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Review

MERS coronavirus: Data gaps for laboratory preparedness

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ABSTRACT

Since the emergence of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, many questions remain on modes of transmission and sources of virus. In outbreak situations, especially with emerging organisms causing severe human disease, it is important to understand the full spectrum of disease, and shedding kinetics in relation to infectivity and the ability to transmit the microorganism. Laboratory response capacity during the early stages of an outbreak focuses on development of virological and immunological methods for patient diagnosis, for contact tracing, and for epidemiological studies into sources, modes of transmission, identification of risk groups, and animal reservoirs. However, optimal use of this core public health laboratory capacity requires a fundamental understanding of kinetics of viral shedding and antibody response, of assay validation and of interpretation of test outcomes. We reviewed available data from MERS-CoV case reports, and compared this with data on kinetics of shedding and immune response from published literature on other human coronaviruses (hCoVs). We identify and discuss important data gaps, and biases that limit the laboratory preparedness to this novel disease. Public health management will benefit from standardised reporting of methods used, details of test outcomes by sample type, sampling date, in relation to symptoms and risk factors, along with the currently reported demographic, clinical and epidemiological findings.

Contents

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1. Introduction

On September 20, 2012, the first confirmed case of a new severe respiratory syndrome caused by a novel coronavirus was reported [1,2]. The patient was a previously healthy male, 60 years old, who lived in Saudi Arabia and died of acute respiratory illness and renal failure [1]. Retrospective analysis of stored samples revealed that the novel coronavirus was also the cause of a severe respiratory disease cluster involving patients and health care professionals in Jordan earlier that year [3]. The isolation of the new virus from the Saudi patient's specimen, and subsequent sequencing and detailed phylogenetic analysis of the virus genome has revealed its close relationship with bat beta-coronaviruses previously found in Asia, Europe and Africa [1,4–7]. Whether this emerging hCoV jumped from bats directly to humans or through other animals as intermediate hosts is still unknown, although the latter scenario is considered to be most likely [2]. The presence of MERS-CoV neutralising antibodies has been detected in dromedary camels in Spain, Oman and Egypt indicating a past infection with MERS-CoV or a highly related virus in these camelids [8,9]. The evolutionary history of coronaviruses shows evidence of recent interspecies jumps [10,11]. One of the first described human coronaviruses, OC43, shares a common recent ancestral history with bovine coronaviruses, suggesting these viruses may have emerged from animals as well [10]. In 2003, Severe Acute Respiratory Syndrome (SARS) emerged and was the first known major outbreak caused by a coronavirus [12]. Now, the emergence of MERS-CoV, and its high fatality rate has triggered new concerns about the potential for a widespread, possibly global outbreak. Challenges for public health are to develop strategies to control this emerging disease, which include early detection of cases for with laboratory diagnosis is of crucial importance.

2. Laboratory preparedness

In outbreak situations, especially with a novel organism causing severe human disease, it is important to understand the full spectrum of disease, as well as how this relates to infectivity, the ability to transmit the microorganism (e.g. virus), and outcomes of laboratory tests. Laboratory response during the early stages of an outbreak therefore focuses on development of virological/microbiological and immunological methods for patient diagnosis, for contact tracing, and for epidemiological studies into sources, modes of transmission, identification of risk groups, screening of potential animal reservoirs, etc. However, optimal use of this core laboratory capacity requires embedding of data needs for laboratory scientists within the outbreak investigations, in order to obtain information needed for assay validation and correct interpretation of test outcomes.

Following the discovery of MERS-CoV, molecular detection methods and antibody detection assays were developed by several groups, and deployed internationally through an international collaborative laboratory response [13–16]. However, in spite of the cutting-edge technological capacity (e.g. deep sequencing, microarray technology), the lack of essential information (e.g. time of sampling, profile of viraemia and shedding, linked to diverse clinical manifestations) severely hampers the use of these techniques in the current outbreak. Here, we review the current knowledge on MERS-CoV and other human coronaviruses against data required for optimal laboratory response for the MERS-CoV.

3. Comparative profiles of coronavirus detection in respiratory and other type of specimens

In an early phase of emerging viral disease outbreaks, typically an initial comparison with clinical manifestations of other viruses from the same taxonomic group is used to develop a sampling strategy. Crucial first questions for choice of sampling and subsequent interpretation of laboratory diagnostics are:

- Which are the kinetics of viral shedding in persons with different disease states (asymptomatic, mild, moderate, severe)?
- What is the concentration of virus (viral load) in various body compartments, fluids and secreta during the progression of the disease?
- How are infection kinetics and loads influenced by host factors (e.g. immunosuppression, co-morbidities)?
- What is the limit of detection of the diagnostic methods used for the different specimens?

3.1. Respiratory shedding, other hCoV's

Nasopharyngeal aspirates (NPA) are the most common respiratory specimens described in the literature for molecular diagnosis of non-SARS and SARS coronaviruses [17–19]. Comparison of data between studies is difficult because specimen sampling is not standardised, with descriptions varying from naso-pharyngeal swab, naso-pharyngeal secretion, throat swab, nasal swab, oro-nasal swab, all reflecting "upper respiratory tract" sampling. Literature review shows that hCoV viral loads peak at different time points during the progression of disease: for non-SARS CoV (e.g. hCoV-NL63), peak viral loads are detected around day 1–2 after onset of disease, with apparent clearance of infection, over the course of 3 weeks in 50% of healthy children [17,20,21]. For SARS-CoV, viral loads gradually increase until day 10 (60–95% of patients) after the onset of symptoms, and then progressively decrease after day 13 (42–90% of patients) [22–24] (Fig. 1). This comparison illustrates that even for viruses belonging to the same family, using a sensitive test (like RT-PCR), the interpretation of a test outcome may be very different: a negative reverse-transcriptase (RT)-PCR in a patient specimen collected early after illness onset for hCoV-NL63 may be used as evidence for ruling out infection. The same assay applied in an early specimen from a patient or contact with recent illness onset after SARS-CoV infection would most likely be false-negative, as viral loads are expected to be low during this phase of illness.

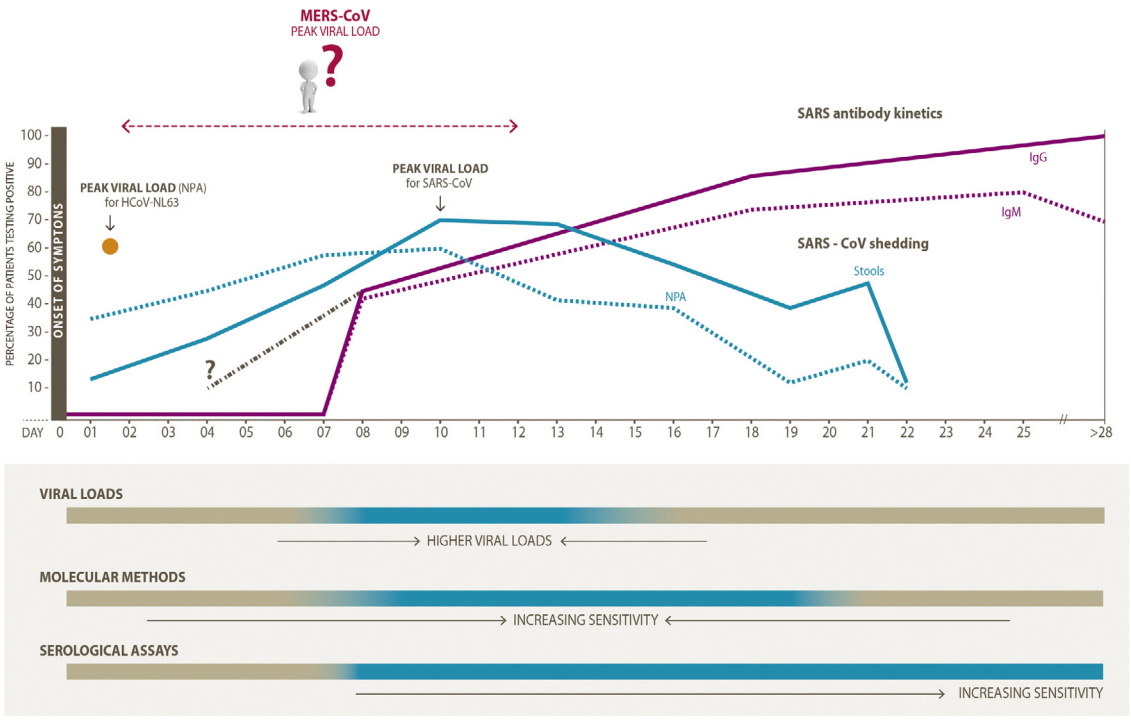

Fig. 1. Top: Schematic representation of comparative profiles of SARS shedding (peak viral load) and antibodies kinetics, based on data described by [24,44–46]. At the top left of the figure, peak viral load information for HCoV-NL63 is given, based on [20]. Bottom: schematic representation of relationship between sensitivity of molecular detection and serology in relation to time of sampling and kinetics of infection.

3.2. Respiratory shedding, MERS-CoV

For MERS-CoV, data on respiratory shedding has only been reported anecdotally, sofar. All the MERS-CoV cases reportedly have developed a respiratory disease, ranging from mild to severe pneumonia, often accompanied by acute respiratory distress syndrome (ARDS) and/or renal failure and/or pericarditis and/or disseminated intravascular coagulation (DIC). Clinical manifestations and severity of MERS seem to be more similar to SARS than other coronavirus infections. However, only one published report has provided data needed for laboratory preparedness, i.e. on sequential sampling, Ct values, and positive AND negative test results [25]. Therefore, at present, viral shedding kinetics can only indirectly be derived (Fig. 2A and B). We plotted a timeline of available data on sampling and test outcome by day of onset symptoms for the MERS-CoV patients diagnosed in UK, Germany, and France, as well as some cases from Saudi Arabia. The data suggests that shedding kinetics may be more similar to what has been observed for SARS than for other human coronaviruses, although very limited information is available for the latter (Figs. 1 and 2B). The review also suggests that the use of upper respiratory specimens for MERS-CoV (e.g. naso-pharyngeal swabs) diagnosis may not be as sensitive as the use of lower respiratory tract specimens (Fig. 2A and B). In agreement with this, viral loads were higher in samples obtained from the lower respiratory tract compared with upper respiratory tract in some MERS-CoV cases [25,26].

The difficulty in interpretation of diagnostic test results is illustrated by two secondary cases in France and in the UK, respectively (Fig. 2A; patients C2 and D2). Both patients were sampled early after onset of symptoms. The NPS specimens were positive in the UK case and negative in the French case, but sputum of the latter was positive. For SARS, although most of the samples tested were NPA, lower respiratory tract specimens were more often PCR positive (76%) than NPA (45%) or other upper respiratory tract samples (24%) [24].

3.3. Gastro-intestinal shedding, other hCoV's

In SARS, diarrhoea was one of the most common extra-pulmonary manifestations in patients, and a progressive viral load peaking around day 10 was found in 70–100% of patients in stool specimens regardless respiratory symptoms (Fig. 1) [22,24]. Observations in children hospitalized for other hCoV suggests that gastro-intestinal shedding is limited, compared with SARS, although all four hCoVs species have been detected in patient stools [32]. However, compared to SARS there is less information about kinetics of shedding and viral loads in stools and respiratory samples.

3.4. Gastro-intestinal shedding, MERS-CoV

Although the major clinical manifestations presented by MERS patients are associated with the respiratory tract, gastrointestinal symptoms including diarrhoea during the course of illness were also observed quite frequently (35%) [25–27,33]. This triggers questions regarding the use of stools to diagnose MERS-CoV. Again, availability of data is too limited for a comparative analysis with other coronaviruses. In the UAE patient who was first treated in an Abu Dhabi hospital on March 19, and later hospitalized in Germany, stool samples tested borderline positive at days 12 and 16 after the onset of symptoms (Fig. 2A, patient B). Later stool samples from patients F from KSA and G, a patient transferred from Qatar to the UK, were negative by RT-PCR [25,28,31]. Whilst diarrhoea has been listed in the clinical picture for other cases, including in two separate clusters in Saudi Arabia no testing has been undertaken [27,34].

3.5. Other specimens used to detect MERS-CoV

Other MERS patient's specimens such as urine, sera/plasma samples, and blood were very rarely tested, not allowing consistent

(a)

Country related with patient treatment (date of publication)	No. of Lab confirmed cases (No. cases cluster)	No. of hospitalized cases ICU / deaths	Patients figure 2 Code	Symptom onset to sample collection days (URT)	URT specimens results	Symptom onset to sample collection days (LRT)	LRT specimens results	Other specimens results by symptom onset to sample collection days	References
KSA (August, 2013)	2 (3)	1/1	*	na	NP (+)	na	TA (+)		33
		0/0	*	na	NP (-)	na	not specify (+)		
Italy (August, 2013)	3	0/0	A1	5d	NP (+)				32
		0/0	A2	3d	NP (+)				
		0/0	A3	1d	NP (+)				
UAE, Germany (May, 2013)	1	1/1	B	16d	ORN (+)	12d, 17d, 18d, 19d 12d, 14d	TA (+) BAL (+)	ST (+): 12d, 16d U (+): 12d, 13d S/P (-): 16d, 18d	25
France (May 29, 2013)	2 (2)	1/0	C1	8d	NP (-)	4d 15d	BAL (+) SP (+)	BL (+): 15d S/P (-): 17d	26
		1/0	C2	1d 3d	NP (Not conclusive)	3d	SP (+)		
KSA (May 29, 2013)	3 (4)	2/2	*			na	2 SP (+) 1 BAL (+)		27, 28
Jordan (May, 2013)	2 (13)	2/2	*	na	NS (+)	na	BAL (+)		6
UK (March, 2013)	3 (3)	1/1	D1	14d	TS (+)				29
		1/1	D2	4d	NP (+)				
		0/0	D3			8d	SP (+)		
Qatar, Germany (Feb, 2013)	1	1/0	E	12 d	NP (+)	20d	BAL (+)		13, 30
KSA (Dec, 2012)	1	1/0	F	na	NS (+)	14d	TA (+)	ST(-): na S/P (-): na	28
Qatar, UK (Oct, 2012)	1	1/0	G	10d 14d 14d	NP (+) TS (-) NS (-)	14d 16d 18d	SP (+) TA (+) BAL (-)	ST (-): 20d BI (-): 20d	31
KSA (Oct, 2012)	1	1/1	H			7d	SP (+) culture		1

(b)

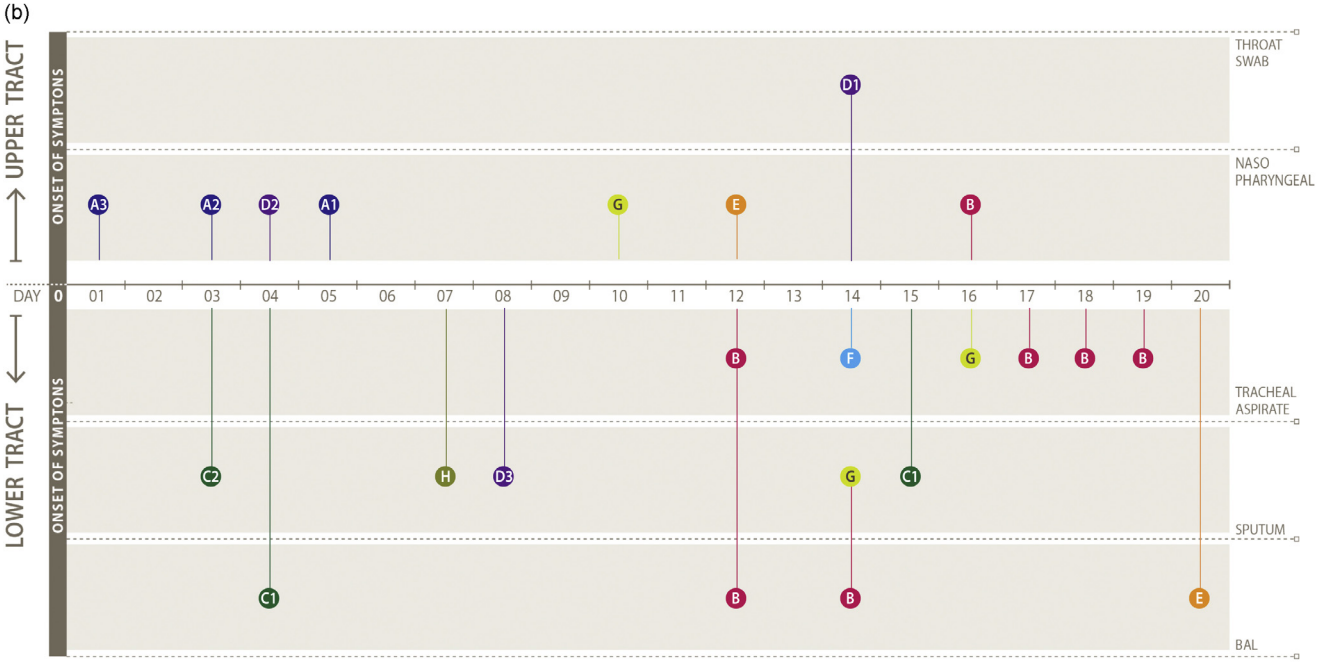

Fig. 2. (A) Summary of case reports with diagnostic information relevant for laboratory preparedness. (naso-pharyngeal swab or aspirate (NP); throat swab (TS); nasal swab (NS); oro-nasal swab (ORS); tracheal aspirates (TA); Sputum (SP); bronchoalveolar lavage (BAL); Stool (ST);Urine (U); serum or plasma (S/P); positive (+); negative (−); upper respiratory tract (URT); lower respiratory tract (LRT); * not present in figure; not available data (na). (B) Summary of data from published literature and reports on MERS-CoV RT-PCR positive respiratory specimens by sample type, and timing of sampling since onset of symptoms (see Ref. [29]).

Table 1
Primers/probes sequences for screening and confirmatory RT-PCR assays for MERS-CoV.

Type of molecular assay	Target region	Genome location	Primers and probes Sequence (5′ > 3′)	Ref.
Screening assays By real RT-PCR	Upstream of Envelope gene (upE)	27,458–27,550[a]	upE-Forward GCAACGCGCGATTCAGTT upE-Reverse GCCTCTACACGGGACCCATA upE-Probe CTCTTCACATAATCGCCCCGAGCTCG	[13]
	Nucleopcapsid gene (N2)	29,424–29,477[a]	N2-Forward GGCACTGAGGACCCACGTT N2-Reverse TTGCACATACCATAAAAGCA N2-Probe CCCCAAATTGCTGAGCTTGCTCCTACA	[57]
Confirmatory assays By real RT-PCR	Open reading frane (ORF) 1A gene	11,197–11,280[a]	ORF1A-Forward CCACTACTCCATTTCGTCAG ORF1A-Reverse CAGTATGTGTAGTGCGCATATAAGCA ORF1A-Probe TTGCAAATTGGCTTGCCCCACT	[14]
	Open reading frane (ORF) 1b gene	18,266–18,347[a]	ORF1b-Forward TTCGATGTTGAGGGTGCTCAT ORF1b-Reverse TCACACCAGTTGAAAATCCTAATTG ORF1b-Probe: CCCGTAATGCATGTGGCACCAATGT	[13]
	Nucleopcapsid gene (N3)	28,748–28,795[a]	N3-Forward GGGTGTACCTCTTAATGCCAATTC N3-Reverse TCTGTCCTGTCTCCGCCAAT N3-Probe ACCCCTGCGCAAAATGCTGGG	[57]
Confirmatory assays By sequencing RT-PCR	RNA-dependent RNA polymerase (RdRp)	15,049–15,290[a]	RdRpSeq-Forward TGCTATWAGTGCTAAGAATAGRGC RdRpSeq-Reverse GCATWGCNCWGTCACACTTAGG RdRpSeq-Rnested CACTTAGGRTARTCCCAWCCCA[b]	[14]
	Nucleocapsid (N) protein gene	29,549–29,860[a]	SeqN-Forward CCT TCG GTA CAG TGG AGC CA SeqN-Reverse GAT GGG GTT GCC AAA CAC AAA C SeqN-Fnested TGA CCC AAA GAA TCC CAA CTA C[c]	[14]

[a] Nucleotide numbering based on human betacoronvirus 2c EMC/2012 strain.
[b] RdRpSeq-Rnested – in cases where no amplification products were obtain a second-round reaction PCR is set up using the same forward primer as in first round, and this reverse primer.
[c] SeqN-Fnested – in cases where no amplification products were obtain a second-round reaction PCR is set up using this forward primer and the same reverse primer as in first round.

conclusions (Table 1). The urine specimens tested were only from the patient transferred to Germany were RT-PCR was positive on days 12 and 13, but not on day 14 after renal failure. Sera/plasma samples collected from three patients (late on disease progression; 16–20 days) were all negative (patients F, C1, B). RT-PCR done on blood of one French patient was positive (15 day).

4. Factors influencing viral shedding kinetics and viral loads

4.1. Host factors and bias for severity in early cases

Establishing relevant cut-off values in tests should also take into consideration that patient groups differ with respect to age, co-morbidities, etc. During the early stages of an outbreak investigation, observations can be biased for severe disease in specific groups of patients (e.g. older patients and with co-morbidities). Sofar, the majority of reported MERS cases have had underlying disease and/or immunosuppression that could be an explanation for the higher case fatality rate (60%) [27,34,35]. In addition, delayed clearance of viral infection in such risk groups could be an alternative explanation for the prolonged shedding observed in the patients described (Fig. 2B). Patients with co-morbidities more often have non-SARS CoV infections (hCoV-229E, hCoV-OC43, hCoV-NL63; hCoV-HKU1) compared with otherwise healthy patients, and may shed virus for prolonged periods of time [36–39]. In SARS, the clinical course among patients less than 12 years of age was milder and less aggressive when compared with adults and teenagers [40,41]. Kinetics of viral loads and the pattern of shedding can differ in these groups of patients and influence interpretation of diagnostic results.

4.2. Mild and asymptomatic cases

While clinical surveillance captures the most severe patients, outbreak containment requires a full understanding of the diversity in clinical presentations, including mild or asymptomatic cases. Assessing shedding kinetics and immunological response in this group is notoriously difficult, as it requires targeted studies and willingness of healthy persons to have samples taken. Without proper studies, it is unclear if for instance asymptomatic persons can contribute to transmission. There are considerably differences among respiratory viruses in the ratio between symptomatic and asymptomatic infections. For instance among children, infections caused by respiratory syncytial virus (RSV) and human metapneumovirus, usually are associated with clinical illness, in contrast with non SARS CoVs, rhinovirus and human bocavirus that are commonly found in asymptomatic children as well [42].

5. Kinetics of antibody response

5.1. SARS-CoV

The literature on kinetics of antibodies in patients with SARS-CoV shows some conflicting results regarding the time of appearance of antibodies. According to some reports, SARS-CoV antibodies (IgG and IgM) are usually not detected within the first 7 days of illness, but increase dramatically in the second week, reaching peak IgG levels within 30 days (Fig. 1) [43,44]. Similarly, IgM antibody levels increased up to 1 month and then declined gradually. This profile has been observed with two different serological assays, assuring that the detection levels of antibodies were not affected by sensitivity of the test. Other studies found specific antibodies (IgG, IgM and IgA) as early as 4 days after the onset of disease [45,46]. IgG and neutralising antibodies may persist in some patients up to 36 months, although titres showed significant decline after four months [47]. Such data is important when planning serosurveys that may take several months to organise due to protocol design, ethical clearance, and planning of study logistics.

The presence of antibodies against SARS-CoV in individuals with no or mild symptoms has been described in healthcare workers [48]. Antibody levels measured in this study were highest for persons with severe illness, indicating that different test cut-offs may be needed for the use of laboratory tests during public health investigations that aim to identify mild cases as well. Based on epidemiological investigations, including serological assessment of

Table 2
Serological assays for MERS-CoV.

Serological Assays	Antigen used	Technical details	Ref.
Indirect Immunofluorescence Assay (IFA)	Whole virus	MERS-CoV infected and uninfected Vero B4 cells For detection of specific IgG and IgM in patient serum	[13,30]
	Recombinant spike and nucleocapsid proteins	Transfected Vero B4 cells expressing recombinant spike or nucleocapsid protein of MERS-CoV For detection of specific IgG and IgM in patient serum (a) Controls of other human pathogenic CoV for differential rIFA should be included. (b) Confirmation by virus plaque reduction neutralisation test (PRNT)	[14,30]
Western Blot	Recombinant spike and nucleocapsid proteins	Transfected HEK-293 T cells expressing recombinant spike or nucleocapsid protein of MERS-CoV (a) Confirmation by virus plaque reduction neutralisation test (PRNT)	[14]
Protein microarray	Soluble S1 subunit of spike protein	Amino-terminal receptor binding spike domain S1, expressed in HEK-293T cells. For detection of specific IgG and IgM in patient serum (a) Controls of other human pathogenic CoV should be included on microarray analysis for differential diagnosis. (b) Confirmation by virus neutralisation test	[16]
Neutralisation test	Plaque Reduction Neutralisation test (PRNT) Whole virus	For serum neutralisation tests Vero B4 cells were used in 24-well plates. Used for testing for MERS-CoV neutralising immunoglobulins in patient serum Require BSL3	[16,25,30]
	Micro Neutralisation Test (MN) Whole virus	For MN test Vero cells monolayers were used in 96-well microtiter plates. Used for testing for MERS-CoV neutralising immunoglobulins in patient serum Require BSL 3	[8,9]
	Pseudoparticle virus (ppNT)	For the ppNT assay, HIV/MERS pseudoparticles containing HIV p24 viral protein were used to infect Vero E6 cells in a single well (96-well plate). eudoparticle virus (ppNT) Used for testing for MERS-CoV neutralising immunoglobulins in patient serum Require BSL2	[9]

the extent of transmission, the conclusion was that only symptomatic patients were efficient spreaders of SARS virus [49].

5.2. MERS-CoV

For MERS-CoV, some asymptomatic infections have recently been identified in health workers and children ($n = 8$) but whether these persons can efficiently transmit infection remains unknown [50,51]. Antibody detection assays have been developed, but their use has been limited. A retrospective study has reported serological testing by immunofluorescence assay of 2400 serum samples from persons seeking medical care at Fakeeh Hospital in Jeddah, Saudi Arabia (where the first case of MERS was diagnosed) in the two previous years [1]. All these patients tested negative for MERS-CoV, whereas a patient with confirmed MERS-CoV infection had a clear antibody response for IgG [1]. Some authors have explained their lack of use of serology by the fact that assays are not yet validated [34]. This argument seems flawed, as the same could be argued for PCR assays that are used universally, with the limitations described above. Serological testing confirmed infection in two MERS-CoV patients (from Abu Dhabi and Qatar), hospitalized in Germany. Both had high titres of antibodies by IFA, that were also confirmed by neutralisation tests and by micro-array testing [16,25,30]. Interpretation of MERS-CoV serology can be hampered by the widespread circulation of the four common hCoVs, especially by hCoV-OC43 and hCoV-HKU1 which belong to the same genus of the *betacoronaviruses*. Cross-reactive antibodies have been shown between SARS-CoV and other coronavirus, depending also on specificity of the assay [52,53].

6. Current WHO recommendations for MERS laboratory testing

6.1. Who to test?

According to the WHO guidance for health professionals, patients should be evaluate for MERS-CoV infection if they develop pneumonia or pneumonitis and fever with a history of travel to, or residence in, the Arabian Peninsula in the 14 days before illness onset; or contact with known confirmed or probable MERS cases in the 14 days before illness onset [54]. Additionally the WHO recommends testing for novel coronavirus of persons, including health care workers, in clusters of acute respiratory infection of unknown aetiology, requiring hospitalisation, or where the respiratory infection is unexpectedly severe.

6.2. What test is required to confirm the case?

According to the MERS case definition (WHO, revised on 3 July 2013) a confirmed case requires laboratory confirmation by molecular methods including a positive real-time reverse-transcription polymerase chain reaction (rRT-PCR) on at least two specific genomic targets (Table 1) or a single positive target with sequencing on a second target [54,55]. A single positive rRT-PCR without confirmation will be considered as inconclusive MERS-CoV laboratory test, and such cases are classified as probable. Several molecular assays are now in widespread use and a two-step approach of screening and confirmation (Table 1) algorithms have been recommended by WHO and CDC [55–57]. In both algorithms a screening PCR targeting a region upstream of the E gene is proposed, sometimes combined with nucleocapsid (N) gene based PCR, to enhance sensitivity for specimen screening [55–57]. For confirmation, a second assay with a different set of primers and probes is recommended (Table 1).

Serological assays for testing antibodies against MERS coronavirus were developed by different laboratory experts and can be used for human diagnostics as well (Table 2). However, no official recommendations are currently available regarding serological tests, and validation has been difficult because of limited availability of human convalescent sera. According to the WHO case definition, a person with an acute febrile respiratory illness of any severity with positive serological test, it will be categorised as probable case of MERS-CoV infection [54]. Whenever possible a paired acute and convalescent sera should be tested, ideally combined with molecular testing of respiratory samples.

6.3. Which specimens to test?

As described above, it is strongly advised that lower respiratory specimens are collected when possible, in addition to nasopharyngeal and oropharyngeal swab specimens should be collected. The WHO also emphasises repeat testing as initial results may be negative. Specimens should be send to a reference laboratory for confirmation.

6.4. Who to notify the case?

Each probable or confirmed case of MERS should be immediately communicated to the national health authorities. Additionally, WHO requests that confirmed and probable cases be reported within 24 h of being classified as such, through the regional Contact Point for International Health Regulations at the appropriate WHO Regional Office.

7. Conclusions

Advances in laboratory techniques over the past decades have led to a continuous improvement of laboratory preparedness for emerging infectious diseases. However, the lack of sufficiently detailed data accompanying patient notifications and publications is an important constraint for developing evidence-based diagnostic support to outbreak investigations. To gain a better understanding of the clinical significance and epidemiology of MERS-CoV, it is imperative to collect detailed data on sampling, laboratory analyses and results, combined with clinical and epidemiological data, in order to improve the quality of the laboratory support during outbreaks. Until full validation of laboratory assays has been done, the combined use of molecular and serological approaches is highly recommended. Acute and convalescent serum samples should be collected from each patient to help define kinetics of seroconversion that will help to confirm or rule out infection in future patients, avoid misdiagnosis (false negative test results) due low sensitivity in an early phase of infection when antibody levels are low, and to control for non-specific reactivity.

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Competing interests

None.

Ethical approval

Not required.

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