REX HEALTHCARE

Laboratory Bulletin...

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Are You Ready for the Flu Season?

The Microbiology Department is pleased to announce that, in time for the upcoming influenza season, we now have a diagnostic rapid test for both Influenza A and B viruses. The test does not distinguish between Influenza A and B. The rapid detection of either virus uses an optical immunoassay technique for the detection of protein antigens unique to Influenza A and B (i.e. nuclear protein). The optical immunoassay is similar to the type of assay used for the detection of group A strep in pharyngeal specimens. The specimen is placed on a surface consisting of a silicon wafer with optical coating that contains immobilized specific antibodies for Influenza A and B that have the capability of capturing the viral antigen in a clinical specimen used for diagnostic testing. The antigen-antibody complexing creates a change in the thickness of the layer, altering the reflective light and ultimately producing a color change.

Proper collection technique is critical for the testing and identification of Influenza A and B viruses in clinical specimens. Test interference may be produced by swabs with wooden shafts, calcium alginate swabs, culturette EZ swabs or cotton tipped swabs. A number of different specimens have been utilized for this particular test and have included: throat and nasopharyngeal swabs, nasal aspirates and sputum specimens. The Rex Microbiology Department recommends nasopharyngeal dacron polyester tipped swabs based on their relevant sensitivity and specificity compared to the other specimen collection devices and specimen types. Swabs with either viral or bacterial transport media should not be used for this testing since the transport media will dilute the specimen and may cause a false negative reaction. The optical immunoassay test is like other diagnostic tests, in which reliable results are dependent on specimen collection and sampling techniques. Since the test detects antigen and not viable viral particles, the optical immunoassay test may produce a positive result when a parallel influenza viral culture is negative. Based on preliminary data, it appears that at least two-thirds of such cases represent true infection, and that antigen detection is more sensitive than culture. Potential interference from medications such as antiviral, antimicrobial, interferon, intranasal steroids and antiasthmatics has not been established.

In one study of nasopharyngeal swab specimens using the optical immunoassay test, the sensitivity was recorded as 83.3% and the specificity 76.2%. In another study (point of care setting), the sensitivity for the nasopharyngeal swab was 95.5% and the specificity was 64.1%. Based on the sensitivity data of these tests, a false negative reaction should be kept in mind in patients presenting with symptoms and physical findings suggestive of Influenza A,B infection. In low incidence populations, a number of positive reactions may ultimately be false positive tests. The Microbiology Department hopes this test provides useful information for infection control with patient isolation procedures and assists in keeping horizontal transmission in the hospital at the lowest possible incidence.

John P. Sorge, M.D.
Pathologist

Rapid HIV-1 test (SUDS) No Longer Available

In a letter dated October 18, 2000, Abbott Diagnostics informed their customers that the Abbott / Murex Single Use Diagnostic System (SUDS) HIV-1 test, the only rapid HIV test currently licensed for the U.S. market, will not be available for an undetermined period of time due to manufacturing problems. The FDA has received assurance from Abbott that the company is working to resolve these problems as quickly as possible. The most significant impact will be on the prompt evaluation of healthcare worker needlestick injuries when the

goal is to start therapy early to prevent transmission of HIV. The Rex Hospital Laboratory has made arrangements to send employee needlestick package samples to UNC Hospitals Laboratory for testing seven days per week. Testing will be performed twice daily, Monday-Friday, and daily on weekends. While the turnaround time will not be as fast as with the SUDS test, results should be available in a relatively timely manner to allow decisions regarding prophylactic therapy for the exposed healthcare worker. This change will be effective immediately. Note that this only applies to employee needlestick packages. All other patient HIV-1 testing will proceed as usual at Rex and will be reported in the usual manner.

Timothy R. Carter, MD
Pathologist

Human Papilloma Virus Typing as an Adjunct to the PAP Smear

Human Papilloma Virus (HPV), in addition to being the causative agent for common warts and genital warts, is closely linked to the risk of developing cervical cancer. It is estimated that greater than 90% of cervical carcinomas are associated with HPV infection. At least 20 million Americans harbor genital infections with this sexually transmitted virus. The great majority of those infected are in the reproductive age group. There are numerous HPV subtypes that have been associated with cervical infection. In addition, recent studies have grouped many of these subtypes into low, intermediate, and high-risk groups for their association with cervical cancer. Certainly not everyone with the high-risk subtypes develops cervical cancer as there are thought to be numerous cofactors involved. These include tobacco use, coexistent herpes virus infections, and, possibly, dietary factors. In addition, it is thought that many HPV infections are cleared over time by the individual's immune response.

The Pap smear, which has been used since the 1940's, is credited with a 70% decrease in deaths due to cervical cancer. HPV cytopathic changes can be well visualized on the Pap smear and thus the Bethesda system for Pap smear reporting includes the designation, HPV Effect, under epithelial cell abnormalities. In the past the presence of HPV effect on Pap smears was often reported synonymously as koilocytotic atypia or condylomatous atypia. Unfortunately the Pap smear changes that allow diagnosis of HPV infection are not present in all cases and when present do not allow for stratification into low or high-risk for the development of cervical cancer. In addition there is the problem of what to do with cases of Pap smears with a diagnosis of Atypical Squamous Cells of Uncertain Significance (ASCUS).

Within the past several years two new tests, both touted as either adjuncts to or replacements for the traditional Pap smear, have received FDA approval. The first of these is the thin-layer, or thin prep, method for performing the Pap test. This methodology utilizes a liquid based medium for the preservation and transportation of Pap specimens as opposed to the traditional technique where the specimen is simply smeared on a glass slide. Several studies have shown an increased sensitivity for the detection of cervical dysplasia when the thin layer method is used. In addition, the thin layer fluid specimen can be retained for possible additional studies including HPV typing.

The second test to receive FDA approval, as an adjunct to the traditional Pap specimen, is the Digene® HPV Hybrid Capture II DNA Assay for HPV typing and subsequent classification into low and high-risk types. HPV typing by the DNA assay can be performed either from a cervico-vaginal sample collected into a Digene® specimen transport kit or can be performed on leftover sample from a ThinPrep Pap Test. It is essential that the results of the DNA assay be interpreted in conjunction with other clinical information such as the presence or absence of cervical dysplasia in a patient, the grade of any cervical dysplasia and any other clinical findings. Very low levels of HPV infection can cause false negative results by the DNA Assay. At Rex Healthcare, samples for HPV DNA typing are forwarded to Mayo Medical Laboratories.

The American Society of Cytopathology currently does not recommend primary, population-based, screening with the HPV Assay due to concerns regarding specificity. However, HPV

testing has been shown to be a useful secondary (adjunctive) test following an indeterminate (ASCUS) cytology result as a means to help triage patients that need colposcopy versus those that can be followed by repeat Pap testing. (JAMA. 1999:281(17):1605-1610.) For patients receiving an ASCUS diagnosis on a traditional Pap smear, a useful strategy would include a repeat Pap test using a thin layer specimen. If the thin layer specimen also were to yield equivocal results then HPV typing using the Digene® assay could be performed off of the residual thin layer specimen. Women with positive HPV typing results would then be triaged to colposcopy with routine Pap testing recommended for those with negative HPV DNA.

If you have any further questions please contact Dr. Keith V. Nance (Medical Director of Cytology) at 784-3286.

Keith V. Nance, MD
Pathologist

The Clot Retraction Test Fades Away

As of November 1, 2000 the Clot Retraction study will no longer be offered at Rex Hospital Laboratory. This decision is unlikely to upset any of you in the medical community since Rex Lab has not received an order in the last five years. Below is a short explanation of the test so you are aware of what is being discarded.

The clot retraction is an in vitro coagulation test that measures the ability of platelets to cause a clot to retract. Normally serum is expressed from a clot and becomes denser over time. Clot retraction begins in 30 seconds after blood has clotted. At the end of 1-hour, appreciable clot retraction occurs and is completed at the end of 4 hours. If the platelet count is below 100,000/ul or there is a qualitative platelet defect, clot retraction is poor or non-existent. Blood diseases such as myeloproliferative and myelodysplastic disorders, Glanzmann's thrombasthenia, and thrombocytopenia are likely to cause an abnormal clot retraction, as will aspirin ingestion.

The Ivy bleeding time and platelet aggregation studies have replaced the clot retraction test. Aggregation studies can pinpoint the platelet abnormality and provide a more precise diagnosis. The aggregation studies are available (by appointment) at the Coagulation Lab (919-966-4264) at UNC Chapel Hill and Ivy bleeding time at Rex Lab.

Stephen V. Chiavetta, MD
Pathologist

Myoglobin D/C'd

For reasons remarkably similar to those discussed in the preceding article, myoglobin testing at Rex will be discontinued in the very near future. Specimens will be referred to an outside laboratory for evaluation.

John D. Benson, MD
Pathologist

The Bethesda System for Pap Smear Reporting

The Bethesda System for reporting of cervical/vaginal cytologic diagnoses was introduced in 1988 as a means to standardize Pap Smear reporting and to encourage the use of specific, descriptive diagnoses. Within the Bethesda System, there are three broad diagnostic categories: 1) Within Normal Limits, 2) Benign Cellular Changes, and 3) Epithelial Cell Abnormalities. With the exception of Within Normal Limits, each of these broad categories is then subdivided into specific diagnostic categories. With the introduction of the Bethesda System, other Pap Smear classification systems, including the old Papanicolau Class System and CIN system, were abandoned. The following list details the major diagnostic categories of the Bethesda System and includes synonymous terminology from prior systems.:

Bethesda System Diagnosis	**Synonym From Previous Classification Systems**
Within Normal Limits	Class I
Benign Cellular Changes	Class II, Inflammatory Atypia
Infection	
Reactive Cellular Changes	
Epithelial Cell Abnormalities	
ASCUS, AGUS	Class II, Squamous Atypia, Glandular Atypia
LGSIL, HPV Effect	Class III. CIN 1, Mild Dysplasia, Condylomatous Atypia, Koilocytotic Atypia
HGSIL	Moderate Dysplasia, Severe Dysplasia, CIN 2 CIN 3, Carcinoma-in-situ (CIS), Class III, Class IV
Invasive Carcinoma	Class V

Explanation of Terms:

Class I, II, III, IV, V - The Papanicolaou Classification
CIN 1, 2, 3 - Cervical Intraepithelial Neoplasia
ASCUS - Atypical Squamous Cells of Uncertain Significance
AGUS - Atypical Glandular Cells of Uncertain Significance
LGSIL - Low Grade Squamous Intraepithelial Lesion
HGSIL - High Grade Squamous Intraepithelial Lesion
HPV - Human Papilloma Virus

For further Information please contact Dr. Keith Nance, the Medical Director of Cytology, at 784-3286.

Keith V. Nance, MD
Pathologist

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