

Lab Updates

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January 2011

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UMassMemorial

Laboratories

Clostridium Difficile Detection

Effective February 9, 2011, the Molecular Diagnostics Laboratory will start testing for *C. difficile* by PCR using the BD-GeneOhm™ C diff real-time PCR assay. The order mnemonic of the test is TOXINB or CDIFFPCR.

This test is being implemented because of the American Society for Microbiology's September 2010 recommendations that PCR as a stand alone diagnostic test can be used to detect *C. difficile* toxin genes.

Inpatients at UMass Memorial Medical Center will only be tested by the PCR method. If the samples reach the laboratory by 1pm, results will be reported the same day.

C.diff PCR assay (TOXINB) will be offered to outpatients in addition to the rapid toxin A/B EIA assay.

Repeat of positive testing by PCR (test of cure) is not recommended since patients may carry toxigenic *C. difficile* for months after clinical cure. Repeat testing following a positive test is appropriate if the patient improves with therapy, but then relapses after the completion of a treatment regimen (clinical relapse).



Photo: Kevin Vance

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Test Description

BD GeneOhm™ Cdiff assay is a rapid in vitro diagnostic test for direct, qualitative detection of *C. difficile* toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having Clostridium difficile-associated disease (CDAD). The test, based on real-time PCR, is intended for use as an aid in diagnosis of CDAD. The test utilizes polymerase chain reaction (PCR) for amplification of specific targets and fluorogenic target-specific hybridization probes for detection of the amplified DNA.

Specimen Collection and Transport

Only patients with diarrhea should be tested for *C. difficile*. It is not necessary to test more than one sample per patient due to the high sensitivity and specificity of the PCR assay. Therefore, only one sample per patient should be tested by PCR method.

Inpatient Sample Collection

1. Stool specimens should be collected in a clean container with a secure lid, labeled, and sent to the laboratory as soon as possible after collection. Mixtures of urine and stool from a bedpan cannot be submitted for testing. Specimens that are liquid or soft, that is, take the shape of the container are acceptable. Specimens that are formed or hard (“moon rocks”) will be rejected. If other tests requiring a stool sample are ordered, the stool samples are first processed by the microbiology lab. For faster Turn-Around-Time, the swab with saline from the PCR kit should be used as described below.

2. The sterile dry swab included with the saline tube in the PCR kit should be dipped and rotated in the liquid or soft stool material, and immediately placed in the saline tube. After tightly closing and labeling the tube it should be sent to the laboratory as soon as possible. The saline sample is sent directly to the molecular diagnostics lab, and it is the preferred sample for faster Turn-Around-Time.

Outpatient Sample Collection

1. Stool specimens should be collected in a clean container with a secure lid, labeled, and sent to the laboratory as soon as possible after collection. Mixtures of urine and stool from a bedpan cannot be submitted for testing. Specimens that are liquid or soft, that is, take the shape of the container are acceptable. Specimens that are formed or hard (“moon rocks”) will be rejected. After tightly closing and labeling the container, it should be sent to the laboratory as soon as possible.

Specimens can be stored up to 5 days at 2-8 °C before testing. Specimens can be kept at room temperature (15-25 °C) up to 48 hours before testing.

Testing Schedule

Specimens will be tested daily, Monday–Sunday.

References:

“A Practical Guidance Document for the Laboratory Detection of Toxigenic Clostridium difficile”. American Society for Microbiology Sept 9, 2010.

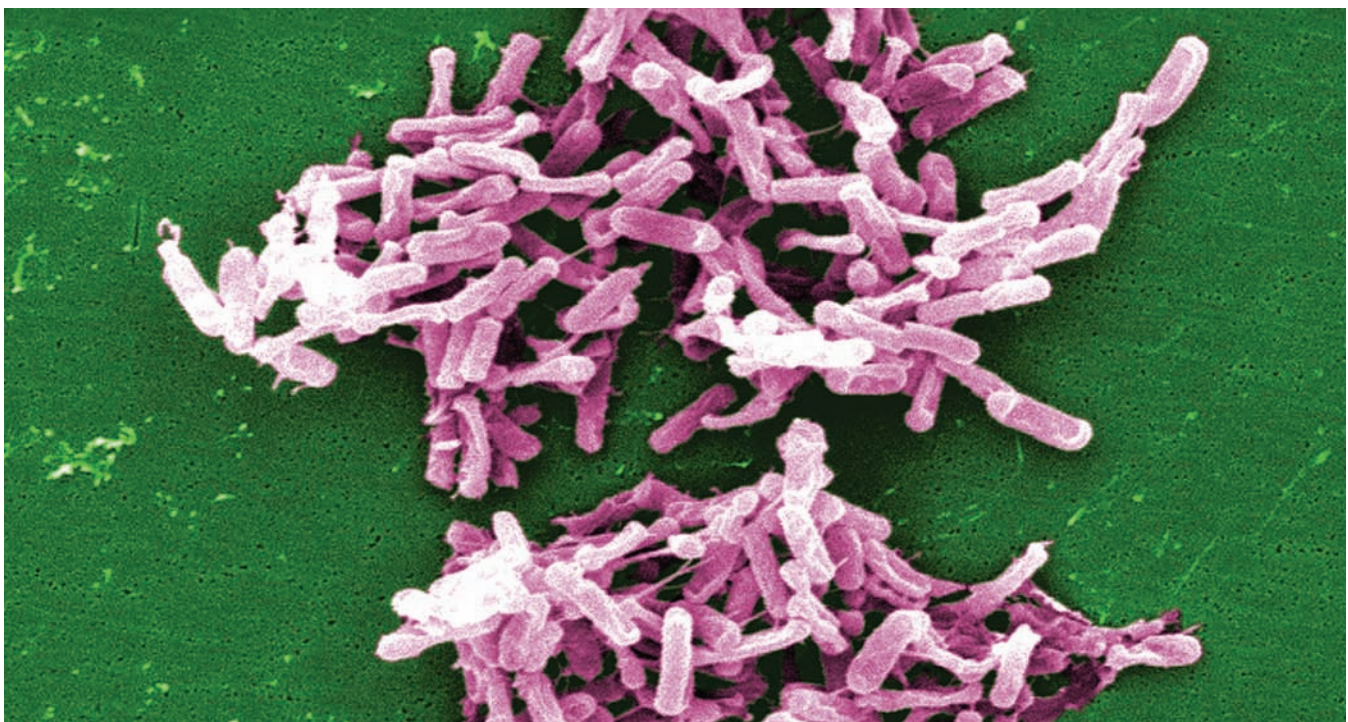


Image Courtesy of Department of Health and Human Services Public Health Image Library (PHIL)

HIV-1 Quantitation by PCR (COBAS®TaqMan®) Test Update

Beginning February 9, 2011, the UMass Memorial Molecular Diagnostics Laboratory will start using the second version for HIV-1 quantitative testing on the COBAS® AmpliPrep/COBAS® TaqMan® system (Roche).

The most important advantage of using version 2 for HIV-1 quantitative viral load assay is that the test now quantifies HIV RNA based on the co-amplification of two distinct regions of the HIV genome: LTR (Long Terminal Repeat) and gag. The dual-PCR target enhances the ability to quantify diverse HIV samples such as HIV-1 group M subtypes and HIV-1 group O. The features and benefits of the HIV-1 version 2 test are summarized in the table below:

For any questions regarding methodology and interpretations, please contact:

- Dr. Edward Ginns at 508-856-8134, or via email at Edward.Ginns@umassmed.edu
- Dr. Marzena Galdzicka at 508-856-4384 or via email at Marzena.Galdzicka@umassmed.edu

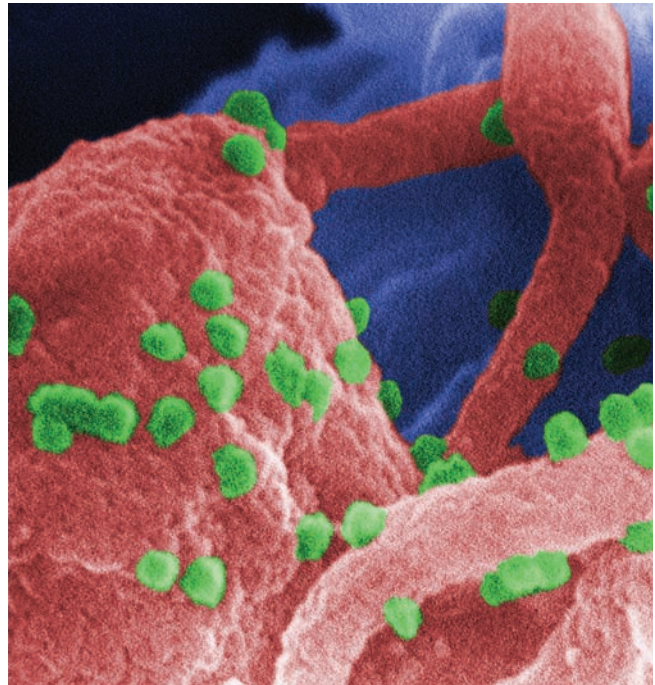


Image Courtesy of Department of Health and Human Services Public Health Image Library (PHIL)

Features	Benefits
Dual-PCR Target	<ul style="list-style-type: none"> • The COBAS® AmpliPrep / COBAS® TaqMan® HIV-1 Test, v2.0 includes a dual PCR target design that simultaneously amplifies and detects two separate regions of the HIV-1 genome, the LTR and gag.
Genetic Coverage	<ul style="list-style-type: none"> • Group M Subtypes A-H, including Circulating Recombinant Forms and Group O • Group O: While group O is still extremely rare and there is no indication of growing prevalence Group O detection is an outcome of our unique PCR dual target design.
Sensitivity	20 cp/mL across Group M subtypes
Linear Dynamic Range	20 – 100,000,000 cp/mL
Clinical Specificity	100%
Precision	< 0.2 log ₁₀ cp/mL S.D. across linear dynamic range
Standardization	Calibrated to the WHO International Standard
Correlation to Previous Methods	95% of samples +/- 0.3 log ₁₀ difference to version 1.0

There are no changes in the assay mnemonic or method of collection and transportation of the specimen.

Cystic Fibrosis CF100 Plus Panel

An extended CF100 plus panel for Cystic Fibrosis CFTR gene mutation screening will be available starting mid February 2011 in the UMass Memorial Molecular Diagnostics Laboratory. This test will be offered in addition to the Invader CFTR InPlex panel (Hologic) that screens for 41 CFTR mutations.

Methodology

This expanded CF100 plus mutation panel tests for 60 mutations using the xTAG[®] Cystic Fibrosis 60 kit v2 (Luminex) and 44 additional mutations developed on the Luminex[®] 200[™] System.

Changes in mutation detection rates depending on the number of tested mutations by ethnicity:

Mutation Detection Rate	Caucasians	Hispanic	African American	Asian	Ashkenazi
ACMG/ACOG 23 mutations (%)	88.3	71.7	64.5	48.9	94
Invader CF 41 mutations (%)	89	74	66	55	94
CF100 Plus test is more than (%)	90.6	80.9	72	55	94



Assay Limitations

1. The results obtained using the CF100 plus assay should be used and interpreted in the context of a full clinical evaluation.
2. This test probes for 104 Cystic Fibrosis transmembrane conductance regulator (CFTR) mutations/variants out of the more than 1300 which have been identified.
3. As with any hybridization-based assay, underlying polymorphisms or mutations in primer-binding regions can affect the alleles being probed and, consequently, the calls made.
4. All homozygous calls except for delta I507 and delta F508 will be confirmed by sequencing (with the exception of when delta I507 and delta F508 homozygous calls are accompanied by I506V, I507V or F508C variants).



5. Genotype-phenotype correlations for rare mutations are based on limited reported clinical cases and can be highly variable and inconsistent, ranging from benign to severe phenotypes; as a result, clinical conclusions should be made with caution.

Mnemonic, TAT and when the assay should be ordered

Mnemonic of the new assay is: CF100PLUS. The assay will be performed once a week with a TAT of 7-10 days. The CF100PLUS assay is designed for screening individuals at high risk:

- a) partners of women who are heterozygous for a CFTR mutation;
- b) individuals with a family history with known or unknown mutations.

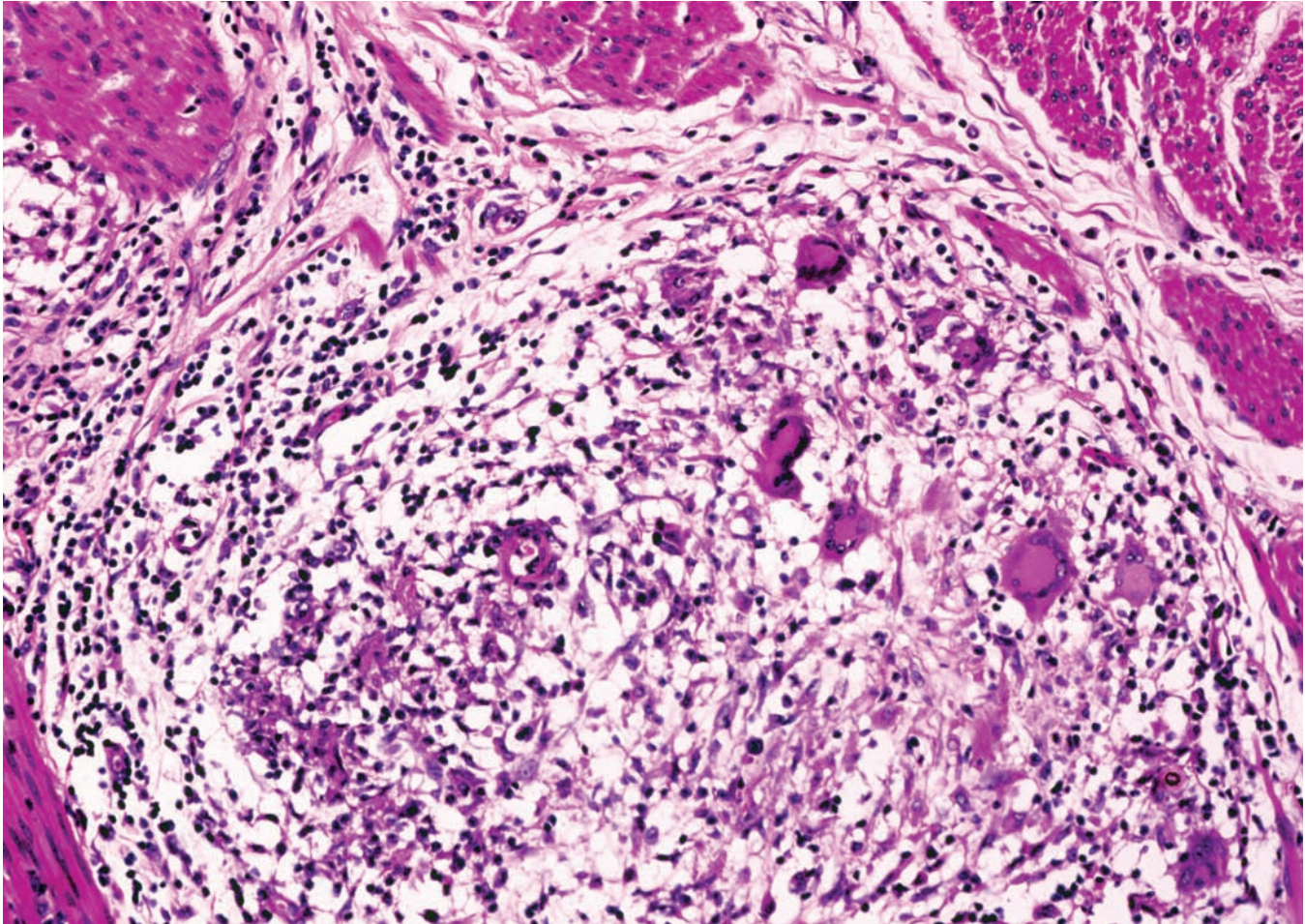
Reports

The report will list all mutations tested by the CF100PLUS test.

For any questions regarding methodology and interpretations, please contact:

- Dr. Edward Ginns at 508-856-8134, or via email at Edward.Ginns@umassmed.edu
- Dr. Marzena Galdzicka at 508-856-4384 or via email at Marzena.Galdzicka@umassmed.edu

Stool Occult Blood Test (FIT): Specimen Collection Requirements



Stool Blood tests are most commonly used to screen the colon for cancer. Stool blood tests are conventionally known as fecal occult blood tests (FOBT) because they are designed to detect the presence of occult blood in stool. FOBT fall into 2 primary categories based on the detected analyte: Guaiac based (gFOBT) and Immunoassay based (FIT).

Fecal Occult blood testing by Immunoassay (FIT) assay has several advantages over the Guaiac based method (gFOBT) including

- ease of collection
- reduction in required number of samples
- no dietary restrictions
- specificity of human hemoglobin

The FIT test detects the intact globin portion of the human hemoglobin and therefore specific for lower GI bleeding. **Blood hemoglobin is NOT stable in untreated stool specimen;** the FIT test sample vial preserves the specimen until it can be tested. The accuracy of this test for the detection of upper GI blood loss is not well established and hence is not recommended. Occult blood tests are not appropriate for the detection of blood in emesis or other body fluids.

- Samples may be obtained during routine physical exam or by use of the personal pack for home collection provided by the laboratory. Testing is performed routinely on a daily basis.
- Please remember to place a completed laboratory requisition in the specimen envelope.

- All specimens must be properly labeled with the patient's first and last name and date of birth or other unique identifier such as a medical record number.
- As soon as the stool sample is collected, a portion of the sample should be placed in the sample vial provided in the kit. FIT testing cannot be performed unless the sample has been preserved in the FIT test vial. This is applicable for those who prefer to use gFOBT testing.
- Effective February 1, 2011, the laboratory cannot accept stool samples for FIT or gFOBT testing that arrive in a plain collection cup. Stool samples submitted in this fashion will be rejected
- Once collected on the sample vial, the specimen is stable up to 14 days refrigerated or 7 days at room temperature.
- Samples may be sent to the laboratory by routine courier pick up for the physician office/site or via US mail in the provided mailer.
- FIT testing cannot be added to stool samples originally sent for any other testing, ie culture.

If you have questions, comments or suggestions, please contact:

- Dr. L.V. Rao, Senior Director of Clinical Lab Operations & Director of Core Laboratories at 774-442-9615 or via email at Lokinendi.Rao@umassmemorial.org
- Ms. Judy Barron, Manager of Automated Chemistry at 774-442-9616 or via email at Judy.Barron@umassmemorial.org



Changes in Prometheus IBS Diagnostic Test



UMass Memorial laboratories would like to notify you of an important change to the availability of Prometheus testing. **Effective January 1, 2011**, Prometheus will no longer be offering the Prometheus IBS Diagnostic test. As of that date, UMass Memorial Labs will no longer process such requests. No alternate tests are available.

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This publication is made possible by Kevin Vance, Senior Director, Business Development and Marketing

We are one of the largest laboratory providers in New England

UMass Memorial Laboratories has opened a Patient Service Center (phlebotomy draw station) at 851 Main Street, Warren, Rhode Island.

The vision of UMass Memorial Laboratories is:

- To be a leading provider of laboratory services throughout New England, meeting the needs of patients and providers in the region, and
- To be one of the top ten academic medical center-based laboratories in the United States



Photo: Kevin Vance

Warren PSC **851 Main Street** **Warren, Rhode Island**

Warren PSC is located at 851 Main Street, Warren, Rhode Island.
The hours are Monday through Friday 7:30am–5:00pm,
Saturday 7:00am–11:00am.
The phone number at Warren PSC is 401-245-0452.