BMJ Global Health

Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia

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To cite: Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. *BMJ Glob Health* 2019;**4**:e001118. doi:10.1136/bmjgh-2018-001118

Handling editor Seye Abimbola

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bmjgh-2018-001118).

Received 15 August 2018
Revised 23 September 2018
Accepted 24 September 2018

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ABSTRACT

Nipah virus (NiV) is an emerging pathogen that, unlike other priority pathogens identified by WHO, is endemic to Southeast Asia. It is most commonly transmitted through exposure to saliva or excrement from the *Pteropus* fruit bat, or direct contact with intermediate animal hosts, such as pigs. NiV infection causes severe febrile encephalitic disease and/or respiratory disease; treatment options are limited to supportive care. A number of in-house diagnostic assays for NiV using serological and nucleic acid amplification techniques have been developed for NiV and are used in laboratory settings, including some early multiplex panels for differentiation of NiV infection from other febrile diseases. However, given the often rural and remote nature of NiV outbreak settings, there remains a need for rapid diagnostic tests that can be implemented at the point of care. Additionally, more reliable assays for surveillance of communities and livestock will be vital to achieving a better understanding of the ecology of the fruit bat host and transmission risk to other intermediate hosts, enabling implementation of a 'One Health' approach to outbreak prevention and the management of this zoonotic disease. An improved understanding of NiV viral diversity and infection kinetics or dynamics will be central to the development of new diagnostics, and access to clinical specimens must be improved to enable effective validation and external quality assessments. Target product profiles for NiV diagnostics should be refined to take into account these outstanding needs.

Summary box

- ► Current diagnostics for Nipah virus (NiV), a WHO priority pathogen endemic to Southeast Asia, include in-house laboratory-based serological and nucleic acid amplification techniques.
- ► This review identified a number of remaining diagnostic gaps, including lack of point-of-care testing, difficulties obtaining clinical specimens and limited understanding of viral diversity, infection kinetics or dynamics, and ecology of the wild life reservoir; target product profiles for NiV diagnostics should be refined accordingly.
- ► Development of more reliable diagnostics will be key to the prevention and management of NiV disease, and to implementation of a 'One Health' approach comprising both human and animal surveillance and intervention.

INTRODUCTION

Nipah virus (NiV) infection is an emerging pathogen in Southeast Asia, first detected in the 1998–1999 outbreaks in Malaysia and Singapore, with seasonal outbreaks in Bangladesh and India since 2001, and a suspected 2014 outbreak in the Philippines.[1–5] In the 1998–1999 outbreak, nearly 300 human cases with over 100 deaths were reported, and more than a million pigs were euthanised.[6] Since 2001, NiV has infected hundreds with an average case fatality rate of 75%.[5] Currently, there are no licensed vaccines or therapies, and only supportive treatment is available.

NiV has been identified as a high-priority pathogen by WHO due to the broad geographical distribution of the NiV host reservoir and its potential for zoonotic and human transmission, as well as limitations in treatment and prevention.[7–9] However, there are few validated and regulated diagnostic tests available for NiV. This landscape analysis provides an overview of the current state of NiV diagnostics for screening, diagnosis and surveillance, highlighting further research and development needs.

Epidemiology

NiV is a member of the *Paramyxoviridae* family, sharing the genus *Henipavirus* because of its similarity and homology to Hendra virus (HeV) and the less pathogenic Cedar virus.[10–12] Both NiV and HeV are carried by the *Pteropus* host endemic to South Asia and northern Australia.[13–15] Infected bats shed NiV virus in their saliva, urine, semen and excreta, but are symptomless carriers. NiV surveillance has found evidence of infection in fruit bats across Southeast Asia,[5 16 17] with seropositivity as far as West Africa[18–20] and Brazil (figure 1).[21] Sporadic outbreaks of human NiV infection have occurred in Malaysia, Singapore, India and Bangladesh since 1999.[1 22–24]

Humans can be infected through direct exposure to *Pteropus* saliva or excrement, particularly through contaminated food.[25]

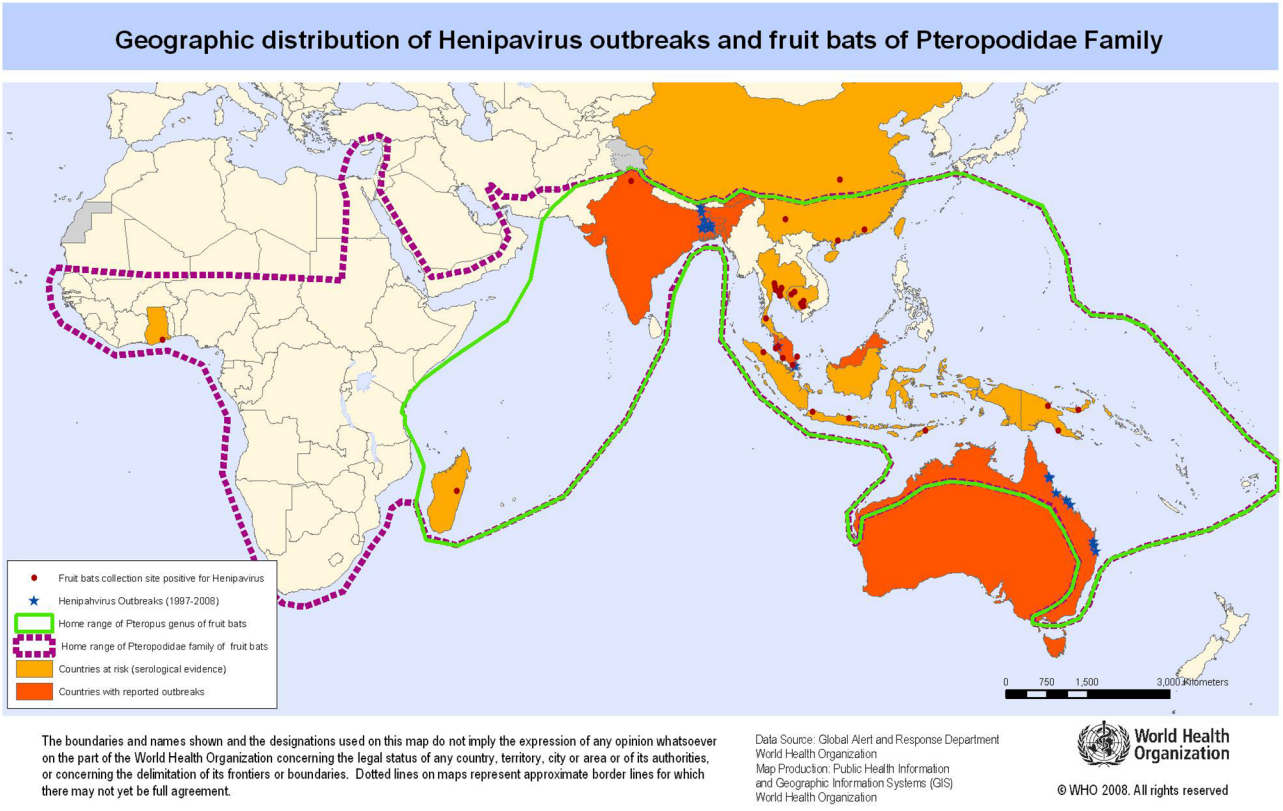

Figure 1 Geographical distribution of henipavirus (Nipah and Hendra) outbreaks. Reprinted from Emergency preparedness and response—Nipah virus infection, WHO, Geographic distribution of henipavirus outbreaks and fruit bats of Pteropodidae family, copyright (2008).

Date palm tree sap is a delicacy in Bengali culture, traditionally harvested by overnight collection which can be contaminated by opportune bats.[14 26 27] Human NiV outbreaks show a strong seasonal pattern during winter and spring months, likely associated with the *Pteropus* breeding season and the date palm sap harvesting season.[25]

Pteropus bats may also infect intermediate hosts, where NiV typically presents as fever and severe respiratory distress.[28 29] Direct contact with NiV-infected pigs was identified as the predominant mode of transmission in the 1998–1999 Malaysia–Singapore outbreaks, where 90% of the infected people were pig farmers.[30 31] There is evidence of NiV seropositivity in other animals including cats, dogs, cattle and horses.[28 30 32–37]

Human-to-human transmission, while uncommon, appears to be possible through exposure to the body fluids of infected patients. Evidence of NiV human-to-human transmission was found in Bangladesh, where indications of respiratory distress were more common than in the Malaysian outbreak.[38 39] Evidence indicated that human-to-human transmission resulted from direct contact with respiratory secretions of severely ill patients.[39–42] NiV can persist on surfaces, posing further risk for fomite-borne NiV transmission.[43]

Clinical indications

NiV causes severe encephalitis in humans, characterised by vasculitis and necrosis in the central nervous system (CNS). The incubation period for NiV is typically 4–14 days.[5 6] NiV primarily affects the CNS via endothelial, vascular, and parenchymal cell infection,[28] with high rates of viral replication in neuronal cells.[44]

At the early stage, NiV infection typically presents as febrile encephalitis or pneumonia, and can be difficult to distinguish from other febrile illnesses. Respiratory distress was a hallmark in approximately 20% of cases in the Malaysia–Singapore outbreak and 70% of cases in Bangladesh–India. Depending on the severity, patients can also present with fever, malaise, headache, myalgia, nausea, vomiting, vertigo and disorientation.

Severe cases include encephalitis with attending drowsiness and disorientation, which can rapidly progress to seizures and coma within 48 hours. Case fatality rates range from 40% (seen in the Malaysian outbreak[7]) to 75% (seen in the Bangladesh outbreak[45]) depending on severity, patient age and underlying health issues. Progression to encephalitis indicates a poor prognosis, with death within a median of 6 days after onset of symptoms.[39]

Approximately 20% of encephalitis survivors sustain neurological dysfunction including persistent seizures, disabling fatigue and behavioural abnormalities.[46] NiV infection can persist as non-encephalitic or asymptomatic infection; late-onset encephalitis and relapse have been observed months following initial recovery.[31 47]

Disease management and prevention

Due to the high pathogenicity associated with henipavirus, NiV and HeV are classified as Biosafety Level-4 (BSL-4) agents.[48] Safe handling of specimens requires physical infrastructure, personal protection equipment and strict operating procedures for both clinical and research operations.[49] As BSL-4 facilities may be limited in many endemic settings, BSL-3 and BSL-2 facilities may be sufficient if the virus can be inactivated following specimen collection.[50]

At present, there are no antiviral drugs or human vaccines available for NiV. Treatment is limited to supportive care.[5 6] Ribavirin has shown some evidence for a reduction in mortality, but its efficacy against NiV disease has not yet been established.[51–53] Several vaccine candidates are in development that employ NiV glycoprotein (G) and fusion (F) proteins to stimulate a protective immune response in preclinical animal models. Some approaches target specific neutralising antibody responses; others have been evaluated for both immune response and efficacy.[54–56] The Hendra G protein-targeted vaccine has been developed in Australia to protect horses against HeV and may offer protection against NiV as well.[57 58]

Phylogenetic diversity

The NiV genome consists of a single-stranded negative-sense RNA of approximately 18.2 kb and encodes for six major structural proteins: F, G, nucleocapsid (N), phosphoprotein (P), matrix protein (M) and RNA polymerase (L) and three accessory proteins (V, W and C).[12 40] Genetic sequencing has shown that the NiV and HeV coding regions for the N, P, C, V, M, F and G proteins have 70%–90% genetic homology, with approximately 92%, 87%, 83% and 68% amino acid homology for the N, L, G and P proteins, respectively.[10 12 40 59 60] Because of this homology, diagnostic tests can be cross-reactive for HeV and NiV, depending on the chosen RNA or antigen targets.

Genetic sequencing confirmed that the NiV strains circulating in Bangladesh and India (Clade I, or NiV-B) were different from the strains identified in Malaysia and Singapore (Clade II, or NiV-M),[23 45 60–62] with >92% genetic and amino acid homology across NiV-M and NiV-B strains.[7 10 44 45 61 63 64] Further analysis confirmed the presence of two distinct NiV-M subclade strains circulating in the north and south, likely arising from distinct hosts.[64 65] Lineage diversity needs to be taken into consideration in order to identify strains specific to a particular region, as well as ongoing genetic mutations to assist detection of all circulating strains (including differentiation from HeV).

NIPAH DETECTION AND DIAGNOSTIC TESTS

Since the symptoms of NiV infection are similar to other febrile diseases, early diagnosis is critical for containment of an outbreak and to facilitate appropriate patient care. Laboratory testing for NiV includes nucleic acid amplification testing (NAAT, eg, PCR and sequencing), IgG/IgM/antigen ELISA, immunofluorescence assay, histopathology, and virus isolation and neutralisation.[50 66 67] Table 1 describes the typical infrastructure, settings and resources required for the various test types.

Currently, the majority of international laboratories use in-house NiV assays; however, the degree of test validation varies widely.[28 50 67] There are a small number of commercial PCR kits available and only one commercial

Table 1 Diagnostic capacity vs setting

Test type	Infrastructure requirements (example)	Training requirements (example)	Test process time	NiV target population	Number of NiV in-house or LDTs*	Number of NiV commercial tests*
Virus isolation, neutralisation	HIGH/BSL-4 (reference laboratory)	HIGH (advanced laboratory technician)	5 days for cell culture	Human, animal	>3	0
NAAT	HIGH (reference laboratory)	HIGH (advanced laboratory technician)	2–3 hours (1–2 hours preparation)	Human, animal	>5	3
NAAT POC	MODERATE (district hospital)	MODERATE (laboratory technician)	1–2 hours	Human, animal	0	0
ELISA, IFA	HIGH to MODERATE (regional laboratory, district hospital)	MODERATE (laboratory technician)	3–4 hours	Human, animal	>5	1 (reagents only)
RDTs	LOW (clinic, health centre, field settings)	LOW (nurse, healthcare worker)	<30 min	Human, animal	0	0

*In-house and LDTs described in sections below; commercial sources listed in online supplementary table S1.
BSL, biosafety level; IFA, immunofluorescence assay; LDT, laboratory-developed test; NAAT, nucleic acid amplification test; NiV, Nipah virus; POC, point of care; RDT, rapid diagnostic test.

Table 2 World Organisation for Animal Health test methods available for diagnosis of henipavirus and their purpose

	Purpose					
Method	**Population freedom from infection**	**Individual animal freedom from infection prior to movement**	**Contribution to eradication policies**	**Confirmation of clinical cases**	**Prevalence of infection—surveillance (no clinical)**	**Immune status in individual animals or populations post-vaccination**
Agent identification*						
Virus isolation	+	+	—	+++		—
RT-PCR and qRT-PCR	+	+	++	+++	++	—
IHC	—	—	—	++	—	—
IFA	—	—	—	++	—	—
Detection of immune response†						
ELISA	+++	+++	+++	+	+++	+++
VNT	+++	+++	+++	+	+++	+++
Bead assays	+++	+++	+++	+	+++	+++

+++, recommended method; ++, suitable method; +, may be used in some situations, but cost, reliability or other factors severely limit application; —, not appropriate for this purpose. Although not all of the tests listed as +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results make them acceptable.
*A combination of agent identification methods applied on the same clinical specimen is recommended.
†One of the listed serological tests is sufficient. Reproduced with permission from *OIE Terrestrial Manual*, 7th ed. (May 2015 update).[70]
IFA, immunofluorescence assay; IHC, immunohistochemistry; RT-PCR, reverse transcriptase PCR; VNT, virus neutralisation test; qRT-PCR, quantitative RT-PCR.

Table 3 In-house NAAT tests for NiV*

Assay type	Target	Reference laboratory	Reference
RT-PCR	NiV P, N genes	ICDDRB and IEDCR, CDC (USA)	7 45
Real-time RT-PCR	NiV N gene	Institut Pasteur (France)	112
SYBR qRT-PCR	NiV N gene	University of Malaya (Malaysia)	113
Duplex nested RT-PCR	NiV N gene	Chulalongkorn University Hospital (Thailand)	114
Nested RT-PCR	HeV/NiV P gene	Bernhard Nocht Institute (Germany), CDC (USA)	18 115
Nested RT-PCR	NiV L gene	CSIRO/AAHL, University of Cambridge, Zoological Society (UK)	71
SYBR qRT-PCR	NiV N gene	CSIRO/AAHL, University of Cambridge, Zoological Society (UK)	71
Real-time RT-PCR	NiV/HeV P, M, N genes	CSIRO/AAHL, University of Cambridge, Zoological Society (UK)	71
Multiplex bead-based real-time RT-PCR	NiV, HeV N and P genes	CSIRO/AAHL (Australia)	89
Multiplex array card real-time RT-PCR	26 multiplex panel including NiV N gene	CDC (Kenya, USA)	90
Multiplex array card real-time RT-PCR	21 multiplex panel including NiV N gene	Kenya Medical Research Institute (Kenya), CDC (Kenya, USA)	91

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AAHL, Australian Animal Health Laboratory; CDC, Centers for Disease Control and Prevention; CSIRO, Commonwealth Scientific and Industrial Research Organisation; HeV, Hendra virus; ICDDRB, International Centre for Diarrhoeal Disease Research, Bangladesh; IEDCR, Institute of Epidemiology, Disease Control and Research; NiV, Nipah virus; RT-PCR, reverse transcriptase PCR; qRT-PCR, quantitative RT-PCR.

source for reagents (not an assembled/validated kit) for ELISA testing (tables 2 and 3, and online supplementary table S1).

While PCR is recommended as the most sensitive method for diagnosis of active NiV infection, NiV-specific IgM ELISA is an alternative serological approach where PCR is not available.[25] Histopathology (immunohistochemistry) is used post mortem to confirm NiV diagnosis in fatal cases, and virus isolation and neutralisation techniques are generally used for confirmation and restricted to BSL-4 facilities with stringent safety precautions.

Specimen collection should be performed in the early stages of disease, typically from throat and nasal swabs, cerebrospinal fluid, urine and blood.[6] In animals, NiV has been detected in respiratory secretions, blood, urine, faeces and organ tissues,[28 37 66] with evidence that NiV initially infects the respiratory system, followed by later stage dissemination into the nervous system.[50 68] There are limited data available for viral kinetics, but throat swabs have indicated 10^3–10^4 genome copies of NiV in severe cases and 10^6 in acute cases in humans.[46] Similar results have been seen in animal blood and serum,[22 69] with up to 10^{10} copies detected in animal brain tissue.[56]

Specifically for animal health, the World Organisation for Animal Health (OIE) has developed the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* to provide internationally agreed diagnostic laboratory methods and requirements for the production and control of vaccines and other biological products, towards the goal of facilitating international trade in animals and animal products and to contribute to the improvement of animal health services worldwide.[70] Details of the specific tests and parameters are described in table 2.

Nucleic acid tests

NAATs such as PCR are often the preferred method for detection of active viral infection as they are highly sensitive (detecting as few as 20 viral genomes); however, the infrastructure required may not be feasible in all endemic settings. Further, these tests can lose sensitivity if the viral genome undergoes significant genetic mutation or if new strains differ in the regions of probe design. To ensure coverage for genetic variants, probes are typically designed for highly conserved (resistant to mutation) sequences present in all known strain sequences for the pathogen of interest.[71] Full genome sequencing has been performed on the NiV-B and NiV-M strains (and substrains) along with the Cambodian and Philippine variants.[7 10 44 45 60 63 64 72]

Reverse-transcriptase PCR (RT-PCR) tests for NiV have targeted the conserved N, M or P genome segments (table 3). Several types of PCR tests for NiV have been developed, including conventional RT-PCR, nested RT-PCR and real-time RT-PCR (also known as quantitative PCR or qPCR). Real-time PCR has been shown to be 1000 times more sensitive as conventional PCR, and is now used almost exclusively.[67]

In addition, next-generation sequencing and deep sequencing enable a direct read of the viral genome, allowing virus and clade identification without prior knowledge of the composition.[73] Complex and expensive, this approach is not practical for screening larger numbers of samples in a diagnostic context; however, this technique provides a comprehensive and unbiased approach to viral analysis. Used in retrospect, it can identify the phylogenetic evolution of viral isolates over time and geography.[74 75]

Serological assays

Serological tests can directly detect NiV antigens, as well as IgM and IgG antibodies raised against NiV antigens. In general, serological tests are less sensitive to minor genetic variation and more broadly cross-reactive within subtypes and strains.[45] Antigen and IgM tests can be used to detect active infection; detection of anti-NiV IgM in serum peaks after 9 days of illness (based on hospital admittance) and can persist for at least 3 months. Since IgG can persist long after convalescence, IgG tests are primarily used for epidemiological studies and surveillance; detection of anti-NiV IgG peaks after 17 days of illness and can persist for more than 8 months.[76] Existing in-house serological tests for NiV are shown in table 4.

IgM ELISA is typically the first-line NiV serological diagnostic test, followed by serum neutralisation or PCR as a confirmatory test.[1 50 67] For increased specificity, recombinant proteins, monoclonal or polyclonal antibodies are used as capture agents. Recombinant NiV M protein has been shown to be highly reactive against antibodies in porcine sera.[77] Taking advantage of the >90% homology between HeV and NiV N and G proteins but ~30% difference between their P proteins, polyclonal antibodies have been developed to capture both NiV and HeV N antigens,[78 79] preferential detection of HeV P antigens,[78] and capture of both NiV and HeV G antigens including live virus.[80] ELISA combinations of these polyclonal antibodies can enable pan-henipavirus (NiV and HeV) detection, as well as differentiation between NiV and HeV. However, it is difficult to compare assay sensitivity due to differences in sample preparation and units of quantitation (pfu/mL vs infectious units vs $TCID_{50}$).

ELISA test reagents can be translated into a lateral flow format to create a rapid diagnostic test (RDT) that can be implemented in a non-laboratory environment, typically paired with minimal specimen processing (blood, plasma, swabs).[81] The lateral flow format enables a faster time to result (10–30 min), although with a lower detection sensitivity than their ELISA counterparts.[82] Currently, there are no NiV (or HeV) RDTs available for humans or animals.

Serological confirmation of NiV infection is typically performed by gold-standard seroneutralisation assays using live NiV, which requires a high-containment BSL-4 facility.[50] To circumvent the need for BSL-4 containment, non-infectious pseudotyped virus particles can be used in neutralisation assays under a BSL-2 environment.[67] NiV

Table 4 In-house serological tests for NiV*

Assay type	Target	Reference laboratory	Reference
ELISA	NiV IgG, IgM	ICDDRB and IEDCR (Bangladesh), CDC (USA)	7
ELISA	NiV neutralising Ab	DVS (Malaysia)	116
ELISA	NiV G protein	Universiti Putra (Malaysia)	117 118
Western blot, ELISA	NiV N, M proteins	Canadian Science Center for Human and Animal Health (Canada)	119
ELISA	NiV N protein	Chinese National Diagnostic Center for Animal Diseases (China)	120
ELISA	NiV N protein	Institute of Tropical Medicine (Japan)	121
ELISA	NiV/HeV P proteins	CSIRO/AAHL (Australia), University of Malaya (Malaysia), China Epidemiology Center (China)	122
ELISA	NiV/HeV N, HeV P proteins	CDC (USA)	78
ELISA	NiV/HeV G protein	National Institute for Infectious Diseases (Japan)	80
ELISA	NiV M protein	MARDI, Universiti Putra Malaysia, Monash University Malaysia	123
ELISA	NiV N protein	National Institute of High Security Animal Diseases/ICAR (India)	79
Multiplex bead-based antibody capture	NiV G, HeV G proteins	CSIRO/AAHL (Australia), Uniformed Services University of Health Sciences (USA)	94

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AAHL, Australian Animal Health Laboratory; CDC, Centers for Disease Control and Prevention; CSIRO, Commonwealth Scientific and Industrial Research Organisation; DVS, Department of Veterinary Services; HeV, Hendra virus; ICAR, Indian Council of Agricultural Research; ICDDRB, International Centre for Diarrhoeal Disease Research, Bangladesh; IEDCR, Institute of Epidemiology, Disease Control and Research; MARDI, Malaysian Agricultural Research and Development Institute; NiV, Nipah virus.

pseudoparticles have been derived from vesicular stomatitis virus or lentivirus (HIV), providing a more convenient laboratory method for diagnostic confirmation.[83–86]

Multiplex panels

As the symptoms of NiV infection can mimic other febrile diseases, severe cases are at risk for fatality with misdiagnosis and delayed treatment. A syndromic approach involves testing for pathogens based on a syndrome such as febrile or encephalitic disease; a shift from individual tests to multiplex panels to quickly identify or eliminate likely pathogens from a single specimen. Consideration must be given to the cost of additional reagents and more sophisticated instrumentation; however, a rapid and efficient diagnosis scheme that impacts infection control may be cost-saving overall.[87 88] As NiV outbreaks can be both regional and seasonal, region-specific panels may be more cost-effective and can be deployed 'as needed' for case detection as well as surveillance of risk factors across patient populations.

Multiplex PCR assays have been developed in the laboratory for differential detection of multiple pathogens or clades, using microarray, microcard or microsphere technologies. Microsphere array (Luminex) technology has been developed for detection and differentiation of HeV and NiV isolates.[89] These microsphere assays achieved differentiation of HeV and NiV, with the sensitivity of HeV detection comparable with individual qPCR. Multiplex TaqMan array card technology, typically a 384-well microfluidic card for simultaneous PCR reactions, has been developed for differential detection of acute febrile illness, including NiV, as 26-member and 21-member real-time RT-PCR panels.[90 91] The clinical performance exhibited an overall 86%–88% sensitivity and 97%–99% specificity compared with the individual real-time PCR assays. The 26-member panel includes 15 viruses, 8 bacteria and 3 protozoa. The 21-pathogen CNS multiple-pathogen assay includes two parasites, six bacterial pathogens and 13 viruses. Microarray technology has been developed for simultaneous interrogation of respiratory and gastrointestinal disease, including as global fever and biothreat agents[92 93]; such automated platforms enable 'sample in, result out' processing, but no panel includes NiV at the present date.

Multiplex immunoassay platforms (Luminex) for differential diagnosis employing microspheres (beads) coated with soluble HeV and NiV G proteins, respectively, have been developed.[94] Two different test formats were evaluated: a binding assay to differentiate between HeV and NiV antibodies, and a blocking or inhibition assay for detection and differentiation of HeV and NiV neutralising antibodies. By comparison, conventional ELISA tests were incapable of antibody differentiation. These bead-based Luminex assays have also been used to detect henipavirus antibodies in fruit bats and pigs.[17 19 95–98] More recently, the Luminex assay was used to assess HeV infection mice,[99] humans,[100] and horses and dogs.[101]

CHALLENGES FOR NIV DIAGNOSTICS

Viral diversity and infection kinetics and dynamics

The ideal NiV diagnostic test would detect all NiV lineages across all regions with equal sensitivity; however, many of the early PCR tests were developed from Bangladesh–India (NiV-B) or Malaysia–Singapore (NiV-M) strains. Thus, NiV diagnostics could benefit from a consensus-driven pan-NiV probe set. Additionally, further information is needed on viral and immune kinetics of NiV to identify the pathogenesis of NiV in blood and non-blood samples. Understanding the course of NiV infection at different stages of the disease would help to identify the window for effective intervention, and to better monitor transmission and recovery.

Clinical validation

For diagnostic test developers, clinical specimens are key to the validation process; however, validation for NiV tests has been limited due to the lack of NiV-positive sera available.[67 78] While animal samples may be used to investigate the performance characteristics of a test, there is a risk that the antigens found in specific animal-sourced NiV infections are not the same as those found in humans or other animals.[78] The WHO International Biological Reference Preparations serve as reference sources for ensuring the reliability of in vitro biological diagnostic procedures and are distributed by the WHO custodian laboratories. As new diagnostic tests are developed, these agencies could perform routine external quality assessment (EQA) monitoring of tests using up-to-date clinical specimen panels and reference standards. This role will be particularly important when test developers have little incentive to seek international regulatory approval.

Point-of-care testing

Given the often rural and remote NiV outbreak settings, sensitive and accurate diagnostics for NiV must be deployable under a range of circumstances, particularly at the point of care (POC). Existing POC and 'near-POC' NAAT platforms have lower infrastructure requirements than laboratory-based diagnostics, with automated sample preparation resulting in fewer training requirements for healthcare workers and cartridge-based formats allowing tests to be self-contained. They can therefore be more easily implemented in decentralised laboratories or field-based settings.[102–104] Given the range of assays already developed for these commercial NAAT platforms, current NiV PCR assays could likely be readily adapted to the POC format.

RDTs are ideal for field testing and low infrastructure settings such as the clinic or home.[81] RDTs have been developed to effectively screen and triage suspected high-risk cases such as Lassa, Ebola and Dengue.[105–107] Similar to Ebola, the pathogenicity of NiV makes specimen processing a challenge in a non-laboratory setting, thus preliminary work in antigen ELISA development[78–80] could serve as a starting point for an antigen NiV RDT.

Surveillance

Education campaigns may be helpful to increase awareness of the risk for NiV infection in the community and promote appropriate care-seeking behaviour, as well as to maintain vigilance for case identification by healthcare personnel.[108–110] Early detection of NiV outbreaks should trigger a coordinated mobilisation of resources for infection control, as well as patient isolation, care and contact tracing.

Surveillance tools for NiV should include reliable laboratory assays for early detection of disease in communities and livestock.[6 70] A One Health approach is key to understanding the fruit bat ecology, NiV disease seasonality and the transmission risk of other intermediate hosts. Without understanding the wildlife reservoir, the risk of reintroduction into animal or human populations cannot be managed.[58 111]

CONCLUSION

In addition to playing a central role in the recognition and control of outbreaks, diagnostic tests can enable a more nuanced understanding of the window of positivity and duration of infection, transmission risk and risk factors for severity for NiV, one of the most widespread agents of febrile encephalitic disease. In particular, diagnostics to support early detection will be critical for 'hot spot' interventions and containment (online supplementary table S2). However, several of the gaps identified in the 2016 WHO R&D Blueprint remain, including a lack of routine EQA, understanding of NiV viral and antibody kinetics, well-characterised and up-to-date proficiency panels, and accurate surveillance data.

Target product profiles for NiV should be refined to include the need to identify all known lineages of NiV, and the benefits of RDT POC diagnostics and syndromic panels. As diagnostics are a key element to achieving the goals of the R&D blueprint, WHO is coordinating research and funding through product development partnerships with groups such as FIND to ensure the development, evaluation and delivery of high-quality, affordable diagnostics for NiV.

Acknowledgements We gratefully acknowledge all those who attended the WHO R&D Blueprint Roadmaps: Consultation on Nipah in March 2018. Editorial assistance for later drafts was provided by Rachel Wright, PhD, funded by FIND, according to Good Publication Practice guidelines (http://annals.org/aim/fullarticle/2424869/good-publication-practice-communicating-company-sponsored-medical-research-gpp3). Publication of this article was funded by FIND.

Contributors LTM contributed to drafting the manuscript and provided background research for the manuscript. CK-C contributed insight into diagnostic needs for outbreak pathogens. All authors reviewed, edited and approved the final version of the manuscript.

Funding FIND was funded for this work by UK aid from the UK Government.

Disclaimer The opinions expressed in this article are those of the authors and do not necessarily reflect those of the institutions or organisations with which they are affiliated.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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