Surface sampling of MERS-CoV in health care settings:
A practical “how to” protocol for health care and public health professionals

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Development of the protocol

This document was conceived at a meeting on the Environmental Contamination Studies of MERS-CoV in Health Care Settings co-hosted by WHO and The University of Hong Kong on 13-14 March 2017. The participants of this meeting are listed at the end of the document. This protocol was developed by Reina Sikkema, Chantal Reusken, Pieter Fraaij, Bart Haagmans and Marion Koopmans from Erasmus Medical Center, Rotterdam, the Netherlands, with input and reviews provided by WHO and a large number of respiratory disease experts (see acknowledgements at the end). This work was supported by the European Commission's H2020 program under contract number 643476 and US Agency for International Development (USAID).

The protocol provides health care and public health professionals guidance on how to investigate the role of environmental contamination during outbreaks of MERS-CoV in a health care setting where a patient infected with MERS-CoV is currently being treated. The results from such studies will be used in risk assessment and risk management of MERS-CoV hospital-acquired infections, but also for guiding the implementation and monitoring of effectiveness of infection prevention and control measures.

Other protocols for MERS-CoV currently available or under development include:

- Cross-sectional seroprevalence study of MERS-CoV infection in presumed high risk populations
- Case-control study to assess potential risk factors related to human illness caused by MERS-CoV
- Seroepidemiological investigation of contacts of MERS-CoV patients

These protocols can be found on the WHO website:


In the event of an outbreak of a novel respiratory pathogen, this protocol could be adapted to assess environmental contamination in the health care setting. In this context, the environmental specimens and laboratory methods would need to be adapted to reflect the characteristics of the novel respiratory pathogen.

Using a standardized protocol such as the protocol described below, environmental data and biological samples can be systematically collected and shared rapidly in a format that can be easily aggregated, tabulated and analyzed across many different settings globally. This is particularly important in the context of emerging respiratory pathogens.

Comments for the user’s consideration are provided in purple text throughout the document as the user may need to modify methods slightly because of the local context in which this study will be carried out.
License

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# Table of contents

Development of the protocol ................................................................. 2
License ........................................................................................................ 3

1. Background and Justification ................................................................. 5
   1.1 Aim, objectives and scope ................................................................. 6

2. Study procedures.................................................................................. 7
   2.1 Background data: Hospital and sample data ...................................... 7
   2.2 Collection sites ............................................................................. 8
   2.3 Timing of sampling .................................................................... 11
   2.4 Sample size considerations .......................................................... 11
   2.5 Sampling methods and procedures ............................................... 11
   2.6 Ethical considerations ................................................................ 12
      Informed consent ........................................................................ 12
      Risks and benefits ..................................................................... 12
      Confidentiality .......................................................................... 13
   2.7 Prevention of MERS-CoV transmission in health care personnel .... 13
      Personal Protective Equipment (PPE) ........................................... 13
   2.8 Detection methods and criteria for confirmation .............................. 14
      RT-PCR .................................................................................. 15
      Virus isolation/culture .............................................................. 15
   2.9 Sequencing .............................................................................. 15

3. Reporting of findings ............................................................................. 17

4. Acknowledgements ............................................................................... 18

5. References ........................................................................................... 19
1. Background and Justification

Middle East respiratory syndrome coronavirus (MERS-CoV) was first detected in humans with respiratory illness in the Kingdom of Saudi Arabia (KSA) and Jordan in 2012. Since then, more than 2000 laboratory confirmed human cases of MERS have been reported to the World Health Organization (WHO)\(^1\). The virus is enzootic in dromedary camels, with occasional spill-overs to humans.\(^2\) Human-to-human transmission is limited, but not negligible, particularly in healthcare settings when infection prevention and control measures are sub-optimal. Health care associated outbreaks can be substantial, and have occurred in several countries including in KSA and the United Arab Emirates (UAE) and the first large scale outbreak outside the Gulf region in the Republic of Korea, following introduction of the virus through a traveler.\(^3\),\(^4\),\(^5\) As a consequence, the majority of notified human cases of MERS have been attributed to human-to-human infections in health care settings.\(^6\)\(^-\)\(^11\)

However, understanding of the mechanisms of transmission of MERS-CoV in health care settings remains limited. The patterns of and risk factors for transmission are important for the development of evidence-based guidelines for infection prevention and control measures. This is not limited to MERS-CoV. Healthcare associated infections (HCAI) are an important contributor to the burden of infectious diseases. Yet measures developed to date targeting infection prevention and control – with the possible exception of blood-borne viral infections (HIV, HBV) have historically focused on bacterial and fungal infections and antimicrobial resistance\(^12\)\(^-\)\(^16\). There is a clear need, however, to improve our understanding of HCAI caused by viruses. HCAI caused by viruses are not uncommon, and may be amplified through infected symptomatic or asymptomatic healthcare workers, visitors, contaminated environmental surfaces and potentially airborne transmission.

During past MERS-CoV nosocomial outbreaks, a number of studies evaluating MERS-CoV virus persistence in health care settings have been carried out in affected hospitals including locations identified as those hosting outbreaks. Additionally, some studies into environmental contamination with infectious MERS-CoV and/or MERS-CoV RNA outside healthcare settings have been published\(^7\)\(^-\)\(^10\),\(^17\),\(^18\). Environmental contamination has been found in several settings, but the extent of environmental contamination and the amount of viable virus that can be isolated are not consistent. Results from available studies have raised questions as to the need for standardization of methods. Studies addressing the role of environmental contamination of MERS-CoV are essential to inform recommendations to prevent and control human infection with MERS-CoV.

On 13-14 March 2017, WHO and The University of Hong Kong co-hosted an informal meeting to bring together public health and academic professionals to discuss previously conducted studies on respiratory virus persistence, and to plan for future observational and experimental studies of environmental and air persistence of MERS-CoV. The participants of this meeting discussed the potential role of environmental contamination and airborne transmission of MERS-CoV in health care settings based on their own research and identified the need for a standardized study protocol. A plan for future research, both experimentally and observationally, was developed for settings in which MERS patients are being treated. One of the outputs of the Hong Kong meeting is this practical “how to” protocol for health care and public health
professionals to systematically collect, store and analyze, in a systematic manner, the appropriate samples to evaluate (viable) virus persistence in hospital settings.

1.1 Aim, objectives and scope

The overall aim is to determine (viable) virus presence and persistence on fomites in various locations within a health care setting in which a patient infected with MERS-CoV is currently being treated, and to relate these observations to risk procedures and transmission events to inform MERS-CoV prevention and control measures.

The specific objectives of this protocol are to:

- Assess the extent and persistence of surface contamination with MERS-CoV in relation to patient care, handling and movement
- Characterize the sequence diversity of MERS-CoV in environmental samples
- Assess the viability of MERS-CoV on inanimate surfaces under different environmental conditions

COMMENT: Air sampling has not been included in this protocol. The current protocol addresses surface sampling which can be implemented by most hospitals reporting a MERS-CoV case, provided there is sufficient personnel. However, to set up a high quality and reproducible air sampling study with the possibility of culturing the virus requires specialist knowledge, sufficient laboratory facilities and costly equipment. Additionally, a best practice for air sampling is yet to be developed. Currently, there is not enough information available on the best equipment and methodology to sample MERS-CoV from air samples, although this is subject to revision as more information becomes available.

COMMENT: Assessing the contribution of environmental transmission is only possible if it is done as part of a comprehensive outbreak investigation and if information obtained by environmental studies is combined with the results of epidemiological, laboratory and sequence data from MERS-CoV patient investigations. They include 5 key steps for an investigation: 1) Preparation, 2) Objectives, 3) Case identification and interview (basic, clinical and exposure information), 4) Case finding, including contact monitoring, and 5) Biological specimen collection and laboratory testing, including molecular and serological testing, viral culture and genetic sequencing. It is important to note that negative environmental testing results cannot exclude the presence of virus within the health care setting.

The following guidelines for the investigation of MERS-CoV outbreaks have previously been published:

1. Investigation of cases of human infection with MERS-CoV:
   http://apps.who.int/iris/bitstream/10665/178252/1/WHO_MERS_SUR_15.2_eng.pdf?ua=1
2. Interview questionnaire of MERS-CoV cases to gather initial information about the potential exposures of a suspected or confirmed case of MERS-CoV infection:
   http://www.who.int/csr/disease/coronavirus_infections/MERS_case_investigation_questionnaire.pdf?ua=1
3. Seroepidemiological investigation of contacts of MERS-CoV patients, including contact tracing and investigation:
4. Investigation of health care exposures, including the assessment of potential risk factors of infection of MERS-CoV among health care personnel in a health care setting, which should be implemented in case of a possible hospital outbreak of MERS-CoV:
http://www.who.int/csr/disease/coronavirus_infections/Healthcare_MERS_Seroepi_Investigatio
n_27Jan2014.pdf

2. Study procedures

2.1 Background data: Hospital and sample data

In order to link data from environmental sampling to outbreak investigations, and to identify risk factors for environmental contamination and for infection in other individuals, it is important to design a study with an extensive collection of background information, including:

1. Link with MERS-CoV outbreak investigation: environmental sampling data provide supplementary information, which needs to be interpreted in the context of the outbreak description, patient sampling and sequencing, and testing of contacts.
2. A detailed plan of the hospital and the patient room layout. This includes: area function (Emergency Department, ward, Intensive Care Unit), placement of major furniture and beds, hospital equipment and ventilation inlets and outlets and the location of other MERS patient(s). The exact sampling locations can be determined using the information on the maps.
3. The routing of the MERS patient and/or the locations that the patient visited previous to being isolated (e.g. elevator, hall, waiting room, X-ray room). Each room where the patient stayed should be noted, with a list of activities done there, and an estimate of the amount of time spent. This information should be known when developing the sampling plan.
4. Information on the routes, patients and treatment procedures that healthcare workers (HCW) in the affected hospital were involved in. For each HCW, the rooms and patients that were visited and treatments that were executed, including dates and time, should be logged.
5. Systematic assessment of the timing and details of factors that can influence the outcomes of environmental sampling.
   - The place, time and duration of aerosol generating procedures should be indicated, including: positive pressure ventilation (bi-level positive airway pressure [BiPAP] and continuous positive airway pressure [CPAP]), endotracheal intubation, high flow nasal cannula, open airway suction, high frequency oscillatory ventilation, tracheostomy, chest physiotherapy, nebulizer treatment, sputum suction and bronchoscopy.\(^\text{19}\)
   - The time, frequency and details (e.g. disinfectant) of the cleaning and disinfection activities should be collected for all sampling locations
     The temperature and humidity of the sampled rooms should be measured and noted daily, as well as the time the bed of the patient was made.\(^\text{20,21}\)
For each sample, at least the date and time of sampling and the exact location should be noted.

2.1 Background data: Patient data

The patient data that should be collected from any infected MERS-CoV patient treated in the health care setting during a MERS outbreak is described in other WHO MERS protocols (see section 2). For this protocol, the essential information from the MERS patient to be collected includes:

1) Patient information
   o Patient ID number/cluster number (if applicable)
   o Demographic information (e.g. date of birth/age, sex)
   o Occupation (including specific classification such as healthcare worker, laboratory worker, and farm worker etc.).
   o Date of sample collection, laboratory testing and specimen type (e.g. nasopharyngeal swab, sputum, etc.)
   o Date of hospital admission and possible previous recent visits to the hospital

2) Clinical information
   o Date of illness onset, hospitalization, isolation
   o Signs and symptoms at initial presentation (Date of symptoms onset, by symptom)
   o Virological outcomes (if available), including duration of MERS-CoV shedding in respiratory tract specimens and extra pulmonary clinical specimens:
     - Date specimen taken
     - Type of test, type of specimen
     - Test results (including CT value) and date of results
     - Name of laboratory performing test
     - Name of national laboratory
     - Name of reference laboratory (if applicable)

COMMENT: The case report form available as part of the protocol ‘Investigation of cases of human infection with MERS-CoV’ can be used to collect this information, which is available from the WHO website: http://apps.who.int/iris/bitstream/10665/178252/1/WHO_MERS_SUR_15.2_eng.pdf?ua=1

2.2 Collection sites

Patients who test positive for MERS-CoV should be moved to an isolated patient room. Any other areas in the health care facility visited by the MERS patient, prior to testing may have been contaminated with MERS-CoV. Therefore it is important to trace back patient movements during his/her visit/admission in the hospital and sample all the areas the MERS patient visited during his/her hospital stay (see also: 3.1.1).
COMMENT: The recommended sampling sites have been based on 1) possible disease transmission routes and 2) current literature of high-touch surfaces. Moreover, standardizing the sampling sites in MERS-CoV surface sampling studies in healthcare facilities will improve the possibilities of comparing the results of multiple studies.

COMMENT: Every health care facility has a different layout. Therefore, the sampling scheme should be adapted to reflect the layout of each health care facility involved in this investigation.
Table 1. Recommended sampling sites based on location in the hospital⁸,⁹,²⁶

<table>
<thead>
<tr>
<th>Possible route of MERS-CoV hospital transmission</th>
<th>Essential sampling sites</th>
<th>Other sampling sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patient (entry) routing</td>
<td><strong>Ambulance</strong></td>
<td><strong>Ambulance</strong></td>
</tr>
<tr>
<td></td>
<td>Medic bag handle, inside of blood pressure cuff, wall next to the patient stretcher</td>
<td>Front of defibrillator, handlebar ambulance ceiling,</td>
</tr>
<tr>
<td>Entrance</td>
<td>Ventilation exits or air purifier filters, guardrails</td>
<td>Entrance, corridor, waiting room</td>
</tr>
<tr>
<td>Corridor</td>
<td>Ventilation exits or air purifier filters, guardrails</td>
<td>Elevator Buttons, Ventilation exits or air purifier filters, guardrails</td>
</tr>
<tr>
<td>Waiting room</td>
<td>Ventilation exits or air purifier filters, guardrails</td>
<td>X-ray room Ventilation exits or air purifier filters, doorknob, light switch, X-ray table, sink, faucet handles</td>
</tr>
<tr>
<td>2. Hospital staff</td>
<td><strong>Staff room</strong></td>
<td><strong>Staff room</strong></td>
</tr>
<tr>
<td></td>
<td>Doorknob, key board, clothes, ventilation exits or air purifier filters</td>
<td>Sink, faucet handles, desk/table, light switch, chairs</td>
</tr>
<tr>
<td>Ante room</td>
<td>Doorknob, light switch, ventilation exits or air purifier filters</td>
<td>Patient room Monitor controls, monitor touch screen, charts</td>
</tr>
<tr>
<td>3. Patient handling and care/patient virus excretion and risk procedures</td>
<td><strong>Patient room</strong></td>
<td><strong>Patient room</strong></td>
</tr>
<tr>
<td></td>
<td>Doorknob, bed rails, bedside table, bed controller, call button, floor (&lt;1meter from the patient, 2m, 3m, etc.), tubing, masks and filters of aerosol generating procedures, control panels</td>
<td>Bedding, IV pole, telephone, chair, curtain, patient clothing, light switch, stethoscope, thermometer, hand soap dispenser, garbage bin, cup, curtains, oxygen flow meter</td>
</tr>
<tr>
<td>Patient bathroom</td>
<td>Doorknob, faucet handles, sink, toilet/bed pan</td>
<td>Patient bathroom Light switch, bed pan cleaner, guard rails</td>
</tr>
<tr>
<td>4. Air flow*</td>
<td><strong>Patient room</strong></td>
<td><strong>Patient room</strong></td>
</tr>
<tr>
<td></td>
<td>Ventilation exits or air purifier filters</td>
<td>Wall (&lt;1meter from the patient, 2m, 3m, etc. if possible)</td>
</tr>
<tr>
<td>Patient bathroom</td>
<td>Ventilation exits or air purifier filters</td>
<td>Patient bathroom Wall (&lt;1meter from the patient, 2m, 3m, etc. if possible)</td>
</tr>
</tbody>
</table>
2.3 Timing of sampling

Ideally, sampling should take place each day, from the day MERS was suspected and/or diagnosed in a patient until at least 7 days after the discharge or passing of the patient\(^8\). In case of aerosol generating procedures (listed above), the environment should be sampled before and after (within 1 hour and 24h later) of each procedure. The sampling sites associated with the patient (entry) routing should be sampled in the period from time of suspicion until transfer to a regular ward or ICU\(^19\).

COMMENT: Patients in ICU may remain hospitalized for extended periods of time and, as such daily sampling may not be possible, particularly if there are multiple MERS-CoV cases within the same health care facility. Feasibility and the outbreak context will determine the frequency and duration of repeated sampling.

2.4 Sample size considerations

The sampling protocol is recommended to be executed as described above. However, in case of an extensive hospital outbreak, the number of samples and the work that is associated with sampling may be too extensive to handle. In that case the sampling interval may be increased from 1 day to sampling every 2-3 days starting on day 1. Moreover, high quality sampling of sufficiently high frequency of one or two patients has priority over sampling all patients involved in the outbreak.

2.5 Sampling methods and procedures

Environmental samples need to be taken using a swab with a synthetic tip and a plastic shaft\(^7,10,17,18\). The swab specimen collection vials should contain 1-3ml of viral transport medium (e.g. protein stabilizer, antibiotics and buffer solution) including neutralizing buffer to counteract the effects of any residual disinfectant (e.g. Tween 80). Viral transport medium is required for virus isolation. However, viral transport medium is not always efficient in case of long shipping times, uncontrolled storage temperature and minute virus concentrations. The use of chaotropic lysis buffers will stabilize viral genomes which is recommended in situations in which storage and transport conditions are not optimal and concentrations of viable virus are expected to be low.

The first step of the sampling procedure is to put sterile, non-powdered nitrile or vinyl examination gloves over the gloves that are part of standard PPE and clothing. Then, remove the swab from the package. Wet the swab with viral transport medium. When applying pressure with the wet swab onto the surface, move in at least two different directions while rotating the swab stick. Avoid letting the swab dry completely. The recommended swab surface area is 25 cm\(^2\). To increase the positive predictive value of the environmental sampling process, each sampling area may require multiple swabs.
After labelling the vial, place in a self-sealing bag and clean the outside of the sealed bag with a 60-80% ethanol, 80% isopropyl alcohol or 5% hypochlorite solution just prior to leaving the contaminated area. Then, place the cleaned sealed bag in another unused similar self-sealing bag.

In each sampling round, a set of control samples also need to be collected. The first set of control samples are handled in the same way as the environmental samples from the potentially contaminated area, including opening the package and removing the swab from the tube, but without sampling any surfaces. The second set of control samples remain sealed, but will be shipped, stored and tested with the surface samples, to exclude contamination later on.

COMMENT: If only a single patient is involved, it would be ideal to include an additional control sample from the room of a non-MERS patient within the same health facility. This would strengthen evidence that any positive specimens from the MERS patient’s room are true positives, and not laboratory or other contamination. However, inclusion of this additional control will need to be determined by feasibility and the outbreak context.

COMMENT: Wipes can also be used for larger surfaces.

2.6 Ethical considerations

Ethical approval must be sought in accordance with local, regional and national authorities prior to the implementation of this protocol.

COMMENT: If an outbreak of MERS-CoV occurs, the study design and sampling guidance found in this document can be modified rapidly to reflect the current outbreak situation. This will have to be submitted for ethical approval, but using this document as a starting point may help minimize delays to the start of investigations.

Informed consent

Informed consent will be sought from all MERS-CoV patients hospitalized in the health care facility willing to participate in the investigation before any procedure is performed as part of the investigation by a trained member of the investigation team. Each participant must be informed that participation in the investigation is voluntary and that s/he is free to withdraw, without justification, from the investigation at any time without consequences and without affecting clinical management within the health care facility.

Informed consent will seek approval to collect demographic data and clinical information related to MERS-CoV and his/her current hospitalization. Informed consent will also indicate that any suspected or confirmed MERS-CoV infection may be notified to the national health authorities under the requirements of the International Health Regulations (IHR).

Risks and benefits

This investigation poses no risk to participants, as no collection of biological specimens is involved. The primary benefit of the study is indirect in that data collected will help improve and guide efforts to
understand the role of environmental contamination in the transmission of MERS-CoV and prevent further spread of MERS-CoV in health care facilities.

Confidentiality
Patient confidentiality will be maintained throughout the investigation. All MERS-CoV patients who participate in the investigation will be assigned a study identification number by research staff for the identification of study questionnaires. The link of this identification number to individuals will be maintained by the health care facility and the Ministry of Health (or equivalent) and will not be disclosed to any other research personnel.

COMMENT: If the data is shared by the implementing organization to WHO or any agency or institution providing support for data analysis, data shared will include only the study identification number and not any personably identifiable information.

2.7 Prevention of MERS-CoV transmission in health care personnel
Before the start of the investigation, all staff involved in the environmental sampling and collection of clinical data from the MERS-CoV patient will be offered training in infection prevention and control procedures (standard contact, droplet or airborne precautions). These procedures will be determined by local or national guidelines, but should include proper hand hygiene and the correct use of surgical or respirators, if necessary, not only to minimize their own risk of infection when in close contact with MERS-CoV infected patients in a health care setting, during home visits and elsewhere, but also to minimize the risk of spread among other HCP and household members.

WHO has produced guidance on infection prevention and control of epidemic and pandemic prone acute respiratory infections in health care:
http://www.who.int/csr/bioriskreduction/infection_control/publication/en

WHO also has guidance on infection prevention and control during health care for probable or confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection:

Key points:
- All health-care workers who collect specimens from patients suspected or confirmed to be infected with MERS-CoV must wear appropriate PPE, e.g. masks, respirators, gowns, gloves and eye protection in all areas with (potential) patient contact
- All those involved in collection and transporting specimens should be trained in safe handling practices and spill decontamination procedures.

Personal Protective Equipment (PPE)
All personnel involved in the environmental sampling should use PPE in accordance with hospital hygiene protocols.
WHO guidance on how to put on and take off PPE is available here: 

2.8 Labeling, shipment and storage of samples
At least two aliquots of viral transport medium (VTM) should be made before the specimens are stored or shipped. One of two aliquots should be stored at -70°C or -80°C as soon as possible.

COMMENT: If the specimens are expected to reach the laboratory within 72 hours, specimens should be stored and shipped at 4°C. If the specimens are expected to reach the laboratory after more than 72 hours, specimens should be stored at -70°C or -80°C and shipped on dry ice or in liquid nitrogen27. Repeated freezing and thawing of specimens must be avoided. It is important that the cold chain (temperature-controlled supply chain) is maintained throughout the whole process of sampling, shipping and storage.

Transport of specimens within national borders should comply with applicable national regulations. International transport of MERS-CoV specimens should follow applicable international regulations, as described in WHO’s Guidance on regulations for the Transport of Infectious Substances for category B infectious substances: http://www.who.int/ihr/publications/who_hse_ihr_2015.2/en/

COMMENT: Key points are the use of a basic triple packaging system, correct marking and labeling of specimens and use of appropriate shipping documents. The receiving laboratory should always be contacted before specimens are shipped.

2.9 Detection methods and criteria for confirmation
Any testing for the presence of MERS-CoV should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on the laboratory biosafety should be followed in all circumstances12.

- Each laboratory should conduct a risk assessment to ensure it is competent to safely perform this testing.
- When handling and processing specimens, including blood for serological testing, laboratory practices and procedures should follow basic to good microbiological techniques (GMT).
- Non-culture diagnostic laboratory work including nucleic acid amplification tests (NAAT) should be conducted adopting practices and procedures described for basic laboratory – Biosafety Level 2 (BSL-2) in the WHO Laboratory Biosafety Manual, 3rd edition: http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/
- Initial processing of all specimens including those for NAAT should take place in a class 2 or class 3 Biosafety cabinet with current certification.
- All technical procedures should be performed in a way that minimizes the generation of aerosols and droplets.
- Appropriate personal protective equipment (PPE) should be worn by all laboratory staff handling these specimens.
- Handling of material with high concentrations of live virus (such as when performing virus isolation or neutralization assays) should be performed only in laboratories capable of meeting additional essential containment requirement including practices recommended for biosafety level 3 (BSL-3) lab.

**RT-PCR**

Three real-time RT-PCR assays for routine detection of MERS-CoV have been developed and their details published. Currently described tests are an assay targeting upstream of the E protein gene (upE) and assays targeting the open reading frame 1b (ORF 1b) and the open reading frame 1a (ORF 1a). The assay for the upE target is considered highly sensitive and is recommended for screening, with the ORF 1a assay considered of equal sensitivity. The ORF 1b assay is considered less sensitive than the ORF 1a assay. An alternative approach involving two rRT-PCR assays targeting the MERS-CoV nucleocapsid (N) protein gene, which can complement upE and ORF 1a assays for screening and confirmation has also been published.

A testing algorithm for investigation of suspected cases of MERS-CoV by NAAT is available in the WHO Guidance on Laboratory testing for Middle East respiratory syndrome coronavirus (MERS-CoV): http://apps.who.int/iris/bitstream/handle/10665/259952/WHO-MERS-LAB-15.1-Rev1-2018-eng.pdf?sequence=1

**Virus isolation/culture**

MERS-CoV isolation and cell culture should only be performed by laboratories with the appropriate experience and biosecurity level 3 capabilities. Therefore, it is recommended to store and/or ship samples for virus isolation to a (inter)national reference laboratory to perform further analyses. (see 4. Labelling, shipment and storage of samples)

**COMMENT:** A limited number of laboratories have the experience and/or biosecurity capacities for virus isolation and culture. Therefore, collaboration between countries and designated reference laboratories is encouraged. Collaboration is at the discretion of Member States conducting the investigation, but WHO is able to facilitate this collaboration and possible shipment for testing, if required.

**2.10 Genome sequencing**

MERS-CoV genome sequencing may provide further details on the genetic relationship of the viruses detected with other viral isolates. A reverse-transcription PCR assay for MERS-CoV targeting a 615 bp spike fragment may already provide a phylogenetic clustering of MERS-CoV variants comparable to that of full-length genomes, but this may often be insufficient for detailed molecular epidemiological investigations. Full genomes obtained by NGS using sets of specific primers to amplify the full genome for instance delivers a more detailed picture of genetic differences between viruses. Virus grown in culture may be used as an alternative source of the viral RNA.
Acquired sequence information should be shared and reported via publicly available databases such as GenBank.

COMMENT: Material and more detailed methods for MERS-CoV sequencing are described in publications\textsuperscript{30-33}.
3. Reporting of findings

Reports of the results of this study should include the number of potentially contaminated samples tested and the number of samples confirmed to test positive for MERS-CoV.

It is also important to fully document the study design, including sampling methods, techniques for detecting MERS-CoV, in order to assist the interpretation of the findings.

COMMENT: The timely dissemination of the results of this study are critical in understanding transmission of the MERS-CoV virus to inform guidance for policy to direct national and international public health response.
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5. References


