

Human Papillomavirus Detection - Adjunctive Testing For Cervical Cancer

Overview

Cervical cancer is a preventable disease associated with significant morbidity and mortality. In 1999, 610 new cases and 170 deaths were reported in Ontario.¹

Papanicolaou (Pap) testing has helped reduce cervical cancer rates dramatically since its implementation in the 1950s. Pap test reporting classifications have evolved and been refined, to the current Bethesda system. The management of equivocal Pap test abnormalities remains a complex clinical challenge. The most common abnormal Pap test result is one of uncertainty, "atypical squamous cells of undetermined significance." (ASCUS).

Clinical Relevance

Several studies have highlighted the causal association between Human Papillomavirus (HPV) and cervical intraepithelial neoplasia (CIN), a precursor of cervical cancer.² Data collected to

date suggests that while 80% of women infected with high risk HPV strains will experience transient infections, 20% will develop CIN. It is felt that a cytotoxic T-cell response allows for resolution of infections and associated lesions in 80% of the latter group. The remainder of women will have persisting or even progressive CIN lesions, which may result in invasive cervical carcinoma.

Recently, a nucleic acid-based HPV assay has become available which may be used as an adjunct to cervical testing, for example, in cases where ASCUS has been reported. In one study, the sensitivity of initial HPV testing for high grade cervical lesions was 89.2% compared with 76.2% for a repeat Pap test. Studies have also shown that the negative predictive value of HPV for high grade cervical lesions is about 99%, confirming that HPV-negative women with ASCUS have a much lower risk of cancer, and can be safely followed.^{3,4}

(to be continued on page 4)

Therapeutic Drug Monitoring and Critical Values


Spurious "critical values" for therapeutic drug levels may result if a blood sample is obtained too close to the time of last dose. This is a particularly frequent problem when digoxin is the drug being monitored and blood is drawn less than six hours after the last dose. Unfortunately, if the sample is collected in a physician's office or nursing home we may not be aware of the time since last dose.

A few simple measures should help avoid an unwelcome call.

- Instruct your patients to take the medication to be monitored in the evening and have blood drawn in the morning, so that they will never present too close to the time of the last dose.
- If this is not possible instruct your patients to take medication in the early morning and pres-



ent for monitoring in the early afternoon.

- If you or your staff draw blood, be sure to write the time since last dose on the requisition.
- Inform your patients they will be asked to return if they present too close to the time of last dose.
- Ensure MDS has the correct contact numbers on your MDS file so that we may contact you for the uncommon, genuine critical value. 

In This Issue

Human Papillomavirus Detection - Adjunctive Testing For Cervical Cancer	1
Therapeutic Drug Monitoring and Critical Values	1
Diagnosis of Thalassemia Trait ...	2
Lab Notes	
Hemoglobin A _{1c} Analysis: Method Update	3
House-Calls	3
Genital Specimens - Important Reminders	3
MDS Contacts	4

Diagnosis of Thalassemia Trait

Thalassemias and hemoglobinopathies are the most common monogenic disorders known to man and, fortunately, are usually easy to detect in the heterozygous state with a few simple tests. There are two main types of thalassemia, alpha and beta, in which there is a reduced production of the respective globin chains, which, along with heme, constitute the hemoglobin molecule.

Beta thalassemia was originally described in persons of Mediterranean origin (thalassa means 'sea' in Greek). It has since been identified in increased frequency in people of any geographic area affected by malaria. High gene frequencies are seen throughout the Middle East, Africa and South East Asia, as well as in Italy and Greece. As many as 25% of some populations are affected.

Alpha thalassemia is probably just as frequent but is primarily observed, in persons of South East Asian origin. In Hong Kong for instance, as many as 5% of the population are carriers (beta thalassemia is also very common in this population).


The importance of detection of the carrier state is two fold:

- Prenatal identification of carriers to identify the possibility of a homozygous fetus. Homozygous beta thalassemia is characterized by profound transfusion dependant anemia. The most serious form of alpha thalassemia is associated with a high-risk pregnancy and possible intrauterine death.
- Evaluation of microcytic hypochromic red cells, often identified on a complete blood count (CBC) as an incidental finding.

CBC abnormalities that may be observed in thalassemia

- MCV < 80 fL (or < reference range in children)
- MCH < 27 pg (or < reference range in children)
- Blood film: microcytes, hypochromia, basophilic stippling, target cells
- Hb normal or slightly decreased

Steps required in the evaluation:

1. Order a **complete blood count (CBC)**
2. Order **ferritin** to rule out iron deficiency.
3. Order **hemoglobinopathy investigations** by high-pressure liquid chromatography (HPLC) to identify abnormal hemoglobins, e.g. Hb S, Hb E and quantitate Hb A₂, which is elevated in beta thalassemia, and Hb F, which is elevated in delta beta thalassemia.
4. Order **hemoglobin H preparation** for alpha thalassemia diagnosis. This test identifies intracellular precipitations of Hb H, a tetramer of beta chains (β_4) that occur in the absence of alpha chains. Sensitivity of this test is only about 50%. A negative Hb H result with a normal Hb A₂, in a patient with suspicious red cell indices, supports a presumptive diagnosis of alpha thalassemia. For a definitive diagnosis, the sample should be submitted for genetic analysis. Please telephone Dr. Brian Sheridan for further information, at extension 4250. 

"Diagnosis of alpha and beta thalassemia trait require unique laboratory evaluation."



References

Weatherall DJ, Science, medicine, and the future: Single gene disorders or complex traits: lessons from the thalassaemias and other monogenic diseases *BMJ* 2000; 321: 1117-1120.

Weatherall DJ and Clegg JB Genetic Disorders of hemoglobin *Semin Hematol.* 1999; 36 (suppl 7): 24-37.



Hemoglobin A_{1c} Analysis: Method Update

MDS Hemoglobin A_{1c} method is a direct automated assay that does not require manual preparation of a hemolysate. The assay has been shown to perform comparable to the designated high performance liquid chromatography (HPLC) reference method and demonstrate minimal interference due to the presence of HbC, HbS, HbH or HbF in the patient specimen.

Unlike the HPLC method however, the automated method **does not** provide information on the presence of variant hemoglobins and is not recommended for hemoglobinopathy work-up.

For hemoglobin variant determination and/or hemoglobinopathy work-up, order **Hemoglobinopathy Investigation** (by HPLC).

For monitoring and/or assessment of glycemic control in diabetics or suspected diabetics, please order **Hemoglobin A_{1c}**.

House-Calls

In many parts of the province MDS is able to provide a limited house-call service for patients who are too sick to visit our Patient Service Centres.¹ House-calls are arranged through Community Care Access Centres (CCACs) or directly with MDS. Because the population of patients served includes those recently discharged from hospital and others with significant illness, our experience is that clinically significant results are more frequently observed as compared to patients who are able to visit our service centres.

House-call service is in increasing demand and we are unable to guarantee that laboratory results will be available by 5:00 p.m. on the day of service. It is, therefore, essential that we have clear identification of the physician responsible for receiving tests results and 24 hour contact numbers for the physician(s) ordering the tests. In cases where the physician responsible for receiving results is not the ordering physician, documentation is required that states that the receiving physician has been contacted and agrees to the associated responsibility.

MDS will require this information prior to initiating service when ordered through a CCAC or directly with MDS. In order to ensure patient safety, we will not provide service to your patients in their homes, until this information is available.

¹ **House calls are not covered by OHIP and are therefore subject to a fee payable by the patient or the CCAC on the patient's behalf.**


Genital Specimens - Important Reminders

Physicians and their staff should ensure that specimen sources are accurately labelled so that laboratories may process the specimens correctly:

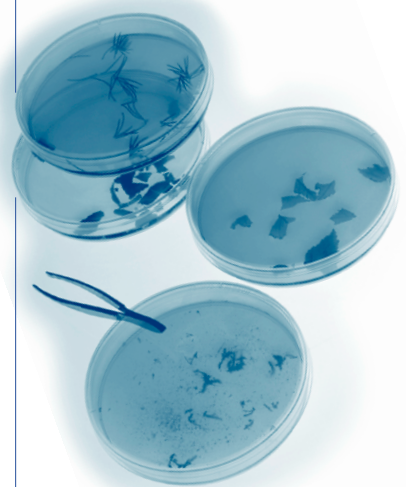
Source	Organisms/Syndrome Routinely Investigated
Cervical	Neisseria gonorrhoeae, Chlamydia trachomatis (dedicated specimen)
Vaginal	Yeast, Bacterial Vaginosis, Trichomonas
Rectovaginal	Group B Streptococcus
Male Urethral	Neisseria gonorrhoeae, Chlamydia trachomatis (dedicated specimen)
Urine	Chlamydia trachomatis

Note:

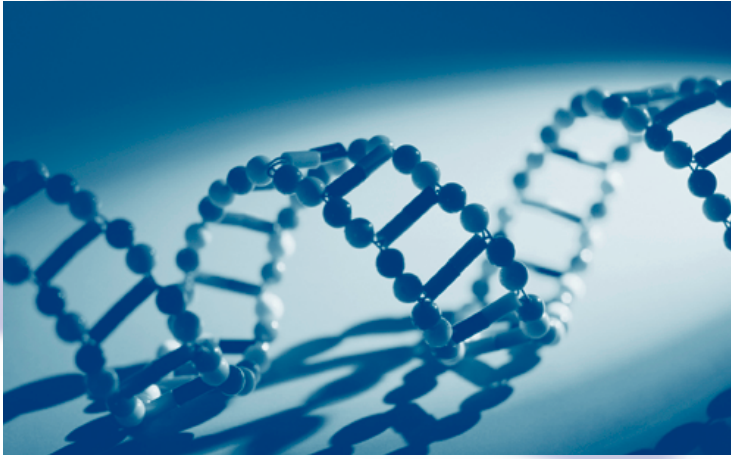
Delay in transport of vaginal swabs may yield a false negative result for Trichomonas vaginalis.

A validated method for the diagnosis of Bacterial Vaginosis in post menopausal women is currently not available. 

"Endocervical swabs are optimal specimens for recovery of *N. gonorrhoeae*."



(continued from page 1)



Testing Recommendations


The literature supports HPV testing when initial PAP test results are positive for ASCUS. "Follow-up" endocervical specimens may be submitted for HPV detection. ASCUS patients who are found to be HPV positive should be referred directly for colposcopy. Those who are HPV negative can be safely monitored as per routine recommendations.

The application of this test for primary cervical cancer screening in women over the age of 30 has also been considered in recent publications.⁵

Specimen Requirements

Using the HPV kit supplied to you by MDS, an endocervical specimen is collected and placed in transport medium. The specimen should be promptly delivered by the usual means to the laboratory for molecular analysis.

Test Availability

In the near future, MDS will be offering physicians nucleic acid-based HPV testing. For further information regarding specimen collection and remuneration for test, please call us at 1-877-849-3637. 

References

- ¹ National Cancer Institute of Canada: Canadian Cancer Statistics, 1999.
- ² Cox JT. Clinical role of HPV DNA testing. In: Lorincz AT, Reid R., editors. Human Papillomavirus 1. 2nd ed. 1996. *Obstet Gynecol Clin NA* 23(3): 811-851.
- ³ Manos MN *et al*. Identifying women with cervical neoplasia. Using Human Papillomavirus DNA testing for equivocal Papanicolaou results. *JAMA* 1999; 281(17): 1605-1610.
- ⁴ Cox JT *et al*. Human Papillomavirus testing by capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol*. 1995 Mar; 127(3): 946-954.
- ⁵ Meijer C *et al*. Screening for cervical cancer: Should we test for infection with high-risk HPV? *CMAJ* 2000; 163(5): 535-538.

4



Winter Issue: February 2001

Labnews

Published by
MDS Laboratory Services
a division of MDS Inc.

Your comments and
suggestions are welcomed.
Please contact us at:

Medical Support
Department
100 International Blvd.,
Toronto, Ontario
M9W 6J6

Toll-free: (877) 849-3637
Fax: (416) 213-4090
www.mdsdx.com

© copyright 2001