Message

From: Adam Rosendorff [/O=THERANOS ORGANIZATION/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=ADAM ROSENDORFD92]
Sent: 10/29/2014 8:43:01 PM
To: arosendorff@[redacted]
Subject: FW: IQP/AAP Decisions and Plan Forward

From: Daniel Young
Sent: Monday, October 13, 2014 11:07 PM
To: Sunny Balwani; Elizabeth Holmes; Chinmay Pangarkar; Nishit Doshi; Suraj Saksena; Adam Rosendorff
Subject: RE: IQP/AAP Decisions and Plan Forward

Please review the attached proposed updates for AAP that Chinmay and I updated based on some of our latest experience and best practices:

This protocol was written for our ELISA assays and is currently being similarly extended to cytometry and then to GC.

Thanks,
Daniel

-----Original Appointment-----
From: Sunny Balwani
Sent: Thursday, October 09, 2014 5:25 PM
To: Sunny Balwani; Elizabeth Holmes; Daniel Young; Chinmay Pangarkar; Nishit Doshi; Suraj Saksena; Adam Rosendorff
Subject: IQP/AAP Decisions and Plan Forward
When: Tuesday, October 14, 2014 5:00 PM-6:00 PM (UTC-08:00) Pacific Time (US & Canada).
Where: CONF.1601.Tricorder

Alternate assessment program (AAP) for Theranos ELISA assays

1. **Purpose**: This document details the standard operating procedures to be followed for Alternate Assessment Program (AAP) of Theranos ELISA assays. The AAP is an internal program designed to demonstrate that performance of Theranos ELISA assays meet assay requirements on a periodic and regular basis. The program is intended to be implemented three times per year. The AAP is intended to substitute for Proficiency Testing (PT) when traditional PT is not able to be performed.

2. **Background:** Proficiency testing (PT) helps ensure comparability of clinical test measurements amongst laboratories. Traditional PT is performed with contrived samples that resemble control materials as a substitute for human samples. Due to matrix effects, different analytical systems are known to respond differently to these PT samples, even though they may respond similarly to actual human samples. Consequently, survey organizations grade performance by peer groups, namely, a group of laboratories using the same analysis method for a given measurand. According to this approach, as long as a laboratory obtains answers comparable to other laboratories using the same method, the conclusion is that the laboratory is running their instruments correctly and the laboratory is deemed proficient. Note that method accuracy/comparability is not assessed in traditional PT programs.

   AAP are developed only when traditional PT programs are not available for the assays offered by the laboratory. This situation arises most commonly where there is no peer group for the analytical method being evaluated. Accordingly, the AAP as described herein was developed to demonstrate adequate ongoing performance of the tests post laboratory developed test (LDT) validation. The predicate methods continue to be active in the CLIA lab and maintain ongoing PT assessments, making them a suitable comparator method for evaluation as described in this protocol.

3. **Scope:** This program covers only quantitative Theranos ELISA assays.

   Comment [DY1]: Will replicate for other assays.

4. **Overview:**
   - 4.1. The program is divided into two main phases:
     - 4.1.1.AAP-baseline: this is intended to be performed once a year
     - 4.1.2.AAP-verification: this is intended to be performed minimally twice a year
   - 4.2. Baseline assessment will have the following outcomes:
     - 4.2.1.Comparability: Comparability of Theranos assay against a predicate will be demonstrated as the bias at the medical decision levels (MDLs) and the mean bias over a set of serum samples. Serum samples are used since it is suitable for both the Theranos assay and the predicate.
     - 4.2.2.Precision: The CV of Theranos assay will be verified for at least two MDLs for each assay.

4.2.3. Matrix comparison: Equivalence of the assay for fingerstick (EDTA plasma) and venous (serum) samples will be demonstrated by comparing paired samples on the Theranos method and the predicate.

4.3. The AAP-verification phase will involve comparing the Theranos method to historical data in order to verify that there is no change in performance including comparability, precision, and matrix effects.

5. Procedure:

5.1. Sample acquisition: This section describes the requirements for acquiring and storing samples to be used for the AAP studies throughout the year.

5.1.1. Acquire at least 50 serum samples that span the analytical range of the assay. The minimum volume of each sample should be 1ml. If, for any sample, the available volume is less than this minimum volume, then samples can be pooled. Pooled samples will be reassigned on the predicate method.

5.1.2. Verify that there are at least 4 samples that are within 50% of the medical decision levels (MDLs). If this condition is not satisfied, then serum samples will be pooled to contrive samples near the MDL. Pooled samples will be reassigned on the predicate method.

5.1.3. Aliquot each sample into a labeled vessel and store these samples at - 80°C. Five aliquots per sample with a minimum volume of uL each is required.

Comment [DY2]: To be updated.

5.2. AAP Baseline: This phase is divided into two studies:

5.2.1. Baseline Comparability and Precision:

5.2.1.1. Assign each sample on predicate assay. The assigned value is a mean of 5 replicates.

5.2.1.2. Select 4 samples which lie closest to each of the MDLs of the assay. If there are not a sufficient number of samples that lie within 50% of the MDLs, then such samples must be contrived by mixing serum samples in (Ib). Each of these samples will be analyzed in quadruplicate on Theranos method. Therefore, 16 data points will be obtained near each MDL.

Comment [DY3]: To be updated.

5.2.1.3. To ensure that 30 samples are analyzed, analyze an additional 22 samples that span the analytical range in singlicate on Theranos method if 2 MDLs are studied. If 3 MDLs are studied, only 18 additional samples are required.

5.2.1.4. Perform method comparison analysis as outlined in section 6 If the comparability study acceptability criteria are not satisfied, run 10 more samples on Theranos method.

5.2.2. Fingerstick and Venous comparison:

5.2.2.1. Select three in-house donors with analyte values within the assay reportable range of the Theranos and predicate assay ranges.

5.2.2.2. From each donor, acquire 6 fingersticks and 1 EDTA-vacutainer. Analyze each pCTN once and each vacutainer sample 6x.

5.3. AAP verification:

5.3.1. Verification of Accuracy:

5.3.1.1. Select 20 previously analyzed serum samples spanning the analytical range from the samples aliquoted in (I).

Comment [DY4]: To be updated

5.3.1.2. Analyze each serum sample once on Theranos method.

5.3.2. Fingerstick and Venous comparison:

5.3.2.1. Select three in-house donors with analyte values within the assay reportable range of the Theranos and predicate assay ranges.

5.3.2.2. From each donor, acquire 6 fingersticks and 1 EDTA-vacutainer. Analyze each pCTN once and each vacutainer sample 6x.

6. Data Analysis:

6.1. Calculation of bias at MDLs and mean bias:

6.1.1. Assign predicate as "method 1" and Theranos as "method 2" if analyzing AAP baseline data

6.1.2. Assign historical assigned value as "method 1" and Theranos as "method 2", if analyzing AAP verification data.

6.1.3. Use methods from CLSI EP-09 to calculate bias at MDLs and mean bias. For verification phase, only mean bias is estimated.

6.1.4. Obtain the 95% confidence interval on the bias estimates.

6.2. Calculation of assay precision:

6.2.1. Pool the 16 data points obtained from 4 replicates of samples near each MDL.

6.2.2. Calculate precision (%CV) for recovery of these data points.

6.3. Acceptance criteria:

6.3.1. Obtain the total allowable error (%TAE) for the assay from DOCXXXXX

6.3.2. Calculate the total allowable bias as (%TAE - %CV)

6.3.3. For acceptance of baseline bias:

6.3.3.1. Bias at MDLs should be less than the total allowable bias AND

6.3.3.2. At least one bound of the CI_{95} should be within the total allowable bias

6.3.4. If condition 5.3.3.1 above is satisfied, but condition 5.3.3.2 is not satisfied, the number of data points collected should be increased by 10 (refer to 4.2.1.4) and the analysis should be repeated.

6.3.5. For acceptance of verified bias:

6.3.5.1. The mean bias for the verified samples should be no higher than the mean bias calculated during the baseline AAP phase as shown by overlapping CI_{95}.

6.4. Analysis of fingerstick vs venous samples:

6.4.1. Perform a paired t-test on 6 replicates each of vacutainer and fingerstick samples.

6.4.2. If the t-test returns a significant result, compare the mean difference to the %TAE. If the magnitude of the difference is within %TEA, than it meets the assay performance metrics.

6.4.3. Results from all three donors should pass. If there is one failure, collect samples from 2 more donors. In this case, four out of the five donors should pass.