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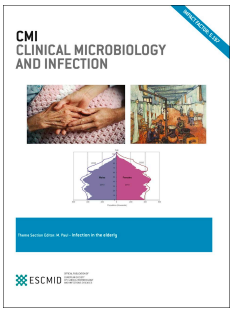
Accepted Manuscript



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Chantal B. Reusken, Margareta Ieven, Louise Sigfrid, Isabella Eckerle, Marion Koopmans

PII: S1198-743X(17)30683-3

DOI: 10.1016/j.cmi.2017.12.010

Reference: CMI 1152

To appear in: *Clinical Microbiology and Infection*

Received Date: 21 October 2017

Revised Date: 8 December 2017

Accepted Date: 12 December 2017

Please cite this article as: Reusken CB, Ieven M, Sigfrid L, Eckerle I, Koopmans M, Laboratory Preparedness and Response with a focus on Arboviruses in Europe, *Clinical Microbiology and Infection* (2018), doi: 10.1016/j.cmi.2017.12.010.

Laboratory Preparedness and Response with a focus on Arboviruses in Europe.

Chantal B. Reusken[1*], Margareta Ieven[2], Louise Sigfrid[3], Isabella Eckerle[4], Marion Koopmans[1]

[1]Department of Viroscience, WHO Collaborating Center for Arboviruses and Viral Haemorrhagic fever Reference and Research, Erasmus University Medical Centre, Rotterdam, the Netherlands

[2] Department of Medical Microbiology, Antwerp University Hospital, Antwerp, Belgium, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium

[3]Centre for Tropical Medicine and Global Health, Nuffield Dept. of Medicine, University of Oxford, Oxford, UK

[4]Department of Virology, Universitäts Klinikum Bonn, Bonn, Germany

*Corresponding author. Chantal Reusken, Department of Viroscience, Erasmus MC, Wytemaweg 80, Rotterdam. e-mail: C.Reusken@erasmusmc.nl.

Abstract

Background. The global health burden of arboviruses is continuously rising which results in increasing pressure on local and (inter)national laboratory infrastructures. Timely and accurate diagnosis of cases is one of the main pillars for public health and clinical responses to an arbovirus emergence.

Aims and Sources. This narrative review aims to summarize recent advances and to identify needs in laboratory preparedness and response activities, with a focus on viruses transmitted by arthropods in Europe. The review is based on evidence extracted from PubMed searches, Public Health and clinical laboratory experiences from the authors and the authors' opinions substantiated by peer-reviewed scientific literature.

Content. We illustrate the importance of inter-epidemic laboratory preparedness activities to ensure adequate Public Health and clinical responses. We describe the status of arbovirus endemicity and emergence in Europe thereby highlighting the need for preparedness for these viruses. We discuss the components and pitfalls of an adequate laboratory preparedness and response and the broader context of the current landscape of international research, clinical and laboratory preparedness networks. The complexity of arbovirus laboratory preparedness and response is described.

Implications. Outbreak preparedness plans need to look beyond national reference laboratories, to include first-line responding onsite hospital laboratories and plans for strengthening of such local capacity and capability as required depending on the nature of the outbreak. In particular, the diagnosis of arbovirus infections is complicated by the existence of geographic overlap of circulation of numerous arboviruses, the overlap in clinical manifestation between many arboviruses and other etiologies and the existence of cross-reactivity between related arboviruses in serology testing. Inter-epidemic preparedness activities need strong national and international networks addressing these issues.

However, the current mushrooming of European preparedness networks requires governance to bring the European preparedness and response to a next level.

Background.

In the past decade arthropod-borne viral diseases have continued their world-wide geographic expansion and thereby exert an increasing pressure on global health [1]. Arthropod-borne viruses (in short arboviruses) are viruses that replicate in and are transmitted by arthropods, such as mosquitoes, ticks and sandflies, between vertebrate hosts. Arboviruses can cause severe disease in humans and/or animals and are maintained in complex multi-component life-cycles. Through globalization of travel and trade, increasing population density, and possibly under influence of climate change (novel) arbovirus diseases have expanded considerably over the past years [2, 3]. Recent examples of large outbreaks in humans resulting from a fast geographic expansion of arboviruses upon introduction in naïve areas with suitable vectors are the emergence of chikungunya virus (CHIKV) and Zika virus (ZIKV) in the New World in 2013 and 2015 respectively [4, 5] , the latter leading to the declaration of a Public Health Emergency of International Concern (PHEIC) by WHO in the period 1 February – 18 November 2016 [6, 7].

During the past decade, arboviruses have been expanding to and within Europe, with autochthonous transmission of dengue virus (DENV) in Croatia, France and Madeira (Portugal), CHIKV in France and Italy, West Nile virus (WNV) in Central and Southern Europe, and the first human cases with Crimean-Congo hemorrhagic fever (CCHF) in Spain [8-17] . In addition in 2016, Usutu virus (USUV), a mosquito-borne bird flavivirus with proven zoonotic potential, has rapidly expanded its geographic coverage in Europe in a multi-country outbreak of multiple virus lineages in birds [18-20]. A recent study in Italy indicated that human USUV infection may not be a sporadic event. USUV infections in patients with or without neurological impairments occurred more frequently than West Nile virus (WNV) infections in a four-year period in Italy [21]. Acute USUV infections have been detected in blood donations in Germany and Austria, raising blood safety concerns [22, 23].

A risk-assessment by WHO-Europe indicated that the risk for an outbreak with ZIKV in Europe should not be underestimated, in particular in countries with established presence of the vectors *Ae. aegypti* and *Ae. albopictus*, [24, 25] although, in contrast to *Ae. aegypti*, field and laboratory evidence do not point to a significant role of *Ae. albopictus* in the transmission of ZIKV [26-31]. While both *Aedes* vectors are established in some parts of South and South-East Europe, other parts of Europe have the established presence of other exotic mosquito vectors [24] in addition to autochthonous vector species e.g. various *Culex* species that vector WNV, USUV and Japanese encephalitis virus (JEV) [32-34]. The 2016 USUV outbreak in North-West Europe showed similarity to the explosive outbreak with the closely related WNV lineage 2, in Central Europe in 2008-2009 and in Greece in 2010 after a few years of limited local circulation [35]. It has been speculated that the expanding emergence of USUV might be a prelude to the emergence of WNV, both with a similar avian-mosquito lifecycle and both being introduced to naïve regions via viremic migratory birds (humans are dead-end hosts for WNV and USUV)[36].

Viremic travelers returning from endemic regions to naïve regions with competent local vectors are thought to have initiated the outbreaks with CHIKV and ZIKV in the America's and the local transmission events with DENV and CHIKV in Europe [9, 16, 17, 37, 38]. Globally the number of yearly travelers has risen from 450 million in 1990 to nearly 950 million in 2010. European Union (EU)Tourism Statistics indicate that in 2014 EU residents above 15 years of age made an estimated 1.2 billion trips (accounting for 2.6 billion nights) , of which 6.2% were to destinations outside the EU. Destinations outside Europe made up 14.6 % of all EU outbound trips: 1.8% to Latin America, 3.6% to North America, 4.7 % to Asia, 0.5% to Oceania and 4.0 % to Africa, although the distributions of travel destinations may differ significantly for travelers from different countries [39]. Outbreaks and/or geographic expansion of arboviruses globally are reflected in (periodic) increases in arbovirus diagnosis in returning travelers. An illustrative example is the increase in reported yellow fever cases (n=4) in European Union travelers in the period August 2016 –March 2017 which reflected the increased activity of YFV in South America

[40]. Some virus infections in returning travelers (e.g. CHIKV, ZIKV, DENV) constitute a risk for further spread if competent vectors are present [2, 16, 17, 41]. The majority of ZIKV cases imported into the EU/EEA (n=2130 since June 2015) were found in France (54%) and Spain(14%) where *Ae. albopictus* has an endemic presence [41, 42], indicated by WHO-Europe as risk factor for autochthonous transmission [25]. One of the other identified factors in an European country's risks for a ZIKV outbreak was the ability of a country to robustly detect ZIKV introduction and local transmission [25].

In addition to the above examples of emergence of arboviruses, several other human pathogenic arboviruses are endemic to Europe, such as the tick-transmitted viruses tick-borne encephalitis (TBEV) and Crimean-Congo hemorrhagic fever virus (CCHFV) and mosquito-borne viruses like Sindbis virus in Northern Europe and WNV in the Balkan and Northern Italy. These show occasional peaks in incidences due to variable local biotic and abiotic drivers of emergence [43-50]. Awareness among clinicians and targeted multi-component surveillance is needed to monitor the epidemiology of these viral infections [51].

The emergence of arbovirus disease in the human population is the result of complex processes usually involving animal reservoirs, arthropods and humans, while in a few cases the pathogen has completely adapted to an urban human-mosquito-human cycle (i.e. CHIKV, DENV, urban YFV and ZIKV)[2]. Although the timing is, the nature and geography of emerging disease events is often not completely unexpected [3, 52, 53] , e.g. the emergence of CHIKV and ZIKV in the America's and the geographic expansion of WNV, USUV and TBEV in Europe. In this light the world might be facing the emergence of YFV in Asia and of JEV in Africa. Indeed, in April 2017 the first case of autochthonous JEV infection was reported from Angola [54]. These continuously changing dynamics of arbovirus emergence and the rise in its global health burden will increasingly exert pressure on local and (inter)national laboratory infrastructures. As

diagnostics are the pillars of surveillance, individual patient care and (clinical) outbreak response, this asks for inter-epidemic laboratory preparedness.

Laboratory response: disease detection

As human arbovirus disease is an endpoint of a complex infection cycle involving vectors and reservoir hosts, timely detection of arbovirus infections requires multidisciplinary collaboration, including ecologists, entomologists, veterinarians, and wildlife disease experts. Laboratory preparedness and response therefore can be seen as a continuum of activities, one of which is the routine diagnostic capacity for evaluation of illness in humans (Figure 1). For common diseases known to be endemic in a region, diagnostic capacity needs to be available in- or rapidly accessible for- routine clinical laboratories. For rare, exotic diseases diagnostics is generally referred to specialized (inter)national reference laboratories. These reference centers have the expertise to support preparedness and response in its broadest sense, including access to diagnostics for rare viruses and laboratories for Risk Group 3 and 4 pathogens, and research-based monitoring of the evolution of viruses to ensure diagnostic accuracy and development of improved diagnostic platforms. For emerging disease threats with epidemic potential, diagnostic capacity available at reference centers ideally would need to be deployable to clinical laboratories to scale up local laboratory capacity.

The laboratory response to an emerging event needs to be timely, i.e. as early as possible, and accurate, i.e. with high sensitivity and specificity [55-58] . *Timeliness* can be assured by thorough preparedness. Laboratory preparedness should comprise a range of inter-epidemic activities in which barriers and challenges for reference laboratories to rapidly implement diagnostics to emerging pathogens could be addressed. For an *accurate* response, the essential basic questions for diagnostic triage (Table 1) need to be known and if (partially) unknown, these knowledge gaps would need to be systematically identified. Awareness of the existing diagnostic knowledge gaps is important to define a proper

133 sampling strategy, for an adequate choice of type of test to use and for a correct interpretation of
134 laboratory results and thus correct confirmation or ruling out of an infection [55-58]. Furthermore it can
135 provide guidance to the clinical and public health response where the identified critical knowledge gaps
136 can be addressed [51]. This requires intensive integration and collaboration between these, traditionally
137 often autonomously operating, disciplines. For example during the first phase of the emergence of ZIKV
138 in the Americas, the lack of knowledge on the infection kinetics of ZIKV in various population groups (i.e.
139 pregnant women) was identified by reference laboratories as a crucial gap to be addressed [57] and this
140 issue was a topic of research in numerous clinical studies during the course of the outbreak [59-63].

141

142 **Laboratory preparedness.**

143 While in theory there is good coverage of clinical diagnostic laboratories and reference centers across
144 Europe [64, 65], a challenge is how to focus the preparedness activities, in view of the expanding list of
145 arboviruses of relevance for Europe and the threat of local outbreaks. Optimal laboratory preparedness
146 constitutes a multi-component approach:

147 *Foresight and the establishment of generic approaches to diagnostic preparedness.* A challenging
148 question is how to prioritize the choice of pathogens to develop toolboxes for. Prioritization exercises
149 like the WHO R&D blueprint that prioritizes diseases likely to cause epidemics in the future could
150 provide guidance to these inter-epidemic activities. The January 2017 blueprint included four
151 arboviruses, i.e. ZIKV, CCHFV, RVFV and Severe Fever with Thrombocytopenia Syndrome virus (SFTS)
152 [66]. Another tool that has been developed to inform preparedness activities is the ECDC on-line tool for
153 the prioritization of infectious disease threats [67]. Furthermore numerous short-lists identifying and
154 classifying emerging virus threats have been published in the past two decades [68-73]. The availability
155 of toolboxes for high risk virus groups would facility the laboratory response to novel emerging viruses

as well, e.g. the genus orthobunyavirus, family *Peribunyaviridae* is known to be prone to yielding novel (re-assorted) arboviruses of importance to veterinary and public health [74-76] while a wide range of studies in bats and rodents has taught us that there is still a lot "out there" to surprise the world [71, 77, 78].

Mapping and overcoming logistic and sharing barriers. While there is widespread capacity to develop primer/probe combinations for (RT-)PCR detection, an obstacle for rapid deployment and implementation of laboratory response to an emerging event are the dissemination logistics for international sharing of materials critical for diagnostic set-up and validation, due to accumulating restrictive regulations fueled by biosecurity concerns ("dual-use") [79] and the Nagoya Protocol on Access and Benefit-sharing [80]. Inter-epidemic preparation of and negotiation on so-called umbrella permits and Memorandums of Understanding together with internationally generally accepted Standard Operating Procedures (SOP) for shipment should facilitate these issues in outbreak situations.

The establishment of sequence data-sharing platforms. With the rapid development of (next generation) sequencing (NGS) approaches, NGS as generic tool for agnostic detection of pathogens has great potential for the emerging infectious diseases field. In this field, sharing of data seems suboptimal, for a range of reasons, including practical, legal, ethical, political barriers [81]. The development and use of data sharing platforms where sequences, preferably linked to essential background information (e.g. date, location, host species, sample type, travel information, clinical manifestation) and bioinformatics workflows are deposited and shared will contribute to an effective laboratory response and overall response to emerging disease events. The sharing of data regarding emerging infectious diseases is not without problems, as it involves multiple stakeholders with different incentives [82,83]. The mapping of barriers to data sharing in order to identify possible solutions is widely debated, with the overall agreement that better systems need to be developed [81-84]. Examples of such data sharing

platforms/networks are the WHO managed DengueNet, the Germany hosted Global initiative for sharing all influenza data (GISAID, [85, 86]), networks managed by national and supranational organizations, and investigator driven platforms for sharing of sequence data and analyses like Genometrack and virological.org [84, 87, 88]. These activities and platforms all share pathogen data and metadata, but the approaches to do so differ greatly.

Quality assurance. Diagnostic laboratories need to comply to accreditation schemes (e.g. ISO15189), which requires extensive validation of assays used, although accreditation requirements differ per country. A specific hurdle to implementation of diagnostics for emerging or newly established infections is that accreditation schemes often do not accept validations done by other laboratories. Clinical samples needed for validation may be difficult to come by when dealing with an emerging disease. In an assessment of the ZIKV laboratory response in European reference laboratories it became clear that although a majority (84%) of laboratories were willing to share their validation data with other laboratories, external validation was only acceptable for 34% of the laboratories [58]. The availability of validation panels and positive controls to assure diagnostic accuracy is generally a major obstacle for a rapid response. Forty-seven percent of the EU/EEA reference laboratories for ZIKV diagnostics indicated that the availability of well-defined serology validation panels was their biggest challenge for implementation of diagnostics closely followed by the lack of positive reference materials (43%) [58]. Of 39 European laboratories responding to an Ebola virus (EBOV) laboratory response questionnaire, 12% indicated the availability of positive reference material as a major obstacle for an adequate response to the EBOV outbreak in West Africa in 2014-2015 (Reusken et al., *in press*). These issues could be addressed during inter-epidemic activities involving general bio-banking of a wide range of well-defined validation cohorts and the establishment of validation data sharing platforms. Bio-banking is addressed for instance by the EU H2020 program EVAg [89]. However, established platforms for timely sharing of validation data are currently lacking. Sharing of such data is mostly done bilaterally between

203 collaborating laboratories or only too late in the response process through peer reviewed publication,
204 while specialized networks like the ECDC Emerging viral disease expert laboratory network EVD-LabNet
205 [94] and the EU Joint Action EMERGE [95] might facilitate. ZIKV emerged in the America's in May 2015
206 and the first publications putting serology test validation data in the public domain appeared > 1 year
207 later, with substantial test comparisons even > 2 years later [90-93].
208
209 *Capability building.* A laboratory's capability for accurate diagnosis of endemic and emerging infectious
210 diseases will benefit from training and External Quality Assessments (EQA, proficiency testing). Both
211 EVD-LabNet and EMERGE provide training courses and twinning partnerships, and run EQAs based on
212 needs indicated by their members [96-107]. The role of the diagnostic laboratory in research, Public
213 Health and clinical response to emerging infectious disease events can be trained, optimized
214 (identification of knowledge/response gaps) and secured in multi-disciplinary outbreak simulation
215 exercises [108-110].
216
217 *Establishment of preparedness networks*. All of the above mentioned inter-epidemic preparedness
218 activities need strong national and international networks addressing these issues. In recent years the
219 European scientific, public health and clinical communities have made substantial progress by
220 establishing a number of international networks like the EU H2020 research networks PANDEM [111],
221 COMPARE [112], ERINHA [113] and EVAg [89], and the Public Health oriented ECDC respectively EC DG
222 Santé-endorsed laboratory response networks EVD-LabNet [94] and EMERGE JA [95]. Clinical research
223 response is addressed in the EU research network PREPARE [114] while the public-private partnership in
224 the Zoonoses Anticipation and Preparedness Initiative (ZAPI,[115]) focuses on the design of new, high
225 throughput manufacturing processes for delivering effective infectious disease control tools. A putative
226 pitfall of this increasing number of preparedness and response networks is the lack of interoperability

227 between these entities. Establishment of collaboration across the disciplines covered by each of these
228 networks would bring the European preparedness and response to a next level.

229 **Laboratory preparedness for arboviruses.**

230 Preparedness and response for arbovirus emergence is quite challenging mainly for three reasons. First,
231 the clinical manifestations of arbovirus infections overlap and are non-specific in the first phase of
232 disease. In general, the broad pallet of arbovirus syndromes are classified in four main syndrome
233 groups: febrile disease, arthralgia and/or rash, hemorrhagic syndrome and neurological syndrome [2,
234 51, 116]. Second, arbovirus circulation overlaps geographically which complicates narrowing down the
235 necessary diagnostic panel. Diagnosis of arbovirus infections is often mainly based on serological testing
236 as viremia is typically short-lived [2, 117-120]. Diagnosis based on serology however has severe
237 drawbacks due to frequent cross-reactivity between antibodies triggered by closely related viruses or
238 their vaccines while secondary infections might boost levels of cross-reactive antibodies due to previous
239 infections/vaccinations which complicates a proper interpretation of test results (76-78). Illustrative is
240 the current co-circulation of DENV and ZIKV in the America's. Overlap in disease spectrum, geographic
241 presence, and widespread yellow fever virus vaccination make interpretation of diagnostic serology very
242 challenging. In Europe co-circulation of multiple neurotropic flaviviruses, like TBEV, WNV and USUV, and
243 sometimes locally high vaccination grades for TBEV, represent similar issues [57, 117, 121, 122].

244 Multiple studies have shown that arbovirus illness is underdiagnosed in returning travelers and in
245 endemic areas [123-127]. A syndromic study among > 2000 Dutch travelers with known clinical and
246 travel history demonstrated that clinicians, irrespective of the likelihood of such an infection, rarely
247 requested arbovirus diagnostics for travelers within Europe and overemphasized arbovirus requests for
248 patients with very severe or very specific presentations while the majority of arbovirus infections
249 present in non-specific syndromes [116]. Although commercially available tools exist to provide clinical

250 laboratories and clinicians with decision support regarding the necessary differential diagnostics [128],
251 the complexities of arbovirus response cannot be reflected in these ranking tools. Therefore an overall
252 underdiagnosis of arbovirus infections is expected while it is simply not feasible (=cost effective) to
253 determine the cause of a disease beyond the most common and treatable etiology.

254 While expert laboratories for BSL3 and BSL4 arboviruses in the two European laboratory preparedness
255 networks EVD-LabNet and EMERGE aim to provide expertise and reference [58, 101, 129, 130], first line
256 arbovirus diagnostics will also be performed in routine, primary, secondary and tertiary health care-
257 associated laboratories especially in case of an epidemic when scale up of testing is needed. Although
258 there is a broad European coverage at the country level for priority arboviruses in reference laboratories
259 and the capability for their diagnostics in European reference laboratories has been assessed in the past
260 [96, 105, 129, 132, 133], the coverage of and capability for such assays in routine, health-care associated
261 laboratories and the existence of pre-arrangements for scale-up need to be assessed as well to address
262 the level of preparedness for larger outbreaks/epidemics. This will affect outbreak response and
263 individual patient care as in large outbreaks (national) reference laboratories will lack capacity to handle
264 diagnostic requests while timeliness is often only assured with onsite testing in absence of a pre-
265 arranged efficient sample transport infrastructure. At the beginning of 2016, the Brazilian government
266 distributed 500.000 PCR kits for molecular testing for ZIKV to 27 laboratories in the country, and in
267 October 2016 3.5 million rapid serology tests were distributed [134]. However, proficiency testing in
268 parallel to the upscaling of diagnostic capacity is crucial, as major differences in assay performance in
269 EQA assessments of emerging infections have been observed [97-99, 133, 135-137]. For instance,
270 although ZIKV diagnostics were widely covered in Europe in the first phase of the outbreak, an EQA
271 showed that the capability for molecular diagnosis of a ZIKV infection lacked in sensitivity [58, 107].

272

Conclusion

The overall global and European health burden of arboviruses results in increasing pressure on laboratory preparedness and response infrastructures. As timely and accurate diagnosis of cases is one of the main pillars for public health and clinical responses to an infectious disease emergence, inter-epidemic activities could ensure such adequate response. (Re)emerging infectious disease outbreak preparedness plans should consider the laboratory pillar and be developed in a collaboration between reference laboratories and hospital laboratories, and include planning of the strengthening of such local capacity and capability when needed e.g. in case of an outbreak overloading the national reference system. The current mushrooming of European preparedness networks requires governance; the establishment of collaboration and alignment across the disciplines covered by each of these networks in order to bring the European preparedness and response to a next level.

Acknowledgements.

This work was funded by the European Commission under H2020 grant number 602525 (PREPARE).

Dr. Reusken reports grants from EU H2020, during the conduct of the study.

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612 **Figure legends.**

613 **Figure 1.**

614 An effective laboratory preparedness and response at reference and clinical laboratory level is the basis
615 for the success of a wide spectrum of disease control measures targeting different phases in the
616 development of an arbovirus disease outbreak. Panel A: Development in time of an arthropod-borne
617 virus enzootic with human spill-over (adapted from [138]). In case of arbovirus infections that transmit
618 from human-to-human there is no involvement of an animal reservoir (green lines). Panel B: the three
619 surveillance pyramids involved in monitoring of arthropod-borne zoonoses (adapted from [139]). In case
620 of a human-mosquito/tick-human transmission there is no involvement of surveillance in animal
621 reservoirs (green pyramid). Early response needs sampling and diagnosis towards the base of the
622 pyramids. Panel C: Two levels, reference and clinical, of laboratory involvement in three scenarios of
623 disease presence: endemic disease, returning travellers and an emerging infectious disease threat. "x"
624 indicates involvement of each of the two levels of laboratory response in the three scenarios. "X*"
625 indicates optional role clinical laboratories in case of common travel-related diseases. Arrows indicate
626 direction of interaction/upscaling of capacity.

627

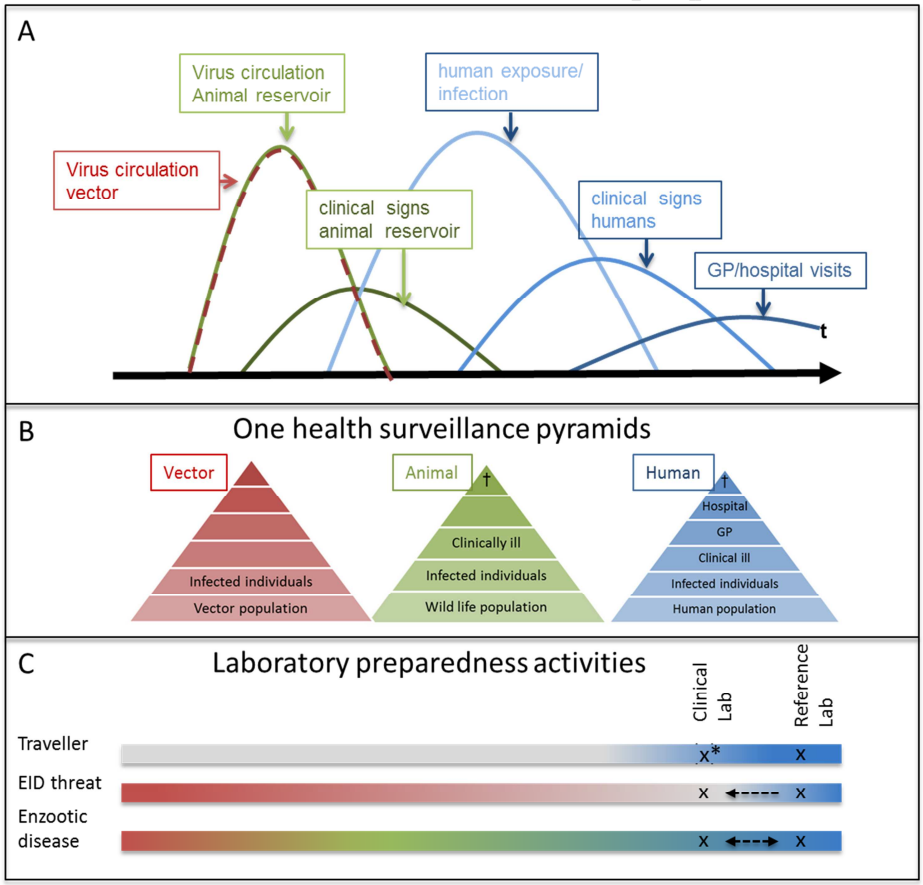
628

629 Table 1. Essential questions during diagnostic triage

What is the time-point during the course of infection when specimens should be collected?
⇨ Which are the kinetics of viral shedding and antibody responses in persons with different disease states (asymptomatic, mild, moderate, severe, acute, convalescent)? ⇨ How are infection kinetics influenced by host factors (e.g. pregnancy, immunosuppression, co-morbidities)?
What are the type of specimens adequate for the suspected pathogen and required for the available diagnostic tests?
⇨ What is the concentration of virus (viral load) in various body compartments, fluids and secreta during the progression of the disease? ⇨ How are viral loads influenced by host factors (e.g. pregnancy, immunosuppression, co-morbidities)?
What are the available in-house and/or commercial laboratory tests to confirm or rule out a diagnosis?
⇨ What is the limit of detection of the various diagnostic methods used for the different specimens and related to stage of illness? Specificity?

630

631 **Figure 1.**



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