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Assays for the Detection of Human Papillomavirus

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Introduction

Human papillomavirus (HPV) is estimated to be one of the most common causes of sexually transmitted diseases in both men and women with the incidence of new infections ranging from 1- 5.5 million per year in the United States (1). HPV is associated with a wide variety of diseases ranging from benign cutaneous warts to cervical cancer (2). Although the majority of HPV infections are transient and clinically non-significant, in certain cases (approximately 10%) infection persists. When persistent infection is due to one or more of the 15-18 HPV high risk (HR) types associated with cervical cancer, in a small percentage of these cases (approximately 8%), the infection progresses to a high grade pre-invasive lesion which tends to persist and can advance to invasive cervical cancer (2, 3).

Several studies have demonstrated that Pap cytology, in conjunction with screening for the presence of HPV, can identify women at increased risk for cervical cancer (3, 4). Women who were HPV DNA positive on a single specimen and those with 2 or more positive specimens tested over time were 16 and 216 times more likely to develop cervical cancer than women who were HPV DNA negative (3). In addition, the ASCUS/LSIL Triage Study (ALTS) demonstrated that HPV testing for cancer-associated HPV types had a greater sensitivity to detect cervical intraepithelial neoplasia 3 (CIN 3) or above in a single cytological test indicating atypical cells of undetermined significance (ASCUS) or above. Studies also demonstrated that women aged 30 years and older who have had both negative cervical cytology and negative HR-HPV DNA test results were at extremely low risk for the developing CIN 2 or CIN 3 during the next 3-5 years.

Based on these and other clinical studies the FDA recently approved the combination of cervical cytology and HPV testing for primary screening for cervical cancer for women aged 30 years and older (5).

HPV Testing

Over the last 10 years a variety of methods, including polymerase chain reaction (PCR), nucleic acid sequence based amplification (NASBA), southern blot, dot-blot, in situ hybridization and solution hybridization, have been developed to detect HPV in cervical scrapings and biopsy material. Each method has distinct advantages and disadvantages, sensitivity and specificity, for identifying women at risk for invasive cervical disease.

In situ hybridization based assays are available which detect either HPV antigens or nucleic acids (DNA or mRNA). In situ assays may be beneficial for samples with equivocal histologic findings and may help clarifying HPV subtypes in low grade squamous intraepithelial lesions (LGSIL), especially in young women, to aid in follow-up and treatment decisions. In situ testing may help to rule out the presence of HPV in histologically benign/negative colposcopic biopsy with a history of ASCUS and above or HR-HPV detected on PAP specimen. In addition, these assays may be used for follow up colposcopic biopsies where HPV was not detected on cytology specimens and a lesion (condyloma/CIN 1) is present histologically.

Monoclonal and polyclonal antibodies (Dako Corp., Carpinteria, CA) are available that detect HPV common antigen, a linear epitope of the major capsid protein. The GenPoint System (Dako, Trappes, France) uses a patented catalyzed signal amplification (CSA) methodology for biotinylated probes that can be designed for the detection of HPV in processed tissue (including formalin-fixed paraffin sections) or cell preparations.

A biotinylated probe is hybridized to the target sequence followed by the binding of streptavidin-horseradish peroxidase (SA-HRP) conjugate. The peroxidase catalyzes the oxidation of biotinyl-tyramide, which immediately forms covalent bonds

with the aromatic groups in the specimen. This results in the deposit of large amount of biotin at the site of hybridization that is used to capture more SA-HRP, resulting in a signal amplification cycle. The signal is developed by adding the chromogenic indicator dye diaminobenzidine (DAB) that is oxidized by the peroxidase enzymes to produce a brown precipitant at the site of hybridization. The assay can detect single copies of HPV 16 DNA in cells in 5 hours. **The Ventana BenchMark™ IHC/ISH/FISH Staining System** is a modular automated platform utilizing multiple technologies including immunohistochemistry, fluorescent and chromogenic in situ hybridization. The walk-away system includes automated baking, deparafinization, cell conditioning and staining. The Ventana INFORM® HPV assay detects high risk HPV types (16, 18, 31, 33, 35, 45, 51, 56, 58, 59, 68, 70) and low risk HPV types (6, 11, 42, 43, 44). The assay can be performed on liquid based pap specimens and tissue biopsies with a six hour run time. The sensitivity of the assay is 10-50 HPV copies per nucleus. The type of signal (confluent, punctate) may reflect either episomal or integrated forms of viral target DNA and the intensity of the signal HPV copy number. The main advantage of the in situ format is the ability to correlate DNA probe results with cellular morphology. A sandwich hybridization reaction, consisting of a fluorescein-labeled probe complementary to the HPV target, a primary biotinylated linking antibody (anti-FITC, anti-biotin or anti-digoxigenin), a secondary biotinylated linking antibody and an alkaline phosphatase conjugated avidin, results in the formation of a blue color in HPV DNA positive cells. Clinical studies have indicated that the assay has a positive predictive value (PPV) for cervical disease of approximately 60 %, a negative predictive value (NPV) of 97 %, sensitivity of 86 % and a specificity of 90 %.

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Currently, the **Digene Hybrid Capture II (HCII) assay (Digene, Gaithersburg, MD)** is the only FDA approved method for the detection of HPV in cervical samples. The HCII HPV DNA test is a nucleic acid hybridization microplate assay for the detection of HPV DNA from both intermediate/high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and low risk types (6, 11, 42, 43, 44) (6). DNA from cervical biopsy material, liquid based cytology samples and cervical brushings are denatured and hybridized with HPV specific RNA probe cocktails. HPV RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA:DNA hybrids.

The immobilized hybrids are reacted with antibodies specific for RNA:DNA hybrids conjugated with several alkaline phosphatase (AP) molecules. Multiple antibodies bind to each substrate resulting in signal amplification detected with a chemiluminescent substrate. The substrate is cleaved by the bound AP and light is emitted that is measured on a luminometer.

Studies have demonstrated that the assay is generally very sensitive (93 %) and has an excellent negative predictive value (99 %). However, the positive predictive value of the assay for disease progression or the presence of invasive cervical disease in the overall population can be quite low (17.2 %) due to the transient nature of the majority of HPV infections. Cross reactivity has been reported with low risk

HPV types and high risk probes. In addition, depending upon individual laboratory criteria for reporting and establishing equivocal zones, between 5 - 10% of samples may give indeterminate results. Procedural changes recently recommended by Digene were designed to decrease the number of indeterminate results.

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The detection of HPV DNA in cervical tissue can also be accomplished by using more sensitive target amplification methods such as PCR. HPV type specific PCR assays are based on sequence variations present in the E6 and E7 genes while general primer PCR uses consensus primers that target conserved regions of the HPV genome such as the L1 capsid (MYB09/MYB11). However, deletions in the L1 capsid gene can lead to false negative results. The sensitivity of the PCR assays are generally in the 10-200 HPV DNA copy range. Until recently most PCR assays have been used in the research setting.

Using HPV prototype reagents provided by Roche Molecular Diagnostics, a U.S. laboratory developed **PCR based assays** using two different formats for the detection of either HR or HR and LR HPV types (7, 8). The microwell plate (MWP) format was found to detect HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and the line blot assay (LBA) was found to detect HR-HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 66, 67, 69, 70, 73, 82 and IS39 and LR-HPV types 6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 71, 72, 81, 83, 84, and CP6108. Genomic DNA was extracted from LBC samples followed by PCR amplification using biotin labeled primers targeting the L1 region of the HPV genome. An internal control primer set targeted the β -globin gene. For the LBA the amplified products were denatured, hybridized with the HPV genotyping strips, followed by the addition of color development substrate. For the MWP format, denatured PCR products were hybridized to the MWP pre-coated with BSA-conjugated capture oligonucleotides with sequences specific for all 13 HR-HPV types and β -globin. Unbound probe is removed, HRP conjugate added, followed by detection after the addition of substrate.

The absorbance of the colored complex is read using an automated plate reader. The PCR based methodology demonstrated increased sensitivity when compared to HCII and 90 % of the HCII HR-HPV indeterminate cervical cell specimens were resolved using the prototype LBA and MWP assays (7, 8). Also in development is an HPV test for use with the Cobas Taqman Analyzer that will couple HPV detection with Roche's real time PCR technology.

Although DNA detection has a high negative predictive value and is an excellent way to rule out the potential for disease, the poor positive predictive value clearly indicates that supplemental testing should be incorporated into the testing algorithm. An alternative approach to DNA detection that may reveal more about the risk of developing dysplasia is the detection of mRNA from the cellular transforming genes E6 and E7 (4). The E6/E7 oncoproteins are required for initiation and maintenance of a malignant cellular phenotype by blocking the tumor suppressor functions of p53 and Rb. HPV E6 binds p53 and can prevent DNA repair and p53 mediated apoptosis. HPV E7 binds and inactivates Rb thereby promoting uncontrolled cell division. Two assays, the

InPath™ In-Cell HPV Test (Molecular Diagnostics, Inc./Invivion, Frankfurt, Mich) and the PreTect HPV-Proofer (Norchip, Norway) have been developed to detect E6 and E7 mRNA.

The InPath™ In-Cell HPV Test is a unique biomolecular based technology designed for the screening of cervical dysplasia and cervical cancer. The system uses a specific combination of proteomic cocktails composed of a variety of markers, stains, permeability and blocking agents that illuminate and map abnormal cells. The two cocktails of the InPath™ System include the Cocktail-CVX and the In-Cell HPV™. The Cocktail-CVX consists of multiple protein-based markers including a transmembrane-associated marker, an enzymatic protein involved with cellular metabolism and a cytoplasmic protein associated with epithelial structure. These markers detect tumor receptor mediated downstream growth and cellular proliferation activity. The cocktail identifies dysplastic cells only. The test can be slide based or applied to a specialized cell collector called the InPath™ e² collector that consists of a balloon that is inflated in the cervix to capture cells from all areas. The assay uses a fluorescence oligonucleotide detection platform that detects the mRNA associated with increased activity within the patient's cells stemming from the replication of HPV. The assay keeps cells intact and retains their morphologic properties.

The PreTect HPV-Proofer assay (NorChip, Klokkarstua, Norway) detects HPV E6/E7 oncogenic activity by targeting E6/E7 mRNA expression from 5 of the most common HR-HPV types (16, 18, 31, 33 and 45) that are present in approximately 89-97% of cervical carcinoma samples (2, 3, 4). The assay combines nucleic acid sequence based amplification (NASBA) technology and real time detection using molecular beacons (9). NASBA is an isothermal process that is highly appropriate for the amplification of RNA.

Amplification is achieved through the coordinated activities of three enzymes (avian myeloblastosis virus reverse transcriptase [AMV-RT], RNase H, and T7 RNA polymerase) and two DNA oligonucleotide primers that are specific for the target sequence of interest. Amplification is based upon primer extension: the E6/E7 mRNA serves as a template for the extension of primer 1 (containing the T7 RNA polymerase recognition site) by Avian Myeloblastosis Virus reverse transcriptase (AMV-RT).

Extension is followed by degradation of the template RNAs by RNase H, synthesis of second strand DNA strand through the extension of Primer 2 by AMV-RT and RNA synthesis by T7-RNA polymerase. With RNA transcription the system enters the isothermal cyclic phase, resulting in the accumulation of wild type RNA amplicates. Added at the start of the amplification reaction are molecular beacons, specific for either one of the 5 HR-HPV types or U1A, a cellular housekeeping gene.

The detection of U1A is used to monitor the efficiency and integrity of the entire test procedure and to eliminate false negatives due to poor sample collection or sample degradation due to inappropriate handling or transport. Three duplex assays (HPV 16 and U1A; HPV 18 and HPV 31; HPV 33 and HPV 45) are performed on each sample. A fluorescent analyzer reads the emitted fluorescence every 10 sec from both the molecular beacons, one labeled with ROX

and one with FAM. The emitted fluorescence is plotted on a graph after the instrument normalizes against background fluorescence.

Studies have demonstrated a high concordance (97%) with HPV DNA and E6/E7 mRNA, indicating stable expression of the HPV oncogenes in cervical cancer (10). In addition, limited data has also indicated that RNA-based tests can reveal a higher prognostic value and a higher specificity than DNA based testing (11). Further studies, currently in progress, will provide more information on the clinical use and prognostic value of E6/E7 mRNA detection.

Conclusions and the Future

The wealth of data currently available on the role of HPV in cervical cancer has been central for establishing guidelines that standardize the management of women with cervical cytological abnormalities. Sensitive and specific HPV DNA testing for ASC-US triage identifies women at risk for invasive disease and accurately correlates with CIN 2/3 and squamous cell carcinoma. Sensitive assays to detect HR-HPV DNA are an excellent way to "rule out" women at risk for HPV related cervical disease and have excellent negative predictive values. However, due to the relatively small number of HR-HPV infected women who actually progress to invasive disease, the positive predictive value of a DNA test can be quite low.

The addition of supplemental testing to the diagnostic algorithm must be further explored so as to more accurately determine which HPV HR positive women are truly at risk for disease progression. The detection of E6/E7 mRNA and the identification and mapping of dysplastic cells by molecular methods can further refine the diagnostic process. While not wanting to let women infected with HR-HPV go undetected, concurrent consideration must be given to reducing the number of unnecessary colposcopies and to providing HR-HPV infected women with reassurance as to the state of their infection.

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New Advances Transform the Management of Women with Abnormal Pap Tests

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Abstract

New advances in Papanicolaou test technology, human papillomavirus DNA testing, and revisions in the Bethesda terminology for cervical cytology have transformed the management of abnormal Pap tests. This approach has been validated by a recent randomized clinical trial, and in some instances can reduce the number of colposcopies by 50%. This article briefly summarizes the new liquid-collection system for thin-layer slide preparation, the new Hybrid Capture 2 HPV DNA test, the 2001 Bethesda reporting system, data from a key trial, and the new management guidelines.

Key Points

- ◆ The new liquid-collection, thin-layer system has improved the quality of Pap smears and the test's sensitivity for diagnosing precancerous cervical lesions.
- ◆ The new Bethesda terminology has clarified the previous ambiguous reporting categories; the category of "atypical squamous cells of unknown significance" (ASCUS) has been divided into two: ASC-US and a higher-risk category called ASC-H.
- ◆ Testing for the DNA of high-risk types of human papillomavirus (HPV) by the Hybrid Capture 2 assay provides a basis for deciding whether women with ASCUS should undergo colposcopy.
- ◆ The ALTS trial clinically validated the value of HPV DNA testing in identifying women at risk for cervical cancer.
- ◆ Women with ASC-US should be tested for HPV and undergo colposcopy if positive. Those with ASC-H should be referred for immediate colposcopy.

New PAP Rest Technique

Up to 90% of false-negative Pap smears are due to limitations of sampling or slide preparation. Accurate interpretation can be hindered by blood, mucus, inflammation, air-drying artifact, or areas of thick cellularity, all of which are common in conventional Pap smears.¹ To address these problems, a new slide preparation method—the liquid-collection ThinPrep system—was developed by the Cytoc Corporation (Boxborough, Mass) and approved by the US Food and Drug Administration in May 1996.²

How the ThinPrep system works

In the ThinPrep system, cells are placed immediately into a fixative (the PreservCyt liquid cytology medium) and transported to the laboratory, where thin-layer slides are prepared using the ThinPrep processor. The machine spins and filters the samples until sufficient cells have been obtained. The cells are then transferred from the filter to a glass slide for Pap staining. The resulting slide contains approximately 50,000 cells, evenly distributed, representing 5% of the total number of cells in the collection in most cases. Sometimes the percentage is higher, eg, in postmenopausal women.

The new system is better

This new Pap test technique has resulted in a statistically significant increase in the cytologic diagnosis of cervical cancer precursors and in specimen adequacy.³ The clarity of the specimen increases the ease of screening by cytology technologists. Most studies have found the liquid-based ThinPrep system to be more sensitive than conventional smears, while its specificity was comparable or a little less. In addition, the captured and preserved cells can later be used for HPV DNA testing. This offers a cost-effective single-sample approach. The ThinPrep technique has been adopted by most clinics in the United States. It has been the standard of care for Pap testing at The Cleveland Clinic since 1999. The cost is approximately \$80.

New HPV DNA Testing by Hybrid Capture Assay

Cervical cancer is strongly associated with HPV infection. Between 93% and 100% of squamous cell carcinomas of the cervix contain DNA from high-risk types of HPV. Testing for HPV DNA has greater sensitivity than cytology for detecting clinically relevant lesions, and it has been a useful adjunct test in cervical cancer screening since the mid-1990s. In April 1999, the FDA approved a breakthrough technology, the Hybrid Capture 2 HPV DNA test (Digene Corporation; Gaithersburg, Md).

How the Hybrid Capture 2 test works

The new test is a sandwich capture molecular hybridization assay that utilizes chemiluminescence to detect one or more of the 13 cancer associated (high-risk) HPV types, ie, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.⁴ Cervical specimens from a ThinPrep collection containing the target DNA hybridize in solution with a specific HPV RNA probe cocktail. The resulting RNA-DNA hybrids are captured onto the surface of a microplate well that is coated with an anti-RNA-DNA hybrid antibody. Captured hybrid is then reacted with an antihybrid antibody conjugated to alkaline phosphatase and detected with a chemiluminescent substrate. When the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured in relative light units on a luminometer. The intensity of the light emitted is proportional to the amount of HPV DNA in the Pap specimen. The new Hybrid Capture 2 test has a high diagnostic sensitivity (85% to 100%) and negative predictive value (99% to 100%).^{4,5} The specificity is about 61%. Older tests were less sensitive and specific.

New PAP Test Terminology

The 2001 Bethesda system was published in the April 24, 2002, issue of *JAMA*, the Journal of the American Medical Association⁶ and more than 20 national and international societies have endorsed it. The 2001 Bethesda System contains several important changes.

- It eliminates the category "benign cellular changes," which had generated confusion about whether women in this category were at higher risk of cervical cancer. In the 2001

updated version, benign changes are more clearly identified as “negative for intraepithelial lesion or malignancy.”

- Probably the most important change: the previous equivocal category of ASCUS has been subdivided into two groups:
ASC-US: atypical squamous cells of undetermined significance
ASC-H: atypical squamous cells, cannot exclude a high-grade lesion. Women with the ASC-H Pap diagnosis are at higher risk of ultimately developing a high-grade lesion and should be referred for colposcopy. Women with ASC-US are at a lower risk of developing a high-grade lesion than those with ASC-H, but they are at a higher risk than those diagnosed “negative for intraepithelial lesion.” This distinction provides further risk stratification in this subset of patients and the foundation for clinical decision-making.⁷

New Evidence From a Clinical Trial

A major issue in cervical cancer screening was how to manage the 3 million women diagnosed with ASCUS each year. Most of these mild cervical abnormalities regress spontaneously without treatment, but physicians had no way to identify clinically significant lesions that represent precancer or cancer and that need treatment. To clarify the management of mildly abnormal cervical cytopathology, the National Cancer Institute in 1996 launched a major randomized, multicenter clinical trial known as the Atypical Squamous Cells of Undetermined Significance/Low-grade Squamous Intraepithelial Lesions (ASCUS/ SIL) Triage Study (ALTS).⁸⁻¹⁰ From 1996 to 1998, a total of 5,060 women with abnormal cervical cytologic findings (ASCUS or LSIL) enrolled at four sites. All underwent HPV testing by Hybrid Capture 2 assay.

Results in women with LSIL

Among 642 women with low-grade squamous intraepithelial lesions, high-risk HPV DNA was detected in 532 (82.9%). The high percentage of HPV DNA positivity in the LSIL population limits the usefulness of HPV testing in clinical decision-making. It was estimated that the cost of HPV testing of all women with a cytologic diagnosis of LSIL would outweigh savings gained from avoiding colposcopy for only 20% to 27% of women. Therefore, in October 1997, the ALTS steering committee decided that women with LSIL would no longer be randomly assigned to a follow-up protocol that used HPV DNA results for triage.⁸

Results in women with ASCUS

A total of 3,488 women with ASCUS were randomly assigned to one of three management strategies - Immediate colposcopy, Colposcopy if HPV-positive (HPV test triage), Repeat Pap smear. The primary end point of the study was histologically confirmed cervical intraepithelial neoplasia grade 3 (CIN 3). High-risk HPV DNA was detected in 1,766 (50.6%) of the 3,488 participants. Overall, 5.1% of women with ASCUS in the trial had histologically confirmed CIN 3. The sensitivity of the HPV DNA test for predicting CIN 3 or cancer was 96.3%, with a negative predictive value of 99.5%. In contrast, the sensitivity of a single repeat Pap test was only 44.1%. About 55% of women with ASCUS would have been referred for colposcopy if the HPV test had been used for triage in all cases. This trial shows that the Hybrid Capture 2 assay has excellent sensitivity for detecting precancerous cervical lesions. HPV testing can help in deciding how to manage women with ASCUS. A positive test suggests that precancer or (rarely) cancer may be present, while a negative test may assure women of the benign nature of their ASCUS. Precancerous lesions were found in 13% of ASCUS cases in which the HPV test was positive. HPV testing reduced

referrals to colposcopy by about 50% compared with immediate colposcopy. Thus, HPV testing is a viable option in triaging women with ASCUS.²⁰

New Guidelines for Abnormal PAP Smears

Pathologists and clinicians have long struggled to deal with the diagnosis of ASCUS, since the significance to the patient was uncertain. Ignoring ASCUS is clearly dangerous, yet referring all women with ASCUS for immediate colposcopy is costly and unnecessary. The recommendations for managing women with abnormal cervical cytology are summarized as follows.

Women with ASC-US should be tested for high-risk HPV DNA. Those testing positive are referred for colposcopy. Women with ASC-US who test negative for high-risk HPV should repeat a Pap test at 1 year.

Women with ASC-H, LSIL, HSIL, (high-grade squamous intraepithelial lesion), or **atypical glandular cells** should be referred for immediate colposcopic evaluation. HPV testing in women with ASCUS should reduce the colposcopy referral rate by 50%. However, only about 5% of cases of ASCUS reflect an underlying CIN 3, an immediate cancer precursor. The less-than ideal specificity of the high-risk HPV DNA test would prevent it from replacing the Pap test in primary screening. Therefore, many women with ASCUS will still undergo unneeded colposcopy. A recent study indicated that the prevalence of high-risk HPV infection declines with age: only 31.2% among women with ASCUS who were 29 years or older, compared with 65% in those age 28 and younger.¹¹ Thus, the reduced referrals with HPV DNA testing in older women may be promising in evaluating the cost-effectiveness of HPV triage and further improving strategies for ASCUS management.

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CANDIDATES FOR PRESIDENT-ELECT



Dr. Gregory Storch is a Professor of Pediatrics, Medicine, and Molecular Microbiology and the Director of Pediatric Laboratory Medicine at the Washington University School of Medicine. He is also the Medical Director of Clinical Laboratories at St. Louis Children's Hospital.

He received his undergraduate degree from Harvard College and MD from NYU Medical School. Dr. Storch did an internship and residency in internal medicine at Washington University Medical Center. Afterwards, he served as an Epidemic Intelligence Officer for the Centers for Disease Control, and was stationed at the Louisiana Department of Health. Since 1981, he has been a member of the Washington University Division of Pediatric Infectious Diseases and Director of Microbiology Laboratories at St. Louis Children's Hospital. He served as Medical Director of Infection Control at St. Louis Children's Hospital from 1982-2002. He also presently serves as Medical Director of Project ARK, a program to provide medical care for HIV-infected children, which he and colleagues founded in 1995.

Dr. Storch has been a member of the Pan-American Society for Clinical Virology for many years, and was elected Councilor in 2001. He is also a fellow of the Infectious Diseases Society of America, and a member of the American Society of Microbiology and the American Academy of Microbiology. He has a long standing interest in diagnostic virology. He is the author of *Essentials of Diagnostic Virology*, published by Churchill Livingstone in 2000, as well as numerous articles in peer-reviewed journals. He continues to be very interested in the development of new viral diagnostic tests and exploring how to use them to achieve maximum clinical utility and to learn more about viral diseases.



Ella M. Swierkosz, Ph.D., Professor of Pathology and Pediatrics, is the Director of the Microbiology and Virology Laboratories of Cardinal Glennon Children's Hospital and Director of the Microbiology Laboratory of St. Louis University Hospital, Saint Louis University (SLU) Health Sciences Center and a member of the St. Louis University Center for

Vaccine Development. She is a Diplomate of the American Board of Medical Microbiology and a Fellow of the American Academy of Microbiology. She is also Co-Chair of the NCCLS Subcommittee on Antiviral Susceptibility Testing and Secretary-Treasurer of the Pan American Society for Clinical Virology. She is an author or co-author of 3 chapters in the current edition (8th) of the *Manual of Clinical Microbiology*. She is a member of the editorial board of the *Journal of Clinical Virology* and a past member of the editorial board of the *Journal of Clinical Microbiology*. In addition, she is Vice-Chair of the Institutional Biosafety Committee of Saint Louis University and is a member of the advisory board of the Missouri State Health Department Laboratory.

Dr Swierkosz received her Ph.D. in medical microbiology from Wayne State University and completed a post-doctoral fellowship in public health and medical microbiology at the University of Rochester Medical Center, Rochester N.Y., before joining the faculty of Saint. Louis University.

Her activities in the Saint Louis University Center for Vaccine Development have included semiannual compilation of viral surveillance data, quantitation of viral shedding after administration of live, attenuated influenza vaccine, which has recently received FDA approval, and monitoring viral shedding in volunteers administered a live, attenuated candidate cytomegalovirus vaccine.



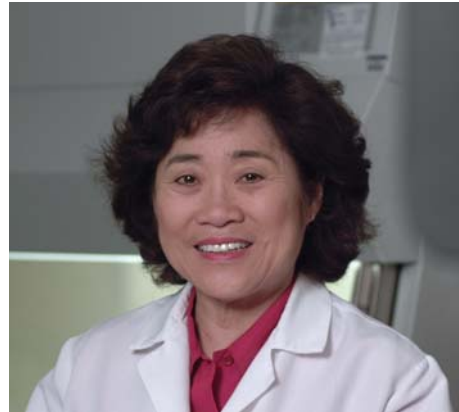
CANDIDATES FOR SECRETARY/TREASURE



Danny L. Wiedbrauk, Ph.D. 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. He created and serves as the moderator for the ASM Division C Listserver and is the alternate Moderator for the ClinMicroNet Listserver. Dr. Wiedbrauk is a co-organizer of the Clinical Virology Symposium and the Molecular Virology Workshop which are held annually in Clearwater Beach, Florida. 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.



Dr. Belinda Yen-Lieberman is an Assistant Clinical Professor in the Department of Clinical Pathology at the Case Western University School of Medicine and the Director of

Clinical Virology, Serology, and Cellular Immunology and a Staff Scientist in the Department of Clinical Pathology and Research Institute at the Cleveland Clinic Foundation in Cleveland, Ohio.

Dr. Yen-Lieberman received her Ph.D. in Immunology/Biochemistry at the University of Arkansas and was a Postdoctoral Fellow in Clinical and Cellular Immunology at Case Western University and the Cleveland Clinic Foundation, respectively. She is a member of the Virology AIDS Clinical Trials Group (ACTG) and a member of the ACTG/NIAID Virology Quality Assurance Subcommittee. Dr. Yen-Lieberman has been a member of the Pan American society for Clinical Virology for numerous years and was elected Councilor in 2001. She is member of the American Society for Microbiology and serves on the editorial board of Clinical and Diagnostic Laboratory Immunology. She was recently appointed as Editor for this journal. Her clinical and research interests include viral diagnostics, HIV, and the development of molecular methods for viral detection. She has published a number of articles in peer-reviewed journals



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PASCV Board Elections

VOTE TODAY!

The PASCV is currently holding elections for the positions of President-Elect, Secretary/Treasurer, and Councilor. Please vote before March 26 and send your ballot to Rick Hodinka at the address below.

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Warde Medical Laboratory
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- Belinda Yen-Lieberman, Ph.D.
Cleveland Clinic Foundation
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Your Name: _____
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Mail ballot to: Richard L. Hodinka, Ph.D.
Clinical Virology Laboratory
716D Abramson Research Center
Children's Hospital of Philadelphia
3615 Civic Center Boulevard
Philadelphia, PA. 19104

Ballots can also be faxed to (215) 590-2556 or emailed to hodinka@email.chop.edu.

2005 Clinical and Diagnostic Virology Awards Nominations

Each year at the Clinical Virology Symposium banquet, PASCV presents two major awards to leaders in our field. The Clinical Virology Award acknowledges an individual whose contributions to clinical virology have had a major impact on the epidemiology, treatment or understanding of the pathogenesis of viral diseases. The Diagnostic Virology Award honors an individual whose contributions to the laboratory diagnosis of viral diseases has had a major impact on the discipline.

If you would like to nominate someone for either of these awards, please send the individual's name and a letter stating why you feel the nominee is deserving of this award to Rick Hodinka at Room 716D Abramson Research Center, Children's Hospital of Philadelphia, 3615 Civic Center Blvd., Philadelphia, PA. 19104 or via email at hodinka@email.chop.edu.

Nominations should be received by December 6, 2004.

Report on the First International Clinical Virology Symposium In Argentina

The First International Clinical Virology Symposium was organized by the Latin American Correspondent from CEMIC (Center for Medical Education and Clinical Investigations) in Buenos Aires, Argentina, November 5-8, 2004.

A total of 34 speakers from Argentina, Brazil, Canada, Chile, Cuba, Uruguay and USA contributed to the high scientific level of the program in round tables, clinical case presentations and plenary sessions.

A total of 118 participants from Argentina, Brazil, Canada, Chile, Cuba, France, Uruguay and USA attended this Symposium. Of the registered participants, 88 received scholarships: 25 given by the Organizing Committee, 10 by the Argentinean Society of Virology and 53 by commercial companies. Participants expressed their satisfaction and enthusiasm with the high scientific level of the program, the organization and the availability of interaction and collaboration with scientists from different countries.

The Symposium had economic support from the following institutions: PAHO, PASCV, Argentinian National Research Council (CONICET), CEMIC, Norberto Quirno Foundation and the Argentinian Society for Virology. In addition, 9 companies from industry exhibited in booths and provided financial support.

The Organizing Committee, formed by Dr. Carballal (President), Dr. Echavarría (Academic Secretary), Dr. Martínez (Technical Secretary), Dr. Videla (Treasurer) and Dr. Novillo, Dr. Mersich, Dr. Zapata, Dr. Herrera (Coordinators), acknowledges the PASCV officers for their support and encouragement from the beginning of this idea and our appreciation to the CEMIC University Hospital. We also want to thank all the invited speakers, the industry sponsors, and all attendees who significantly contributed to the success of this first meeting in Argentina.

Prof. Guadalupe Carballal, MD
Latin American Correspondent of PASCV

For further collaborative studies please contact: gcarballal@cemic.edu.ar.
For more information regarding CEMIC University Hospital visit: www.cemic.edu.ar

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The Officers and councilors welcome suggestions and comments concerning the PASCV. It is intended that the Newsletter be a forum for exchange of views and information that will be beneficial to the membership. In particular, the experiences of members with reagents, techniques, and new products applicable to rapid viral diagnosis are solicited and will be published in the newsletter.

PAN AMERICAN SOCIETY FOR CLINICAL VIROLOGY MEMBERSHIP FORM

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