

Message

From: Adam Rosendorff [/O=THERANOS ORGANIZATION/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=ADAM ROSENDORFD92]
Sent: 2/23/2014 8:22:52 PM
To: Mark Pandori [/o=theranos organization/ou=exchange administrative group (fydibohf23spdlt)/cn=recipients/cn=mark pandori16d]
Subject: RE: Morning HDL Study Tecan Dilution Results

Thanks you too

Sent from my Windows Phone

From: Mark Pandori
Sent: 2/23/2014 12:09 PM
To: Adam Rosendorff
Subject: RE: Morning HDL Study Tecan Dilution Results
Yes, ok.

I hope you are doing well, this weekend.

Mark

From: Adam Rosendorff
Sent: 2/23/2014 12:05 PM
To: Mark Pandori
Subject: RE: Morning HDL Study Tecan Dilution Results
Yes but this relationship is preanalytic and these analytes are unlinked ex vivo

Sent from my Windows Phone

From: Mark Pandori
Sent: 2/23/2014 11:51 AM
To: Adam Rosendorff
Subject: RE: Morning HDL Study Tecan Dilution Results
Yea, ok.

Seems correct though, in terms of patient profiles too. People whose hdl are really higher, might actually have lower ldl.

I mean, if you had to predict.?

Just talking here.

Mark

From: Adam Rosendorff
Sent: 2/23/2014 11:48 AM

To: Mark Pandori

Subject: RE: Morning HDL Study Tecan Dilution Results

LDL would go down and the rest would go up if we switched. We should look at the LDL QC data to see if ADVIA 3 QC is consistently above the mean?

Sent from my Windows Phone

From: Mark Pandori

Sent: 2/23/2014 11:45 AM

To: Adam Rosendorff

Subject: RE: Morning HDL Study Tecan Dilution Results

Seems switching instruments would resolve virtually all of it.

QC bias remains a mystery, but that on top of instrument bias might explain phenomenon.

?

Mark

From: Adam Rosendorff

Sent: 2/23/2014 11:42 AM

To: Mark Pandori

Subject: RE: Morning HDL Study Tecan Dilution Results

Hi Mark

For sure the instrument bias is exacerbated with the p-protocols.

I think the qc bias has been quite steady since at least 9/29

Adam

Sent from my Windows Phone

From: Mark Pandori

Sent: 2/23/2014 11:27 AM

To: Adam Rosendorff

Subject: RE: Morning HDL Study Tecan Dilution Results

Has the Qc data revealed more bias in Feb than December?

My sense is that instrument bias is the major cause, and that for some reason, p protocols are more susceptible to the problem.

Mark

From: Adam Rosendorff

Sent: 2/22/2014 9:55 PM

To: Mark Pandori

Subject: FW: Morning HDL Study Tecan Dilution Results

Hi Mark

Forgot to cc: you on this email.

Cheers,

Adam

From: Adam Rosendorff

Sent: Saturday, February 22, 2014 9:49 PM

To: Daniel Young; Nicholas Haase; Paul Patel; Rose Edmonds; Xinwei Sam Gong; Curtis Schneider; Nishit Doshi

Subject: RE: Morning HDL Study Tecan Dilution Results

Daniel

Thanks for your analysis. I have gone ahead and calculated an instrument bias and a "method" bias. To eliminate instrument bias from confounding the method bias analysis, ADVIA 3 p-protocols (FS) were compared with paired data also from ADVIA 3 for both p-protocols (venous) and predicate protocols (venous.) So the Method Bias is all from the same instrument.

The data is summarized below:

Instrument Bias

	A3-A1	%Bias (A3-A1/A3)
p-Chol FS	-15.3	-8.1
p-HDL FS	-9.3	-16.4
p-LDL FS	8.3	6.5
p-TRIG FS	-16.9	-19.8
p-Chol V	-11.5	-6.1
p-HDL V	-8.1	-8.1
p-LDL V	11.6	8.7
p-TRIG V	-16.1	-20.7
Chol V	-3.8	-1.9

HDL V	-6.3	-10.9
LDL V	-1.4	-1.4
TRIG V	-1.96	-2.7

Method Bias

	p-Chol V	Chol V	p-HDL V	HDL V	p-LDL V	LDL V	p-TRIG V	TRIG V
p-Chol FS	0.4	2.9	NA	NA	NA	NA	NA	NA
p-HDL FS	NA	NA	-2.3	1.1	NA	NA	NA	NA
p-LDL FS	NA	NA	NA	NA	-0.1	17.5	NA	NA
p-TRIG FS	NA	NA	NA	NA	NA	NA	8.1	15.2

Conclusions:

(1) Instrument bias:

In general, instrument bias is greater than method bias. In terms of p-protocols (FS as well as venous) ADVIA 3 underreports Chol, HDL and Trig values and overreports LDL values. The differences between ADVIA 3 and ADVIA 1 diminish significantly when looking at predicate protocols for all four analytes. Thus simply moving from ADVIA 3 to ADVIA 1 may not be the answer as 3 assays would increase (Chol, HDL and Trig) and one would decrease (LDL). I'd like to understand more about why the p-protocols exaggerate instrument to instrument variability (at least shown by the current data). Please note that all 3 HDL QC levels for ADVIA 3 (has been used exclusively for FS patient samples) and for ADVIA 1 (has been used exclusively for venous samples) have both been running on the low side, thus low QC cannot explain ADVIA 3 HDL underreporting versus ADVIA 1.

(2) Method bias

The p-protocol values (FS and venous), for Cholesterol and HDL match very well with the predicate values. However, what is most interesting is that the p-protocol values (FS and venous) for Triglycerides and LDL show a significant positive bias relative to their respective predicate values. One can speculate on why this might be the case.

(3) Spate of HDL values in 20's and 30 in February

The recent spate of HDL values in the 20's and 30's, and the fact that the February p-HDL average is now 46.1 (excluding obviously reruns) as opposed to around 65 for December and January, could be the result of the following:

- (1) Hemolysis (bilirubin quenches H2O2 that is measured in the Siemens HDL assay)- perhaps due to preanalytic factors such as specimen collection or transportation.
- (2) Statistical aberration (only 17 patients have been tested so far as opposed to ~30 for Dec and Jan)
- (3) Subtle changes in Siemens HDL reagent that would not affect the predicate assay but would affect the p-protocols (eg surfactant concentration, azide concentration).

Thanks all for looking into this. Please let me know if you have other comments. I am attaching Daniel's spreadsheet together with my additional analysis.

Adam

From: Daniel Young

Sent: Friday, February 21, 2014 6:32 PM

To: Nicholas Haase; Adam Rosendorff; Paul Patel; Rose Edmonds; Xinwei Sam Gong; Curtis Schneider; Nishit Doshi

Subject: RE: Morning HDL Study Tecan Dilution Results

See plots in the attached. My summary is:

- 1) p-HDL on fingerstick and Venous are very comparable
- 2) p-HDL on fingerstick compares very well to predicate on Venous (better for Advia 3 than on Advia 1)
- 3) p-HDL and predicate method both on Venous show some differences at the high HDL concentrations, but are well correlated (performance is worse on Advia 1)
- 4) Advia 1 is reading 10% to 15% higher than Advia 3 for all sample types and HDL protocols
- 5) We do not see a problem of abnormally low HDL results
- 6) There was one low result for one replicate; this may have been a Tecan related issue since it affected all the lipid assays similarly; Nick is checking into this

Please let me know if you have any comments.

Thanks,

Daniel

From: Nicholas Haase

Sent: Friday, February 21, 2014 5:03 PM

To: Daniel Young; Adam Rosendorff; Paul Patel; Rose Edmonds; Xinwei Sam Gong; Curtis Schneider; Nishit Doshi

Subject: Morning HDL Study Tecan Dilution Results

Everyone,

Attached are the tabulated results from the Tecan-diluted Fingerstick and Venous samples from this morning's study, as well as the predicate venous results.

The key is as follows: P-xxxx = P-protocol; A3-1 = Advia 3 rep 1 (and so on).

The last three subjects were not fasting, hence the coloration of their patient numbers. Only one sample returned an HDL result below 35 mg/dL, and that one was low for all analytes. This is due to over-dilution, from either the Tecan picking up gel or hitting some plasma on the sidewall of the pCTN.

Let me know if anyone would like calculations and/or plots of anything in particular.

Overall, we did not see any low HDL samples, omitting the aforementioned exception. Advia 3 tends to report lower values than Advia 1, but there are different reagent lots on the instruments.

Let me know if anything else is needed. We are currently doing manual sample dilutions.

Regards,

Nick H.