World Health Organization

EMERGENCY GUIDANCE

Selection and use of Ebola in vitro diagnostic (IVD) assays

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Contents

Acknowledgements

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Objective

To provide interim guidance to Ministries of Health and other organizations on factors to consider in the selection and use of available in vitro diagnostic (IVD) assays for diagnosis of Ebola virus disease (EVD).

Key messages

Selection of appropriate diagnostic assays for Ebola requires consideration not only of technical criteria but also the social and medical implications of test results. Given the consequences of a misdiagnosis, WHO recommends that only diagnostics that have undergone independent, comprehensive assessment of quality, safety and performance are used in diagnosing infection with Ebola virus. As incidence and prevalence of Ebola disease continues to decrease, assays with high specificity are required. This is because, in the context of low disease prevalence, widespread use of low specificity assays will generate more false positives than true positives. WHO recommends that nucleic acid testing using technologies such as polymerase chain reaction (PCR) should be the method of choice. A rapid antigen test that has reasonable sensitivity in patients with high concentrations of Ebola virus in the blood may have utility in settings without laboratory infrastructure if the benefits and limitations of the test are understood and appropriately managed.

1. Introduction

The role of laboratory diagnosis in response to the Ebola outbreak in West Africa has been critical. Early identification of infected patients assists in the provision of rapid access to health care as well as interruption of the chain of transmission by timely isolation of infected patients. Detection of viral nucleic acid using nucleic acid tests such as PCR technology is the recommended technique for laboratory diagnosis of EVD[1]. PCR methods in common use for this epidemic are highly sensitive and detect only Ebola virus, but are often complex to perform. In contrast, rapid tests for Ebola antigen are much more easily performed, but more often generate false signals. In October 2014, WHO issued a Target Product Profile for the development for rapid, safe and cost effective EVD IVDs[2]. Since then, a number of novel diagnostic assays including those using PCR and also lateral flow (rapid) tests that detect viral antigen have been developed for use in the current outbreak. Few of these have been reviewed by stringent regulatory authorities.

Due to the need for enhanced biosafety and biosecurity when handling viruses like Ebola, as well as the social and medical implications of test results, careful selection of appropriate diagnostic assays for use in the African context is vital.

Consideration must be given to the design and performance of the diagnostic assays, to ensure testing is safe and effective. The intended use of the diagnostic also influences selection of assay; methods used during an outbreak in which there is widespread transmission may be different to those selected for routine screening as part of a national surveillance programme. As many of the affected countries are now faced with the task of building and strengthening the national public health laboratory network, the selection of diagnostic assays should take into account the need for human resources, training, infrastructure, cost effectiveness, biosafety and infection control and prevention. The following document is designed to help guide decision makers, technical staff and ministries of health to select the most appropriate in vitro diagnostic solutions for procurement and use.

[1] 'Laboratory diagnosis of Ebola virus disease', Geneva, World Health Organization, September 2014. Available online at http://who.int/csr/resources/publications/ebola/laboratory-guidance/en/.

[2] 'Target Product Profile for Zaïre ebolavirus rapid, simple test to be used in the control of the Ebola outbreak in West Africa', Geneva, World Health Organization, October 2014. Available online at http://www.who.int/medicines/publications/target-product-profile.pdf?ua=1.

2. Overview of Ebola IVDs

Current routine diagnostic methods for Ebola rely on detection of viral components (proteins (antigens) or nucleic acids) in blood.

Molecular testing for Ebola virus nucleic acid (nucleic acid tests/NAT) using a technique known as PCR has become the standard method for Ebola virus detection in outbreaks. Properly designed tests of this type are highly accurate, and may be able to detect very low concentrations of virus. **Traditional NAT** methods are often well suited to a reference laboratory setting, for diagnostic purposes and for research. Traditional NAT systems can offer high throughput and flexibility to test for many different pathogens. The systems can utilise both commercial assays and those produced in-house (i.e. not for commercial distribution). However they are often complex and can be time-consuming methods to perform (around 3 to 5 hours). Special care needs to be taken to avoid false positive results due to contamination of the work area with by-products produced in the process, and so the different steps in the method often need to be performed in dedicated rooms to avoid this problem. Additionally, it is essential that these tests are performed by experienced laboratory scientists.

Recently, several companies have developed **automated NAT** methods that simplify molecular testing (including PCR and similar assays), increasing reliability, and decreasing the likelihood of false results and the number of ancillary reagents and equipment needed. Several such assays have been developed for Ebola, or are in the process of being developed. These assays may use cartridges pre-loaded with all necessary reagents, some are in a temperature-stable format, and are relatively simple to perform with the accompanying specific equipment. The other advantage of these systems is that the user of the test does not need the same level of understanding of molecular methods as is required for traditional NAT. This provides the opportunity for testing to move out of the reference laboratory to hospital based services.

An alternative to nucleic acid testing is direct detection of Ebola proteins (antigens). Antigen-detection tests are generally less sensitive than PCR methods. However, given the large amounts of virus present in most Ebola patients after several days of symptoms, these sensitivity limitations may not be critically important in some situations. Currently, these tests are available in a form similar to an over-the-counter pregnancy test. These **rapid antigen detection tests** (RDTs) theoretically can be performed anywhere, and without ancillary equipment. However, with these tests, false-positive results are harder to predict and control, and may be more common. This makes clinical study results, as well as an understanding of the probability of true Ebola disease in a given patient or cohort, critical to understand the utility of such tests. A number of these IVDs have recently been developed, and studies are ongoing to compare their performance. In a recent guidance document[3], WHO recommended that antigen detection RDTs for Ebola have no role in the routine management of Ebola in settings where PCR (molecular) testing is available; however, they may have utility in settings without laboratory infrastructure if their benefits and limitations are understood.

Tests detecting Ebola viral antigen in what is known as an ELISA format have been used for many years. These IVDs are sensitive in patients with high levels of virus present. However, most **ELISA antigen detection tests** are not available commercially. They require comprehensive laboratory infrastructure and skilled laboratory scientists to perform the assays. As such, these assays are generally most useful in a reference laboratory setting. None of these assays have been listed under the WHO EUAL mechanism (see section 3).

Serology tests that detect antibodies (IgM or IgG) produced by an infected patient have a role in epidemiologic or vaccine research, but not in routine clinical diagnosis or case management. These tests are most suited to a research setting. None of these assays have been listed under the WHO EUAL mechanism.

It is important to remember that all IVDs have the potential for incorrect results. This can be due to the inherent design of the test, how the test is performed, the disease stage, or if they are used in a manner other than stated by the manufacturer (e.g. a different specimen type).

A graphic summary of diagnostic assays available in different settings is presented in Figure 1.

[3] 'Interim guidance on the use of rapid Ebola antigen detection tests', Geneva, World Health Organization, March 2015. Available online at http://www.who.int/csr/resources/publications/ebola/ebola-antigen-detection/en/

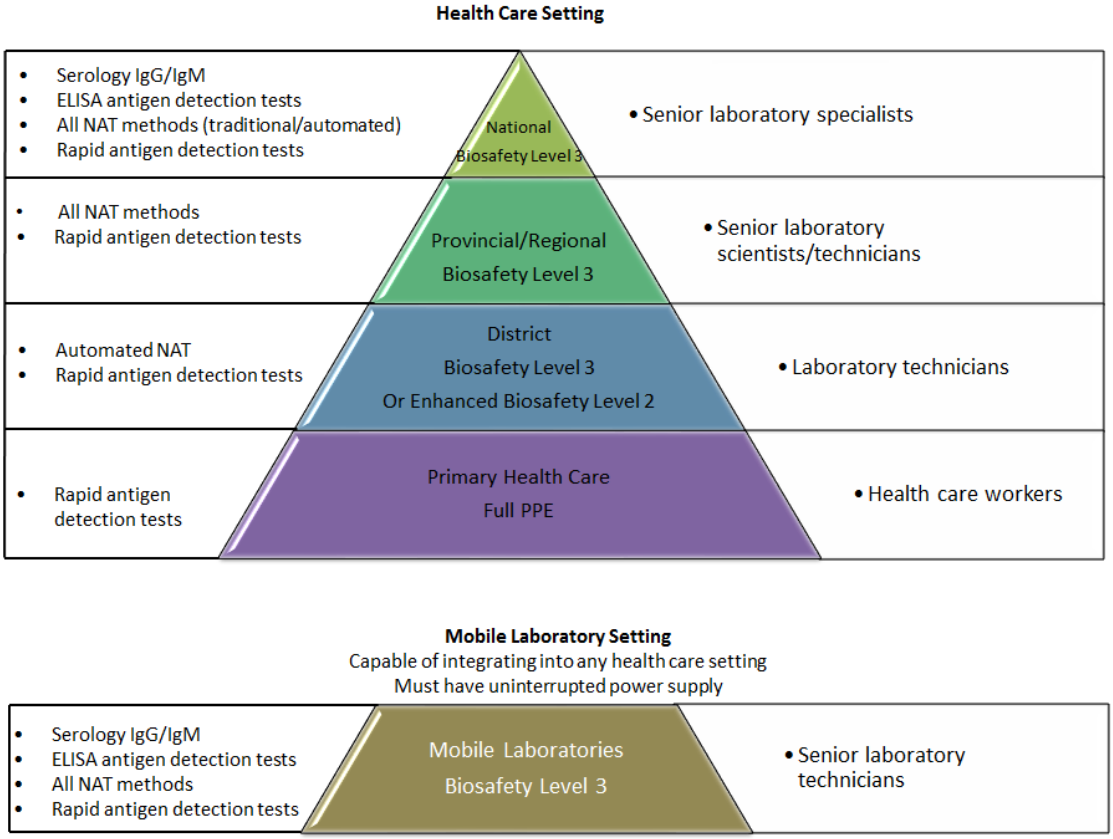

Figure 1. Diagnostic assays available in different settings

3. Description of regulatory approval processes (e.g. US FDA, WHO EUAL, CE marking)

Many EVD IVDs have not had any regulatory assessment of quality, safety or performance. Regulation of IVDs is normally the role of the national regulatory authority (NRA) but few NRAs have assessed EVD IVDs. Additionally, each NRA may have a different approach to IVD regulation, from comprehensive premarket assessment to only requiring a manufacturer to certify that the product meets regulatory requirements and obligations (as is the case of CE marking for EDV IVDs where there is no independent review).

As full regulatory review may be lengthy and the need for quality IVDs is urgent, some jurisdictions have established special regulatory processes based on minimal essential requirements during an emergency. An example of this is the United States Food and Drug Administration (US FDA) Emergency Use Authorization