



To: Review Committee Chair

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Sponsor/Product: Instituto Grifols, S.A. /Fibrin Sealant Grifols (FS Grifols) (Human), Product Name: VeraSeal

Subject: In-support testing for Licensing Action of Fibrin Sealant Grifols (Human), STN 125640 using the following tests:

1. Fibrinogen Potency by Total Clottable Protein
2. Thrombin Potency by Clotting Assay
3. Fibrin Sealant Identification and Verification of Functionality

Summary

The fibrinogen and thrombin potencies of three lots of VeraSeal (Fibrin Sealant Grifols (Human)) drug product (STN 125640) were measured using the fibrinogen potency by total clottable protein assay and thrombin potency by clotting assay respectively. The thrombin potency was measured against an in-house reference standard supplied by the manufacturer. The identity and ability of VeraSeal to form a fibrin clot were measured using the fibrin sealant identification and verification of functionality tests. All three lots were within the proposed specifications for the three tests examined.

Background

A request was made by the review committee Chair to measure the fibrinogen potency, thrombin potency, and ability of fibrin clot formation of three lots of VeraSeal drug product using the fibrinogen potency by total clottable protein assay, the thrombin potency by clotting assay and the fibrin sealant identification and verification of functionality test.


1. Fibrinogen by Total Clottable Protein

Method

The fibrinogen contents of three lots (IBND6L3MP1, A4YCB00021 and B4YBB00021) of the drug product were measured at DBSQC/CBER using the Assay for Fibrinogen Potency by Total Clottable Protein method, TM000771 version 02. A WHO international standard (IS) for fibrinogen concentrate (NIBSC Fibrinogen Reference Standard, 09/242) is used as the control.

One determination of each lot of drug product and assay control was measured.

(b) (4)



The total clottable protein in the sample is calculated from (b) (4)



Results

The assay validity criteria and the results obtained are as follows:

- Tests of lot IBND6L3MP1 and lots A4YCB00021 and B4YBB00021 were performed on two different occasions.
 - The potency of the assay controls (WHO IS for Fibrinogen Concentrate; NIBSC 09/242) were 78% and 81% of the assigned Total Protein content. The results were within the DBSQC qualified acceptance range of 71% – 94% of the NIBSC assigned Total Protein content. The %RSD of the assay control measurements were <1%, which met the acceptance criteria, ≤10%.
- The fibrinogen contents of the three lots of VeraSeal, drug product are summarized in Table 1. The proposed BLA specification is (b) (4). Table 1 shows that all results from the three lots were within the proposed specification limits.

Table 1: Fibrinogen potency measurements at CBER using the fibrinogen potency by total clottable protein assay

Sponsor's Specification: (b) (4)

Presentation: 80 mg/mL

Lot Number	Presentation (mL)	¹ Sponsor Results (mg/mL)	CBER Result (mg/mL)	CBER/Sponsor Results Ratio (%)
IBND6L3MP1	5	79	79	100.0
A4YCB00021	3	79	73	92.4
B4YBB00021	2	74	71	95.9

¹Sponsor's fibrinogen potency values were taken from the Certificate of Analysis in 125640/0.14 Section 1.11.1 Quality Information Amendment (Lot Number IBND6L3MP1) and 125640/0.27 Section 1.11.1 Quality Information Amendment (Lot Number A4YCB00021, B4YBB00021)

2. Thrombin Potency by Clotting Assay

Method

The thrombin potency of three lots of VeraSeal drug product was measured at LACBRP/DBSQC/CBER using the thrombin potency by clotting assay as described in DBSQC Document ID # 000785, version 2. The method differs from the manufacturer's described method as detailed in Table 2.

Table 2: Differences between the sponsor's and DBSQC's methods

Differences between methods	Sponsor method	DBSQC method
Sample Dilution Buffer	(b) (4)	10 mM Trisodium citrate dehydrate, 150 mM NaCl, 0.5% BSA, 0.1% Tween 20, pH 5.0
Standard	In-house (b) (4) thrombin standard	WHO 2 nd International Standard for Thrombin, 01/580
Control	In-house thrombin control	Thrombin (Sigma)
Range of Assay (IU/mL)	(b) (4)	20 – 3.75
Instrument	(b) (4)	STA Compact System (Diagnostica Stago)

The in-house secondary standard and control provided by the sponsor was used instead of the WHO International standard (IS), 01/580 and Sigma Thrombin positive control, however, all others steps were carried out as detailed in

Document ID # 000785. Single measurements of the dilution series of standard and two concentrations of the control sample, as well as three independent measurements of one concentration of test sample were carried out within the testing range. The results are reported as the mean of the final activity of two determinations of control and three determinations of test sample. The assay was carried out once.

Reagents supplied by the sponsor

- In-house secondary thrombin standard, batch number (b) (4), calibrated against the (b) (4) Standard for thrombin, (b) (4), is a VeraSeal drug product with an assigned potency of (b) (4).

-In-house control, batch number (b) (4), calibrated against the (b) (4) Standard for thrombin, (b) (4), is a VeraSeal drug product with a labeled potency of (b) (4) (specification limits (b) (4)).

Results

The standard curve was established by linear regression analysis of the plot of log activity (IU/mL) vs log clotting time (sec) of the standard. The results of the assay validity criteria are shown in Table 3.

Table 3: Assay Validity criteria

Assay Validity Criteria (TM 000785)	CBER Results for Assay Validity Criteria
R ² standard (b) (4)	0.986
Potency Control sample (Qualified Control Range)	652 IU/mL (583 – 713 IU/mL)
%RSD triplicate measurements of sample (b) (4)	3.42, 3.71, 3.09 %

Table 3 shows that the R² value of the standard curve, the potency of the control and the %RSD of the triplicate measurements for each lot met the respective acceptance criteria.

The results for the thrombin potency are presented in Table 4.

Table 4: Thrombin potency measurements at CBER using the thrombin potency by clotting assay

Sponsor's Specification: (b) (4)

Presentation: 500 IU/mL

Lot Number	Presentation (mL)	¹ Sponsor Results (IU/mL)	CBER Result (IU/mL)	CBER/Sponsor Results Ratio (%)
IBND6L3MP1	5	455	559	122.9
A4YCB00021	3	498	584	117.3
B4YBB00021	2	494	526	106.5

¹Sponsor's thrombin potency values were taken from the Certificate of Analysis in 125640/0.14 Section 1.11.1 Quality Information Amendment (Lot Number IBND6L3MP1) and 125640/0.27 Section 1.11.1 Quality Information Amendment (Lot Number A4YCB00021, B4YBB00021)

Table 4 shows that the results from all three lots were within the proposed specification. The ratio of CBER's results to that of the manufacturer is 106.5% – 122.9%. In addition, the %RSD of triplicate measurements of test samples ranged from 3.09 – 3.71% which met the acceptance criteria of (b) (4).

3. Fibrin Sealant Identification and Verification of Functionality Method

The identity and fibrin clot formation ability of three lots of VeraSeal drug product was measured at LACBRP/DBSQC/CBER using the Fibrin Sealant Identification and Verification of Functionality assay as described in STN 125640 Document ID # IG_MA-000664_ING, version 6.0. The identity was examined (b) (4)

(b) (4)

For each test, three independent measurements of each lot were made and the results reported as the mean of the three determinations. The assay was carried out once.

Results

The results for the identity and functionality tests are presented in Table 5.

Table 5: Identity and fibrin clot formation measurements at CBER using the fibrin sealant identification and verification of functionality

Method

Sponsor's Specification:

Identity Test: Clot formation

Functionality: Clot formation (b) (4)

Lot Number	Identity Test	Functionality Test
		(b) (4)
IBND6L3MP1	Clot formation	(b) (4)
A4YCB00021	Clot formation	
B4YBB00021	Clot formation	

Table 5 shows that the results from all three lots were within the proposed specification for both tests.

Conclusions

The results presented in Tables 1, 4 and 5 show that all three lots of VeraSeal drug product tested using the fibrinogen potency by total clottable protein assay, the thrombin potency by clotting assay and the fibrin sealant identification and verification of functionality test were within specifications for the respective assays.