected with predominantly non B virus, in whom the HIV load is underestimated using one, two or all three assays. Access to different assay methods may be required to evaluate patients from heterogeneous cohorts.
P-112

Antiretroviral therapy in an urban clinic (Slovakia). Risk factor of virologic failure studies
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Object: To contribute to the optimalization of antiretroviral treatment (ART) of HIV infected individuals diagnosed in Slovak Republic, clinical, immunological and viral status were regularly investigated in them. In attempts to reveal possible factors of the virological failure of the treatment the HIV resistance to administered drugs has been examined.

Patients and Methods: CD4 counts (flow cytometry, Becton-Dickinson) and viral load (Monitor HIV1 Amplicor, ROCHE) have been measured in 29 patients in various stages of HIV infection. During monitoring period (range from 14 to 50 months) 6 patients received bi- or 11 triple combination therapy (mostly the nRTi’s and Indinavir), 12 patients were not treated (3 refusing and 9 not fulfilling criteria for treatment initiation). Resistance was tested by genotyping of the RT HIV 1 genom domain in 5 treated and 4 untreated patients and in 16 patients by the INNO-LIPA HIV1-RT (Innogenetics).

Results and Conclusions: Antiretroviral impact demonstrated by viral load decrease and by an increase or a stabilisation of CD4 counts was observed in 4 (24%) of 17 treated patients. ART did not affect significantly viral load levels in 13 (76%) of patients. Appearance of mutations associated with AZT was found in all of AZT administered patients except of one, as well as in 4/4 ART naive patients studied. Nevertheless, valid compliance with therapeutic regime was noticed and clinically favourable status was sustained. Other reasons influencing the virological failure of ART given are under investigation (in collaboration with Dr. V.Calvez, Dept.of Virology, C.E.R.V. 1. Pitie-Salpetriere, Paris).

P-113

Detection of herpes simplex virus antigen and infectious HSV at different stages of genital HSV-infection
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Purpose: Comparative analysis of HSV antigen and infectious HSV in the urethra cells of patients at different stages of genital HSV-infection: acute stage, epithelization and remission.

Methods: Clinical materials (urethral scrapings) from patients with HSV-infection were studied by two methods: indirect immunofluorescence (iIF) of patient cells on the cytological preparations and rapid culture method (RCM). Revealing of HSV antigen was carried out by mixture of monoclonal antibodies (MAB) to two different HSV proteins (VP22 and gB), prepared in our laboratory. It was shown earlier that mixture of MAB revealed the same number of HSV infected cells as Sanofi test system and intensity of specific fluorescence was higher.

Results: 63 samples taken at different stages of HSV -infection were studied. The most number of positive results was obtained at the acute stage: 61.9% by iIF and 95.1% by RCM. 58.3% and 66.7% of positive samples were revealed at the epithelization stage by iIF and RCM, accordingly. At the stage of remission (no clinical manifestations of the infection), number of positive samples was 33.3% by iIF and 44.4% by RCM.

Conclusion: Both methods could be used for laboratory diagnosis at any stage of HSV -infection. The effectiveness of HSV revealing by two methods was different. Maximal discrepancy (30%) in acute phase of HSV-infection was obtained. iIF of the smears can be helpful as a screening method on condition that
quality of smears is satisfactory. RCM is more reliable and informative and should be employed as
cumbersome in-house technique using restriction fragment digested nested PCR products. Several commercial techniques are now available, like Line Probe Assay (Inno-Lipa, Gant, Belgium). This technique is based on reverse hybridization of HCV amplified PCR products with short oligonucleotides, immobilised on paper strips.

Purpose: To compare both genotyping technics.

Methods: We carried out genotyping on 47 frozen plasma samples obtained from as many HCV positive patients. Both techniques were applied to each plasma. HCV viral load (VL) was determined as well. Results: There was a 83% correlation between both techniques. 5 samples gave undetermined results with RFLP and 4 with Line probe Assay, with 1 sample unidentified by both techniques. 2 samples had different results according to the technique used. Some genotypes were easier to identify than others by RFLP, whereas Inno-Lipa results were not influenced according to the genotype involved. RFLP is a
more time consuming technique than Line Probe Assay. Cost of both techniques was similar, considering the technical time involved with RFLP.

**Conclusion:** Both techniques gave similar results with an 83% correlation, and involved similar cost, although RFLP is somewhat more tedious and more time consuming.

P-116

**Development of an automated test for quantifying HIV-1 RNA**

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**Purpose:** To develop an automated test for determining HIV 1 viral load based on target capture, TMA (transcription-mediated amplification) and VIDAS detection.

**Methods:** HIV 1 RNA plus internal control (IC) RNA were captured onto paramagnetic particles. The particles were resuspended, transferred to a unit dose VIDAS PROBE strip (containing all the reagents necessary for amplification and detection) and the particle-bound RNAs automatically amplified by TMA in the AMPstation. The strips were transferred to the VIDAS instrument for automated detection of the amplicon products. Quantitation was achieved relative to a master calibration curve which was adjusted by run-specific calibrator results.

**Results:** Preliminary results with a manual TMA format demonstrated accurate quantitation of all HIV-1 subtypes, compatibility with plasma anticoagulants, and agreement with PCR based methods for quantifying clinical specimens. Initial results with the automated format, using the AMPstation instrument, revealed particle clumping with some clinical specimens and relatively poor precision at low viral loads. Recent optimizations of the particle resuspension buffer and the TMA reagent have eliminated clumping of the paramagnetic particles and improved assay reproducibility. Intra-assay precision studies showed CVs of <30% for HIV 1 RNA inputs = 1000 RNA/ml, <40% for viral loads 100 to 1000 RNA/ml, and <60% for inputs = 50 RNA/ml. IC results were also reproducibly generated (33% CV) for negative samples. Linearity was observed from 25 to > 100 000 RNA/ml, with 100% positivity at all levels. An evaluation of this automated format is on-going with clinical specimens relative to a PCR-based method.

**Conclusion:** The automated TMA/VIDAS format under development should prove easy to use and provide valuable quantitative HIV 1 viral load results useful for monitoring HIV infected patients

P-117

**Value of markers for cytomegalovirus (CMV) infection for the occurrence of CMV disease in human immunodeficiency virus (HIV) infected patients receiving highly active antiretroviral therapy: the predivir study**

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The aim of the study was to assess the thresholds of CMV antigenemia and -DNAemia predicting CMV disease in patients receiving HAART. 198 HIV-infected adults, CMV-seropositive, free of CMV disease
were enrolled in the Predivir cohort with CD4 counts <100/mm$^3$ or <200/mm$^3$ under HAART. Blood culture, pp65 antigenemia assay, plasma CMV DNA PCR (Amplicor* PCR Diagnostics Roche), late mRNA RT-PCR, peripheral blood leukocyte (PBL) CMV DNA quantification were performed every 4 months. CMV DNA was quantified from PBL by PCR (Cobas Amplicor CMV Monitor and a semiquantitative assay using ELOSA* Kit from Valbiotech) and bDNA hybridisation assay (Quantiplex CMV, Bayer). At inclusion, median CD4+ cell count was 77/mm$^3$ and 85% of the patients were receiving HAART. The median follow-up was 719 days and 100% of the patients had been receiving HAART since month 12. CMV disease was diagnosed in 11 patients (incidence 3.1 per 100 patient/years). Concordance between DNA markers was the most demonstrative at month 8 in patients with CD4 <75/mm$^3$: it was 0.84, 1.00, 0.87, 0.84 for PCR Valbiotech/plasma PCR, bDNA/plasma PCR, PCR Roche/PCR Valbiotech and bDNA/PCR Valbiotech respectively. The best predictors of occurrence of CMV event within 4 months were PCR Roche > 100 copies (cps)/1.5 $\times$ 10$^5$ PBL, PCR Valbiotech > 10 cps/1.5 $\times$ 10$^5$ PBL, plasma DNAemia, antigenemia > 100/2 $\times$ 10$^5$ PBL and bDNA > 104 cps/106 PBL in order of increasing probability. The tests with higher predictive value had lower sensitivity. All these markers appeared suitable for defining a group of patients at risk for CMV disease.

P-118

**HCV qualitative assay (TMA)-a new standard for improving patient management**

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Recent improvements in the therapeutic regimens available to individuals infected with Hepatitis C require improved methods for detection and the assessment of a patient’s response to therapy. Our laboratory recently evaluated a new assay from Bayer Diagnostics based on Transcription Mediated Amplification technology (TMA) for the qualitative detection of HCV in clinical specimens. The performance characteristics of this assay (described below) will improve the ability for clinicians to detect HCV earlier following infection, assess a patient’s response to therapy throughout treatment, and identify those patients with a sustained response.

Sensitivity was evaluated using multiple replicates of a 7-member dilution panel with value assignments of 500, 164, 82, 61, 41, 20 and 8 HCV RNA copies/mL. The analytical sensitivity was observed to be 98% down to 41 copies/mL. ($n=226$, 96.8% down to 20 copies/mL ($n=250$), and 92.7% down to 8 copies/mL. By comparison with the WHO International HCV Standard (96/790), 50 copies/mL correlates to approximately 5 IU/mL-representing a one log (10X) improvement over the sensitivity of other commercially available products for qualitative HCV testing.

Specificity was evaluated using multiple replicates of a 6-member panel derived from individual seronegative donor pools. The results demonstrated specificity of > 99% ($n=228$ replicates).

Interestingly, in a population of 59 clinical specimens, we observed 5 discrepant cases where the result was HCV positive by TMA and negative by Roche Cobas Amplicor PCR. Upon retest, 2/5 specimens were positive by PCR. The remaining 3/5 specimens retested as PCR negative, and were likely below the PCR assay’s level of detection. These discrepant samples may represent either relapsers or cases where modifications to therapy were needed to improve the likelihood of response. An analysis of the results from larger populations of breakthrough patients, relapsers and sustained IFN-responders will be presented. We feel that the use of the more sensitive HCV QL TMA Assay allows greater clarity and will help clinicians make better decisions for patient management.
P-119

Monitoring of recurrent HCMV disease in an immuno-com promised child using a real-time PCR procedure
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Human Cytomegalovirus (HCMV) infections are frequent in immuno-compromised children. Real-time PCR procedures are now available for the monitoring of these infections by quantification of HCMV genome given. We report the case of a child with a combined immune deficiency that underwent bone marrow transplantation. This child presented with a severe immuno-compromission and a chronic enteroviral infection. HCMV genome quantification was carried out in sera samples. The bone marrow transplantation was carried out the end of May 1999. Two weeks after engraftment, he had fever and symptoms of reject. A peak of genome load of 7300 DNA copies/ml. Simultaneously, HHV6 DNA was detected in sera. He was successfully treated with 21 days of ganciclovir; both HCMV and HHV6 genomes becoming not detectable. The patient relapsed with both viruses 3 times, with higher HCMV genome peaks (up to 22 800 DNA copies/ml), and was treated successively with foscarnet and ganciclovir. During the last relapse, he presented with bilateral retinitis. A long term cidifovir therapy was started, yielding low HCMV genome load. Treatment improved the clinical status of the child. This surveillance using HCMV genome load is still carried out.

In this case, the measurement of HCMV genome load using real-time PCR procedure has been helpful for both monitoring and managing antiviral therapy.

This measurement of HCMV genome load should be widely used to adapt the doses and duration of antiviral treatments during HCMV disease in immuno compromised patients.

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Development of a real-time PCR procedure including an internal control for measurement of HCMV genome load
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Human Cytomegalovirus (HCMV) infections are frequent in immuno-compromised patients. The recent development of real-time PCR procedures that allows the rapid quantification of genome load are going to be helpful for the accurate monitoring of these infections. First, two extraction procedures were evaluated using 30 different blood samples that were processed pure and diluted (1/10th). Secondly, repeatability and reproducibility of the quantitative PCR procedure using an internal control for amplification were analysed, and its sensitivity compared to a qualitative PCR procedure using 50 HCMV positive blood samples (HCMV positive by culture). The real-time PCR and qualitative PCR procedures were positives in 46 and 48 of the positive samples that were tested, respectively. The discrepancies were observed in samples with a low genome load. The sensitivity of the real-time PCR procedure was 500 HCMV DNA copies per ml of sera. The use of an internal control concomitantly processed during the HCMV quantification was not altering the sensitivity of the procedure, and was relevant for the detection of putative PCR inhibitors that may interfere with the amplification process.

This procedure was used to measure genome load in 2 bone marrow transplant patients with HCMV disease, confirming that this new PCR procedure should be widely used to diagnose and monitor HCMV infection in transplant patients.
Asymptomatic HBV infections in vaccinated hemodialyzed patients during outbreak of viral hepatitis B
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An explosive outbreak of HBV infection occurred in patients of a hemodialysis unit. Sequencing and epidemiological analysis identified a single source of HBV. Out of 42 patients of the unit HBsAg occurred in 16 patients during 4 months. There were 38 patients completely vaccinated against VHB before outbreak. The aim was to study development of HBV infection and changes in humoral immunity during one year followup of all patients of the unit.

Sixteen infected HBsAg and HBV DNA positive patients were found in HD unit four months after the entry of chronic hepatitis B patient later recognised as source of HBV infection. Four patients had clinical symptoms. Elevated ALT were found in 13 patients. Twelve patients (75%) remained HBsAg positive after 8 month of follow-up. Only two patients seroconverted to anti-HBs. There was a group of ten patients with booster of anti-HBs. Half of them developed also anti-HBc during first 3 months of follow-up. Anti-HBs levels before occurrence of anti-HBc were 21, 818, 39, 3, and 602 ml.U./ml. Anti-HBs booster in these patients was in range from 2 times to 330 times. HBsAg, HBe and anti-HBe were negative. In two of these patients anti-HBc IgM were detected. There is serological evidence that at least 27 out of 42 (64%) patients were exposed to HBV.

Conclusions: Efficacy of vaccination against HBV is limited in hemodialyzed patients. Spectrum of asymptomatic HBV infections were recognised during the outbreak. High chronicity rate (75%) was found in hemodialyzed patients.

The additive effect of high-dose IFN to lamivudine therapy in chronic hepatitis B patients
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With interferon (IFN) or lamivudine monotherapy, viral clearance is obtained in the minority of patients. Combination of virus suppressive and immunostimulatory agents seems logic. Lamivudine was used to suppress viral replication. At a point of maximal viral suppression, high dose IFN was added to enhance virus eradication.

Methods: Twenty-five patients were included. In 3 patients IFN was stopped prematurely due to drop-out (n = 2) and collapse (n = 1). In 1 patient both IFN and lamivudine were withdrawn after 4 weeks of combination therapy. Twenty-one patients, 9 previous IFN non-responders, were included in the per protocol analysis (9 female, 12 male) Median (range) age was 29 (19 61) years, HBV DNA at baseline was 1,1 6 10⁹ (1,5 10⁶–2,8 10¹⁰) geq/ml. One patient was HBeAg negative before start of lamivudine therapy. Patients received lamivudine monotherapy for more than 16 weeks. IFN therapy was added in an initially high-dose, 10 MU daily for the first 4 weeks, thereafter IFN was continued at a lower dose, 10MU tiw, for at least 12 weeks. Lamivudine therapy was withdrawn after at least 8 weeks of combination therapy. Follow-up was 16 weeks. HBV DNA was measured by Digene HCS II, if negative by quantitative or qualitative PCR.

Results: HBV DNA levels decreased during lamivudine monotherapy (16 74 weeks) by 3,3 (range 1,8 6,7) logs, followed by a further median decrease of 1,5 (range –0,11 2,7) logs after addition of IFN. Withdrawal of lamivudine therapy after more than 8 weeks of combination therapy was accompanied by
a median increase in HBV DNA levels by 3.5 (0.4, 4.4) logs. During the follow-up period, HBV DNA increased further by median 0.9 (–1.8, 6.0) logs. HBeAg and HBV DNA negativity (Digene HCSII) was sustained until the end of follow up in 2 patients. In 4 patients re-introduction of lamivudine was necessary because of a severe hepatitis flare (ALT 10 × ULN) 9, 13, 16 and 20 weeks after withdrawal of IFN monotherapy. This was respectively 27, 25, 40 and 28 weeks after withdrawal of lamivudine.

Conclusions: High dose IFN therapy had an additive effect on lamivudine monotherapy in suppressing HBV DNA levels. An additional reduction in HBV DNA levels of more than 2 log was achieved in 7/21 patients. Sustained viral clearance however was achieved in only 2 patients.

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Utilisation of the immunoperoxidase technique for titration of a Dengue 1 virus strain
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Application of the immunoperoxidase technique for the quantitative titration of Dengue virus has improved some of the disadvantages of the plaque formation in cell cultures titration technique which is considered a fastidious method due to the number of factors involved in the viral plaque formation. In the literature, the reduction on time to obtain the results was reported, as the principal advantage of the immunoperoxidase technique, but nothing is said about its precision. Our purpose was to standardise the immunoperoxidase to titrate Dengue 1 virus in our lab.

The procedure described by Okuno et al. in 1985 for BHK 21 cells was followed, modified by the fixation of infected cells at 5, 7, and 10 days and blocking with PBS-Tween 20-BSA 1%, during 1 hour, after it. The working dilution for the human sera was 1/500 and for the polyclonal ascitic fluid (AF) obtained in mice against Dengue 1, was 1/100; for the anti human and anti mouse peroxidase conjugates were useful 1/200 for the first and 1/1000 for the second.

In that conditions, we observed characteristic foci produced by Dengue virus and it could be quantified at 7th and 10th days but not at 5th. The Variation Coefficient was < 30%. We chosen the 7th day advantageous to titrate the virus, earlier than by the plaque method (10th day). Significant differences between viral titration obtained by both techniques were not found.

Key words: Dengue 1, immunoperoxidase, viral titration.

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HPV detection and genotyping in cervical cells taken from women attending a colposcopy clinic for persistent, mild, abnormal cytology
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The aim of this study was to genotype HPV which had been detected as part of a routine service provided by UCLH.

253 women attending the colposcopy clinic with mild cervical atypia had initial samples taken for HPV detection by Digene Hybrid Capture II (HC II). From a small aliquot of this sample, DNA was extracted (Qiagen) and any HPV DNA was amplified by PCR using the broad spectrum SPF10 primer set.

253 women attending the colposcopy clinic with mild cervical atypia had initial samples taken for HPV detection by Digene Hybrid Capture II (HC II). From a small aliquot of this sample, DNA was extracted (Qiagen) and any HPV DNA was amplified by PCR using the broad spectrum SPF10 primer set.
Amplification was confirmed using a microtiter based hybridization assay and typed by a Line Probe Assay (LiPA).

94 initial samples gave a positive result by the manufacture’s criteria for high risk (HR) HPV in HC II with test control ratios (TCR) ranging from over 3000–1.14. 28 samples with a TCR < 10 to > 1.0 were repeated and 21 continued to be reactive. 87 HR HPV positive samples (repeat reactive by HC II) were tested using SPF10 primers. In this group HPV amplicons were detected in 81 samples and HR HPV genotypes were demonstrated by LiPA in 73. The LiPA detected 18 different HPV genotypes (II HR) in this cohort of patients, with 36% having multiple infections. Follow up samples were taken from 5 women who had HRHPV detected in their cervical cells by HC II. Two still had the same HR genotype while two had different HR genotypes and one had no HPV DNA detected in the second sample. The differences in the detection of HPV by these two methods show it will be important to determine the role of sensitivity and selectivity in HPV detection. Both the Digene Hybrid Capture and the SPF10 PCR/LiPA system can be used to determine presence of HR HPV. LiPA can genotype to determine if the same HRHPV type persist or whether the viral population has changed.

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Experience on quantitative plasma PCR in the diagnosis of clinically significant CMV infections in transplant patients

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Introduction: Cytomegalovirus (CMV) infection is a common complication in transplant patients. The specific antiviral treatment is based on the rapid, usually quantitative, laboratory tests and clinical symptoms. In CMV pp65 antigenemia test, high antigenemia levels correlate with CMV disease. Although PCR methods are already commonly used in CMV diagnostics, the clinical correlation of the findings is not clear. Here we report our experience on a quantitative plasma PCR test and compare it with the pp65 antigenemia assay in the diagnosis of CMV infection in transplant patients.

Materials and methods: Altogether 253 consecutive blood samples of liver and kidney transplant patients were evaluated. The quantitative DNA PCR Cobas Amplicor CMV MonitorTM Test (Roche) was used to detect CMV in the plasma. The positive results were expressed as copy numbers/ml plasma. The standard CMV pp65 antigenemia test was used in parallel. The positive results of pp65 antigenemia were expressed as positive cells/50000 leukocytes.

Results: From the total 253 blood specimens, CMV was detected in 89 samples by one or both methods and 164 were negative by both assays. PCR detected 78 (range 287 165000 copies) and the pp65 test 79 (range 1 1500 positive cells) of the CMV positive findings. The sensitivity and specificity for PCR were 89% and 100% and for pp65 90% and 100%, respectively. The 11 PCR negative/pp65 positive samples were of low-level antigenemia (1–5 positive cells). On the other hand, 6 out of the 10 PCR positive/pp65 negative samples were obtained during ganciclovir treatment, and the others a few days before the patients developed antigenemia. The PCR detected all clinically significant CMV infections (> 5 positive cells in the antigenemia test) which required antiviral treatment. The correlation between the two tests was almost linear and the patients with high antigenemia levels (> 200 positive cells) also demonstrated high levels of copy numbers (> 10000 copies) in plasma.

Conclusions: The PCR method was as sensitive as the pp65 antigenemia test in detecting clinically significant CMV infections. The correlation between the two tests was almost linear, and the blood specimens with high antigenemia levels demonstrated also high copy numbers in the plasma. The quantitative plasma PCR could be another alternative in the diagnosis of clinically significant CMV infections.
Comparison of pp65 antigen detection in leucocytes to an in house real Time PCR from serum or plasma in CMV monitoring of immunocompromised patients
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In immunocompromised patients reactivation of CMV can lead to serious complications. Therefore monitoring after transplantations is important to recognise a reactivation in its praeclinical state in order to begin a preemptive antiviral therapy.

In our retrospective study we compared the quantitative results of our in house real time PCR for CMV DNA in serum or plasma to those of the pp65 assay in leucocytes, we also compared them with the qualitative real time-PCR from whole blood. The study included 42 patients, 18 after bone marrow transplantation, 9 after kidney transplantation, 9 after heart transplantation and 6 others. We examined 336 pairs of leucocytes and serum or plasma. In 265 samples quantitative PCR was positive, in only 105 of the corresponding samples pp65 antigen was detectable. In 3 samples pp65 antigen was positive and quantitative PCR negative, but qualitative PCR from whole blood was weakly positive. So the real-time-PCR is more sensitive than the pp65 assay. Regarding the follow-ups-the longest lasting 10 months -, there were 58 reactivations identified by PCR, only 35 of them with positive pp65 antigen. In average, PCR became positive 9 days before pp65 antigen and remained positive 16 days longer. During 8 reactivations pp65 antigen was not continuously detectable while PCR remained positive. Therefore real-time-PCR is reflecting the virus presence better than pp65 antigen which sometimes is correlated with clinical symptoms as we demonstrate in some case reports.

According to these results, we propose to use real time PCR from serum or plasma for monitoring CMV reactivations instead of the pp65 assay.

Comparison of different methods for cytomegalovirus (CMV) surveillance
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Despite numerous available diagnostic methods for the surveillance of patients at risk of cytomegalovirus (CMV) infection and disease, there is little data concerning their comparative performances in the diagnostic setting.

We evaluated eight bone marrow transplant (BMT) recipients at high risk for developing CMV disease for 7 to 20 weeks post transplant, using spin-amplification rapid urine culture, pp65 antigenaemia assay, qualitative and quantitative in-house plasma CMV DNA PCR (ih-PCR and qPCR) and four commercial systems: NucliSens pp67 mRNA NASBA (mRNA, Organon Teknika), Hybrid Capture System DNA Assay (HC, Digene), and Amplicor CMV Monitor PCR (Roche) on plasma (Mon pl) and on whole blood (Mon wb).

Four patients (50%) suffered CMV reactivation during follow-up, but presumably thanks to the use of pre-emptive therapy, only one developed CMV disease. Out of 103 sample dates, 26 (25.2%) yielded a positive CMV result in at least one assay. Whilst viruria was positive only once and pp65 antigenaemia in 6/103 cases (5.8%), the molecular assays yielded a higher percentage of positive results: ih-PCR 16.5% (17/103), qPCR 56.6% (30/53), mRNA 12.2% (11/90), Mon-wb 26.9% (14/52), Mon-pl 18.8% (18/96), and HC 16.7% (16/96).
While we demonstrated once more the superiority of molecular assays over conventional ones for BMT recipients, there were significant differences between the sensitivities of the former, despite good agreement as regards definition of reactivation episodes. The qPCR, by utilising a large volume of plasma, combines the highest sensitivity with a potentially high predictive value which could allow a better targeting of preemptive treatment strategies.

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The evaluation and standardisation of a PCR-based method for the quantification of cytomegalovirus load in patients undergoing allogeneic bone marrow transplantation

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Purpose: This study describes a quantitative CMV PCR assay with retrospective and prospective evaluation in patients undergoing allogeneic bone marrow transplantation.

Methods: 200ml of plasma was “spiked” with murine CMV (MCMV). Common primers were designed to amplify both the target human CMV and the MCMV “spike”. Separate detection of the amplicons was done using alkaline phosphatase-labelled specific probes with a chemiluminescent detection system. A retrospective and prospective analysis comparing the results obtained from the whole blood qualitative CMV PCR (1 ml and 10 ml input) with those of the qPCR was conducted. Results obtained were compared with clinical outcome and use of antiviral therapy.

Results: In the retrospective analysis 139 samples were examined from 14 patients, 107 of which showed concordant results. The correlation of viral load with initiation and discontinuation of antiviral therapy was biologically plausible. Prospective analysis of 138 samples showed concordant results in 116 of 138 samples tested. Of the two discrepant samples not detected by qPCR, one was inhibitory (“no test”) and one had detectable CMV DNA on the qualitative PCR using a 10 ml input only. Nine samples had detectable CMV DNA by qPCR only, 7 of which 12 copies/ml only (lowest level of detectability).

Conclusion: This study demonstrates the feasibility of using plasma as an analyte; the exclusion of cell-free CMV DNA may increase clinical relevance. The application of a standardised method for the determination of “CMV load” will allow for a more rational approach for the initiation, monitoring and discontinuation of antiviral therapy.

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Evaluation of different laboratory methods in diagnosis of cytomegalovirus (HCMV) infection in infants and newborns

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Objectives: HCMV is an important pathogen for infants. The tests for diagnosis of HCMV infection include serology, detection of pp65-antigen in PBMC (pp65-antigenemia), virus isolation from blood or urine, tissue histopathology and CMV DNA detection. However, in some cases these methods may give discrepant results which are difficult to interpret. The aim of this study was to evaluate diagnostic significance of various methods for detection of active CMV infection in children patients.
Methods: A total of 48 patients were analyzed. HCMV-specific IgM antibodies were determined by ELISA. Shell vial cultures were used for the virus isolation from urine. HCMV DNA was detected in PBMC, plasma and urine using qualitative and quantitative PCR. In some patients, the pp-65 antigenemia test was performed, too.

Results: Congenital or perinatal CMV infection was diagnosed in 11 infants (group A). It was symptomatic in 10 out of them. Later-acquired CMV infection was proved in another 15 infants (group B). In 22 children, active CMV infection was not confirmed. The results of laboratory testing are as follows:

| IgM anti-CMV antibodies                  | Group A: 60% | Group B: 87% |
|-----------------------------------------|-------------|-------------|
| CMV DNA in PBMC                         | 78%         | 55%         |
| CMV DNA in plasma                       | 67%         | 70%         |
| CMV DNA in urine                        | 60%         | 83%         |
| CMV isolation from urine                | 60%         | 44%         |
| pp-65 antigenemia                       | 0%          | 22%         |
| CMV DNA load > 10^3 copies/2 × 10^4 PBMC| 75%         | 40%         |
| CMV DNA load > 2 × 10^4 copies/1 ml plasma| 44%    | 50%         |

Conclusions: The presence of CMV DNA in PBMC was the most sensitive marker of congenital or perinatal CMV infection. Detection of IgM anti-CMV antibodies and CMV DNA in plasma were the most sensitive and specific methods for diagnosis of acquired CMV infection. Pp-65 antigenemia was negative in the most cases. Congenitally infected children had higher virus load in peripheral blood than the children with acquired infection.

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A unique case of patient-to-provider-to-patient transmission of hepatitis C virus

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Background: Five individuals developed hepatitis C virus (HCV) infection after surgical treatment in a Western European municipal hospital and involvement of staff member(s) in this outbreak was suspected. The objective of the current study was to determine the source of infection and possible ways of virus spread in this setting.

Methods: A comprehensive survey of the HCV outbreak was conducted including epidemiological evaluation, hygienic inspections, as well as cloning, sequencing, and phylogenetic analysis of HCV hypervariable region 1 (HVR 1) from all virus isolates.

Results: An anaesthesiology assistant was identified as the only common denominator in all cases. Our epidemiological look-back investigations suggested that he himself had contracted HCV from a chronically infected patient and subsequently transmitted the virus during the incubation period of his disease lasting for about six weeks to the five patients. Most probably the virus was spread via a wound on the third finger of the assistant’s right hand since he usually did not wear gloves. The homology of the HVR
sequences of virus isolates obtained from the assistant and the patients was more than 95%. Upon a phylogenetic analysis, these sequences formed a monophyletic group, thus confirming the data of the epidemiological survey.

**Conclusions:** The unique case of HCV patient-to-provider-to-patient transmission described here, demonstrates for the first time that non-surgical staff members might be involved in such incidents and stresses the continuing necessity to adhere to universal precautions in all medical settings.

**P-131**

**Qualitative detection of hepatitis C virus RNA by TMA nucleic acid testing**
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**Background:** The detection of hepatitis C virus (HCV) RNA by nucleic acid amplification techniques is the method of choice for monitoring HCV infection. In this study, we have evaluated the performance characteristics of a newly developed Transcription Mediated Amplification (TMA)-based assay (Bayer Diagnostics, Emeryville, USA) designed to qualitatively detect HCV RNA.

**Methods:** Samples tested by the TMA assay included: dilutions of a HCV genotype 1a panel 164 to 8 HCV RNA copies/ml, dilutions (100 to 1 IU/ml) of the WHO HCV RNA standard 76/790, dilutions of a HCV genotyping panel, 100 HCV antibody negative sera, and 150 specimens that tested positive for HCV RNA by Amplicor (Roche Diagnostics, Mannheim, Germany). Most of these samples have been obtained from interferon-treated patients and individuals after liver transplantation.

**Results:** The TMA-based assay demonstrated an analytical sensitivity of 100% at 41 HCV RNA copies/ml (genotype 1a panel) and 5 IU/ml (WHO Standard), respectively. All genotypes were detected equivalently. Clinical specificity of the assay was greater than 98%. Qualitative RNA detection by diagnostic Roche Amplicor PCR and TMA was in agreement in 95% of all 150 clinical samples tested. No cross-contaminations were observed.

**Conclusions:** In our hands, the TMA-based assay proved to be a sensitive and specific method for qualitative HCV RNA detection that meets the manufacturer’s claims. The test might turn out to be an attractive alternative to already established techniques for HCV RNA amplification in routine clinical laboratories.

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**Molecular diagnostics in the management of HIV-2 infections**
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**Objectives:** To determine the impact on management of HIV-2 induced disease of data obtained with recently developed assays for HIV-2 quantitative plasma viral load and genotypic resistance.

**Results:** All HIV-2 seropositive individuals with more than 500 CD4+ cells per µl (n = 6) had less than 500 HIV-2 RNA copies per ml of plasma. No HIV-2 plasma viral loads above 30.000 copies per ml were detected in individuals with more than 200 CD4+ cells (n = 3). The mean plasma HIV-2 viral load in patients with less than 200 CD4+ (n = 8) proved to be significantly (0.7log10) lower than the
HIV-1 plasma viral load in patients with less than 200 CD4+. Analysis of pre-therapy HIV-2 prot and RT sequences suggested that HIV-2 is resistant to non-nucleoside RT inhibitors and the protease inhibitors Nelfinavir and Amprenavir, due to natural sequence variation. Furthermore, due to the presence of multiple secondary resistance associated mutations for protease inhibitors HIV-2 may be expected to develop resistance to this class of drugs more rapidly than HIV-1. Of the 14 HIV-2 infected patients receiving therapy seven failed due to inadequate therapy (one or two drug regimens or nelfinavir containing regimen) or because of non-compliance due to undesired drug side effects. Of the seven compliant patients receiving an adequate drug regimen (AZT, 3TC, Indinavir) no therapy failure was observed (mean follow-up > 1 year). Within the patients failing on therapy the 3TC associated mutation M184V and the multi-nucleoside analog RT inhibitor mutation Q151M were mostly observed. These mutations seriously limit the choice of a salvage regimen in these patients.

**Conclusions:** Because of the low HIV-2 plasma RNA load especially in the end-stage of the disease and the limited choice in effective drugs for first choice and salvage regimens, we recommend to offer therapy for HIV-2 infected patients later than for HIV-1 infected patients.

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**Quantitation of HIV viral load in AIDS patients with ophthalmic opportunistic infection**

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**Purpose:** To study the clinical relationship between the change of HIV load and the incidence of ophthalmic opportunistic infections in AIDS patients in Taiwan.

**Methods:** In the present study, we examined and followed up the ophthalmic conditions of a total of 389 HIV-infected patients during the period from March 1994 to December 1999. HIV RT-PCR (Roche kit) were used to detect and count HIV load in plasma from 189 AIDS patients. The ophthalmic diagnosis was based on ophthalmoscope examination and fluoresceine angiography. The correlation of clinical ophthalmic findings to HIV load in plasma by Rt-PCR were analysed by multiple regression method.

**Results:** Cytomegalovirus retinitis was the most commonly seen opportunistic ocular infection, occurring in 34 (18.1%) of 189 AIDS patients. At the time of diagnosis, the mean plasma HIV virus load was 235,089 copies/ml in patients with CMV retinitis (n = 34). The mean plasma HIV virus load was 28,105 copies/ml in patients (n = 22) with HIV related retinopathy (cotton wool spots and retinal hemorrhage). 28,105 copies/ml. The mean plasma HIV virus load was 480,975 copies/ml in patients (n = 4) with toxoplasmosis retinitis.

**Conclusions:** Ocular manifestations have been reported in up to 30% of individuals infected with HIV in Taiwan, and it is becoming increasing apparent that these ocular manifestations almost invariably reflect extent of higher HIV virus load and progression of the disease.

**P-134**

**Evaluation of amplicor HIV-1 monitor-, nuclisensu QT- and quantiplex BDNA-assays to quantitate different HIV-1 subtypes**

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Background: In HIV-1 infection viral load monitoring is a standard clinical management tool. Recommendations to start or to change antiretroviral therapy are based on the viral load level and/or changes in the viral load of a patient. Currently there are three commercially available quantitative assays to measure HIV-1 viral load in plasma. Two of the tests, the Amplicor HIV-1 Monitor (Roche Diagnostics Systems) and the NucliSens QT (Organon Teknika), are based on template amplification and the Quantiplex HIV-1 RNA assay (Chiron Diagnostics) on signal amplification. However, the genetic heterogeneity between different HIV-1 subtypes can vary from 15% in the gag coding sequence to 30% in the envelope sequence doing the selection of universally conserved primer and probe binding regions for template amplification and detection a difficult task.

Methods: We compared the performance of the HIV-1 Monitor, NucliSens and Quantiplex assays for quantitating a number of different HIV-1 subtypes circulating in Finland. In addition, the efficiency of the tests to measure the HIV-1 RNA plasma load in A/B recombinant viruses prevalent in Kaliningrad, Russia was investigated. All the quantitated viruses were further studied by sequencing the primer binding sites.

Results: The accuracy of all the three assays was comparable among most most of the subtypes studied. However, the amplification efficiency was significantly decreased in subtype A/B HIV-1 recombinant virus with the NucliSens QT-assay compared to the Amplicor HIV-1 Monitor and Quantiplex assays. A number of primer and probe mismatches occurring in the gag-gene region of the A/B recombinant virus was found.

Conclusions: The continuous control of new emerging HIV-1 subtypes is essential for ensuring of valid viral quantification.

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Quantitation of polyomavirus DNA by a competitive nested polymerase chain reaction
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The polymerase chain reaction (PCR) is a highly and specific method for detection of nucleic acids and a useful tool for their quantitation in clinical samples. A new method to quantitate small amounts of DNA in clinical samples is described. The method, a nested competitive polymerase chain reaction (ncPCR), is able to quantitate between 10 and 106 copies per tube of polyomavirus and shows good reproducibility when clinical samples are analysed. Throughout the whole procedure, an internal standard (IS) competes for the primers with the target DNA. The IS, a heterologous sequence containing the four primer recognition sites, was constructed using a modification of the “MIMIC” approach that is useful for obtaining competitor sequences for any viral, bacterial or eukariotic target. The ncPCR method for polyomavirus was applied to cerebrospinal fluids (CSF) from AIDS patients with progressive multifocal leukoencephalopathy (PML) and urine specimens from bone marrow transplant patients affected by haemorrhagic cystitis (HC).

The ncPCR we have developed shows a very high sensitivity and a broad range of linearity without the need of any post-amplification methodology. Furthermore, the approach used to obtain the competitor DNA can be applied to convert any qualitative nested PCR into a quantitative nested PCR en a few days.
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Investigation of viral genetic determinants of prolonged pain following herpes zoster virus reactivation
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Aim of the study: To investigate the virological factors in varicella zoster virus (VZV) infection which correlate with the development of persistent zoster associated pain (ZAP) or post herpetic neuralgia (PHN).

Methods: Patients with a clinical diagnosis of zoster were referred from GPs in the East London area to the Department of Virology, RLHNT. In all cases, the diagnosis was confirmed by immunofluorescence and Polymerase Chain Reaction (PCR) of vesicle fluid virus. The presence of VZV DNA in peripheral blood lymphocytes (PBMCs) was determined by nested PCR designed to amplify either gene IE 63 or E29. A demographic questionnaire was completed and pain was formally assessed and scored. Patients were seen at 6 weeks, 3, 6 and 12 months and evaluated for continuation of their pain. Pain of longer than 6 weeks duration was defined as PHN.

Results: 185 patients with a confirmed diagnosis of shingles have been entered into this prospective study. Of these, 106 have been followed up for 12 months. PCR of PBMCs at the time of diagnosis (baseline) was positive in 66 and negative in 40. Preliminary data suggest a trend towards a correlation between positive baseline PCR and PHN at 12 months, with 9 cases in the PCR positive group versus 1 in the PCR negative group ($p = 0.06$). PHN was correlated with age as expected ($p < 0.004$); however, there was no relationship between age and PCR positivity at baseline ($p = 0.7$). Virus load studies are now planned.

Conclusion: ZAP is known to be directly related to age and severity of pain at presentation, and inversely related to serum levels of specific antiviral drugs. In addition, our results suggest that presence of detectable VZV DNA in blood at baseline may be an independent predictor for the development of PHN. This could be an indication for a viral factor in the pathogenesis of persistent pain following shingles.

P-137

NucliSens® Easyq; a new, high throughput HIV-1 viral load analysis assay based on real-time detection with molecular beacons
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The advance of HIV-1 clinical therapies and emergence of new virus strains required viral load assays to be accurate and sensitive at low RNA levels (e.g. 50 copies/ml) with broad subtype reactivity of group M (A-H). In addition, the assay should be simple and fast.

We have implemented a homogeneous fluorescent detection technology based on Molecular Beacons which enables amplification based on NASBA (gag primer set) and detection to take place in a single tube. Analysis of 48 samples requires less than 1 hour with only 1 minute per sample hands-on time. Automated extraction of 1 ml plasma is performed on the NucliSens® Extractor using the Boom chemistry.

The performance was tested in a pre-clinical study. The linearity and precision of the assay was checked with a VQC HIV-1 type B standard of the CLB The Netherlands. Sensitivity was shown to be 50 copies/ml. The average assay precision was 0.17 log over the range of 100–300 000 copies/ml at 9 tested concentrations ($n = 264$).
All subtypes of a panel of 15 culture quantified HIV-1 gag typed clades A-H could be accurately quantitated. The specificity was 100% on non HIV-1 samples HIV-2, HTLV-1 and HTLV-2. The specificity on negatives was 97% (225/232). The assay robustness was checked on VQC controls 99.8% (473/474) with an overall robustness of 98% (1125/1148).

In conclusion, the new real-time NucliSens® assay meets the state-of-the-art HIV-1 viral load performance requirements combined with a high level of user convenience.

P-138

The Eclipse CMV DNA Detection Kit: a quantitative and rapid test for the detection of human cytomegalovirus (HCMV)
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Introduction: Early and rapid diagnosis of CMV infection is of great importance in avoiding over treatment with immunosuppressive drugs and in guiding antiviral therapy. Conventional methods for isolation of CMV from blood are sensitive, but may not yield results for several weeks. There are several requirements for an optimal assay for CMV monitoring. The most important characteristics are: (1) A high sensitivity and specificity for early detection in individuals at high risk for disease, (2) The potential to quantify the results to measure viral load, (3) Rapidity of the test, (4) High degree of reproducibility.

Eclipse Q-PCR Test system: Competitive DNA Amplification: The ECLIPSE Test system is designed to amplify and detect CMV target DNA through the performance of two real competitive steps. A competitive DNA amplification procedure in combination with a competitive microwell system offers the sensitivity and quantitative results for clinical diagnosis of viral CMV DNA.

Data Acquisition: Data analysis of the graph expressing the CMV OD signal: Standard OD signal ratio as a function of the Standard ICS copy number will give the input CMV Target DNA copy number at the ratio of 1. Inter-assay data obtained with CMV DNA extracted from blood samples of patients, demonstrated that the ECLIPSE Test system can be used to obtain reproducible quantitative results with CV’s of < 25%.

P-139

Genotyping of hepatitis C virus isolates by trugene HCV 5'NC
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Background: Determination of hepatitis C virus (HCV) genotypes and subtypes has become increasingly important for the clinical management and prognosis of HCV infections. The aim of the current study was to assess the specificity and reliability of a newly developed, commercially available HCV genotyping kit (TRUGENE HCV 5'NC Genotyping Kit).

Methods: TRUGENE HCV 5'NC Genotyping Kit utilises PCR fragments previously generated by diagnostic Roche Amplicor HCV test, which are subsequently subjected to simultaneous PCR amplification and direct sequencing (CLIP) of the 5' non coding region (5'NCR). HCV isolates from 100 randomly chosen patients were analysed by both TRUGENE HCV 5'NC Genotyping Kit and DNA enzyme immunoassay (DEIA).
Results: Typing results obtained by both methods were in complete concordance in 91% of all cases. HCV RNA from the samples with discordant genotype assignment in both assays was additionally amplified with primers from the HCV core and NS513 regions. Phylogenetic analysis of the obtained sequences showed that as a result of the high conservation of 5’NCR TRUGENE HCV 5’NC Genotyping Kit in some cases could not correctly differentiate between types 1 and 2 subtypes. Since, however, not a single misclassification between HCV genotypes 1 and non-1 types occurred, the results obtained with this system are in general reliable for clinical practice.

Conclusions: TRUGENE HCV 5’NC Genotyping Kit in our hands proved to be a fast and convenient technique that might be an attractive option for HCV genotyping in laboratories already using the Roche Amplicor HCV test for diagnostic RT-PCR.

P-140

Improving diagnosis of CMV disease in organ transplant recipients by using quantitative CMV DNA measurement
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Study purpose: In this study, we evaluated several techniques for diagnosing CMV infection and disease: pp65 CMV antigen detection, Hybrid capture DNA assay on whole blood and quantitative PCR on plasma samples.

Patients: In this prospective longitudinal study, we included all patients receiving an organ allograft in 1998. The three different assays were performed at different time-points after transplantation. The study group consisted of 43 kidney transplant recipients (including 9 children) and 18 heart transplant recipients. Different immunosuppressive and prophylactic regimens were used for the different groups.

Results: Twenty of the 61 patients suffered from CMV disease. The incidence was highest in the adult kidney transplant recipients (40%), who did not receive any CMV prophylaxis. In patients with CMV disease usually all three assays became positive, though in three cases pp65 detection remained negative. The value of the assays depended on donor and recipient CMV status. In CMV negative recipients receiving a CMV positive organ, all three assays had a high sensitivity in diagnosing CMV disease. Reactivity in the hybrid capture assay on whole blood not only preceded reactivity in the other tests, but also the development of clinically apparent CMV infection. In all patient groups the molecular techniques had a high negative predictive value for CMV disease. The positive predictive value of these assays in CMV IgG positive recipients was relatively low, though the actual CMV DNA load could contribute to the diagnosis of CMV disease.

Conclusion: Quantitative measurement of CMV DNA in organ transplant recipients can improve early diagnosis of CMV disease allowing pre-emptive treatment and allows the clinician to discriminate between CMV disease and other causes of post transplant fever and accompanying symptoms.

P-141

Application of real time PCR in the detection of viruses from nasal aspirate samples
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A fluorogenic real time PCR method (TaqMan™ PCR) was used to quantify viral nucleic acid in clinical samples and a comparison of the sensitivity and specificity made with conventional PCR. Respiratory viruses have been implicated in episodes of acute exacerbation of asthma in children (Johnston et al. 1995). Retrospective real time PCR analysis was performed using nasal aspirate samples from a cohort of 116 children with a history of wheeze or persistent cough. The nucleic acid was extracted and real time PCR was performed on samples that had previously been tested for the presence of respiratory viruses using conventional RT-PCR. Real time assays were developed to quantify respiratory syncytial virus, influenza A and B, picornavirus, coronavirus OC43 and 229E, parainfluenza III and I, and adenovirus. Real time PCR was found to be comparable in specificity to conventional RT-PCR, but was less sensitive for respiratory syncytial virus. Further work indicated that freezing and thawing of this virus significantly reduced viral load, suggesting that respiratory syncytial virus might potentially be under represented in retrospective PCR analyses of study samples. Those viruses detected in samples by real time but not conventional PCR had low mean viral loads compared with those that were detected by both methods. Real time PCR was found to be more sensitive than conventional RT-PCR, or comparably sensitive for most of the viruses tested.

P-142

Serological and molecular biological analysis of herpes simplex virus (HSV) and varicella zoster virus (VZV) reactivation in Dutch Bell’s palsy patients

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Background and methods: It has recently been suggested that Bell’s palsy is associated with HSV reactivation (Furuta et al., J. Med. Virol. 1998; 54: 162.) In a pilot-study in 14 Dutch patients with Bell’s palsy, visiting our hospital within ten days after onset of symptoms, and 4 control persons, reactivation of HSV and VZV was analyzed serologically (IgG, IgM, complement fixation tests (CFT)), as well as by detection of viral DNA in throat swabs using internally controlled PCRs targeted at the HSV TK gene and VZV gene 29 (J. Clin. Microbiol., in press).

Results: In none of the patients and controls serological or molecular evidence of VZV reactivation was demonstrated. Three patients were HSV-seronegative. In 1 of the remaining 11 patients HSV-DNA and an increased CFT titer (1:64) was found. In 6 other patients high HSV CFT-titers but no HSV-DNA was detected. In control persons neither HSV DNA nor a significant HSV CFT titer was found. Conclusions: In agreement with previous studies, our serological analysis suggests an association between HSV reactivation and Bell’s palsy in a substantial proportion of patients. However, in contrast to Furuta et al, HSV DNA in throat swabs could only be detected in 1/14 patients. The reason for this discrepancy needs to be addressed to validate the role of PCR in the diagnosis and therapeutic decision-making in Bell’s palsy patients.

P-143

Cytomegalovirus (CMV) gB genotyping in HIV-infected patients: correlation between CNS pathogenicity and genotype between pathogenicity in central nervous system and gB genotype

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Abstracts

Background and objectives: CMV pathogenicity and localisation might be correlated with gB genotype. To evaluate the possible presence of neurotropic CMV strains, we analyzed gB subtype in cerebrospinal fluid (CSF) and blood of HIV-infected patients with CMV encephalitis (CMV-E) or other CMV-associated diseases. In particular, we 1) investigated the distribution of gB genotypes in patients with CMV-E; 2) Compared CMV genotypes in blood and CSF from same patients; 3) assessed the variation of gB genotypes in CSF of individual patients over time.

Patients and Methods: gB sequences were amplified from CSF, plasma or culture surnatants using a nested polymerase chain reaction. Amplicons were digested by two restriction enzyme systems: MaelI and Hinfl-Rsal.

Results: 1) Among 17 patients with CMV-E, the distribution of gB genotypes was gB1 = 23%, gB2 = 29%, gB3 = 6%, gB4 = 6%, mixed gB type = 35%, that was not significantly different from that observed in 34 patients without CMV-E. 2) Identical gB genotypes between blood and CSF were demonstrated in 21/24 patients. 3) The same genotype was present in 5/6 patients with serial CSF samples drawn over 2-12 months.

Conclusion: In CMV-E, genotypes gB1 and gB2 were found more frequently than gB3 and gB4, however a surely neurotropic CMV genotype was not identified by gB analysis. Since the majority of patients had identical genotype in CSF and plasma, CMV is likely to spread to the brain from the blood, and with no evidence of compartmentalisation.

P-144

The amino acid sequence of the Pkr-eIF2α phosphorylation homology domain (PePHD) of HCV E2 and response to interferon

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It was recently reported that a region of the HCV E2 protein, the PePHD may be important in interferon resistance [1]. This study was designed to determine whether a PePHD variant with prognostic significance could be isolated from pre-treatment serum.

The PePHD was sequenced from the stored pre-treatment serum of 14 genotype 3 infected patients treated with 6MU Interferonα for 12 weeks and 3MU for a further 36 weeks if RT-PCR negative for HCV RNA at 12 weeks. 4 patients were non-responders, 4 had a breakthrough after week 12, 3 were relapers and 3 were sustained responders. Only one patient (a non-responder) had a PePHD variant.

Interferon resistant PePHD variants present at low titre in pre-treatment serum should be selected by interferon treatment. To investigate this the PePHD was also sequenced from serum collected during after treatment failure in five patients. We found no difference between the pre and post treatment PePHD sequences.

Conclusion: Pretreatment sequencing of the PePHD would not provide useful information for clinicians on who would respond to therapy. Interferonα treatment does not select for variants which have an altered PePHD in HCV genotype 3.

Reference:
- DR Taylor, ST Shi, PR Romano, GN Barber, MMC Lai. Inhibition of the Interferon Inducible Protein Kinase PKR by HCV E2 Protein; Science 1999; 285:107 110.

P-145

The role of “Norwalk-like viruses” in outbreaks of gastroenteritis in Spain (1997–1999)

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“Norwalk-like viruses” (NLVs) have emerged as the most common cause of outbreaks of acute non-bacterial gastroenteritis (GE) world-wide. To investigate the role of NLVs in GE outbreaks in Spain, all non-rotaviral outbreaks \((n = 54)\) that were submitted for viral characterisation to the central Spanish public health laboratories from 1997 to 1999, were examined by electron microscopy (EM). In addition, stool samples from 5 outbreaks were analyzed by immune-EM using paired serum samples. The NLV-positive outbreaks by EM were confirmed by RT-PCR and the genetic variability of the strains was determined by reverse line blot hybridisation (RLB) and sequence analysis. By EM, NLVs were detected in 17 outbreaks, corresponding to 20% of the outbreaks reported in 1997 and 1998 and, after using standardised protocols, to 47% of the outbreaks reported in 1999, showing the significance of NLVs in outbreaks of gastroenteritis in Spain. Of these outbreaks, 58% occurred in homes for the elderly. Genotyping of NLV positive samples by RLB and sequence analysis revealed that the majority of strains belonged to genogroup II NLVs. This is the first study confirming the importance of NLVs as a cause of outbreaks of gastroenteritis in Spain.