Message

From: Elizabeth Holmes [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=EHOLMES]
Sent: 3/6/2009 7:45:16 PM
To: Cullen, Constance [constance.cullen@spcorp.com]
Subject: RE: Follow up to our call

Hi there,
Take a look at the attached as per our conversation – if we go down this path there are two sets of documents that will complement this summary – one is the detailed protocol for running these experiments, the other is the Cartridge Inserts that detail performance specifications.
Elizabeth.

From: Cullen, Constance [mailto:constance.cullen@spcorp.com]
Sent: Wednesday, February 25, 2009 8:42 AM
To: Elizabeth Holmes
Cc: Carolyn Balkenhol
Subject: RE: Follow up to our call

Elizabeth,

It's good to hear. I am free after 1:00 PM eastern.

Thanks. Connie

> -----Original Message-----
> **From:** Elizabeth Holmes [mailto:eholmes@theranos.com]
> **Sent:** Tuesday, February 24, 2009 7:13 PM
> **To:** Cullen, Constance
> **Cc:** Carolyn Balkenhol
> **Subject:** RE: Follow up to our call
>
> Connie,
> I had a good call with Jim McLeod today. Let me know a convenient time to follow up with you and I'll give you a call tomorrow,
> Elizabeth.

theranos
redefining healthcare

Theranos™ System Full Validation Protocol: (hs)CRP, IL-6, TNF-a multiplex

References/Background

- ICH Q2 (R1) "Validation of Analytical Procedures"
- FDA CDER "Bioanalytical Method Validation"
- Binodh DeSilva et al. Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules. J. *Pharmaceutical Research, Vol. 20:1885-1900 (2003)*
- Previous programs for development of diagnostic systems undergoing regulatory approval

Generally recommended parameters for investigation

1. Calibration Curve
2. Accuracy
3. Precision
4. Specificity
5. Quantitation limit
6. Linearity
7. Range
8. Stability

Reference methods

Proposed reference method for each assay --TNF-alpha, IL-6 and CRP (both high and low sensitivity) -- is R&D Systems kits. Performance specifications are included in the Cartridge Inserts in each Cartridge box.

Proposed analysis

Detailed analysis protocols will be sent prior to evaluation initiation.
The calibration curve (1) can be assessed using 8 points (one blank, one zero spike and 6 spikes at levels, including LOQ's) in triplicate on each instrument. This can be done over three days (not necessarily consecutive) to generate data on reliability.

For accuracy (2), precision (3), and specificity (4), we recommend using 10 instruments and 120 cartridges per instrument. 20 cartridges will be run at each of the LLOQ, medium range and ULOQ levels for each sample type (plasma & whole blood). This will yield a strong dataset for statistical analysis of accuracy, precision and specificity.

For quantitation limit (5) verification, one can run 6 levels of spiked plasma around the stated limits of quantitation. We propose testing three levels above and three below the LLOQ and ULOQ, respectively, in triplicate on each instrument.

For linearity (6), we recommend using plasma and spiked analytes and testing 5 levels in triplicate on each instrument.

For range (7), we recommend testing 6 points around the claimed LLOQ and ULOQ to gauge response at the boundaries of the assay(s). This will also be done in triplicate on all instruments.

For stability (8), we test under 2-8℃ conditions, for time points 0, 1, 2, and 3 months and run tests in triplicate at each of the LLOQ, mid-range and ULOQ levels of the assay(s). We recommend using plasma for these experiments as it can be stored for the duration of testing.

NOTE: The aforementioned numbers assume statistical significance for three analytes as each cartridge is multiplexed. The processing requirement is for each sample tested to contain a mix of all three analytes to be measured, in known quantities. Such samples may include archived clinical samples, spiked samples, or may be purchased. Each cartridge requires ~20uL of sample.

Summary of runs per instrument to achieve statistical significance

Calibration Curve	Accuracy/Precision					
In plasma	In plasma			In whole blood		
8 point calibration	At Mid-range	At LLOQ	At ULOQ	At Mid-range	At LLOQ	At ULOQ
72	20	20	20	20	20	20

Linearity	Range around Limits		Stability (3months)		
In plasma	In plasma		In plasma		
	at LLOQ	at ULOQ	At Mid-range	At LLOQ	At ULOQ
15	18	18	12	12	12

Estimated schedule

Standard Theranos instrument run-time will be accelerated for this program.
Total number of cartridges provided will be 2,790 of the TNF-alpha, IL-6, CRP multiplex. Up to 10 additional instruments will be shipped. The total expected runtime (not including sample load, reference testing and the stability duration) is around 1 month. The total human capital commitment will only be 5 days over the entire program duration as it only takes up to 10 minutes to prepare and load a sample on a single instrument, after which the instruments run on their own.