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UROLOGY - REVIEW

Advantage of urine based molecular diagnosis of Zika virus

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Abstract Zika virus (ZIKV) infection is an emerging global health concern, and diagnostic recommendations are currently developing based on new information. Several case or small sample size studies using both urine and blood samples suggest that ZIKV RNA can be detected at higher levels and for a longer time after onset of infection in urine compared to blood. We recommend that urine and serum collection for molecular testing be a standard part of evaluating patients for ZIKV infection, and that urine is a good alternative testing sample when blood collection is problematic.

Keywords Zika virus · Urine biomarker · Molecular diagnostic · Zika infection

Introduction

Infection with Zika virus (ZIKV) is of growing concern since it is suspected with causing brain defects in newborns including microcephaly [1] and, more recently, having potential neurological and autoimmune complications, such as Guillian–Barré syndrome and acute disseminated encephalomyelitis (ADEM) [2, 3]. ZIKV is anticipated to spread throughout the United States and globally within the next year, as it is transmitted by infected *Aedes* mosquitos, which are present on all continents except Antarctica. ZIKV has also been reported to be transmitted sexually [4, 5].

ZIKV infection is characterized by mild fever, arthralgia, myalgia, headache, retroorbital pain, conjunctivitis, and cutaneous maculopapular rash. It is difficult to diagnose ZIKV infection based on clinical signs and symptoms alone due to overlaps with other arboviruses that are endemic to similar areas [6], and infection is asymptomatic in most (60–80 %) adult healthy patients [7, 8].

Methods

Search strategy and selection criteria

References for this article were identified through searches of PubMed for articles published from 1973 to 10 April 2016, by use of the terms "Zika virus", "ZIKV", "urine", "saliva", "semen", "diagnosis". Articles resulting from these searches and relevant references cited in those articles were reviewed.

Current Zika virus diagnostic criteria

ZIKV infection can also be misdiagnosed during the acute (viremic) phase because of nonspecific flu signs and symptoms. At this time, the Food and Drug Administration (FDA) has issued emergency use authorization for two commercial tests for ZIKV detection: the

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Table 1 Detection of ZIKV RNA in various sample types (adopted from [42])

	Detection (days) after symptom onset	Viral RNA load (copies/mL)	Reference
Blood	1–11	30–8.1 × 10e6	[13, 36–38, 43, 44, 48, 50]
Urine	2 to > 20; one report 29 days	30–2.2 × 10e8	[36–38, 43–46, 48]
Saliva	1–8; one report 29 days	90–3 × 10e6	[37, 48, 50, 54]
Semen	21–62	1.1 × 10e8–4 × 10e8	[4, 5, 51–53, 55]
Breast milk	3–8 after delivery	Up to 2.1 × 10e6	[37, 56]
Amniotic fluid	17–31 weeks gestation (4–18 weeks after possible maternal infection)	Not reported	[40, 41, 57]
Cerebrospinal fluid	Not detected, but recommended to be tested if obtained for other reasons	Not applicable	[45, 58]
Nasopharyngeal swab	Tested day 6 only	Not reported	[44]
Buccal	Not reported		

RealStar® Zika virus RT-PCR Kit (available from Altona Diagnostics) and the Zika virus RNA Qualitative Real-Time RT-PCR (available from Quest Diagnostic Tests). There are currently several diagnostic tests available for ZIKV. Quantitative reverse transcription PCR (RT-PCR) of ZIKV RNA in serum or plasma samples is the primary approach. However, this method can give a negative result as soon as 3–5 days after symptom onset [8, 9], which does not exclude ZIKV infection. IgM antibody detection by ELISA can be done 4 days after onset but cannot distinguish between ZIKV from Dengue Fever virus, Yellow Fever virus, and possibly to vaccines against flaviviruses [8, 10–12]. Plaque-reduction neutralization test (PRNT) can measure virus-specific antibodies, but cross-reactivity also exists.

Advantages of non-invasive sample collections

Urine, saliva, nasopharygeal, and/or buccal (cheek swab) testing are non-invasive and are an attractive sample for diagnostic testing in which blood collection can be difficult. This may include young children, neonates, elderly, patients with hemorrhagic syndromes, patient refusal, or patients who present with small, dehydrated, or elusive veins. Sample collection can also be done at field locations where trained medical personal or facilities may be lacking, and allow for self-collection within communities for surveillance or epidemiology studies during an outbreak. Lastly, ZIKV has been reported to be transmitted through blood donation [13].

Diagnosis of other viral infections in urine and saliva samples has been reported for human immunodeficiency virus (HIV), Hepatitis A, B, and C, and rubella [14–20], and more recently in other flaviviruses, West-Nile virus (WNV) [21–23] and Dengue (DENV) serotypes -1, -2, -3, and -4 [24–35]. WNV can be detected longer in urine than serum [21] and can be isolated from urine [22, 23]. Some studies also suggest that like WNV, DENV can be detected for a longer time in urine than saliva or serum [25, 29, 31, 32].

Urine may have a longer detection window for molecular diagnosis than serum or plasma samples

Kutsuna and colleagues first reported that ZIKV RNA could be detected by RT-PCR in the urine but not the serum of an infected patient [36]. ZIKV has also been reported to be detected in urine of adults [37–39] and at least one neonate [37]. It is also detectable in the amniotic fluid (comprising mostly of fetal urine) of fetuses with microcephaly or fetal brain abnormalities [40, 41]. The diagnostic utility of urine was best characterized in a more extensive study of six infected patients from a 2014 ZIKV outbreak in New Caledonia, from which urine and serum from the same patients at multiple comparable time points were investigated [38]. Importantly, ZIKV is detectable for a longer time frame post-infection in urine than serum (7 or more days after becoming undetectable in serum) [38] and at a higher viral load in urine (up to 220 × 10^6 copies/mL) than in corresponding serum samples (up to 8.1 × 10^6 copies/mL) [42]. Other groups have also reported either a longer detection window or an increased ZIKV detection in urine over other bodily fluids on a case study basis (Tables 1 and 2) [30, 37, 43–46], although there have been cases where ZIKV was detected in the serum but not urine [47]. A larger cohort of paired serum and urine samples would be beneficial to support these findings. ZIKV was even detected in urine up to 15–21 days after onset of Guillian–Barré syndrome in two patients; ZIKV was not detectable in plasma or CSF for either patient and detection may not be related to neurological symptoms [45]. Isolation and sequencing of ZIKV from urine samples has also been documented [39, 44, 48]. The Centers for Disease Control and Prevention (CDC) now recommends that urine samples be collected

Table 2 Published reports using urine as a sample for RT-PCR detection of ZIKV

Reference (PMID)	Study summary	Sample comparison
Besard et al. 2014 (PMID 24721538)	2 ZIKV infected women and their newborns. Mothers tested positive in serum within 2 days of delivery, and had ZIKV also detected in breast milk. Both newborns tested positive for ZIKV in serum and evolved favorably	Mother 2 tested positive for ZIKV in urine when undetectable in serum on day 8. Newborn 2 also had positive urine sample. Neither Mother 1 nor Newborn 1 had urine tested
Fonseca et al. 2014 (PMID 25294619)	First case of ZIKV infection in Canadian traveler returning from Thailand	Urine, Nasopharyngeal swab, and serum all collected on day 6 were positive for ZIKV. ZIKV was successfully isolated from the urine. There was enough RNA isolated from urine and nasopharyngeal samples to sequence most of the genome (GenBank KF993678)
Kutsuna et al. 2014 (PMID 24507466)	Two cases of ZIKV in Japanese individuals returning from French Polynesia	Case 1 was detected in serum on day 4; urine was not tested. Case 2 was negative for ZIKV in serum but positive in urine on day 6
Gourinat et al. 2015 (PMID 25530324)	6 ZIKV cases from the 2014 New Caledonia outbreak with sequential serum and urine samples collected over several weeks	ZIKV was detected in urine at a higher load and with a longer duration than in the serum. In serum, it was only detected within first 3 days of infection, and only in 4/6 (67 %) of the cases. In urine, ZIKV was detected up to 10-29 days after infection and in all 6/6 cases (100 %)
Barzon et al. 2016 (PMID 26987769)	Case study of Italian women returning from Dominican Republic. Plasma, urine, and saliva were collected sequentially for 1 month post infection	Plasma had lowest ZIKV detection, several logs less than detected in urine or saliva, and was undetectable after day 15. ZIKV in urine was detectable at higher levels than saliva days 11–23, and was undetectable after day 24. ZIKV in saliva was more detectable than urine days 5–9 and 25–29; ZIKV was undetectable after day 29 in saliva. ZIKV was sequenced from saliva and urine samples (GenBank KU853012) and isolated from saliva (GenBank KU853013)
Brasil et al. 2016 (PMID 26943629)	A study following 88 pregnant women in Rio de Janeiro. 72/88 (82 %) tested positive for ZIKV in blood, urine, or both. Pregnancy outcomes were observed	ZIKV was detected in 60/88 (68 %) of the serum samples and 46/88 of the urine samples (52 %). 34/88 (39 %) had ZIKV detected in both urine and serum samples; 12 were positive in urine only, and 26 positive in blood only. ZIKV detection was at 29.0 (IQR 26.0–31.8) cycles for urine and 33.0 (IQR 30.0–34.0) for serum
de M. Campos et al. 2016 (PMID 26401719)	Paired serum and urine samples were tested for ZIKV in seven Brazilian patients	ZIKV detected as early as 2 days after onset in serum, and 4 days in urine. However, higher levels of ZIKV RNA were observed in urine compared to serum. ZIKV could be detected in urine up to 14 days
Korhonen et al. 2016 (PMID 26794427)	Case study of Finnish man returning from Maldives	Serum and urine were collected on day 7—serum was negative but urine tested positive for ZIKV. ZIKV was partially sequenced from urine sample. Seriological tests in serum showed cross-reactivity with DENV
Rozé et al. 2016 (PMID 26967758)	Two cases of Guillian–Barré syndrome in Martinique	Blood, cerebrospinal fluid (CSF), and urine samples were collected from 2 patients. Blood and CSF were negative for both patients. ZIKV was detected in urine of both patients, and in patient 2 up to 21 days after onset of neurological symptoms
Shinohara et al. 2016 (PMID 26782128)	Case study of Japanese man returning from Thailand	ZIKV was detected by RT-PCR in day 7 urine, but not serum, sample. Seriological tests in serum showed cross-reactivity with DENV
Mansuy et al. 2016 (PMID 26949027)	Case study of French man returning from Brazil and French Guyana	2 weeks after infection, ZIKV was detected in semen, urine, and serum. The viral load was significantly higher (100,000×) in the semen sample
Musso et al. 2015 (PMID 25625872)	Case study of Tahiti patient who presented with hematospermia and may have had two previous ZIKV infections	Serum, urine, and semen were tested approximately 10 weeks after initial symptoms. Serum was negative for ZIKV. Urine and semen samples were positive; viral load was significantly higher in semen

<14 says after onset of symptoms in patients with suspected ZIKV infection and that molecular testing of urine be performed in conjunction with serum testing [49]. The CDC Trioplex rRT-PCR assay is the only ZIKV diagnostic test authorized by the FDA for urine. It is important to note that urine may not work as well as blood samples for assays other than RT-PCR, including IgM assays. Lastly, the majority of studies used only the primers described in Lanciotti et al. [12].

Other non-invasive samples

ZIKV has also been reported to be detected in saliva of both neonates and adults [37, 39, 50] and ZIKV can be isolated from saliva [39]. In a study comparing paired saliva and serum samples ($n = 182$) from the 2013–2014 French Polynesia outbreak, saliva had increased rate of molecular detection but not increased window of detection [50]. The authors noted that ZIKV RNA could be negative in some saliva samples while still positive in blood samples, and since blood samples are required for other laboratory tests that saliva could not act as a replacement.

ZIKV has also been detected in semen of a few men, and supports the idea that ZIKV can be spread by sexual transmission [5, 51–53]. In the few cases ZIKV has been evaluated in semen, the infectious load was considerably high and persisted over 8–10 weeks past symptom onset [5, 51–53]. Viral load was several logs of magnitude higher than corresponding urine samples, and not detectable at all in serum. This area merits more investigation, especially given the potential for ZIKV sexual transmission and poor pregnancy outcomes, but may be a good option for late diagnosis in men.

Implications for policy and practice

Taken together these studies suggests that urine samples should be collected in addition to blood for molecular testing of ZIKV especially if samples are not collected within first few days of symptom onset. This may result in an increased number of laboratory confirmed cases. Urine samples may also allow for easier monitoring of potentially exposed individuals who are at high risk for ZIKV infection complications, such as pregnant women, or individuals were collection of blood is problematic. Lastly, molecular testing of urine samples may aid in large epidemiological or surveillance studies.

Conclusion

Several studies have now been published using paired urine and serum or plasma samples, suggesting that overall, ZIKV RNA can be detected at higher levels and for a longer time after onset of infection in urine compared to serum. Semen may also allow late detection in men. Together, this suggests that ZIKV may be shed through the urine and may have a reservoir or even be actively replicating in the genito-urinary tract. In summary, the results of these studies suggests that urine samples should be considered for collection in addition to blood for molecular testing of ZIKV, and may result in an increased number of laboratory confirmed cases. Urine samples may also allow for easier monitoring of potentially exposed individuals, especially pregnant women, couples wanting to conceive, or individuals with suspicious symptoms.

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Compliance with ethical standards

Conflict of interest Drs. Lamb and Chancellor have a pending provision patent on urine biomarkers using proteins and RNA in urine for infectious and other urologic diseases. For the remaining authors none were declared. No funding was received for this work.

Ethical approval This article does not contain any studies with human participants and animals performed by any of the authors.

Informed consent None.

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