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Molecular Detection of Herpes simplex virus

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Introduction

Herpes simplex virus (HSV) is the viral agent most commonly isolated from clinical specimens in the diagnostic laboratory. Rapid and accurate laboratory diagnosis of HSV infection is important for several reasons, including confirmation of clinical impressions for pregnant women near term, detection of subclinical HSV reactivation at parturition, for preventing serious complications of HSV infections in immunocompromised patients and for facilitating prompt initiation of informed therapy (1).

The reference method or "gold standard" for laboratory confirmation of HSV infection is isolation and/or detection of the virus in cell culture (6). The presence of virus is usually detected by observation of characteristic viral cytopathic effect (CPE) in cell culture. A variety of cell culture systems have been used for HSV isolation, with a mean detection time of 2.5 days (1, 4, 6). Several modifications of cell culture detection of HSV such as centrifugation culture and genetically engineered host cells have reduced detection times to 24 to 48 hours (2, 4)

For nearly a decade PCR has been shown to be a highly sensitive and specific assay for the rapid detection of central nervous system infections caused by HSV (5). This method is now considered to be the "gold standard" for diagnosis of HSV encephalitis and meningitis. Recently, real-time PCR assays have been developed. Several studies have shown that real-time PCR is more sensitive and specific than cell culture for the rapid detection of HSV from all clinical sources (3, 7).

The objective of this study was to compare standard HSV culture to real-time PCR for the direct detection of HSV from clinical specimens other than CSF.

Methods

Specimens, Controls and Extractions: A total of 1303 specimens were submitted for HSV viral culture or HSV PCR. All specimens submitted for culture were inoculated into MRC-5 and A549 cells and observed 5 to 7 days for typical HSV CPE. Known HSV 1 and HSV 2 controls (Advanced Biotechnologies Incorporated) were used to validate the PCR assays. All samples tested were extracted using the QIAamp DNA blood mini kit from Qiagen (Valencia, CA).

Real-Time Taqman assays: Primers and probe were made using Primer Express software (Applied Biosystems,

Software 2.0, Foster City, CA). For HSV detection, the primers and probe were made from the highly conserved region of the herpesvirus group DNA polymerase gene UL 30. In validation studies, these primers and probe detected both HSV 1 and HSV 2 isolates and controls. HSV primers and probes specific for HSV 1 and HSV 2 were kindly provided by Dr. Eva Thomas and Laura Book of the Children's and Women's Health Centre, Vancouver, BC. The HSV 1 primers and probe detect the gG region (US4) of HSV 1 and the HSV 2 primers and probe detect the gD region (US6) of HSV 2. Primers and MGB probes were made using the ABI PRISM Primer Express software. The HSV 1 probe was labeled with VIC and the HSV 2 probe was labeled with FAM.

All PCR amplification reactions were performed in a 50- μ l volume containing 2x TaqMan Universal Master Mix (MM, Applied Biosystems), 10 μ l of isolated DNA and nuclease free water. For the HSV assay both primers were used at a final concentration of 900nM (0.45 μ l of each primer per test), and the final concentration of probe was 200 nM (0.1 μ l of probe per test). For the type specific HSV 1 & 2 assays, primers were used at a final concentration of 900nM (0.45 μ l of each primer per test), and the final concentration of probe was 200 nM (0.1 μ l of probe per test). Additionally, Albumin was used as a housekeeping gene for inhibition control. Amplification and detection was carried out in a 96-well plate and performed using the ABI Prism 7000 sequence detection system (Applied Biosystems). The PCR cycling program consisted of 40 two-step cycles of 15 s at 95°C and 60 s at 60°C. Real-time measurements were taken, and a Ct value for each sample was calculated.

Results

Of the 1303 specimens tested, 419 (32.2%) were considered positive for HSV by one or both assays.

A total of 340 (26.1%) specimens were HSV positive by both culture and real-time PCR

A total of 78 (6.0%) specimens were PCR positive only and 1 (0.08%) specimen was culture positive only (Table 1).

Of the 78 specimens positive by PCR only, 45 (57%) were re-extracted and tested again using the same primers and probe as above and also tested with the primers and probes specific for HSV-1 and HSV-2. Of these, all 44 were detected and considered positive for HSV using the type specific primers and probes. One specimen was unable to be typed (Table 2).

The 1 specimen positive in culture only, when retested by PCR, was still negative by the HSV PCR assay but was

positive using the HSV-2 PCR primers and probe. This isolate was also typed as a HSV-2 from culture. Of the 418 PCR positive specimens, 379 (91.0%) were detected between cycle 15 and 35 (See Figure).

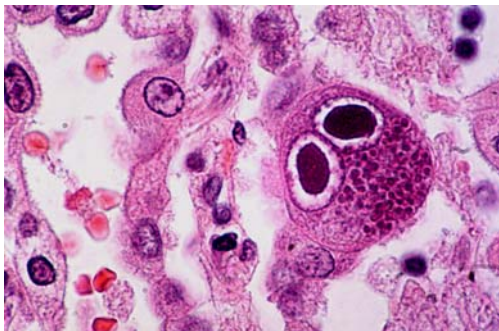
Conclusions

In this study the sensitivity of real-time PCR exceeded traditional cell culture for the detection of HSV from clinical specimens. The data here confirm the results seen in other HSV real-time PCR studies (3, 7).

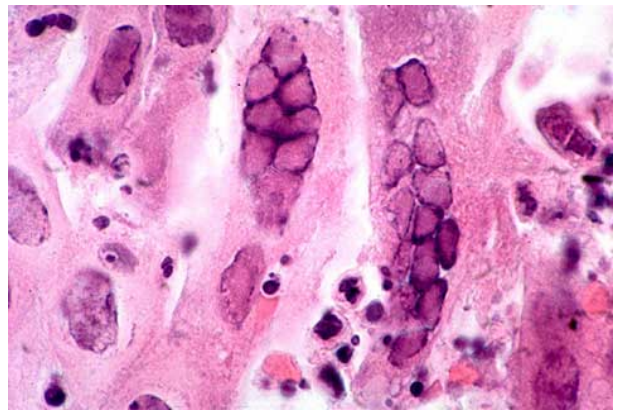
Detection of HSV in cell culture continues to be the standard that all other detection methods are measured by. However, we conclude that the data in this study and as seen in other studies supports the use of real-time PCR replacing cell culture for the routine diagnosis of HSV infections in the diagnostic laboratory.

Literature Cited

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H&E stained lung section showing typical owl-eye CMV inclusions (480X)



H&E stained section showing multi-nucleated cells in a patient with HSV esophagitis.

PASCV Council Notes

Lifetime Memberships

The PASCV Council decided in 2005 that they would no longer accept Lifetime Membership applications. Current lifetime members will continue in their Lifetime Member status.

PASCV Dues Notices

In the past, membership dues were payable 12 months after receipt of the first membership payment. Your renewal month was the month you first became a PASCV member. This policy of sending renewal notices each month has become difficult to manage as our numbers have increased. Effective immediately, dues reminders will be sent to all members in December of each year. PASCV members who paid their dues late in 2005 will receive credit for 2006 membership and they will not receive a 2006 dues notice. Once you have paid your dues, you will receive a receipt (as a pdf form) via e-mail. Please contact Dan Wiedbrauk [dwiedbrauk@wardlab.com] if you have any questions.

Newsletter Delivery

Newsletters, ballots, and other PASCV communications will be distributed to the membership via E-mail. This change will save postage costs and it will facilitate communications with our international members. To make this work, it is very important that the Society has an up-to-date E-mail address for every member. Members without an E-mail address should send a letter or telefax to Dan Wiedbrauk. Dr. Wiedbrauk's contact information is listed elsewhere in this newsletter. Newsletters will also be posted on our website www.virology.org

PASCV Awards

Information on sending in nominations for the Young Investigators Award, Travel Awards or other specific awards may be found on our website www.virology.org. Look under the heading "PASCV Awards"

2005 PASCV Award Recipients

Mario Escobar Award

The Mario Escobar travel award recognizes the best abstract from a resident of Latin America. This special award is sponsored by the Clinical Virology Symposium in memory of Mario Escobar, an outstanding teacher and scientist. Dr. Escobar had a special interest in supporting scientific studies in Latin America. The 2005 Mario Escobar award was presented to **Juan M. Pascale, Ph.D.** from the Gorgas Memorial Institute, Panama and School of Medicine, University of Panama for his abstract on the "*Clinical Significance of Soluble Mediators of Inflammation in Patients with Hantavirus Pulmonary Syndrome (HPS)*."

Edwin Lennette Award

The Edward Lennette travel award recognizes one of the two best Abstracts submitted to the Clinical Virology Symposium. This Award is sponsored by Diagnostic Hybrids, Inc. in memory of Dr. Edwin H. Lennette and his many contributions to Diagnostic Virology. The 2005 Edwin Lennette Award was presented to **Janie Trepanier, Ph.D.** from Sainte-Justine Hospital Research Center and the Departments of Microbiology and Pharmacology, University of Montreal, Montreal, Canada for her abstract involving "*In vitro Studies on the Use of Deoxyribozymes as a Potential Therapeutic Against Hepatitis C Virus*"

Edith Hsuing Award

The Edith Hsuing travel award recognizes one of the two best abstracts submitted to the Clinical Virology Symposium. This award is sponsored by Diagnostic Hybrids and honors Dr. Edith Hsuing, an outstanding teacher and scientist, and celebrates her lasting legacy in Diagnostic Virology. The 2005 Edith Hsuing Award was given to **Charlotte A. Romain, BS MT (ASCP)** from the University of Minnesota and Fairview-University Medical Center, Minneapolis, Minnesota for her abstract on "*Monitoring the Quality of Epstein-Barr Virus (EBV) in Whole Blood (WB) Samples of Patients at a University Medical Center.*"

PASCV Travel Awards

The PanAmerican Society for Clinical Virology sponsors several travel awards to help defray some of the cost of attending the Clinical Virology Symposium. These merit-based awards are presented to selected students, postdoctoral fellows, and technologists who submit outstanding abstracts to the Clinical Virology Symposium.

2005 PASCV Travel Awards

Luisa Montecinos

Infectious Disease and Virology Laboratory and Department of Pediatrics, Medical School, Catholic University, Santiago, Chile

Abstract: Subtyping of Human Metapneumovirus During Respiratory Season in Santiago, Chile, 2004.

Arthur Bagabag, Ph.D.

Department of Laboratory Medicine, Virology Division, University of Washington and The Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, Seattle, Washington

Abstract: Comparison of the Abbott/Celera Real-Time ASR vs. RFLP for HCV Genotyping.

Kimberly S. M. Benschop

Dept of Human Retrovirology, Academic Medical Center, Amsterdam, The Netherlands

Abstract: Human Parechovirus 3 in Culture Isolates from Dutch Infants.

Gwen A. Crist, S.V. (ASCP)

ARUP Laboratories, Inc., Salt Lake City, Utah

Abstract: Comparison of Five Commonly Used Cell Lines for Isolation of Enterovirus and Distribution of Enteroviruses Over a Three-Year Period.

A Veloz

Infectious Disease and Virology Laboratory and Department of Pediatrics, Medical School, Catholic University, Santiago, Chile

Abstract: First Virus Isolation of Human Metapneumovirus in Santiago, Chile.

Frank Zhang, B.S.

North Shore - Long Island Jewish Health System Laboratories, Lake Success, New York

Abstract: Purification of Nucleic Acids by Magnetic Extraction and the MiniMag Instrument for a variety of Molecular Applications.

Yagna B. Joshi, B.S.

The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Abstract: Real-Time Reverse Transcriptase PCR for Detection of Human Metapneumovirus in Infants and Young Children.

2006 Council Elections

In 2006, the term of office will expire for three PASCV council members (Dr. David Myerson, Dr. Christine Robinson, and Dr. Angela Calendo) and Dr. Richard Hodinka will step down as Past-President. The membership will elect three new councilors and a new President Elect. Ballots (see page 5) should be sent to the current president (Dr. James Mahony) and the winners will be announced at the PASCV Business Meeting in Clearwater Beach, Florida.

The new councilors will serve for four years (until 2010). The President-Elect will serve for two years as President-Elect, two years as PASCV President, and two years as Past-President (until 2012). Photographs and biographical sketches for the President-Elect candidates are shown below.

Candidates for President-Elect



Angela Caliendo is an Associate Professor of Pathology and Laboratory Medicine and an Associate Professor in Medicine at Emory University School of Medicine. She serves as the Vice Chair Pathology and Laboratory Medicine, Director of Emory Medical Laboratories, and the Medical Director of the Microbiology and Molecular Diagnostics Laboratories at Emory University Hospital. She received a PhD (Biochemistry) and MD from Case Western Reserve University School of Medicine and completed an internship and residency in Internal Medicine at Brigham and Women's Hospital in Boston, MA and an Infectious Diseases fellowship at Massachusetts General Hospital, Boston MA.

Dr. Caliendo is a member of the CLSI Subcommittee on Genotyping for Infectious Diseases and has served as a member of the Subcommittee on Quantitative Molecular Diagnostics for Infectious Diseases, and an Advisor on the Subcommittee on Antiviral Susceptibility Testing. In 2004, she served as the President of the

Association of Molecular Pathology and currently is a member of the Professional Relations Committee. Dr. Caliendo is actively involved in PASCV and currently serves as a Member of Council.

For the past several years she has been co-director of the ACTG Quantitative CMV working group and the Quantitative HCV working group, which have evaluated the performance characteristics of CMV and HCV viral load assays. Dr. Caliendo is an Associate Editor for the Journal of Clinical Virology, a member of the Editorial Boards for the Journal of Clinical Microbiology and the Journal of Molecular Diagnostics, a Virology Section Editor for the Manual of Clinical Microbiology (9th Ed), and the Infectious Diseases Section Editor for Molecular Pathology and Clinical Practice. Her research interests include the evaluation of HIV-1 RNA burden and the development of antiretroviral resistance in the plasma and cervical secretions of HIV-1 seropositive women, and the development of molecular diagnostic tests for various infectious diseases.



Danny L. Wiedbrauk is the Scientific Director of Virology and Molecular Biology at Warde Medical Laboratory in Ann Arbor. Dr. Wiedbrauk received his Ph.D. degree from the University of Notre Dame and was a staff fellow in the Laboratory of Persistent Viral Diseases at the NIH Rocky Mountain Laboratory in Hamilton, Montana. Dr. Wiedbrauk currently serves on the editorial boards of the Journal of Molecular Diagnostics and Clinical and Diagnostic Laboratory Immunology and is a reviewer for NIH/NIAID Study Sections ZRG1 SSS-K (10) and ZRG1 IDM-B (12). Dr. Wiedbrauk established the ASM Division C listserv and is currently a moderator for this discussion forum. He is also a co-organizer of the Clinical Virology Symposium and the Molecular Virology Workshop. Dr. Wiedbrauk has authored 49 papers and book chapters, written two books and edited one book. His

PASCV activities include service as a councilor (1997-2001) and Secretary-Treasurer (2004-present). Dr. Wiedbrauk's clinical and research interests include viral diagnostics, medical informatics, and the application of nucleic acid amplification and sequencing technologies to the diagnosis of infectious diseases.

PASCV Council Elections

VOTE TODAY!

The PASCV is currently holding elections for the positions of President-Elect, and Councilor. Please vote before March 26 and send your ballot to Dr. James Mahony at the address below.

PRESIDENT-ELECT (select one)

_____ **Angela Caliendo, M.D., Ph.D.**
Emery University Hospital
Atlanta, GA

_____ **Danny Wiedbrauk, Ph.D.**
Warde Medical Laboratory
Ann Arbor, MI.

COUNCILOR (select three)

_____ **Caroline Alferi, Ph.D.**
University of Montreal
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_____ **Kirsten St. George, Ph.D.**
New York State Dept of Health
Albany, NY

_____ **Henry H. Balfour Jr., M.D.**
University of Minnesota
Minneapolis, MN

_____ **C. Worth Clinkscales, Ph.D.**
Vironostics Laboratories
Tulsa, Ok

_____ **Marcela Echavarria, Ph.D.**
CEMIC University Hospital
Buenos Aires, Argentina

_____ **Alexandra Valsamakis, M.D.**
Johns Hopkins Medical University
Baltimore, MD

Your Name: _____
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E-mail ballot to: mahonyj@mcmaster.ca

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The officers and councilors welcome suggestions and comments concerning the PASCV.

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Please type or print all information

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