Production of Shelf-Stable PCR Reagents that are Deployed at Ambient Conditions Strengthening Supply Chain Stability

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NMRC BDRD MOLECULAR DIAGNOSTICS LAB

- Sole ISO 17034 Accredited DoW Real Time PCR Reagent Production Facility, established in 2006 to provide validated assays to all USG entities
- Wet reagents available for any Navy, DoW, USG laboratories, and collaborators
  - *Developed over 70 QPCR assays for 30+ biothreat agents (bacterial, viral, toxin, and simulants)*
- Extensive Quality Assurance Program in place
  - *Meets or exceeds industry standards for reagent production*
  - *Nearly 40 detailed SOPs written and refined*
  - *Certificates of Analysis provided with mix detailing performance and platforms*
  - *Annual internal and external audits*
- Eliminates the need of commercial vendors, shortening the supply chain and mitigating the risk of commercial/business timelines and product availability

COLD CHAIN TO SHELF STABILITY

- Production, storage, distribution, and use of wet formulations involve an extensive **cold chain** requirement
  - -20ºC freezers required for storage by end user
    - Problematic in forward deployed, mobile, austere, spatially challenged, or difficult to supply laboratory environments
  - Cold chain shipping and handling of PCR reagents is cumbersome and costly
    - Ice packs, cold shippers, overnight shipping
- **Shelf-stable** reagents shipped and stored under ambient conditions
  - Negates the cold chain burden, enhancing supply chain resilience
  - Reduces shipping and handling costs and simplifies logistics
  - Facilitates stockpiling in forward deployed or logistically difficult locations (Ships, Special Forces, etc.)

PROOF OF CONCEPT

- Shelf stable or lyophilized PCR reagents are currently available through various vendors
  - Pouches, cartridges, master-mixes
    - *Locks end-user into commercial assays of unknown targets and performance*
    - *Reliance on vendor production timelines*
    - *Risk – emerging novel pathogen or pandemic outbreak response may be delayed*
  - Develop an independent government-controlled production/supply pipeline
- HDTRA CB11283 – Transition of (Defense Biological Products Assurance Office (DBPAO) assays to field platform and development of shelf-stable formulations
  - Successfully demonstrated current wet formulations perform equivalently on field platforms
  - Identified three avenues of shelf stabilization (vitrification) in limited pilot studies
    - *Addition of excipient to current wet formulations*
    - *Leverage existing primer/probes with a commercial lyophilized buffer/taq*
    - *Novel vitrification platform that employs porous scaffold and excipient*

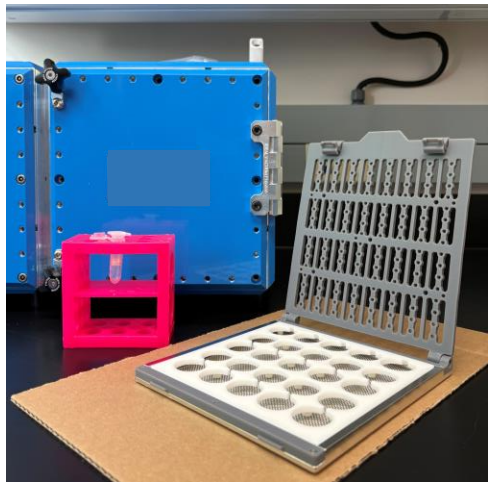
VITRIFICATION METHOD 1
LYOPHILIZED CAKE (LC)

- **Addition of DBPAO assay primers/probes to commercially produced LC**
  - Re-lyophilize and store at specified conditions
  - One-year proof-of-concept
    - Singleplex *Bacillus anthracis* assay stored under ambient conditions
  - Demonstrated comparable sensitivity and functionality at 365 days post-production

Instrument	Field Based PCR		Lab Bench PCR	
Time	Day 0	Day 365	Day 0	Day 365
100x Ave Ct	21.12	24.53	23.51	23.28
10x Ave Ct	25.17	27.79	26.96	26.94
1x Ave Ct	28.76	31.20	30.29	30.27
0.1x Ave Ct	32.11	35.87	33.74	33.59
0.01x Ave Ct	36.40	37.67	36.71	37.25

VITRIFICATION METHOD 2
POROUS SCAFFOLD SYSTEM

- **Scaffolding: Freeze-free stabilization in a rapid time-frame**
  - Porous scaffold that prevents boiling
    - Eliminates the need to freeze prior to application of vacuum drying
    - Master mix assays demonstrated an immediate loss of sensitivity post-vitrification
    - Identified interactions between scaffold and reporter dyes
      - *Revisions to scaffolding, buffer, Taq polymerase currently undergoing evaluation in consultation with collaborators*
    - Positive control material indicates no loss of efficacy at 5 months post-vitrification



1x PC	BRK		TULA		TULB	
Time	Production	5 Month	Production	5 Month	Production	5 Month
Ave Cts	28.90	29.25	28.39	28.39	28.68	28.59

VITRIFICATION METHOD 3 – EXCIPIENT BUFFER(EB)

- **Current DBPAO Assay Formulation with EB Stabilization Additive**
  - Excipient stabilization buffer was incorporated into the current BA3 wet formulation
    - *Minimal decrease in sensitivity after one year stored under ambient conditions*
    - *Negative even at high concentrations without excipient*

Assay	BA3					
Instrument	Field Based PCR			Laboratory Bench PCR		
Time	Day 0	Day 365	Day 365	Day 0	Day 365	Day 365
Formula	MM + EB	MM + EB	MM	MM + EB	MM + EB	MM
100X	22.15	24.53	Neg	24.01	26.65	Neg
10X	26.50	27.79	Neg	28.26	29.74	Neg
1X	29.62	31.20	Neg	32.19	32.88	Neg
0.1X	33.01	35.87	Neg	35.64	36.40	Neg
0.01X	36.98	37.67	Neg	38.13	40.21	Neg

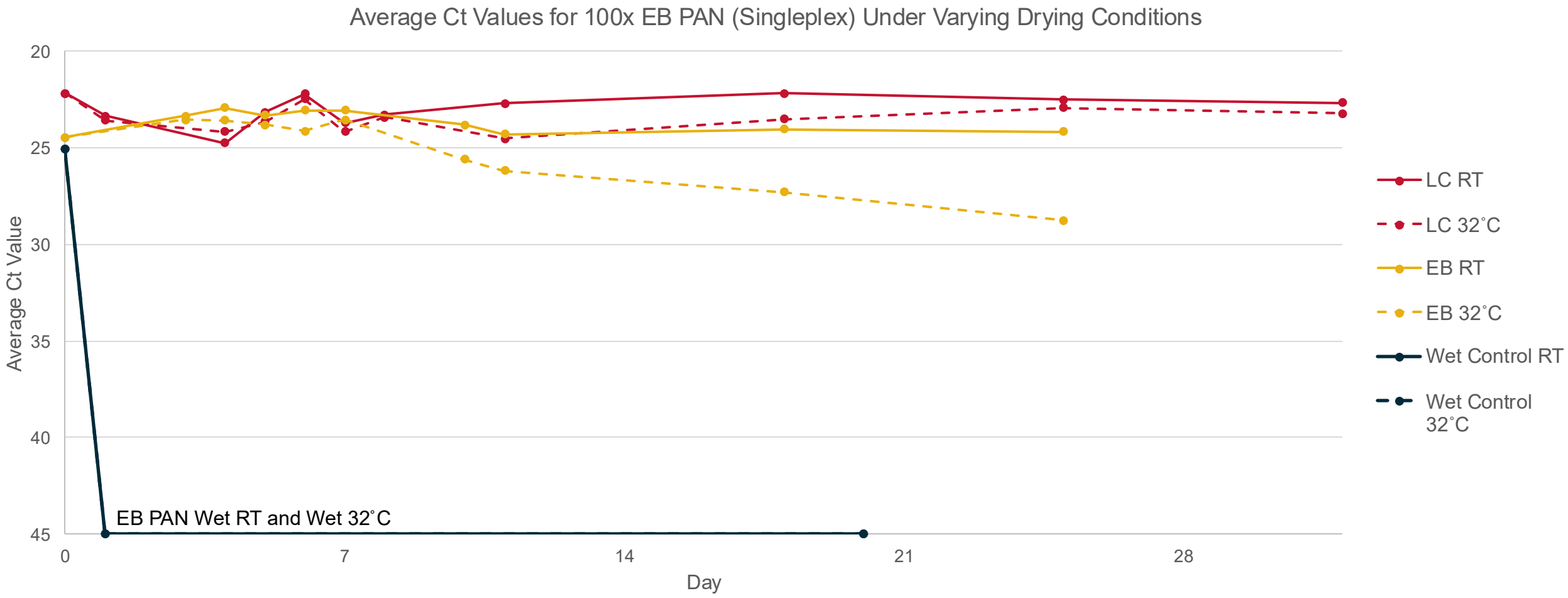
EXTENSION OF PILOT STUDY

- Produce large lots of representative assay types for vitrification methods 1 and 3
  - *DNA Singleplex*
  - *DNA Multiplex*
  - *RNA Singleplex*
  - *RNA Multiplex*

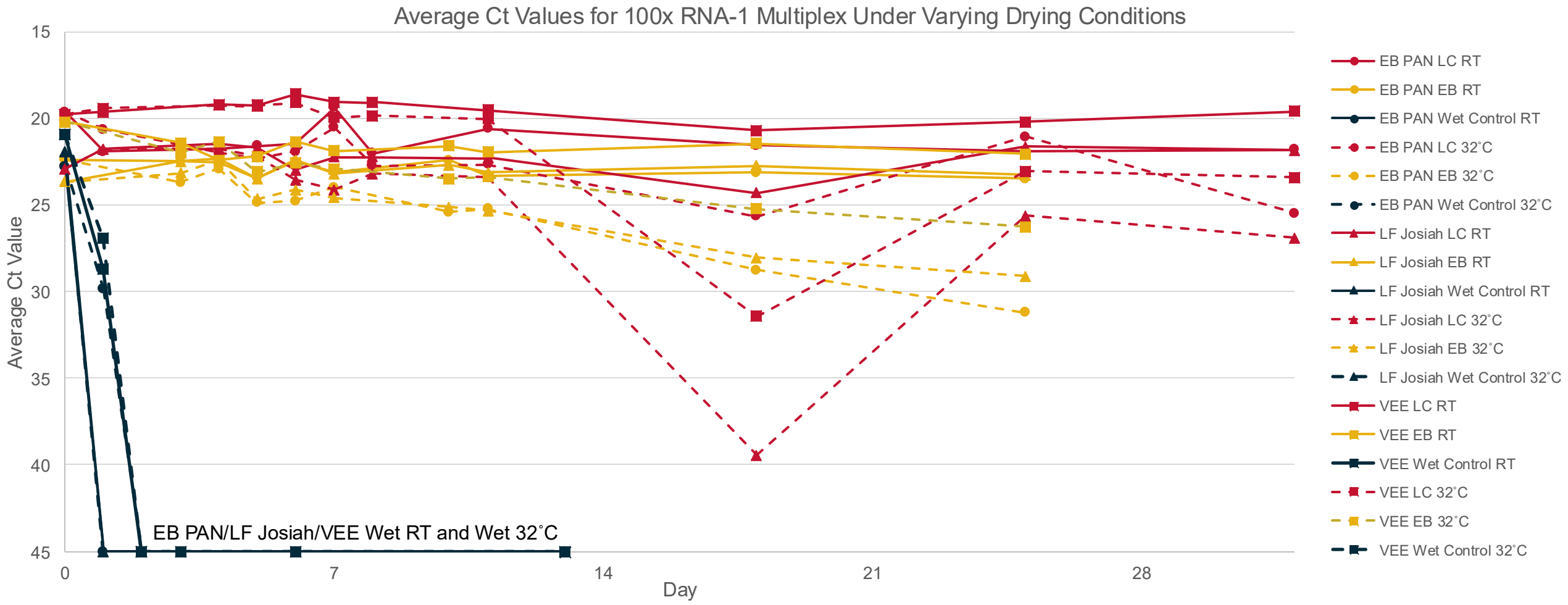
- Produce a lot of wet control for each assay type
  - *Standard wet formulation with Taq polymerase added*

- Store at either room temperature or 32°C
  - *Collect standard curve data at various time points*
  - *320fg, 32fg, 3.2fg, 320ag, 32ag positive control plasmid (100X to 0.01X)*
  - *2, 2, 2, 8, 8 replicates*

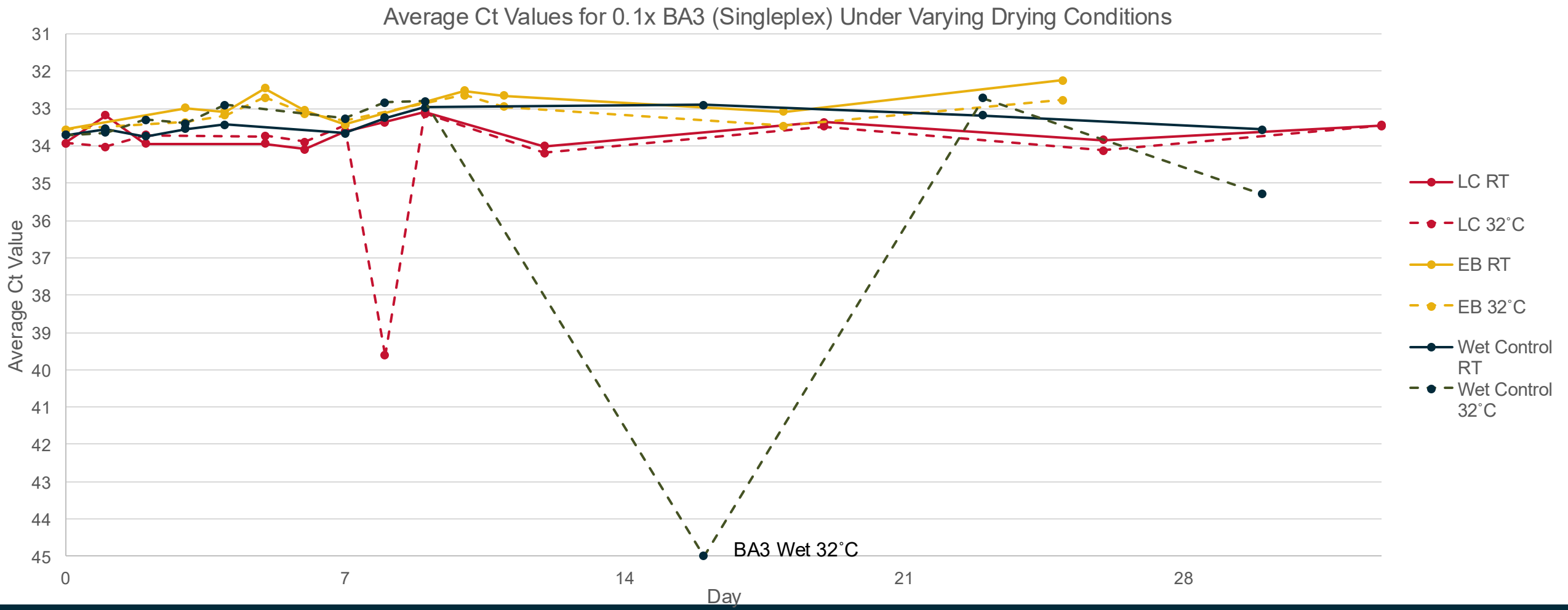
Average Ct Values for 100x EB PAN (Singleplex) Under Varying Drying Conditions



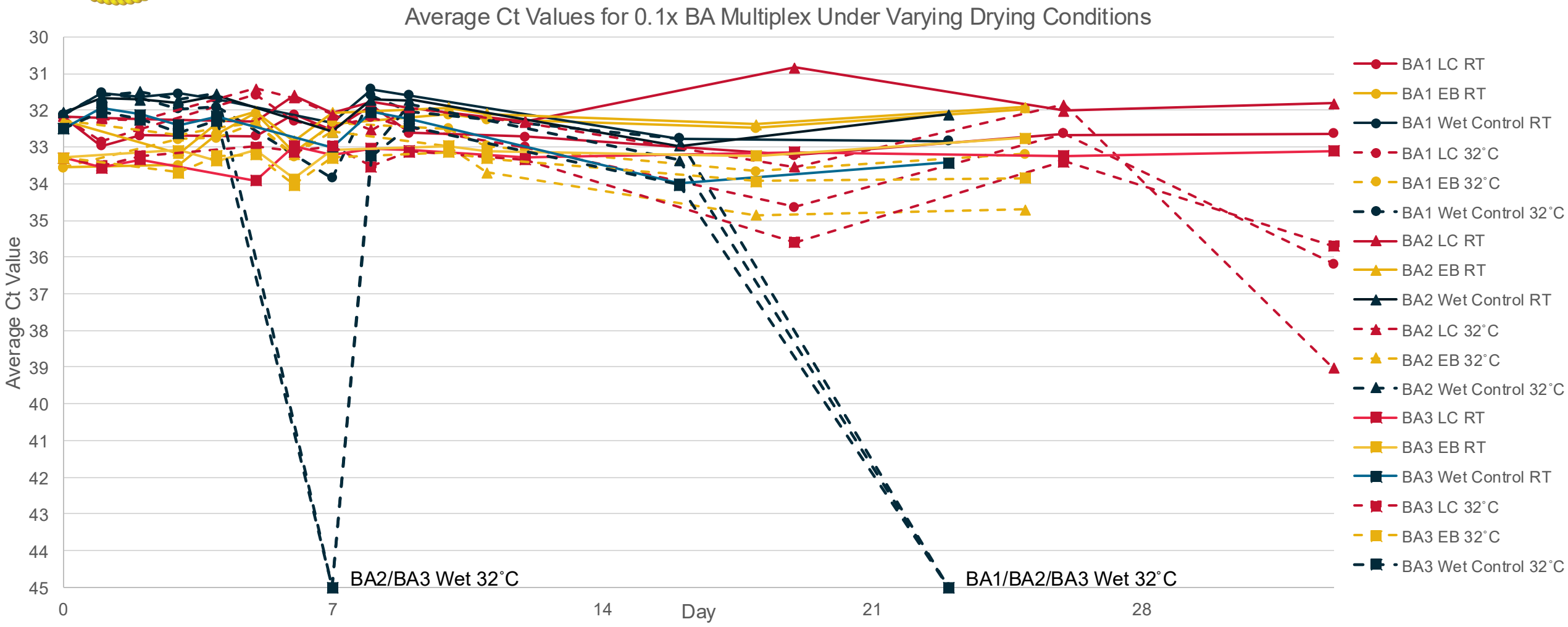
Average Ct Values for 100x RNA-1 (Multiplex) Under Varying Drying Conditions



Average Ct Values for 0.1x BA3 (Singleplex) Under Varying Drying Conditions



Average Ct Values for 0.1x BA Multiplex Under Varying Drying Conditions



NEXT STEPS

- **Evaluation of defined common operational environmental conditions**
  - Produce large single lots of representative assay types
    - *All three vitrification methods*

      Collaborators have developed and improved scaffold material and enhanced excipient
  - Produce large lots of Positive Controls
    - *Scaffolds*
    - *Direct lyophilization with and without excipients*
  - Environmental chambers maintain defined temperature and humidity profiles.
  - Evaluate performance characteristics at incremental time points
    - *Determine the operational longevity of shelf-stabilized mastermix and positive controls*
- **Investigate alternative excipients**
  - Move away from proprietary commercial formulations and platforms

REAL WORLD TEST AND EVALUATION

- **Deployment of shelf-stabilized reagents**
  - Field exercises will evaluate vitrified PCR assays under field conditions
    - *The Expeditionary Laboratory Operations Division (ELOD) of NMRC provides biological defense and expeditionary detection capabilities*
    - *Capabilities are sustainably available, rapidly deployable, and operationally effective in support of U.S. military missions*
    - *Deploy field stable reagents for test and evaluation under real life deployment and exercise conditions*

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QUESTIONS?

- **Ordering System for Critical Assays and Reagents (OSCAR)**
  - *OSCAR is a resource managed entity within the Defense Biological Product Assurance Office (DBPAO), under the Joint Project Lead for CBRND Enabling Biotechnologies, and provides critical assays and reagents to DOD and Other Government Agency (OGA) customers.*

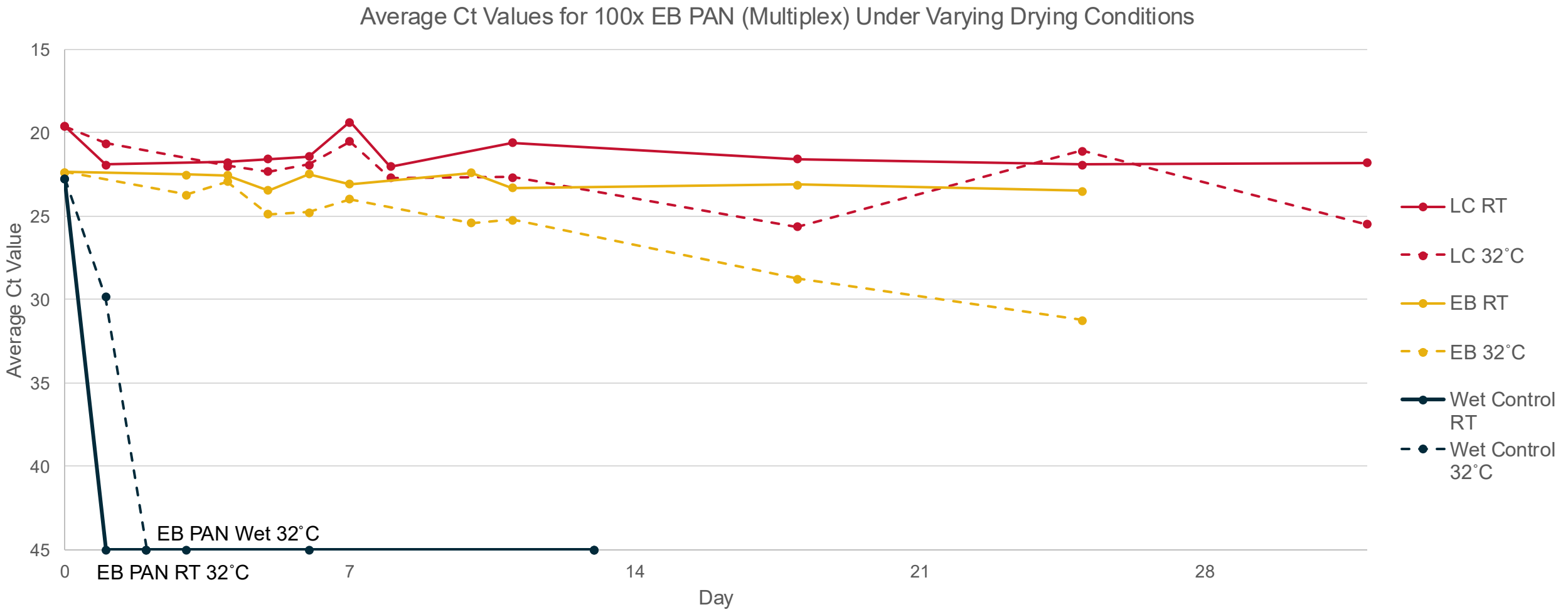
  - *Customers can purchase products through the web-based OSCAR database. DOD and OGA customers may register for an OSCAR account by completing the online registration form at https://jacks.jpeocbrnd.army.mil/. All others may inquire about access by contacting DBPAO at usarmy.detrick.dod-jpeo-cbrnd.mbx.crp@army.mil. Please allow one full business day for a response.*

Product Catalog:

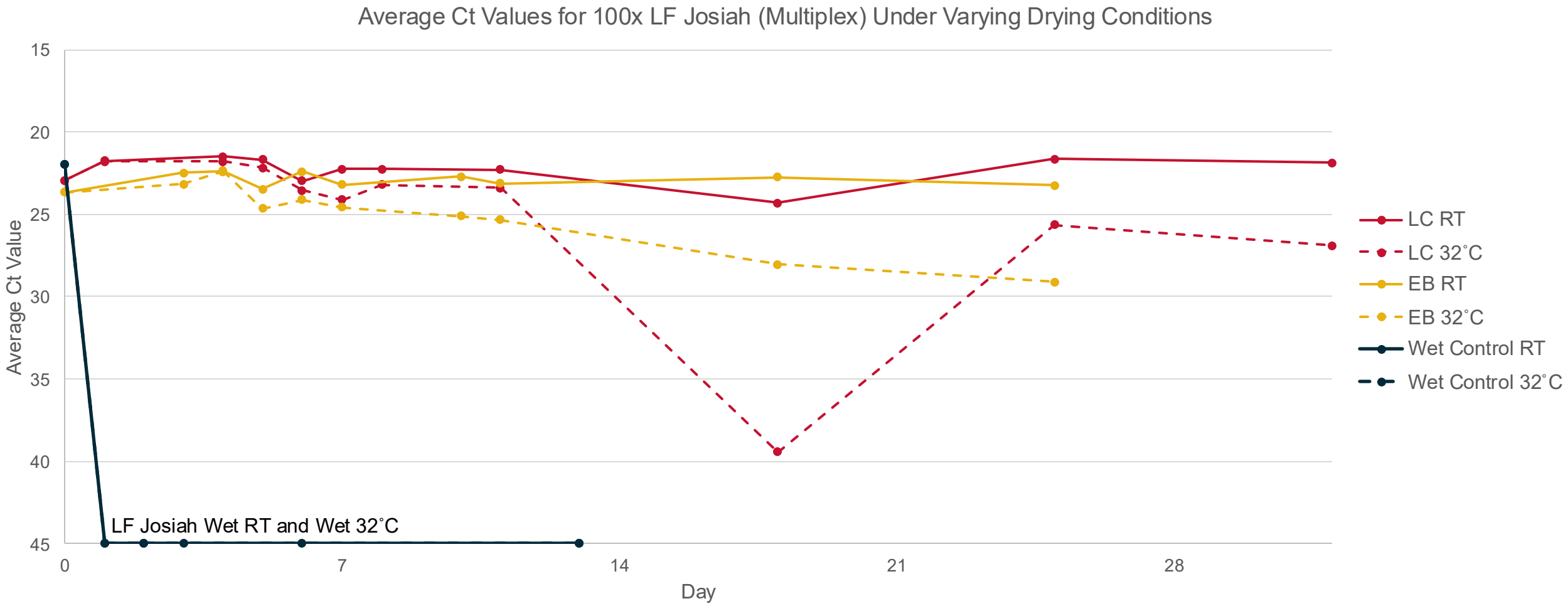
https://pki.jacks.jpeocbrnd.army.mil/OSCAR

Additional Data

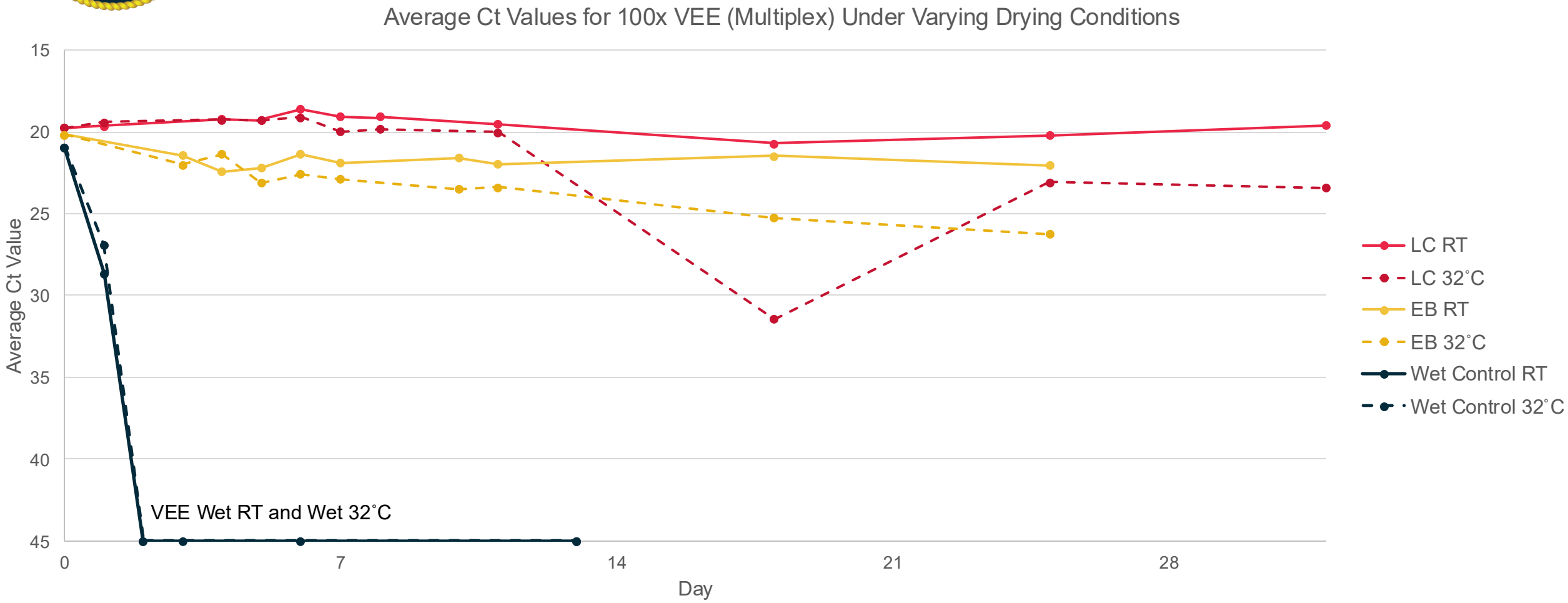
Average Ct Values for 100x EB PAN (Multiplex) Under Varying Drying Conditions



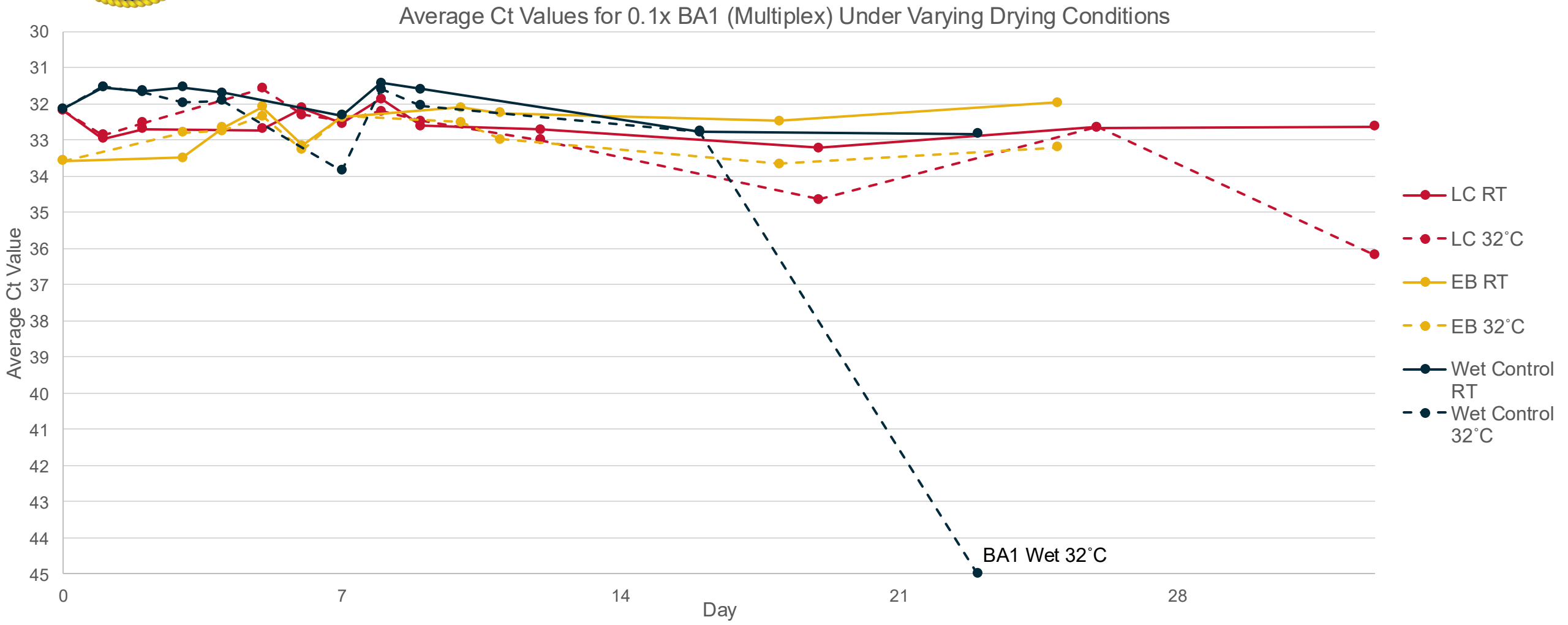
Average Ct Values for 100x LF Josiah (Multiplex) Under Varying Drying Conditions



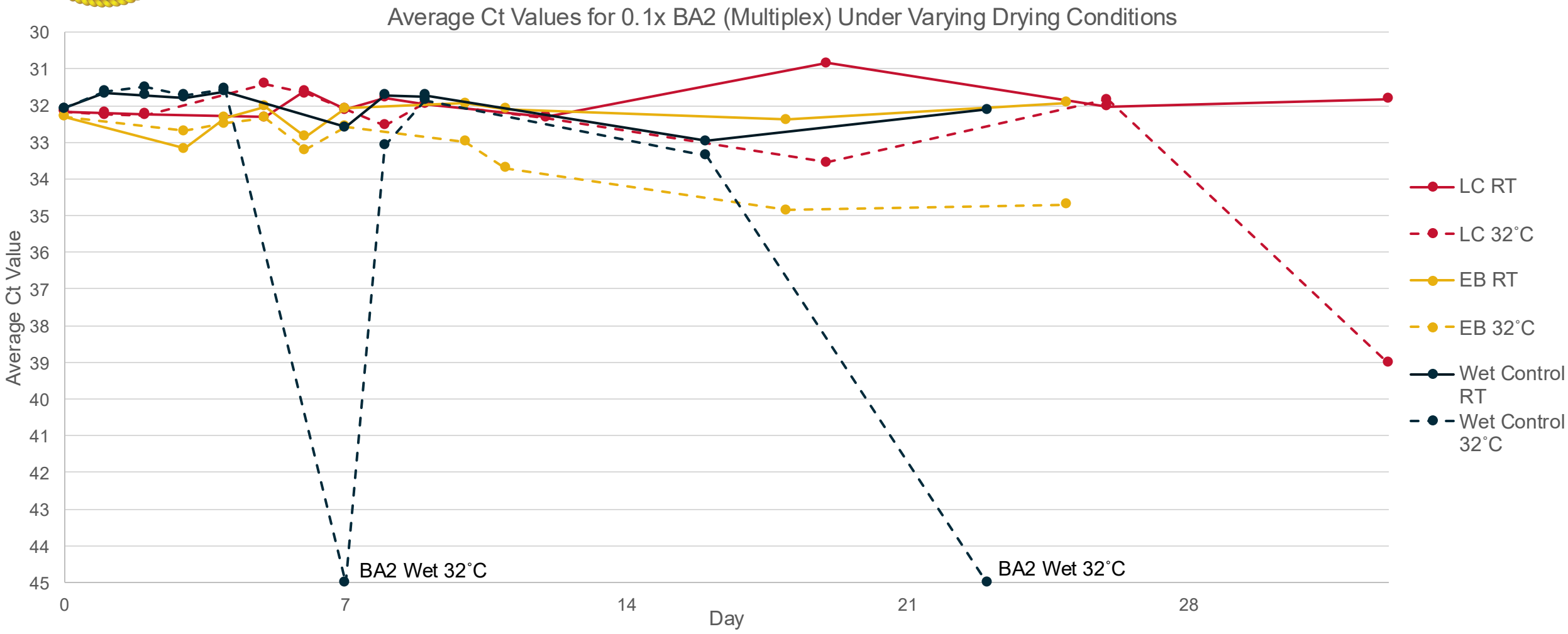
Average Ct Values for 100x VEE (Multiplex) Under Varying Drying Conditions



Average Ct Values for 0.1x BA1 (Multiplex) Under Varying Drying Conditions



Average Ct Values for 0.1x BA2 (Multiplex) Under Varying Drying Conditions



Average Ct Values for 0.1x BA3 (Multiplex) Under Varying Drying Conditions