Labeling, Packaging, Handling and Transport of Biological Specimens for In-Country Transport
Learning Objectives

• Review the importance of in-country transport
• Timeliness, cold chain
• Biosafety
• Specimen aliquots
• Strategies for optimizing success
• **Group exercise**: Discuss strategies for timely delivery of specimens from rural sentinel sites to your laboratory
Pre-analytical phase

- Often overlooked.
- Perhaps the most critical phase that labs have little experience with.
- Specimen storage, timeliness, appropriate transport media, maintenance of cold-chain, accurate documentation.

The Rule of Thumb: Garbage in = garbage out!
Outbreak Response Activities

- Bacterial culture
- Virus culture
- PCR testing
- Serology
- Microscopy

Important to consider the type of transport media which can effect the stability of the pathogen.

Consult with the laboratory is essential for questions regarding appropriate specimen types.
Viral Specimens

• Optimal Timing
  – Respiratory swab specimens should be collected within 3 days of onset (maximum 7 days).
  – Whole blood/plasma, CSF collected as soon as possible after onset.
  – Lower respiratory (BAL, sputum)
  – Other specimens may depend upon the disease course and area of replication

• Choose the appropriate virus transport medium
  – Consider those that do not require refrigeration for storage
Bacterial Specimens

• Wide variety of sample types
  – Stool, whole blood, CSF, sputum, urine, autopsy tissues

• Transport mediums considerations important (VTM may not be appropriate)

• Specimen collection prior to antimicrobial therapy

• Shipping temperatures may be different!


Serology specimens

• Refrigeration (4-8C) for 24-48hrs.
• -20C for longer periods.
• Some assays have specific requirements, so check the package insert or SOP.
• Make sure that acute and convalescent serums are labeled as such.
Adequate Volumes

- PCR tests generally require <1.0ml.
- Culture tests can require considerably more.
- 3.0ml of VTM is appropriate.
- Consider collecting enough volume for repeat testing or further characterization assays.
- Some specimen types should NOT be placed in transport media; undiluted is optimal.
- Despite convenience of swabs, sometimes overall detection is enhanced by increasing specimen volume [1]
  - Nasopharyngeal washes vs. swabs
What are some commonly used transport medias/stabilizers?
Specimen Stabilizers

• Bovine Serum Albumin (BSA), gelatin for viruses.
• Lysis buffer, glycerol for nucleic acid.
• Commercial reagents* (DNA/RNA shield, RNAsable, etc.)
• Amies, Stuarts, Carey-Blair common for bacteria.

*Important to evaluate new technologies to verify performance before putting into use.
Are the type of swabs used for specimen collection important?
Flocked swabs vs. nasal washes

Improved Detection of Respiratory Viruses in Pediatric Outpatients with Acute Respiratory Illness by Real-Time PCR Using Nasopharyngeal Flocked Swabs [Kenya]
Patrick Kiio Munywoki et al., (2011) JCM; 45(9): 3365-3367

<table>
<thead>
<tr>
<th>Respiratory virus (n = 299)</th>
<th>No. of viruses detected by:</th>
<th>% sensitivity (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both NW and NFS</td>
<td>NW only</td>
<td>NFS only</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>50</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>RSV</td>
<td>61</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>RSV-A</td>
<td>26</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>RSV-B</td>
<td>35</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>PIV</td>
<td>25</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>7</td>
<td>3</td>
<td>12</td>
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<tr>
<td>Coronavirus</td>
<td>16</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>hMPV</td>
<td>12</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total#</td>
<td>172</td>
<td>26</td>
<td>52</td>
</tr>
</tbody>
</table>

* PIV, parainfluenza virus types 1, 2, 3, and 4.
* One-sided 97.5% CI reported if sensitivity was 100%.
* Virus positive by either NW or NFS was considered the true positive for the sensitivity analysis.
* Exact McNemar's significance probability values comparing sensitivities for NW and NFS.
* Includes one coinfection of RSV-A and -B.
* Includes one coinfection of PIV type 3 (PIV3) and PIV4.
* Shows all viruses detected, which includes one influenza A infection detected by both NFS and NW.
Biosafety

What are common precautions that staff use when collecting patient specimens?
Biosafety Considerations

• Emergence of Ebola virus impacted specimen collection practices.
  – Re-emphasized the importance of protections for the person collecting samples.
    • Gloves, impermeable gown, face shield, respirator for collecting samples suspected of containing high consequence and novel pathogens.
    • Other practices included using plastic whole blood tubes, automated re-sheathing needles, sharps containers
    • Training

Perform a risk assessment!
Which image shows proper collection of a respiratory swab?


Image source: www.youtube.com
Specimen Labeling

- Best practice to consider having two unique identifiers
- Standardized labeling practices
- Labeling should be performed at the time of specimen collection!

Image source: www.remel.com
Specimen Collection Logs

• Keep accurate logs of specimen collection data
  – Patient name, DOB, Patient ID
  – Specimen source
  – Date of Collection
  – Location
  – Time of Collection
  – Lot#, Expiration date of transport medium
  – Person performing collection
  – Pertinent information useful for the laboratory
Does this image show an appropriately packaged specimen?

Specimen Packaging

- Disinfect the outside of the specimen container.
- Use a sealable, leakproof bag.
- Absorbent material.
- One specimen per bag.
- Do NOT leave swab shafts outside the cap.
- Consider using parafilm or similar sealer.
- Include specimen submission forms.

Cold chain maintenance

• For viral specimens requiring refrigeration
  – Store immediately post-collection 4°C
  – Received by the lab ≤72 hours.
  – If delayed, freeze at -70°C or below.

• Shipping frozen with dry ice or liquid nitrogen is optimal for respiratory specimens, but likely not feasible.

• Use a portable insulated cooler that can be easily decontaminated.

Avoid wet ice if possible!
Effects of time and temperature on influenza virus

Fig. 1. Infectivity decay curves for type A/Port Chalmers-like (H3N2) virus at different storage temperatures in (●) veal infusion broth, (×) Hanks balanced salt solution, and (▲) charcoal viral transport media. Concentration of virus at time zero was $3.7 \log_{10}$ per ml.

Baxter et al., (1977)
Confirm cold chain maintained

• Check the contents visually.
• Record the condition of specimens when received (e.g. cold, warm, frozen, leaking).
• Temperature indictors?
  – Visual
  – Indicates exposure temp.
  – Relative time

Image source: www.3m.com
Should specimen aliquots be prepared at the time of collection?
Specimen aliquots

• Aliquots
  – Multiple test requests.
  – Testing performed in different departments or labs.
  – Testing is performed at different times
    • PCR
    • Virus isolation

• Referenced in SOP or Policy
Specimen aliquots

**Best practices**

- Only one specimen will be uncapped and manipulated at a time.
- Do not place aliquots back into the original container.
- Label aliquots with lab ID# and date prepared.
- Make aliquots when received for specimens where testing is delayed.
- Others?
Shipping totes for in country transport

- Insulated
- Non-porous surface
- Labeled appropriately
- Meets any national standards
In-country transport

• Specimen boxes should be secured inside the vehicle.
• Spill kit including disinfectant (bleach).
• If possible, serums should be separated from the clot to avoid hemolysis.
In-country transport

• Not subject to IATA; However, training is essential.
• Must follow all local and/or national policies and laws.
• Frequently one of the major challenges in low resource settings.
Mailed specimens

• Diagnostic specimens must be sent in conformance with all relevant national and postal service requirements.

• Contact postal officials prior to sending specimens
  – To ensure their ability to ship
  – To inquire about regulations
  – Timeliness considerations
In-country transport

Using a bus service for transporting sputum specimens to the Central Reference Laboratory: effect on the routine TB culture service in Malawi

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What are some of the challenges you face when transporting specimens from sentinel sites?
Key Points

• In-country transport must follow national regulations.
• Develop SOP’s for specimen requirements, packaging and transport.
• Risk assessment.
• Training, refresher with documentation.
Useful Resources


Questions?