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Laboratory diagnosis of Ebola virus disease

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Introduction

An outbreak of Ebola virus disease (EVD) began in Guinea in December 2013, and by 21 November 2014, 15,531 cases had been identified with 5,429 associated deaths primarily in Guinea, Liberia, and Sierra Leone [1, 2]. Case fatality rates during the current outbreak are around 70 % among patients with known outcome [1]. As treatment options are limited and largely supportive, preventative measures are paramount in the control of this disease. Rapid and reliable diagnosis of EVD is critically important for patient management, infection prevention, and control measures, and optimization of use of healthcare resources. This article briefly reviews new developments in the diagnosis of EVD.

General considerations

Although a presumptive diagnosis of EVD may be made in the appropriate clinical context based on exposure history, symptoms, signs, and findings on routine laboratory evaluation, confirmation using specific tests is required [3, 4]. Clinically EVD may be confused with or co-diagnosed with a number of other important illnesses occurring in West Africa including malaria, dengue, typhoid fever, influenza, and other viral haemorrhagic diseases [5].

A point of emphasis regarding all testing of patients with suspected EVD is that specimen acquisition, transport, and processing must be performed with the appropriate bio-containment procedures. The World Health Organisation (WHO) and the Centers for Disease Control (CDC) have issued recommendations regarding these aspects (http://apps.who.int/iris/bitstream/10665/134009/1/WHO_EVD_GUIDANCE_LAB_14.1_eng.pdf?ua=1 and http://www.cdc.gov/vhf/ebola/hcp/interim-guidance-specimen-collection-submission-patients-suspected-infection-ebola.html). Hospitals should have protocols ready in accordance with regional healthcare policies to handle specimens from patients with suspected EVD (including use of personal protective equipment, transportation, and decontamination).

Specific diagnosis

Specific laboratory diagnosis of EVD can be achieved either by detection of the virus or viral particles in the blood or by measurement of the antibody response to the infection [6] (Fig. 1). Most studies that evaluated these diagnostic methods were conducted during previous outbreaks of EVD, which were relatively small and also occurred in resource-limited countries.

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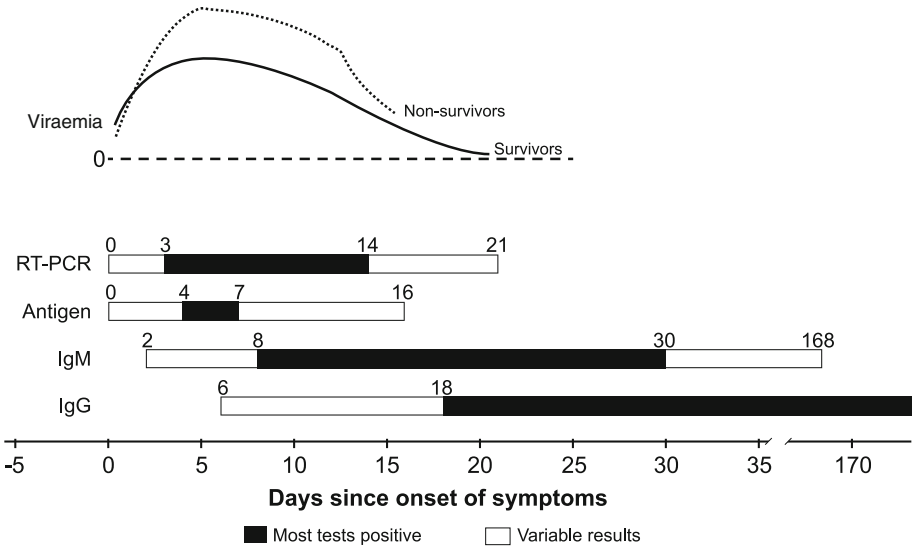

Fig. 1 Average viraemia in survivors and non-survivors of Ebola virus disease and expected diagnostic laboratory test results in relation to number of days since onset of symptoms. Most fatally infected patients fail to develop an IgG antibody response

Culture

Viral culture is limited by the need for tissue culture capability within a Biosafety Level 4 containment facility. Because viral growth is slow (usually achieved within 14 days), it is not clinically useful for direct patient management [7].

Polymerase chain reaction (PCR)

RT-PCR targeting viral nucleic acid is a rapid and sensitive technique to detect Ebola virus. There are a number of commercial and in-house PCR assays for detection of Ebola virus with different targets [8]. The WHO recommends that tests performed outside of a reference laboratory be sent to a WHO Collaborating Centre for secondary confirmation such as the Institut Pasteur de Lyon (France) and the Bernhard-Nocht Institute for Tropical Medicine (Germany). WHO has established an emergency mechanism to assess in vitro diagnostics. As of 11 December 2014, only one product, the RealStar Filovirus Screen RT-PCR kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany), has been approved [9]. For its part, the US Food and Drug Administration (FDA) has recently issued an Emergency Use Authorization for an Ebola virus nucleoprotein (NP) real-time RT-PCR assay (http://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM418810.pdf). Sensitivity in the acute phase is reported to be excellent ($\approx$100 %) but false negative results (specificity $\approx$97 %) [10] are possible and can arise from multiple causes such as too early or improper sample collection, improper shipping or storage, or inadequate PCR technique. Virus is not detected during the incubation period and experience has shown that PCR might be negative until 72 h after the onset of the symptoms [11, 12]. False positives are also possible, mostly attributed to cross-contamination during the PCR technique. Although individual samples can be analysed in less than 6 h using either the Altona PCR kit or the FDA approved kit, rapid testing of multiple samples in an epidemic setting will require rapid antigen detection cassettes that have not yet been approved. Quantitative RT-PCR has been developed and could possibly be used to predict outcome. Some data suggest that very high viral loads might be associated with worse outcomes such as death [13, 14]. In survivors, viraemia usually clears by the end of the second week of illness [4, 15] (Fig. 1). Virus can also be detected in other body fluids such as saliva, sweat, and semen. According to the WHO, oral swabs can be collected from deceased patients or in situations where blood collection is not possible, but sensitivity is reported to be lower. The viral dynamic has been less extensively studied for specimens other than blood. It is possible to detect viral RNA up to 3 months after the onset of symptoms in the semen of some survivors [6].

Antigen detection

Antigen detection ELISA was the mainstay to diagnose EVD in pre-2000 epidemics [16], but has since been largely replaced by RT-PCR which is slightly more sensitive and furthermore can be deployed more easily in epidemic settings [15]. Antigen detection is also very sensitive in the acute phase ($\approx$93 %) [10] but becomes less sensitive further in the infection with disappearance of the antigen at 7–16 days [17], a few days before PCR becomes negative [15]. It is expected that PCR will detect cases more rapidly than antigen capture and this has been demonstrated in one study from the Uganda outbreak of EVD with the Sudan strain in 2004 [15]. Several rapid antigen detection tests are currently being evaluated and may advantageously complement RT-PCR in future epidemics by their sensitivity and ease of use [18].

Serology

Specific IgM antibody can be detected by capture ELISA in the first week after the onset of symptoms and peaks during the second week of illness [4, 16, 17]. It disappears at variable rates between 30 and 168 days after onset. IgG antibody appears soon after the IgM, 6–18 days after onset of symptoms, and persists for many years [17]. Diagnosis using IgG requires paired serum samples. Most fatally infected patients fail to develop an IgG antibody response [4]. Thus, presence of antibody might be associated with a more favourable outcome. This suggests that serology might be less sensitive in the ICU setting. Most publications on Ebola ELISA serology do not show any cross-reaction with other virus associated with viral haemorrhagic fever [11]. Given the limited sensitivity and time to positivity, serologic tests have limited clinical utility in the management of critically ill EVD patients.

Conclusion

Laboratory studies of suspected EVD patients represent a critical part of the management of the current epidemic. Specific diagnostic tests are sensitive enough to rule out EVD if the patient experienced symptoms for more than 72 h. In the case of negative Ebola tests and absence of alternate diagnosis, consideration to repeat the test in 48–72 h might be warranted, especially if tests were performed early after the onset of symptoms. As there is a risk of transmission of the infection to healthcare workers, a protocol must be ready to collect and handle laboratory specimens from these patients to perform Ebola-specific tests and other laboratory tests.

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. WHO Response Team (2014) Ebola virus disease in West Africa–the first 9 months of the epidemic and forward projections. N Engl J Med 371:1481–1495
2. WHO (2014) Ebola response roadmap-situation report update. http://www.who.int/csr/disease/ebola/situation-reports/en/. Accessed 20 Nov 2014
3. Tattevin P, Durante-Mangoni E, Massaquoi M (2014) Does this patient have Ebola virus disease? Intensive Care Med 40:1738–1741
4. Kortepeter MG, Bausch DG, Bray M (2011) Basic clinical and laboratory features of filoviral hemorrhagic fever. J Infect Dis 204(Suppl 3):S810–S816
5. Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, Jalloh S, Momoh M, Fullah M, Dudas G, Wohl S, Moses LM, Yozwiak NL, Winnicki S, Matranga CB, Malboeuf CM, Qu J, Gladden AD, Schaffner SF, Yang X, Jiang PP, Nekoui M, Colubri A, Coomber MR, Fonnie M, Moigboi A, Gbakie M, Kamara FK, Tucker V, Konuwa E, Saffa S, Sellu J, Jalloh AA, Kovoma A, Koninga J, Mustapha I, Kargbo K, Foday M, Yillah M, Kanneh F, Robert W, Massally JL, Chapman SB, Bochicchio J, Murphy C, Nusbaum C, Young S, Birren BW, Grant DS, Scheiffelin JS, Lander ES, Happi C, Gevao SM, Gnirke A, Rambaut A, Garry RF, Khan SH, Sabeti PC (2014) Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science 345:1369–1372
6. Knipe D, Howley P (2013) Fields virology. Lippincott Williams & Wilkins, Philadelphia
7. Rollin P, Nichol S, Zaki S, Ksiazek T (2011) Arenavirus and filoviruses. In Versalovic J (ed) Manual of clinical microbiology, 10th edn. ASM Press, Washington, pp 1514–1525
8. Grolla A, Lucht A, Dick D, Strong JE, Feldmann H (2005) Laboratory diagnosis of Ebola and marburg hemorrhagic fever. Bull Soc Pathol Exot 98:205–209
9. WHO (2014) Update on the WHO emergency quality assessment mechanism for in vitro diagnostics for Ebola virus disease, December 11th 2014. http://www.who.int/diagnostics_laboratory/procurement/141211_evd_idvs_update.pdf?ua=1. Accessed 16 Dec 2014
10. Leroy EM, Baize S, Lu CY, McCormick JB, Georges AJ, Georges-Courbot MC, Lansoud-Soukate J, Fisher-Hoch SP (2000) Diagnosis of Ebola haemorrhagic fever by RT-PCR in an epidemic setting. J Med Virol 60:463–467
11. Chertow DS, Kleine C, Edwards JK, Scaini R, Giuliani R, Sprecher A (2014) Ebola virus disease in West Africa—clinical manifestations and management. N Engl J Med 371:2054–2057
12. Feldmann H, Geisbert TW (2011) Ebola haemorrhagic fever. Lancet 377:849–862
13. Bah EI, Lamah MC, Fletcher T, Jacob ST, Brett-Major DM, Sall AA, Shindo N, Fischer WA, Lamontagne F, Saliou SM, Bausch DG, Moumie B, Jagatic T, Sprecher A, Lawler JV, Mayet T, Jacquerioz FA, Baggi MF, Vallenas C, Clement C, Mardel S, Faye O, Faye O, Soropogui B, Magassouba N, Koivogui L, Pinto R, Fowler RA (2014) Clinical presentation of patients with Ebola virus disease in Conakry, Guinea. N Engl J Med 372(1):40–47
14. Schieffelin JS, Shaffer JG, Goba A, Gbakie M, Gire SK, Colubri A, Sealfon RS, Kanneh L, Moigboi A, Momoh M, Fullah M, Moses LM, Brown BL, Andersen KG, Winnicki S, Schaffner SF, Park DJ, Yozwiak NL, Jiang PP, Kargbo D, Jalloh S, Fonnie M, Sinnah V, French I, Kovoma A, Kamara FK, Tucker V, Konuwa E, Sellu J, Mustapha I, Foday M, Yillah M, Kanneh F, Saffa S, Massally JL, Boisen ML, Branco LM, Vandi MA, Grant DS, Happi C, Gevao SM, Fletcher TE, Fowler RA, Bausch DG, Sabeti PC, Khan SH, Garry RF, KGHLF Program C Viral Hemorrhagic Fever WHOCR Team (2014) Clinical illness and outcomes in patients with Ebola in Sierra Leone. N Engl J Med 371:2092–2100
15. Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, Vincent M, Lee WF, Spiropoulou CF, Ksiazek TG, Lukwiya M, Kaducu F, Downing R, Nichol ST (2004) Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. J Virol 78:4330–4341

16. Ksiazek TG, Rollin PE, Williams AJ, Bressler DS, Martin ML, Swanepoel R, Burt FJ, Leman PA, Khan AS, Rowe AK, Mukunu R, Sanchez A, Peters CJ (1999) Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis 179(Suppl 1):S177–S187

17. Rowe AK, Bertolli J, Khan AS, Mukunu R, Muyembe-Tamfum JJ, Bressler D, Williams AJ, Peters CJ, Rodriguez L, Feldmann H, Nichol ST, Rollin PE, Ksiazek TG (1999) Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis 179(Suppl 1):S28–S35

18. Vogel G (2014) Infectious diseases. Test New Ebola Test Sci 345:1549–1550