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/

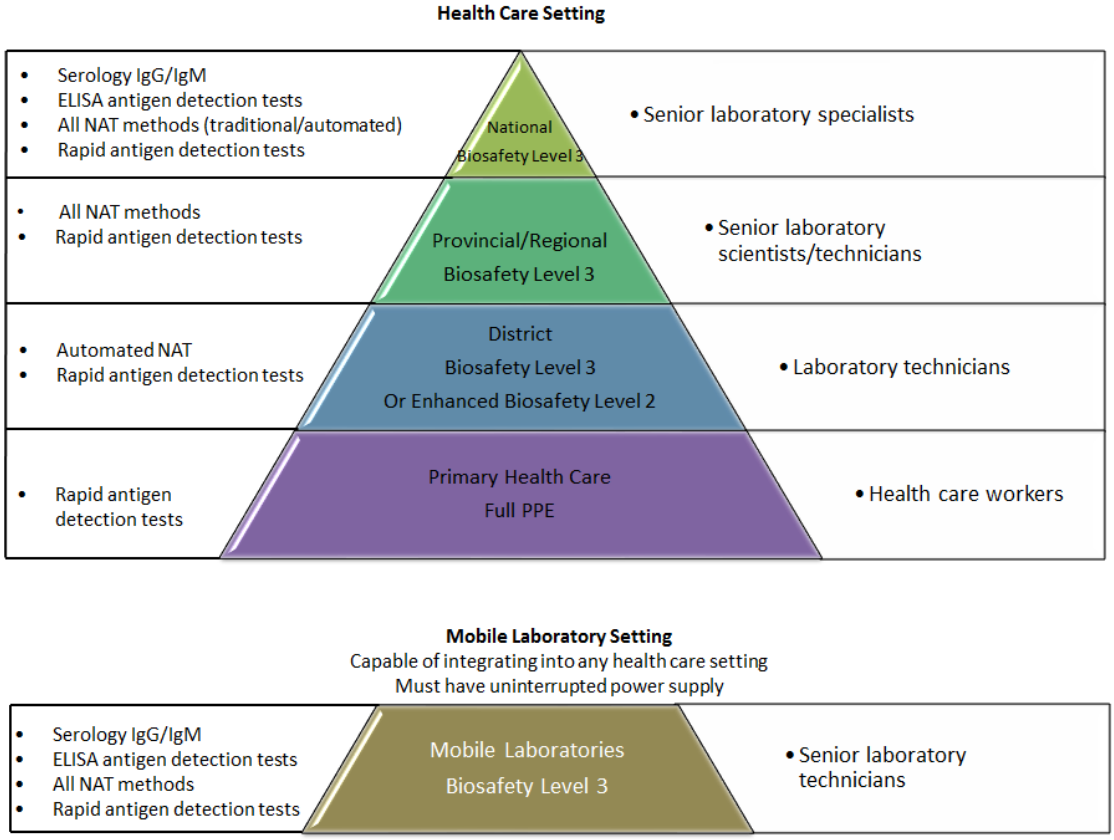

Figure 1. Diagnostic assays available in different settings

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 (EUA) authority. This risk based authorization is time limited and includes restrictions to the use of the IVD. With some authorizations such as the US FDA EUA, restrictions may extend to who can perform the test and/or where it can be performed and obligations for the manufacturer to report performance problems (see Annex 2).

In response to the need for quality assured IVDs for EVD, WHO has also developed an emergency use assessment and listing procedure (EUAL). The EUAL procedure assesses if there is sufficient evidence to demonstrate that the benefits of using the IVD for EVD outweigh the foreseeable risks in the context of the current outbreak. The EUAL adopts many FDA EUA requirements but has further assessment steps, including verifying test sensitivity (LoD) by independent testing, and assessing manufacturing capacity. As for FDA, WHO listing also obliges the manufacturer to report performance issues.

Given the consequences of misdiagnosis, WHO highly recommends that only diagnostics that have undergone independent, comprehensive assessment of quality, safety and performance are used in diagnosing infection with Ebola virus (see Annexes 2 and 3).

4. Considerations for the selection and use of Ebola IVDs

The following aspects should be considered when choosing appropriate IVDs for EVD. The order in which the characteristics are presented does not represent a prioritization listing since priorities will be context-specific.

General characteristics of the test

These may include:

- Performance
  - The ability of the assay to correctly identify all infected individuals in different stages of the disease. Where clinical trial data are not available analytical sensitivity (limit of detection) may be used as a general indicator of clinical sensitivity
  - The ability of the assay to correctly identify all non-infected individuals (diagnostic specificity)
- Targets
  - The spectrum of analytes which can be measured (e.g. if the diagnostic detects only Zaire strains of Ebola, or other Ebola species too)
  - Susceptibility of the assay to genetic changes of the organism over time period (genetic drift) for example whether nucleic acid detection tests target one or two genes
- Test procedure controls
  - Presence of appropriate test procedure controls supplied with the test kits to ensure that each step of the testing process has performed correctly.
  - For lateral flow devices, the presence of a control line and provision of positive and negative controls by the manufacturer,
    - For nucleic acid detection assays, decontamination controls, Internal controls (added to every specimen), provision of positive and negative controls ,
    - For automated nucleic acid detection assays, processing and amplification controls, and sample adequacy controls
    - Availability of external Quality Assessment (proficiency) Control specimens (provided by a party other than the manufacturer)
- Transport and shelf-life
  - Clearly defined specimen storage and transportation requirements
  - Shelf-life and storage conditions for all reagents supplied or required for the test
- Read-out
  - The time required (including specimen processing) to obtain results
  - Whether results are subjective and transiently available versus objective and permanent
  - Whether results are qualitative or quantitative
  - Connectivity of the technology including remote communication for data transfer.

Anticipated use

When considering the type of assay to be selected for use, consideration should be made of the following:

- Setting of intended use, taking into account
  - Whether centralized or decentralized testing is preferred
  - The minimal qualifications of the users and training needs e.g. whether staff with molecular biology expertize are needed to operate the test; for RDTs training in performing the test and training and practical experience in PPE are needed
  - The minimum number of specimens which can be tested per time period (e.g. per day or shift)
  - The need for instrument(s) to conduct the testing; whether such equipment is in place, or whether new instrumentation is needed
- Intended use of the assay
  - Clear understanding of the intended use of the tests including whether there is a need to undertake confirmatory testing

- Antigen detection assays in general are less sensitive or specific compared to NAT, although both can be used to establish the laboratory diagnosis of EVD
- As incidence and prevalence of the disease continues to decrease, assays with higher specificity are required. In the current epidemiological context, widespread use of RDTs will generate more false positives than true positives, (see Annex 1 for an explanation). Widespread use of RDTs is therefore not recommended. Operational research is however encouraged to investigate strategies to address this issue.
- Need to detect markers for other related infections.
- How EVD testing will be used in the context of diagnostic testing for fever patients. Except in areas of ongoing EVD transmission, other causes of fever, such as malaria, will be more likely.
- Because of the overlapping symptomatology between Ebola and malaria, and until the Ebola outbreak is declared officially over, WHO's current guidance on use of malaria RDTs in Ebola affected areas is to suspend their use UNLESS they are performed with PPE[4].

Infection prevention and control

Based on the fact that Ebola viruses are associated with a high case-fatality-ratio, specific steps must be taken to minimize chance of acquiring the disease during the testing process. This should cover the process of specimen collection, preparation, storage and waste disposal. Aspects to consider include:

- Whether manual specimen manipulation is required e.g. centrifugation and plasma separation
- Whether testing must occur in a setting of high biosecurity level equipment.
- Whether venipuncture is required to collect the specimens (venous whole blood, serum or plasma) or if fingerstick testing may be performed
- The need to decontaminate equipment after use
- Need for appropriate disposal of laboratory wastes
- If pipettes are required, plastic pipettes rather than glass pipettes should be used.

Regardless of the setting of use, testing should always be performed under strictly applied universal biosafety precautions by trained and properly equipped personnel. During an Ebola epidemic, health workers should be adequately equipped and trained in both performance and disposal of the test and donning and doffing of personal protective equipment.

Infrastructure requirements

Infrastructure factors which should be considered may include:

- The dedicated space required for the instruments including the need for reinforced benches
- Design of laboratories to prevent potential contamination from high titre specimens or amplification products.
  - Conventional PCR methods usually require separated laboratory rooms or space for the different steps of the process (extraction/amplification)
  - Fully automated system may use different compartments inside the instrument, obviating the need for physical separation of the steps.
  - Availability of glove boxes or biosafety cabinets
- Other requirements in this context are additional instruments needed for assay performance (e.g. centrifuges) or need for storage of assay reagents and specimens (e.g. refrigerators).
- Availability of electricity, as a number of the EVD diagnostics requires an uninterrupted power supply. Electricity is also required for air conditioning of rooms to prevent overheating of some instruments and for refrigeration of some reagents. Some dedicated technologies and point-of-care technologies can run on direct current such as portable car batteries.

Cost

Regarding the cost of the test, consideration should include cost of reagents, equipment and accessories that are not supplied with the kits. Other factors include:

[4] 'Guidance on temporary malaria control measures in Ebola-affected countries', Geneva, World Health Organization, November 2014. Available online at http://www.who.int/malaria/publications/atoz/malaria-control-ebola-affected-countries/en/

- the cost of PPE, and waste disposal and management requirements
- the cost of maintenance of instrument and the requisite infrastructure,
- the cost of servicing and maintenance of the diagnostic
- the cost of labour (hands-on times for specimen collection, transport, test performance etc)
- Simplicity of supply and procurement, e.g. local support capacity of vendor or representative; number and type of accessories or reagents not provided in the kit.

Regulatory status

As noted in section 3, it is especially important to know if the claims of quality, safety and performance made by the manufacturer have been verified by a stringent independent assessment. Where regulatory approval exists, it is important to understand what assessment has occurred for the approval to have been given. Conditions may apply, such as restrictions on the use of the diagnostic, including who can perform the test and where it can be performed.

5. Fact sheets of each WHO EUAL-listed IVDs and US FDA EUA-listed IVDs

The Fact Sheets are available on the WHO website. This is to facilitate updates as needed, whilst the core document can remain unchanged. Please refer to Annexes 2 and 3 for these documents.

It is important to note that there is no priority implied by order of tests listed and also that the list will grow as new tests are developed and evaluated.

6. Criteria to take into account in setting up an EVD IVD testing laboratory network

An EVD laboratory test should be used within the context of a laboratory network system. Additional criteria need to be taken into account by decision makers when setting up a diagnostics network (as distinct from decision-making for the individual laboratory), making it as useful as possible for other diseases , not just Ebola, including technical input to decision-making, and consideration of national/regional surveillance.

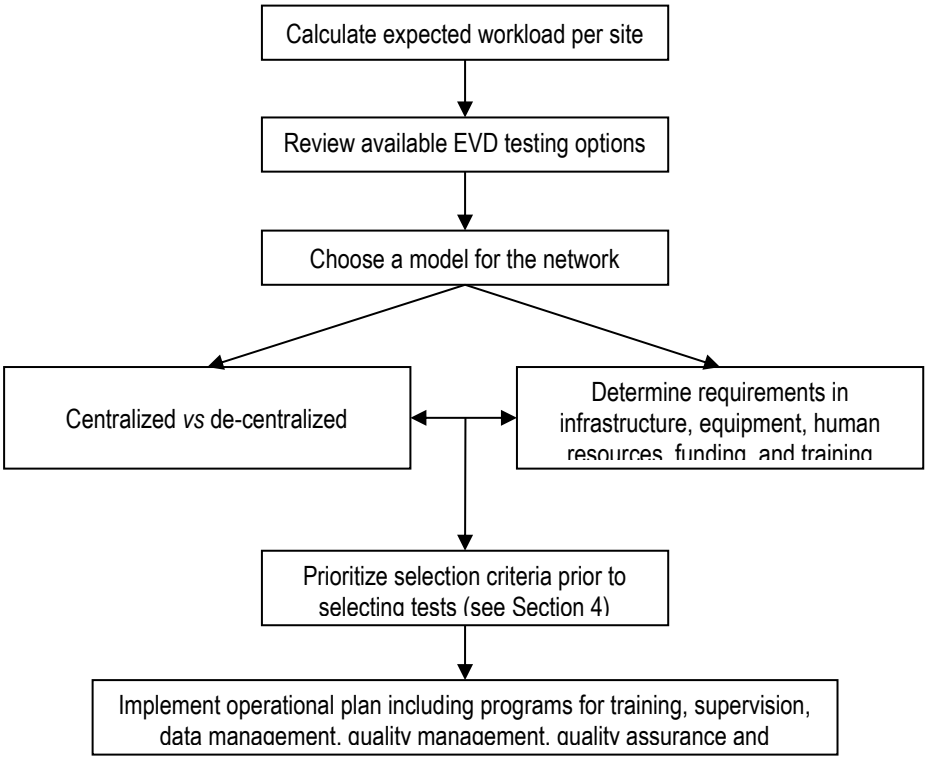

Figure 2. Criteria to take into account in setting up an EVD IVD testing laboratory network