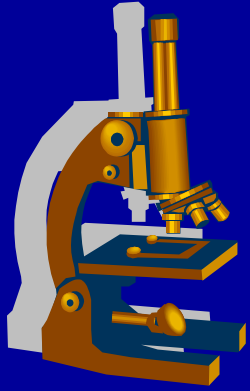


تجميع العينات/ التشخيص المعملية والأمان الحيوي

Specimen Collection, Laboratory Diagnostics, and Biosafety Issues



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دورة تكوين فرق الاستجابة السريعة لمكافحة جائحة انفلونزا الطيور
المختبر المرجعي للأمراض السارية
المركز الوطني للوقاية من الأمراض السارية والمتوطنة ومكافحتها
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Learning Objectives

- **Prepare and maintain collection kits**
- **Collect and transport specimens safely and correctly**
- **Manage laboratory specimens safely**
- **Interpret laboratory data**

Session Overview

- **Specimen collection**
- **Specimen storage, handling, shipping**
- **Disposing infectious waste in the field**
- **Safety precautions in the laboratory**
- **Working with reference laboratories**
- **Managing laboratory data**

Specimen Collection Kit

- Collection vials with viral transport media (VTM)
- Polyester fiber-tipped applicators
- Tongue depressors
- Secondary container
- Ice packs
- Items for blood collection
- Personal protective equipment
- Field collection forms
- A pen or marker for labeling samples or cryolabels

What is Viral Transport Medium?

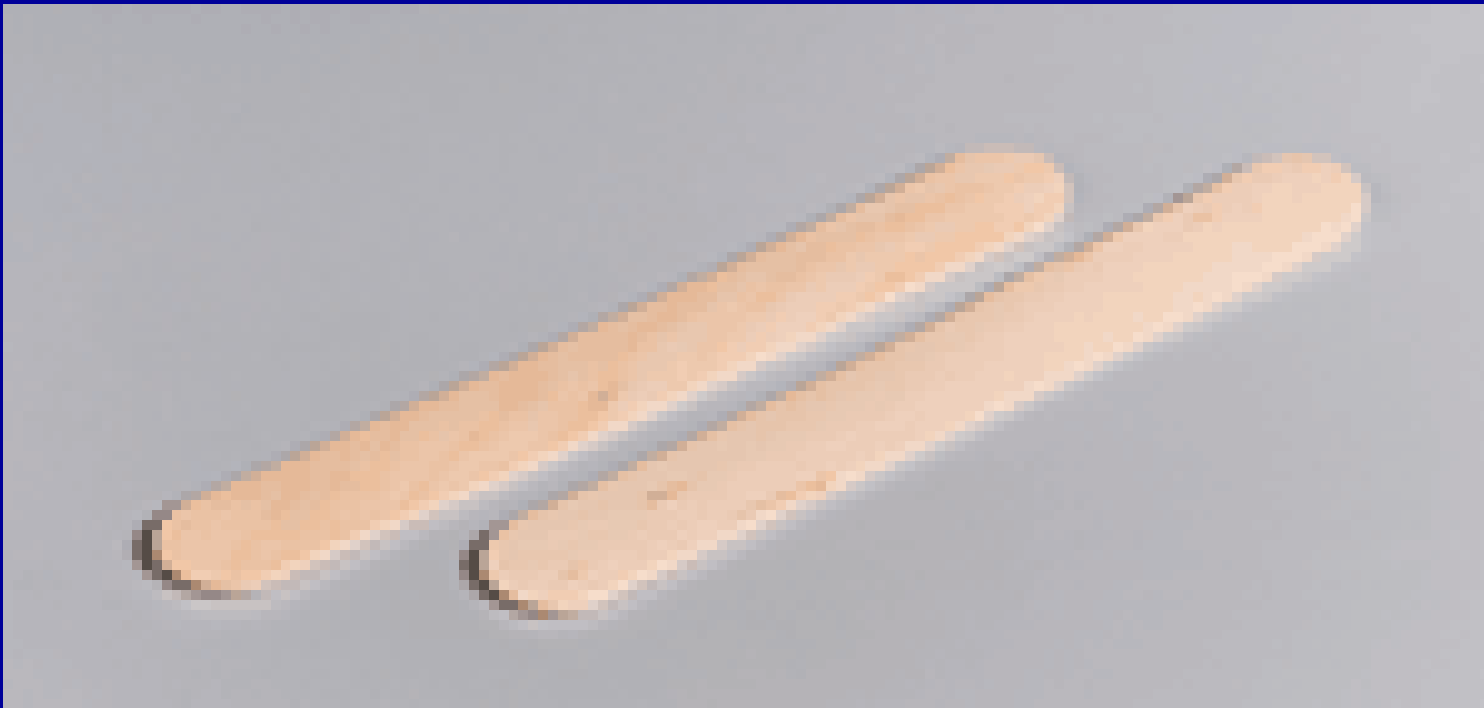
- **Used in the collection of samples for viral isolation and testing**
- **Essential for virus survival**
- **Prevents specimen from drying out**
- **Prevents bacteria and fungi growth**

Polyester Fiber-Tipped Applicator



- Should be drayon, rayon, or polyester-fiber swabs
- Do **not** use cotton swabs.

Tongue Depressors



How to Manage Kits

Storing VTM

- Sterile collection vials containing 1-2 ml of VTM
- Vials can be stored in a freezer at $-20\text{ }^{\circ}\text{C}$ until use
- Vials can be stored for short periods of time at $4 - 6\text{ }^{\circ}\text{C}$



How to Manage Kits

- **Store specimen collection kits “except VTM” in a dry, clean place**
- **Store specimen collection kit where it will be accessible after hours and on weekends**

How to Safely and Correctly Collect Samples

Clinical Specimen Sources

Be prepared to collect specimens before you leave for the field

- **Suspected cases**
 - Symptoms consistent with influenza
- **For Contacts, no specimen should be taken systematically:**
 - Contacts should be followed-up daily for 7 days and checked if AI symptoms develop. If AI signs develop, the contact is then considered as a suspected case and therefore specimen should be taken.

What to Collect

From an Ambulatory patient

- Throat swab *and*
- Nasal swab
- Can be collected into the same VTM

From an Intubated patient

- Lower respiratory aspirate

When to Collect Respiratory Specimens

- **As soon as possible after symptoms begin**
- **Before antiviral medications are administered**
- **Even if symptoms began more than one week ago**
- **Multiple specimens on multiple days could be collected if you have access to patient**

Serological Samples

Paired serum samples are most useful

Acute sample

Within 7 days after symptom onset

Convalescent sample

More than 21 days after symptom onset

Personal Protective Equipment

- **Masks (N-95 or N/P/R-100)**
- **Gloves**
- **Protective eye ware (goggles)**
- **Hair covers**
- **Boot or shoe covers**
- **Protective clothing (gown or apron)**

How to Collect Specimens

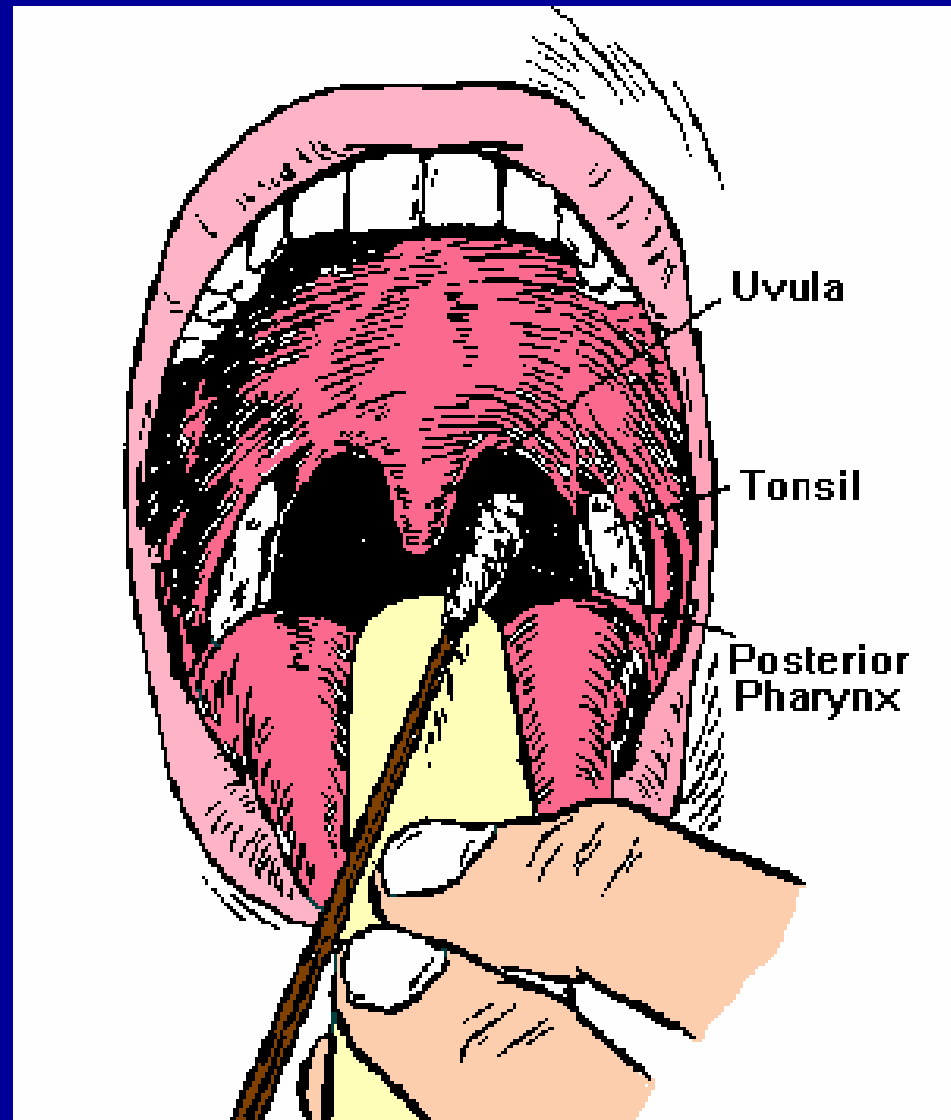
Methods of Collection

- **Oropharyngeal (Throat) swab**
- **Nasopharyngeal swab**
- **Nasopharyngeal aspirate**
- **Blood sample**

Oropharyngeal (Throat) Swab

- **Easy to do**
- **Highest yield in detecting avian influenza in suspected cases**
- **Have the patient open his/her mouth wide open.**
- **The patient should try to resist gagging and closing the mouth while the swab touches the back of the throat near the tonsils.**





Field Data Collection Form

General patient information

Name:
 Address:
 Country:
 County:
 City/town/village:

Tracking record number

Date of Birth (dd/mm/yyyy):
 Sex: M F
 Nationality:
 Occupation:

Date of onset of illness (dd/mm/yyyy):

Clinical specimens

| Unique ID No. | Type | Date of collection | Clinical diagnosis | Health status when specimens collected | Remarks |
|---------------|------|--------------------|--------------------|--|---------|
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Post-mortem specimens

Date of death(dd/mm/yyyy): ___/___/___

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Name of person completing form: _____
 Institutional affiliation: _____
 Contact details: _____
 Date(dd/mm/yyyy): ___/___/___

How to Label Samples

Use pre-printed barcode* labels:

- On the specimen container
- On the field data collection form
- On the log book

* If barcode labels are not available use Marker.

Label each specimen with:

- Subject's name
- Subject's unique identification number



Specimen Tracking System

Maintain a database to track:

- Identification number
- Subject information “including date of onset”
- Specimen collection date
- Specimen collection location
- Diagnostic test date and results

Specimen Storage, Handling, Packaging and Transportation

How to Store Specimens

For specimens in VTM:

- Transport to laboratory as soon as possible
- Store specimens at 4 °C before and during transportation within 48 hours
- Store specimens at -70 °C beyond 48 hours
- Do not store in standard freezer – keep on ice or in refrigerator
- Avoid freeze-thaw cycles
 - Better to keep on ice for a week than to have repeat freeze and thaw

How to Store Specimens

For sera:

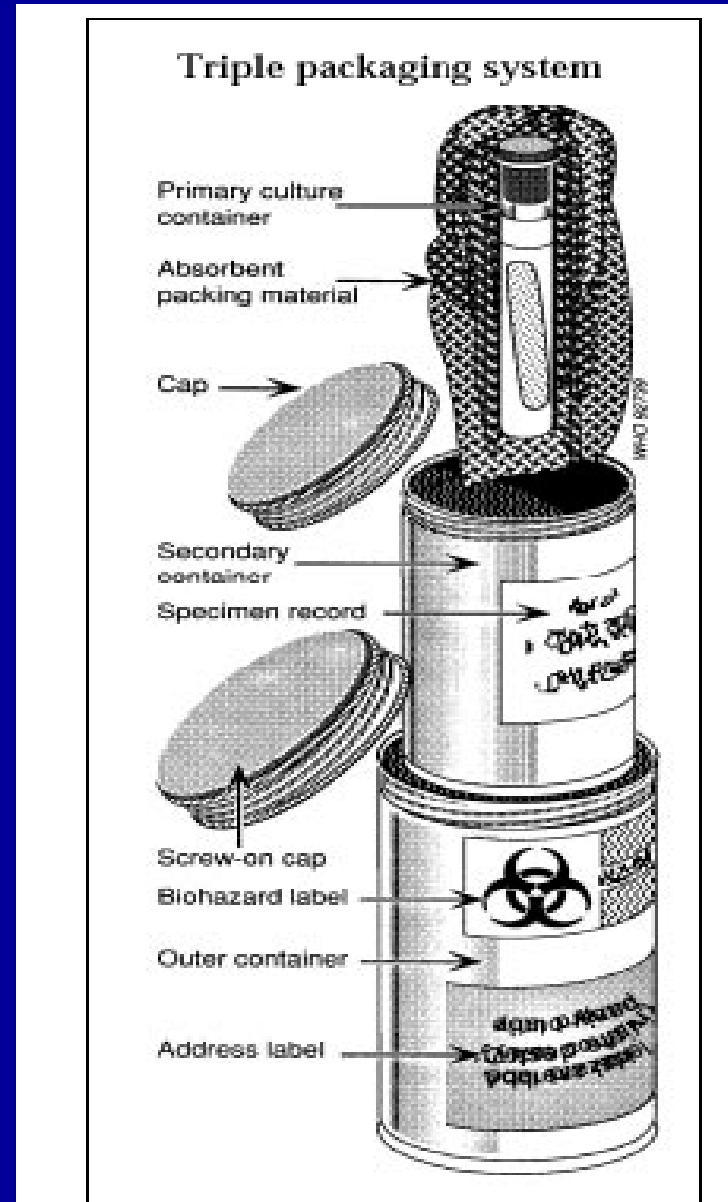
- Store specimen at 4 °C within 48 hours
- Store specimens at -20 °C beyond 48 hours
- Avoid repeated freeze-thaw cycles

Transporting Specimens

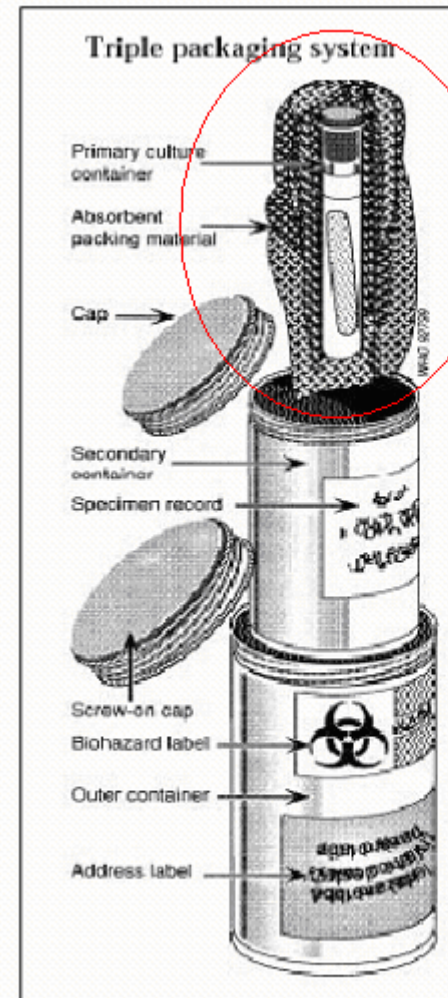
- **Refer to WHO guidelines for the safe transport of infectious substances and diagnostic specimens**
- **Follow local regulations on the transportation of infectious material**
- **Coordinate with the laboratory**

Packing Specimens for Transportation

- Use three packaging layers



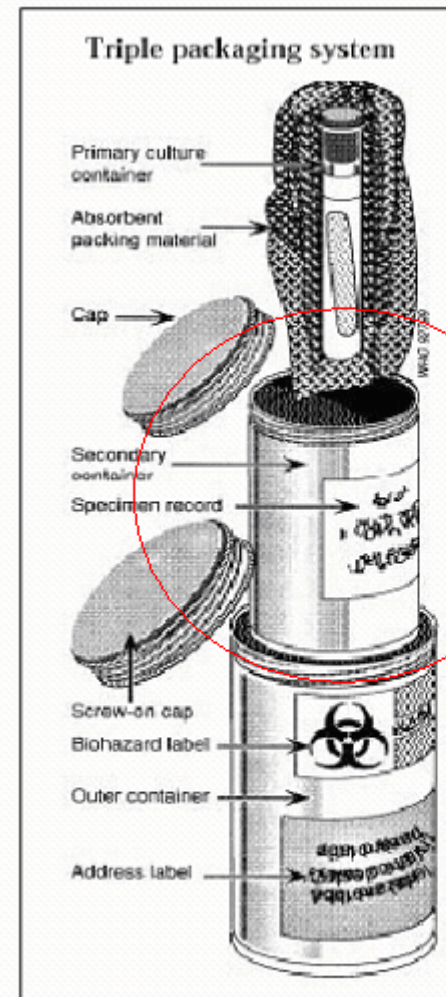
- This primary Container must be leak proof, labeled, capped, and sealed with parafilm
- Wrap specimen container with absorbent material or enough tissue/paper to prevent breakage and absorb contents in case of leakage.



- Secondary packaging must be watertight.

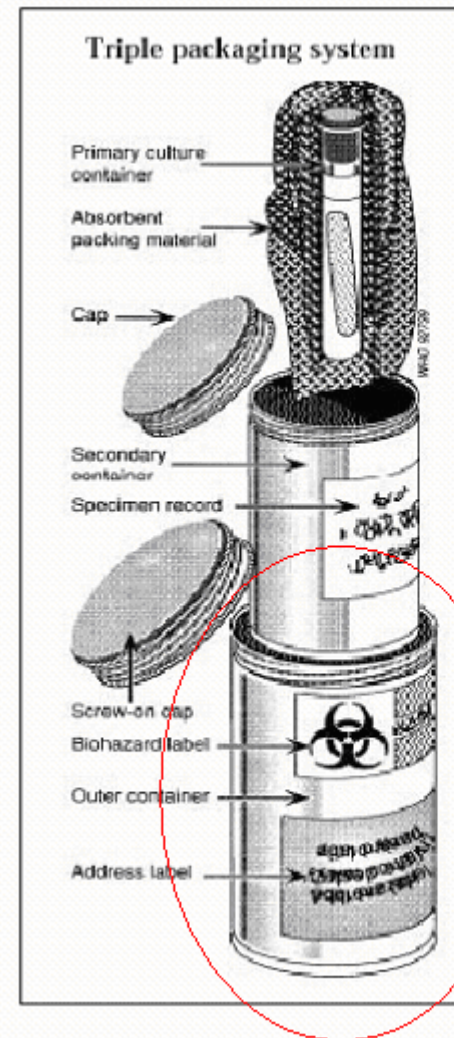
- If transporting with wet ice, surround secondary container with ice in sealed plastic bags (to prevent leakage,).

- Place an itemized list of contents in a sealed plastic bag between the secondary container and the outer packaging.



The third layer protects the secondary packaging from physical damage while in transit.

Specimen data forms, letters and other types of information that identify or describe the specimen and identify the shipper and receiver, and any other documentation required, must also be provided





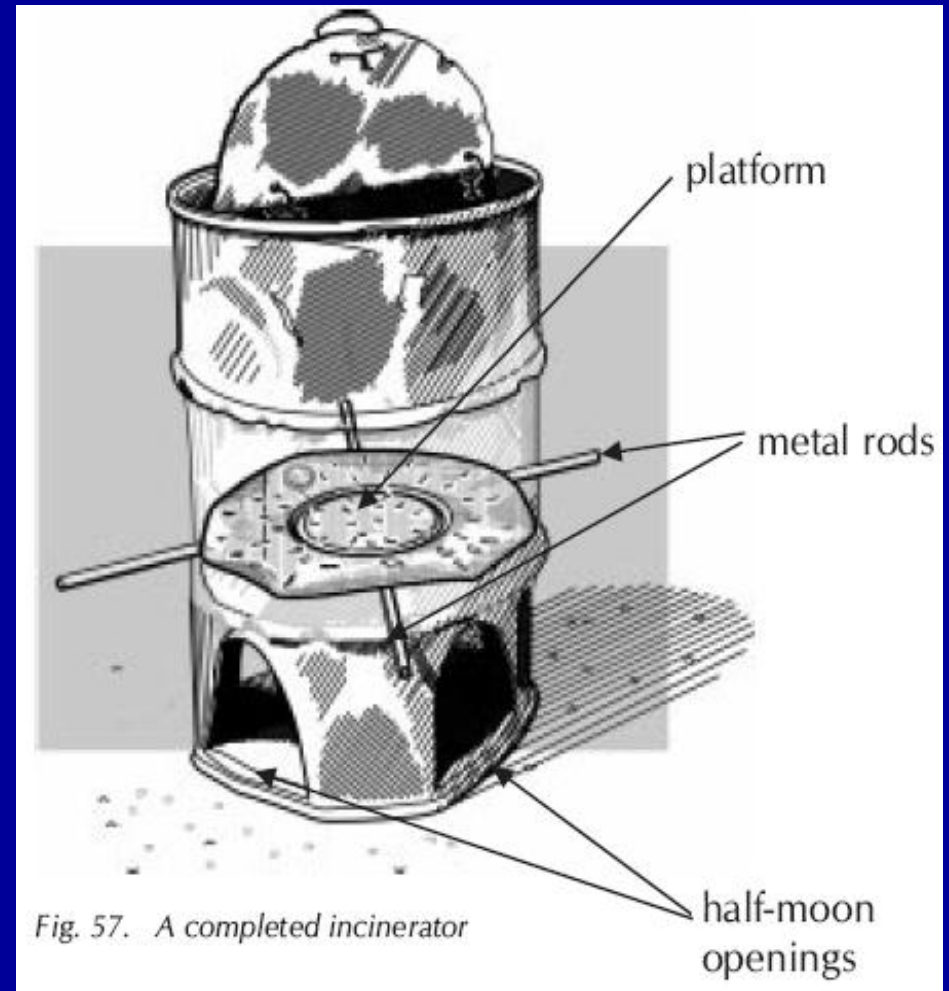
Waste Disposal in the Field

Items Requiring Disposal

- **Infectious blood, body fluids, leftover biological samples**
- **Disposable needles and syringes**
- **Disposable or non-reusable protective clothing**
- **Disposable or non-reusable gloves**
- **Used laboratory supplies**
- **Used disinfectants**

Building an Incinerator

1. Cut out one end of a 220 liter drum
2. Cut out 3 half-moon openings
3. Cut 4 side holes and insert metal rods to form an X
4. Cut away half of other end of drum to form lid
5. Insert first end cut as a waste platform



How to Burn Waste in An Incinerator

1. Place waste on platform inside of the drum
2. Soak waste in one liter of fuel
3. Light the fire carefully
4. Watch the fire and frequently mix the waste with the metal bars to be sure all of the waste is burned
5. When the fire has gone out, empty the ashes into a pit

Note: You must replace the incinerator if large holes burn through the sides.

Disposal Using a Pit

- **Can be used for disposal of:**
 - Liquids, if no isolation latrine or toilet is available
 - All other flammable items such as clothing
- **Building a Pit:**
 - Locate pit far from foot traffic flow
 - Dig 2 meters deep, wide enough to hold all contaminated waste material

How to Burn Waste in a Pit

1. Place disinfected waste in the pit
2. Pour fuel (diesel) over waste
3. Carefully start fire
4. Observe burning process
5. Repeat fire if waste remains after burning
6. Cover ashes with soil
7. Bury a nearly full pit with $\frac{1}{2}$ meter of soil
8. Dig a new pit for more waste

Disposal Safety Precautions

- **Use safe handling practices for infectious material**
- **Locate disposal site on health facility grounds, away from traffic flow and public view**
- **Maintain disposal site security by roping off, posting signs, and stationing a guard**
- **Do not leave unburned waste in an incinerator or pit**

Guidelines for Potential Avian Influenza Specimens

- Use **BSL 2** laboratory with **BSL 3** practices for:
 - Diluting specimens
 - Nucleic acid extractions
 - Diagnostic testing that does not involve culturing
- Use **BSL 3** laboratory with **BSL 3** practices for:
 - Culturing avian influenza virus

Laboratory Tests

Laboratory Diagnosis for Avian Influenza

Tests for respiratory samples:

- PCR-based techniques
- Virus isolation
- Immunofluorescence
- Rapid antigen detection

Blood used for:

- Measurement of specific antibodies (Most common for influenza, sera is used)
- Viral isolation (whole blood if viremia is a consideration)
- PCR-based techniques (sera)

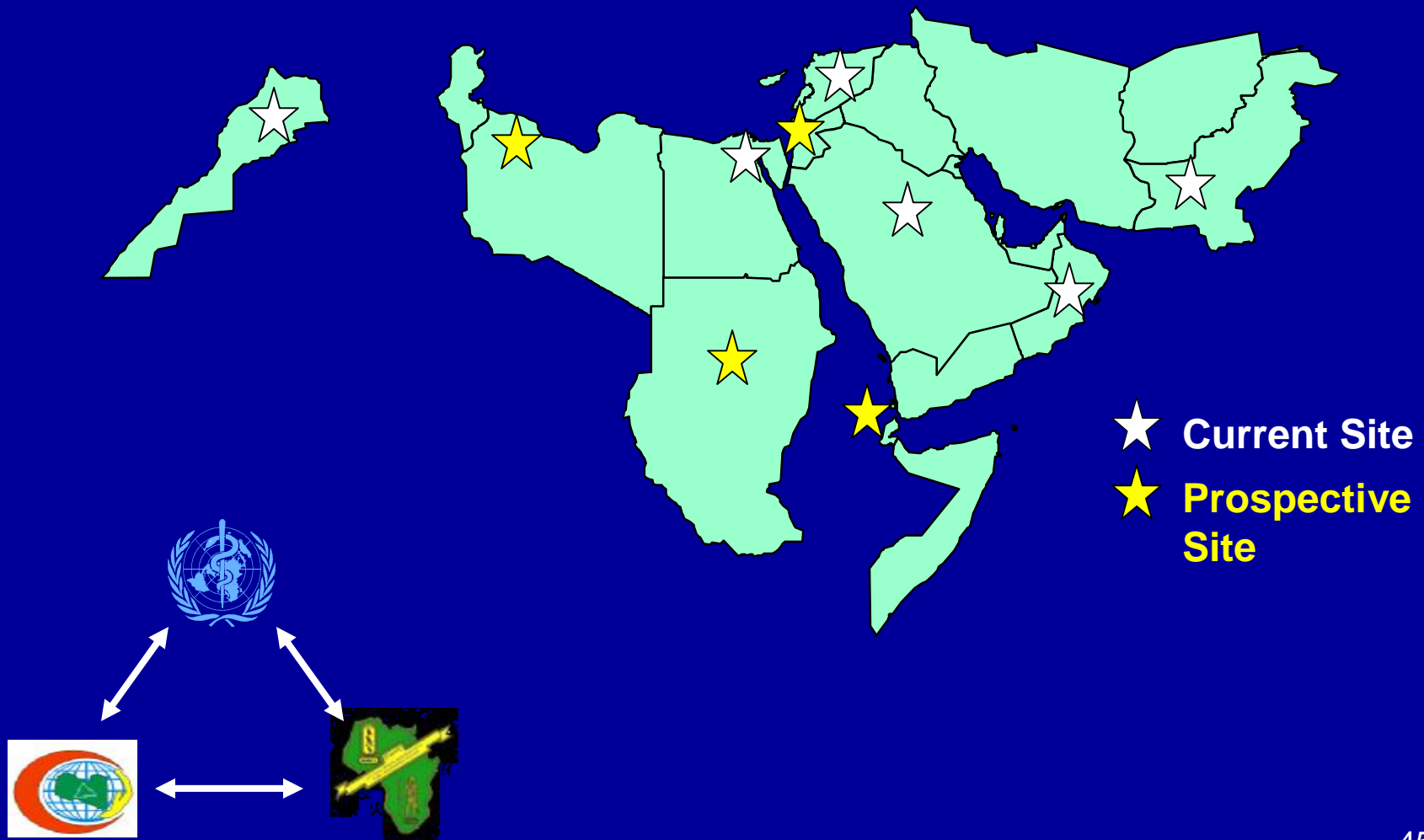
Laboratory Diagnostic methods for Avian Influenza

- **Most commonly applied methods in EMRO labs:**
 - PCR
 - Possibly Cell culture
- **Recommendation:**
 - PCR if there is equipment, BSL-2 lab
 - If positive, send specimen for cell culture at BSL-3 lab

Laboratory Tests for Avian Influenza A (H5N1)

- **PCR Based Techniques- recommended**
 - Sensitivity depends on the particular test, the influenza strain, and the type of specimen used
- **Virus Isolation**
 - Technically difficult
 - Requires a BSL-3 laboratory with enhancements

Tri-lateral Agreement: A Collaborative Approach to Influenza Surveillance



EMRO Logistics - Influenza

Lines of Communication

Ministry of Health
Ministry of Agriculture

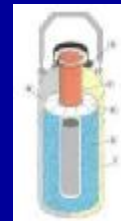


Regional Adviser, Emerging Diseases
Communicable Disease Surveillance,
Forecasting and Response
Regional Office for the Eastern
Mediterranean
World Health Organization



NAMRU-3 Cairo, Egypt

Specimen Movement



WHO



Reporting

Managing and Analyzing Laboratory Data

Data Management Rules

- **Double check data entry accuracy**
- **Include unique identification numbers**
- **Keep subject names confidential**
- **Track testing dates and results**
- **Back up the database**

How to Present Results

Report:

- Time and place of the infections
- Prevalence of infection

(Prevalence = number of cases tested positive / by the total number of the people)

- Clinical information about cases
- Epidemic curve

Share results with local health officials and the WHO Global Influenza Program

Summary

- **Maintain adequately stocked specimen collection kits and store them properly when they are not in use.**
- **Oropharyngeal (throat) swabs are the easiest and best specimens to collect for suspected cases of avian influenza. Nasal swabs are easy to collect as well and should be done to increase yield.**
- **Collect multiple specimens (respiratory and blood) on multiple days.**

Summary

- **Proper specimen storage, handling, and shipping is vital to a successful laboratory test.**
- **Keep track of information on the specimens collected in a database or logbook.**
- **When handling infectious materials in the laboratory or in the field, take safety precautions.**
- **Properly dispose of any infectious material.**

Summary

- **Most countries will be able to perform some basic influenza identification tests on a clinical specimen, but more specific testing may need to be done at a higher level “regional or global laboratory”.**
- **When you report on the progress of an outbreak investigation, share investigation results with local health officials and the WHO Global Influenza Program.**

References and Resources

- Recommended laboratory tests to identify avian influenza A virus in specimens from humans. World Health Organization, June, 2005.
http://www.who.int/csr/disease/avian_influenza/guidelines/avian_labtests2.pdf
- WHO guidelines for the collection of human specimens for laboratory diagnosis of avian influenza infection, 12 January 2005.
http://www.who.int/csr/disease/avian_influenza/guidelines/humanspecimens/en/index.html
- Infection control for viral haemorrhagic fevers in the African health care setting. WHO/EMC/ESR/98.2 Section 6: Dispose of Waste Safely
http://www.who.int/csr/resources/publications/ebola/WHO_EMCSR_98_2_EN/en/index.html