To: Tina Noyes[tnoyes@theranos.com]; Sharada Sivaraman[ssivaraman@theranos.com]
From: Surekha Gangakhedkar
Sent: Mon 5/6/2013 5:25:47 PM
Importance: Normal
Subject: FW: ELISA data for today
Received: Mon 5/6/2013 5:25:49 PM

From: Surekha Gangakhedkar
Sent: Monday, May 06, 2013 10:21 AM
To: Samartha Anekal; Elizabeth Holmes; Daniel Young; Michael Chen; Paul Patel; Chinmay Pangarkar; Timothy Smith
Subject: RE: ELISA data for today

Hi Sam,

- I believe the sample processing for the centrifuge and the ELISA comes from separate aliquots; so even though the centrifuge tube failed the ELISA sample should have been picked up from the nanotainer. Given that we have been seeing dark counts each time the Omniplex has been run (for PSA and A1c) indicated that there is a separate issue impacting those assay positions. It may best to observe the Omniplex protocol to confirm given that all the calibrations are on a different protocol and have given a response. Is there a reader that can be used for this ?

- There is also an observation that different readers have different run times for the same protocol. See table below for a quick summary. Where are the differences in the elapsed time coming from ?

Device	13A	21A	21A
Log file	L2_C3_ML13A_OmniCal.log_05_03_11_16_38_AM	L1_C2_ML21A_OmniCal.log_05_03_11_29_18_AM	L4_C2_ML
First time stamp	5/3/2013 17:10	5/3/2013 17:08	5/3/2013 1
Last time stamp	5/3/2013 18:16	5/3/2013 18:29	5/3/2013 2
Elapsed Minutes	**66**	**81**	**81**

-Surekha

From: Samartha Anekal
Sent: Friday, May 03, 2013 11:53 PM
To: Elizabeth Holmes; Surekha Gangakhedkar; Daniel Young; Michael Chen; Paul Patel; Chinmay Pangarkar; Timothy Smith
Subject: RE: ELISA data for today

Please see below. As far as I know, we only ran one omniplex run on 5B1, which had the defective centrifuge tube. We were not able to run another run on 5B1 since the blade had to be cleaned up. So, the results which Surekha is reporting below is from the failed run.

From: Elizabeth Holmes
Sent: Friday, May 03, 2013 9:20 PM
To: Surekha Gangakhedkar; Daniel Young; Samartha Anekal; Michael Chen; Paul Patel; Chinmay Pangarkar; Timothy Smith
Subject: RE: ELISA data for today

Sam:

Please comment on your read on root cause for these issues.

Elizabeth

From: Surekha Gangakhedkar
Sent: Friday, May 03, 2013 9:16 PM
To: Elizabeth Holmes; Daniel Young; Samartha Anekal; Michael Chen; Paul Patel; Chinmay Pangarkar
Subject: ELISA data for today

Attached is summary for today:

Calibration

- Vit D – Very noisy, Calibration curve cannot be generated

We have to look into this closer. I know Vitamin D can be a finicky assay, and the timing might be different in this multiplex. We really don't have any understanding of the kinetics of this assay.

- PSA, HbA1c – A few outliers, but response looks encouraging

- TSH –No response, troubleshooting issues

We narrowed it down to either coated tips which have become bad (this is the same batch from which we ran previous 4-plex assays), or the conjugate is inactive, or both. Runs on the M5 showed no signal. Tina is looking closer into this.

- 7 errors in 20 runs; 2 due to round vessel pick up; 1 – color strip dropped; 4 – unknown

The first failures in the morning were due to an error in manually changing the protocol. The dropped color strip is because of this as well. Karthik and I have already worked this out with Sekhar, and starting next week, this will be rolled into the protocol automatically.

Regarding the round vessel pickup, the moment we have a sufficiently large batch of the new round vessels, we will transition over to the new cartridges which should eliminate this issue. Currently, the number of available round vessels is too small for us to make this transition.

Demo data:

- First run on 5B1 – RLU only for Vitamin D; PSA & A1c counts below 100. Something must be different with the multiplex protocol (including the CBC), because with the calibration protocol (GC+ ELISA) we see a response for the 3 assays. Similar issues were seen in previous demos.

L00|VitD|1|pmt|PMT	L02|PSA|1|pmt|PMT	L04|HbA1c|1|pmt|PMT	L06|TSH|1|pmt|PMT
6366	44	66	188

Again, I think this is the run with the leaky centrifuge vessel, which explains all the dark counts. Vitamin D gives positive count even if there is no sample present (competitive assay?). We have to repeat this run before we conclude that the protocol is faulty.

- Demo sample run on ML6B1 kept failing during the CBC sample prep step. Omniplex protocol was not run before on ML6B1, may need optimization ?

ML 6B was not configured (PXE, Normandy app, etc.) to run the full omniplex. It doesn't even have a cytometer. Ameet tried to make some changes so that the imaging portion would be ignored and we could run the rest of the multiplex, but it still erred out. I will check with the cyto team on how to bypass imaging so that we can run it in future.

- Second demo cartridge on Minilab5B1 not run – due to blood spill inside the blade so that device is down for cleaning and sanitization.

-Surekha