Appropriate Specimen Types and Handling Methods
Learning Objectives

Participants will be able to understand:

- Types of specimens used for meningitis diagnosis
- Proper procedures for specimen storage, package and transport
- Procedures for packing and shipping specimens and bacterial isolates
- Proper processing of bacterial meningitis specimens
Overview

- Meningitis
- Specimen collection from meningitis cases
- Meningitis specimen handling and processing
- Isolate transport
- Summary
Meningitis
Meningitis

- Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges.

- Most common symptoms
  - Fever, headache, and neck stiffness
  - Other symptoms include confusion, altered consciousness, vomiting, and reduced tolerance to light and loud noises.

- Non-treated cases can reach up to 50% lethality. Even with proper diagnosis 5 -10% patients may die within 24 - 48h.

Source: [www.cdc.gov/meningitis/index.html](http://www.cdc.gov/meningitis/index.html)
What are the Causes of Meningitis?

- Bacterial organisms
- Viruses (HIV, mumps virus, varicella zoster virus)
- Parasites (Cryptococcus neoformans)
- Autoimmune conditions
- Trauma
- Neoplastic disorders (brain tumor)
Bacterial Meningitis

- **Most common causes** (beyond the newborn period):
  - *Neisseria meningitidis* (*G*-, diplococcus)
    - 12 serogroups
      - A, B, C, W, X, and Y cause the great majority of infections in people.
  - *Streptococcus pneumoniae* (*G*+, diplococci/chain)
    - More than 93 serotypes
      - 1 and 5 predominant in developing countries.
  - *Haemophilus influenzae* (*G*, coccobacillus)
    - 6 serotypes (a-f)
      - Hib causes most cases in developing countries.

*Source: WHO Manual, 2011*
Bacterial Meningitis

- Epidemiology is geographically different.
- High disease burden in the African Meningitis Belt
  - Seasonal outbreaks punctuated by periodical epidemics in 8-12 years.

Specimen types for bacterial meningitis

- **Cerebral Spinal Fluid (CSF) preferred**

- **Other specimens are acceptable** *(Will not be discussed):*
  - Blood
  - Joint Fluid
  - Tissues (i.e., brain)
  - Sputum
  - Nasopharyngeal swabs
CSF Collection from Meningitis Patients
Cerebral Spinal Fluid (CSF)

- A clear, colorless body fluid that surrounds the brain and the spine, and is found between meninges in the subarachnoid space, the central canal of the spinal cord, and the four ventricles (the cavities in the brain).

- CSF is formed continuously in the ventricles.

- Function: Buoyancy, protection, homeostasis (nutrition, waste clearance)

- CSF is rich in nutrients, poor in immune cells.
  - Important for clinical outcome. Once infected, bacteria multiply very rapidly)
Meninges and CSF

- Cerebrum
- Skull bone
- Subarachnoid space (contains cerebrospinal fluid)
- Cerebellum
- Spinal cord
- Subarachnoid space of spinal cord
- Dura mater
- Arachnoid mater
- Pia mater
- Cranial meninges
- Blood vessel
- Spinal meninges

http://classes.midlandstech.edu
Collection of CSF

- CSF is collected by a medical procedure known as lumbar puncture (LP).
- A needle is inserted into the spinal canal between vertebrae L4-L5.
- LP is an invasive procedure and should be performed by experienced personnel under aseptic conditions.
Specimen Tubes and Volumes

- Collect CSF into 3 tubes as follows (if possible):
  - 1 - 2 ml biochemistry/Rapid Tests
  - 1 - 2 ml for cytology
  - 1.0 ml for microbiology
    - Culture or PCR

https://meds.queensu.ca

http://blog.timesunion.com
Specimen Labeling

- Label each tube with
  - Date of collection
  - Name of the patient (if possible)
  - EPID number (CCC-RRR-DDD-YY-NNNN)
  - Time collected
  - Any other necessary identifiers

- All clinical specimens and samples should be accompanied by a case investigation form
Meningitis Specimen Handling and Processing
Why emphasizing proper specimen handling and processing?

- To reduce the risk of exposure to humans and the environment
- To maintain the integrity of the specimen
- To ensure accurate diagnosis and save lives
Important Considerations

- Always use safe laboratory practices and aseptic techniques when handling specimens.
- Treat all specimens as highly infectious.
  - *Unknown pathogens in clinical specimens!*
- Adhere to local and international regulations when transporting specimens.
- Coordination between the sender and recipient laboratories to ensure proper specimen transfer.
Overview of CSF Handling

Aliquot and freeze/keep on ice packs 250-500 ul

Aliquot 1-2 ml and transport at room temperature for microbiological tests (culture, RDT and gram stain)

Inoculate 0.5 -1 ml in T-I microbiology, rt-PCR, sequencing

Transport at room temperature
If transport delayed by > 1 h

For proper culture results, CSF specimens must be plated within 1 hour

*Adapted from WHO Manual

CSF Handling for rt-PCR

- rt-PCR is the confirmatory test of choice for meningitis.
- Direct rt-PCR is now used for meningitis testing.
- Aliquot between 250 – 500 ul CSF into cryotubes using aseptic conditions.
- Freeze immediately (if possible) or keep refrigerated and transport in triple packaging with ice packs to reference lab.
- rt-PCR can also be performed on inoculated T-I.
CSF Handling for **Microbiological Tests**

- Do not refrigerate the CSF specimen
  - Meningitis bacteria are fragile and die quickly at room or low temperatures (except deep freezing in proper media).

- Transport the specimens to the laboratory immediately within an hour.

- Coordinate with the receiving laboratory to ensure the immediate processing of the specimens once it arrives.
CSF Packaging and Transport

- Place specimens inside a clear biohazard bag along with a liquid absorbent pad that will quickly absorb the bodily fluids in case there is leakage.

- The bag should have a zipper closure to allow repeated access to specimen and be imprinted with biohazard symbol.

- Place all specimens and case report forms in a biohazard cooler and transport immediately to lab (if possible) at room temperature within one hour.

- **DO NOT** add in ice packs.


CSF Processing

If transport to laboratory <1 hour

Transport at RT

At the lab

Centrifuge at 1000x g for 10-15 minutes

- Supernatant
  - Rapid diagnostic test (RDT)
- Sediment
  - Primary plating on chocolate and blood agar
  - Gram stain

For proper culture results, CSF specimens must be plated within 1 hour

*Adapted from WHO manual
CSF Handling: T1 Inoculation

If unable to transport CSFs to a Reference Laboratory within 1 hour:

- Inoculate CSF into T-I media.
  - T-I is a biphasic medium that is useful for the primary culture and transport of meningitis bacteria
- T-I media should be stored at 4°C once received.

- Warm up T-I to room temperature (25°C or higher) before inoculation to avoid bacterial cold shock.

- Lift up metal lid and disinfect w/ 70% ethanol.

- Inoculate 0.5 -1.0 ml of CSF into T-I media, disinfect stopper with 70% ethanol.

- Label tube and invert several times to mix.
T-I Inoculation, Packaging, and Transport

- Do not vent if transport to lab is within 24 h.
- If transport times exceeds 24 h, vent.
- Insert a sterile cotton plugged needle to vent the T-I media (*Do not touch media*) and incubate at 35-37°C with 5% CO₂.
- If transport not possible within 4 days, remove from incubator and place at room temperature.
- **Remove needle**, disinfect with 70% ethanol and replace metal cap **before transporting**.
- Use triple packaging for shipping.
Inoculate T-I

*Vent (if delay >24h)*

- **incubate** at 35 - 37° C, 5% CO₂

→ **Transport Unvented @RT**

Aliquot

- Liquid phase → **Direct rt-PCR**
- 50-100ul liquid phase

At the lab

- Primary plating on CAP & BAP
- Gram stain

*Adapted from WHO manual*
Shipping and Packaging of Isolates
Importance of Bacterial Isolates

- **Surveillance of meningococcal antimicrobial resistance**
- **Whole genome sequencing**
  - Assess the genetic similarity at the strain level by phylogenetic analysis and understand the evolution of the strain
  - Track the spreading and transmission of infectious diseases through comparing the genetic fingerprints of different strains
  - Trace the origin of the epidemic strains and estimate the emerging time of the most recent ancestor
- **WHO Collaborative Centers (CDC Atlanta, NIPH Oslo, and IPP) play essential roles in molecular surveillance**
Media for Shipping Bacterial Isolates

I. **media** (for short term transport only)
   - Ship at room temperature
   - Isolate can survive up to 1 week

- **Silica gel sachets**
  - Shipped at 4°C (preferable) or at room temperature (25°C).
  - Isolates can survive for up to 2 weeks at 4°C

- **Blood or Chocolate agar slants**
  - Shipped at room temperature (25°C).
  - Isolate can survive for at least 1 week

- **Skim milk with 15% glycerol**
  - Shipped with dry ice
  - Isolates can survive for more than 1 week

- **Other media used:**
  - Skim Milk, Tryptone Glycerol, Glucose (STGG)
  - Tryptone soya broth plus glycerol (TSB)
  - Transported on dry-ice
International Shipping
(Will be covered in details in other lectures)

- **Leak-proof primary receptacle(s)**
- **Leak-proof secondary packaging including sufficient absorbent**
- **Rigid outer packaging**

Summary
## Transport Conditions

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Purpose</th>
<th>Transport Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF specimens</td>
<td>Microbiology/biochemistry</td>
<td>Room temperature (RT) to laboratory within 1 hour</td>
</tr>
<tr>
<td>CSF aliquot</td>
<td>Real-time PCR (rt-PCR)</td>
<td>-20°C or on ice packs within 7 days</td>
</tr>
<tr>
<td>T-I media</td>
<td>Microbiology/biochemistry rt-PCR, whole genome sequencing</td>
<td>Ship at RT, unvented, within 1 week or as soon as possible</td>
</tr>
<tr>
<td>Silica gel sachets</td>
<td>Microbiology/biochemistry rt-PCR, sequencing</td>
<td>Ice packs (preferable), RT is ok, within 1 week</td>
</tr>
<tr>
<td>Agar slants</td>
<td>Microbiology/biochemistry rt-PCR, sequencing</td>
<td>Room temperature within 1 week</td>
</tr>
<tr>
<td>Skim milk with 15% glycerol</td>
<td>Microbiology/biochemistry rt-PCR, sequencing</td>
<td>Dry ice with ice packs</td>
</tr>
</tbody>
</table>
Key Points

- Label specimens properly with key information.
- Include a case investigation form with each specimen.
- Transport the CSF immediately to the laboratory for microbiology tests.
- Ensure that there is someone available to receive the specimen prior to transporting.
- Proper collection, handling, storage, and transport conditions are all critical to the survival of the organism, reduction of contamination, and prevention of DNA degradation.
- Any problems with these areas can affect downstream testing and lead to false-negative and/or false-positive reporting.
References


Questions