Message

From: Adam Rosendorff [/O=THERANOS ORGANIZATION/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=ADAM ROSENDORFD92]
Sent: 1/16/2014 7:54:07 PM
To: Sharada Sivaraman [ssivaraman@theranos.com]; Erika Cheung [echeung@theranos.com]
Subject: RE: QC for EDISONS

Thanks-

What should we do in cases where we get dark counts with the QC runs? Then we would be more limited in chosing which cartridges to disregard.

Adam

From: Sharada Sivaraman
Sent: Thursday, January 16, 2014 11:52 AM
To: Adam Rosendorff; Erika Cheung
Subject: RE: QC for EDISONS

Yes I do agree with you that intra Cvs are not relevant. I was highlighting to you that when evaluated independently each cartridge has tight Cvs in both Conc and RLU regimes which indicates that the assay itself does not have imprecision.

At this time our outlier removal procedure is manual (we can remove 2 out of the 6 data points) and it also depends on the dataset. See example below from the original dataset that Erika sent out.

I chose the cartridges in green for exclusion since they improved conc CVs.

Patient ID/Gender	Date/Time	Barcode	Reader	All Tips		Dark Exclusion		Intra-Cartridge		Inter-Cartridge		Calibration	Concentration		
				Tip 1	Tip 2	Tip 1	Tip 2	Mean	%CV	Mean	%CV		Tip1	Tip2	Mean
QC Level 1	41649	928245663500300317	E00101	65593	6850	59 3	85 0	672 21.5	0.03 426 1	633 88.33	0.06 940 6	3	15.1 485 2	11.9 912 7	17.2 344 4
		928245663500300318	E00230	57474	6619 3	47 4	19 3	618 33.5	0.09 970 8			3	22.8 275 2	14.5 758 5	
		928245663500300319	E00312	59005	6321 5	00 5	21 5	611 10	0.04 871 4			3	21.3 677 4	17.3 976 9	

			E00		71	67	71	694	0.05		0.12		13.7	8.80	19.5
QC Level	4164	928245663	023	670	93	01	93	72.	010	609	567		898	538	162
1	9	500300329	0	11	4	1	4	5	7	64	9	3	1	3	2
			E00		61	50	61	558	0.13				29.7	19.3	
		928245663	010	505	19	50	19	45.	534				574	033	
		500300330	1	01	0	1	0	5	2			3	3	6	
			E00		59	56	59		0.03				24.1	21.3	
		928245663	031	561	00	14	00	575	519				118	658	
		500300331	2	41	7	1	7	74	9			3	9	4	
			E00		34	25	34	300	0.19	326	0.10		66.0	50.2	52.7
QC Level	4165	928245663	010	258	14	87	14	09.	507	35.3	470		542	970	647
2	0	500300341	1	70	9	0	9	5	6	3	2	3	2	9	2
			E00		35	34	35	348	0.01				49.6	48.7	
		928245663	031	345	13	55	13	42.	166				590	714	
		500300342	2	55	0	5	0	5	9			3	3	2	
			E00		32	33	32		0.00				51.7	52.3	
		928245663	023	332	88	22	88	330	735				844	525	
		500300343	0	26	2	6	2	54	9			3	9	8	

I believe an algorithm for outlier removal has been incorporated in the software app process when evaluating clinical samples in Normandy.

Karthik in Daniel's group can give you details of this protocol/algorithm.

Thanks,

Sharada

From: Adam Rosendorff
Sent: Thursday, January 16, 2014 11:14 AM
To: Sharada Sivaraman; Erika Cheung
Subject: RE: QC for EDISONS

Sharada

Since 3 devices are being used as one testing system, for the purposes of QC we must consider them as one testing system/device. Therefore the within cartridge CV is not relevant. As you are aware, we are calculating the mean of the 6 measurements (or minimum 4) to come up with the assay value, and the SD between the means to come up with the %CV (SD/mean).

In considering QC, if the CVs are very high or the ranges overlap, which 2 data points (which 1 out of 3 cartridges) should we remove from the dataset for QC going forward? IS there a protocol for this?

Thanks,

Adam

From: Sharada Sivaraman
Sent: Thursday, January 16, 2014 9:39 AM
To: Adam Rosendorff; Erika Cheung
Subject: RE: QC for EDISONS

Hi Adam,

The Cvs are high between devices, if you see for the data in the daily QC the within cartridge Cvs are really tight.

Since we are allowed to remove 2 data points from 6 (1 cartridge out of 3), I have attempted to do that with both the level 1 and 2 data and it improves things. See attached file and

also see below table with revised QC ranges. We still pass.

	Mean	SD	2SD	Low	Hi	%CV
Level 1	18.49	5.009772	10.01954	8	29	27
Level 2	45.40	5.390087	10.78017	35	56	12

Erika please confirm but it appears that we have used the calibration in tab "Dexter 3" that is the calibration for these set of readers (101, 230, 312).

If this is correct then precision/accuracy at LLOQ and ULOQ are within acceptance criteria. See below:

Model Type	**LogLin 4PL**					Date	**10-Jan-14**
Model Equation	**log10(RLU) = b1+(b2-b1)/(1+(Conc/b3)^b4**					Name	**UD**
Calibration Equation						Cartridge Lot	**Set #1**
						Analyte	**Vitamin D**
						Units	**ng/mL**
						Cartridge Id	**Set #1**
	If RLU <= SignalMax & RLU >= SignalMin Then						
	Conc = b3 * (((b2 - b1) / (log10(RLU) - b1)) - 1) ^ (1 / b4)					Dilution	**1.00**
	End If						
						Reagent Lot	**1**
						desired LLOQ	**10.00**
						desired ULOQ	**300.00**
						max LLOQ	**10.00**
LLOQ	**10.00**	**ng/mL**	Model Parameters		SE	min ULOQ	**300.00**
ULOQ	**300.00**	**ng/mL**	**b1**	**3.497**	**0.056**		
desired LLOQ	**10.00**	**ng/mL**	**b2**	**4.890**	**0.025**	Data Grouping	**by tip**
desired ULOQ	**300.00**	**ng/mL**	**b3**	**101.363**	**5.866**		
LLOD	**Not Reported**	**ng/mL**	**b4**	**1.523**	**0.124**	Precision criteria for estimation of LLOQ	**On**
LLOQ accuracy	**122**	**%**	**b5**	**#N/A**	**#N/A**		
LLOQ precision	**12.2**	**%**					
Average Residuals	**6**	**%**					
Error in prediction: Best case	**10**	**%**				Preferred models (if any)	
Error in prediction: Expected	**10**	**%**					
Signal Min	**4827**	**RLU**				fingerstickmultiplier	**1.00**
Signal Max	**72664**	**RLU**				venousmultiplier	**1.00**
						plasmamultiplier	**1.00**

Outliers detected based on within-standard variance and manual detection	Outliers		ULOQ accuracy	**100**	%	srerun_lo	**250**
	Conc	RLU	ULOQ precision	**9.0**	%	srerun_hi	**50000.0**
Statistically Detected ■	**10**	**56319**					
Manually Selected ■	**10**	**80350**					
	50	**46538**					

50 41310

Let me know if this is acceptable.

Sharada

From: Adam Rosendorff
Sent: Wednesday, January 15, 2014 6:24 PM
To: Erika Cheung
Cc: Sharada Sivaraman
Subject: RE: QC for EDISONS

Erika

The CV% at 18 ng/mL is way to high (~33%)- according to our validations it should be around 20%. The CV at the LLOQ (5) should not even be this high. This is messing up our QC ranges:

Level	mean	SD	CV%	2SD	lo	hi
Level 1	17.69	5.82175	32.9045	11.6435	6.05	29.34
Level 2	45.14	5.64918	12.5144	11.2984	33.84	56.44

I am concerned that the high value for level 1 is quite close to the lo value for level 2.

@Sharada- can you weigh in on a possible solution?

Thanks,

Adam

From: Erika Cheung
Sent: Wednesday, January 15, 2014 6:02 PM
To: Adam Rosendorff
Subject: RE: QC for EDISONS

I just figured out we didn't get the QC range established for the new set of Vitamin D. The data is attached for vitamin D under the tab QC. Can you send me this information too. I need the mean as well has the high low values. I'll create a standard place to put all the QC information now in the J drive.

Thanks,

Erika

From: Adam Rosendorff
Sent: Wednesday, January 15, 2014 5:44 PM
To: Erika Cheung
Subject: RE: QC for EDISONS

Erika

Do you happen to have the Vitamin D ranges? And can we put the active and prior QC data in a dedicated folder in CLIA?

Thanks,

Adam

From: Erika Cheung
Sent: Wednesday, January 15, 2014 5:40 PM
To: Adam Rosendorff
Subject: RE: QC for EDISONS

Beautiful, thanks Adam!

From: Adam Rosendorff
Sent: Wednesday, January 15, 2014 4:59 PM
To: Erika Cheung
Subject: QC for EDISONS

Level	Lo	Hi
Level1	101.39	116.63
Level2	419.28	566.32
Level3	759.36	931.21

Total Testosterone

Hepatitis C Virus

CTRL	average	SD	2SD	lo	hi
2 (POS)	2.55	0.40	0.79	1.76	3.34
3 (NEG)	0.94	0.13	0.27	0.67	1.21
5 (POS)	1.57	0.15	0.30	1.27	1.88

Free T4

Level	Lo	Hi
Level 1	1.008265	2.088112
Level 3	4.28746	5.936169

TSH

Daily QC	Mean	Low	High	CV%
Level 1	0.557	0.38	0.734	15.883
Level 2	4.549	3.403	5.695	12.591
Level 3	22.741	17.153	28.328	12.284

tPSA

	Mean	1 SD	2SD	Low	High	%CV
Level 1	1.06	0.19498	0.389959	0.67	1.45	18.44082
Level 2	4.39	0.607102	1.214205	3.17	5.60	13.83328
Level 3	35.97	7.246318	14.49264	21.48	50.46	20.14532

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