



ViroMed Laboratories Cell Culture Bulletin

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This **annual newsletter** highlights advances in the cell culture industry and new offerings from **ViroMed Laboratories**, a LabCorp Center of Excellence.

ViroMed to Present Posters at Clinical Virology Symposium

ViroMed Laboratories' scientists conduct research throughout the year to develop innovative technology applications. ViroMed will present the 2 posters described below at the 26th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology in **Daytona Beach, Fla, April 25-28, 2010.**

Increasing Prevalence of Norovirus GII.12 in the United States, 2009-10

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Noroviruses (NoV) are a group of small, single-stranded, positive-sense RNA viruses that constitute an important etiologic agent of acute gastroenteritis in humans. These viruses are commonly associated with large-scale outbreaks in hospitals, cruise-ships, schools and daycare centers. Two genogroups (I and II) are primarily responsible for human disease, with a specific genocluster (designated GII.4) being recognized as the most commonly implicated virus in both sporadic and epidemic norovirus infections worldwide. A diagnostic PCR assay for detection and discrimination of NoV, utilizing MultiCode-RTx[®] technology (EraGen Biosciences, Madison, WI), is performed at ViroMed Laboratories. The assay targets the highly conserved ORF-1/ORF-2 junction of these viruses, with confirmation of positive results being achieved via comparison of peak T_m values of amplified products with that of ultrameric control targets. During routine QA review of NoV-PCR data (December, 2009) it was noted that several samples containing high concentrations of NoV-GII target RNA (based on assay C_t value) produced amplicons with peak T_m values 0.6-1.0°C below the T_m value of the control amplicon. Retrospective review of past data revealed this to be a highly unusual occurrence and prospective analysis of positive samples was initiated. During the period November 1, 2009 through February 22, 2010, a total of 675 NoV-PCR tests were performed at ViroMed Laboratories on samples originating from 35 states. Of these, 307 (45.5%) were positive for NoV, with 3 (1%) samples positive for NoV-GI and 306 (99%) positive for NoV-GII. Further analysis of the data for GII positive samples revealed that 231 samples (75.5%) generated amplicons with peak T_m values within 0.5°C of the control amplicon (Variant A), 65 samples (21.2%) generated amplicons with peak T_m values 0.6-1.0°C below the control amplicon (Variant B), and 8 samples (2.3%) generated amplicons with peak T_m values 0.6-1.0°C above the control amplicon (Variant C). The 3'-region (282bp) of the capsid protein gene (ORF2) was amplified

from representative members of each of the observed variants, and phylogenetic analysis performed. All analyzed viruses in the Variant A and C group belonged to the GII.4 cluster and were related to major circulating strains identified in the US over the past 5 years. All Variant B viruses, however, belonged to GII.12, and were most closely related to a virus (NSW199U) implicated in a norovirus outbreak in Sydney, Australia in 2006. Retrospective analysis of data from the corresponding period of the previous winter season (November, 2008 - February, 2009) revealed that of 723 NoV positive samples (45.9% of the 1,575 samples tested), 57 (7.9%) were NoV-GI positive and 666 (92.1%) NoV-GII positive. Of the GII positive samples, 628 (94.5%) were Variant A, 13 (2.0%) Variant B, and 25 Variant C (3.8%). These data indicate a dramatic increase in the prevalence of GII.12 NoV infections in the US in the winter of 2009-10, and also demonstrate the epidemiologic value of utilizing diagnostic PCR assays capable of detecting and identifying viral variants.

Analytical and Clinical Performance Characteristics of a Multicode-RTx[®] PCR Assay for the Quantitative Detection of Human Herpesvirus 6 DNA in Plasma Samples

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Human Herpesvirus 6 (HHV-6) is a lymphotropic, beta-herpesvirus, known to frequently reactivate in immunosuppressed solid organ and hematopoietic transplant recipients. Although the causative role of HHV-6 in any specific disease manifestation in these patient populations is yet to be definitively established, HHV-6 reactivation is clearly associated with increased risk of adverse clinical events in transplant recipients. Monitoring for the presence of circulating HHV-6 DNA by quantitative PCR may, therefore, be a useful tool for detecting at risk patients and evaluating the success of therapeutic interventions. We report here on the performance characteristics of a quantitative PCR assay for HHV-6 (qHHV6-PCR) that utilizes Multicode-

RTx® technology (EraGen Biosciences, Madison, WI) for detecting PCR products in real-time. One of the advantages of Multicode-RTx is that, since tagged-primers rather than probes are used for amplicon detection, amplification efficiency is the sole variable influencing the kinetics of signal generation resulting in improved precision of target quantitation. The qHHV6-PCR assay described enables multiplexed detection of HHV6-DNA and an extractable internal control target. Quantitation is accomplished via interpolation of results into externally-generated, lot specific, calibration curves. We have established the analytical performance metrics of the

qHHV6-PCR assay, including the lower limits of quantitation (LLOQ) and detection (LOD), and delineated the sample storage requirements, reproducibility, specificity and internal control performance of this novel assay. The results of these experiments indicate that this assay is capable of highly reproducible quantitation of HHV-6 DNA in plasma samples over a range of at least 5 logs. Utilizing samples submitted for quantitative monitoring of CMV infection, we were also able to examine the prevalence and viral dynamics of HHV-6 infection in patients with and without concomitant CMV infection.

Molecular Infectious Disease Testing

Molecular infectious disease testing has added greatly to the laboratory's ability to quickly detect viruses, especially in circumstances in which there may be minute quantities of a specific pathogen present, the patient may have received antibiotics prior to specimen collection, or the etiology may require unusual culture conditions.

ViroMed offers many molecular infectious disease assays that complement the services of hospital laboratories.

Test Name	Test #	Test Name	Test #
Adenovirus Detection, PCR	138164	Hepatitis C Virus (HCV), RT PCR, Quantitative (Graph)	550070
<i>Anaplasma phagocytophilum</i> DNA PCR	138172	Herpes Simplex Virus (HSV) Types 1 and 2, DNA PCR	138651
<i>Babesia microti</i> , Real-time DNA PCR	138318	Herpes Simplex Virus Types 1 and 2, Amniotic Fluid	138594
<i>Bartonella</i> , DNA PCR	138350	HIV-1, Quantitative, Real Time PCR (Non-Graphical)	550430
BK Virus Quantitative, Real-time DNA PCR	138962	HIV-1, Quantitative, Real Time PCR (Graph)	550420
BK Virus Quantitative, Real-time DNA PCR, Urine	138880	Human Herpesvirus 6 (HHV- 6), DNA PCR	138479
<i>Bordetella pertussis</i> & <i>B parapertussis</i> DNA PCR	138677	Influenza A and B, Real-time RT- PCR	186221
<i>Chlamydia (Chlamydophilia) pneumoniae</i> , PCR	138263	Influenza A and B PCR w/ Reflex to Influenza A, H1N1	186270
<i>Chlamydia trachomatis</i> , NAA	188078	Influenza A, H1N1 (Novel), Real-time RT-PCR	186205
<i>Chlamydia/Gonococcus</i> , NAA	183194	JC Virus DNA, PCR	139210
Cytomegalovirus (CMV) Qualitative, PCR	138693	Lyme Disease (<i>Borrelia burgdorferi</i>), Real-time PCR	138685
Cytomegalovirus (CMV), Amniotic Fluid, PCR	138701	<i>Mycoplasma pneumoniae</i> , DNA PCR	138420
Cytomegalovirus (CMV), Quantitative, Plasma, PCR	139149	<i>Mycoplasma/Ureaplasma</i> , Real-time PCR	138778
Cytomegalovirus (CMV), Quantitative, Urine, PCR	139144	<i>Neisseria gonorrhoeae</i> , NAA	188086
<i>Ehrlichia</i> Profile, DNA PCR	138412	Norovirus Detection, Real-time PCR	138307
<i>Ehrlichia chaffeensis</i> , DNA PCR	138168	Parvovirus B19, DNA PCR	138644
Enterovirus, Real-time PCR	138636	Parvovirus B19, DNA, Amniotic Fluid	138719
Epstein-Barr Virus (EBV), Qualitative, PCR	138289	<i>Plasmodium</i> Species, Real-time DNA PCR	138852
Epstein-Barr Virus (EBV), Quantitative, PCR	138230	Respiratory Virus Panel (RVP) PCR	139250
Hepatitis B Virus DNA Quantitative, PCR, Non-graph	551610	<i>Toxoplasma gondii</i> , PCR	138602
Hepatitis B Virus DNA Quantitative, PCR, Graphical	551620	<i>Toxoplasma gondii</i> , Amniotic Fluid, PCR	138586
Hepatitis C Virus (HCV), RNA, Qualitative, PCR	550400	Varicella Zoster Virus (VZV) DNA PCR	138313
Hepatitis C Virus (HCV), Real Time PCR, Quantitative	550080		

Contact Information

For more information about ViroMed's testing services, call Client Services at 800-582-0077, or e-mail cshelpdesk@LabCorp.com.



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