

Recommended Additional EUA QC checks for Zika MAC-ELISA

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Current Plans

- 3 Phase approach to standardize the Zika MAC-ELISA
 - Phase 1: Distribution of instructions for additional primary (plate) and secondary (clinical sample) acceptance criteria
 - Goal – enhance precision and accuracy of the assay across all testing laboratories
 - Phase 2: Evaluation and distribution of Standardized Calibration Control Serum and an independent negative control
 - Timeline – September 2017
 - Phase 3: Distribution of a manufactured Zika-MAC-ELISA kit
 - Timeline – before the end of 2017

Requested follow-up

- Response required to eocevent278@cdc.gov for the following:
 - Acknowledgement of receipt of these additional recommendations within 5 working days
- In the future (within the next 4-6 weeks) we will be asking all labs to provide the mean P/N ratio and confidence interval (upper and lower range) of both the Positive control as well as the Negative Calibration control within 30 days

Definitions

- **Calibration Control Serum** - previously referred to as normal/negative control sera in the IFU, this is normal human sera that has tested negative for Zika antibodies. This material is represented as (N) in the plate layout presented in the IFU. The mean OD for this material reacted with the Zika Viral Antigen is the denominator of patient P/N calculations. When screening candidate serum samples for use as a Calibration Control, the ratio of the [mean OD of sample with Zika antigen] / [mean OD of sample with normal antigen] should be < 1.5 .
- **Negative Control** – a new addition in the May 3 version of the IFU, this is normal human sera that has tested negative for Zika antibodies and comes from a different lot of materials that is used for the Calibration Control Serum. This sera is run alongside patient specimens on each plate, treated the same way as a patient specimen.

Recommendations

- Establish mean, standard deviation and confidence intervals for controls (Positive control, Calibration Control Serum, Negative Control)
 - A minimum of 5 separate experiments should be used to establish mean and standard deviation
 - 99 % confidence interval (CI) approximates to 2.58 SD around the mean
 - Update limits until a minimum of 30 individual runs have been completed

Recommendations (cont)

- Plate (primary acceptance criteria)
 - OD of background wells should be checked for outliers ($>$ upper bound 99% CI of established values)
 - Outliers should be excluded prior to taking the mean to subtract from test wells
 - High outliers can potentially cause false positive results
 - In one CDC lab this has been seen approx. 1% of the time and can potentially be due to a bubble in the well
 - OD of Calibration Control Serum reacted with Zika viral antigen (Calibration Control) should be checked for high Coefficient of Variation ($CV = SD/mean$)
 - If Calibration Control CV is greater than 60% plate should be rejected
 - Observed within CDC laboratory and can potentially cause false negatives due to an increased threshold

Recommendations (cont)

- Plate (primary acceptance criteria) cont.
 - The mean OD of both the Calibration Control Serum and the Positive Control should be within 99% of the upper and lower bounds of the pre-established values.
 - Reject plate if not within 99% CI
 - Observed within CDC laboratory; depending on which direction this could result in false positives or false negatives
 - The positive control P/N ratio should be within 99% CI of the upper and lower bounds of the pre-established values.
 - Reject plate if not within 99% CI
 - Observed within CDC laboratory; depending on which direction this could result in false positives or false negatives
 - The negative control P/N ratio should be less than 2.0 and within 99% CI of the upper and lower bound of the pre-established values.
 - Reject plate if both requirements are not met.
 - Since this sample is a known negative any positive result should trigger a rejection

Recommendations (cont)

- Clinical sample (secondary acceptance criteria)
 - If the CV of triplet ODs for the Clinical Sample with Zika antigen is $>30\%$ and the range of these ODs (i.e., OD range 0.1-0.4) spans 2 times the mean OD of the Calibration Control Serum reacted with Zika viral antigen (Negative/Equivocal threshold) (i.e., OD of 0.2), the mean Clinical Sample OD (i.e., OD of 0.22) may move across this threshold and cause a false positive result.
 - Reject sample if this occurs.
 - A high CV can mean that there is at least one outlier within the triplicate OD. If this is near the threshold then the outlier could potentially alter the specimen result.

Recommendations (cont)

- Clinical sample (secondary acceptance criteria)
 - If the sample interpretation is Negative, but the ratio of the [mean clinical sample OD with Zika antigen] / [mean clinical sample OD with normal antigen] is > 2 , the result should be reviewed to make sure it is not a false negative due to either an outlier Calibration Control Serum OD (CV $>60\%$) or an outlier in the clinical sample ODs
 - In populations that are naïve to Flavivirus infections this may be an indication of possible false-negative, but in areas with endemic Flavivirus exposure it may be more common in the true negative population.