In the Name of God

Overview of influenza laboratory diagnostic technology: advantages and disadvantages of each test available

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Diagnosis includes:

• 1) Sampling
  - collection of high-quality specimens

• 2) Transportation
  - rapid transport to the laboratory and appropriate storage

• 3) Diagnosis in the laboratory
Case Selection

- Select cases based on WHO case definition
  “Fever >38°C, cough and sore throat”
- Quality is more important than quantity
- **Suspected cases**
  - Symptoms consistent with influenza case definition
- **For Contacts, NO specimen should be taken systematically:**
  - Contacts should be followed-up daily for 7 days and checked if symptoms develop. If symptoms developed, the contact is then considered as a suspected case and therefore specimen should be taken.
Timing

Days after onset of illness

-5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

incubation

virus shedding

RT-PCR

antigen detection / culture

antibody detection

antibody response
Posterior pharyngeal swab (throat swab)

Hold the tongue out of the way with a tongue depressor. Have the subject say "aahh" to elevate the uvula.
How to Store Specimens

• Store specimens at 4 °C before and during transportation within 48 hours

• beyond 48 hours store specimens at -70 °C and transport using liquid nitrogen tank or dry ice

• **Never** 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
Influenza Diagnosis

Diagnosis of influenza can be achieved by:

- Directly - detecting the virus or viral products
- Indirectly - detecting an immunological response to virus (Ab detection)
Direct Diagnosis of Influenza

- Virus isolation
- RNA detection
- Antigen detection
Virus isolation
Virus isolation can be done by:

1- Tissue culture
   MDCK Cell line, Siat MDCK Cell line

2- Embryonated egg
A successful virus isolation depends largely on viral viability of the specimen

Important variables include:

- The timing of the specimen collection in relation to the illness.
- The quality and amount of specimen material obtained
- The time and conditions of transport to the laboratory.
Advantages:

Virus isolation has a higher priority because it can be used for:

a: vaccine preparation
b: Antiviral resistance studies
d: future studies
Virus Isolation in MDCK cell line

**Disadvantages:**

- Require specialized facilities
- Require expert personnel
- relatively expensive
- prolonged time to isolate
- Is not sensitive enough to isolates some strains
- The growth of viruses in cell culture is often associated with minimal CPE and needs to be detected by Hemagglutination, Hemadsorption or IF tests.
MDCK Cell Culture

A. non Infected MDCK
B. Influenza Infected MDCK
Hemagglutination test (HAT)

- RBCs of different species can be used for haemagglutination Test.
- Avian: turkey or chicken
- Mammalian: guinea pig or human type “O” Rhesus Monkey, Horse RBC(H5)

Problem:

a) The sensitivity of RBCs to agglutinate virus is Changing due to antigenic changes of HA
b) Elution of virus from RBCs due to activity of NA Could result in false negative in HAT
Haemagglutination Assay

| HA titre | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | 2056 |

Slide from Vicky Gregory
Haemadsorption

Haemagglutinin

+ 

RBC

Cell monolayer

haemadsorption

Slide from Vicky Gregory
Hemadsorption test

Slide from Simin Abbasi
Detection of Antigen by IF

Influenza A IF

Slide from CDC
Isolation in embryonated egg (4)

**Advantages:**
Easier and faster than tissue culture
- Virus isolates are suitable for vaccine production

**Disadvantage:**
- Obtaining embryonated egg (SPF) is not an easy task
- Some of the subtypes such as (H3N2) is not easily isolated
• The development of monoclonal antibodies in the 1970s increased Rapid viral diagnosis during the 1980s as monoclonal antibody-based assays to detect a wide variety of viral antigens directly in clinical specimens.

• The introduction of molecular techniques into the diagnostic virology laboratory accelerated with the development of PCR, in 1985, and further with the development of real-time PCR methodology in the late 1990s.
RNA Detection
The most common variations of standard PCR used in influenza diagnosis are:

- Conventional PCR (RT-PCR)
- Nested PCR (n-PCR)
- Multiplex PCR (m-PCR)
- Real-time PCR
Multiplex and Nested PCR

Multiplex PCR

• More than one target sequence can be amplified by including more than one pair of primers in the reaction.

Nested PCR

• PCR is performed on the primary PCR product using a new set of internal primers For increased sensitivity
The advantages of n-PCR are:

- Its increased specificity (specific binding of 2nd primer pair).
- Increased sensitivity (2nd round of PCR amplification)
- n-PCR is used to detect organisms present in low copy Numbers
Disadvantages of n-PCR

• n-PCR takes too long

• Risk of contamination is high
**Multiplex PCR**

- M-PCR is a rapid method of detecting multiple targets in a single reaction.

- Major advantage is the reduction in test processing time
Multiplex PCR for Typing
Multiplex PCR for Subtyping
Real time PCR

Advantages:

• contamination is decreased, because the system is closed.
• Rapid cycling times (1 hour).
• Very sensitive
• Of great importance, quantitation of PCR targets.
Conclusion

- Most of the labs prefer to do PCR for virus detection but PCR is an easy test however when a problem occurs it takes a long time to solve the problem.
- PCR is expensive
- Sensitivity is high
- Decrease the time
- The lack of requirement for virus viability
- Reduce virus isolation
Antigen Detection

Source: CDC, USA
Antigen Detection

- Antigen detection methods can provide diagnostic information within a few hours of the receipt of the specimen in the laboratory.

- The lack of requirement for virus viability is important advantage compared with viral culture, especially when specimen transport time is prolonged.

- Methods used for viral antigen detection include
  - FA staining, immunoperoxidase staining, and enzyme immunoassay, …
Immunofluorescence test

The main advantage:
- Direct detection of Ag in patient specimen within the few hours
- Decrease the time
- The lack of requirement for virus viability

The main disadvantage:
- Equipment
- Experienced personnel
- Require M-Ab
- Lack of virus
Near Patient Test

• “Any investigation carried out in a clinical setting or the patient’s home for which the result is available without reference to a laboratory and perhaps rapidly enough to affect immediate patient management”

• Hobbs.....British Medical Journal 1996
Rapid diagnostic tests (1)

Advantages:
• Speed
• Unskilled
• Early warning
• Aid management/infection control

Disadvantages:
• Expensive
• Reduce virus isolation
• Sensitivity/specificity is low
Indirect Method
Indirect Method

• Antibody detection/ Serology
• Hemagglutination Inhibition test (HIT)
• Neutralization Test (NT)
• Single Radial Hemolysis(SRH)
• Plaque Reduction Test (PRT)
Haemagglutination Inhibition Assay (HAI)
SeroLogic Assays (1)

Binding Assays:
• directly measure binding of antibodies to viral antigens. Include HI, EIA, radioimmunoassay (RIA), and the indirect immunofluorescent antibody assay (IFA).

Immunobinding Assays:
• The western blot
Haemagglutination Inhibition Assay
Sero logical diagnosis (2)

• Shows presence or absence of antibody against influenza in human sera
• Antigen characterization
• HAI can distinguish between
  Type e.g. A & B
  Subtype e.g. H1 & H3
Haemagglutination Inhibition Assay (3)

• WHO Influenza Kit is used to identify the following antigens and antibodies (to these viruses) in sera:
  
  A (H3)
  
  A (H1)
  
  B (usually more than one lineage)
Haemagglutination Inhibition Assay
Common problems (4)

1 - Non-specific inhibitors present in antisera
   Appears as false positive which could lead to incorrect typing or subtyping

2 - Non-specific agglutinins present in sera (non-specifically bind RBC) could result in false negative in HIT, especially when HI titers are low

3 - Elution of virus from RBCs
   Could result in false positive in HIT
Non-specific inhibitors in sera (5)

- Cross-reaction of virus with non-specific antisera in HI

- Appears as false positive which could lead to incorrect typing or subtyping

- Non-specific inhibitors can be removed by RDE treatment
Non-specific agglutinins in sera (6)

- Agglutinating proteins present in the sera non-specifically bind to RBCs
- Identified by agglutination in control sera
- Could appear as false negative in HI, especially when HI titers are low
- Non-specific agglutinins can be removed by adsorption of antisera to RBCs
Haemagglutination Inhibition Assay
Elution of RBCs (7)

• RBCs elute from the HA protein and reduces agglutination (due to NA)

• This can be overcome by reading results as soon as normal RBCs have settled to a compact button

• Incubation of plates at 4°C also slows down elution
Haemagglutination Assay
Avian RBCs (8)

• Turkey RBCs are preferred to chicken
• Currently circulating human influenza, particularly H3s, bind better to turkey RBCs than chicken RBCs
• Chicken RBCs are more readily available
• Some recently circulating human influenza viruses, particularly H3, bind better with guinea pig RBCs than avian RBCs
Plaque Assay

Slide from Simin Abbasi
Plaque Reduction test

- Golden test for Ab detection specially for H5
- Golden test for Ab detection after vaccination
- Golden test for drug resistancy
- Takes time
- Requirement viable virus
- Experienced staff
Number of specimens positive for seasonal and non-seasonal [A(H1N1) and H5] influenza
Future Concern

If a novel virus with the transmissibility of H1N1 and even a fraction of the virulence of H5N1 emerged, the consequences would be devastating.