Sample Selection, Collection, Transport: Issues & Challenges

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LEARNING OBJECTIVES

1. Identify factors to consider when deciding the optimal specimen and timing of collection for viral nucleic acid testing.
2. Discuss the methods used to stabilize viral nucleic acid during specimen transport and storage.
3. Understand the regulatory requirements that pertain to specimen collection and transport.
PRE-ANALYTIC PHASE

• Up to 75% of all testing errors occur in the pre-analytic phase
  o Hemolyzed sample
  o Insufficient volume
  o Incorrect sample
  o Incorrect ID
  o Wrong transport conditions

• Affects up to 1 in every 164 lab reports

PRE-ANALYTIC PHASE

• Many people involved
  • Physicians: writing orders, instructing patients/staff
  • Nurses/Phlebotomists/RTs: collection, preservation, transport
  • Lab staff: storage, processing
    ▪ Often specimen management and not technical staff who initially receive specimen
PRE-ANALYTICAL CHALLENGES

- Most time and steps
- Most people
- High urgency and stress
- Most variation in work environment, technique, and training

% of Time Spent

- Pre-analysis: 60%
- Analysis: 25%
- Post-analysis: 15%
PRE-ANALYTIC FACTORS TO CONSIDER

PATIENT

- Which test?
- Which specimen?
- How to collect?
- When to collect?
- How to transport?
PRE-ANALYTIC FACTORS TO CONSIDER

VIRUS

- Incubation period?
- Duration of infection?
- Tissue tropism?
- Latency?
- Causality?
TEST SELECTION
WHICH TEST TO SELECT?

- Depends on signs & symptoms, clinical syndrome
  - Differential diagnosis – many viruses may be associated with the same clinical syndrome
- Other factors: immune status, age, travel, exposures, season, etc.
- Screening recommendations
  - e.g. HPV, post-transplant viral load testing
- Qualitative vs. quantitative
- Diagnosis vs. monitoring
# MYOCARDITIS/PERICARDITIS

<table>
<thead>
<tr>
<th>Virus</th>
<th>Specimen Sources</th>
<th>Test Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviruses</td>
<td>Blood, respiratory, tissue</td>
<td>Culture, histology, IA, NA</td>
</tr>
<tr>
<td>CMV</td>
<td>Blood, tissue, urine</td>
<td>Culture, histology, NA</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>Blood, respiratory, stool, tissue</td>
<td>Culture, NA</td>
</tr>
<tr>
<td>Influenza viruses</td>
<td>Respiratory, tissue</td>
<td>Culture, IA, NA, serology</td>
</tr>
<tr>
<td>Parechoviruses</td>
<td>Blood, respiratory, stool, tissue</td>
<td>Culture, NA</td>
</tr>
</tbody>
</table>
SPECIMEN TYPE
WHICH SAMPLING SITE/SPECIMEN TYPE IS BEST?

- Guided by test best suited to establish a particular diagnosis
- Usually the anatomic site affected
- Dependent on viral factors/pathogenesis
- Disseminated vs. localized infection
- Permissive vs. non-permissive sites
  - e.g. enterovirus in stool
WHICH SAMPLING SITE/SPECIMEN TYPE IS BEST?

• Choice also depends on compatibility with downstream test methods
  • NA extraction and amplification platforms
  • Follow manufacturer’s recommendations to be on-label
WHICH SAMPLING SITE/SPECIMEN TYPE IS BEST?

• Viral respiratory tract infection
• EBV viral load testing
# OPTIMAL SPECIMEN FOR VIRAL RTI?

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Positives</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5 yrs.</td>
<td>169</td>
<td>NP aspirate &gt; nasal swab</td>
</tr>
<tr>
<td>≤ 18 yrs.</td>
<td>Flu: 41</td>
<td>NP aspirate = NP flocked swab</td>
</tr>
<tr>
<td></td>
<td>RSV: 39</td>
<td>NP aspirate &gt; NP flocked swab</td>
</tr>
<tr>
<td>≤ 18 yrs.</td>
<td>FluB: 65</td>
<td>NP swab &gt; OP swab</td>
</tr>
<tr>
<td></td>
<td>PIV: 355</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FluA: 256</td>
<td>OP swab &gt; NP swab</td>
</tr>
<tr>
<td></td>
<td>Adeno: 679</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSV: 328</td>
<td>NP swab = OP swab</td>
</tr>
<tr>
<td>&gt;18 yrs.</td>
<td>251</td>
<td>NP wash &gt; NP swab &gt; OP swab</td>
</tr>
</tbody>
</table>

(NP wash + OP swab = 94% sens.)

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Q: What is the primary specimen type recommended by your lab for molecular respiratory virus testing?

1. NP swab
2. NP aspirate
3. Nasal wash
4. Oropharyngeal swab
5. Mid-turbinate swab
OPTIMAL SPECIMEN FOR MONITORING EBV-ASSOCIATED LYMPHOPROLIFERATIVE DISORDER?

- Pediatric HSCT pts.

<table>
<thead>
<tr>
<th>N</th>
<th>PBMC</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>420</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>119</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>39</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

- Significant correlation between PBMC and WB ($r=0.81; P<0.001$)

- Kinetics show PBMC positive earlier in $\frac{1}{3}$ of patients

OPTIMAL SPECIMEN FOR MONITORING EBV-ASSOCIATED LYMPHOPROLIFERATIVE DISORDER?

- Pediatric heart transplant pts.

- PBMC & WB more sensitive vs. plasma
- Plasma more specific for PTLD

OPTIMAL SPECIMEN FOR EBV VIRAL LOAD?

Q: Which matrix does your lab recommend for EBV viral load testing?

1. Plasma
2. Whole blood
3. PBMC
4. Combination of two or more of these
5. Depends on underlying disease/transplant type
TIMING OF COLLECTION
TIMING OF COLLECTION

• Collect when higher titer of virus expected
  • Generally within 3-7 days after onset of symptoms
  • But viral replication may peak prior to onset of symptoms
• Varies with syndrome and matrix selected
• Disseminated disease or immune compromised
  • Prolonged viral shedding
• Repeat collections may be needed
  • e.g. HSV in CSF
WNV: Virologic & Serologic Response

DAYS POST ONSET

1 2 3 4 5 6 7 8 9 10

#pfu/ml

WN viremia

IgM

IgG

ELISA P/N

illness
TIMING OF COLLECTION

• Plan for appropriate staffing if sample must be processed or aliquoted within specific time frame indicated by package insert
  • e.g. HIV plasma viral load ≤ 6 hrs.
• Based on best practice recommendations or consensus guidelines for patient management
  • CMV load testing post-transplant
    • For preemptive strategy - viral load monitored at least weekly for 3 to 6 months after transplantation
    • Use the same assay and same matrix
SAMPLE STABILITY AND TRANSPORT

• Maintain NA integrity prior to processing
  • i.e. prevent false negatives

• Method of transport
  • Hand delivered, pneumatic tube, courier, shipped

• Liquid transport media (e.g. VTM)
  • Useful for split testing
  • Most methodologies compatible with VTM

• Transport 4°C (short delay) or -70°C (longer term)
INHIBITORS & INTERFERING SUBSTANCES

- Heme
- Nucleases
- Inhibitors in urine, CSF, sputum
- Phenol, EDTA, SDS, heparin, chaotropes
- Polyamines (spermine, spermidine)
- Certain acid and plant polysaccharides
- Calcium alginate
TRANSPORT MEDIA

- Liquid samples (e.g. blood, urine, CSF, BAL) do not generally need VTM
- Swabs in VTM or other buffered solution
- Nucleic acids may be stable in desiccated state
  - Longer storage at room temp
  - Reduced biohazard risk
  - Simple & inexpensive ambient shipping
## SHORT-TERM STORAGE

<table>
<thead>
<tr>
<th>Virus</th>
<th>Specimen</th>
<th>Days stored</th>
<th>RT % loss</th>
<th>4°C % loss</th>
<th>-80°C % loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>Serum</td>
<td>16</td>
<td>80%</td>
<td>50%</td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td>EDTA WB</td>
<td>16</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>CSF</td>
<td>16</td>
<td>82%</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Influenza A</td>
<td>NP aspirate</td>
<td>14</td>
<td>74%</td>
<td>70%</td>
<td>66%</td>
</tr>
</tbody>
</table>

VIRAL RNA STABILITY IN BLOOD

• Manufacturer recommendations for separation of plasma or serum (HIV, HCV, HBV)
  • Typically within 4 to 6 hrs.
• Plasma HIV-1 RNA levels stable up to 30 hrs in whole blood stored at room temp
  • SD range 0.035 to 0.269 log units
• Plasma HCV RNA levels stable at least 24 hrs in whole blood stored at room temp

REGULATORY CONCERNS
REGULATORY CHALLENGES

- The FDA-cleared platform does not include specimen types that need testing

MIC.64810  Test Performance - Manufacturer's Instructions

Tests are performed and results reported as specified in package inserts without substitution of reagents or modification of testing protocol.

- Alternative Specimen or Collection Device
- Change in Test Procedure

YES

LDT or Modified FDA Test

NO

Inspect as FDA/Extensive Validation Required

Texas Children's Hospital

Affiliated with Baylor College of Medicine
REGULATORY ISSUES

✓ COM.06000   Specimen Collection Manual
  ▪ Written procedures describing methods for patient identification, patient preparation, specimen collection and labeling, specimen preservation, and conditions for transportation, and storage before testing.

✓ COM.06300   Specimen Rejection Criteria
  ▪ Written criteria for rejection of unacceptable specimens, instructions for the special handling of sub-optimal specimens, and records of disposition of all unacceptable specimens in the patient report and/or quality management records.

✓ MIC.13275   Specimens for Molecular Amplification
  ▪ Written procedures for the handling of specimens that will be tested using molecular amplification methods.
REGULATORY ISSUES

✓ MIC.63318 Specimen Handling Procedures
  ▪ Written procedures to prevent specimen loss, alteration, or contamination during collection, transport, processing and storage.

✓ MIC.63328 Specimen Processing/Storage
  ▪ Patient samples are processed promptly or stored appropriately to minimize degradation of nucleic acids.

✓ MIC.63324 Residual Samples
  ▪ If residual samples are used for amplification-based testing, policies and procedures ensure absence of cross-contamination of samples.
PROCEDURE MANUAL

• Provide clearly defined methods of collection, handling and delivery
  • Timing of collection
  • Specimen type and quantity
  • Collection & transport devices

• Procedures in place to maintain target nucleic acid integrity and prevent cross-contamination
  • Transport and storage time, temperature and conditions
SUMMARY

• Pre-analytical errors can lead to harm
• A bad sample is worse than no sample – specimen collection/handling can have a significant effect on test results & patient management
• Accurate diagnosis depends on appropriate specimen and test selection AND collection AND transport
THANKS

COMMENTS/QUESTIONS?