



## Consultation on the documentary evidence and independent performance evaluation requirements for the Emergency Use Assessment and Listing procedure for Zika virus in vitro diagnostics

Geneva, 14-15 March 2016

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### Consultation background

The recent increase in reported cases of microcephaly and Guillain-Barré Syndrome potentially associated with Zika virus (ZIKV) has highlighted the urgent need to identify individuals infected with ZIKV. In order to do this, in vitro diagnostics (IVDs) of assured quality, safety and performance are required. Therefore, on 5th February, 2016, WHO opened the Emergency Use Assessment and Listing (EUAL) procedure for IVDs for ZIKV in order to determine their eligibility for procurement by WHO and other partners. The EUAL procedure takes into account that many IVDs are not yet commercially available and as such allows for an assessment decision based on a minimum data set provided by the manufacturer.

The EUAL assessment process includes the following sequential steps:

- Step 1:** review of the manufacturer's quality management system documentation;
- Step 2:** review of the documentary evidence of safety and performance, including labelling and product performance, and associated verification and validation studies;
- Step 3:** performance evaluation of limited scope to verify analytical and clinical performance characteristics.

The purpose of the consultation was to identify and formalise the minimal technical requirements required for the documentary evidence (refer Step 2) and to invite expert review of the WHO protocols for the performance evaluations (refer Step 3). Additionally, the consultation provided advice to WHO on how to best leverage available evidence of product safety and performance through an abbreviated EUAL assessment.

Finally, it was noted in the Ebola outbreak that national regulatory processes were sometimes bypassed for import of IVDs in response to the outbreak. The outcomes from this consultation should therefore be useful for national regulatory authorities (NRAs) from the affected region to learn about the WHO EUAL and consider leveraging the efforts undertaken under this mechanism.

## **Objective 1 - to finalise the minimal technical requirements and acceptance criteria for the documentary evidence submitted to the EUAL for Zika virus IVDs**

It was agreed that the FDA requirements be adopted with the following extra requirements, that take into account the broad “jurisdiction” of WHO and also the evolving knowledge of the virus and its associated clinical conditions.

It was agreed that for manufacturers of NAT reagents that may be run using different extraction and amplification platforms, that the documentary evidence includes sufficient information of the performance of these reagents from initial steps of extraction of RNA to generation of results and interpretation. These platforms should be noted in the product instructions for use.

WHO requirements, in addition to FDA requirements include:

1. For NAT assays, additional specimen types (for example, urine, amniotic fluid, foetal tissue, semen, saliva, CSF) can be accommodated as more is known about ZIKV pathology but limit of detection (LoD) must still be determined for each different specimen type.
2. For NAT assays, WHO will accept manufacturers using Probit analysis to calculate the 95% percentile.
3. For a multiplex NAT assay (for example, Zika/dengue/chikungunya) there is the need to determine the LoD of each viral target claimed.
4. For antigen (Ag) detection assays, LoD should be established with live and inactivated material, as well as the recombinant target material. After equivalence has been established, other analytical studies may then be undertaken using only the recombinant material.
5. For serology (e.g. Ab, Ag), precision data (repeatability/reproducibility) must be submitted.
6. For NAT assays, when undertaking cross-reactivity of yellow fever virus (wet testing required), it is only necessary to test either the vaccine strain or native wild virus.
7. FDA requirements state that cross-reactivity should be tested in specimens CHIKV, DENV, yellow fever virus (YFV), West Nile virus, St Louis encephalitis, and Japanese encephalitis. The following organisms should also be tested (in silico analysis at a minimum) for cross reactivity:
  - a. Rocio virus,
  - b. Ilheus virus,
  - c. Iguape virus
  - d. tick borne encephalitis
  - e. Mayaro virus.

8. For serology IgM assays, specimens to test for interference with yellow fever virus (YFV) may be sourced from individuals vaccinated for YFV.
9. WHO will require each assay to be calibrated/tested against international standards (IS) (biological reference material) as they become available:
  - a. WHO-supplied ZIKV interim International Standard for NAT assays, estimated availability April-May 2016. This interim standard may be used by assay developers before final status of IS attained. (For those manufacturers who have already submitted to the EUAL, calibration studies with this standard are still required and results need to be submitted to the EUAL as soon as possible).
  - b. PEI-supplied Chikungunya virus (CHIKV) reference standard for NAT assays, estimated availability in 2017.
  - c. FDA/CBER-supplied dengue virus (DENV) 1, 2, 3, and 4 reference standard for NAT assays, available now.
  - d. NIBSC-supplied reference standard for DENV antibody (serology), available now.
10. Clinical specificity
  - a. For NAT and serology assays, testing of populations outside of the Americas is acceptable, but must include at least 25 (NAT) / 50 (serology) specimens from pregnant women (commercial panels are available).
  - b. For serology assays, specimens from patients with both primary and secondary DENV infection should be tested, acknowledging that these specimens will be difficult to source.
  - c. For serology assays, use paired acute and convalescent phase specimens from DENV infections when possible.
  - d. For NAT assays, contrived specimens may be used, but the dilution matrix must be derived from the settings of the intended use population (the Americas, as well as returning travellers).
11. For serology assays developed using viral lysate to bind antibody, if a cell line of human origin is used, the manufacturer should investigate possible interference from human leukocyte antigen (HLA).
12. Establishing performance:
  - a. Manufacturers should attempt to demonstrate performance with different strains, if possible by using specimens sourced globally.
  - b. Inactivation of sample by heat inactivation (serology) or lysis (NAT) may be considered.
  - c. For serological assays, when assigning clinical truth for all specimens, optimally specimens obtained from patients with seroconverting illness should be used (i.e. from patients with a pattern of nucleic acid detectable only for early bleeds with results of serial bleeds demonstrating the rise of antibodies (in the absence of nucleic acid). Acute and convalescent phase specimens should be chosen and ideally

tested in parallel. In the absence of such specimens, verification of ZIKV status should be confirmation using PRNT for ZIKV, DENV, CHIKV, and West Nile virus. Evidence of effectiveness of this testing strategy should be demonstrated by verification of the PRNT using rabbit immune serum.

## **Objective 2A - to agree on the protocol for the evaluation of NAT based assays for Zika virus RNA**

### **Benchmark assay:**

The benchmark assay selected for the evaluation of NAT assays was the CDC RT-PCR protocol, authorised on 17 March 2016 by the US FDA<sup>1</sup>, using primers and probes as described in Lanciotti et al (2008)<sup>2</sup> spanning the region 1086-1162. All participating laboratories must use the same protocol and source the same reagents and instrumentation for the benchmark assay.

### **Limit of Detection (LoD):**

Verification of LoD will be carried out with the same material for all assays under evaluation with the aim of achieving comparability of results. Preferably, the interim international standard under development at the Paul Ehrlich Institute (PEI) on behalf of WHO will be used. The testing must also include the extraction step. The experiment will be as follows:

- log step dilution series of cell culture supernatant spanning concentrations around the expected LoD of the assay under evaluation will be spiked in the claimed specimen of intended use. A minimum of 2 replicates of each dilution concentration will be tested.
- In a second experiment, 0.5-log step dilution series spanning the LoD will be tested with a minimum of 5 replicates.
- The LoD will be the lowest concentration that can be consistently detected in >95% of specimens tested under routine conditions.

Alternatively, results from the WHO/PEI Collaborative Study to develop the interim international standard may be considered.

### **Clinical sensitivity:**

- Will be determined by comparison of results to the benchmark assay.

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<sup>1</sup> FDA Emergency Use Authorisation , accessed 24 March 2016.

<http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm182568.htm>

<sup>2</sup> Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, Stanfield SM, Duffy MR. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis.* 2008 Aug;14(8):1232-9. doi: 10.3201/eid1408.080287.

- A total of 50 positive specimens will be required (minimum of 25 clinical and the rest can be contrived specimens in the claimed specimen matrix).
- The contrived specimens, if used, should be prepared by spiking live or inactivated virus into negative clinical matrix from individual patients (50% at or near the LoD and the remaining 50% across the detection range of the assay).
- Two by two tables will be constructed.
- 95% confidence intervals will be provided.

**Analytical specificity (cross-reactivity):**

A minimum of 30 specimens, ideally 50 specimens should be used. These may be contrived. These will include:

- Clean negative specimens
- ZIKV negative, DENV positive (1,2,3,4)
- ZIKV negative, yellow fever vaccine strain positive
- ZIKV negative, CHIKV positive

**Specimen matrix:** the evaluation will be limited to the specimen matrix claimed by the manufacturer but cannot be carried out on all of them. Serum/plasma and urine will be the priority.

**Discrepant results:** Resolution of discrepant results was not discussed. A suggestion will be made in the protocol and distributed to participants for comment.

**Objective 2B - to agree on the protocol for evaluation of serology assays for Zika virus**

- There will be specific serum/plasma panels for both the IgM and IgG assays.
- The panel will be characterized using the following assays where applicable CDC Zika IgM assay (MAC-ELISA), ZIKV, DENV/YF PRNT and ZIKV DENV/YF NAT assay for IgM panel. For the case of IgG panel in absence of Zika IgG assay, specimens should be tested with PRNT.
- Each WHO Zika positive panel will consist of minimum 50 specimens from individuals with laboratory evidence of Zika seroconversion (detection of both RNA and Antibodies The panel should include at least 30% of the specimens which are dual Zika and Dengue positive. When the number of specimens from individuals with laboratory evidence of Zika seroconversion is not adequate, then other specimens characterized using the assays mentioned above will be included.
- The Zika negative specimen panel will include a minimum of 100 specimens ( $\geq$  50 specimen- Zika negative only,  $\geq$  30 Zika Ab negative specimen + Dengue Ab positive and  $\geq$  specimen 30 Zika Ab negative + Yellow fever Ab positive) .
- WHO will repeat the index test if there is a suspected false negative result which may have implications on volumes required.

- FioCruz will confirm whether they can assist as they already have a substantial quantity of appropriate specimens but noted that further recruitment (double) is required for larger volumes and the logistics required. Currently recruit 10/week.
- Erasmus lab can assist in providing follow up clean samples without Dengue background from returning travellers and can contribute to ZIKV convalescent panel but would like an indication on the volume required –to see whether this is feasible – 10 ml was suggested as an optimum volume by WHO.
- CDC PR can assist with specimens as they can collect a set of paired convalescent serum/plasma samples. They are not available to assist immediately but open to participate and share when time comes.
- Several laboratories indicated their willingness to participate in the evaluation of assays.
- PAHO is willing to facilitate the coordination of transportation of Zika specimens to the laboratory which will conduct the laboratory evaluation once a particular country has agreed to contribute to the evaluation panel based on the country specific regulations.
- FioCruz has ethical clearance for a multicentre ethics clearance. They have just sent amendments to be able to collect urine. They have already published protocols, and are willing to make amendments.
- Regarding performance acceptance criteria there was a general consensus that it would be more practical for WHO to develop a guidance for countries on the requirements on specificity and sensitivity for various implementation settings.

### **Objective 3 - to agree on the circumstances for an abbreviated EUAL assessment procedure.**

It is proposed that where possible, the EUAL procedure will take into account equivalent activities performed by other bodies where possible to abbreviate the assessment. The following was agreed:

1. WHO will always undertake the review of the QMS and production capacity (Step 1 of the assessment)
2. Because of the alignment of FDA EUA and WHO EUAL documentary evidence requirements, if a product is authorized under the FDA EUA, there will be no requirement for WHO to assess those aspects in the documentary evidence submitted under the WHO EUAL. Only the few additional requirements of WHO will be assessed.
3. There was a general consensus in the meeting that it was of great value to undertake the performance studies using the same evaluation protocol and specimen panels, therefore there will not be the opportunity to accept other studies in place of the WHO-led laboratory evaluation.

## Acknowledgments

The following institutions' representatives participated in the consultation:

- Agência Nacional de Vigilância Sanitária (Anvisa) (Brazil);
- Centers for Disease Control and Prevention (CDC) (Porto Rico);
- Erasmus MC (The Netherlands);
- Federal Service on surveillance in healthcare (Roszdravnadzor) (Russia);
- Fundação Oswaldo Cruz (FioCruz) (Brazil);
- Institut Pasteur (France);
- Institute of Tropical Medicine (ITM) (Belgium);
- Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE) (Mexico);
- London School of Hygiene & Tropical Medicine (LSHTM) (United Kingdom);
- Pan-American Health Organization (PAHO);
- Paul-Ehrlich-Institute (PEI) (Germany);
- The National Institute for Biological Standards and Control (NIBS) (United Kingdom);
- UNICEF Supply Division (Denmark);
- U.S. Food and Drug Administration (FDA) (USA);
- World Health Organisation (WHO) (Switzerland).