



HPA Protocol for UNDIAGNOSED SERIOUS ILLNESS: A MICROBIOLOGICAL APPROACH TO INVESTIGATION

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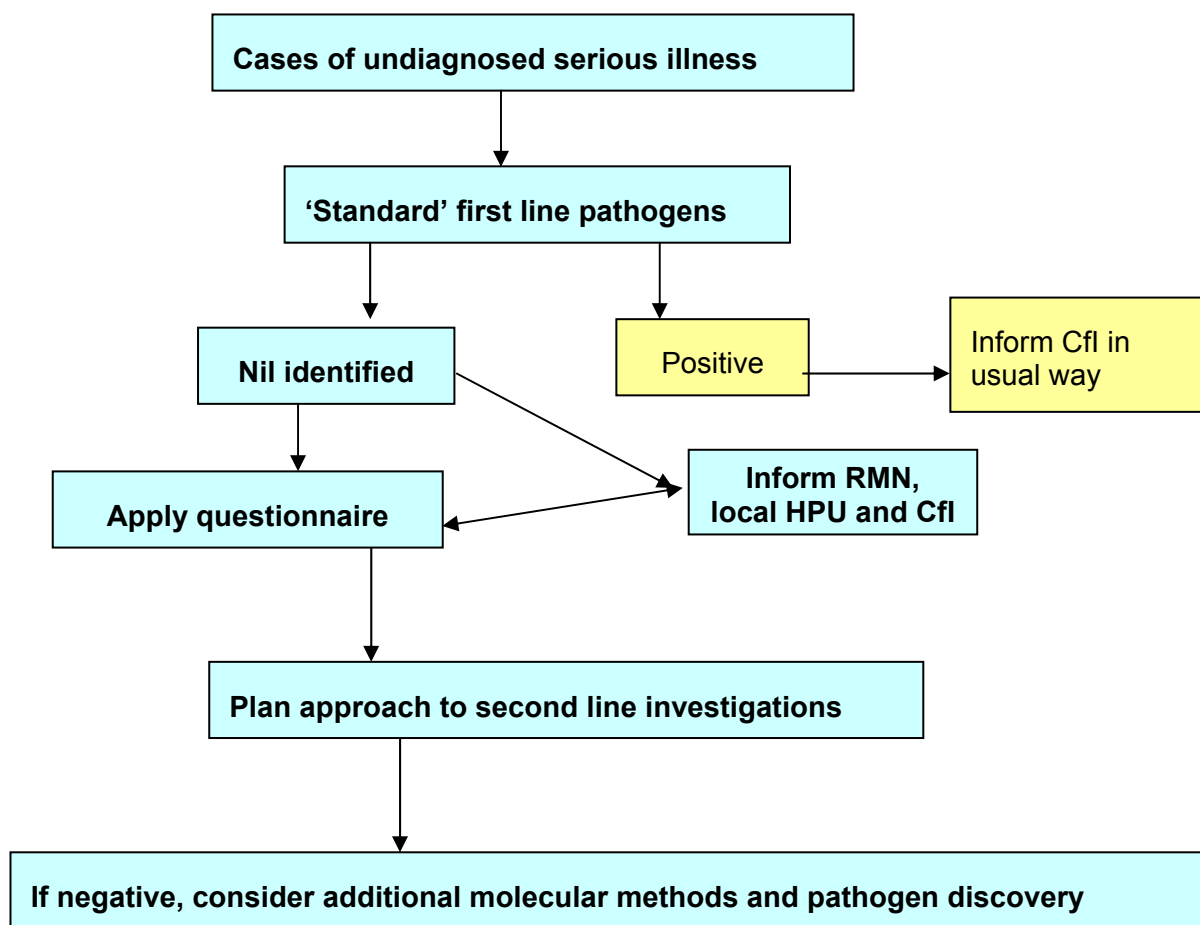
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Introduction

Outbreaks and incidents of “unusual” illnesses might have any of a number of causes, including infectious, chemical, nutritional, or radiological. The aetiological agent may remain undetermined, it may be that a novel organism is responsible, or that there has been an accidental or deliberate release of a chemical, biological or radiological agent. This document outlines the approach for the microbiological investigation of cases or clusters of serious undiagnosed illness.

Failure to identify a “conventional aetiology” should result in a direct approach to the Regional Microbiologist and to the Duty Officer at the local HPU, and then to the HPA Centre for Infections (CfI). The first points of contact at CfI for reporting the existence of a patient(s) who has presented with an unexplained illness of suspected infectious aetiology are given below. Specific microbiological or public health advice should be obtained from the relevant experts (see page 3). Clinical and epidemiological observation together with consultation and expert advice should result in one or more presumptive diagnoses, which can then be investigated appropriately.

The following diagram indicates the proposed investigation algorithm.



At CfI, please inform Mandy Walsh, Emerging Infections and Zoonoses Section (☎ 020 8327 7483), or if out of hours, the Duty Doctor at CfI (☎ 020 8200 4400)

Case definitions

For the purpose of focusing microbiological investigations and surveillance for an “unknown” infectious agent, it is essential to document in detail the clinical and epidemiological features of suspected cases. A working case definition should also be developed based on the initial clinical/epidemiological observations at the time. The following ‘starting point’ case definition encompasses the type of patient for which this guidance is appropriate. A cluster is defined here as two or more cases related in time and space. Single cases may be considered if they are severely ill, as may clusters of very severe or prolonged gastrointestinal disease.

A suitable case is :-

- aged between 1 and 60 years
- previously healthy
- has died or been admitted
- with a serious illness suggestive of an infectious process*
- with signs and symptoms relevant to these syndromes of interest
 - Neurological:** meningitis, encephalitis, encephalopathy, or neurological disturbance
 - Respiratory:** pneumonia, infiltrates, pneumonitis, or ARDS
 - Sepsis:** acute fulminating septicaemia, haemorrhagic disease, or shock
 - Hepatitis/Jaundice:** fulminant hepatitis, hepatic failure, or serious illness with jaundice

* Including for example: fever or history of fever, leucocytosis or leucopaenia, raised CRP or other marker of infection, histopathological evidence of an acute infectious process, or a physician-diagnosed syndrome consistent with an infectious aetiology.

Avian Influenza guidelines

Cases of severe respiratory disease may require to be investigated in order to rule out avian influenza. Appropriate algorithms for this can be found here:

http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1202115578855 (history of travel to endemic area)

http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1202115579653 (if not travelled to endemic area)

Recognising cases of unusual illness

The laboratory may become involved in investigating cases of unusual illness either before or after those cases have been recognised clinically as being unusual. If the laboratory is first to recognise such cases, please do the following:

- Liaise with the senior clinician in charge of the case and between you ensure that you have informed the local HPU, the Regional Microbiologist, and Mandy Walsh at Cfl (☎ 020 8327 7483)
- Inform the infection control team, and ensure that appropriate infection control procedures are in place
- If appropriate, seek expert advice concerning the management of any potentially exposed laboratory staff. If relevant, make a list of staff who may have been exposed (name, age, address, contact details, their GP contact details, and type of exposure)
- Advice on the **initial investigation and management of outbreaks and incidents of unusual illnesses** is available on the HPA website. Sections of this document provide advice specifically for laboratories. Available at: http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1201265888951
- Cases may be first recognised through post-mortem examinations, either coronial or routine. Section 7 of the above document provides advice on sampling by histopathology departments.

First line investigations

On receipt of enquiries about, or clinical material from, patients with 'undiagnosed' serious illness, the laboratory will naturally work through its own standard first line list of likely pathogens. This may require referral of samples, for example, for virology or PCR. Lists of first and second line pathogens are included overleaf for each of the clinical syndromes. *Please note that these lists are NOT intended to be a complete list of every possible pathogen, but rather some examples of rarer diagnoses to be considered.*

If first line diagnostic investigations are negative, use of the checklist *(below) and questionnaire (appendix 2) may guide the direction of further laboratory diagnostic testing, and ensure that small volume samples are reserved for the most appropriate tests.

Checklist

(see Appendix 2 for full questionnaire)

a) The patient	b) The samples
<ul style="list-style-type: none"> • What is the syndrome? <ul style="list-style-type: none"> ▪ Neurological :- meningitis, encephalitis, encephalopathy, or neurological disturbance ▪ Respiratory:- pneumonia, infiltrates, pneumonitis, or ARDS ▪ Sepsis:- acute fulminating septicaemia, haemorrhagic disease, or shock syndrome ▪ Jaundice/Hepatitis:- fulminant hepatitis, hepatic failure, or serious illness with jaundice • Rapidity of onset? Onset date? Duration of illness to date? (or to death) • Age, sex, and geographic location? • Contact with other sick people, at home or at work? • Occupational exposure? What do they do and where? How long have they worked there? • Hobbies or recreational exposure? Doing what, when, and where? • Animal exposure? Which animals/birds, where, and duration of exposure? Were the animals/birds sick or healthy? • Consumption of unpasteurised or 'unusual' food items, or home-processed foods? What, where, and how produced? When consumed? • National or foreign travel? If so where, how long for, and what type of exposure is likely to have occurred? • Compromised immune status, underlying disease or other risk factors? 	<ul style="list-style-type: none"> • What samples are available? Or can be retrieved? • What tests have already been done? Negative on standard tests/cultures so far?* • What are now thought to be the most likely pathogens? • Is there a possibility of a BT agent? (particularly anthrax, smallpox, plague, tularemia, VHF, botulism) - may require urgent referral to relevant labs at CFI or CEPR • Consider referral of samples to other specialist or reference laboratory • Consider additional media or cell lines, atmosphere, range of temperatures, prolonged incubation etc. • Retain all samples <p>* If an 'unidentifiable' organism has been isolated, refer to relevant reference lab (if known), or to laboratories at Cfl for identification (see below).</p>

* Identification of unknowns – please refer isolates to Cfl:

1. Laboratory of Health Care Associated Infections for cellular fatty acid analysis (MIDI) and for 16S ribosomal DNA sequencing. (☎ 020 8327 7233)
2. Molecular Identification Services Unit (☎ 020 8327 7869)

Further diagnostic investigations

Once the first line pathogens have been excluded, samples can be referred as appropriate to a range of reference laboratories, and/or to laboratories at Cfl for additional expertise. In order that surveillance for Serious Undiagnosed Illness may be carried out more formally, and so that scientific/clinical advice and support may be given, please always notify one of the following:

Initial contact: Mandy Walsh, Emerging Infections and Zoonoses Section (☎ 020 8327 7483)

Virology: Dr. David Brown, Virus Reference Department (☎ 020 8327 6017)

Bacteriology: Dr. Robert George, Respiratory & Systemic Infections Department (☎ 020 8327 7222)

Public health: Dr. Dilys Morgan, Gastrointestinal, Emerging & Zoonotic Infections (☎ 020 8327 7474)

Molecular testing and a range of pathogen discovery options are also available at Cfl. The latter may be considered if there is a cluster of cases for whom a diagnosis cannot be reached. These options require further discussion with the Directors of the VRD and RSID (as above), and with the Molecular Identification Services Unit (☎ 020 8327 7869).

Reference laboratories are listed on page 7.

Specimen collection and storage guidance is in Appendix 1 on page 8.

1. Neurological :- meningitis, encephalitis, encephalopathy, or neurological disturbance

Likely 1st line

Streptococcus pneumoniae
Neisseria meningitidis
Haemophilus influenzae
Listeria monocytogenes
Staphylococcus aureus
 β -haemolytic streptococci
 Enterobacteriaceae, including
Salmonella spp

Herpes simplex virus
 Varicella-Zoster virus
 Enteroviruses
 Parechovirus
 Adenovirus
 HHV6/7
 Influenza viruses A&B

Cryptococcus neoformans

Mycobacterium spp

Clostridium botulinum

Examples of 2nd line organisms (depending on immune status, underlying disease, and possible exposures including travel history). *Note: this is not intended to be a complete list of possible aetiologies.*

Mycoplasma pneumoniae
Treponema pallidum
Borrelia spp
Leptospira spp
Nocardia spp

Measles virus
 Mumps virus
 CMV
 EBV
 HIV

Toxoplasma gondii
Acanthamoeba & *Naegleria* spp
Angiostrongylus cantonensis
Gnathostoma spinigerum
Baylisascaris procyonis

Brucella spp
Rickettsia spp
Ehrlichia spp
Bacillus anthracis
Yersinia pestis

Lyssaviruses
 Poliovirus
 Arboviruses

 LCMV
 JC virus
 Herpes B virus

Candida spp
Histoplasma capsulatum
Coccidioides immitis

2. Respiratory:- pneumonia, infiltrates, pneumonitis, or ARDS

Likely 1st line

<i>Streptococcus pneumoniae</i>	Influenza viruses A&B	<i>Pneumocystis jiroveci</i>
<i>Haemophilus influenzae</i>	Parainfluenza viruses	<i>Cryptococcus neoformans</i>
<i>Moraxella catarrhalis</i>	RSV	<i>Aspergillus fumigatus</i>
<i>Staphylococcus aureus</i>	Adenoviruses	
Enterobacteriaceae	HSV	
	CMV	
<i>Mycoplasma pneumoniae</i>		
<i>Legionella pneumophila</i>		
<i>Mycobacterium</i> spp		

Examples of 2nd line organisms (depending on immune status, underlying disease, and possible exposures including travel history). *Note: this is not intended to be a complete list of possible aetiologies.*

<i>Chlamydomphila pneumoniae</i>	Measles virus	<i>Ascaris lumbricoides</i>
<i>Chlamydomphila psittaci</i>	VZV	Hookworms
<i>Coxiella burnetii</i>	EBV	<i>Strongyloides stercoralis</i>
<i>Pasteurella</i> species		<i>Paragonimus westermanii</i>
<i>Neisseria meningitidis</i>	Hantaviruses (e.g. Sin Nombre virus) [hantavirus pulmonary syndrome]	<i>Candida</i> species
<i>Fusobacterium necrophorum</i> and other anaerobes		<i>Histoplasma capsulatum</i>
<i>Nocardia</i> spp	SARS coronavirus	<i>Coccidioides immitis</i>
<i>Actinomyces</i> spp		<i>Blastomyces dermatitidis</i>
<i>Bacillus anthracis</i>		<i>Paracoccidioides brasiliensis</i>
<i>Yersinia pestis</i>		<i>Cryptococcus gattii</i>
<i>Francisella tularensis</i>		
<i>Burkholderia pseudomallei</i>		

3. Sepsis: Acute fulminating septicaemia, haemorrhagic disease, or shock

Likely 1st line

Escherichia coli
Streptococcus pneumoniae
Staphylococcus aureus
Other Enterobacteriaceae
Neisseria meningitidis
 β -haemolytic streptococci

See 2nd line list – the possible viral agents require a relevant travel history

Pseudomonas aeruginosa and other non-fermentative GNRs

Haemophilus influenzae

Clostridium novyi and other clostridia

Examples of 2nd line organisms (depending on immune status, underlying disease, and possible exposures including travel history). *Note: this is not intended to be a complete list of possible aetiologies.*

Anaerobes including <i>Fusobacterium</i> (Lemierre's syndrome)	Dengue haemorrhagic fever/ Dengue shock syndrome
<i>Leptospira</i> spp	
<i>Salmonella</i> Typhi & Paratyphi	VHFs (CCHF, Lassa, Ebola, Marburg, New World Arenaviruses, etc)
<i>Salmonella</i> spp	
<i>Brucella</i> spp	
<i>Vibrio</i> spp	
<i>Mycobacterium</i> species	Hantaviruses (Puumala, Dobrava, Seoul, Hantaan) [haemorrhagic fever with renal syndrome]
<i>Listeria monocytogenes</i>	
<i>Streptococcus bovis</i>	
<i>Bartonella</i> species	
<i>Bacillus anthracis</i>	
<i>Yersinia pestis</i>	
<i>Francisella tularensis</i>	
<i>Burkholderia mallei</i> and <i>B. pseudomallei</i>	

4. Hepatitis/Jaundice: Fulminant hepatitis, hepatic failure, or serious illness with jaundice

Likely 1st line

<i>Leptospira</i> spp	HAV
	HBV
Pyogenic liver abscess (Enterobacteriaceae, <i>Staph aureus</i> , Anaerobes, Streptococcus 'milleri')	HCV
	HEV (Note: does NOT require a history of travel)
	CMV
	EBV

Examples of 2nd line organisms (depending on immune status, underlying disease, and possible exposures including travel history). *Note: this is not intended to be a complete list of possible aetiologies.*

<i>Coxiella burnetii</i>	HSV	<i>Entamoeba histolytica</i>
<i>Brucella</i> spp		<i>Toxoplasma gondii</i>
<i>Rickettsia</i> spp	Haemorrhagic fever viruses	<i>Plasmodium falciparum</i>
<i>Treponema pallidum</i>	Yellow fever virus	
<i>Borrelia burgdorferi</i>	Paramyxoviruses	
<i>Salmonella</i> Typhi		

Specialist / Reference Laboratories

Links to all the laboratory-specific information can be found here <http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1153846673361?p=1153846673361>, or by selecting "Laboratories and Reference Facilities" from the left hand menu on the Infectious Diseases page on the HPA website www.hpa.org.uk

1. Colindale

- Virus Reference Department (VRD)
- Respiratory and Systemic Infections Laboratory (RSIL)
- Laboratory for Enteric Pathogens (LEP)
- Food Safety Microbiology Laboratory (FSML)
- Laboratory Health Care Associated infections (LHCAI)
- Sexually Transmitted Bacteria Reference Laboratory (STBRL)

2. Porton

- Special Pathogens Reference Unit (SPRU)

3. Other

- Anaerobe Reference Lab (Cardiff)
- Brucella Reference Unit (Liverpool)
- Leptospira Reference Unit (Hereford)
- Lyme Reference Unit (Southampton)
- Meningococcal Reference Unit (Manchester)
- Mycology Reference Laboratory (Bristol)
- Mycobacterium Reference Unit (London)
- Parasitology Reference Laboratory (London)
- Q fever Reference Laboratories (SPRU and Bristol)
- Toxoplasma Reference Laboratory (Swansea)

Appendix 1: Specimen Collection Protocol and Storage

The following is a guide for taking specimens from potential case-patients. In addition to examination in the local laboratory, it is likely that specimens will be referred to other laboratories for further microbiological analysis. A key objective of this guidance is to maximise the potential to identify an infective cause.

In the investigation of an unknown infection, in addition to culture techniques, it is likely that DNA amplification techniques to detect microbial specific nucleic acid (eg PCRs for eubacterial 16/23S rDNA, and specific/generic viral nucleic acids) will play a major role. Thus, normally sterile site samples are preferred wherever possible, providing they are relevant to the signs/symptoms displayed. Other investigations may include electron microscopy and serology.

The order of priority for specimen collection will in large part depend upon the clinical presentation.

Specimens should be collected and transported to the clinical microbiology laboratory as rapidly as possible for testing, storage, and possible subsequent transfer to a reference laboratory.

Samples

Blood cultures: for extended aerobic and anaerobic culture; ideally at least three sets of blood cultures collected over one hour, with at least one set prior to antibiotic administration. Consideration should be given to immediate post-mortem blood cultures if none have been collected in the previous few hours. Bottles negative after the standard incubation period should be retained for possible examination by PCR or other testing methods. Aliquot into 3x1ml containers and store at -70°C (or lowest temperature available).

Respiratory tract samples: sputum, broncho-alveolar lavage or similar, for standard cultures and virology. Nose and throat swabs may be appropriate depending upon signs and symptoms.

Biopsy tissues collected aseptically from local inflammatory lesion, necrosis or abscess, if surgical debridement is performed.

- as many samples as possible from multiple areas; quantity is important
- tissue placed in sterile containers for direct culture (aerobic and anaerobic) and storage
- formalin-fixed (10% buffered formalin) or paraffin embedded

Pus and vesicle fluid or swab of local lesion

- pus - as large a volume as feasible, placed in sterile containers for microscopy, aerobic and anaerobic cultures, and storage
- if no pus available, swab of lesion put immediately into transport medium
- vesicular fluid - should be swabbed and placed into viral transport medium and/or dried onto a microscope slide

Sera: acute (admission) and convalescent (near time of death, or on discharge) samples.

- optimal volume - 10 ml spun serum

Whole blood: acute and convalescent samples

- optimal volume - 10 ml whole blood (EDTA tube)

Urine: clean catch collection into sterile container

- optimal volume greater than 20ml

Body fluids: if clinically indicated - cerebrospinal, pleural, or pericardial fluids, or other specimens taken as part of the clinical workup.

Faeces/Stools. If ingestion of contaminated food or water is considered as a possible route of exposure, then faeces/stool samples should be collected for culture/microbial toxin detection whether GI symptoms are present or not.

Sample storage

Specimen	Requirements
Blood cultures	Negative blood culture bottles should be aliquoted into 3x1ml containers and stored at -70°C or lowest available temperature
Serum	Acute and convalescent samples. Store at -70°C or lowest available temperature
Whole blood in EDTA	Acute and convalescent samples, 2 x 5mls each if possible. <ul style="list-style-type: none"> ▪ Separate one, and keep both plasma and cell deposit. ▪ Store the other unseparated. All to be stored at -70°C or lowest temperature available
Respiratory samples	A portion of sputum, BAL or other respiratory sample, stored frozen at -70°C or lowest available temperature
Pus and tissue samples Tissues e.g.: <ul style="list-style-type: none"> • local inflammatory lesions or abscess material • liver • spleen • lung • kidney • heart • enlarged lymph nodes • bone marrow • other organs with gross pathologic changes • vitreous 	Collect duplicate tissue fragments measuring ~ 1cc Pus or <i>non-fixed</i> tissue – store at -70°C or lowest available temperature Fixed samples (10% buffered formalin for 24 hours of fixation, and subsequent paraffin embedding. Antigenicity decreases for immunohistochemical assays with prolonged formalin fixation), may be stored at room temperature
Stained and unstained slides	Stained slides may be kept at room temperature. Unstained slides should be frozen at the lowest available temperature
Urine	5mls, store at -70°C or lowest available temperature
Faeces/ stools	Store at -70°C or lowest available temperature
Other body fluids	e.g. cerebrospinal fluid, pleural fluid, pericardial fluid and other sterile site specimens. Store at -70°C or lowest available temperature

Appendix 2: Questionnaire

Completed by:
 HPU / RMN / other
 Date.....

a) The patient

- What is the syndrome?
 - **Neurological** :-
 Meningitis Encephalitis Encephalopathy Neurological disturbance
 Details/other.....
 - **Respiratory**:-
 Pneumonia Infiltrates Pneumonitis ARDS
 Details/Other.....
 - **Sepsis**:-
 Acute fulminating septicaemia Haemorrhagic disease Shock syndrome
 Details/other
 - **Jaundice/Hepatitis**:-
 Fulminant hepatitis Hepatic failure Serious illness with jaundice
 Details/other
- Age/DOB.....Sex: Male / Female... Ward.....
- Hospital
- HPU.....Region
- Where does the patient live ?
- Onset date? Rapidity of onset?
- Does the onset date relate to a possible exposure?
- Duration of illness to date? (or to death)
- **Contact with other sick people? Y/N**
 If yes, Who?
 Where?
- Is their diagnosis known? Y/N If yes, please specify.....
- Type of contact?
- Duration of exposure?
- **Contact with sick animals or birds? Y/ N**
 If yes, what animals/birds? (specify please)
 Where?
 What type of sickness did they have?
 Type of contact.
 Duration of exposure.....
- **Contact with healthy animals or birds? Y / N**
 If yes, what animals/birds?
 Where?
 Type of contact.....
 Duration of exposure
- **Occupational exposure? Y / N**
 What occupation?
 Where?
 How long have they worked there?

• **Hobbies or recreational exposure? Y / N**

Doing what?

When?

Where?

• **Consumption of unpasteurised or 'unusual' food items, or home-processed foods? Y / N**

What?

How produced?

Where consumed?When consumed?

• **National travel Y / N**

Foreign travel? Y / N

Where?Where?

How long for?How long for?

What type of exposure likely?

• **Underlying disease or risk factors? Y / N**

(Please specify).....

b) The samples (NB. Requirement for chain of evidence documentation if a deliberate release)

▪ What samples were taken?

Blood culture Urine Sputum CSF Pus Plasma Clot Buffy coat Other

If other, please specify.....

▪ Which samples are still available?

Blood culture Urine Sputum CSF Pus Plasma Clot Buffy coat Other

If other, please specify.....

▪ What tests have already been done?

Bacterial culture TB culture Viral culture Antigen detection PCR Serology

Results?.....

.....

▪ Negative on standard tests/cultures at source laboratory? Y / N

▪ Optimal methods used? Y/N

▪ What are now considered the most likely pathogens?

.....

▪ Likelihood of BT agent? (particularly anthrax, smallpox, plague, tularemia, VHF, botulism) Y / N

Note:- may require urgent referral to relevant labs at HPA CfI or HPA Porton (SPRU)

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▪ Likelihood of an 'atypical' pathogen including zoonotic infections? (e.g. Q fever, chlamydiosis, leptospirosis, brucellosis, etc.)

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▪ What samples/cultures have to be referred to other Specialist / Reference laboratories?

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