Nucleic Acid and Antibody Integrity in Clinical Samples on Dried Blood Stabilization Products During Ambient Temperature Shipment

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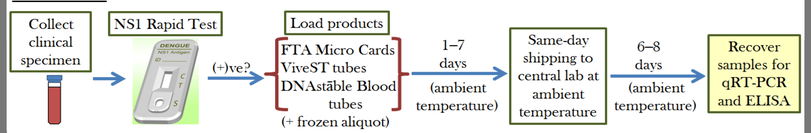
Abstract:

Degradation of RNA and antibodies during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of RNA-based pathogens. This is particularly true when shipping may require more than a day of transit, as cold-chain is not always available in low-resource settings. In this study, we used dengue as a model RNA virus to compare the performance of three down-selected commercially available nucleic acid-stabilization products: Biomātrica DNAstāble Blood tubes, ViveBio ViveST tubes, and Whatman FTA Micro Cards. Whole blood specimens collected from acute dengue fever patients (Days 0-4 Post Onset of Symptoms) during routine febrile surveillance in Iquitos, Peru were applied to the nucleic acid-stabilization products and dried overnight. At various time points, the stabilized specimens were shipped under ambient conditions to a diagnostic testing laboratory in Lima, Peru. Viral RNA levels and anti-dengue antibodies were then tested via qRT-PCR and ELISA, respectively, and compared to unloaded frozen matched samples. Pearson coefficients with unloaded frozen matched samples were: 0.796 RNA, 0.983 IgM, and 0.984 IgG (DNAstāble Blood); 0.752 RNA, 0.979 IgM, and 0.966 IgG (ViveST); and 0.877 RNA, 0.962 IgM, and 0.925 IgG (FTA Micro Cards). Despite high correlation, FTA Micro Cards demonstrated a three log loss in RNA copy number during recovery, whereas DNAstāble Blood and ViveST tubes showed a less-than-one log loss. Other considerations were also evaluated in this study and should influence any decision to incorporate commercial sample stabilization products into a diagnostic testing workflow.

Introduction:

- According to the WHO, about one-half of the world's population is at risk for dengue (1). Current estimates indicate ~390 million dengue infections worldwide annually (2), resulting in approximately 21,000 deaths (3). Accurate diagnosis informing careful clinical management is needed to increase patient survival.
- Serological and molecular detection methods complement each other in diagnosing dengue among other co-circulating febrile syndromes; however, cold chain resources are not always available to maintain sample integrity (4,5). Specifically, degradation of RNA during specimen transport from collection site to advanced laboratory facility is a major problem affecting diagnostic accuracy.
- Previously, we spiked whole blood with high-titer human anti-dengue IgG serum and dengue virus, simulating low viremic antibody-positive samples. We used these clinical surrogates to test four commercially-available nucleic acid-stabilization products over a period of 4 weeks at conditions that simulate loss of cold chain (6).
- In this study, we tested down-selected products in the field by using them to ship clinical dengue-positive whole blood samples from Iquitos to Lima, Peru for analysis. Stabilization of viral RNA was evaluated by quantitative reverse-transcription PCR (qRT-PCR); stabilization of anti-dengue IgM and IgG was evaluated by ELISA.

Methods:



- **Sample collection:** Whole blood was collected into purple-top EDTA tubes. Samples testing positive for NS1 using the SD BIOLINE Dengue Duo (Dengue NS1 Ag + IgG/IgM) immunochromatographic rapid test (Alere) were included in the study. Dengue was later confirmed by RT-PCR and qRT-PCR (7,8).
- **Sample processing and shipping:** A 200 µL reference aliquot from each sample was maintained at -80 °C. Samples were loaded onto each product according to the manufacturers' instructions, except 500 µL was loaded onto ViveST tubes and 200 µL was loaded onto FTA Micro Cards. Samples were dried overnight (16-18 hours) in a biosafety cabinet. Dried samples were stored at room temperature for 1-7 days, sent in two shipments at ambient temperatures with a 2c\temp-RH temperature and humidity data logger (Marathon Products), and held an additional 6-8 days before sample recovery.
- **Sample recovery:** Samples were rehydrated according to manufacturers' instructions for DNAstāble Blood and ViveST tubes, except ViveST-loaded samples were rehydrated with 1 mL water. For RNA recovery from FTA Micro Cards, ¼ of each blood spot was minced and incubated in 200 µL AVL buffer (QIAgen) for one hour at 23 °C; for antibody recovery , ¼ of each blood spot was minced and incubated in 200 µL PBS-0.05% Tween® 20 for 30 min at 37 °C; for both, supernatant was collected after centrifuging at 17,000xG at for 5 minutes.
- **RNA Extraction and qRT-PCR:** RNA extractions were performed using the QIAamp Viral RNA Mini kit (QIAgen) according to the manufacturer's instructions. qRT-PCR reactions were performed using a modification of McAvin et al. (9) using the TaqMan EZ RT-PCR Core Reagent kit (Life Technologies). The master mix included 0.7 µM each of forward and reverse primers, 0.27 µM probe.
- **IgM and IgG ELISAs:** IgM levels were measured using the Dengue Virus IgM Capture DxSelect ELISA kit (Focus Diagnostics); IgG levels were measured using the Dengue Virus IgG DxSelect ELISA kit (Focus Diagnostics). Both ELISA kits was used according to the manufacturer's instructions.

Results:

Figure 1

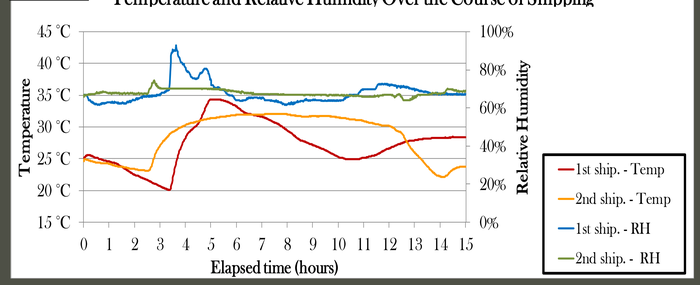


Figure 2

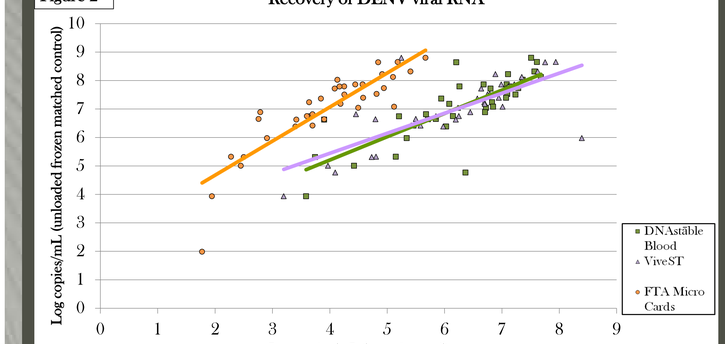
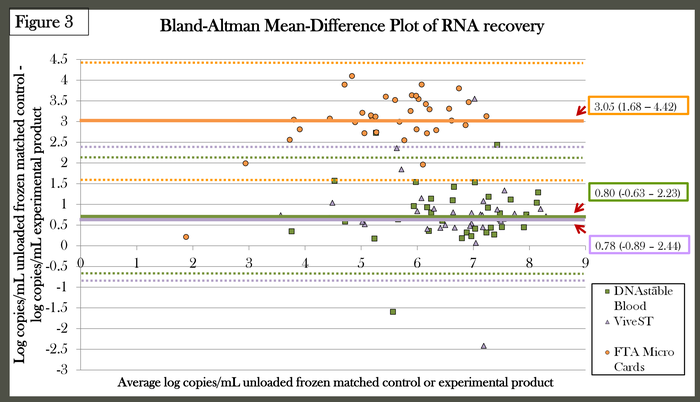


Figure 3



Product:	DNAstāble Blood	ViveST	FTA Micro Cards
Company	Biomātrica	ViveBio	Whatman
Volume Held	200 µL	1 mL	200 µL
Sample Recovery	• Add 300 µL sterile H_2O • Shake at 1200 RPM at room temperature for 1 hour	• Place matrix in syringe • Add 1 mL sterile H_2O • Incubate for 10 min. • Replace plunger and depress to recover	• Mince ¼-spot • Add appropriate buffer • Shake at optimized temperature/time • Recover supernatant
Mechanism	Will stabilize DNA in whole blood in dry form	Will stabilize RNA in plasma in dry form	Chemically treated paper that lyses cells and protects nucleic acids
Price	$4.50/tube	$8.00/tube	$2.93/card

Figure 4

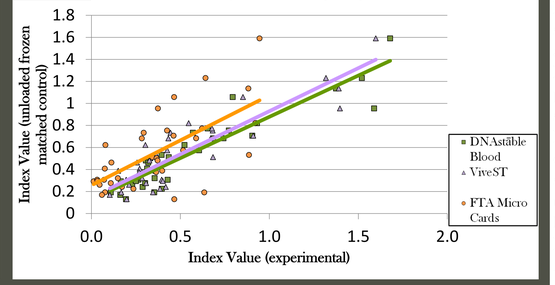


Figure 5

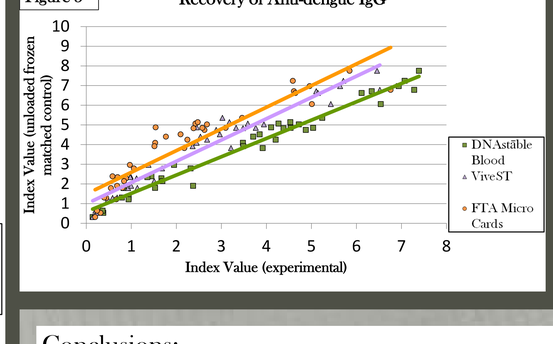


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Figure 1: *Temperature and relative humidity.* Two-shipment representative variation in ambient temperature and relative humidity during same-day shipping.

Figure 2: *DENV viral RNA recovery.* Linear regression plots comparing DENV log copies/mL from stabilized specimens vs. frozen matched controls.

Figure 3: *Bland-Altman plot of RNA recovery.* Agreement of RNA copy number recovered from clinical specimens stabilized on each product with RNA extracted from unloaded, frozen matched controls. Average bias (solid line) ±2 standard deviations (dashed lines) shown.

Figures 4 and 5 *Anti-dengue IgM and Anti-dengue IgG recovery.* Linear regression plots comparing anti-dengue IgM or IgG Index Values from stabilized specimens vs. frozen matched controls.

Table 1: *Pearson coefficients.* Linear correlations between each experimental product compared to the unloaded, frozen matched control, for RNA, IgM, and IgG recovery.

Table 1: Pearson correlation coefficients	DNAstāble Blood	ViveST	FTA Micro Cards
RNA	0.796	0.752	0.877
IgM	0.983	0.979	0.962
IgG	0.984	0.966	0.925

Conclusions:

- Ambient temperature and relative humidity vary widely during shipping. In two representative shipments from Iquitos to Lima, Peru, we noted temperatures ranging from 20.1 to 34.3 °C, and relative humidities ranging from 61.4 to 92.6 %.
- Dengue viral RNA was able to be recovered from all three products. RNA recovery for each product was correlated with unloaded, frozen matched samples, with agreement highest for the DNAstāble Blood and ViveST tubes.
- Bland-Altman Mean-Difference analysis (10) of the three products shows an average loss of less-than-one log of viral RNA copy number during storage and recovery on DNAstāble Blood (0.80 ± 1.43) and ViveST (0.78 ± 1.66) tubes. This is contrasted with storage and recovery on FTA Micro Cards (3.05 ± 1.37), which results in a loss of almost three logs of viral RNA copy number.
- Previous investigations have determined that some liquid nucleic acid stabilizers in blood-collection tube format are incompatible with downstream serological testing (11). All three products investigated here show comparable ability to stabilize both human anti-dengue IgM and human anti-dengue IgG; however, methods to recover nucleic acid from FTA Micro Cards are not optimized for recovery of antibodies (data not shown).
- As all three products demonstrated ability to stabilize physiologically- relevant levels of viral RNA and anti-dengue antibodies, other considerations such as cost, sample volume required, and ease-of-use should also influence any decision to incorporate commercial sample stabilization products into a diagnostic testing workflow.

Future Directions:

- Examination of these products for the ability to stabilize dengue protein biomarker NS1 and putative predictors of severe disease.
- Continued optimization of a single-method recovery of RNA, antibodies, and other biomarkers from Whatman FTA Micro Cards.
- Investigate commercially-available RNA-stabilization liquids for the ability to stabilize dengue RNA and anti-dengue antibodies, as well as dengue NS1 and other biomarkers and putative predictors of severe disease.

References:

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