

**OLB20**

**2-hour Oral Session**

**Late breaker session: Other**

### **Analytical Sensitivity and Specificity of the VERSANT Zika RNA 1.0 Assay (kPCR)**

Hong Huang\*<sup>1</sup>, Tom Battersby<sup>1</sup>, Albert J Chmura<sup>2</sup>, Anita Lal<sup>2</sup>, Ashleigh Miller<sup>1</sup>, Joern Mosner<sup>2</sup>

<sup>1</sup>*Siemens Healthcare Diagnostics, Berkeley, United States*

<sup>2</sup>*Siemens Healthcare Diagnostics Inc., Berkeley, United States*

**Background:** Zika virus (ZIKV) is a mosquito-borne virus of the family Flaviviridae related to yellow fever, dengue and west Nile viruses. ZIKV was first isolated in 1947 from Rhesus macaques in Uganda. The first ZIKV outbreak outside Africa and Asia occurred in 2007 in Yap Island (Federated States of Micronesia) and the largest one from October 2013 to March 2014 in French Polynesia, Pacific. Clinical presentation of ZIKV infection is non-specific; common symptoms include rash, fever, arthralgia, myalgia, asthenia and conjunctivitis. Patients usually report mild symptoms and some are asymptomatic. The WHO recently declared that the recent cluster of microcephaly cases and other neurological disorders reported in the Americas, where an outbreak with ZIKV is ongoing, constitutes a Public Health Emergency of International Concern.

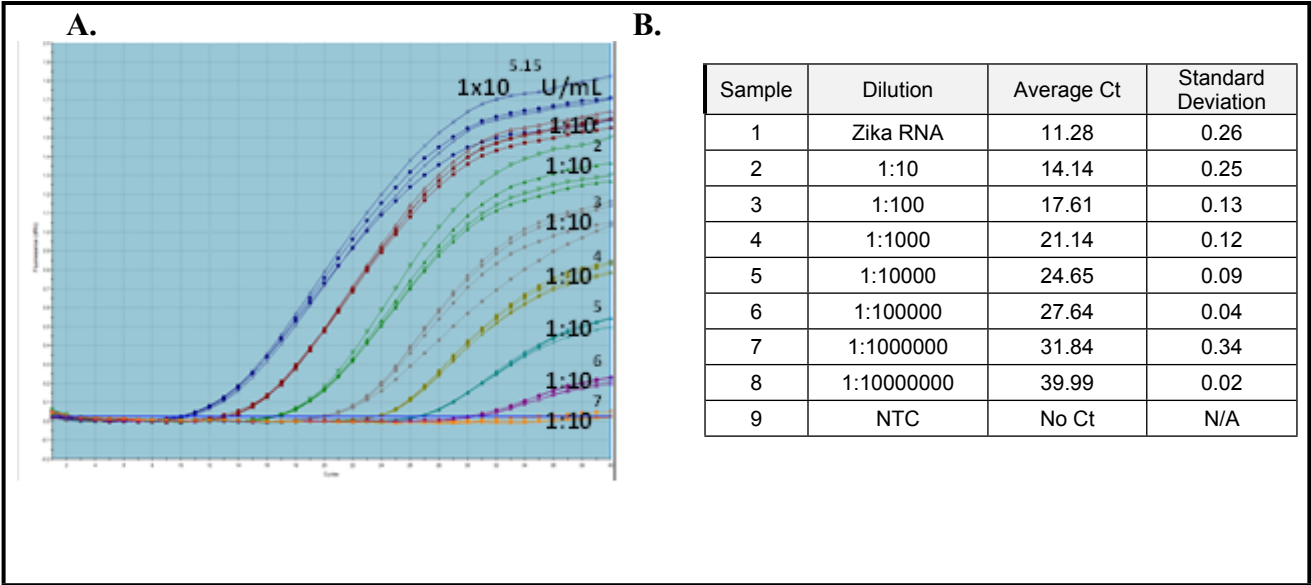
Laboratory ZIKV diagnosis is challenging because there is no “gold standard” diagnostic tool. We present the analytical sensitivity and specificity of a qualitative diagnostic real-time PCR assay, the VERSANT® Zika RNA 1.0 Assay (kPCR).

**Material/methods:** The VERSANT Zika RNA 1.0 Assay (kPCR) qualitatively detects ZIKV RNA. ZIKV RNA is extracted with either the QIAamp Viral RNA Kit (Qiagen) or the Siemens VERSANT Sample Preparation 1.0 Reagents. Ten microliters of RNA was amplified on either Siemens' VERSANT AD Module, ThermoFisher's QuantStudio5™, BioRad's CFX96 Q™ Thermocyclers and AppliedBiosystem's 7500 Fast Real-time PCR

Inclusivity of the assay was tested in silico using a BLAST search. Specificity of the assay was evaluated by testing RNA from dengue, yellow fever, chikungunya and west Nile viruses. Analytical sensitivity of the assay was evaluated by testing 10 fold serial dilutions of the ZIKV culture ( $1 \times 10^{5.15}$  U/mL, determined by TCID50 Endpoint Dilution Assay by ZeptoMetrix Corporation).

**Results:** The sequence of the VERSANT Zika RNA 1.0 Assay (kPCR) amplicon showed a match with 95% of published Zika sequences. ZIKV cultures (titer  $1 \times 10^{5.15}$  U/mL) showed positive amplification and had a Ct of 11 while negative ZIKV samples showed no amplification. Positive amplification was detected up to a  $1:10^7$  dilution of ZIKV RNA (Figure 1) indicating that the assay was very sensitive. The assay was negative with other family Flaviviridae viruses.

Figure 1: Analytical Sensitivity of the VERSANT Zika RNA 1.0 Assay (kPCR): PCR growth curve (A) and the associated Ct (B).



**Conclusions:** The VERSANT Zika RNA 1.0 Assay (kPCR) qualitatively detects ZIKV RNA. This assay recognizes a broad spectrum of published ZIKV RNAs in silico, has high analytical sensitivity and is specific to ZIKV among the Flaviviridae viruses.