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An updated roadmap for MERS-CoV research and product development: focus on diagnostics

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ABSTRACT

Diagnostics play a central role in the early detection and control of outbreaks and can enable a more nuanced understanding of the disease kinetics and risk factors for the Middle East respiratory syndrome-coronavirus (MERS-CoV), one of the high-priority pathogens identified by the WHO. In this review we identified sources for molecular and serological diagnostic tests used in MERS-CoV detection, case management and outbreak investigations, as well as surveillance for humans and animals (camels), and summarised the performance of currently available tests, diagnostic needs, and associated challenges for diagnostic test development and implementation. A more detailed understanding of the kinetics of infection of MERS-CoV is needed in order to optimise the use of existing assays. Notably, MERS-CoV point-of-care tests are needed in order to optimise supportive care and to minimise transmission risk. However, for new test development, sourcing clinical material continues to be a major challenge to achieving assay validation. Harmonisation and standardisation of laboratory methods are essential for surveillance and for a rapid and effective international response to emerging diseases. Routine external quality assessment, along with well-characterised and up-to-date proficiency panels, would provide insight into MERS-CoV diagnostic performance worldwide. A defined set of Target Product Profiles for diagnostic technologies will be developed by WHO to address these gaps in MERS-CoV outbreak management.

INTRODUCTION

The Middle East respiratory syndrome-coronavirus (MERS-CoV) is an emerging virus associated with severe respiratory illness, first detected in 2012 in Saudi Arabia.[1] As of 30 October 2018, the WHO has been notified of more than 2254 laboratory-confirmed cases of MERS-CoV infection from 27 countries, including 800 deaths (figures 1 and 2).[2] MERS-CoV is one of the high-priority pathogens identified by the WHO R&D Blueprint because of its high fatality rate (~35%) for severe cases, large geographical range of the reservoir and lack of medical

Summary box

► The Middle East respiratory syndrome-coronavirus is a high-priority pathogen identified by the WHO R&D Blueprint because of its high fatality rate, large geographical range of the dromedary camel reservoir and lack of medical interventions.

► Accurate and accessible diagnostic tests are essential to outbreak containment and case management, as well as surveillance in both humans and animals, but available diagnostic tests are limited by inconsistent quality assessment, specimen acquisition issues and infrastructure requirements.

► Diagnostic research and development (R&D) needs to include point-of-care testing options, syndromic panels for differential diagnosis, a greater understanding of viral and antibody kinetics, improved access to clinical specimens, and establishment of international reference standards.

countermeasures, with critical knowledge gaps in veterinary and human epidemiology, immunity and pathogenesis.[3 4] Currently, there are no licensed vaccines or therapies specific to MERS-CoV.

The WHO R&D Blueprint for Action to Prevent Epidemics is a global strategy and preparedness plan to strengthen the emergency response to highly infectious diseases, including MERS-CoV, by fast-tracking the development of effective medical technologies that can be brought to patients during epidemics.[4] This landscape analysis, complementary to the recent Food and Agriculture Organization (FAO)-Office International des Epizooties (OIE)-WHO MERS Global Technical Meeting report,[5] provides an overview to the current status of MERS-CoV diagnostics, including feedback from subject matter expert and developer interviews on the common challenges with test development and implementation, and identifies gaps for further research and development (R&D).
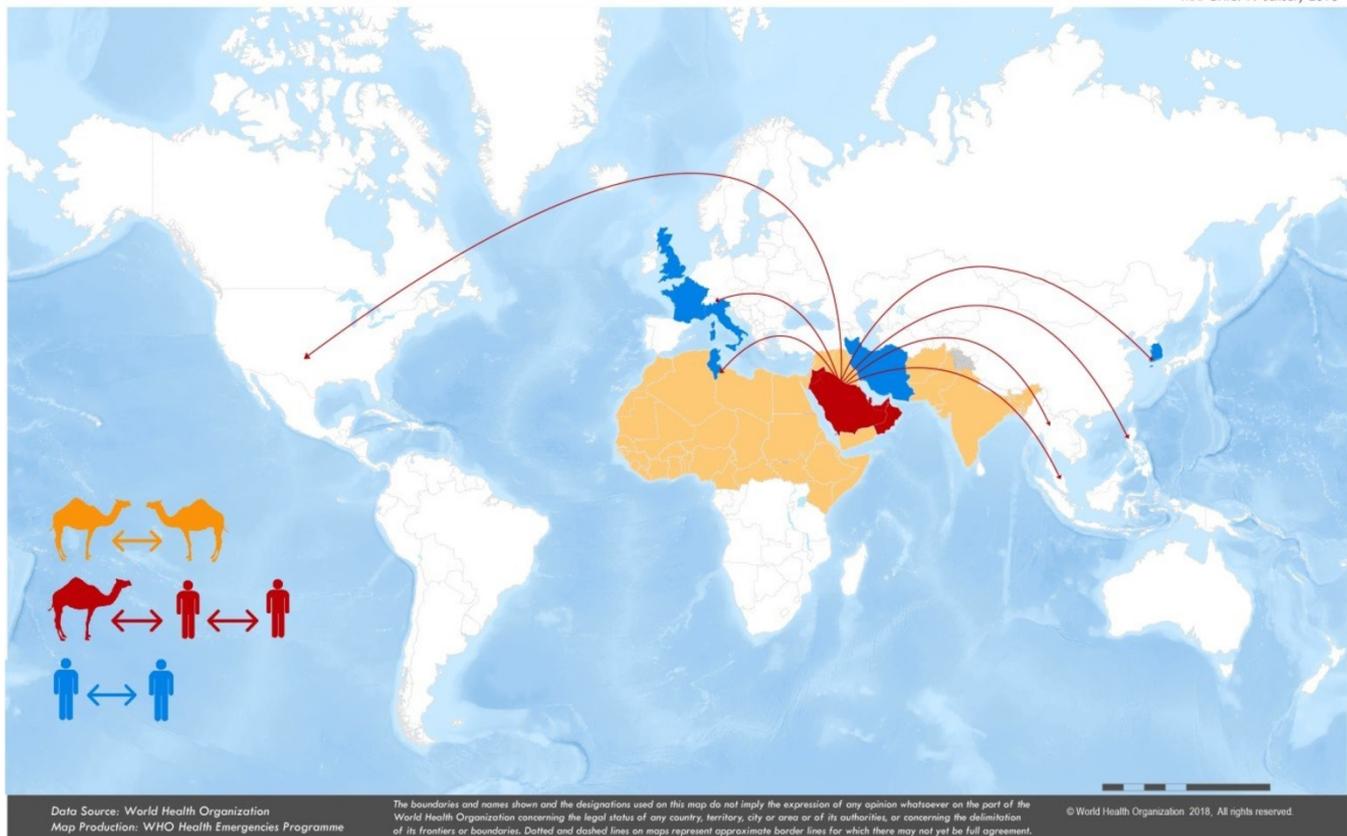
Figure 1 MERS-CoV transmission and geographical range. Reprinted from emergencies preparedness, response—Middle East respiratory syndrome-coronavirus (MERS-CoV), WHO, MERS-CoV transmission and geographical range. Copyright (2018).

MERS-CoV reservoir

MERS-CoV is a zoonotic virus, and dromedary camels (*Camelus dromedarius*) are the reservoir host and the source of zoonotic transmission to humans.[6–8] Dromedaries appear to be only mildly symptomatic following infection and present a significant reservoir risk for spillover events.[2 6 9] MERS-CoV RNA has been detected in dromedary camels in a number of countries, including Egypt, Oman, Qatar and Saudi Arabia, with evidence suggesting that MERS-CoV is also widespread in the Middle East, Africa and South Asia.[5 8 10–35] Infection in camels is notifiable to the OIE.[36] Individuals with close and frequent contact with dromedaries are at a higher risk for MERS-CoV infection than the general population.[37 38]

Clinical indications and management

Coronaviruses are a family of viruses that can cause diseases in humans, ranging from the common cold to severe acute respiratory syndrome (SARS). The clinical spectrum of MERS ranges from no symptoms (or asymptomatic infection), mild symptoms including fever, cough, gastrointestinal illness and shortness of breath, to severe disease including pneumonia, acute respiratory

distress syndrome and death.[2 39] Severe cases of MERS can result in respiratory failure, requiring mechanical ventilation and support in intensive care. Risk factors for severe disease include a weakened immune system, older age (>60 years), and comorbidities such as diabetes, cancer, renal disease and chronic lung disease.[40 41] Human-to-human transmission spreads through close and unprotected human contact, and more than half of reported MERS cases have occurred through nosocomial transmission.[42–45] To prevent nosocomial infections, WHO and others recommend using standard infection and prevention control measures when caring for patients.[46–48] WHO also recommends that contact tracing of all symptomatic and asymptomatic close contacts of the primary patient should be conducted routinely.[49]

Molecular epidemiology

The molecular epidemiology for MERS-CoV has not changed significantly since the initial human cases were detected in 2012. The current virus remains 99% identical to the sequences seen in the first human cases from 2012 as well as archived camel sera from 1983, with no increase in pathogenicity observed in the animal host.[50–52] As genetic mutations could impact detection,
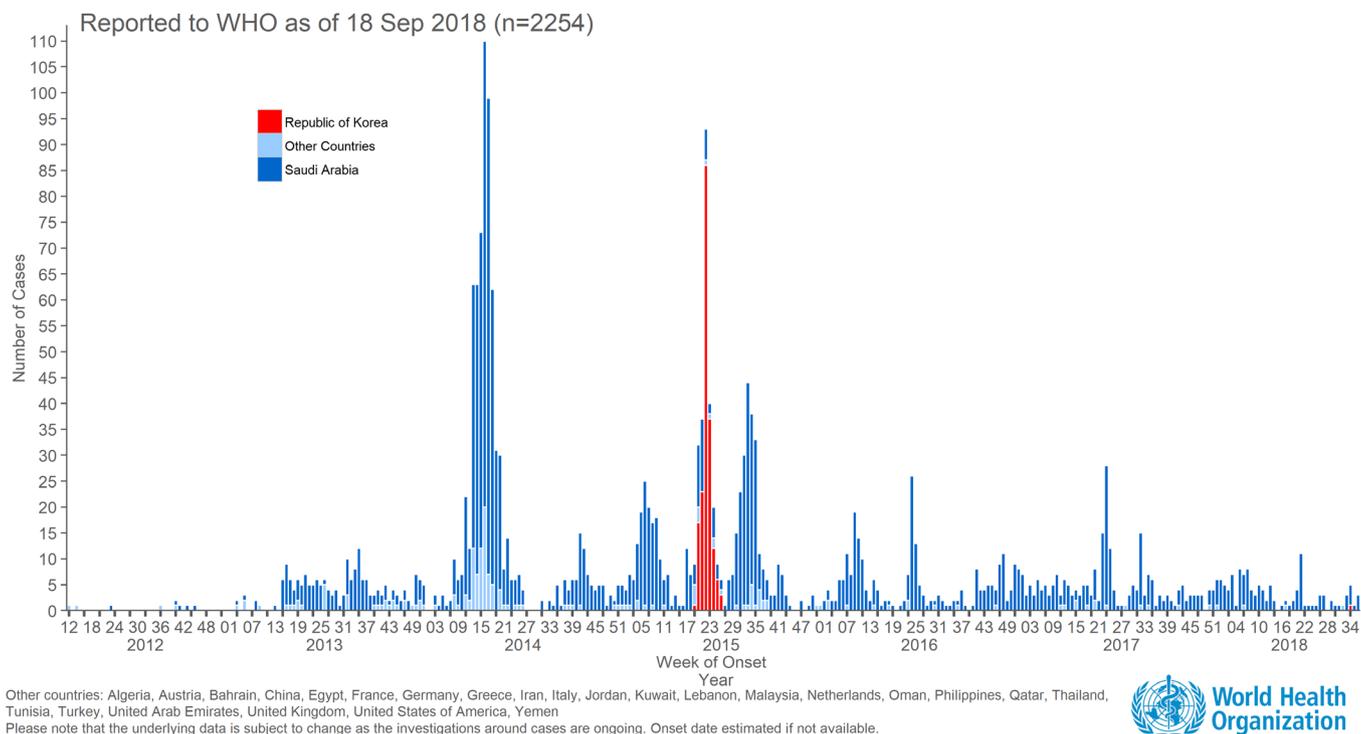
Figure 2 Confirmed global cases of MERS-CoV as of 18 September 2018. Reprinted from emergencies preparedness, response—Middle East respiratory syndrome-coronavirus (MERS-CoV), WHO, MERS-CoV transmission and geographical range. Copyright (2018).

immunotherapy and vaccine development efforts,[53] sequencing of MERS-CoV strains from camels and humans (after a zoonotic spillover) is important and is regularly being conducted in affected member states (WHO, personal communication, 2018).

Therapeutic and vaccine efforts

There are currently no prophylactic or therapeutic interventions of proven efficacy for any coronavirus infections. Without a specific therapy for MERS, treatment is supportive.[5 54 55] Effective MERS therapeutics are still in the early stages of research and evaluation. Several broad-spectrum antiviral agents including nitazoxanide,[56] viral methyltransferase inhibition[57] and nucleotide prodrugs[58] have shown in vitro activity against MERS-CoV. Early results for novel MERS-specific therapeutics that inhibit viral replication or have specific neutralising activity are promising.[47 59 60]

The WHO R&D Blueprint for MERS has called for three types of vaccines: (1) dromedary camel vaccine to prevent zoonotic transmission, (2) human vaccine for long-term protection of persons at high exposure risk and (3) human vaccine for reactive use in outbreak settings.[55 61] MERS-CoV vaccines are in the early stages of development,[55 62 63] with one candidate vaccine in phase I clinical trials (NCT02670187).[64] Neutralising monoclonal antibodies have been designed to target the MERS-CoV spike protein,[53 65] with ChAdOx1 and modified vaccinia Ankara vectors also strong vaccine candidates,[60 66] but none have yet advanced to clinical trials. To accelerate the process, the Coalition for Epidemic Preparedness Innovation has recently launched a call for proposals for the development of a human MERS-CoV vaccine in order to engage with developers interested in supporting these efforts.[67]

MERS-COV DIAGNOSTICS
Specimens and sampling

The WHO laboratory guidelines recommend nucleic acid amplification tests (NAAT) for diagnosis, using serology for diagnosis only when NAAT is not available.[68] In suspected patients, a single negative test result does not exclude diagnosis. Repeat sequential sampling and testing is strongly recommended. The kinetics of MERS-CoV infection has been shown to vary widely across cases,[40 69–72] prompting a more detailed investigation of viral and antibody dynamics across the broad range of sample types, disease states and host factors.[73 74]

The best NAAT test sensitivity is achieved using specimens from the lower respiratory tract (sputum, tracheal aspirates or bronchoalveolar lavage), where MERS-CoV replication occurs at higher and more prolonged levels of MERS-CoV RNA, typically between 10^6 and 10^{10} copies/mL.[72 75] MERS-CoV viral load is generally higher for severe cases, with more prolonged viral shedding than mild cases. Viral load concentrations, which may be undetectable at early-stage infection, generally peak in the second week after symptom onset, and then drop to undetectable in survivors by the fourth week from onset.

Table 1 Diagnostic capacity vs setting

	Infrastructure requirements (example)	Training requirements (example)	Test process time	MERS-CoV target population	MERS-CoV inhouse or LDTs (n)	MERS-CoV commercial tests (n)
Neutralisation	High/BSL-3 (reference laboratory)	High (advanced lab technician)	5 days	Human, camel	>3	0
NAAT reference	High/BSL-3 (reference laboratory)	High (advanced lab technician)	2–3 hours (1–2 hours prep)	Human, camel	>3	>15
NAAT POC	Moderate/BSL-2 (district hospital)	Moderate (laboratory technician)	1–2 hours	Human	1	0
ELISA, IIFT	High to moderate (regional lab, district hospital)	Moderate (laboratory technician)	3–4 hours	Human, camel	>5	>5
RDTs	Low (clinic, health centre, field settings)	Low (nurse, healthcare worker)	<30 min	Human, camel	3	2

BSL, biosafety level; IIFT, indirect immunofluorescence test; LDT, lab-developed test; MERS-CoV, Middle East respiratory syndrome-coronavirus ; NAAT, nucleic acid amplification test; POC, point of care; RDT, rapid diagnostic test.

Upper respiratory tract specimens (nasopharyngeal or oropharyngeal swabs) may also be used, but demonstrate 100×-1000× lower viral load and can test negative for mild cases.[76 77] If possible, both upper and lower respiratory tract sampling are advised. Specimens outside the respiratory tract are not recommended for diagnosis, as they can test negative in both severe and mild presentation. Viral RNA has been detected in stool samples (10^4 copies/mL), serum samples (10^3 copies/mL) and urine (10^2 copies/mL), more likely an indicator of severity as it typically precedes a poor clinical outcome.[71 76 78]

Serological diagnosis can be made using paired samples, more often used for research rather than diagnostic purposes, preferably with the initial sample collected in the first week of illness and the second collected 3–4 weeks later. If only a single serum sample can be collected, this should occur at least 3–4 weeks after onset of symptoms for determination of a probable case.

Table 1 presents an overview of the implementation requirements for MERS-CoV diagnostics (detailed commercial product information is presented in online supplementary tables S1 and S2). Molecular diagnostics such as NAAT (eg, PCR) typically require sophisticated laboratory infrastructure including biosafety cabinets,[79] while most serological tests (ELISA, indirect immunofluorescence test (IIFT)) can be run on the benchtop in a more modest laboratory environment, depending on sample preparation precautions.[80 81] Point-of-care (POC) tests are designed to be used outside of a traditional laboratory; near-POC tests are defined for rapid use in a laboratory near the patient, but are more automated and easy to use than the traditional laboratory test.[72 75] POC tests such as low-complexity rapid diagnostic tests (RDTs) can be used at the bedside, typically with non-invasive samples after minimal training. Inhouse

tests are described in sections below; commercial sources are listed in online supplementary tables S1 and S2.

Molecular diagnostics
NAATs are currently the standard for MERS-CoV diagnosis, as these tests (typically reverse transcriptase PCR (RT-PCR)) have the highest sensitivity at the earliest time point during the acute phase of infection. Following the WHO guidelines, two different targets on the MERS-CoV need to be detected by RT-PCR to confirm a case. MERS-CoV assays to detect the upstream envelope gene (upE) followed by confirmation of open reading frame 1A (orf1a), 1B (orf1b) genes or nucleocapsid (N) genes for confirmation have been developed.[55 82] Most commercial PCR tests perform parallel screening for the upE gene with confirmation by the orf1a, orf1b or N genes (most commonly upE + orf1a).

Initial NAAT tests for MERS-CoV were developed as inhouse tests, following the first detection of MERS-CoV in the Middle East.[83–86] Inhouse tests are not necessarily subject to quality control or regulation, and may not be rigorously validated; in some cases, inhouse tests are eventually developed into commercial products through collaboration and licensing efforts.[50 83 84 87–89] Commercial assays may undergo an international and/or incountry regulatory process; once on the market they can be independently evaluated for sensitivity, specificity and limit of detection.[78 90] As of 2018, there are several commercial NAAT tests available for MERS-CoV, including duplex and multiplex panels (see online supplementary table S1).

Serological assays
Serology is not widely performed for diagnosing acute MERS-CoV infection; however, it has been a useful tool

to determine the extent of infection around clusters and in seroepidemiological studies in animals and humans. Seroconversion typically occurs during the second and third week after symptom onset; data suggest that low antibody titre in the second week or delayed seroconversion is more closely associated with mortality than high viral load.[71 74] MERS-CoV seroconversion may not be observed for some patients, notably with mild or asymptomatic infection, and can show cross-reactivity with antibodies to other coronaviruses.[42 69]

Serological methods for the detection of antibodies against MERS-CoV include ELISA, IIFT and neutralisation tests. MERS-CoV serological assays can employ commercial reagents or proprietary monoclonal antibodies as capture agents.[87 91 92] Many MERS-CoV ELISA tests are labelled for research use only, with little or no clinical validation data available. Similar to the ELISA, IIFT is used when it is difficult to evaluate specific antigens individually by enzyme immunoassays or there is a preference for broader analysis of an immobilised specimen. IIFT microscopy assay can probe the entire antigen spectrum of the specimen, and is often designed for simultaneous detection of antibodies against biochemically distinct antigens. Neutralisation is a method for detecting anti-MERS-CoV antibody activity via inhibition of infection or replication,[69 93] performed as plaque reduction neutralisation, microneutralisation (MN) and pseudoparticle neutralisation (ppNT). MN is labour-intensive and slow, requiring at least 3–5 days for results; neutralisation techniques other than ppNT require biosafety level 3 containment as they involve live virus cultures.[94]

RDTs can leverage the same antibody/antigen capture agents as ELISA but in a lateral flow strip cartridge.[95] This enables a fast 10–30 min time to result, but with a 100-fold lower detection sensitivity than ELISA.[91 92] Follow-up confirmatory testing is therefore required. RDTs are typically paired with minimally invasive specimen collection (blood, oral fluid, nasal swabs) so that they can be used with minimal training outside of laboratory settings. Early prototypes for MERS-CoV RDTs have been developed,[87 92 96] with commercial RDTs for detection of MERS-CoV in camels and humans available (online supplementary table S2). The human MERS-CoV RDT does not appear to be widely used, perhaps due to the more invasive processing required for lower respiratory specimens, as well as sensitivity issues for upper respiratory specimens. The camel MERS-CoV RDT is used with upper respiratory specimens; however, test sensitivity varies depending on specimen sampling and infection kinetics.[97]

Multiplex panels

At the early stages, the symptoms of MERS-CoV infection can mimic diseases such as influenza, pneumonia, SARS and other respiratory infections. A syndromic approach involves testing for pathogens based on a syndrome such as fever or acute respiratory distress; a shift from individual tests to multiplex panels can quickly identify or eliminate likely pathogens from a single specimen. For analysis of circulating reservoirs, multiplex microbead-based immunoassays have been used to detect IgG antibodies for multiple pathogens.[98 99] Multiplex, syndromic panels that include MERS-CoV have been demonstrated using PCR-based panels including MERS-CoV, showing similar limits of detection to single assays.[89 100 101] Commercial respiratory panel tests including MERS-CoV have also recently been developed (see online supplementary table S1).

CHALLENGES FOR MERS-COV DIAGNOSTICS
Harmonisation and communication

There is a need for international consensus and adoption of minimum standards for tests used in diagnosis, surveillance and research, following WHO's recommended algorithm for human cases[82] and OIE recommendation for animal health.[36] Harmonisation of the testing process can be achieved by building consensus and capacity across international and incountry laboratories. In order to enable and sustain the capacity for a rapid outbreak response, laboratories must have access to high-quality reagents and instrumentation, along with technical support and cold-chain transport when necessary. In addition, international reference panels would achieve a more standardised training for external quality assessment (EQA) and quality control. Building on mandatory case reporting,[102] an international MERS-CoV data sharing platform that includes case exposure history and sequence data would greatly facilitate the knowledge base across the MERS-CoV community.[103–106]

Clinical validation

Understanding MERS-CoV viral dynamics across a broad range of specimen types is critical to establishing the limits of detection and timing of diagnostics in order to make the greatest impact for diagnosis, case management and surveillance. Ensuring a test has appropriate sensitivity and specificity is a major challenge in the development of diagnostics for novel and rare pathogens, as there is often a very limited supply of well-characterised clinical material. Especially during the early stage of an outbreak, clinical evaluation must often be performed in the affected countries by laboratories working closely with the Ministries of Health. Typically only a small number of patient specimens are shared outside of the affected countries due to strict import and export regulations, particularly for 'dual-use' pathogens.[107 108] Specifically, the provisions of the Nagoya protocol have significant impact on the access to genetic materials for both commercial and non-commercial applications.[109 110]

In particular, the development and validation process for new diagnostics could be accelerated if well-characterised specimens and reference standards could be more easily obtained. EQA can be useful for evaluation of test performance, as shown with evaluations of both inhouse and commercial assays for MERS-CoV,[111–113] and

more recently a global proficiency testing programme used to assess laboratory detection of MERS-CoV.[114] Even after validation, a substantial amount of reference material is required for quality control; often manufacturers must develop their own calibration standards to maintain supply and to control lot-to-lot variability. International reference standards and qualified specimen panels can accelerate the development and validation of diagnostic tests. In particular, the WHO International Biological Reference Preparations (as provided by member states) serve as reference sources for ensuring the reliability of in vitro biological diagnostic procedures used for diagnosis of diseases and treatment monitoring, including MERS-CoV. Several international institutes also provide specimens for validation; these groups typically have a defined pathogen/disease focus with a corresponding archive of biological reference materials; however, the supplies may be limited (see online supplementary material 1).

POC testing

Currently, MERS-CoV diagnosis by PCR requires a laboratory with sophisticated facilities and biosafety cabinets. The turnaround time to receive a test result can take days to weeks, depending on laboratory proximity, sample transport options and laboratory processing capacity,[72 75] and infrastructure requirements place most PCR systems in reference laboratories, which may not be ideal for diseases like MERS-CoV that recommend immediate isolation for infections detected across a variety of settings.[81 115 116] A more nimble approach is needed for MERS-CoV case detection and triage,[92 117] and at border crossings for animal surveillance, quarantine and targeted vaccination.[11 21 87 118] The FAO-OIE-WHO MERS Technical Working Group has given a clear call for the development of an RDT to improve identification and isolation of primary human cases in healthcare facilities.[5]

Serological RDTs are ideal for low infrastructure settings such as a primary health clinic, home or field testing. However, specimen collection remains a key challenge for MERS-CoV, as the recommended lower respiratory specimens are difficult to obtain outside of a hospital setting. Upper respiratory specimens such as nasal swabs are easy to obtain and work well in conjunction with RDTs for camels, but these specimens generally have low virus titre in humans, thus limiting current use of RDTs to animal testing.[87 92 96] Improvement of the current RDT detection chemistry, if feasible, may support the future use of these tests in humans, at least for rapid triage in highly infectious cases.

POC and near-POC microfluidic platforms enable a more flexible, but still highly sensitive approach for near-patient NAAT testing in decentralised settings. Near-POC NAAT platforms are compact and self-contained, with automated sample preparation for processing in minimal laboratory settings, which most healthcare workers can be trained to operate within a day.[119–121] Recent publications describe MERS-CoV assays designed for POC PCR,[89] loop-mediated isothermal amplification assay[122] and paper-based sensor detection[123]; however, no MERS-CoV assays are currently available for the existing near-POC platforms. Given that PCR is now the standard for MERS-CoV diagnosis, it would be highly desirable to have an automated, self-contained NAAT assay that can be readily deployed in a field or clinic setting.

Syndromic approach

Syndromic testing can be valuable during the early stages of an outbreak, in order to distinguish MERS-CoV from other respiratory infections or identify cases of coinfection.[100 124] A syndromic panel could be effective in expediting pathogen and outbreak identification, especially with technologies that can screen for multiple pathogens simultaneously.[125] Using the panel approach, a definitive diagnosis could enable timely decisions about triage, treatment, infection control and contact tracing.[126] While the per-test cost rises with test complexity, including additional reagents and more sophisticated instrumentation, a rapid and efficient diagnosis scheme can impact intervention and infection control and can be cost-saving overall.[127 128] As respiratory diseases are both regional and seasonal,[129–131] region-specific panels may be more cost-effective.[132] Multiplex panels offer the alternative for a 'bundled' testing paradigm; however, if not routinely used (if the market is small), then developers may be reluctant to support the test for diagnostic use, which requires additional investment for validation and regulation.

Surveillance

Surveillance can be an effective method to identify the initial stages of outbreak, but it requires routine and effective sampling. The impact of surveillance testing depends on the test sensitivity and specificity, sampling rates, kinetics of the disease, and whether the target is animal or human populations. Most surveillance sampling is performed in the field, either through population-based or 'hot spot' sampling. For MERS-CoV, it may be difficult and expensive to implement routine surveillance in dromedary camel stock, as they represent a significantly large reservoir but may suffer only mild effects from MERS-CoV infection, if any. The ideal surveillance tool would be a highly sensitive and field-appropriate screening test. Per-test cost is also an important factor along with ease of implementation.

CONCLUSION

This review has identified diagnostics currently available for MERS-CoV and highlighted ongoing challenges caused by critical gaps in diagnostics to support outbreak management. RDTs offer the potential for rapid POC screening for MERS-CoV; however, there are practical limits to implementing lower respiratory sample acquisition outside of a hospital setting, limiting feasibility. POC or near-POC NAAT platforms provide an opportunity for implementation of automated, self-contained

testing in hospitals and clinics with limited training in endemic-prone areas. Expansion of test menu options for existing POC or near-POC NAAT platforms will strengthen incountry response capacity to endemic diseases and simultaneously ensure countries are prepared for future pandemics. Syndromic multiplex panels may expedite differential diagnosis of MERS-CoV from other endemic respiratory diseases, but further analysis is needed to inform implementation and cost-effectiveness in the context of regional and seasonal detection. There is also a need for more sensitive serological assays with lower cost and minimum cross-reactivity that can be used as surveillance tools.

A more detailed understanding of MERS-CoV viral and antibody kinetics is needed across the broad range of sample types in order to optimise the use of existing assays and to address ongoing technical challenges in the detection of mild and asymptomatic infections. Surveillance continues to be important for the detection of MERS-CoV spillover events; however, questions remain on the cost-effectiveness of routine screening of the large reservoir camel population. In addition, support towards sample biobanks with well-characterised specimens and reference standards will facilitate diagnostic development and quality assurance for MERS-CoV diagnostics worldwide. In order to achieve the goals of the R&D Blueprint efforts, WHO is identifying key Target Product Profiles for diagnostics in order to mobilise funding and resources to support the development and implementation of the most critically needed tests.

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