Meeting PT Requirements in a
Clinical Virology Laboratory

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Regulation of Clinical Laboratories

• Centers for Medicare and Medicaid Services regulates laboratory testing performed on humans in the U.S.
  – http://www.cms.hhs.gov/clia/

• Mechanism of regulation is the Clinical Laboratory Improvements Amendment of 1988
Why NYS?

- In 1965 NYS became the first state in the nation to establish a licensure program for laboratories performing clinical testing
- Public Health Law established the Clinical Laboratory Reference System
- Administered through the Wadsworth Center which is the NYS public health laboratory - Clinical Laboratory Evaluation Program (CLEP)
Overall aim……

Promote public health by ensuring quality laboratory testing

**Quality assurance**: A comprehensive set of policies, procedures and practices necessary to ensure the laboratories results are reliable.

In contrast to……

**Quality control**: A set of laboratory procedures designed to ensure that the test method is working properly

PT is just one part of QA!
PT is just one part of a QA plan

- Monitoring overall quality of testing
- Ensuring accurate, reliable and prompt reporting of results
- Assuring staff competence
- Identifying and resolving problems
- Monitoring and evaluating Proficiency Testing and other QC data

- PT is required by
  - CLIA ’88
  - NYS Standards
  - CAP accreditation
NYS PT Program Objectives

• Ensure the accuracy and reliability of clinical laboratory testing
• Provide applicant laboratories with validation and certification (special PT) panels
• To offer technical help and support to participating laboratories
• Over 400 Proficiency Panels are sent to 254 laboratories 3 times per year (Jan., May, & Sept.)
NYS PT Process

• Recent circulating NYS isolates are chosen from NYS Virus Reference and Surveillance Laboratory (VRSL)
• The isolate is re-amplified, re-identified, and confirmed by PT Lab
• Blinded Panels are confirmed by 10 volunteer reference laboratories
• Samples are used only if 100% concordant
• Isolates are then amplified on large scale and dispensed into hundreds of individual vials.
CLIA requirements for Virology PT

- Two types of virus laboratory and PT panels
  1. Direct detection in cells derived from infected tissue or in fluid secretions
  2. Performing isolation and identification as well as direct detection

The laboratory should process the PT samples in the same manner it would process clinical samples
CLIA approved PT providers - 2004

- http://www.cms.hhs.gov/clia/ptlist
  - Accutest
  - American Academy of Family Physicians
  - American Association of Bioanalysts
  - American Proficiency Institute
  - California Thoracic Society
  - College of American Pathologists
  - External Comparative Evaluation for Laboratories
  - Medical Laboratory Evaluation Program
  - New Jersey Department of Health and Senior Services
  - Commonwealth of Pennsylvania DOH
  - Puerto Rico DOH
  - Wisconsin State Laboratory of Hygiene
  - Maryland Department of Health and Mental Hygiene
  - New York State Department of Health
Direct detection of viral antigen

Direct detection in cells derived from infected tissue or in fluid secretions

- Point-of-care FDA-approved kits
- Immunofluorescence-based detection assays
Direct viral antigen detection panel

- Accutest
- American Academy of Family Physicians
- American Association of Bioanalysts
- American Proficiency Institute
- College of American Pathologists
- External Comparative Evaluation for Laboratories
- Medical Laboratory Evaluation Program
- Wisconsin State Laboratory of Hygiene
- New York State Department of Health
Laboratories performing isolation and identification

Shell vial and conventional tube culture coupled with secondary identification method e.g fluorescence, EIA, PCR.

CPE of human metapneumovirus in LLC-MK2 cells
Virus isolation panels

**CLIA-approved provider**
- College of American Pathologists
  - [http://www.cap.org](http://www.cap.org)
- New York State Department of Health
  - [http://www.wadsworth.org/labcert/](http://www.wadsworth.org/labcert/)

**Non-CLIA-approved provider**
- Diagnostic Hybrids Inc., Athens, OH
  - Enterovirus, herpes simplex and respiratory virus panels
    - [http://www.dhiusa.com](http://www.dhiusa.com)
CLIA &NYS requirements for Virology PT cont…

- Three testing events per year
- Both direct detection and isolation must be tested
- Five samples per event
- Should include viral species currently in circulation and must include important emerging viruses
CAP Virology PT

• **Isolation panel**
  – Adenovirus, influenza, PIV, RSV, CMV, HSV, enterovirus, VZV and *Chlamydia trachomatis*
  – Lyophilized specimens

• **Direct detection**
  – Immunofluorescence panel: Adenovirus, CMV, HSV, Flu A and B, PIV2, RSV, VZV
  – EIA-based: Adenovirus, HSV, Flu A and B, RSV, Rotavirus
NYS Virology PT

• **General panel:**
  – Adenovirus, Coxsackievirus A and B, CMV, Echovirus, Influenza A and B, PIV 1,2 and 3, RSV, HSV I and II, VZV
  – Frozen virus samples

• **Limited to Herpes Group**
  – HSV I and II, VZV and CMV

• **Direct detection:**
  – Flu A, Rota, RSV and Herpes
NYS Program is evolving!

- CLIA ’88 somewhat static but laboratory science is evolving
- A detailed survey will shortly be sent to all regulated labs to see what needs we don’t meet
- Need to anticipate where the future lies
- Should be an interactive process
What happens when there is no PT available?

• For all tests with no available PT the laboratory:
  1. Shall have a system for verifying the reliability and accuracy of test results
  2. Shall perform this verification twice a year.

  • Split sample comparisons
  • Evaluation of clinical outcomes
  • Blind testing of specimens with known results
Where are the holes in PT programs?

- Inclusion of other agents, especially newly emerging viruses
- Newer technologies and molecular testing generally
- Detection of viruses that don’t grow well in cell culture – e.g. HPV, HHV-6 and HCV
- Viral genotyping – HIV and HCV
- Others????
Newly emerging viruses

- Examples are new coronaviruses, and human metapneumovirus
  - CDC panels exist for SARS-CoV & WNV using inactivated virus
  - Exchange blinded samples with other labs
  - Internal panel of previously tested agents
  - Buy specific reagents e.g. human metapneumovirus isolates available from Emerging Pathogens Laboratory, Univ. of Iowa
Molecular detection

• General NYS category description
  – “This category is for laboratories that perform virus isolation and identification or molecular techniques for any of the viral agents normally encountered in a clinical virology laboratory.”
  – This means the isolation panel can be used, in parallel as a molecular panel

• Use of CAP or DHI isolation panels
• Use of previously analyzed clinical samples verified by another method
• CAP has a nucleic acid amplification panel (CMV, entero, HBV, HSV, HPV)
QCMD: Quality Control for Molecular Diagnostics

- [http://www.qcmd.org](http://www.qcmd.org)
- Evolved out of European Union Concerted Action for Quality Control
- Global program
- Panels offered in HSV I and II, VZV, enteroviruses, HIV drug resistance testing
- Proposed panels for JC/BK, WNV, HPV, ParvoB19, respiratory viruses, norovirus & HCV genotyping
Molecular detection cont…..

- NYS is beginning to develop a molecular virology PT program
- Plasma spiked with virus at a range of concentrations
- Needs to evaluate extraction as well as detection steps
- Issues: What matrices to use? Sequence variation!
Other solutions….

• Commercial control reagents that can be used to set up “internal” panels or to spike clinical matrices
• Virus spiking versus nucleic acid spiking

  – Boston Biomedica Inc. HIV-1, HCV, HBV
    • http://www.bbii.com
  – Zeptometrix – NATrol standards for hCMV, HIV-1, HBV, HCV, WNV
    • http://www.zeptometrix.com
  – Acrometrix – nucleic acid test standards for HIV-1, HBV, HCV
    • http://www.acrometrix.com
  – Advanced Biotechnologies Inc. – nucleic acid test standards for many viruses
    • http://www.abionline.com
  – Others?????
Using the same reagents to validate molecular assays

- **What is necessary?**
  - Verification - are the results correct?
  - Assay range - high and low
  - Reproducibility - inter- and intra-assay
  - Sensitivity - what is the lowest amount of target that can be detected?
  - Specificity - must not detect related organisms
  - Validation - using (spiked) clinical samples
PT specimens for viral genotyping

• Infectious molecular clones
  – Add specific mutations to a reference clone by site-directed mutagenesis
  – Linearize plasmid and transfec into cell line
  – Prepare high titer stocks of infectious virus and dilute to desired level
  – May mix different stocks to achieve nucleotide mixtures at different positions

• May not have the sequence diversity characteristic of real clinical specimens
Availability of genotyping panels

• Acrometrix – HIV and HCV
• QCMD
• College of American Pathologists
  – one sample as part of HIV and HCV viral load panels

• Other options?
  – Split samples with another clinical lab
Genotyping PT Results reporting

• Needs to be compatible with multiple methodologies
• HIV: usually the resistance mutations detected are reported
  – Sequence-based assays: should nucleotide sequence concordance be included?
  – What about interpretation?
• HCV: usually genotype only is reported
  – Allows one PT to be used for different assay methods
Summary

- Current PT programs are not changing as fast as laboratory needs
- PT programs should be responsive and educational in their approach
- PT programs are expensive
- “One size does not fit all”
- Laboratory managers need to be innovative to determine ways to meet regulations
Contact Information

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