

**A11****Detection of *Chlamydia trachomatis* DNA in Urine and Swab Specimens by ABI PRISM™ 7000 SDS PCR after Semi-Automated Extraction Using the ABI PRISM® 6100 Nucleic Acid PrepStation**<sup>1</sup>M. Eickhoff, <sup>1</sup>T. Laue, <sup>2</sup>S. Thamm, <sup>2</sup>G. Michel<sup>1</sup> artus GmbH, Hamburg, Germany<sup>2</sup> Abbott GmbH & Co. KG, Wiesbaden-Delkenheim, Germany

**Background:** In the past decade, diagnostic testing for genital chlamydia infection has changed. New highly sensitive real-time PCR tests have been developed to allow non-invasive specimen collection. To create new opportunities for innovative screening programs a change from manual DNA extraction methods to automated systems will be necessary.

**Objectives:** We evaluated a semi-automated DNA extraction system, the ABI PRISM® 6100 Nucleic Acid PrepStation (Applied Biosystems, USA) as effective replacement for a manual method with the goal of implementing a standardized system for high throughput processing of clinical specimens.

**Study design:** After optimizing the DNA extraction protocol 357 for *C. trachomatis* negative samples (192 urines, 165 swabs) and 270 samples spiked with 1 copy/μl *C. trachomatis* plasmid-DNA (180 urines, 90 swabs) were purified with the ABI PRISM® 6100 Nucleic Acid PrepStation and analyzed on the ABI PRISM™ 7000 SDS (Applied Biosystems, USA) with the RealArt™ *C. trachomatis* TM PCR Kit (artus GmbH, Germany).

**Results:** The semi-automated extraction method showed a high DNA recovery rate (all 180 urine and all 90 swab samples were detected positive) and no membrane clogging after optimizing the extraction protocol. The inhibition rate for swab samples was 0.8%, for urine samples 0%. Therefore the total inhibition rate was 0.3%.

**Conclusions:** Our results indicate that the ABI PRISM® 6100 Nucleic Acid PrepStation DNA extraction method provides a standardized, reproducible method for the processing of high numbers of urine and swab specimens for *C. trachomatis* PCR on the ABI PRISM™ 7000 SDS.

**A12****HIV-1 gp41 Genotyping Using the TruGene® Platform**

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Mutations in gp41 have been linked to T-20 (enfuvirtide, FUZEON) treatment failure. The genetic complexity of HIV in the envelope region, which includes the gp41 transmembrane and gp120 surface glycoprotein coding regions, makes routine sequencing challenging. Bayer's TRUGENE® HIV-1 Genotyping Kit and OpenGene™ DNA Sequencing System were the first to be approved for detection of HIV genomic mutations as an aid in monitoring and treating HIV infection. DNA sequencing assays can detect known resistance associated mutations can contribute to critical in patient care and clinical trials of new antiretroviral compounds. The application of the TRUGENE genotyping platform was considered for sequencing the first heptad repeat region of gp41 (HR1). HIV envelope specific amplification and sequencing primer sets were designed and evaluated on the

OpenGene DNA Sequencing System. An approximately 3 kilobasepair fragment including gp41 was amplified using RT-PCR. Bidirectional sequencing results utilizing CLIP™ technology were analyzed with GeneObjects 3.2 software. Automated report generation was tested using a user defined gene library and processing template. M group samples including tissue cultured virus (non B clades) and patient derived plasma (B clade) were analyzed. Recent genotyping results from patients failing salvage therapy with T-20 are presented.

**A13****Geniom® Technology: The First Benchtop Microarray Facility**

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Febit's geniom one instrument represents a new fully integrated approach towards monitoring gene expression profiling and mutation analysis employing DNA-microarrays. Starting from genomic sequence data, which is stored in digital form in public databases, any individual array may be fabricated within few hours in a fully automated fashion. This integrated and fully flexible approach puts the researcher into the position not only to design and fabricate arrays for his individual field of interest but also to hybridize and analyze these arrays in a fully automatic fashion within less than one day. The geniom technology therefore allows the researcher to put his resources on the design and analysis of the experiments and not on the logistics, production and handling of the necessary arrays. The geniom technology opens up new possibilities in research not only for the prominent organisms like human, mouse etc., but especially in the field of pathogens and bacteria and DNA- microarray based clinical diagnostic tests. For example several strains of the human papillomavirus (HPV) could be easily distinguished from other HPV strains, an approach which may find application in cervix cancer diagnosis. The presentation will give a short introduction about the geniom technology and demonstrate the performance of this new technology. First results showing the versatility of the geniom one technology for expression profiling and genotyping experiments will be discussed.

**Session Pathogens 1 (A14–A18)****A14****SARS: Isolation and Molecular Detection of a Novel Emerging Virus**<sup>1</sup>W. Preiser, <sup>2</sup>C. Drosten<sup>1</sup> Institute of Medical Virology, Johann Wolfgang Goethe University Frankfurt am Main, Germany<sup>2</sup> Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

A novel respiratory infectious disease emerged in southern China in late 2002 and rapidly spread to different areas of the globe. Clinical specimens from patients with severe acute respiratory syndrome (SARS) were searched for unknown agents using different techniques. Three laboratories within a World Health Organization (WHO)-coordinated network were – independently

and simultaneously, using different approaches – able to isolate and characterize a novel coronavirus in SARS patients. After Koch's postulates had been fulfilled, WHO officially declared on April 16, 2003 that this virus never before seen in humans is the cause of SARS. Its complete genome had been sequenced only weeks after the first isolate of the virus had become available. Although diagnostic tests based on the detection of SARS-CoV RNA were developed, the SARS case definition still remains based on clinical and epidemiological criteria. When the outbreak of SARS came to an end in July 2003, it had caused over 8000 probable cases worldwide and more than 700 deaths. The origins of SARS are still unknown, and it is unclear whether it will reemerge. The agent's environmental stability, methods suitable for inactivation and disinfection, and potential antiviral compounds were studied, and development of vaccines and immunotherapeutics is ongoing. Despite its grave consequences in humanitarian, political and economic terms, SARS may serve as an example of how much can be achieved through a well-coordinated international approach, combining the latest technological advances of molecular virology with more "traditional" techniques carried out to an excellent standard.

### A15 Subtyping and Investigation of Transmission Events of Rotavirus Type A Using Phylogenetic Analysis

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**Background:** Rotavirus infection is associated with human gastroenteritis and is the major cause of infantile gastroenteritis worldwide. Infections in the hospital environment are frequently found. Outbreaks have mainly been analyzed using serological or PCR subtyping methods (VP7 and VP4 gene).

**Objectives:** We describe a phylogenetic analysis including 25 VP4 and 29 VP7 GenBank sequences using a single PCR product to determine rotavirus A subtypes and to investigate possible nosocomial outbreaks.

**Methods:** Rotavirus RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). RT-PCR was performed using the OneStep RT-PCR Kit (Qiagen) with primers Con2/Con3m and Beg9m/End9m for the VP4 and VP7 gene, respectively. PCR products were sequenced with specific primers Con3m and Beg9m, and analysed on an ABI 377-96 sequencer (Applied Biosystems, Darmstadt, Germany). Sequence alignments were done using the MegAlign module of the LaserGene software (DNASTAR, Madison, WI, USA), and imported into the Mega2 software (<http://www.megasoftware.net>) for phylogenetic analysis.

**Results:** The different sero- and genogroups of the GenBank isolates were clearly distinguishable in the phylogenetic analysis for both the VP4 and VP7 gene. Patient isolates could definitely be assigned to a specific geno- or serotype defined by a subtype-specific cluster of the GenBank isolates. The patient isolates had no common source of infection, as they were clearly unrelated in the phylogenetic trees.

**Conclusions:** Our results show that subtyping of Rotavirus sero- and genotypes can be performed very easily using phylogenetic analysis, and that investigations of nosocomial outbreaks can be done in parallel to the subtyping procedure.

### A16 West Nile Virus PCR in Austrian Blood Donors

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**Background:** West Nile virus (WNV) is an infectious agent, which has been present in Europe for several decades infecting birds and horses only. Nevertheless, during past years, strains, which can also be dangerous for humans, emerged in the USA. When the first case imported to Europe was reported in summer 2003, we amended a molecular test for the definition of WNV.

**Study design:** A previously published real-time PCR assay, which is capable of detecting the NY99 and European strains, was extended by adding an internal control into each reaction. An automated extraction procedure using 1–1.5 ml plasma on a Tecan Genesis RSP 150 pipettor preceded by a concentration step was implemented to increase the sensitivity of the assay. We also started a retrospective testing of pooled plasma samples of summer 2001, 2002 and 2003 and HCV indeterminate RIBA samples.

**Results:** The detection limit of the assay was determined to be below 13 copies DNA/reaction. No single WNV RNA positive sample has been detected so far.

**Conclusion:** We have implemented a sensitive and rapid assay for WNV RNA in order to meet a possible need for testing in Austria as well as to investigate possible epidemics of this virus.

### A17 Evaluation of Automated Sample Preparation and Quantitative PCR LCx Assay for Determination of Human Immunodeficiency Virus Type 1 (HIV-1) RNA

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**Objectives:** To evaluate a new molecular assay consisting of automated HIV-1 RNA extraction on the MagNA Pure LC instrument and the Abbott LCx HIV RNA Quantitative assay. To compare the new assay to the standard version of the LCx HIV RNA Quantitative assay including a manual nucleic acid extraction protocol.

**Study design:** Accuracy, linearity, interassay and intra-assay variations of the new assay were determined. Additionally, clinical samples were tested with both of the assays.

**Results:** When accuracy of the new assay was tested, all results were found within  $\pm 0.5$  logs of the expected results. Determination of linearity resulted in a quasi-linear curve over at least 3 logs. For determination of interassay variation, coefficients of variation were found between 21 and 66% for the new assay and between 10 and 69% for the standard assay. For determination of intra-assay variation, corresponding numbers were 7 to 25% (new assay) and 7 to 19% (standard assay) with manual sample preparation. When clinical samples were tested with the