Comparing Detection Methods for the Zika Virus: Saliva and Blood

Author: Chelsey Tiger   
Major: Biological Sciences  
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

**Key Words:**

Zika Virus, Zika, World Health Organization, arbovirus, detection methods

**At the time of this study in 2015, there was no set detection method best used for detecting the Zika Virus (ZIKV). An infant who was suspected of being infected with the Zika Virus was tested via a blood sample, but after the result came back negative, physicians tested the same infant again, but using a sample collected from saliva. The Zika Virus was positively tested from the saliva sample, which prompted a team of scientists to conduct an experiment to compare the detection methods between saliva and blood to find the most accurate detection method [1].**

**Those researchers found that the Zika Virus was detected more frequently in saliva than in blood, but had a shorter window of time for detection. The conductors of this experiment suggest that saliva is an effective and noninvasive source for sampling in neonates and when blood is not available, but using both blood and saliva would lead to the most accurate testing result. [1]**

**Introduction**

The Zika Virus was first discovered and isolated in Uganda in 1947. Since then, the arbovirus stayed in the African region with rare and sporadic cases until it was found on Yap Island in 2007. Before the current outbreak in Brazil, the largest Zika Virus epidemic occurred in French Polynesia between March 2013 and October 2014. The virus has since spread to South and North America, where it has been linked to cause microcephaly in fetuses whose mothers are infected. The Zika Virus closely resembles dengue fever, with symptoms including conjunctivitis (red eyes), fever, arthralgia (joint pain), myalgia (muscle pain), asthenia (weakness or loss of energy) and rash. [1] However, due to the severity of the microcephaly cases, the World Health Organization has called the virus a “public health emergency” as of February 1, 2016. [2]

At the time of this study, the main method of detection was from a blood sample which was tested for the virus through real time reverse transcription PCR (ZIKV RT-PCR). There were reports of people who showed Zika Virus symptoms from the French Polynesia outbreak, but when tested for the virus with RT-PCR from samples collected from blood, the results were negative. However, when the patients were tested using RT-PCR from a saliva sample instead, the results came back as positive. This prompted this team of researchers to conduct a study on saliva samples for RT-PCR and from blood. [1]

**Recent Progress**

Between the months of October 2013 and March 2014: 1,067 samples were collected from 885 patients exhibiting Zika Virus symptoms. Out of these samples, 748 were samples collected from blood, 319 were samples collected from saliva. Out of the 885 patients, 182 had both blood and saliva samples collected at the same time. Additionally, a questionnaire was given to most of the patients that outlines their symptoms and the number of days from initial symptom onset. The blood samples were collected intravenously, while the saliva samples were collected from a cotton swab in the patient’s mouth. [1]

Using the NucliSENS® easyMAG® System (BioMerieux), RNA was extracted in order to detect ZIKV in the saliva samples. NucliSENS® easyMAG® System is a system that extracts nucleic acid from saliva, in this case, it was used to extract RNA. [3] The RNA was extracted when the cotton swab taken from the saliva samples were vortexed with 2 milliliters of lysis buffer, then eluted with 50 microliters of elution buffer, leaving RNA isolated. 5 microliters of the RNA was then used for amplification and then detected using real time reverse transcriptase. [1]

182 out of 319 (57.1%) of saliva samples tested positive for the Zika Virus, while 210 out of 748 (28.1%) tested positive for the virus in the blood sample. For the 182 patients who were tested from both blood and saliva, thirty-five patients tested positive for the virus (19.2%) in saliva but negative from the blood. Sixteen patients (8.8%) tested positive in blood but negative for saliva. [1] This difference between the 19.2% who tested positive in saliva but negative in blood and the 8.8% who tested positive in blood but negative in saliva is statistically significant as found using the McNemar test (p=.0017). [1]

Of the 137 patients with only saliva samples, 95 tested positive for ZIKV, with significant data collection at 3 days following the onset of symptoms in the patient, meaning the most positive test results were yielded when patients reported having symptoms of the Zika Virus for 3 days. Blood was detectable in the patients tested, however, up to 8 days after patients reported having symptoms of the Zika Virus. [1]

The researchers also compared symptoms between the patients who gave saliva samples and the patients who gave blood samples. The purpose of this is to test whether Zika Virus RNA was correlated to a specific clinical explanation. The researchers found that the symptoms were not statistically significant using the Chi-Square test (p=0.2889 for asthenia, p=0.8548 for fever, p=0.3054 for rash, p=0.5545 for conjunctivitis, and p=0.9054 for arthralgia). This suggests that regardless of sampling methods, the symptoms of the virus were the same. [1]

**Discussion**

With the new epidemic of the Zika Virus in South and Central America, it’s important to develop accurate detection methods in patients suspected of having the Zika Virus. The results of this study found that collecting saliva samples from people and then running real time reverse transcription PCR is an effective and noninvasive method of detecting the Zika Virus when blood samples are not available. It could also be used as a primary detection method for children. [1] The study found that the ability to detect ZIKV RNA in saliva was higher compared to blood, but when used together, blood and saliva gave the most accurate results for detection. [1] Saliva sampling is effective, cheap for developing countries, noninvasive to the patient (as compared to venous blood sampling), and the recommended detection sampling method for children and infants.

However, there is a third detection method that they did not test, urine sampling. Even though saliva sampling is an effective method for detection, it has a short window of testing time (within a week) compared to testing from urine samples. Blood and saliva samples are accurate for testing for the Zika Virus from a short onset of time (where all the patients in this study reported viruses within a week), but urine sampling is used for patients who have reported viruses after a week. A further study is needed to compare in depth how urine sampling compares to blood and saliva sampling. The timing window has separated the detection methods in this study. [1]

Since the World Health Organization declared the virus a “public health emergency,” it is imperative to conduct research to combat the virus and stop its spreading. More research is needed to learn more about this virus, which has recently been linked to causing microcephaly in infants, with more research being needed to figure out how this occurs, and what can be done to stop it. [2]

Even though an accurate detection of the Zika Virus is established, finding a cure for this virus has not yet been accomplished and is open to research. There is still no new information on the prevention for microcephaly in pregnant women infected with this disease and is still yet to be researched and discovered. As this virus has been declared a “public health emergency” by the World Health Organization [2], more research should be conducted on the epidemiology of this virus.

**References**

1. Musso, Didier, Claudine Roche, Tu-Xuan Nhan, Emilie Robin, Anita Teissier, and Van-Mai Cao-Lormeau. "Detection of Zika Virus in Saliva." *Journal of Clinical Virology* 68 (2015): 53-55. Web.

2. "Latest Zika Situation Report." *World Health Organization*. Web. 06 Feb. 2016.

3. “NucliSENS® easyMAG® Count the Ways” *Biomerieux.* Web. 06. May. 2016