



Microbiology Samples Management

WCSC Molecular Approaches to Clinical Microbiology in Africa,

30 August–6 September 2024

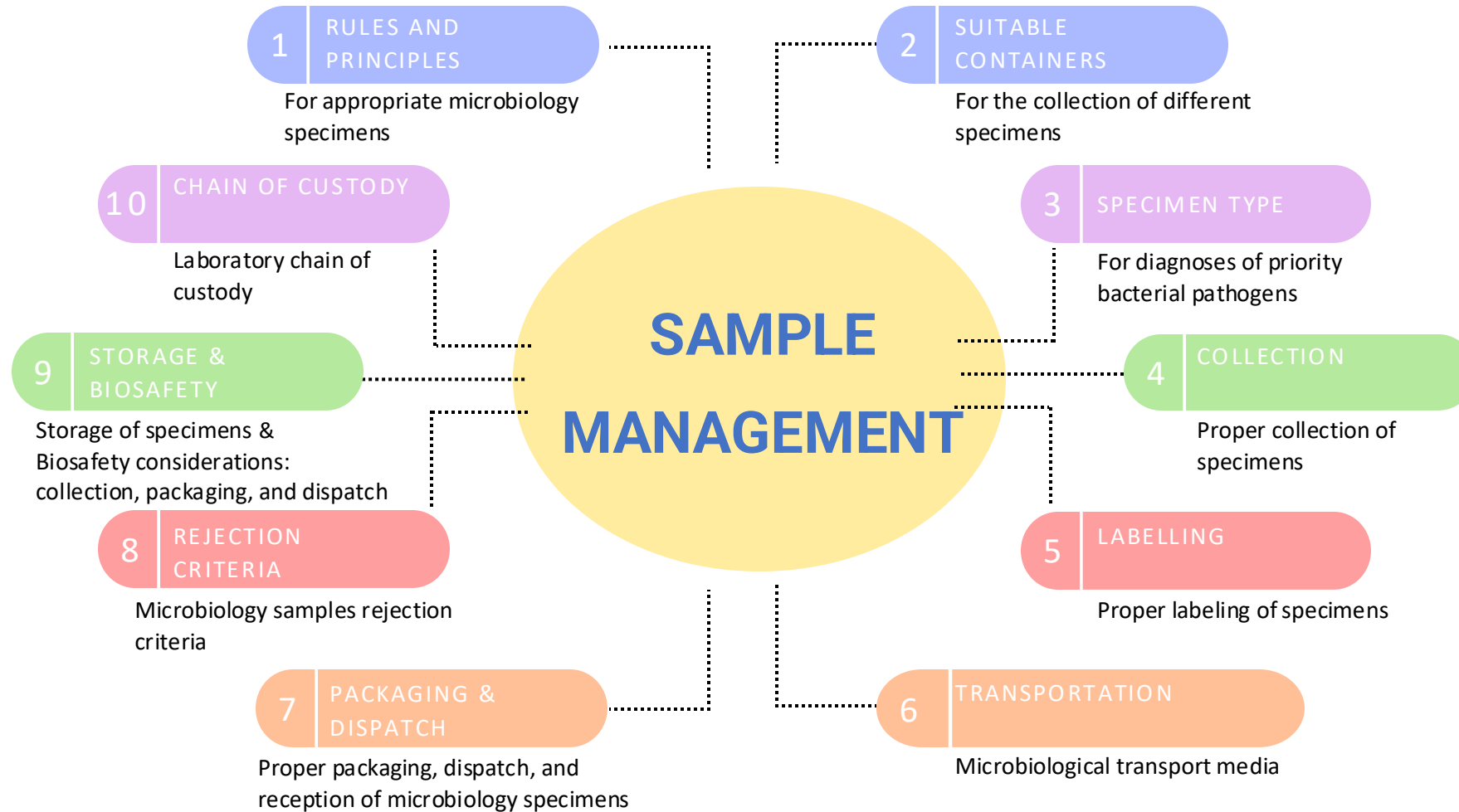
Stellenbosch University, Cape Town, S.Africa

Robert Onsare, *PhD*

OVERVIEW



OUTLINE



EXERCISE

- What are the most common Microbiology samples?



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- What are the most frequent causes of samples rejection?



Microbiology Specimen Collection Rules

- 1. Collect Before Antibiotic Therapy:** Obtain specimens before starting antibiotics to avoid altering the pathogen profile.
- 2. Target the Infected Site:** Collect specimens from the specific area where the infection is suspected for accurate results.
- 3. Observe Aseptic Techniques:** Use sterile equipment and follow proper hygiene practices to prevent contamination.
- 4. Consider Disease Stage:** Take into account the infection's stage, as it affects pathogen presence and specimen type.

Additional Rules for Specimen Collection

- 5. Instruct Patients Clearly:** Provide clear, specific instructions on specimen collection and delivery to ensure proper procedures
- 6. Use Proper Containers and Transport Media:** Choose the correct containers and media to maintain specimen viability during transport.
- 7. Deliver Specimens Promptly:** Ensure timely delivery to the laboratory to preserve specimen quality and accuracy of results.
- 8. Provide Sufficient Information:** Include comprehensive patient details, specimen source, and clinical history with each specimen for accurate analysis.

Specimen Collection Guidelines

- **Specimen Type:** Choose based on target pathogen (e.g., cervical swab for *N. gonorrhoeae*).
- **Containers and Preservatives:** Use appropriate sterile containers and transport media.
- **Timing:** Collect specimens before antibiotic therapy and at optimal disease stages.
- **Aseptic Techniques:** Use sterile tools and follow hygiene protocols to avoid contamination.
- **Labeling:** Clearly label with patient details, specimen type, and collection time.
- **Storage and Transport:** Keep specimens at 2–8°C; ensure proper conditions to maintain viability.
- **Urgent Processing:** Prioritize urgent specimens and have arrangements for after-hours processing.

Specimen Collection Kit

- Personal protective equipment (PPE)
- Collection containers with appropriate media
- Items for blood collection
- Secondary container or cooler
- Ice packs
- Request forms
- A pen or marker for labeling samples
- Labels

Examples of Sample Collection Containers



I. Blood Sample Collection for Culture

- **Indication for Blood Culture:** Required when **bacteraemia (septicaemia)** is suspected.
- **Timing:**
 - **Before Antimicrobials:** Collect blood **before starting antimicrobial treatment** whenever possible.
 - **During Fever:** For recurring fever, collect blood **as temperature begins to rise.**
- **Collection Technique:**
 - **Aseptic Method:** Employ **strict aseptic techniques** to avoid contamination.
 - **Disinfection:** Thoroughly disinfect the skin before drawing blood.
 - **Volume and Timing:** Ensure an **adequate volume** is collected and follow appropriate timing for accurate results.
- **Key Factors:**
 - Disinfection
 - Adequate Volume
 - Appropriate Timing

TYPES OF SAMPLES & KEY CONSIDERATIONS

I. Blood Sample Collection: Key Considerations

- **Avoid IV Line Contamination:**
 - **Caution:** Blood from indwelling IV lines may have **surface contaminants**.
 - **Issue:** Contaminants can cause **false-positive culture results**.
 - **Recommendation:** Draw blood directly from a fresh puncture site.
- **Optimal Collection Strategy:**
 - **Two Sets:** Collect **two sets** of blood cultures.
 - **Media:** Use both **aerobic and anaerobic media** for each set.
 - **Timing:** Collect at **different times** to improve organism detection.
- **Procedure for Accuracy:**
 - **Sites:** Preferably draw from **different sites**.
 - **Timing:** Collect each set at **different times** for better results.

Collection Guidelines for Venous Blood

- **Storage of Blood Culture Bottles:**
 - **Follow Manufacturer Instructions:** Store bottles as directed.
 - **Pre-Warming:** If stored at 4°C, **pre-warm** to room temperature (25°C) or 37°C before use.
- **Site Preparation:**
 - **Disinfect Stopper:** Clean the **rubber stopper** of the culture bottle.
 - **Vein Palpation:** **Palpate the vein** before disinfection of the venipuncture site.
 - **Disinfect Site:** Use **70% alcohol** to disinfect the collection site and allow it to **dry**.
 - **Avoid Palpation with Non-Sterile Gloves:** Do not palpate the vein again without **sterile gloves**.
- **Collection and Handling:**
 - **Collect Blood:** Draw the blood into the bottle.
 - **Mix Thoroughly:** Gently **invert the bottle** to mix.
 - **Remove Iodine:** After venipuncture, remove any iodine residue with **alcohol**

Transport and Storage Guidelines for Blood Cultures

- **Transport Time and Conditions:**
 - **Time:** Ensure transport is completed within **2 hours** at **room temperature (RT)**.
 - **Position:** Transport bottles **upright** with cushioning to **prevent hemolysis**.
 - **Protection:** Wrap bottles in **absorbent cotton** for added protection.
- **Storage Conditions:**
 - **Avoid Refrigeration:** **Do not** place inoculated bottles in the refrigerator.
 - **Temperature Range:** Protect from temperature extremes, ensuring temperatures **not $<18^{\circ}\text{C}$ or $>37^{\circ}\text{C}$** .

2. Cerebrospinal Fluid (CSF)

Guidelines for Lumbar Puncture and CSF Collection

- **Notify the Laboratory:**
 - **Pre-Procedure:** Inform the laboratory **before performing a lumbar puncture** to ensure readiness and appropriate processing.
- **Additional Sample:**
 - **Blood Culture:** Obtain blood samples for culture in addition to cerebrospinal fluid (CSF) to aid in diagnosis.
- **Timely Processing:**
 - **Examination Delay:** Minimize delay in examining CSF to **improve pathogen isolation.**
 - **Plating Time:** Plate **CSF** samples within **1 hour** of collection.

Use	Volume (Adults)
Bacterial culture	>1 ml
Fungal culture	8-10 ml
Molecular test	>1 ml
Mycobacterial culture	8-10 ml
Viral culture	>2 ml

Cerebrospinal Fluid (CSF) (cont.)

- If Trans-Isolate (T-I) media is available, use for **transportation** (useful for the primary culture of meningococci and other etiological agents of bacterial meningitis (*S. pneumoniae* and *H. influenzae*) from CSF
- **Transport time:**
 - Immediately within 15 minutes at RT. CSF for bacterial culture should never be refrigerated.
 - CSF collected in T-I media; up to 4 days at RT. Vent the T-I bottle by inserting a needle through the rubber stopper. No need to vent if bottle is sent to the lab same day.
- **Storage:**
 - At RT for up to 24 hours.
 - Never refrigerate CSF for bacterial culture, do not expose to extreme cold, excessive heat, or sunlight. For virus culture and other analysis store at 4°C for 48 hours, or -70°C for longer durations.

3. Stool Sample Collection and Handling

- **Preferred Sample:**
 - **Stool:** Always superior to a **rectal swab** for bacterial testing.
 - **Rectal Swab:** If the only option, insert **deep enough** into the rectum to contact stool (swab should appear brown).
- **Swabs:**
 - **Use:** Generally not recommended for routine pathogens except for **infants**.
 - **Transport Media:** Always submit swabs in **transport media** to preserve pathogen viability.
- **Collection Tips:**
 - **Multiple Samples:** Collecting more than one stool sample is usually **not productive** for bacterial isolation.
 - **Visual Examination:** Preferably sample areas of stool with **blood, pus, or mucus**.
 - **Formed Stools:** Testing **formed stools** is less effective for bacterial isolation.
- **Routine Cultures:**
 - **Hospitalization:** Avoid routine stool cultures for patients hospitalized for **more than 3 days** without a diagnosis of **gastroenteritis**.

Stool Sample Collection

Sample Patient Instructions



Label a stool container with patient details



Instruct patient to poop on a clean towel



scoop stool at different points especially areas with blood, pus



Screw back the cap tightly with the scoop attached

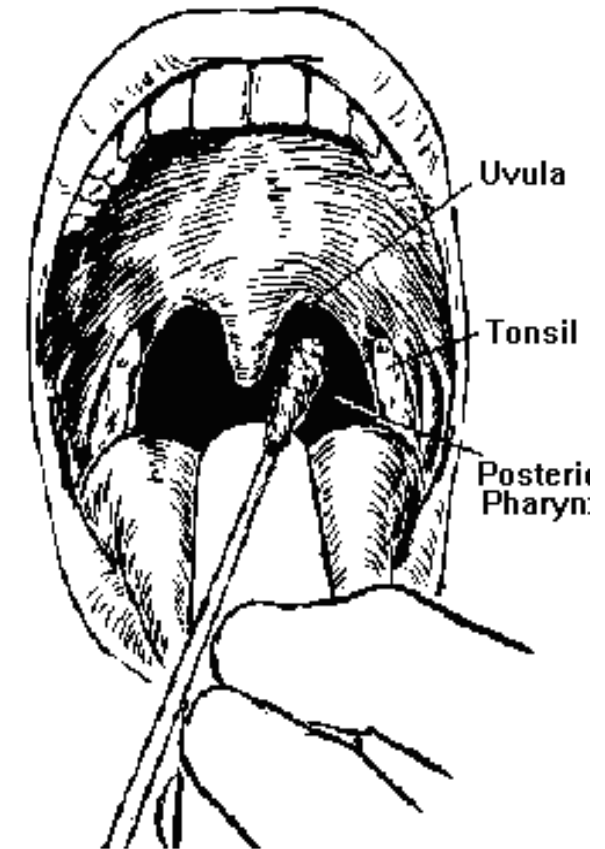
Please note: Proper instructions to the patient is important to avoid receiving samples that are contaminated with water, urine and other substances.

Stool Sample Transport and Storage Guidelines

- **Transport Time:**
 - **Immediate Delivery:** Deliver samples to the laboratory without delay.
 - **Viability at Room Temperature:** Bacteria are viable for up to **2 hours** at RT.
 - **Anticipated Delays:** Transport samples in **Cary-Blair medium** to maintain viability for up to **24 hours** at RT.
- **Storage:**
 - **Unpreserved Stools:** Store at **4°C** only if testing within **24 hours** (not recommended).
 - **Transport Media:** Stool samples in transport media can be stored at **4°C** for up to **48 hours**.

4. Throat Swab (Posterior Pharyngeal Swab)

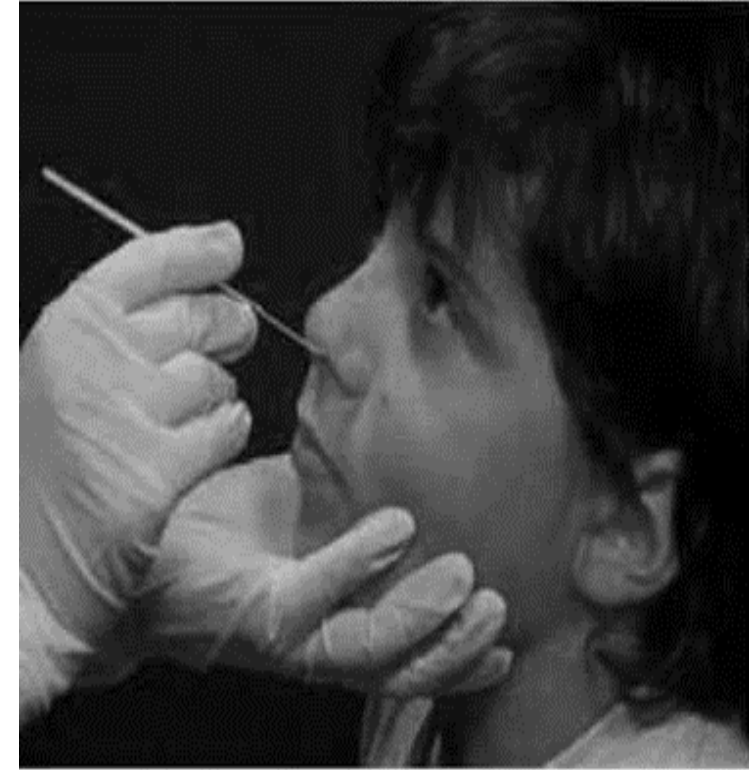
- Throat swabs are contraindicated for patients with epiglottitis.
- **Collection guidelines:**
 - Hold tongue away with tongue depressor.
 - Locate areas of inflammation and exudate in posterior pharynx, tonsillar region of throat behind uvula.
 - Avoid swabbing soft palate; do not touch tongue.
 - Rub area back and forth with sterile cotton or Dacron swab.
- **Transport time:**
 - Dry swabs, with or without silica gel, can be transported to the lab within 2 hours at RT.
 - Swabs collected in transport media (Amies or Stuart) are acceptable for up to 48 hours at RT.
- **Storage:**
 - Less than 24 hours at RT.



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5. Nasopharyngeal Swab Collection Guidelines

- **Purpose:** Used to identify carriers of ***S. aureus***, ***N. meningitidis***, and other pathogens.
- **Collection Procedure:**
 - **Head Position:** Tilt the patient's head **backwards**.
 - **Swab Insertion:** Insert a **flexible, fine-shafted polyester swab** into the nostril, advancing to the nasopharynx.
 - **Moistening:** Swab may be moistened in **sterile normal saline** if needed.
 - **Contact Duration:** Leave the swab in place for a **few seconds** to ensure proper sampling.
 - **Nasal Pressure:** Gently **press the nose inward** during collection to maximize swab contact.
 - **Withdrawal:** Remove the swab **slowly** using a **rotating motion**.



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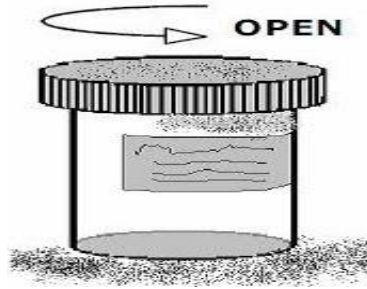
Transport and Storage Guidelines for Nasopharyngeal Swabs

- **Transport Time:**
 - **Inoculated Plates:** Plate can be **inoculated at the bedside** and **delivered to the lab within 15 minutes** at room temperature (RT).
 - **Swabs with Transport Medium:** Swabs in **Amies or Stuart's medium** are acceptable for **up to 48 hours** at RT.
 - **Swabs without Transport Medium:** Transport to the lab **within 2 hours**.
- **Storage:**
 - **Inoculated Plates:** Store at RT for **up to 24 hours**.
 - **Unpreserved Swabs:** Store at **4°C** for **up to 24 hours**.
 - **Swabs in Transport Medium (TM):** Store at RT for **up to 48 hours**.

6. Sputum Samples



1. Gargle or rinse mouth with water and spit out.



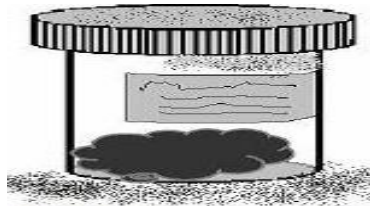
2. Open the sample container.



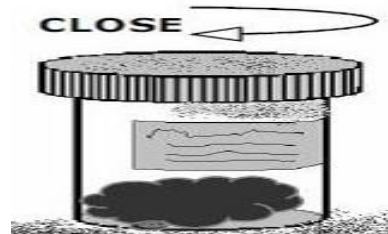
3. Hold the container to your mouth with your lips inside it.



4. Take a deep breath as much as you can and cough into the container. **Do not just spit saliva.**



5. The sample you cough should look thick, yellow, or green.



6. Close the container lid tightly



7. Take the sample to your caregiver without delay.

Note: .Expectorated sputum is the best non-invasive sample for diagnosis of pneumonia, TB (Pathogens: M. tuberculosis, S.pneumoniae, H.influenza, among others .It can be expectorated or induced sputum.

Sputum Samples Collection and Transportation

- **Sputum Sample Collection and Transport**
- **Transport Time:**
 - **Immediate Delivery:** Deliver the sputum sample to the lab with **minimal delay**.
 - **Critical Timing:** Organisms like **S. pneumoniae** and **H. influenzae** need culturing **within 1 hour** at room temperature (RT).
 - **For Delays Beyond 2 Hours:** Transfer a purulent part of the sputum to a **cotton swab** and place it in a **tube with Amies transport medium**.
- **Storage:**
 - **Short-Term Storage:** Keep at **4°C** for **less than 24 hours**.
 - **Important Note:** Specimens for **S. pneumoniae** and **H. influenzae** **MUST NEVER BE REFRIGERATED**.

7. Genitourinary Tract Sample Collection:

(i) Urine Samples

- **Diseases:** Urinary tract issues include **prostatitis**, **urethral syndrome**, **cystitis**, and **pyelonephritis**.
- **Collection Methods:**
 - **Midstream Collection:** Collect urine midstream to minimize contamination.
 - **Catheterization:** Use straight (in-out) or indwelling catheters for collection if necessary.
 - **Other Methods:** Alternative methods may be used based on clinical needs.
- **Optimal Sample:**
 - **First-Voided Morning Urine:** Best for detecting bacterial growth as bacteria have been multiplying in the bladder overnight.

Urine Sample Collection Guidelines

- **Cleansing:**
 - **Effectiveness:** Cleansing of periurethral areas does not significantly improve urine culture quality.
 - **If Cleaning:** Use **soap** rather than disinfectants; disinfectants can **inhibit microorganism growth**.
- **Collection Containers:**
 - **Use:** Sterile, leak-proof **wide-mouthed tubes or cups** for collection and transport.
- **Collection Guidelines:**
 - **Females:** **Hold labia apart** during voiding to minimize contamination.
 - **Males:** **Retract the foreskin** (if not circumcised) and keep it retracted during collection.
 - **Midstream Collection:**
 - Allow a few milliliters of urine to pass.
 - **Collect the midstream portion** in a sterile container.

Urine Sample Collection from Indwelling Catheters

- **Procedure:**
 - **Clean Port:** Wipe the **catheter collection port** with a **70% alcohol swab**.
 - **Sterile Technique:** Use **sterile technique** to avoid contamination.
 - **Needle and Syringe:** Puncture the collection port with a **needle attached to a syringe**.
 - **Aspirate and Transfer:** **Aspirate** the urine and transfer it to a **sterile container**.
- **Important Note:**
 - **Bacterial Presence:** Patients with indwelling catheters often have **bacteria in their bladders**.
 - **Symptomatic Collection:** Only collect urine from these patients if they are **symptomatic**.

Urine Sample Collection: Straight Catheter

- **Collection Procedure:**
 - **Clean Area:**
 - **Urethral Opening:** Clean with **soap** and rinse with **water**.
 - **Females:** Clean the **vaginal vestibule** as well.
 - **Catheter Insertion:**
 - Use **sterile technique** to insert the catheter into the bladder.
 - **Initial Collection:**
 - Discard the first **15 to 30 ml** of urine.
 - **Sample Collection:**
 - Collect the urine from the **mid- or later flow** in a **sterile container**.
- **Transport Time:**
 - **Timeliness:** Transport to the laboratory so that it is **plated within 1 hour** of collection.
 - **Peripheral Facilities:** Refrigerate at **4°C** for up to **24 hours** before plating.
- **Storage:**
 - **Refrigeration:** Store at **4°C** for **up to 24 hours** before plating.

Genitourinary Tract Sample Collection .. Cont'd:

(ii) Urethral Swabs: Collection and Transport Guidelines

- **Urethral Swabs: Collection and Transport Guidelines**
- **Collection Procedure:**
 - **Timing:** Collect at least 1 hour after the last urination.
 - **Preparation:** Remove any old exudate from the urethral orifice.
 - **Discharge Collection:**
 - **Penile Discharge:** Massage the urethra to collect discharge on a swab.
 - **No Discharge:** Wash periurethral area with **povidone-iodine soap**, rinse with water, then insert a **small swab 2–4 cm** into the urethra. Rotate and leave in place for **at least 2 seconds**.
- **Transport Time:**
 - **Timeliness:** Transport the swab to the lab in **less than 2 hours** at room temperature (RT).
 - **Amies Medium:** Swabs collected in **Amies transport medium** are viable for up to **24 hours** at RT. Inoculate the swab before placing it in the transport medium.
- **Storage:**
 - **Duration:** Store at RT for **less than 24 hours**.

Genitourinary Tract Sample Collection .. Cont'd:

(iii) Swabs: Collection Guidelines

- **Vaginal Swabs**
- **Consideration:** Often less informative; **cervical** and **high vaginal swabs** are preferred.
- **Procedure:**
 - **Speculum:** Use a speculum **without lubricant**.
 - **Collection:** Obtain secretions from the **mucosa high in the vaginal canal** with a **sterile swab**.
 - **Transport:** Place the swab in an appropriate **transport system** (e.g., Amies medium).
- **Cervical Swabs**
- **Lubrication:** Use only **warm water**, avoid other lubricants.
- **Procedure:**
 - **Speculum:** Insert and visualize the **cervix opening**.
 - **Mucus Removal:** Use a **cotton ball** to remove excess mucus.
 - **Swab Insertion:** Insert a **dacron swab** into the distal portion of the cervix, **rotate gently**, and leave for **10 to 30 seconds**.
 - **Transport:** Place the swab in **Amies transport medium** and transport at **ambient temperature**.

8. Abscess Sampling Guidelines

- **Preferred Sample:**
 - **Tissue or Aspirate:** Always superior to a swab for accurate results.
- **Swabs:**
 - **Culture:** Perform **aerobic culture only**.
 - **Number of Swabs:** Collect **two swabs**—one for culture and one for **Gram staining**.
 - **Preservation:** Place swabs in **Stuart's** or **Amies medium**.
- **Preparation:**
 - **Surface Exudate:** Remove by wiping with **sterile saline** or **70% alcohol**.
- **Collection Guidelines:**
 - **Open Abscesses:**
 - **Aspirate** if possible.
 - Alternatively, pass a swab **deep into the lesion** to sample the “**fresh border**”.
 - **Closed Abscesses:**
 - **Aspirate** abscess material using a **needle and syringe**.
 - Aseptically transfer all material into an **anaerobic transport device**.

Abscess Sample Transport and Storage

- **Transport Time and Conditions:**
 - **Timeframe:** Transport samples within **less than 2 hours** at **room temperature (RT)**.
 - **Conditions:** Use an **anaerobic transport device**.
- **Storage:**
 - **General Storage:** Keep at **4°C** for **less than 24 hours**.
 - **Swabs in Transport Medium:**
 - **RT or 4°C:** Viable for up to **4 days**.

9. Fluid Sample Collection and Handling

- **Examples of Fluids:**
 - **Pleural, Ascitic, Synovial, Pericardial, Peritoneal** fluids.
- **Collection:**
 - **Professional Collection:** Recommended to be collected by a **clinician or physician**.
- **Transport:**
 - **Prompt Delivery:** Deliver to the laboratory **immediately** for testing.
 - **Delayed Analysis:** If analysis is delayed **beyond 2 hours, refrigerate** the specimen.
- **Note:**
 - **Handling Variations:** Handling may differ depending on the **suspected pathogen**.

Labeling Specimens: Key Considerations

- Patient's name
- Age
- Sex
- Clinical specimen type
- Unique ID number (Research/Outbreak)
- Specimen type
- Date, time, and place of collection
- Name or initials of collector

Microbiological Samples Transportation

- **General Guidelines:**
 - **Prompt Delivery:** Samples should be transported **immediately** after collection.
 - **Inoculation:** Inoculate bacterial culture media within **24 hours**, even if appropriate holding media or refrigeration is used.
- **Special Considerations:**
 - **Bacterial Sensitivity:** Some bacteria are sensitive to **ambient conditions**.
 - **Immediate Processing:** Reliable detection requires **immediate processing**.
 - **Transport Media:** Delays up to **6 hours** can lead to loss of **colony-forming units (CFU)**; **longer delays** cause significant loss of organisms.
- **Packaging and Labeling:**
 - **Adherence:** Follow **specimen packaging and labeling instructions** for safe transport between laboratories.

Transport Media for Microbiological Samples

- **Transport Media for Microbiological Samples**
- **Purpose:**
 - **Preservation:** Transport media preserve **bacterial viability** during transport without allowing **multiplication**.
 - **Composition:** Typically include buffers, carbohydrates, peptones, and nutrients (excluding growth factors). May also contain **antibiotics** or **glycerol**.
- **Examples:**
 - **Cary-Blair Medium:** Used for **stool samples**.
 - **Amies Medium with Charcoal:** Ideal for isolating **Campylobacter**.
 - **Amies Medium without Charcoal:** Suitable for **Mycoplasma** and **Ureaplasma**.
 - **Stuarts Medium:** For **throat, wound, and skin swabs** that may contain **fastidious organisms**.
 - **Sach's Buffered Glycerol Saline:** Used for **bacillary dysentery stool samples**.
 - **Anaerobic Transport Medium (ATM):** e.g., **Thioglycolate broth** for anaerobes.
 - **Trans-Isolate (T-I):** Ideal for **CSF samples**.

Clinical Samples Packaging and Dispatch

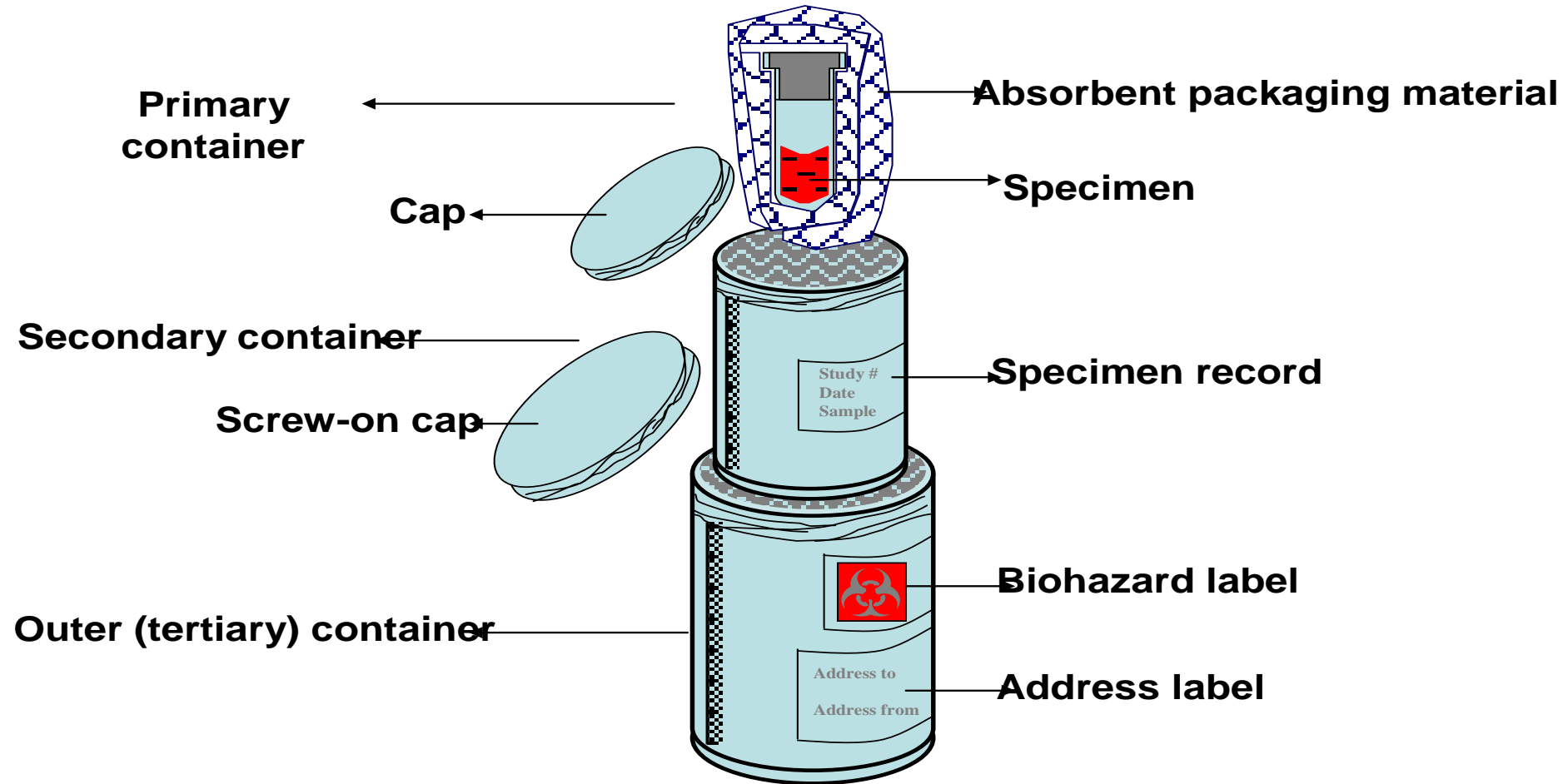
- **Unpreserved Specimens:**
 - **Temperature:** Deliver at the **appropriate temperature** for the target organism.
- **Packaging:**
 - **Triple Packaging:** Adhere to **triple packaging requirements** for safe dispatch:
 - **Primary Container:** Leak-proof container holding the sample.
 - **Secondary Container:** Durable outer container with absorbent material.
 - **Tertiary Packaging:** Outermost packaging for transport, including labeling and documentation.
- **Record-Keeping:**
 - **Sample Register:** Maintain a **register of samples** sent to the testing laboratory for tracking and verification.

IATA Regulations for Sample Transportation

- **IATA Regulations for Sample Transport**
- **Sample Categories:**
 - **Category A:**
 - **Definition:** Infectious substances capable of causing **permanent disability, life-threatening, or fatal disease** to humans or animals.
 - **Category B:**
 - **Definition:** Infectious substances that do not meet **Category A** criteria.
 - **Shipping Name:** "Biological substance, Category B."
- **Exemptions:**
 - **Minimal Risk:** Samples with minimal likelihood of containing pathogens may be exempt from stringent regulations.
- **Packaging Requirements:**
 - **Triple Packaging:** Essential for all potentially hazardous materials to ensure safe transport:
 - **Primary Container:** Leak-proof container holding the sample.
 - **Secondary Container:** Durable outer container with absorbent material.
 - **Tertiary Packaging:** Outermost packaging for transport with appropriate labeling.

IATA Requirement: Triple Packaging

Packing & shipping infectious material



Steps for Triple Packaging

1. Don Personal Protective Equipment (PPE):

1. **Gloves:** Recommended to don **2 pairs of gloves** for added protection.

2. Prepare the Primary Container:

1. **Labeling:** Ensure the specimen container is **well labeled**.

2. **Closure:** Check that the container is **tightly closed**.

3. Wrap the Primary Container:

1. **Absorbent Material:** Wrap the primary container with **absorbent material** to manage any potential leaks.

4. Place in Secondary Container:

1. **Secondary Container:** Put the wrapped primary container inside a **bigger secondary container**.

2. **Closure:** Close the secondary container securely.

Steps for Triple Packaging (cont.)

Remove and Dispose of Outer Gloves:

1. **Gloves:** Remove the outer gloves and **dispose of them appropriately.**

Prepare the Tertiary Container:

1. **Place Secondary Container:** Put the **secondary container** into a **bigger tertiary container.**
2. **Request Form:** Place the **patient request form** in a plastic bag and insert it into the tertiary container.
3. **Close:** Securely **close the tertiary container.**

Labeling:

1. **Addresses:** Label the outer container with **referring and referral laboratory addresses** and **contact persons.**
2. **Biohazard Label:** Add the **biohazard label** to the outer container.

Reference Resources

- Video: **Steps for Triple Packaging**
- 1. <https://www.youtube.com/watch?v=GJK9FRT4IXM>
- 2, <https://www.youtube.com/watch?v=p99WYyB5o64>
- 3, <https://www.youtube.com/watch?v=04PLtLyd0l8>

Samples Reception

- **Importance:**
 - Proper sample reception is crucial for maintaining the **integrity of the entire workflow**. Ineffective procedures can compromise results.
- **Documentation:**
 - **Well-Documented Procedure:** Ensures **traceability** and proper handling of samples through reception and rejection.
- **Key SOP Components:**
 - **Acceptance/Rejection Criteria:** Clear criteria for **accepting or rejecting samples** based on quality and compliance.
 - **Logging Samples:** Instructions for **logging samples** in **physical or electronic registers**.
 - **Lab IDs:** Procedure for **assigning laboratory identification numbers** to samples.
 - **Urgent Samples:** Guidelines for **handling urgent samples** to prioritize processing.
 - **Clinical Data:** Requirements for **clinical data** that may be needed for specific investigations.

General Samples Rejection Criteria

- **General Sample Rejection Criteria**
- **Unlabeled or Improperly Labeled Specimens:**
 - **Issue:** Samples must have correct and complete labels.
- **Label Mismatch:**
 - **Issue:** Labels must match details on the **request form**.
- **Incomplete Request Forms:**
 - **Issue:** Forms should include complete **patient information, tests requested, and clinical details**.
- **Wrong Container:**
 - **Issue:** Specimens must be collected in the **correct container**.
- **Leaking Samples:**
 - **Issue:** Leaking samples cannot be processed.

General Samples Rejection Criteria .. Cont.

- **Missing Request Forms or Specimens:**
 - **Issue:** Each request form must be accompanied by the appropriate **specimen** and vice versa.
- **Unavailable Test:**
 - **Issue:** The requested test must be **available** in the laboratory.
- **Canceled or Incorrect Test Orders:**
 - **Issue:** Tests ordered must match those requested; **canceled orders** or **wrong orders** lead to rejection.
- **Specimen Delays:**
 - **Issue:** Samples delayed **in transit** may be rejected.
- **Insufficient Volume:**
 - **Issue:** Specimens must have **sufficient volume** for the requested test.

Specimen Rejection Criteria

Sample	Rejection
Stool	Formed stools
	Sample collected after more than 72 hours of admission and gastroenteritis was not the reason for admission
	Multiple samples collected from the same patient, same day
	Sample received after 2 hours without transport media
	If specimen in transport medium is delayed for more than 3 days at 4°C or is delayed for more than 24 hours at 25°C
	Rectal swabs for adults
Foley Catheter Tips	Unsuitable sample
Sputum	> 25 squamous epithelial cells/lower power field
	Saliva in place of sputum
Dry Swabs	If received in the lab after 1 hour
Swabs	Received when transport media is dried
	Collection site not stated
Blood	Cracked bottle
	Over-filled or under-filled bottles
	Received more than 12 hours after collection
	Clotted blood

Storage and Disposal of Samples

- **Written Policies:**
 - **Description:** Define:
 - **Which Samples:** Which samples need storage.
 - **Retention Time:** Duration for which samples should be kept.
 - **Location:** Storage location specifics.
 - **Conditions:** Storage conditions and temperature.
 - **Organization System:** System for organizing stored samples.
- **Monitoring:**
 - **Freeze/Thaw Cycles:** Critical to monitor to prevent sample deterioration.
- **Storage Before Testing:**
 - **Temperature:** Most specimens stored at **2–8°C**.
 - **Pathogen Dependence:** Storage conditions may vary based on the **suspect pathogen**.
- **Storage Post Testing:**
 - **Temperature:** Store at **2–8°C**.
 - **Duration:** Typically **7 days** or as per **institution policies**.
- **Disposal:**
 - **Type:** Dispose of as **infectious waste** according to safety protocols.

Storage of Isolates: General Guidelines

- **Regular Use:**
 - **Short-Term Storage:** Bacterial cultures used **daily/weekly** can be stored:
 - **On Agar Plates** or
 - **In Stab Cultures**
 - **At 4°C** in a standard refrigerator.
- **Long-Term Storage:**
 - **For Cultures Not Used for Weeks:**
 - **Consider Long-Term Options** to ensure maximum bacterial viability.
 - **Methods:** Options include:
 - **Freezing** at ultra-low temperatures.
 - **Lyophilization (Freeze-Drying).**

Condition	Temp (°C)	Time (approx.)
Agar plates	4	4–6 weeks
Stab cultures	4	3 weeks–1 year
Standard freezer	-20	1–3 years
Super-cooled freezer	-80	1–10 years
Freeze dried	≤4	15 years+

Storage of Isolates for Long-Term Viability: Key Considerations

- **Cryoprotectants:**
 - **Purpose:** Additives used to:
 - **Lower Freezing Point:** Protect bacterial cells during freezing.
 - **Minimize Damage:** Reduce detrimental effects of ice crystal formation and increased solute concentration.
 - **Common Cryoprotectants:**
 - **Glycerol & Dimethylsulfoxide (DMSO).**
 - **Concentration:** Typically used at **5–15% (v/v)**.
- **Steps for Freezing Samples:**
 - **Prepare Cryoprotectant:**
 - **Autoclave Glycerol** and add to bacterial suspension.
 - **Mix:**
 - **Vortex** for even mixing.
 - **Aliquot:**
 - Place the suspension into **cryogenic screw cap vials**.
 - **Freeze:**
 - Store vials in a **freezing container** at **-20°C to -80°C** overnight.
 - **Transfer to Liquid Nitrogen** for long-term storage.
- **Alternative Method:**
 - **Freeze-Drying (Lyophilization):**
 - Use **lyophilization medium** for preservation.



Biosafety in Sample Collection and Handling

1. Protect the Patient:

- **Single-Use Equipment:** Always use **single-use needles and syringes**.
- **Disinfection:** Properly **disinfect the sample collection site**.
- **Clean Area:** Conduct procedures in a **clean, dedicated area**.

2. Protect Yourself:

- **Personal Protective Equipment (PPE):** Wear appropriate PPE to ensure safety.
- **Needle Disposal:** Discard used needles immediately into **sharps containers** to prevent needle-stick injuries.
- **First Aid:** Keep a **first aid kit** readily accessible for emergencies.
- **Avoid Reuse:** **Do not reuse contaminated equipment**.

Biosafety .. Cont'd : Protect others and the Environment

- **Protect Others and the Environment**
- **Sample Packaging:**
 - **Appropriate Packaging:** Ensure samples are **packaged correctly** for safe transport.
- **Decontamination:**
 - **Spills:** Decontaminate spills using **10% bleach**.
 - **Equipment:** Soak contaminated non-disposable equipment in **5% household bleach** for up to **30 minutes**.
- **Disinfection:**
 - **Working Areas:** Disinfect with **5% household bleach** daily.
- **Waste Management:**
 - **Biohazard Bags:** Place waste in **leak-proof biohazard bags**.
 - **Final Management:** Ensure **safe final disposal** of biohazard waste.

Chain of Custody (COC)

- **Definition:**
 - **Traceable Records:** Unbroken control over documents, raw data, or samples from **initial collection** to **final disposal**.
- **Requirements:**
 - **Quality Management:** Essential for compliance with **GLP, ISO 15189, and ISO 17025**.
- **Historical Practice:**
 - **Paper Documents:** Traditionally managed through extensive **paper documentation**.
- **Modern Practice:**
 - **LIMS: Laboratory Information Management Systems** are increasingly replacing manual systems.
- **Purpose:**
 - **Quality Maintenance:** Ensures sample integrity during **collection, transportation, and storage** before and after analysis.
- **COC Documentation:**
 - **Communicates Instructions:** From reference lab to testing lab.
 - **Permanent Record:** Provides a record of sample handling.
 - **Evidence of Handling:** Shows that samples were handled by **authorized, trained personnel**.

Chain of Custody .. Cont'd)

Paper-Based COC Documentation

1. Sample Transport Logbook

- 1. Records details of sample transport, including date, time, and personnel involved.

2. Referral Register

- 1. Logs details of samples referred to other locations or laboratories.

3. Reception Registers

- 1. Tracks the receipt of samples at different stages or locations.

Electronic COC

1. Types of Electronic COCs

- 1. **Sample-Based COCs:** Tracking is centered around individual samples.

- 2. **Container-Based COCs:** Each container has a unique, unalterable ID and description recognized by the Laboratory Information Management System (LIMS). The unique ID and user-friendly description ensures security of sample containers.

Chain of Custody .. Cont'd)

- **Electronic COC .. Cpnt'd**

3. Location-Based COCs: Utilizes Radio Frequency Identification (RFID) to track containers with embedded microchips and antennas. RFID readers monitor samples in and between locations.

4. Tracking Sample Volumes

I. Electronic systems can also track sample volumes.

5. Electronic Signatures

I. Authenticate user identity for sample acceptance, release, transfers, and disposals

Sample Tracking and Custody Procedures

- **Labeling and Documentation**

1. Sample Labeling

1. **Unique Identifiers:** Each sample must have a unique identifier.
2. **Additional Details:** Include sampling location, date, time, and the person who collected the sample.

2. Request Form

1. Capture essential details about the sample including unique identifiers, collection information, and collector details.

- **Record Keeping**

1. Referral Register

1. **For Dispatched Samples:** Record all pertinent information when samples are sent out.

2. Reception Register

1. **For Testing Labs:** Log all relevant details upon receipt of samples in the lab.

- **Chain of Custody (COC)**

1. Transfer Documentation

1. **COC Form:** Each transfer of custody must be documented with a signature, date, and time.

2. Custody Responsibilities

1. **Transport and Storage Conditions:** Ensure samples are kept under the correct conditions as specified.

Summary of Key Messages

- **Specimen Collection**

1. **Timing of Collection**

1. **Before Antibiotic Therapy:** Collect specimens before initiating antibiotic treatment to avoid compromising test results.

2. **Aseptic Techniques**

1. **Key in Microbiology:** Employ strict aseptic techniques during sample collection to prevent contamination.

- **Specimen Packaging**

1. **International Guidelines**

1. **Infectious Materials:** Follow international guidelines for the packaging of specimens for dispatch to reference laboratories.

- **Documentation and Retention**

1. **Request Forms**

1. **Completion:** Ensure all samples are accompanied by well-completed request forms.

2. **Sample Retention**

1. **Post-Testing:** Retain tested samples for a designated period before disposal, as per regulatory requirements.

- **Chain of Custody (COC)**

1. **Importance**

1. **Quality Tracking:** COC documentation is crucial for tracking sample quality, adhering to biosafety and biosecurity standards.

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THANK YOU