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National Laboratory Training Network Public Health Series Course: Molecular Diagnostic Techniques for the Public Health Laboratory

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- ▶ The National Laboratory Training Network (NLTN), in cooperation with faculty from academia, federal and state public health laboratories, and industry, developed and presented a Public Health Series Course on molecular diagnostics in the clinical laboratory for scientists working in state health laboratories.
- ▶ The course content included theory and application of selected nucleic acid-based procedures.
- ▶ Participants reported that their understanding of and ability to perform molecular diagnostic assays improved as a result of attending the course.
- ▶ Information from the course was used to make changes in the operations of the participants' home laboratories.

Molecular diagnostic testing has shifted dramatically in the past decade from the research arena to the clinical arena. The success of the Human Genome Project, forensic applications, genetic identification of various disease-causing microbes, establishment of the Laboratory Response Network (LRN) for detection of bioterrorism agents,¹ and expanded public health epidemiology and surveillance activities have all contributed to the incorporation of molecular diagnostics into the routine practices of medical and public health laboratories at a rapid speed. Personnel in clinical laboratories around the world are being asked to provide rapid identification of emerging and reemerging disease-causing agents associated with "common" disorders

and bioterrorism preparedness activities. The clinical laboratory has always been an evolving environment in which personnel are constantly challenged to implement new diagnostic tests designed to provide more sensitive and specific tests for detecting and monitoring disease.² These laboratory scientists are being challenged yet again by the introduction of complex molecular diagnostic techniques that were formerly performed only in research settings.

Historically, the prevention, control, and treatment of infectious disease are improved by early and accurate identification of the causative pathogenic organism. Many detection procedures require the pathogen to be grown in culture, followed by analytical testing in differential media for proper identification. These tests, although usually effective, can be slow and costly. Furthermore, the organisms (especially bacteria and parasites) can be fastidious or cannot be cultivated at all, leading to severe limitations in pathogen detection, and ultimately, delayed patient treatment. To overcome these major constraints, molecular diagnostic techniques are being developed and introduced into routine laboratory practice.³ For a molecular diagnostics approach to succeed in a clinical setting, it is critical that technologists, residents, and clinicians are well trained in performing, troubleshooting, and interpreting the assays. They must understand the limitations (eg, false positives, false negatives, cross-reactivity, contamination issues, inhibition) of both the technology and the results produced from molecular diagnostic tests.²

To aid in this undertaking, the National Laboratory Training Network (NLTN), a training system sponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL), and faculty from academia, federal and state health laboratories, and industry conducted training for state public health laboratory personnel during the fall of 2003. The more than 60 applications from state laboratories nationwide indicated a compelling interest in a comprehensive, wet-laboratory course in molecular diagnostics. Due to space restrictions however, the course was limited to 30 students. A pre-course survey, sent to these 30 applicants, asked about their current practices, confidence levels, and experience in molecular diagnostics. In addition, course participants were asked to complete a course feedback evaluation immediately upon completion of the workshop and again 3 months post-workshop to evaluate the content, format, and effectiveness of the training. At the 3-month follow-up, participants reported higher levels of ability when performing selected molecular diagnostic laboratory processes and procedures. All respondents reported multiple changes to their home laboratory operations and increased networking with colleagues in similar positions across the United States. These reports indicate that activities such as this workshop and other ongoing advanced training courses can contribute to strengthening the capability of the nation's laboratories to perform routine molecular diagnostic testing.

Background

A training-needs assessment, conducted by APHL with the nation's state public health laboratory directors in 2001, indicated the need for a comprehensive course in molecular methods for public health laboratory staff. In response, the NLTN formed a small workgroup tasked to further investigate and report back to APHL with a proposed action plan. In January 2002, a 1-page survey was sent to 50 state public health laboratories via the State Training Coordinator (STC) list serve with a request that the STCs route the survey for completion by the appropriate molecular laboratory staff person. Of the 26 states that responded to the survey, 92% indicated that polymerase chain reaction (PCR) was performed in their laboratory, and 100% indicated that their staff needed more training or information on specific molecular methods. The survey summary results were forwarded to the APHL Training Committee and NLTN management for follow-up. In the summer of 2002, the APHL Training Committee and NLTN management recommended that a Public Health Series course on molecular diagnostic techniques be developed and conducted in 2003. The CDC's Public Health Practice Program Office agreed to provide funding for the course and the California Department of Health Services (CDHS) agreed to provide the facility in which to conduct the course.

The central goals of the course were to: 1) provide participants with background theory and information on a variety of nucleic acid-based procedures; 2) provide hands-on training on selected nucleic acid-based procedures so as to increase the participants' skills and confidence in performing the procedures; 3) teach proper laboratory quality control and containment procedures for PCR; 4) provide guidance in compliance with applicable regulations; 5) enhance participants' PCR troubleshooting skills; and 6) demonstrate a general "how to" approach for developing a new PCR assay and bringing it online. In addition, all participants were expected to convey the information provided in the course to other

technical staff in their laboratories and to use the course information to improve molecular diagnostic testing in their home laboratories as needed. The course was also to provide participants an opportunity to begin networking with and learning from their peers in other states.

Materials and Methods

Early in 2003, the NLTN assembled a team of instructional designers, content experts, and educators to begin development of a molecular diagnostic course for public health laboratory personnel. To provide participants with a comprehensive treatment of molecular diagnostic techniques, the team recruited faculty and staff from the public health, clinical, academic, and commercial arenas (see acknowledgements). Each of the selected faculty members has extensive experience in molecular biology applications and is recognized as an expert in molecular diagnostics.

One of the most difficult aspects of conducting this course was the assembly of sufficient, often expensive, molecular diagnostics equipment and supplies for a class of 30 students. Fortunately, the team was able to call upon the CDHS for loan of their spacious, new training facilities. The state public health community, CDC, and APHL provided consultation, protocols, and unique reagents. Molecular diagnostic vendors provided various types and models of equipment as well as many of the required reagents. The NLTN provided logistical support and overall coordination. Without this team approach, the molecular diagnostic workshop could not have been conducted.

The training was divided into 2 parts: a Web-based pre-study and a 1-week hands-on wet workshop conducted at the CDHS training facility in Richmond, Calif. The first phase of training involved participants reviewing various Web sites that were chosen based on responses to the training needs assessment profiles [T1]. The participants' pre-study objectives were to 1) recognize structure and biochemistry of nucleic acids, 2) discuss organization and regulation of gene expression, 3) describe DNA and RNA structure and replication, and 4) explain transcription,

Course Participant Profile (n=30)

T1

	%
Current Function	
Supervisor	30%
Senior bench technologist	37%
Bench technologist	30%
Other (post-doc)	3%
Experience in Molecular Diagnostics	
0-1 year	62%
2-5 years	28%
>5 years	10%
Education Level	
Bachelor's degree or less	67%
Master's degree	20%
Doctorate degree	13%

translation, and post-translational modification. We asked the participants to quiz themselves on their mastery of the materials from the Web by completing a 24-item self-assessment. We planned that the students would review basic molecular biology concepts in a self-study format so that we could proceed directly to more complex material in the live class.

The 1-week wet workshop was conducted during the fall of 2003. It consisted of approximately 15 hours of lecture and 20 hours of laboratory activities, including both demonstration and hands-on exercises. In addition, we incorporated approximately 4 hours of informal discussions between faculty and participants.

Course faculty members were from state public health laboratories, the CDC, universities, and private laboratories. Lectures included the following topics: 1) molecular methods overview; 2) prevention of contamination and equipment maintenance; 3) developing a PCR assay; 4) real-time PCR; 5) sequencing theory and applications; 6) molecular subtyping techniques; 7) supervisory and management issues; 8) verification of a new procedure; 9) basics of microarrays; and 10) bioterrorism agents by microarrays. Laboratory exercises included: 1) detection of *Bordetella pertussis* by conventional PCR with both agarose gel and probe capture visualization; 2) detection of *Varicella Zoster Virus* (VZV1) by real-time PCR; and 3) DNA sequencing demonstrations and practice.

Laboratory professionals from 30 state public health laboratories, 1 clinical laboratory, and an APHL fellow participated in the course. The typical course participant was a traditionally trained microbiologist or chemist with interest or training in molecular methods but generally without education at the doctoral level. The public health laboratory participant profile is summarized in **T1**, and participants' pre-course experience in specific applications is summarized in **T2**. The students selected for the course had a range of experience in molecular diagnostic testing. Sixty-two percent had less than 2 years of experience in a molecular diagnostics laboratory at the time of the course. Sixty-seven percent did not have a graduate degree and characterized themselves as bench technologists [**T1**]. Many participants indicated that prior to the course they had little or no experience in developing and troubleshooting molecular diagnostic assays. Specifically, the areas of primer selection for PCR, PCR protocol optimization, and PCR protocol validation approached the 50% level of no experience. In fact, a student noted that they had been "put in charge" of the molecular biology section of the public health laboratory because of personnel shortages and other human resource concerns. Conversely, 55% of participants reported that they had sufficient experience with DNA amplification to train others [**T2**].

Two evaluation instruments were used to gather feedback and data about the success of the training. The first was an overall course feedback form completed by the participants on the last day of the course. Measurements were made by using a series of question sets including 1) post-course ability to perform specific molecular diagnostic applications, and 2) opinions of lecture and laboratory formats and content. We also provided space for individual comments. The participants' responses are summarized in **T3**.

Three months post-training, we asked the 30 course participants from public health laboratories to complete a short survey that was designed to evaluate the effectiveness of the course in reaching the goals stated above.

Twenty-three of 30 participants responded to the mailed survey for a response rate of 80%. In the survey, we presented participants with a list of 16 possible changes they might have implemented as a result of attending the course and asked them to check which of these had been made in their home facility. We also asked them to indicate any barriers to change they might have encountered by choosing from a list of potential barriers. In addition we asked about transfer of information from the course to others at their facility and about their networking activities with other students, faculty, and related groups. We asked them to assess any change in their confidence in performing and troubleshooting molecular assays and in bringing a new assay on line that had occurred as a result of

attending the course. The responses are summarized in **T4**, **T5**, **T6**, **T7**, **T8**.

Results

Immediately upon conclusion of the 5-day course, participants' reported ability to perform specific molecular diagnostic applications reached 100% for 1) conventional and real-time PCR, 2) troubleshooting of problems with PCR procedures, and 3) appropriate quality control (QC) and containment procedures related to PCR [**T3**]. Additionally, the categories of bringing a new PCR assay online and applying the appropriate regulatory guidelines for molecular diagnostic applications resulted in reported ability levels of 97% and 88%, respectively. Ninety-four percent of the students indicated that they would recommend changes in

Participants Self-reported, Pre-course Experience in Specific Molecular Diagnostic Applications (n=30)

T2

Applications	None	Some	Experienced/Able to Train Others
Protein extraction and purification	40%	43%	17%
DNA amplification by PCR	7%	38%	55%
Reverse transcription	34%	38%	28%
Real time PCR	24%	45%	31%
Primer selection	47%	43%	10%
PCR protocol optimization	45%	41%	14%
PCR protocol validation	47%	47%	6%
Molecular testing QA/QC	39%	54%	7%
Gel electrophoresis	31%	31%	38%
Sequencing	70%	20%	10%

Participants Immediate Overall Course Feedback (n=32)

T3

	% who agreed or strongly agreed
Respondents' self-reported post-course ability to:	
Successfully perform conventional and real-time PCR.	100
Troubleshoot problems with PCR procedures.	100
Perform activities necessary to bring a new PCR assay online.	97
Perform appropriate quality control and containment procedures for PCR.	100
Apply appropriate regulatory guidelines for application of MD in a clinical setting.	88
Lecture and laboratory presentation format:	
The teaching methods were appropriate to learning.	97
The level of material was appropriate for my background.	91
The content covered was new or updated information.	81
The material presented will help me perform my job better.	100
I will recommend changes at work based on this workshop.	94
The pre-study material adequately prepared me for the course.	91
The amount of lecture time was sufficient for learning.	100
The amount of laboratory time was sufficient for learning.	69
The training facility was appropriate for learning.	94
I was able to interact with faculty and other participants.	97
Overall, the course was worth the time and money invested.	100
The pace of the course was about right to hold my interest.	94

Responses to the Request to Mark Any Changes That Had Been Made in the Participant's Laboratory as a Result of Attending the Course (n=23)

T4

Potential Change	% Who Selected Change
Move PCR assays toward real-time and away from gel-based detection	35
Be more likely to exchange protocols with other laboratories	78
Offer cross-training in molecular diagnostics for other personnel in my lab	65
Improve hiring and purchasing decisions	26
Use freeware to design primers and probes	30
Implement or expand sequencing	35
Implement enhanced QA/QC methods	61
Take advantage of opportunities to perform research	22
Strengthen compliance with new and existing CLIA regulations	52
Begin or improve validation and verification processes	74
Improve overall documentation	35
Streamline work flow	43
Run additional or other controls as needed	43
Network more with other laboratorians who perform molecular assays	78
Develop more of a team approach with persons who perform molecular assays in my laboratory	26
Add additional assays	17

workflow and procedures, and use appropriate molecular diagnostic controls as needed. Many participants also indicated that they would begin developing various molecular diagnostic applications in their laboratory and planned to offer cross-training opportunities for other laboratory personnel.

Participants indicated that the well-organized take-home materials would be extremely helpful in their laboratory. Seventeen (53%) participants also noted that they appreciated the opportunity provided by the open format to network with colleagues, allowing them to share ideas, experience, and protocols. Overall, the participants agreed that the course was excellent, particularly in that the faculty was comprised of well-informed and interesting speakers, active in their field, who presented both the technical and theoretical information in an interesting manner.

We were pleased to find that after 3 months, many of the goals we had set for the course were being achieved. All 23 respondents to the post-course survey indicated they had made changes in molecular diagnostic procedures and processes as a result of attending the course. The number of changes that were checked ranged from 3 to 15, with an average of 7.2 changes per laboratory site [T4]. Greater than one-half of the respondents indicated they had implemented enhanced QA/QC methods, and/or strengthened compliance with CLIA regulations, and/or improved assay verification. We can reason that those changes improved the quality of molecular diagnostic test results in their laboratories. Other reported changes (streamlined work flow, assay changes, cross-training) are indicators of the improved molecular diagnostic testing the instructors sought to achieve.

The respondents' indications of barriers to change are tabulated in T5. Lack of time, money, and personnel were seen as the 3 most powerful barriers. All respondents had communicated with at least 1 of the listed groups [T6] and many indicated increased networking activities due to the workshop experience [T4]. All respondents had in some way attempted to increase knowledge

Responses to the Request to Indicate any Barriers to Making Changes (n=23)

T5

Constraint	% Who Selected Constraint
Resistance by management	26
Lack of personnel	48
Resistance by staff	13
Too busy	57
Financial constraints	52
Other	17

Responses to the Request to Indicate Whether Participant Had Communicated With Any of the Following as a Result of Attending the Course (n=23)

T6

Group	% Who Selected Group
Fellow student(s)	91
Clients	4
Faculty member	48
Bioterrorism staff	43
Epidemiology staff	13
Other	13

Responses to the Question: Have You Done Any of the Following to Increase Knowledge and Skills in Molecular Diagnostics in Your Facility's Personnel? (n=23)

T7

Activity	% Who Selected Activity
Recommended purchasing a textbook	17
Purchased a textbook	22
Accessed Web sites recommended in the course	65
Conducted 1-on-1 laboratory training	35
Gave a molecular diagnostics inservice to your colleagues	26
Shared handout material you received at the course	87

Participants Description of Changes in Confidence as a Result of Attending the Course (n=23)

T8

Laboratory Practice Area	% Somewhat or Much More Confident than Before the Course
Performance of molecular assays	87%
Bringing a new assay online	96%
Troubleshooting (PCR) assays	96%

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laboratory operations when they returned home. Specifically mentioned were plans to strengthen their compliance with CLIA regulations, including their documentation, validation, and

verification procedures (17 students). Eight students indicated that they would use information from the training to implement stronger quality assurance (QA) and QC methods, streamline

and skills in molecular diagnostics in their home laboratory [T7]. Of note is the fact that 20 (87%) of the respondents shared the handout material they received at the course with others in their facility.

Most of the participants (87%) stated that their confidence in performing the molecular diagnostic applications covered in the course increased [T8]. Ninety-three percent indicated that they were more confident in bringing a new assay online and troubleshooting PCR assays. These findings are significant, considering the range of molecular diagnostic backgrounds of the students. While a number of the students came to the course with an intermediate to high level of molecular diagnostic experience, others had little actual hands-on experience and/or almost no skill in interpreting molecular diagnostic data.

Several students commented that they liked the combination of lectures, laboratories, and demonstrations. The most common participant recommendations called for more time for laboratory work, more hands-on exercises using other platforms, and more equipment to allow for independent work. Based on the open-ended comments, it is recommended that in future courses, more

discussion time should be allocated for: 1) examining data from laboratory exercises; 2) using proper laboratory controls; 3) brainstorming and discussion of CLIA regulations and NCCLS standards; and 4) studying the feasibility of a molecular diagnostic user group.

Overall, it was clearly evident that this molecular diagnostic training workshop increased awareness among these laboratory professionals of the complexity and intricacies of nucleic acid-based technologies and enhanced their abilities to perform the assays.

Discussion

Technology improvements and understanding of molecular biology have facilitated the fast-paced development of molecular diagnostics. Many tests are now common practice in medical settings (eg, HIV-1 viral load, DNA testing for genetic disease). Sequencing of the human and many pathogen genomes has opened the door for basic and clinical sciences to combine forces to identify new molecular markers that can be used for the diagnosis of many infectious, genetic, and neoplastic diseases as well as for forensic science and tissue typing. Molecular diagnostic testing is still in its infancy. Growth and change in this area of laboratory practice will be a common

feature of clinical testing for years to come.⁴ It is important to note that many public health and clinical laboratory scientists with considerable years of service have not been part of the “genetic technology explosion” during the last 2 decades. Thus, molecular diagnostic workshops like the one described here are critical for the continued advancement and education of past, present, and future laboratory workers.

In conclusion, the NLTN molecular diagnostic Public Health Series Course was an example of successful delivery of a comprehensive wet-laboratory course to public health scientists that enhance their understanding of this new and rapidly advancing field.

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List of Equipment/Instrument Manufacturers

Affymetrix, Santa Clara, CA (800.223.5281) www.affymetrix.com
 Applied Biosystems, Foster City, CA (800.874.9868)
www.appliedbiosystems.com
 artus Biotech USA, San Francisco, CA (415.512.7887) www.artus-biotech2.com
 Bayer HealthCare Diagnostics Division, Tarrytown, NY (510.705.5915)
www.bayerhealthcare.com
 Beckman Coulter, Fullerton, CA (800.742.2345) www.beckmancoulter.com
 BioRad Laboratories, Hercules, CA (800.876.3425) www.bio-rad.com
 Cepheid, Sunnyvale, CA (408.541.4191) www.cephheid.com
 Pyrosequencing, Westborough, MA (877.797.6767)
www.pyrosequencing.com
 Embi Tec, San Diego, CA (800.255.1777) www.embitec.com
 PerkinElmer, Life Sciences, Fremont, CA (800.446.0035)
www.perkinelmer.com
 Promega Corporation, Rancho Cucamonga, CA (909.481.3539)
www.promega.com
 Qiagen, Fairfield, CA (800.426.8157) www.qiagen.com
 Roche Diagnostics, Indianapolis, IN (800.428.5074) www.rocheusa.com

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