Evaluation of Stability of Reference Materials for Infectious Disease Testing at Elevated Temperatures in Liquid and Dry States

Catherine Huang[1], Rajeswari Vemula[1], Anita McClernon[2], Timothy Murray[3] and Bharathi Anekella[1]
SeraCare Life Sciences, Inc., Gaithersburg, Maryland[1]
bioMONTR® Labs[2] and ViveBio, LLC[3]

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INTRODUCTION

Biological reference materials for molecular diagnostics tests typically require cold chain logistics and storage for stability. However, refrigerated or frozen storage may not be available in resource limited settings. Even in industrial nations where cold storage is available, it adds to the cost and therefore limits accessibility of testing. To improve standardization and access to diagnostic testing, room temperature and elevated temperature storage of reference materials should be evaluated. SeraCare tested analytes such as viruses and bacteria at ambient temperature and elevated temperature in both liquid format and dry storage including lyophilization and ViveST™ [4] Sample Storage devices.

MATERIALS AND METHODS

Preparation of recombinant Ebola stability study material:
SeraCare developed AccuPlex™ rEbola GP/NP Reference Material, which targets the GP/NP regions. The reference material is formulated in defibrinated plasma with a fill volume of 250µL, and can be stored at 2-8 ˚C. Some of these vials were lyophilized and stored at room temperature and 42 °C. ViveST™ samples were prepared by adding 250 µL of virus onto absorbent matrix and allowing it to dry overnight [1,2]. These vials were stored at room temperature, 2-8 °C, and 42 °C. At each time point, the samples were tested in triplicates on an in-house developed TaqMan® assay to assess the concentration in copies/mL.

Preparation of CRE OXA stability study material:
Heat inactivated CRE OXA bacteria in commutable matrix was prepared at 5.0E+05 cfu/mL and aliquoted into microcentrifuge vials at 200µL fill volume. ViveST samples were prepared by adding 200 µL of virus onto absorbent matrix and allowing it to dry overnight. Liquid vials were stored at 2-8 °C and room temperature, whereas ViveST™ vials were stored at room temperature and 42 °C. At each time point, the samples were tested in duplicates on Cepheid GeneXpert® instrument to assess the variation in Cycle Threshold (Ct)

Preparation of *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) stability study material:
Heat inactivated CT/NG bacteria in commutable matrix was prepared at 5.0E+05 copies/mL and aliquoted into microcentrifuge vials at 1.1mL fill volume. ViveST samples were prepared by adding 1.1mL of bulk onto absorbent matrix and allowing it to dry overnight. Liquid vials were stored at 2-8 °C and room temperature, whereas ViveST vials were stored at room temperature and 42 °C. Some vials were lyophilized at 1.1mL fill volume and stored at room temperature and 42 °C. At each time point, the samples were tested in duplicates on Cepheid GeneXpert instrument to assess the variation in Cycle Threshold (Ct) using Cepheid Xpert CT/NG assay.

Figure 1a: ViveST rEbola Matrix

Figure 1b: Lyophilized rEbola Material

RESULTS AND DISCUSSION

Recombinant Ebola Stability Study Result: Comparison of results across different storage systems showed that recombinant Sindbis virus was stable as lyophilized material and on ViveST absorbent matrix at room temperature (< two-fold difference in concentration across 3 months of storage). Surprisingly, the virus was also stable in liquid form (<25% difference across 3 months); this stability may be due to the proteinaceous nature of the matrix and the presence of an enveloped virus coat. Lyophilized viral material was stable at 42 °C without decrease in concentration but there was a decline in concentration on ViveST matrix at 42 °C. This is the due to the inherent nature of storage system for virus or bacteria where viral concentration reduces by a log and is then stabilized[3].

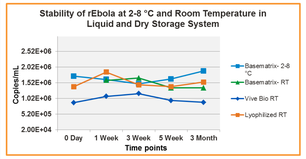


Figure 2a: Stability study data of rEbola reference material in liquid form, on ViveST matrix and in lyophilized form at 2-8 °C, room temperature and 42 °C tested on In-house developed TaqMan assay in triplicates

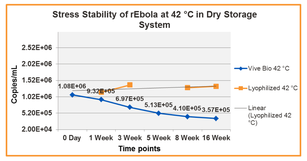


Figure 2b: Stability study data of rEbola reference material on ViveST matrix and in lyophilized form at 42 °C tested on In-house developed TaqMan assay in triplicates

ΔCt values compared to 2-8 °C zero time point		ΔCt values compared to RT ViveST zero time point		ΔCt values compared to RT lyophilized zero time point	
Log10(copies/mL)		Log10(copies/mL)		Log10(copies/mL)	
2-8 °C Liquid (zero timepoint-Non stressed)	6.24	RT ViveST (zero time point-Non stressed)	5.95	RT Lyophilized (zero time point-Non stressed)	6.15
RT Liquid (3 Months)	6.13	RT ViveST (3 Months)	5.95	RT Lyophilized (3 Months)	6.19
Δ Log10(Copies/mL)	**0.10**	**Δ Log10(Copies/mL)**	**0.00**	**Δ Log10(Copies/mL)**	**-0.04**
		42 °C ViveST (16 Weeks)	5.55	42 °C Lyophilized (16 Weeks)	6.13
		Δ Ct	**0.40**	**Δ Ct**	**0.02**

Table 1: Comparison of rEbola final time point data at each condition for each storage system with zero time point data on In-house developed TaqMan assay

Heat-Inactivated CRE OXA Bacteria Stability Study Result: For CRE OXA, comparison of test results in Figure 3 and Table 2 at different conditions showed that the bacteria was stable in liquid state and also on ViveST™ absorbent matrix at room temperature (< 2 Ct difference across 3 months of storage). A decline of 1.5 Ct was observed after 16 weeks stress at 42 °C, which is close to half a log variation at 42 °C.

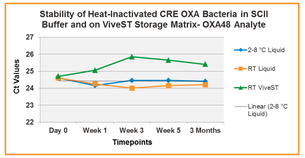


Figure 3a: Testing of CRE OXA on Cepheid Xpert Carba-R Assay. Stability study data of CRE OXA material in commutable liquid matrix and on ViveST storage matrix at 2-8 °C and room temperature tested in duplicates.

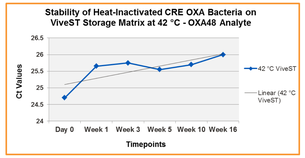
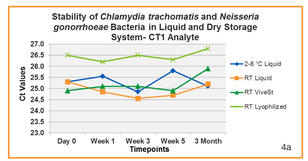


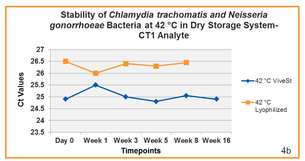
Figure 3b: Testing of CRE OXA on Cepheid Xpert Carba-R Assay. Stability study data of CRE OXA material on ViveST storage matrix at 42 °C tested in duplicates.

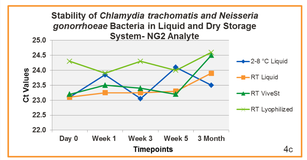
ΔCt values compared to 2-8 °C zero time point		ΔCt values compared to RT ViveST zero time point	
	OXA48 Ct		OXA48 Ct
2-8 °C Liquid (zero time point- Non stressed)	24.6	RT ViveST (zero time point-Non stressed)	24.7
RT Liquid (3 Months)	24.2	RT ViveST (3 Months)	25.4
Δ Ct	**-0.2**	**Δ Ct**	**0.3**
		42 °C ViveST (16 Weeks)	26.0
		Δ Ct	**1.3**

Table 2: Comparison of CRE/OXA final time point data at each condition for each storage system with zero time point data on Cepheid Xpert Carba-R assay

CT and NG Stability Study Result: *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were tested in liquid form, lyophilized form and on ViveST storage matrix. Data presented in Figure 4 and Table 3 look consistent at all conditions and storage systems without any significant decline (< 2 Ct difference across 3 months of storage). Table 3 data compares zero time point for each storage system with final time point. Even on ViveST storage system at 42 °C, no decline in concentration was observed.







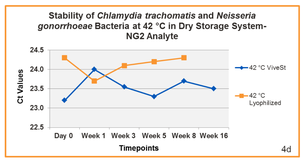


Figure 4: Cepheid Xpert CT/NG Assay. Stability study data of CT and NG material in commutable liquid matrix, on ViveST matrix and as lyophilized material at 2-8 °C, room temperature and 42 °C. At each time point, material was tested in duplicates. Cepheid assay tests for three different analytes: CT1, NG2 and NG4 for each sample. 4a and 4b shows CT1 trend, 4c and 4d shows NG2 trend.

ΔCt values compared to 2-8 °C zero time point			ΔCt values compared to RT lyophilized zero time point			ΔCt values compared to RT ViveST zero time point		
	CT1	NG2		CT1	NG2		CT1	NG2
2-8 °C Liquid (zero time point- non stressed)	25.3	23.1	RT Lyophilized (zero time point- non stressed)	26.5	24.3	RT ViveST (zero time point-non stressed)	24.9	23.2
RT Liquid 3 Months	25.2	23.9	RT Lyophilized 3 Months	26.3	24	RT ViveST 3 Months	25.9	24.5
ΔCt	**-0.1**	**0.8**	**ΔCt**	**-0.2**	**-0.3**	**ΔCt**	**1**	**1.3**
			42 °C Lyophilized 16 Weeks	26.5	24.3	42 °C ViveST 16 Weeks	25.1	23.7
			ΔCt	**0**	**0**	**ΔCt**	**0.2**	**0.5**

Table 3: Comparison of CT/NG final time point data at each condition for each storage system with zero time point data on Cepheid Xpert CT/NG assay

CONCLUSIONS

- Virus and bacteria were stable at 42 °C in both the lyophilized state and on the ViveST dry storage matrix. Since temperatures of 40 °C or higher are not uncommon in many African and Asian countries, these might be appropriate mechanisms for transport and storage.
- rEbola GP/NP reference Material was evaluated as a representative RNA virus. It is stable in liquid and dry storage form at room temperature. It is also stable in dry storage at elevated temperatures.
- Bacteria such as CRE OXA, *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are stable at room temperature in liquid and dry storage form and also at elevated temperatures in dry storage form.
- On the ViveST Storage system, a steady reduction in copies/mL or Ct values was observed for rEbola and CRE OXA at 42 ˚C. A similar reduction in values has been previously reported with other analytes and recovery was observed to have a high degree of precision and reproducibility.[1]
- Lyophilized material of rEbola and CT/NG is stable at room temperature and 42 °C.
- CT/NG was highly stable at elevated temperatures when applied to the ViveST storage system and could help support diagnostic testing where cold chain logistics are not available.
- Adoption of dry storage for quality control materials could help standardize and improve the quality of diagnostic testing in developing countries and could also drastically cut cost associated with cold storage worldwide.

REFERENCES

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