



Accurate Detection of Zika Virus in Semen using Aptima® Zika Virus Assay on the Fully Automated Panther® System



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BACKGROUND

- ❑ Zika virus (ZIKV), causes the infectious disease Zika fever, and is a positive-sense RNA virus
- ❑ ZIKV
 - Is transmitted by mosquito-bite and also by Sexual intercourse (up to 41 days after onset of initial symptoms)
 - Has been detected in semen over 188 days post infection, much longer than the initial symptoms
 - RNA can persist in the semen after its disappearance from blood
- ❑ Clinical labs test ZIKV in frozen or refrigerated serum and/or urine. Its detection in semen samples are done on fresh or frozen specimens. However, to the best of our knowledge there is no commercially, FDA cleared test available for use with Semen
- ❑ To mitigate exposure, a validated clinical assay designed to detect ZIKV in semen should be implemented to the infectious screening performed for potential fathers through natural conception or ART

OBJECTIVE

To evaluate Aptima® ZIKV assay on the fully automated Panther system from Hologic, Inc. off-label to detect ZIKV RNA from semen samples

MATERIALS AND METHODS

- ❑ **Patients and Specimen:**
Semen samples were collected from a total of 207 male patients.
The samples were tested neat for ZIKV. Following their negative result, semen spiked with SeraCare Life Sciences AccuPlex™ recombinant ZIKV
-107 neat semen samples spiked at 3 viral copies/ml run a total of 238 times on 6 days.
- Another set of 100 semen were spiked with 5 viral copies per ml and added to Aptima® Urine Specimen Transport Tubes off label and tested immediately. The experiment was repeated three times (3x100=300)

- ❑ **Limit of Detection:**
The Limit of Detection (LoD) was determined by testing semen specimens spiked with a recombinant Zika Accuplex RNA from SeraCare. We tested 3, 5, 10, 15 and 20 viral copies per mL and 24 replicates of each target level on 3 different days.
- ❑ **Semen Processing:**
We spiked negative semen with SeraCare Life Sciences AccuPlex™ recombinant ZIKV and mixed with Aptima Urine Transport Medium at the ratio of 1:1 (2 mL of semen was added into Aptima Urine Specimen Transport Tube, which contained 2 mL of urine transport medium). Testing of neat semen spiked with recombinant ZIKV was also done.
- ❑ **Aptima® Urine Specimen Transport Tubes:**
The Aptima® Urine Specimen Collection tube from Hologic, Inc. is intended to be used for the collection and transport of male or female urine specimens to be used with Aptima assays. After collection, the processed specimens can be transported at 2°C to 30°C and stored at 2°C to 30°C until tested.
- ❑ **Aptima® ZIKV assay:**
The Aptima® ZIKV assay was released under the FDA's emergency use authorization on the fully automated Panther system from Hologic, Inc.,. It is a transcription-mediated amplification test, was used off-label to detect ZIKV RNA from semen samples. The assay was intended for the qualitative detection of ZIKV RNA in human serum, plasma and urine specimens. The assay incorporates an internal control (IC) to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.
- ❑ **Cross Reactivity:**
Sera from individuals seroconverted for West Nile Virus, Chagas and Dengue were tested along with recombinant Dengue Virus, recombinant Chikungunya Virus, Human Papilloma Virus, Herpes Simplex Virus 1, Herpes Simplex Virus 2, Chlamydia, and Gonorrhea were tested for cross reactivity.

RESULTS

- The 5 viral copies per mL was chosen as the Limit of Detection (LoD) for this study following the evaluation of 3, 5, 10, 15 and 20 copies.
- At 3 viral copies, there were 7 false negatives, but upon repeating 4 came positive

- ✓ Five negative control semen samples were run in each experiment all were negative, and none of the controls tested showed reactivity with ZIKV assay
- ✓ Neat semen is often viscous and some have gelatinous filaments that blocks the pipetting or interfere with the panther fluidics, false negative samples when rocked for five minutes and retested some became positive

Sample type	Count	Positive	Negative	Agreement %
Semen w Zika @ 3 copies / ml of neat ejaculate	238	231	7	97.05
Semen w Zika @ 5 copies / ml of processed ejaculate in Aptima Urine transport Kit	300	300	0	100

CONCLUSIONS

- ❖ The assay was found to be 100% sensitive and 100% specific to ZIKV RNA from semen samples at 5 viral copies per mL of semen
- ❖ Based on our study it is now possible to detect ZIKV reliably from semen
- ❖ ZIKV screening for all semen samples prior to ART procedures, in sperm banking and couples at risk of Zika attempting natural conception is highly recommended

REFERENCES

- ❖ <https://www.cdc.gov/zika/index.html>
- ❖ <http://www.hologic.com/products/clinical/diagnostics/asays-and-tests/aptima-zika-virus-assay>
- ❖ http://www.hologic.com/sites/default/files/packagage%20in%20serts/502260-IFU-PI_002_01.pdf
- ❖ <https://www.seracare.com/products/controls-and-reference-materials/molecular-reference-materials-ruo/accuplex-zika-reference-material/>
- ❖ <http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCM/uses/ucm494615.htm>

ACKNOWLEDGMENTS

Hologic, Inc, Marlborough, MA 01752 USA; Sera Care Life Sciences, Milford, MA 01757; Dr. Charles E. Miller, Advanced IVF Institute, Naperville, IL; Reproductive Medicine Institute, Oakbrook, IL