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Laboratory Validation of the Sandfly Fever Virus Antigen Assay

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Abstract

Sandfly fever group viruses in the genus *Phlebovirus* (family *Bunyaviridae*) are widely distributed across the globe and are a cause of disease in US military troops. We assessed the laboratory viability of the Sandfly Fever Virus Antigen Assay (VecTORTest Systems Inc., Thousand Oaks, CA), a rapid dipstick assay designed to detect Sandfly Fever Naples virus (SFNV) and Toscana virus (TOSV), against a panel of phleboviruses. The assay detected SFNV and TOSV, as well as New World phleboviruses Aguacate, Anahanga, Arumowot, Charges, and Punta Torro viruses. It did not detect Sandfly Fever Sicilian, Heartland, Rio Grande, or Rift Valley fever viruses. It did not produce false positive results in the presence of uninfected sandflies (*Lutzomyia longipalpis*) or Cache Valley virus, a distantly related Bunyavirus. Results from this laboratory evaluation suggest that this assay may be used as a rapid field-deployable assay to detect sandflies infected with TOSV and SFNV, as well as an assortment of phleboviruses found in the New World.

Keywords

Phlebovirus; Toscana virus; sandfly fever; assay; dipstick

The Sandfly fever (SF) virus group (Bunyaviridae: *Phlebovirus*) is comprised of arboviruses primarily transmitted by phlebotomine sandflies in the genera *Lutzomyia* and *Phlebotomus* (Diptera: Psychodidae) although some, like Rift Valley Fever virus (RVFV) and Arumowot virus, are transmitted by mosquitoes (Tesh et al. 1988). The most clinically significant members of the SF virus group are RVFV, Toscana virus (TOSV), Sandfly fever Sicilian virus (SFSV), Sandfly fever Naples virus (SFNV), and Punta Toro virus (Tesh 1988, Alkan et al. 2013). These viruses pose a threat to US military personnel in tropical and temperate regions (Brett-Major and Claborn 2009). In particular, TOSV (a serotype of SFNV) is one of the primary causes of SF disease among US troops (Brett-Major and Claborn 2009) and is a common cause of meningitis in Mediterranean and southern European countries during the vector season (Braito et al. 1997). SFSV also causes sporadic epidemics of Pappataci fevers in humans (Brett-Major and Claborn 1997). RVFV and Arumowot virus are transmitted by mosquitoes and cause disease in humans (Tomori and Fabiyi 1976, Tesh 1988). RVFV is of particular military concern because it could be used as a biological weapon (Dudley and Woodford 2002). There are SF viruses in the New World, including Punta Toro, Rio Grande, Augacate, Anhanga, and Chagres viruses, and several of these are also known to cause

serious disease (Tesh 1988). Chagres virus has caused human disease in residents of Panama (Srihongse et al. 1974) and has also been isolated from US military personnel stationed there (Peralta et al. 1965).

Rapid field assessments of sandflies for phleboviruses have been previously unavailable. The available tests are virus isolation or RT-PCR, which require appropriately equipped and staffed laboratories, and several days to weeks to receive test results. The SandFly Fever Virus Antigen Assay (SFFVA) (Product number SFFVA-K020; VecTOR Test Systems Inc., Thousand Oaks, CA) is a dipstick assay that is easy to perform and interpret, and has the potential to be field deployable. Samples are processed in a proprietary Grinding Solution provided with the kit that facilitates wicking of viral antigens up the assay stick. The dipstick is added to an aliquot of the homogenized sample supernatant and allowed to incubate for 15 minutes. The appearance of a red band at the both the test and control zones indicate a positive result, while the appearance of a red band at only the control zone indicates a negative result. The appearance of no bands at all indicates test failure and a re-test of the sample is required. While the SFFVA assay was designed to detect TOSV and SFNV in field collected sandflies by serologically detecting the partially conserved viral N protein, we also evaluated its ability to detect other phleboviruses in the SF group.

We conducted a laboratory evaluation of the SFFVA dipstick assay on an assortment of antigenically distinct SF viruses. These included the Old World viruses – TOSV, SFNV, and SFSV. We selected several New World SF viruses including Rio Grande virus, which is the only known phlebovirus transmitted by sandflies in the continental US (Endris et al. 1983), and Aguacate, Anahanga, Charges, and Punta Toro viruses from Central and South America, which were chosen based on previously described human infections and published antigenic variability (Peralta et al. 1965, Sather 1970, Srihongse et al. 1974, Tesh et al. 1975).

We also tested the SFFVA against arthropod-borne phleboviruses which are not members of the SF virus group (Heartland virus) or which are members of the SF virus group but not known to be transmitted by sandflies (RVFV and Arumowot virus). Heartland virus (HRTV) is a North American phlebovirus known to cause febrile illness in people (McMullen et al. 2012), and was recently shown to be transmitted by ticks (Savage et al. 2013). We tested Cache Valley fever virus (*Bunyaviridae*: *Orthobunyavirus*) as a negative virus control, because it is a distantly related genus of *Bunyaviridae*. Finally, uninfected pools of sandflies, *Lutzomyia longipalpis* (Diptera: Psychodidae), were used to confirm that the test strips would not produce false positive results in the presence of homogenized insects.

Viruses were purchased from the American Type Culture Collection (Manassas, VA) or were provided by the Centers for Disease Control and Prevention or US Department of Agriculture. Viruses were propagated and tittered using VERO cells. Dead *L. longipalpis* were acquired from colonies maintained at the Walter Reed Army Institute of Research (Silver Springs, Maryland). A voucher specimen was deposited at the Museum of Biological Diversity, Columbus, Ohio.

Negative controls consisted of either 250 μl of uninfected VectorTest Grinding Solution, pools of 25 homogenized *L. longipalpis* in 200 μl of Grinding Solution, or Cache Valley

virus stock diluted in Grinding Solution to a final volume of 250 μl (Table 1). All other virus samples were produced by serially diluting each virus in VectorTest Grinding Solution in a final volume of 250 μl. Samples containing TOSV, RVFV, and all New World SF group viruses also contained 25 homogenized *L. longipalpis*. SFSV, SFNV, and HRTV samples did not contain sandflies as they were unavailable at the time of testing. Positive and negative results were interpreted according to the presence of one or two bands as described above. To control for possible bias, the dipsticks were examined and scored positive or negative by a student that had no knowledge of the contents of each tube and who was uninvolved with any other aspect of the study.

Results of the SFFVA are listed for each virus tested (Table 1). None of the negative controls produced positive results. No positive results were detected for HRTV, RVFV, Rio Grande virus, or SFSV. Failure to detect RVFV was expected as this virus is not closely related to Toscana virus (Charrel et al. 2009). Rio Grande virus was characterized as a phlebovirus by serology but its phylogenetic relationship to other *Phlebovirus* is unknown (Calisher et al. 1977). Heartland virus is a tick-borne phlebovirus and thus a distant relative. The SFFVA dipstick assay did not detect SFSV, which is antigenically different from SFNV (Sabin 1955, Alkan 2013).

All of the other phleboviruses produced positive SFFVA dipstick assay results (Table 1). The assay detected a minimum titer of $10^{2.43}$ tissue culture infectious dose $(TCID)_{50}$/ml of TOSV and a minimum titer of $10^{3.5}$ plaque forming units (PFU)/ml of SFNV. Aguacate, Anahanga, Arumowot, Chagres, and Punta Torro viruses produced positive bands; their respective sensitivity limits are listed in Table 1. The presence of homogenized sandflies in the virus positive samples did not produce false negative results.

Viral loads in wild caught *Lutzomyia* and *Phlebotomus* are poorly known; however a study of laboratory infected *Phlebotomus* and *Lutzomyia* indicated that the titers of SFNV and TOSV can range from 10^3 – $10^{4.5}$ and 10^3 – $10^{4.7}$ PFU per insect, respectively, 5–7 days post-infection (Tesh 1984). The SSFVA dipstick assay detected the target viruses (TOSV and SFNV) in a laboratory environment; however, field testing is needed to determine if this assay will be useful for threat assessments of these phlebotomine-borne viruses. The incidental ability of the SFFVA dipstick assay to detect the New World SF phleboviruses listed above may prove useful in detecting infected sandflies where those viruses are known to circulate.

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Table 1.

Results of the laboratory evaluation of the SandFly Fever Virus Antigen (SFFVA) dipstick assay. Sensitivity results are presented as the lowest detectable titers of viruses that tested positive with the dipsticks. Specificity of the assay is demonstrated by viruses that were undetected.

Viruses detected by the SFFVA assay	**Strain**	**Lowest titer detected**
Toscana virus	Unknown	$10^{2.43}$ $TCID_{50}$/ml
Sandfly Fever Naples virus	Original	$10^{3.5}$ PFU/ml
Aguacate Virus	VP 175 A	$10^{3.93}$ $TCID_{50}$/ml
Anhanga Virus	Be An 46852	$10^{5.5}$ $TCID_{50}$/ml
Arumowot Virus	AR 1284–64	$10^{6.1}$ $TCID_{50}$/ml
Chagres Virus	JW 10	$10^{6.77}$ $TCID_{50}$/ml
Punta Toro Virus	Unknown	$10^{5.9}$ $TCID_{50}$/ml
Viruses not detected by the SFFVA assay		Highest titer tested
Sandfly Fever Sicilian Virus	Original	$10^{5.2}$ PFU/ml
Heartland Virus	MO12–75	10^{7} PFU/ml
Rift Valley Fever Virus	MP12	$10^{7.97}$ $TCID_{50}$/ml
Rio Grande Virus	Unknown	$10^{6.59}$ $TCID_{50}$/ml
Cache Valley Virus	89B-7060	$10^{6.6}$ $TCID_{50}$/ml