

Establishing a Molecular Diagnostic Testing Quality Assurance Program for Detection of Infectious Diseases- Validation of Molecular Tests at the North Carolina State Laboratory of Public Health

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Abstract

High-quality clinical laboratory services are critical for public health. Accurate and timely laboratory results are important in identifying, tracking, controlling, and preventing public health threats. For the past 20 years, molecular diagnostic assays have been available for infectious disease detection. These technologies have played and continue to play a critical role in clinical laboratories. Molecular diagnostic testing offers laboratories the ability to provide high-quality services that provides rapid test results, thus enabling for effective patient management and establishment of infection control and prevention measures. Public health laboratories are including molecular diagnostic testing in routine offered laboratory services. The majority of public health laboratories' clinical activities must be compliant to the Clinical Laboratory Improvement Amendments (CLIA) which define most basic quality systems for clinical laboratories. However, these regulations provide no specific guidance on molecular diagnostic testing. Public health molecular laboratory directors are being faced with the task of establishing quality assurance programs that are conducive with the mission and scope of their respective laboratories. The North Carolina State Laboratory of Public Health (NCSLPH), like many state public health labs, has been offering an increasing number of molecular diagnostic tests for preliminary detection of infectious disease agents. Prior to this study the Laboratory's each technical area developed a quality assurance program resulting in quality inconsistencies and important regulations being overlooked. To better maintain CLIA compliance, a molecular diagnostics quality assurance program was established. This study project presents the development and implementation of the quality control procedures, which assure the accuracy and reliability of tests results and subsequent reports, for method validation of molecular diagnostic testing.

1. Introduction

According to the National Center for Health Statistics (www.cdc.gov/nchs/fastats/infectis.htm), almost 6 million cases of infectious disease related illnesses occurred in 2004. Detection, control, and prevention of infectious diseases are critical for the public health of our communities. Rapid diagnosis of infectious diseases provides information that can be utilized to determine treatment, infection control, and prevention measures. Conventional bacterial and viral laboratory diagnostic tools, that are traditionally utilized for pathogen identification, have several limitations including time consuming culture based assays, prolonged assay times for fastidious pathogens, requirements for additional testing and wait times for pathogen identification, and decreased sensitivity due to administration of antiviral or antibacterial agents. Moreover, certain pathogens can not be cultured. A rapid method for pathogen

identification is invaluable for patient care and management; it is also critical in determining the best infection control and prevention methods to establish for ensuring communities' public health.

Molecular diagnostics, which are nucleic acid based assays, provide a means for rapid and accurate identification of infectious diseases. It has been suggested that molecular testing will eventually render conventional laboratory testing obsolete (1). The first nucleic acid based assays used for rapid pathogen identification were a DNA probe technology that detected microbial targets in culture and clinical specimens (1). This reduced the reporting time and provided information needed for prompt and appropriate patient management.

Over the past 20 years, molecular diagnostic assays available for infectious disease detection have become more sensitive and rapid. Additionally, these technologies are becoming the "gold standard" for detection of some clinically significant pathogens that are uncultivable, such as Norovirus, the causative agent of gastroenteritis, or pathogens that have extremely fastidious characteristics (requiring several days or weeks of growth or specialized growth media and conditions) such as *Mycobacterium tuberculosis*. In clinical laboratories, several different molecular technologies are utilized for diagnosis of infectious diseases. Polymerase chain reaction (PCR) amplification is the most extensively used method. PCR is an enzyme driven process that amplifies targeted regions of DNA. Using amplification technologies, such as PCR, reverse-transcriptase (RT)-PCR, nucleic acid sequence based amplification (NASBA), or transcription based amplification (TMA), the identification of infectious disease agents has been reduced from several days/weeks to 1-2 days. Real-time PCR, in which amplification and fluorescent detection is carried out in a single tube, has further reduced pathogen identification to a few hours.

In 1988, the Clinical Laboratory Improvement Amendments (CLIA '88), were ruled into law to regulate laboratory testing (except research) performed on humans (2). The objective of the CLIA program is to ensure quality laboratory testing. Subsequently in 1995, the Clinical and Laboratory Standards Institute (CLSI) published MM3-*Molecular Diagnostic Methods for Infectious Diseases* (3). This publication was the first approved guideline to be used by clinical laboratories for the new field of molecular microbiology which used nucleic acid methods to diagnose and manage patients with infectious diseases. The second edition of MM3-A2-*Molecular Diagnostic Methods for Infectious Diseases* (4), published in 2006, provides guidance on clinical applications; amplified and non-amplified nucleic acid methods; selection and qualification of nucleic acid sequences; establishment and evaluation of test performance characteristics, inhibitors, and interfering substances; controlling false-positive reactions; reporting and interpretation of results; quality assurance; regulatory issues; and recommendations for manufacturers and clinical laboratories. An additional publication, CUMITECH 31 *Verification and Validation of Procedures in the Clinical Microbiology Laboratory* (5), provided guidance on producing consistent accurate and precise results in the clinical microbiology laboratory.

As molecular testing becomes a routine diagnostic tool in public health laboratories, public health molecular laboratory directors have the task of establishing quality assurance programs. CLIA (2) and CLSI (4) provide assistance in establishing quality assurance programs for molecular testing. Additionally, College of American Pathologists (CAP) Molecular Pathology Checklist (2007) and the Microbiology Checklist (2007) (6), which includes a molecular microbiology section, ask questions to enable laboratories to assess compliance with CAP rules and regulations to prepare for CAP inspections (for CAP accredited laboratories). The CAP checklists reference both CLIA '88 and CLSI publications.

A critical component and one of the most common practices in quality assurance is the practice of quality control. Quality control is only one part of a quality assurance program. These procedures assure the accuracy and reliability of tests results and subsequent reports. The purpose of the North Carolina State Laboratory of Public Health (NCSLPH) Quality Assessment Plan (QAP) for Laboratory Services (7) is to define the systematic and ongoing processes for monitoring, assessing and improving the quality of laboratory services by the NCSLPH.

The mission of the NCSLPH is to provide certain medical and environmental laboratory services (testing, consultation and training) to public and private health provider organizations responsible for the promotion, protection and assurance of the health of North Carolina citizens (8). In 1999, as part of epidemiologic surveillance programs, the NCSLPH offered its first molecular tests, the detection of Norovirus by RT-PCR and pulse-field gel electrophoresis for analysis of bacterial isolates associated with food-borne outbreaks. Since then, the Laboratory has offered, as part of medical laboratory services, several different molecular diagnostic tests with each technical area establishing a quality assurance program plan based on Good Laboratory Practices (9), CLIA (2), and CAP (6) recommendations. It was determined that a Laboratory molecular testing quality assurance program needed to be established.

2. Purpose

The primary goal of this study is to establish a Laboratory wide molecular diagnostic quality assurance program including quality control procedures. The focus of this study is to design quality control procedures for molecular diagnostic testing for infectious diseases that are consistent in all technical areas and CLIA compliant.

3. Methods

3.1. Study Partners

The NCSLPH Quality Assurance Team, Mycobacteriology, Special Bacteriology, Viral Culture, and HIV technical areas participated in the development and implementation of a quality assurance program for molecular methods at the NCSLPH. The Laboratory's director, quality assurance manager, unit supervisors, and technical

supervisors were consulted on method validations for new molecular tests offered at the NCSLPH.

3.2 *Study design*

Incorporating the established QAP (7) with CLIA rules (2), CLSI guidelines (4), CAP checklists (6), and CUMITECH 31 (5) recommendations, method validation procedures for molecular testing of infectious diseases were established. Following consultation with the technical area and technical review of the nucleic assay system, recommendations were made on designing and performing validation studies. The molecular tests evaluated and verified for this study utilized amplification methodologies and the appropriate controls for each assay. Each validation study was conducted using test matrix that will be the routine testing clinical sample. Validation of molecular diagnostic tests included: real-time PCR detection of *Bordetella pertussis* (causative agent of whooping cough; homebrew) utilizing *B. pertussis* specific primers and probes; real-time PCR for detection of *Mycobacterium tuberculosis* complex (causative agent of TB; homebrew) utilizing *M. tuberculosis* complex specific primers and probes; real-time RT-PCR for influenza virus utilizing influenza A and B specific primers and probes, an assay for HIV (modified FDA clear or approved test) utilizing HIV specific reagents, and an assay for human papillomavirus (HPV the causative agent of genital warts; FDA clear or approved test) utilizing HPV specific reagents which is still in progress.

3.3 *Assessment of performance characteristics of new molecular test method*

Validation studies were carried out according to CLIA regulations (2) and CSLI guidelines (4). Briefly, for an un-modified FDA cleared or approved systems (in this case kit based), the following performance characteristics were performed: accuracy, precision, reportable range, and reference range (normal values). For a modified US Food and Drug Administration (FDA) or non-FDA cleared or approved system (usually home brew or modified FDA cleared system), the following performance characteristics were performed: accuracy, precision, reportable range, reference range (normal values), diagnostic/analytic sensitivity, diagnostic/analytic specificity, and detection limit.

Un-modified test systems performance characteristics were verified to be consistent with the performance characteristics stated by the manufacturer. For modified FDA or non-FDA cleared or approved assay, performance characteristics were determined from the data obtained during the method validation study.

3.3a The real-time PCR *B. pertussis* validation study included a year long comparative study of a PCR assay modified from the Virginia State Laboratory protocol (10), direct fluorescent antibody (DFA) staining, and culture which is considered the “gold standard”. The PCR results from the study were compared to culture results to determine accuracy, sensitivity, and specificity. Reportable range and limit of detection were determined using DNA extracted from 10-fold dilutions of enumerated bacteria (colony forming units; CFU). Diagnostic/analytic specificity was determined using other *Bordetella* species.

3.3b The validation of the tuberculosis complex real-time PCR was carried out using the PCR assay modified from the Illinois Department of Public Health – Division of Laboratories, Chicago Molecular Laboratory protocol (11). The PCR results from the study were compared to culture results to determine accuracy. Diagnostic/analytic sensitivity, reportable range, and limit of detection were determined using DNA extracted from 10-fold dilutions of enumerated *M. tuberculosis* bacteria (McFarland). Diagnostic/analytic specificity was determined using nucleic acid isolated from influenza A and B, parainfluenza, adenovirus which are common respiratory pathogens. Additionally, *Mycobacterium avium-intracellulare*, *Mycobacterium kansasii*, *Mycobacterium gordonae*, and *Mycobacterium fortuitum* group were studied to investigate cross reactivity of TB complex specific primers and probes. Isolates used for sensitivity studies were from stocks maintained by the NCSLPH Viral Culture or the Mycobacteriology technical areas.

3.3c The real-time influenza RT-PCR validation study included a seasonal influenza cycle in which a CDC-modified RT-PCR assay and cell culture results were compared to determine accuracy. Diagnostic/analytic sensitivity, reportable range, and limit of detection were determined using RNA extracted from 10-fold dilutions of known viral particles (vp) supplied by Advanced Biotechnologies Incorporated (Columbia, MD). Diagnostic/analytic specificity was determined using RNA isolated from respiratory pathogens influenza A or B, adenovirus, parainfluenza, and herpes simplex virus (HSV) I and II, rhinovirus, respiratory syncytial virus (RSV), and enterovirus (coxsackie B). All isolates were viral stocks maintained by the NCSLPH Viral Culture technical area.

3.3d The validation study for HIV was performed with modifications to the GEN-PROBE® APTIVA® HIV-1 RNA Qualitative Assay technical bulletin insert. Briefly, the GEN-PROBE® APTIVA® HIV-1 RNA Qualitative Assay (Gen-Probe Incorporated, San Diego, CA) kit procedure was modified to reflect the use of human serum for diagnosis of HIV-1 instead of the intended use of diagnosis of HIV-1 in human plasma. Diagnostic/analytic sensitivity, reportable range, and limit of detection were determined using negative serum spiked with known amounts of HIV virus. Diagnostic/analytic specificity was determined using RNA isolated from hepatitis A virus (HAV) which was generously donated by Dr. Lee-Ann Jaykus (Department of Food Science, North Carolina State University, Raleigh, NC), hepatitis B cloned DNA (HBV) obtained from the American Type Culture Collection (ATCC; Manassas, VA), hepatitis C virus (HCV) which was generously donated by Dr. Lizzie J. Harrell (Department of Molecular Genetics and Microbiology, Duke University, Durham, NC), and cytomegalovirus (CMV) obtained from stocks maintained by the NCSLPH Viral Culture technical area.

3.3e The evaluation of the Digene Hybrid Capture®² High-Risk HPV DNA Test® (Digene Corporation, Gaithersburg, MD) validation study is in progress

Results and discussion

Over the past several months, several molecular method validation studies have been conducted. As a result, the technical areas involved in this study, with the exception of the HPV assay, have completed validation studies and either implemented or are preparing to implement and offer molecular testing. The results of the validation studies were gathered upon recommendations based on the NCSLPH QAP (7), CLIA rules (2), CLSI guidelines (4), CAP checklists (6), and CUMITECH 31 (5). The outcome of the validation studies were used to develop a Laboratory wide molecular diagnostic quality assurance program that will be consistent between technical areas and CLIA compliant.

During the study, each technical area was faced with challenges. To be CLIA compliant, validation studies must be carried out in the matrix submitted as the clinical specimen. For the *B. pertussis* real-time PCR validation study, nasopharyngeal swabs containing bacteria associated with the nasal cavity were submitted for PCR testing. Once received in the Laboratory, the bacterial containing swab was suspended in buffer to remove the bacteria. This suspension was processed for DNA isolation. Since the PCR data from the swabs could only be compared to the culture results for accuracy, the challenge was how to design the sensitivity study to determine the reportable range and limit of detection. A sensitivity study was designed that performed 10-fold dilutions of enumerated bacteria in the suspension buffer used to remove the collected bacteria from the swab. This approach was also utilized to perform the sensitivity studies of the influenza and TB complex molecular validation studies.

Another instance of a design challenge was the entire validation of the GEN-PROBE® APTIVA® HIV-1 RNA Qualitative Assay (Gen-Probe Incorporated, San Diego, CA). This FDA cleared/ approved intended use of this assay is to diagnosis of HIV-1 in human plasma and all the manufacturer's performance characteristics are based on plasma results. Since serum instead of plasma, is the matrix for HIV testing at the NCSLPH, this is considered a modified test. The interpretation of CLIA regulations, 42 CFR Section 493.1253 (2), requires that performance characteristics of a modified test system be conducted in the sample matrix that will be the routine testing clinical specimen. Therefore, the HIV validation study was performed using HIV negative serum spiked with virus.

The challenge that all of the technical areas experienced was determining the pathogens to include in cross reactivity studies to investigate assay specificity. The participating technical areas are involved in ongoing study or surveillance programs. These programs establish the criteria for the participating patient population. Thus, the demographics of the patient populations to be tested assisted with the determination of the pathogens to be investigated for each molecular assay.

The *B. pertussis* real-time PCR was the first attempt in designing a molecular diagnostic test validation study loosely based on CLIA rules (2), CLSI guidelines (3), and CAP checklists (6), and CUMITECH 31 (5). This study has resulted in a method validation for molecular testing of infectious disease that provides CLIA compliant

protocols and consistency between technical areas. Using the existing quality assurance resources available at the NCSLPH and the results of the conducted method validation of molecular tests study, a manual, "Quality Assurance Program for Molecular Testing at the North Carolina State Laboratory of Public Health", and a workshop presentation, "Validation of Molecular Methods at the North Carolina State Laboratory of Public Health", has been prepared as resources for the technical areas at the NCSLPH. PowerPoint presentations presented by Dr. Denise Toney (Commonwealth of Virginia, Division of Consolidated Laboratory Services, Richmond, VA) and Dr. Christina Egan (Biodefense Laboratory, Wadsworth Center, New York State Department of Health, Albany, New York), at the 2007 Association of Public Health Laboratories Annual Meeting were used as templates for the workshop presentation that will accompany the molecular testing quality assurance manual. The manual will be reviewed at least annually and modified as needed to ensure that newly added molecular tests and regulatory updates are reflected in the program.

I have recommended to the NCSLPH Quality Assurance Manager that we prepare and instruct a quality assurance workshop that includes information on the molecular testing quality assurance program and require all NCSLPH unit and technical supervisors participate. Hopefully, the workshop will be on the quality assurance team meeting calendar, which meets every first Monday of the month, early in 2008.

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