theranos	LDT Validation Report	Theranos Erythrocyte Sedimentation Rate (ThESR), pCTN	Rev:
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Description	Validation Report for Theranos Laboratory Developed Test for Erythrocyte Sedimentation Rate (pCTN)		
Originator: Samartha Anekal		Effective Date: 12/19/2013	Page 1 of 7

Theranos Erythrocyte Sedimentation Rate (pCTN) Assay Validation

Author(s):

Signature:	Date: 09/05/2015
Name: Samartha Anekal, PhD.	Title: Director, Systems Integration and V&V

Reviewer(s)

Signature:	Date: 9/5/2015
Name: Daniel Young, Ph.D.	Title: Vice President

Approver(s):

Signature:	Date: 9/9/2015
Name: Adam Rosendorff, M.D. Hoda A Yamdar for lab director	Title: Laboratory Director

9/19/15

I. Summary

ThESR description

The ThESR assay is intended to report Erythrocyte Sedimentation Rate (ESR) values comparable to the modified Westergren method. The assay is performed with 60-80 uL of EDTA fingerstick blood in standard Theranos Nanotainers. The ThESR assay is performed starting with a well-mixed sample of EDTA blood in standard Theranos Nanotainers, and centrifuging the sample at a low g-force (40g) for a short time duration (90s). The nanotainer is then imaged at high resolution and the images processed to determine the air/plasma and plasma/red cell interface. These are used to extract an accelerated sedimentation rate, to which a calibration is applied to obtain the actual sedimentation rate.

This report describes data collected to validate the ThESR method against a predicate. The predicate method is the Streck ESR Auto Plus, run by a CLIA-certified technician in the Theranos CLIA facility.

Sample details

The data summarized below involves the following samples:

1. Venous blood collected in a standard vacutainer and pipetted into Theranos Nanotainers.
2. Venous blood spiked with fibrinogen and pipetted into Theranos Nanotainers.
3. Capillary blood collected in a Theranos Passive Capillary and Nanotainer (pCTN) system.
4. ESR controls (Vital Diagnostics Inc.) pipetted into Theranos passive Nanotainers

Method details

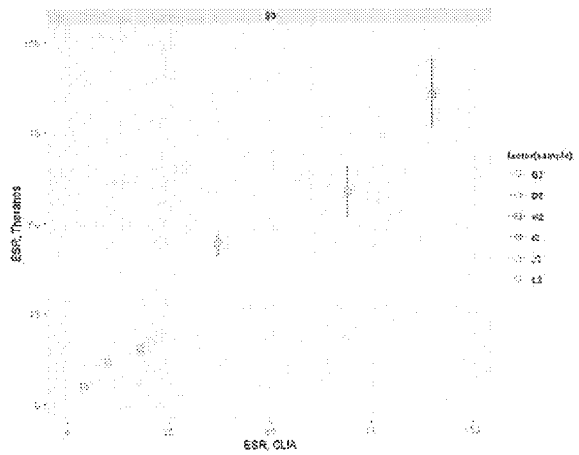
See ThESR SOP document () for a detailed description of the ThESR method.

II. Validation data

The data validation consists of four parts:

1. Precision across replicate measurements done on the same day
2. Precision across replicate measurements done across multiple days
3. Clinical correlation between ThESR and predicate method
4. Reference range verification for Male and Female patients

1. Same day precision



Sample	ESR, CLIA, mm/hr	Mean, Theranos, mm/hr	CV(%), Theranos	Replicates
B2	37	44.1	8.2	20
D2	13	15.3	6.8	20
H2	60	85.1	16.9	20
I2	69	98.3	12.3	20
J2	4	4.6	6.5	20
L2	16	11.5	3.8	20

Figure 1. Same day precision data for venous samples with and without spiked Fibrinogen.

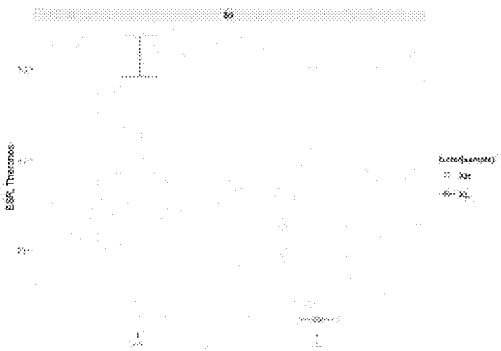
6 EDTA stored anti-coagulated venous blood samples were collected in vacutainers. Blood samples consisted of both fresh draws as well as samples refrigerated for 2-5 days. Some samples were spiked with Fibrinogen to get a range of elevated ESR values. Samples from vacutainers were aliquoted into nanotainers and Streck ESR tubes. The samples in nanotainers are run on the ThESR system, and the Streck samples are run on the predicate instrument. The predicate runs were done in singlicate, and the ThESR runs were done with 20 replicates each.

The plot in Figure 1 shows precision data for the 6 samples described below. Sample SF2 is unspiked. The remaining samples are all spiked with varying levels of fibrinogen to achieve different ESR values. The square points are the mean value of ESR over 20

replicates as calculated on the Theranos system against the predicate value for the corresponding sample. Error bars correspond to one standard deviation calculated over the 20 replicates.

The table in Figure 1 shows the data in more detail. The CV for all samples is less than 15% for all samples, demonstrating good measurement precision.

2. Precision across multiple days



Sample	Mean, Theranos, mm/hr	CV, Theranos, %	Replicates
XH	62.9	7.1	20
XL	4.8	5.3	20

Figure 2. Precision across 5 days.

For precision across multiple days, ESR controls (Vital Diagnostics Inc.) were used as samples. The ESR controls were chosen for inter-day precision as opposed to real samples since the ESR controls are designed to yield a stable ESR value over several weeks. 20 replicates of Control 1 and Control 2 were run on the ThESR system across 5 days (4 replicates per day). Figure 2 shows this data both as a plot and in tabular form. The inter-day precision is less than 10% for both controls, demonstrating good inter-day precision.

3. Clinical correlation

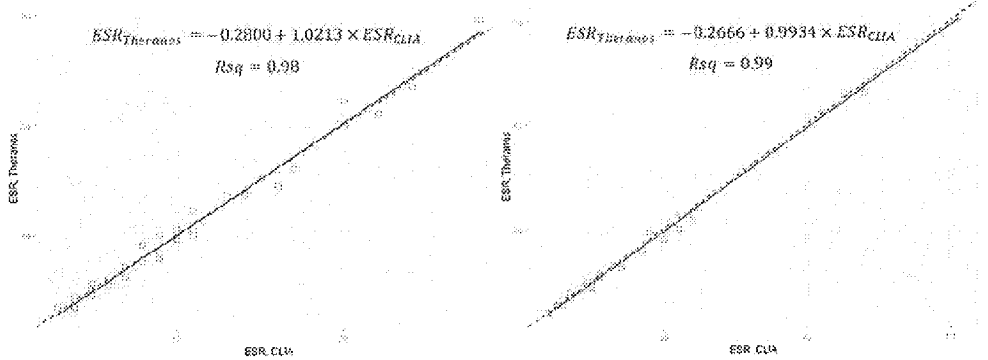


Figure 3. Comparison between Theranos ESR and predicate-measured ESR values for fingerstick (L) and venous (R) blood samples. Solid lines represent linear regressions. Dashed line is the equity plot.

Venous blood samples, both fresh draws and samples refrigerated for 2-5 days were included in this study. Some venous samples were spiked with fibrinogen to simulate ESR values outside of normal clinical range. In total, 34 venous samples were used in this study. For the Theranos ESR method, samples are pipetted into Theranos Passive Nanotainers. For the predicate method, samples are pipetted into Streck ESR tubes.

Fingerstick samples (in Theranos pCTN units) and paired venous samples from 40 subjects were collected. Venous samples were pipetted into Streck ESR tubes for the predicate method.

Method comparison

6 replicates (venous samples) and 2 replicates (fingerstick samples) were processed using the Theranos ESR method. The mean value for the Theranos method was calculated. Corresponding samples were run on the predicate device, and the ESR values were noted. Figure 3 shows the correlation plot for fingerstick (L) and venous (R) samples. The correlation is good, quantified by a coefficient of determination of 0.98 and 0.99 for fingerstick and venous samples respectively. The correlation bias at medical decision levels of 13mm/hr and 20mm/hr are -2.7% and -2.0% for the venous samples and -0.02% and 0.73% for the fingerstick samples.

Fingerstick samples

Fingerstick blood samples(40 samples, 2 replicates each) and venous blood samples (34 samples, 6 replicates each) were taken for this study. The venous samples consisted of both normal and fibrinogen-spiked samples. Fingerstick samples were run on the ThESR system, and the corresponding paired venous samples were run on the predicate device. Venous samples were aliquoted and run on both the ThESR system and on the predicate.
Some of the venous samples were spiked with fibrinogen to obtain elevated ESR values. The correlation for both fingerstick and venous samples is good, as quantified by Rsq>0.98.

4. Reference range verification

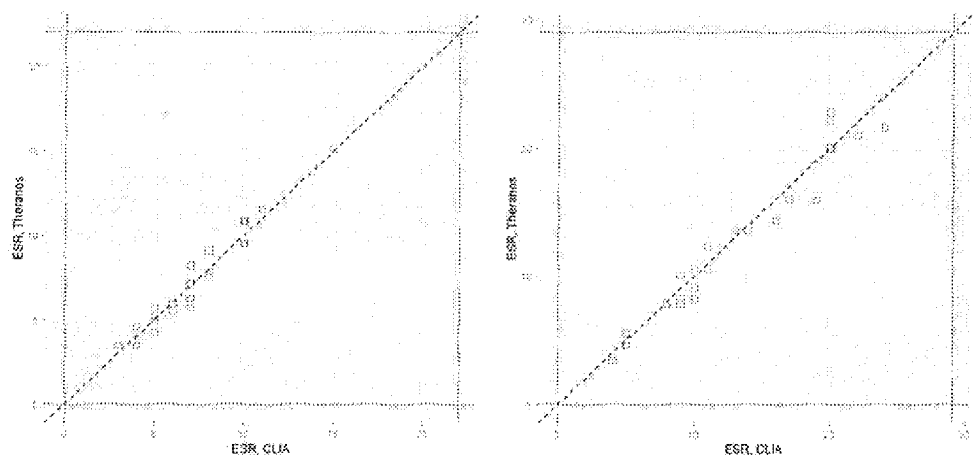


Figure 4. Reference range verification for Male (L) and Female (R) samples. Dotted lines represent reference ranges. Dashed line is the equity plot.

The reference range for the predicate instrument is as follows:

0 – 22mm/hr for men
0 – 29mm/hr for women

Using the correlation reported in Section 3., the reference range for the Theranos ESR method is determined to be:

0 – 22mm/hr for men
0 – 29mm/hr for women

To verify reference range, normal fingerstick blood samples (20 Male and 22 Female), were collected in Theranos pCTN units. Paired venous samples were collected and transferred to Streck ESR tubes for determining the predicate value. Figure 4 shows predicate and Theranos ESR values plotted for these samples. Dotted lines represent reference ranges reported above, and the dashed line is the equity plot. 100% of samples for both Male and Female subjects lie within the reference range.

Revision History			
Revision Level	**Effective Date**	**Initiator**	**ECO/DCO Number**
A	12/20/2014		CL-RPT-15012
Section Number	**Description and Justification of Changes**		