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Application Note

Station One Thaw Station

Genomic Assay Validation Case Study

Abstract:

The Station One thaw station provides fast, safe and reproducible sample thawing across a broad spectrum of life sciences applications. This customer case study highlights the use of the Station One in the customer's efforts to develop a new biomarker assay. Utilized in multiple capacities throughout the assay process, the Station One helped improve process efficiency, data resolution and process control en route to successful validation of the assay.

Background:

The target assay is a cancer diagnostic assay based on qPCR and a correlative algorithm. The assay process consists of extraction of RNA from tissue samples, RNA quantification, reverse transcritpion, and quantitative PCR against a panel of biomarker genes.

While these core processes were already well established within the company, additional development tasks included:

-Discovery of the most correlative biomarkers
-Manufacturing of test plates for the selected gene panel
-Establishing assay LLT
-Validation of revised RNA quantification process.

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Method:

Biomarker Discovery – Over 3000 potential biomarkers were evaluated for their diagnostic affinity. Primer probe pools for each gene were created and tested against known specimens to evaluate their diagnostic capability.

Early in the discovery phase the customer discovered that roughly 30% of their oligonucleotide stocks had drifted from their initial concentrations due to shortcomings in their sample handling procedures. It was determined that improper thawing and mixing of master stocks had resulted in wide concentration disparities of aliquots taken from these stocks. As a result, data from several large sample arrays was corrupted resulting in redundancy in labor and a potentially missed deadline.

The subject oligonucleotide stocks were stored in Matrix 96 tube racks, and pooled into Eppendorf 96 well microplates. As such four Station One units were purchased for both their speed and capacity for both sample configurations. After running some basic evaluations an optimized thaw/mix procedure was implemented for all handling of oligonucleotide stocks and qPCR reagents.

Points of Focus:

The customer had already established procedural thawing protocols in their manufacturing processes. As such their aim was to apply procedural thawing to component processes of the assay as a QbD measure. Three areas of focus were identified as targets for improvement through addition of procedural thawing:

-Biomarker discovery

-RNA extraction

-RNA quantification

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RNA Extraction – One challenge the customer faced was the small sample size of the tissue specimens received for testing. As such, the most efficient RNA extraction possible was critical to the success of the assay. While the customer's RNA extraction process was highly automated and highly efficient they nonetheless supplemented it with two Station One units, noting its a single thaw capacity of 52 of the 1.7ml tubes they used for housing the accessioned samples. Furthermore Station One offered fast, reproducible thawing of samples from -80C storage to process temperature , helping technicians keep pace with the downstream process while providing more consistent inputs.

RNA Quantification -With the challenge of limited sample quantities also impacting RNA quantification, procedural thawing was applied as part of improving the LLT of their RNA quantification assay. Believing that an optimal thaw protocol would reduce RNA aggregation and improve data resolution, two Station One units were added to the new procedure for thawing the 1.7 ml RNA specimen tubes.

Results:

Biomarker Discovery – After applying the new thaw/mix protocol, randomly tested oligonucleotide aliquots showed improved homogeneity of concentration throughout the data discovery process. A corresponding improvement in data resolution was additionally observed. With handling times and data resolution improved the needed biomarker data was delivered on schedule and yielding a gene panel that ultimately passed assay validation.

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Results:

RNA Extraction – Application of the Station One for thawing accessioned tissues prior to RNA extraction also yielded the desired results. A sustained level of high extraction efficiency was observed in validation trials despite the smaller sample size. The extracted samples yielded RNA quantities safely above the LLT of the quantification assay at a rate exceeding validation targets. A 10% improvement in process speed was also attained.

RNA Quantification – The combination of improved extraction efficiency and reproducible thawing of extracted samples enabled viable data resolution for quantifying extracted RNA. Furthermore low variation between sample replicates revealed improved sample integrity. Sufficient quantities of specimen, and accurate quantification were fundamental to the successful validation of downstream qPCR processes, and ultimately the assay itself.

Notes:

Throughout the development process, the efficiency, versatility and reproducibility afforded by Station One proved indispensable . By yielding high sample integrity the result was enhanced data resolution, decreased variability and tighter process control. To date thousands of patient samples have been successfully tested with this assay with the Station One remaining a core component of the success of the assay and the accurate diagnoses it provides patients worldwide.

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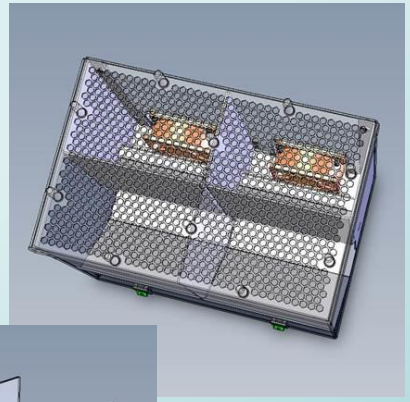
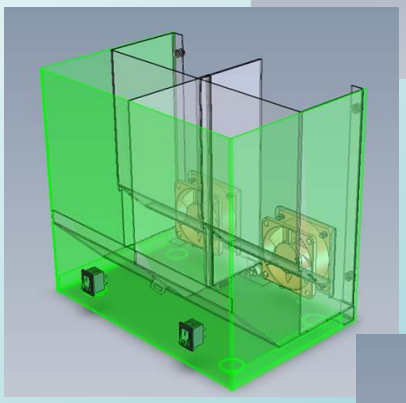
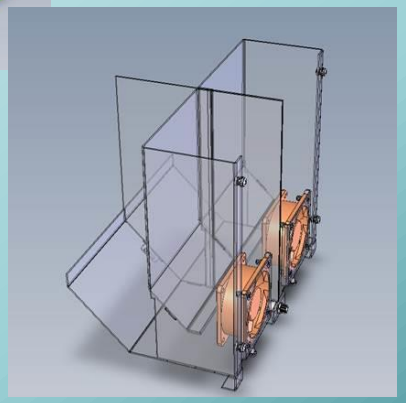
Station One Specifications:

Product:	Station One thaw station
P/N:	1-300-0725-01
Dimensions (cm):	
L	23
W	15.5
H	25.5
Power Source:	120VAC to 12VDC/600mA wall supply
Power Requirements:	12VDC/300mA
Fans	2 x 60mm
I/O	independent fan control switches
Other modes:	none
Max fan airflow (cfm):	38
Weight (lbs):	6
Shipping container:	corrugated box/cut foam insert
Packaged weight (lbs)	14
Package Contents:	Station One unit, small tube accessory (1), large tube accessory (1), manual, power supply (boxed)
Container Dimensions(cm):	
L	43.5
W	37
H	24







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