Stability of IgG and Viral RNA in Dried Whole Blood in Commercially-Available RNA-Stabilization Products

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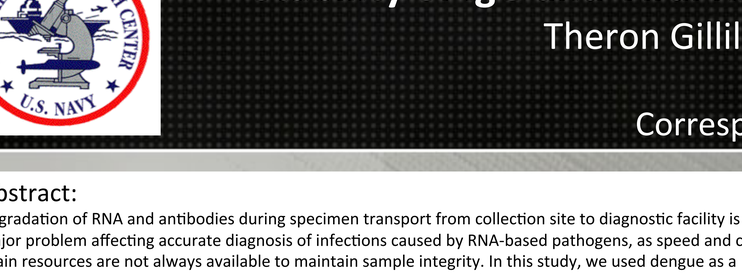
Abstract:

Degradation of RNA and antibodies during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of infections caused by RNA-based pathogens, as speed and cold chain resources are not always available to maintain sample integrity. In this study, we used dengue as a model RNA virus to compare the performance of four commercial-off-the-shelf RNA-stabilization: Biomātrica DNAstable tubes, Biomātrica RNAstable tubes, ViveBio ViveST tubes, and (commonly-used) Whatman FTA Micro Cards. We tested these products at conditions simulating antibody-positive low-viremia samples (1:500 pooled high titer human anti-dengue IgG serum and 10^2 plaque-forming units/mL dengue 1 virus spiked into whole blood) subjected to a loss of cold chain in a tropical environment (exposed to 37 °C for 0 or 24 hours, then restored to room temperature), conditions that may be experienced during shipping. Of the four products tested, our results show that DNAstable and ViveST tubes performed best by maintaining IgG and RNA integrity for up to 4 weeks after either initial temperature exposure.

Introduction:

- According to the WHO, over 2.5 billion people--~40% of the world's population--are now at risk for dengue (1), and current estimates indicate nearly 400 million dengue infections worldwide annually (2). Because of potentially lethal complications, such as development into severe dengue, early clinical diagnosis and careful clinical management by experienced physicians and nurses are needed to increase patient survival.
- Molecular methods complement serological tests in the diagnosis of dengue among other co-circulating endemic febrile syndromes. However, several studies in the field document that speed and cold chain resources are not always available to maintain sample integrity (3,4). Degradation of RNA during specimen transport from collection site to diagnostic facility is a major problem affecting diagnostic accuracy.
- In this study, we spiked human whole blood with high titer human anti-dengue IgG serum and dengue virus, simulating antibody-positive samples with low viremia. We tested four commercially-available RNA-stabilization products over a period of 4 weeks. Stabilization of IgG was evaluated by ELISA; stabilization of viral RNA was evaluated by quantitative reverse-transcriptase PCR (qRT-PCR).

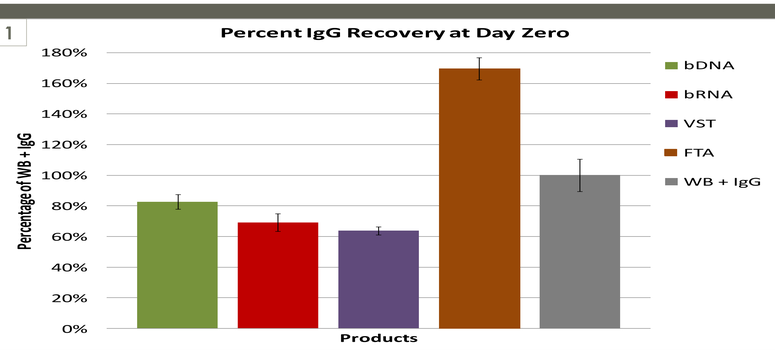
Methods:



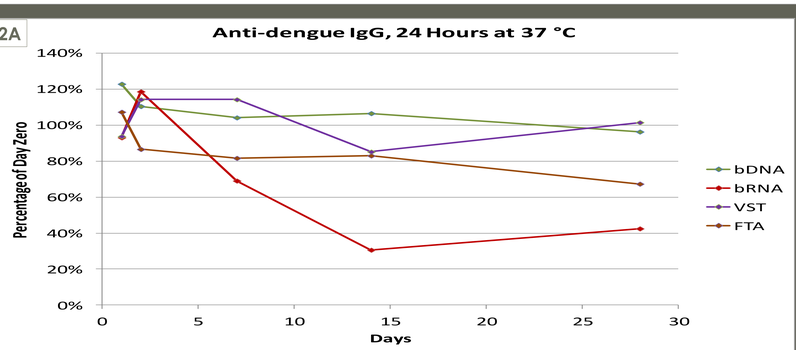
- **Sample recovery:** Samples were rehydrated according to manufacturer's instructions, with the exception of Whatman FTA Micro Cards. For those, ¼ of each blood spot was finely minced and incubated in 700 µL sterile water for 15 minutes at 95 °C (RNA recovery), followed by supernatant recovery after centrifuging 17,000 x G at for 5 minutes.
- **IgG ELISA:** IgG levels were measured using the Dengue Virus IgG DxSelect ELISA kit (Focus Diagnostics) according to manufacturer's instructions, except that samples were diluted 1:20 in diluent prior to use.
- **RNA Extractions and qRT-PCR:** RNA extractions were performed using the QIAamp Viral RNA Mini kit (Qiagen) according to manufacturer's instructions. All qRT-PCR reactions were performed using a modification of McAvin *et al.* (5) 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.

Results:

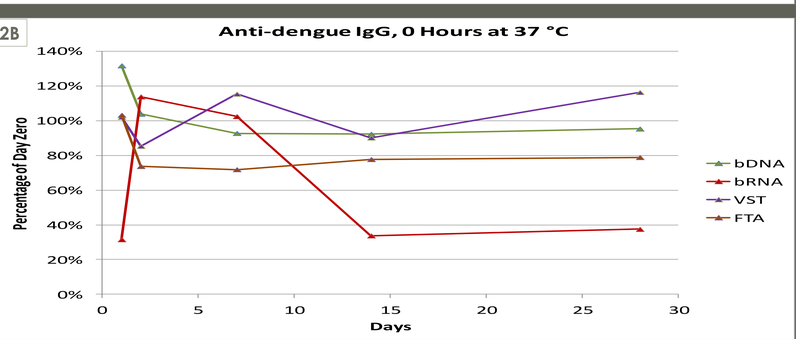
1



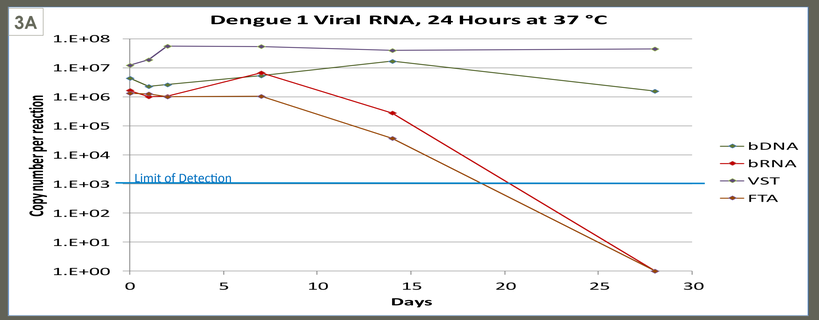
2A



2B



3A



3B

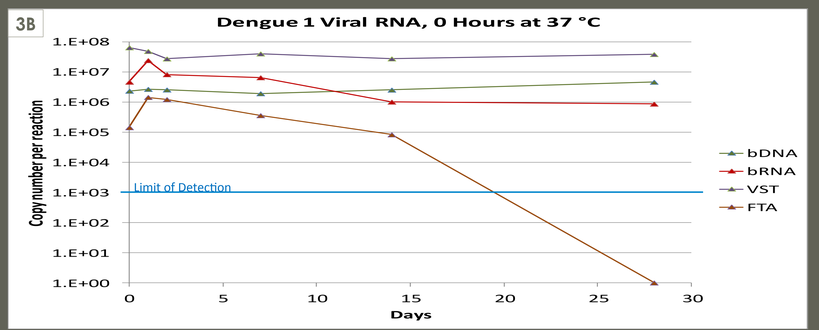


Figure 1: *Percent IgG Recovery.* IgG was detected by the Dengue Virus IgG DxSelect ELISA kit and corrected for theoretical dilution factor. Index Values were calculated using a cut-off calibration control, then normalized to unprocessed IgG-spiked whole blood. A comparison was made between unprocessed IgG-spiked blood, unprocessed blood alone, and IgG-spiked blood loaded onto the various products (N=3).
Figures 2A and 2B: *Anti-dengue IgG.* IgG was detected by the Dengue Virus IgG DxSelect ELISA kit, and Index Values were calculated using a cut-off calibration control. IgG was measured up to 28 days following 24 hours at 37 °C (2A) or 0 hours at 37 °C (2B) and compared to day zero (N=3).
Figures 3A and 3B: *Dengue 1 Viral RNA.* RNA was detected by qRT-PCR using a modification of McAvin *et al.* RNA was measured up to 28 days following 24 hours at 37 °C (3A) or 0 hours at 37 °C (3B), and copy number was calculated by standard dilution curve (N=3).

FIGURES LEGEND:

- **bDNA** = Biomātrica DNAstable
- **bRNA** = Biomātrica RNAstable
- **VST** = ViveBio Vive ST
- **FTA** = Whatman FTA Micro Cards
- **WB** = Whole Blood-CPDA

Product:	DNAstable	RNAstable	ViveST	FTA Micro Cards
Company	Biomātrica	Biomātrica	ViveBio	Whatman
Volume Held	200 µL	200 µL	1 mL	200 µL
Sample Recovery	•Add 300 µL sterile H_2O •Shake at 1200 RPM for 2 hours	•Add 200 µL sterile H_2O •Vortex lightly to mix	•Place matrix in syringe •Add 1 mL sterile H_2O •Incubate for 10 minutes •Replace plunger and depress to recover	•Mince ¼-spot •Add 700 µL sterile H_2O •Incubate at 95 °C, 15 min •Centrifuge 17K x G for 15 min.; recover SUP
Mechanism	Will stabilize DNA in whole blood in dry form	Capable of storing previously extracted RNA in dry form	Will stabilize RNA in plasma in dry form	Chemically treated paper that lyses cells and protects nucleic acids
Manufacturer's claim	DNA in whole blood for up to 8 years.	RNA stable at RT for up to 29 months (simulated up to 12 years).	RNA is stable at RT for up to 2 months.	Genomic DNA is stable for up to 17 years at RT

Conclusions:

- Antibody was recoverable at high levels from all four products; future studies will investigate the reason for seemingly higher-than-predicted antibody yield from FTA Micro Cards.
- DNAstable, ViveST and FTA Micro Cards products were able to stabilize anti-dengue IgG for at least 4 weeks, while RNAstable tubes demonstrated a time-dependent loss of antibody. Previous investigations have demonstrated that some RNA stabilization products are incompatible with downstream serological testing (6). Our data shows that all four products tested here are suitable for IgG detection via ELISA.
- DNAstable and ViveST tubes yielded detectable viral RNA for at least 4 weeks regardless of temperature exposure, while RNA exposed to 37 °C for 24 hours while stabilized on Biomātrica RNAstable tubes became undetectable after 2 weeks. All products were an improvement over FTA Micro Cards, which only yielded detectable viral RNA for up to 2 weeks.
- Based on superior performance in stabilizing both IgG and viral RNA, we will proceed with DNAstable tubes and ViveST tubes in studies involving clinical specimens. These products may also be examined for ability to stabilize dengue protein biomarker NS1 and cytokine IL-10, a putative predictor of severe disease. Other future studies will investigate commercially-available RNA-stabilization liquids not prepackaged in blood collection tubes.

References:

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Disclaimer: *This work was performed when Mr. Theron Gilliland, Jr. was employed by the Henry M. Jackson Foundation for the Advancement of Military Medicine. The view expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. This work was supported by work unit number MIDRP 6000.RAD1.L.A0311. This was prepared as part of the author's official duties. Title 17 U.S.C. §105 provides that 'Copyright protection under this title is not available for any work of the United States Government'.*