



Harborview Medical Center, Seattle, Washington

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Two Premier Seattle Hospitals Opt for Rapid *Clostridium difficile* Results



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The sister Seattle hospitals Harborview Medical Center (HMC) and the University of Washington Medical Center (UWMC) have their similarities and differences, but they agree that implementation of the Xpert® *C. difficile* assay will improve care for their patients. Both facilities are among the nation's

leading academic medical centers, serving as training sites for the University of Washington Medical School and other allied health professional schools in the region.

HMC's specialties include neurosurgery, eye care, vascular medicine, rehabilitation medicine, sleep medicine, and spine care. The facility serves as the region's primary Level One adult and pediatric trauma and burn center, serving patients from Alaska, Montana, and Idaho as well as Washington State. HMC has a longstanding tradition of caring for the underserved and indigent in the

community, including an outstanding and broadly inclusive HIV/AIDS treatment program. UWMC provides highly specialized care in cardiology, high risk pregnancy and neonatal intensive care, oncology, orthopedics, and organ transplantation. Their patient referral area also extends beyond Washington to Wyoming, Alaska, Montana, and Idaho.

The clinical microbiology laboratories in these two hospitals, located 4.3 miles

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ON-DEMAND

REPORT

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Two Premier Seattle Hospitals

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apart, are both administered by the University of Washington Department of Laboratory Medicine. Both perform broad diagnostic testing for inpatients, outpatients, and reference laboratory business. To capitalize on their proximity, they have agreed that the HMC microbiology laboratory performs all of the nucleic acid amplification assays for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, as well as specific mycobacterial testing. Mycobacteria, *Streptococcus pneumoniae*, and *N. gonorrhoeae* isolates are identified using commercial molecular assays. The



Laboratory Director
Dr. Ferric Fang

UWMC laboratory performs basic serological assays, special antimicrobial susceptibility testing, and the bulk of the more esoteric molecular diagnostic assays for their shared patient population, including identification of pathogens and resistance factors using nucleic acid sequencing developed in-house.

The world-famous Fred Hutchinson Cancer Research Center, staffed by University of Washington professionals, brings additional patients from across the globe and locally. The UWMC laboratory supports those patients via the Seattle Cancer Care Alliance. The UWMC laboratory, in fact, has a nationwide re-

From the Editor



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In this issue we highlight two laboratories from opposite sides of the world and performing in radically different settings, both of which have recently enhanced their capabilities by adding a GeneXpert® assay. The unique needs of their patient population informed the type of test that each laboratory chose. In South Africa, with an extremely high incidence of multi-drug resistant tuberculosis, often in the context of co-infection with HIV

(see new World Health Organization Report at http://whqlibdoc.who.int/publications/2010/9789241599191_eng.pdf), the Lancet Laboratory chose the Xpert® MTB/RIF assay. In Seattle, where concern about *Clostridium difficile* infection in both inpatients and in the community is increasing, Harborview Medical Center moved from a two- and three-part testing algorithm to the "one and done" philosophy of the Xpert® *C. difficile*. We focus on some of the challenges and surprises experienced by these two different facilities as they implemented their new tests.

Opt for Rapid *Clostridium difficile* Results

ferral business using uniquely developed molecular assays for detecting and identifying bacteria, fungi, and mycobacteria directly from tissue samples and even from specimens in paraffin blocks in some instances (see the website <http://depts.washington.edu/mol-micdx/>). Most specimens received for virology testing are sent to the separate laboratories of the Virology Division.

The Harborview Clinical Laboratory is fortunate to have approximately 17 clinical laboratory scientists, 8 technicians, and a half-time laboratory assistant working exclusively in Microbiology under the leadership of Manager Jean Houk and Director Dr. Ferric Fang, Professor of Laboratory Medicine and Microbiology at the University of Washington. They receive around 350 total specimens daily. Dr. Fang, in addition to a distinguished record of publications on pathogenesis and the host immune response, has directed an academic clinical microbiology laboratory since 1992, and has had a long-standing interest in *C. difficile* infection (CDI) for almost that long. As far back as 1994, Dr. Fang and colleagues published a rebuttal to an article in the *New England Journal of Medicine*, disputing the article's contention that the cytotoxin assay alone was the most sensitive method for diagnosis of CDI⁴. Fang and Madinger pointed out that the case definition itself included cytotoxin positivity. Two years later, with Dale Gerding and Lance Peterson, two of the nation's most influential clinicians regarding *C. difficile* diagnosis, he co-authored another response to a publication in the *Annals of Internal Medicine*; Fang and his colleagues emphasized that toxigenic culture is the most sensitive method for diagnosis of CDI and additionally that such cultures



Xpert *C. difficile* assay implementation team (left to right: Veronica Crisologo, Tom Smith, Dave Nowowiejski, Ann Larson, Cheryl McMillan, Jean Houk and Carolyn Wallis).

yield isolates that are useful in epidemiological studies³.

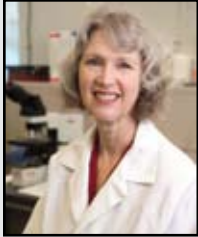
Prior to March, HMC laboratory had been testing stool samples submitted for *Clostridium difficile* testing using a 3-step algorithm, the benefits and cost estimates of which were summarized in a paper just published this year⁵. Briefly, the samples were first tested for the presence of glutamate dehydrogenase antigen using a lateral flow enzyme immunoassay (Quik Chek C Diff EIA; GDH-Q, Wampole/TechLab, Blackburg, VA). This antigen is found on the surface of both toxigenic and non-toxigenic *C. difficile*, and had been thought to be detectable in all stools containing *C. difficile*. Because presence of GDH is not specific, however, positive samples needed another test. At HMC, samples positive by GDH-Q were tested for toxins A and B using the lateral flow device Tox A/B Quik Chek (AB-Q; Wampole/TechLab). No toxin assay, however, is sensitive enough to be the final confirmatory test. Therefore, GDH-positive, toxin-negative stool samples were reflexed for an additional assay using an in-house developed direct PCR reaction to detect a sequence unique to the toxin B gene (tcdB) of *C. difficile*. Spe-

cial amplification controls were built into the PCR assay to detect potential inhibitors. As a final confirmatory assay as part of a research study, a subset of GDH-negative samples were tested by the in-house PCR method, and an additional 1.9% were considered to be true positives, as confirmed by toxigenic culture. Their overall rate of positivity was around 10%.

The EIA had been performed as a batch, twice daily, resulting in a turnaround time (TAT) of up to 18 hours for specimens that did not require PCR testing. When PCR was needed, results were available within approximately 25 hours, except on Sundays when PCR was not done. This could extend their TAT to 49 hours for some samples received on weekends. Technologists were spending 4-5 hours a day doing testing for *C. difficile* alone. Although only one sample per patient per day was tested and samples from patients positive for *C. difficile* were not retested for 10 days, there was no policy on retesting from patients yielding negative results the first time. Over a 7 month period they received 194 repeat specimens, only 6

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Lancet Laboratories, South Africa, renovates its TB *CE IVD Marked Product* (not available in the United States)



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Lancet Laboratories is one of the largest private laboratory groups in South Africa. With facilities in four countries (South Africa, Botswana, Swaziland, and Zimbabwe), two major reference laboratories, several additional larger facilities, and >100 smaller laboratories and depots (specimen collection stations) in different locations, they process specimens from >15,000 patients daily. Lancet Laboratories provides full-service, performing chemistry, toxicology, hematology, cytogenetic, histopathology, and other diagnostic and occupational health tests in addition to microbiology, virology, parasitology, and mycobacteriology. The main laboratory in Richmond, located on a major highway near Johannesburg on the way to Durban (Figure 1), serves as the mycobacterial reference laboratory for many of the smaller laboratories and depots. They employ 3 medical technologists, 6 medical technicians, 3 laboratory assistants, 7 student technicians, and 5 administrative staff who work exclusively in the TB laboratory. Numerous additional employees work in the reference microbiology laboratory. Medical Technology training in South Africa appears to be similar to that in the U.S., with university coursework culminating in a National Diploma in Biomedical Technology, followed by on-site internship training, and then passing a certifying board examination as prerequisites to obtaining a license from the Health Professions Council of South Africa.

Sharmila Naidoo is the laboratory manager who was the guiding force in implementation of the new assay and the recent successful renovation of their laboratory (Figure 2). Dr. Henry Booker, Lancet Head of Microbiology, is the pathologist in charge of the TB Laboratory. The TB Laboratory receives between 600–1200 samples for TB testing every day. Lancet takes the safety of their workers seri-



Figure 1. Lancet Laboratory reference laboratory facility in Richmond, South Africa

ously. TB Laboratory staff work in biological safety cabinets located within a state-of-the-art P3 facility, one with limited access, anterooms, a closed interior surface that can be completely disinfected, and negative air pressure to the rest of the building (Figure 3). Workers wear gloves, gowns, and protective foot covers in the P3 area (Figure 4). The samples come from patients seen in doctors' offices and those collected in private hospitals and delivered to Lancet depots for transport to the main facility. Most of the time, patients submitted two or three samples, just as they do in the U.S.

Prior to Xpert implementation, samples were treated in a conventional manner. They were concentrated and smears were made, first examined with auramine fluorescent stain, and con-



Figure 2: Sharmila Naidoo, TB Laboratory Manager

processing with implementation of rapid PCR Assay



Figure 3. Access to the TB Laboratory is limited



Figure 4. Processing samples in specimen handling area

firmed after restaining with Ziehl-Neelsen acid fast stain. At this facility, smear results were usually available within 24–48 hours. Cultures were incubated in liquid media on the BD BACTEC MGIT 960 instrument (BD Diagnostic Systems, Franklin Lakes, NJ). Positive cultures were identified either by the GenProbe AccuProbe system (GenProbe, San Diego, CA) with a 24 hour TAT, or the Hain GenoType MTB test (Hain Lifescience, Nehren, Germany), for which samples were sent out to the Lancet Molecular Biology Laboratory for testing. Negative results were reported at 6 weeks, but whenever acid fast organisms were detected, the secondary tests were initiated. Susceptibility testing was performed in the MGIT, with a 14–21 day turnaround time (TAT).

Initial comparison and validation of the Xpert® MTB/RIF test involved 1140 samples processed by the laboratory using MGIT and AccuProbe compared with Xpert MTB/RIF. In less than 21 days they found 686 Xpert positive results, of which 9% were rifampin resistant. To workers in U.S. laboratories, those numbers are astounding. For both smear-positive and smear-negative samples, there was 99% correlation between culture and Xpert results. Ms. Naidoo and Dr. Booker were pleasantly surprised at the good correlation between Xpert and their conventional

results. In some cases, samples were positive by the Xpert MTB/RIF test but negative in culture. On further exploration, these samples often came from patients with previous or subsequently positive cultures. They also tried some non-respiratory secretion samples including cerebrospinal fluids (CSFs) and pleural fluids but the Xpert results were not as reliable as conventional cultures. Right now 6 people perform the Xpert assay, including medical technologists and technicians. The laboratory setup is ideal, with the GeneXpert® right next to the processing cabinet



Figure 5. Technologist Donovan Miller working in the GeneXpert® dedicated BSC with the GeneXpert on the workbench immediately to the right side.

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Lancet Laboratories, South Africa, renovates its TB processing with implementation of rapid PCR Assay

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(Figure 5). Batches are run anywhere from 2 to 8 times a day, depending on the day and the number of samples received (Figure 6). Spent cartridges are currently autoclaved and then sent to biohazardous waste for incineration. *Editor's note: the organisms in the cartridge are likely all non-viable. Studies of this issue are in process.*




Figure 6. Sharmila Naidoo showing a typical batch being run on the Xpert® MTB/RIF.



Figure 7. TB Laboratory staff (left to right: Akira Maharaj, Leonie Jacobs, Alisha Siripal, Juanita Brown, Avril van der Berg, Natasha Dyzell, Ester Havenga, Collen Mohamme, Howard Hashe, Donovan Miller, Kashmeel Maharaj, Maureen Mkhwanazi, Melissa Dawson, Chantal Deonarain, and Manager Sharmila Naidoo).

The TAT for positives has been reduced drastically from an average of weeks to an average of 48 hours. Although multiple samples from each patient are still submitted, only the first one is tested by the Xpert MTB/RIF. The time freed up with the use of the Xpert moderate complexity-type assay allows staff more time for conventional testing, culture processing (which still must be performed, of course), and other activities. The entire staff was surprised and positively impressed by the ease of use and rapid TAT that the Xpert provided (Figure 7). Their biggest challenge was the high cost of the assay and the fact that cultures continue to be necessary, particularly because isoniazid susceptibility testing is still required by the South African government *M. tuberculosis* testing protocol. However, physicians have expressed happy disbelief that their results are returning so quickly, and the volume of requests is growing every month to approximately 1000/month currently. The laboratory has also modified its standard protocol to include more samples for GeneXpert testing.

The entire staff at Lancet are delighted with their new test, and other areas of the Lancet organization are already using Xpert® *C. difficile*, Xpert® EV (enterovirus for CSF), and the BCR-ABL/ABL (CE IVD marked product, not available in the US) assays. As additional applications become available, particularly expanded *M. tuberculosis* resistance tests, especially isoniazid and second-line drugs, and perhaps identification of mycobacteria other than tuberculosis, the Lancet lab staff will be eager to try them. 

Rapid *Clostridium difficile* Results

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(3%) of which were positive, and all of those were received ≥ 4 days after the first sample. This complex time-consuming algorithm, unfortunately, was only 83.8% sensitive compared to performing their own PCR directly on all specimens (sensitivity 97.1%), as detailed in their publication⁵.

The results of the HMC algorithm were remarkably similar to those published this year by Novak-Weekley and colleagues, who performed their own algorithm study at Kaiser-Permanente Laboratories in Southern California⁶. Novak-Weekley's studies yielded a sensitivity of 83.1% for an algorithm that started with the TechLab GDH-60 microwell EIA followed by the Premier Toxin A&B microwell EIA (Meridian Bioscience, Inc., Cincinnati, OH) and finally testing the toxin-negative stools by a tissue culture cytotoxicity neutralization assay using Vero cells (Diagnostic Hybrids, Athens, OH) as the indicator cell line and antitoxin from TechLab (Blacksburg, VA) for neutralization of cytopathic effect. Performing the Xpert *C. difficile* (Cepheid, Sunnyvale, CA) alone showed a sensitivity of 94.4% compared with the gold standard toxigenic culture including broth-enriched culture results, the most stringent comparator assay possible¹.

Dr. Fang and his laboratory scientists, using their in-house PCR, were able to document faster and more reliable results (missing only 2.5% of true positives) with PCR testing up front. They also showed that costs (labor, supplies, and overhead) and TAT would be minimized with that strategy, with an estimated cost per sample of \$40.57 but cost per positive sample of \$363. In contrast, their 3-step algorithm cost a virtually identical \$40.68 per speci-

men, but \$425 per positive sample and still missed approximately 14% of true positive patients with CDI. However, the resources to perform their PCR often enough to improve clinical care were not available. Therefore, the Xpert *C. difficile* assay, with its 45 min TAT and minimal hands-on time, was evaluated to see if its performance and ease of use matched their needs. After a thorough appraisal of workflow, performance, and cost, the decision to convert all *C. difficile* testing to Xpert was made. The Xpert *C. difficile* assay is the only *in vitro* diagnostic assay that was compared to the gold standard toxigenic culture to accumulate data for the FDA clearance, demonstrating excellent sensitivity and specificity (see the product insert) versus toxigenic culture, acknowledged by experts to be the most sensitive and reliable test available¹.

To convince administrators and departmental leaders, the laboratory first showed that testing with the Xpert *C. difficile* assay would be cost-neutral with regard to labor. Offering the home-brew PCR assay six days a week required the dedicated staffing of a half-time Research Scientist and three technologists, as well as the addition of a technologist to each weekend shift. With the Xpert assay, the daily 4 hours of technologist time could be cut dramatically and the half-time Research Scientist could be assigned to other, more pressing developmental activities. Savings occurred beyond the laboratory as well. Some publications estimated the daily hospital costs associated with CDI to be between \$3791 and \$6959 per episode, so that earlier treatment and thus shortening length of stay for a patient or, even better, prevention of CDI altogether, is cost-effective^{2,7}. Dr. Fang's group noted that even if dai-

ly costs attributable to CDI were only \$1300-\$2000, the one-day sooner diagnosis based on PCR results for cases called negative by EIA would save their hospital >\$200,000 per year. For physicians and infection control personnel, other arguments were also necessary. A key point was that faster and more accurate reporting of positive patients would allow interruption of nosocomial *C. difficile* transmission. Improved outcomes for individual patients is another factor, less quantifiable but critical to the healthcare mission. For example, Larson and co-authors described a patient with serious disease and repeated negative results from GDH and toxin immunoassays. Positive PCR and toxigenic culture results (days later) were required to convince the physicians of the true etiology of the patient's symptoms and allow the initiation of appropriate therapy.

An HMC laboratory team (some members shown on Page 3) is in the process of performing their internal validation of Xpert *C. difficile* for routine testing using a GX 16 module, 4-color instrument. They plan to test at least 50 specimens, some frozen (-75°C) and some fresh, including at least 16 positives. Discrepancies between Xpert results and previous results will be resolved using their in-house PCR and/or toxigenic culture. Their toxigenic culture protocol involves inoculating stools onto pre-reduced agar with and without alcohol-shock (to select for spores) and testing for the toxin B gene from phenotypically appropriate isolates using their PCR assay. Their sample handling requires use of a biological safety cabinet for molecular test preparation of samples subjected to the home-brew PCR, and use

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Highlights from Previous Issues:


Good medicine is good economically, too
Volume 3, Issue 1, Winter 2010

Outbreak! The New *Clostridium difficile*
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of a fume hood for performing the enzyme immunoassays. Workers wear a laboratory coat, gloves, and eye protection. All technologists and technicians will be trained to set up the Xpert assay. A benefit of this moderate complexity assay is the ease with which anyone can be trained to do it, saving more hands-on time for actual patient testing. In fact, the staff have not noticed any technical challenges in adopting the assay and were actually surprised at how easy it is. Once the assay is on-board, HMC plans to batch samples four times daily. Cartridges are placed into biohazard bins for disposal. *Editor's note: Some states require that the cartridges be disposed of as chemical waste due to some components of the assay. Please observe universal precautions for all biological specimens and, when in doubt, please check with your local authorities on proper disposal requirements.*

Clostridium difficile has been called the "new Superbug," having surpassed methicillin-resistant *Staphylococcus aureus* (MRSA) as the most prevalent healthcare-associated infection over the past two years in some institutions, as stated in a talk given by Dr. Becky Miller (Duke University, Durham, NC) at the Fifth Decennial International Conference on Healthcare-Associated Infections on March 20 in Atlanta, Georgia. The two Seattle hospitals discussed here have taken a step closer to better control of this scourge by implementing routine use of the fastest, most accurate technology available for *C. difficile* detection in stool. Their clinical colleagues had already voiced their appreciation when the laboratory revised their *C. difficile* testing algorithm and developed their home-brew PCR several years earlier, which improved some aspects of TAT and certainly improved reliability of results. The laboratory leadership at Harborview Medical Center anticipates that there will be even more enthusiastic support from their infectious diseases clinicians and infection control staff once the improved overall TAT is realized. 

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