Laboratory testing for novel coronavirus

Interim recommendations

21 December 2012

1. INTRODUCTION

The purpose of this document is to provide interim recommendations to laboratories and stakeholders involved in the laboratory testing for novel coronavirus (see http://www.who.int/csr/disease/coronavirus_infections/en/index.html for the most recent updates on the event). These recommendations are based on the current understanding of this novel coronavirus.

The recommendations have been prepared by WHO and reviewed by laboratory experts, including those with experience handling this virus and other coronaviruses, and also those with expertise in the development of diagnostic assays for coronaviruses. Part of this review process included a global conference call in late November 2012. WHO is closely monitoring developments related to this virus and will revise these recommendations when necessary. Unless revisions are made, this document will expire on 31 March 2013.

2. INDICATIONS FOR TESTING

WHO recommends that clinicians, epidemiologists and laboratory scientists consult the WHO case definition (http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html), which will be updated as needed, to determine which patients should be tested. Should resources permit, other potential causes of respiratory infections should be ruled out using routinely available laboratory tests as recommended in local management guidelines for community-acquired pneumonia, to determine the presence of other potential primary aetiologies. Examples of other aetiologies include *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, *Legionella pneumophila*, influenza virus, and respiratory syncytial virus. A number of human coronaviruses are known causes of respiratory tract

infections; these include the betacoronaviruses hCoV-OC43 and hCoV-HKU1 and the alphacoronaviruses hCoV-229E and hCoV-NL63.

A triage process should ensure that testing for the novel coronavirus is undertaken only when there is clinical or epidemiological evidence that this virus may be the cause in an individual or cluster of patients so as to avoid the inappropriate use of scarce resources, the generation of false positives and the risk of overwhelming the health system by unnecessary activation of hospital-based and public health response teams.

3. SPECIMEN COLLECTION AND SHIPMENT

Whenever specimens are collected from cases under investigation, infection control guidelines should be followed. WHO has published guidelines here: http://www.who.int/csr/resources/publications/swineflu/WHO_CDS_EPR_2007_6/en/

Based on current but limited information, lower respiratory tract specimens (such as tracheal aspirates and bronchoalveolar lavage; see Table 1) appear to have the highest virus titre. Upper respiratory tract specimens are also recommended, especially when lower respiratory tract specimens cannot be collected. Paired serum samples (collected at least 21 days apart, with the first being collected during the first week of illness) should also be collected and stored pending the availability of serological assays.

Specimens being sent to another laboratory for testing for the novel coronavirus should preferably be tested to exclude the presence of known respiratory pathogens before dispatch.

Table 1 lists the specimens that can be collected as well as their storage and transport requirements.

Specimens should reach the laboratory as soon as possible after collection. The importance of proper handling during transportation cannot be overemphasized. When there is likely to be a delay in the laboratory receiving respiratory tract specimens or serum, it is strongly advised to freeze them on dry ice.

Table 1. Types of specimens for testing for the presence of novel coronavirus and advice on handling

Specimen type	**Transport medium**	**Transport to laboratory**	**Dangerous goods shipping category**	**Comment**
Naturally produced sputum*	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	Biological substance, Category B	Need to ensure the material is from the lower respiratory tract
Bronchoalveolar lavage	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	There may be some dilution of virus but still a worthwhile specimen
Tracheal aspirate	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Nasopharyngeal aspirate	no	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Combined nose/throat swab	Virus transport medium	On ice.	As above	Virus has been detected in this type of specimen
Nasopharyngeal swab	Virus transport medium	On ice.	As above	
Tissue from biopsy or autopsy including from lung	Virus transport medium or saline	On ice. If a delay in testing of > 24 hours consider freezing with dry ice	As above	
Serum for serology or virus detection: always collect paired samples if possible. Acute – first week of illness	no	On ice **or** frozen	As above	

Convalescent - ideally 3 to 4 weeks later				
Whole blood	EDTA anticoagulant	On ice	As above	For virus detection, particularly in the first week of illness

* The collection of induced sputum samples may pose an additional infection risk for health care workers.

Transport of specimens within national borders should comply with applicable national regulations. International transport of novel coronavirus specimens should follow applicable international regulations as described in the *WHO Guidance on Regulations for the Transport of Infectious Substances 2011-2012 (Applicable as from 1 January 2011*) available at: http://www.who.int/ihr/publications/who_hse_ihr_20100801/en/index.html

LABORATORY TESTING METHODS AND ALGORITHM

Routine confirmation of cases of novel coronavirus infection will be based on detection of unique sequences of viral RNA by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and sequencing.

In certain circumstances, but not routine diagnosis, laboratories with the appropriate experience and containment facilities, may attempt to isolate the virus in cell culture. These recommendations do not cover virus isolation procedures.

Any testing for the presence of this virus should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. Individual Member States will decide which, if any, of their laboratories should perform these tests.

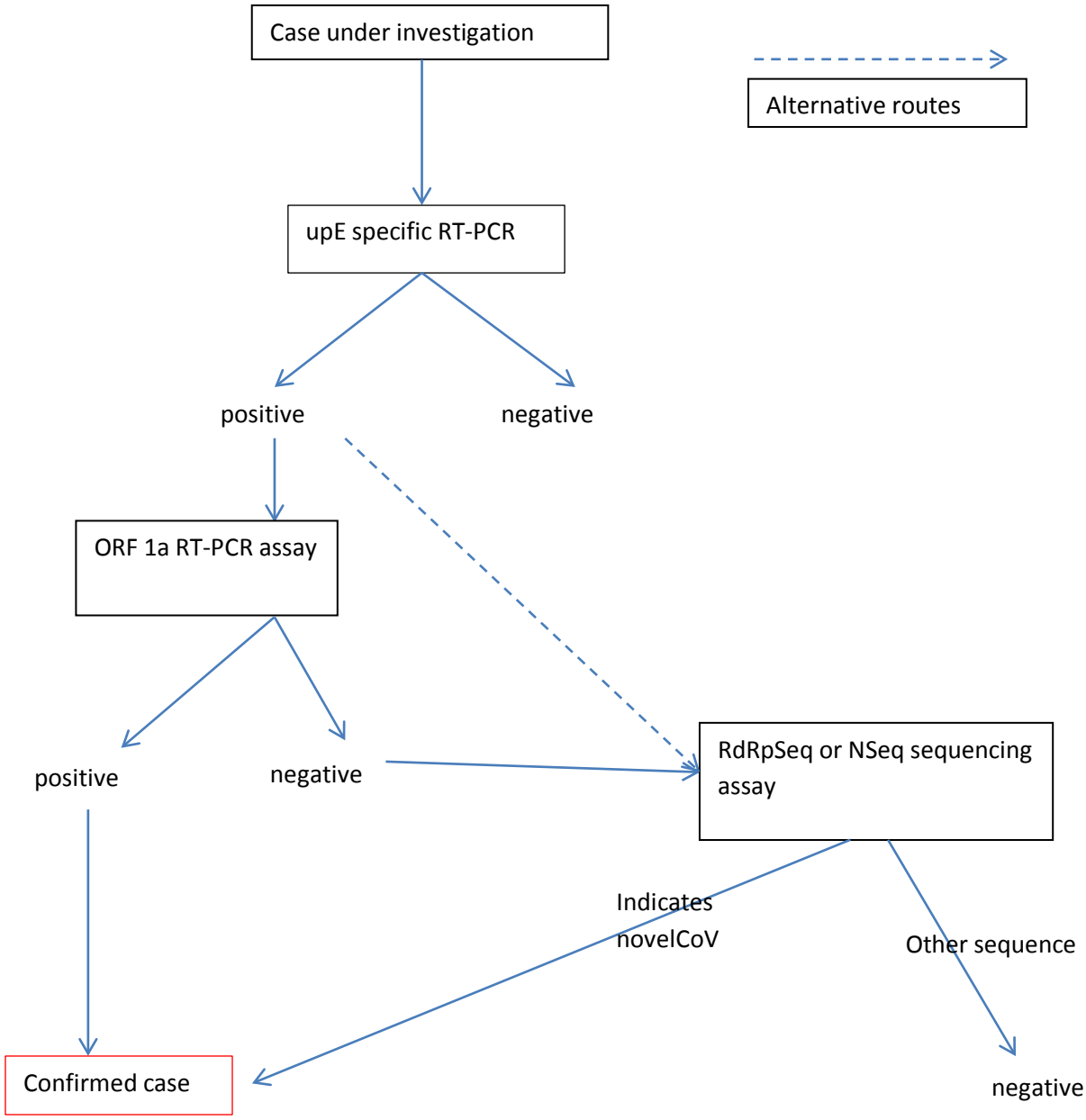
A number of RT-PCR assays that are specific for the novel coronavirus have been developed and published. Currently described tests include an assay targeting upstream of the E protein gene (*upE*)[1] and assays targeting the open reading frame 1b (*ORF 1b*) gene[1] and the open reading frame 1a (*ORF 1a*) gene[2]. The assay for the *upE* target is considered highly sensitive, with the *ORF 1a* assay considered of equal sensitivity. The *ORF 1b* assay is considered less sensitive than the ORF 1a assay but may be more specific. The papers cited in references 1 and 2 contain detailed descriptions for performing these assays.

In addition, several target sites on the novel coronavirus genome suitable for sequencing to aid confirmation have been identified. These are in the RNA-dependent RNA polymerase (*RdRp*) and nucleocapsid (*N*) protein genes.[2]

Further information on these assays is available at the website of the Institute of Virology, Bonn, Germany: http://www.virology-bonn.de/index.php?id=40.

Figure 1 shows a testing algorithm for the investigation of cases.

Figure 1: Testing algorithm for cases under investigation for novel coronavirus



When there are discordant results with two assays targeting unique sites on the novel coronavirus genome, sequencing of an amplicon generated from an appropriate PCR assay to confirm the test result should be performed. These sequence data, in addition to providing confirmation of the presence of the virus, can also provide valuable information to help understand the origins of the virus and whether it has one or several sources. Hence, sequencing of nucleic acid from as many positive specimens as possible is recommended.

A description of reagents to use in an assay to detect antibodies to the novel coronavirus by immunofluorescence has recently been published[2]. When serological testing becomes widely available, recommendations on its use will be included in later versions of this document. .

It is important to remember that a series of negative results should not rule out the possibility of infection in a patient with clinical symptoms. A number of factors could result in false-negative results, including:

- poor quality of specimen, such as a respiratory tract specimen containing primarily oropharyngeal material
- the specimen was collected late or very early in the illness
- the specimen was not handled and shipped appropriately
- technical reasons inherent in the test, e.g., virus mutation or PCR inhibition

When the clinical presentation and epidemiology suggest an infection with novel coronavirus despite negative PCR results, serological testing may be useful to confirm infection. This highlights the importance of collecting paired serum samples from cases under investigation.

While laboratories are familiarizing themselves with the new assays and reagents, they may wish to obtain confirmation, through sequencing, of positive results in other laboratories with greater experience in handling material containing this virus. WHO Headquarters and Regional Offices can assist Member States that wish to access the services of laboratories in other countries.

4. INTERPRETATION OF LABORATORY RESULTS

To consider a case as laboratory-confirmed, one of the following conditions must be met:

positive PCR assays for at least two different specific targets on the novel coronavirus genome

OR

one positive PCR assay for a specific target on the novel coronavirus genome and an additional different PCR product sequenced, confirming identity to known sequences of the new virus[3].

A positive PCR assay for a single specific target without further testing is considered presumptive evidence of novel coronavirus infection. Final classification of cases will depend on clinical and epidemiological information combined with laboratory data. Case definitions can be found at: http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/index.html

Member States are requested to immediately notify WHO of initially positive laboratory results even before completion of all testing and final confirmation.

5. REAGENTS

As the primer and probe sequences for the PCR assays for the novel coronavirus have been published, laboratories can order these from their usual suppliers. Alternatively, laboratories may consult: http://www.virology-bonn.de/index.php?id=40.

Positive control material for the upE and 1A specific RT-PCR assays can be ordered from the European Virus Archive portal (http://www.european-virus-archive.com/Portal/produit.php?ref=1386&id_rubrique=9).

6. BIORISK MANAGEMENT

It is recommended that all manipulations in laboratory settings of samples originating from suspected or confirmed cases of novel coronavirus infection be conducted according to WHO recommendations available at: http://www.who.int/csr/disease/coronavirus_infections/NovelCoronavirus2012_InterimRecommendationsLaboratoryBiorisk/en/index.html

7. GLOBAL LABORATORY NETWORKING

Access to timely and accurate laboratory testing of samples from cases under investigation is an essential part of the surveillance of this emerging infection. All countries should have access to reliable testing either internally or in laboratories in other countries that are willing and able to perform primary detection or confirmatory testing. WHO, through its Regional Offices, can assist Member States to access testing internationally should the need arise. Member States may wish to sign Material Transfer Agreements (MTA) covering such topics as ownership of clinical material and intellectual property rights with international laboratories before shipping specimens.

For further information on the response of WHO to the novel coronavirus please see: http://www.who.int/csr/disease/coronavirus_infections/en/index.html

REFERENCES

1. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, van Boheemen S, Gopal R, Ballhause M, Bestebroer TM, Muth D, Müller MA, Drexler JF, Zambon M, Osterhaus AD, Fouchier RM, Drosten C (2012) Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill 17: pii=20285.
2. Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, Kreher P, Lattwein E, Eschbach-Bludau M, Nitsche A, Bleicker T, Landt O, Schweiger B, Drexler JF, Osterhaus AD, Haagmans BL, Dittmer U, Bonin F, Wolff T, Drosten C. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 2012;17(49):pii=20334.
3. Van Boheemen S, et al. 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio 3(6): e00473-12. doi:10.1128/mBio.00473-12.