

## Microbiological guidance for taking and handling specimens from individuals at risk of avian influenza

*Investigation of cases can be discussed with Dr Maria Zambon (tel 020 8327 6269).*

### Introduction

This guidance is to assist in the decision making process of management and sampling of a possible case of avian influenza in the UK. Avian influenza has been in the news since 2004, with reports of fatal cases from Turkey, East and South East Asia. It is possible that enhanced awareness of avian influenza will lead to clinical enquiries regarding potential of H5N1 infection in travellers returning from affected areas. It should be remembered that the risk of avian to human transmission is very low, and the likelihood of a case of avian influenza presenting in the UK is extremely low.

There have been outbreaks of avian flu in the last 20 years with human infections. The outbreaks recorded since 1996 are given in table 1.

**Table 1: Outbreaks of Avian Influenza with human infections in the last 20 years**

<b>Year</b>	<b>Country</b>	<b>Influenza A</b>	<b>Cases</b>	<b>Deaths</b>	<b>Carriers</b>	<b>Human to human transmission</b>
1996	UK	H7N7	1	0	Ducks	No
1997	Hong Kong	H5 N1	18	6	Chicken, Ducks & Geese	Yes (extremely limited dead-end transmission)
1999	Hong Kong	H9 N2	2	0	Chicken	Uncertain
2003	Hong Kong	H9 N2	1	0	Chicken	No
2003	Hong Kong	H5 N1	2	1	Chicken	No
2003	Netherlands	H7 N7	89	1	Chicken	Yes*
2004-2006	Various	H5 N1	Updated numbers are available from <a href="http://www.hpa.org.uk/infections/topics_az/influenza/avian/situation_update.htm">http://www.hpa.org.uk/infections/topics_az/influenza/avian/situation_update.htm</a>			

\*Evidence of conjunctivitis in family members.

(modified from [http://www.europa.eu.int/comm/health/ph\\_threats/com/Influenza/avian\\_influenza\\_en.htm](http://www.europa.eu.int/comm/health/ph_threats/com/Influenza/avian_influenza_en.htm)):

This guidance is broadly divided into three sections;

- clinical features of avian influenza,
- risk assessment of individuals presenting with illness, and
- testing, biosafety and transport of specimen.

The section on clinical features list the signs and symptoms that are recognised to date in human cases from SE Asia from 1997-2004. **It is essential to obtain a clear clinical history with appropriate epidemiological information including travel history and contact history.** This information allows a risk assessment to be carried out. With clinical information and baseline laboratory tests, a probability of infection can be deduced. Based on the probability of infection, the risk category and appropriate biosafety level for handling specimens can be finalised.

Samples should be tested in the local/regional laboratories for appropriate causes of infection; in particular rapid tests for normal human influenza should be carried out as this is

highly likely to be part of the differential diagnosis. If Influenza A is diagnosed in a high risk individual, then the sample may need to be transferred to the Virus Reference Division (VRD), HPA Colindale for further testing.

## About the person

The probability of avian influenza infection should be considered in the context of appropriate clinical findings, relevant travel and contact history along with abnormal baseline investigations. The information that may be useful in assessing a case is given below.

## Clinical Presentation

The exact clinical presentation in avian influenza is still unclear. In a report on 10 cases from Vietnam, the median incubation period is around 3 days (range 2 – 4 days) [1]. The median time to death after onset of symptoms is around 13 days [2]. The available information along with the presentation seen in the Hong Kong outbreak in 1997 is presented in the table 2. The main clinical features that have been reported are listed below.

- a. Fever
- b. Cough
- c. Sore throat
- d. Rhinorrhoea
- e. Myalgia
- f. Conjunctivitis
- g. Watery diarrhoea
- h. Severe unexplained respiratory illness

**If there is no fever, it is highly unlikely that avian influenza infection has occurred.**

## Case definition for suspected avian influenza (H5N1) infection.

### Clinical Presentation:

Fever ( = 38 ° C) **OR** history of fever

**AND** respiratory symptoms ( cough or shortness of breath requiring hospitalization.

### Travel and Contact history

History of travel in the 7 days prior to onset of symptoms, travel to an area affected by avian influenza A (H5N1) **AND** close contact (within 1 meter) with live or dead domestic fowl, wild birds or swine in any setting, including bird markets.

**OR** one of the following

- Close contact (touching/speaking distance) with other case(s) of severe respiratory illness or unexplained death from the above areas)
- Part of a health care worker cluster of severe unexplained respiratory illness.
- A laboratory worker with potential exposure to influenza A (H5N1).

A list of countries where poultry are currently affected can be obtained from:

<[http://www.hpa.org.uk/infections/topics\\_az/influenza/avian/algorithm.htm#H5N1list](http://www.hpa.org.uk/infections/topics_az/influenza/avian/algorithm.htm#H5N1list) >

The link given below gives a map of affected areas:

<[http://www.europa.eu.int/comm/health/ph\\_threats/com/Influenza/images/pathogenic.jpg](http://www.europa.eu.int/comm/health/ph_threats/com/Influenza/images/pathogenic.jpg)>

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### **Baseline Investigations**

The following baseline tests are recommended as they help in the risk assessment of the case.

- a. Chest X-ray
- b. Total and differential counts (for lymphopenia)
- c. Liver function tests

**Based on published data, these are highly likely to be abnormal in cases of avian influenza. If laboratory tests and CXR is normal, this is unlikely to be avian influenza**

**Table 2: Summary of clinical findings in cases of H5 avian influenza:**

Year	Vietnam [1]		Thailand [2]		Hong Kong [3]	
	2004		2004		1997	
Number of patients described	10 (M=6,F=4)	%	5 (M=4, F=1)	%	12 (M=5, F=7)	%
Age (Median)	12.5		6.5		9	
Time from exposure to illness (Median)	3 days		NA		NA	
Time from exposure to illness (Range)	2 – 4 days		NA		NA	
Fever >38°C	Yes	100	Yes	100	Yes	100
Cough	Yes	100	Yes	100	Yes	67
Shortness of breath	Yes	100	Yes	100	NA	
Hypoxia (in those who died)	Yes	> 90	Yes	100	NA	
Crackles	Yes	90	NA		NA	
Chest X-ray changes	Yes*	100	Yes	100	Yes	58.3
Sore throat	No		Yes	80	Yes	33.3
Rhinorrhoea	No		Yes	40	Yes	58.3
Myalgia	No		Yes	40	No	
GIT symptoms (diarrhoea / loose stools, abdominal pain / vomiting)	Yes	70	No		Yes	50
<b>Conjunctivitis</b>	No		No		No	
Rash	No		No		No	
Lymphopenia	Yes	100	Yes	100	Yes	58.3
LFT abnormality	Yes (n = 6)	100	Yes (n = 4)	80	Yes	60
Death	Yes	80	Yes	100	Yes	41.7
Time to death after onset of illness (Median)	9 days		18 days		7 days	
Time to death after onset of illness (Range)	6 – 17 days		8 – 29 days		5 – 25 days	

\*Chest X-ray abnormalities were non-specific and included diffuse, multifocal or patchy infiltrates. Some cases showed segmental or lobular consolidation with air bronchogrammes.


**About the risk: should avian influenza be considered?**

The next step is to assess the risk of being infected with avian influenza. In the presence of an appropriate travel and contact history, the probability of infection (a weighting given to clinical presentation and baseline investigations) is assessed. Then, the risk category for the individual is assessed. Respiratory sample processing should follow risk assessment.

**Probability of Infection:**

Clinical presentation and the results of baseline investigations are considered to measure the probability of infection. Table 3 gives the suggested probability of infection:

**Table 3: Probability Of Infection:**

				
	LOW	Increasing probability of infection		HIGH
	Suspect	Possible	Probable	
Clinical presentation	Any 2 symptoms from (a-f)	Suspect + g	Fever + Severe respiratory illness ± Any other symptom	
Baseline Investigations	Normal	Borderline	Abnormal	

**Risk Category:**

In the presence of a:

- Positive travel history, **AND**
- Positive contact history,

Consult table 4 to find the risk category:

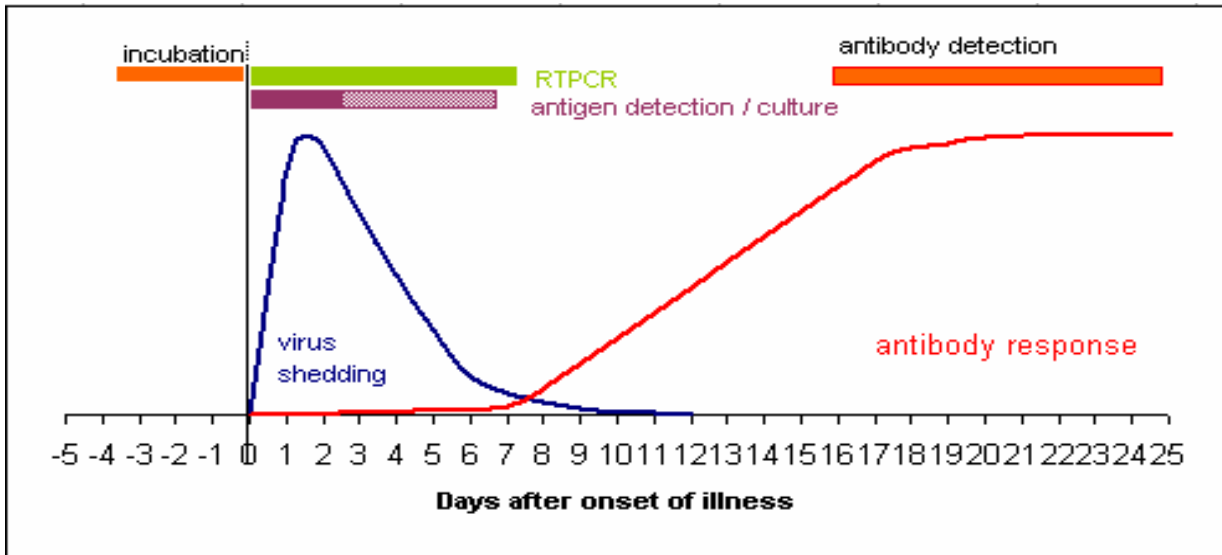
**Table 4: Risk Category:**

Baseline investigation	Clinical presentation		
	Suspect	Possible	Probable
Normal	Low	Low	Low
Borderline	Low	Low	Medium
Abnormal	Low	Low	High

## About Samples

Data is insufficient on the duration of virus shedding from the current outbreak. Figure 1 illustrates the viral shedding and antibody response to the infection following H5 influenza A infection.

**Figure 1: Viral Shedding and antibody response following Influenza A H5 infection.**



The most sensitive test is RT-PCR for the detection of viral RNA. Viral culture and antigen detection is useful in the first few days after onset of symptoms. Rapid EIA tests and immunofluorescence (IF) tests are aimed at the nucleoproteins of influenza A, and are capable of detecting avian influenza. Commercial IF reagents available in the UK **are capable** of detecting H5 influenza, and as DIF is the most commonly used rapid influenza diagnostic test used, it should be part of the investigation of a suspect case of avian influenza. The list of samples required and the tests that can be performed depending on the duration after onset of illness are given in table 5:

**Table 5: Tests that can be performed on samples collected at different stages of illness**

Detection of:	Tests recommended	Samples	Days from onset of illness											
			1	2	3	4	5	6	7	8-14	>14			
Antigen	Immunofluorescence or Rapid EIA tests*	• NPA												
		• BAL	√	√	√	√	√	√	√					
		• ETA												
		• NTS												
Nucleic Acid	RT-PCR	• NPA												
		• BAL	√	√	√	√	√	√	√	???				
		• ETA												
		• NTS												
Virus	Culture	• NPA												
		• BAL	√	√	√	√	√							
		• ETA												
Sero-response	Neutralisation test	Acute sera	√	√	√	√	√	√	√					
		Convalescent sera												√

NPA- nasopharyngeal aspirate; BAL-Bronchoalveolar lavage; ETA-Endotracheal aspirate; NTS-Nose and Throat swab

\*- Directigen Flu A (Becton Dickinson), Quick Vue (Quidel)

If the travel history includes a geographical area where SARS could re-emerge, samples should also include EDTA blood, stool and urine samples in the acute stage. Please see guidance on SARS for further information. Local laboratories should be able to undertake tests for influenza in suspect avian influenza cases, using their influenza testing algorithms with consideration to biosafety (see below). If influenza A is detected, and it is considered that avian influenza is a serious consideration, specimens can be referred to the Virus Reference Division (VRD) for specialist testing

### Handling specimens from a potentially infected individual

Highly pathogenic avian influenza (H5N1 / H7N7) has been classified as a Containment Level 3 (CL3) organism by the advisory committee on dangerous pathogens (ACDP). The following guidance is for clinical laboratories – modified from ACDP guidance <<http://www.hse.gov.uk/infection/diseases/avianflu.htm>>

- A clinical risk assessment must be performed (See above)
- Results of the risk assessment must be communicated to lab staff before work starts on the sample
- All staff handling specimens potentially containing these viruses should be given detailed information on hazard and instructions on measures to reduce risk of exposure to the agent

### Containment Levels for Processing Clinical Samples:

- Low / Medium risk - CL2
  - High risk - CL3
  - Known positive - DEFRA Cat 4
- (CL 3 - either class I or class III biosafety cabinet can be used).

The containment levels and the tests that can be performed depending on the risk category are given in table 6:

**Table 6: Containment levels by test and risk category**

	Low / Medium risk	High risk
Immunofluorescence	CL 2	CL 3
Rapid tests	CL 2	CL 3
RT-PCR	CL 2	CL 3*
Culture	CL 2	**

\* Sample handling prior to inactivation for nucleic acid extraction.

\*\* Wild type avian influenza H5N1 is an ACDP category 3 organism, but a DEFRA category 4 organism. However, DEFRA regulations take precedence on avian influenza work, hence work should only be performed in DEFRA 4 laboratories. If there is a strong suspicion that avian influenza strains (uncharacterised) are present, a high risk specimen should be handled at CL3 prior to confirmation that influenza A is present,

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- In a known positive case, if a DEFRA level 4 facility is not available, samples have to be sent to a lab which has the facility. If the containment facility is not available in the regional laboratory, samples have to be sent to the Influenza laboratory at the Virus Reference Division (VRD), HPA, Colindale (contact number 020 8200 1295, or contact Dr Maria Zambon 020 8327 6269 for discussion about case).
- Risk of handling specimens for biochemical/hematological tests: virus shedding is primarily in the respiratory tract, and there is no evidence of viraemia or shedding in other body fluids. Hence there is no need for specialist handling of these samples.

### **Transport of specimens**

All samples should be transported as per UN 602, and instructions are available in [http://www.blood.co.uk/foi/08\\_Human\\_resources/Carriage\\_and\\_Packaging\\_Of\\_Dangerous\\_Goods111003.pdf](http://www.blood.co.uk/foi/08_Human_resources/Carriage_and_Packaging_Of_Dangerous_Goods111003.pdf) >

If specimens have to be transported to a regional or reference laboratory, specimens should be transported on wet ice. It is preferable to courier the specimen, after discussion with the receiving laboratory. Serum samples for serology can be sent to VRD through Hays Dx. All samples that are sent to VRD should be marked "For the attention of Dr. Maria Zambon", and should be sent only after telephone discussion with the lab personnel (contact number: 020 8327 6269).

### **References:**

1. Hien TT, Liem NT, Dung NT, *et al.* Avian Influenza A (H5N1) in 10 Patients in Vietnam. *N Engl J Med* 2004,350:1179 - 1188.
2. Avian influenza A (H5N1). *Wkly Epidemiol Rec* 2004,79:65-76.
3. Yuen KY, Chan PK, Peiris M *et al.* Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet* 1998,351:467-471.

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