Prepared by Utah Public Health Laboratories
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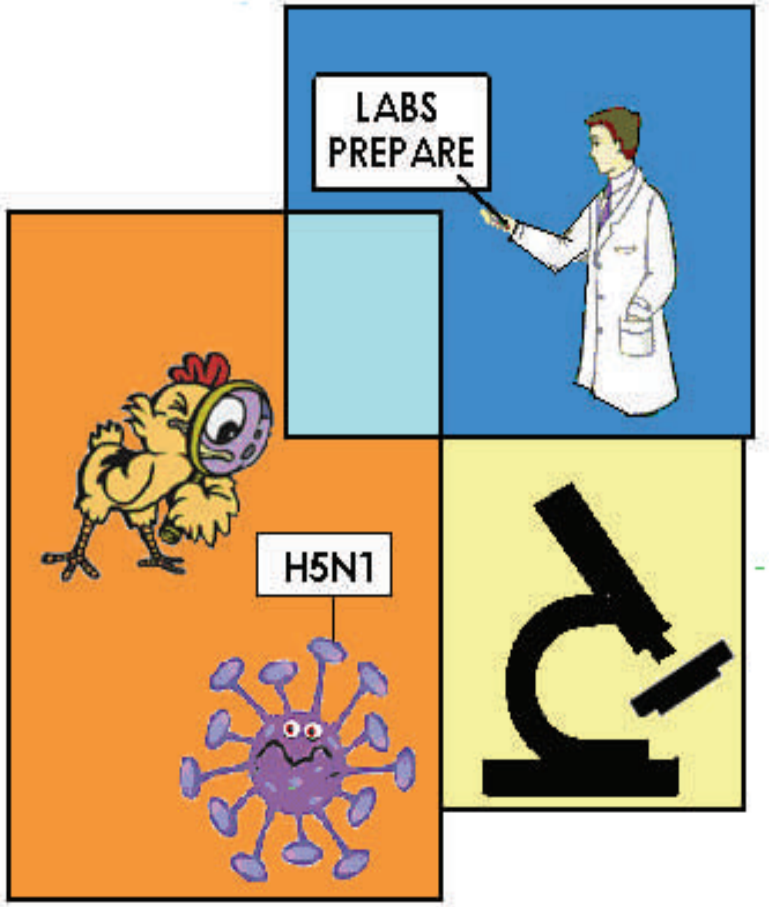
Association of Public Health Laboratories

Lab Clues for Flu



Preparing Laboratories For Pandemic Influenza

Introduction

Because Influenza mimics many other respiratory illnesses, laboratory diagnosis is important for the appropriate clinical diagnosis and treatment of patients. Complexity of laboratory testing goes from simple rapid testing done in "point-of-care" facilities to expertise-dependent testing, such as viral culture and molecular genetic tests, done in appropriate bio-safety secured facilities. The information in this brochure is meant for all levels of laboratory and health care personnel involved in Influenza testing to assist in preparing for a possible Influenza pandemic. Use this brochure for review or to learn new information.

About Influenza A Virus[1]

- There are other influenza viruses but Influenza A virus strains are the most virulent pathogens to humans and cause the most severe illness.
- Influenza A virus has 8 RNA genes that code for 11 proteins. The two best-characterized proteins are hemagglutinin (HA or H) and neuraminidase (NA or N).
- Hemagglutinin and neuraminidase proteins act as antigens and viral subtyping is done by targeting NA or HA with antibody reagents in invitro lab testing. The response of the virus antigens NA or HA to different serological antibody reagents gives rise to the various subtypes.
- Thus, the current avian influenza A of concern reacts to H(5) antisera and N(1) giving the familiar H5N1.

References:

1. Sengamalay, G.E. et al. (2005, October 5). Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution. *Nature,* 437, pp.1162-1166.
2. Influenza symptoms and diagnostic procedures. (2006, March 6). Retrieved March 26, 20006 from Centers for Disease Control and Prevention website: http://www.cdc.gov/flu/professionals/diagnosis/labprocedures.htm
3. Fact Sheet: Rapid diagnostic testing for influenza. (2006, December 4). Retrieved March 26, 2006 from Centers for Disease Control and Prevention website: http://www.cdc.gov/flu/professionals/diagnosis/rapidclin.htm
4. Respiratory protection: OSHA standards. (n.d.). Retrieved March 26, 2007 from U.S. Department of Labor website: http://www.osha.gov/SLTC/respiratoryprotection/standards.html
5. Questions and answers about the influenza A (H2N2) panels: Destruction of the panels. (2005, April 22). Retrieved March 26, 2007, from Centers for Disease Control and Prevention website: http://www.cdc.gov/flu/h2n2panelsqa.htm
6. Collecting, preserving, and shipping specimens for the diagnosis of avian influenza A (H5N1) virus infection. (2006). Retrieved March 26, 2007, from the World Health Organization website: http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1.pdf

- In general, infectious agents must be shipped in three layers: 1) primary or specimen tubes, wrapped with Parafilm-like material to prevent leaking 2) all primary specimens up to 50 ml. are placed in a secondary container. Absorbent material is packed between the primary and secondary container, 3) The primary and secondary containers are put in a United Nations certified third container for shipping.
- Questions may be addressed to UPHL for further information on shipping or to inquire about shipping & packing trainings for your facility.

Contact Information

Utah Public Health Laboratories
(main desk for paging and referral) 801-584-8400

Virology (DFA & Viral culture) 801-584-8235
or 801-584-8454

Molecular Biology (RT-PCR) 801-584-8449

Customer Support/
Specimen Shipping & Receiving UPHL 801-584-8417
or 801-584-8286

24/7 Emergency Response
(leave message for call back in < 15 Min.)
1-888-374-8824
(1-888-EPIUTAH)

About Influenza A Virus

- A common subtype for annual influenza that is currently circulating in North America is H3N2. There are currently 16 H subtypes and 9 N subtypes of influenza A.
- New influenza viruses are constantly being produced by mutation or by reassortment. Mutations can cause small changes in the hemagglutinin and neuraminidase antigens on the surface of the virus. These mutations create an increasing variety of strains over time until one of the variants eventually achieves higher fitness, becomes dominant, and rapidly sweeps through the human population – often causing an epidemic.
- In contrast, when influenza viruses re-assort, they may acquire new antigens — for example by reassortment between avian strains and human strains; this is called antigenic shift. If a human influenza virus is produced with entirely novel antigens, everybody will be susceptible and the novel influenza will spread uncontrollably, causing a pandemic.

Types of Laboratory Testing Available for Influenza

It is important in any kind of laboratory testing that all manufacturer and/or laboratory standard operating procedures are followed. This includes collection of the appropriate specimen and using the appropriate collection swab or tools. Deviation from these procedures may give erroneous results. **Never test human and animal/bird influenza A specimens in the same laboratory.**

Influenza Diagnostic Testing Table

Procedure	Influenza Types Detected	Time For Result	Comments
Viral Culture	A and B	3-10 days	This is the gold standard and provides information for virus characterization and vaccine formulation. Viral culture **IS NOT** recommended if a novel or highly pathogenic avian influenza strain is suspected unless the lab has BSL3+ facilities and appropriately trained scientists. UPHL will assist in sending specimens to CDC.
Immuno-Fluorescent DFA Antibody Staining	A and B	2-4 hours	These kits may also be used for other respiratory virus detection such as Parainfluenza, RSV & Adenovirus.
RT-PCR	A and B	2-4 hours	May be used for H and N characterization in some laboratories with the capacity. Most public health laboratories will have H subtyping capacity.
Enzyme Immuno Assay (EIA)	A and B	2-3 hours	Sensitivity and specificity of test kits may vary.
Serology	A and B	>2 weeks	Requires paired sera (acute and convalescent) and has greatest value in epidemiological population studies.
Rapid Diagnostic Tests	Must refer to kit Inserts to see if Both A&B are detected and if they can be differentiated from each other.	<30 minutes	Most kits have an average of 70% sensitivity and 90% specificity. It is recommended to confirm rapid test results with another method such as viral culture or PCR. Check with UPHL for free options.

UPHL asks that Influenza testing be done at local clinical and hospital labs and point-of-care testing facilities where appropriate biosafety cabinets and trained personnel are available. UPHL will confirm any referred influenza specimens free of charge. Testing at UPHL for avian influenza or a suspected novel strain must first be authorized by local public health or state epidemiology.

Packing and Shipping Key Points[6]

- Nasopharyngeal (NP) swabs and viral transport media are available from UPHL (NP swabs are preferred for annual influenza/ an oropharyngeal or throat swab is preferred if avian influenza H5N1 is suspected). An emerging new type of influenza might have different specimen collection requirements. Please check with UPHL if you have questions.

- Swabs to be used for RT-PCR testing must be Dacron or Rayon. DO NOT use cotton swabs or calcium alginate swabs or swabs that have wooden shafts. These may have inhibiting substances for PCR testing.

- All Department of Transportation (DOT) and International Air Transport Association (IATA) regulations must be followed for shipping suspected infectious agents, such as influenza specimens.

- All individual specimen tubes must be marked with the patient's name and/or unique identifier, the specimen source, and the specimen collection date.

Clinical Lab & Point-of-Care Help Needed for Public Health Surveillance

During outbreaks of respiratory illness when influenza is suspected, some respiratory samples should be tested by both rapid tests and by viral culture. The collection of some respiratory samples for viral culture is essential for determining the influenza A subtypes and influenza A and B strains causing illness, and for surveillance of new strains that may need to be included in the next year's influenza vaccine. During outbreaks of influenza-like illness, viral culture also can help identify other causes of illness.

The Utah Department of Health's Office of Epidemiology, along with the Utah Public Health Laboratories participates in year-round influenza surveillance. Clinical laboratories and other influenza testing sites can assist the important work of public health by sending to UPHL for testing influenza specimens from those patients who are hospitalized or extremely ill, or who have specific travel or work histories that put them at risk for novel or avian influenza strain infections. Influenza hospitalizations or deaths are reportable diseases to local or state health authorities.

Testing at the Utah Public Health Laboratories

- Utah Public Health Laboratories has laboratory testing capability for Direct Fluorescent Antibody staining for Influenza A & B, Parainfluenza I, II, III, Adenovirus, & RSV, RT-PCR for influenza A and B, as well as the A "H" subtyping. UPHL has viral culture capability to be used for annual influenza surveillance. For novel and suspected avian influenza testing, UPHL will do RT-PCR subtyping. These samples will then be forwarded to CDC for viral culture and characterization of the virus

Special Guidelines for the Use of Rapid Influenza Test Kits[3]

- Median sensitivities of rapid diagnostic tests are approximately 70-75% when compared with viral culture, but median specificities of rapid diagnostic tests for influenza are approximately 90-95%.
- False-positive (and true-negative) results are more likely to occur when disease prevalence in the community is low, which is generally at the beginning and end of the influenza seasons.
- False-negative (and true-positive) results are more likely to occur when disease prevalence is high in the community, which is typically at the height of the influenza season.
- Use rapid diagnostic tests with high sensitivity and specificity.
- Collect specimens as early in the illness as possible (within 2-5 days).
- Follow manufacturer's instructions, including handling of specimens.
- Consider sending specimens for viral culture to confirm results of rapid tests especially when community prevalence of influenza is low and the rapid diagnostic test result is positive and when the rapid diagnostic test result is negative but disease prevalence is high. (Contact your local or state health department for information about influenza activity).

Guidelines for Personnel Safety for Laboratory Testing of Influenza Specimens

- All specimens of unknown respiratory illness should be treated as if they potentially contain influenza virus.
- Respiratory specimens should not be tested on bench tops where there is no appropriate biosafety cabinet.
- Specimens suspected of being routine annual influenza samples should be tested using Biosafety Level (BSL) 2 conditions. (See Biosafety in Microbiological and Biomedical Laboratories 5th Ed. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm
- Personal Protective Equipment used should include, gowns, gloves, respirator masks (N-95 or N-100 recommended) and goggles or face shields (personal prescription glasses are not adequate). **OSHA respiratory protection rules require respirator masks to be fit-tested.** [4]
- If a novel or highly pathogenic avian strain is suspected, testing should be done only in a facility that has BSL-3+ conditions. Cell culture should not be attempted.
- It is highly recommended that laboratory and other healthcare staff testing for influenza receives the yearly influenza vaccine.
- Laboratories and other facilities doing testing for influenza should have a fever watch program in place for employees doing the testing.
- In a pandemic, it is estimated that up to 40% of staff may not be available for work. Laboratories should have plans in place to prioritize testing and establish surge capacity protocols in order to efficiently rotate workers for their health and safety.

Sterilization of Discarded Influenza A Materials[5]

- The following parameters may be used in a gravity displacement autoclave: 121 degrees C, 15-19 pounds of pressure, for at least 20 minutes.

- These general parameters may be used for sterilization with dry heat in a dry-heat oven: 170 degrees C for 1 hour, or 160 degrees C for 2 hours, or 121 degrees C for at least 16 hours.

- If sterilization of influenza A material by using an autoclave or dry heat oven is not possible and on-site incineration is not available, chemical disinfection is an alternative. A 1:10 dilution of household bleach (containing 5250 - 6000 ppm) can be used; a contact time of at least 10 minutes is recommended.

- It is not considered safe practice to use a sterilizer dedicated for sterile supply preparation (as found in operating rooms and central sterilization departments) for decontamination purposes. Use a sterilizer earmarked as a decontamination sterilizer.