

MEMORANDUM

To: Administrative file for STN 125640/0
Natalya Ananyeva, PhD, Chair of the review committee, OTAT/ DPPT/ HB
Yu Do, Regulatory Project Manager, OTAT/DRPM/RPMBI

From: Svetlana Shestopal, PhD, OTAT/ DPPT/ HB

Through: Andrey Sarafanov, PhD, OTAT/ DPPT/ HB
Tim Lee, PhD, Acting Branch Chief, OTAT/ DPPT/ HB
Basil Golding, MD, Director, OTAT/ DPPT

Applicant: Instituto Grifols, S.A.

Product: Fibrin Sealant (Human)

Subject: Final review of the *Control of materials, Excipients, Analytical procedures, and Reference Standards or Materials* sections

1. Executive summary

This memorandum summarizes the review of several aspects of the Chemistry, Manufacturing and Controls in an original Biologics License Application (BLA) and amendments under STN 125640/0 submitted by Instituto Grifols, S.A. (Grifols) on November 4, 2016, for Fibrin Sealant (Human) (FS). I have reviewed the following sections of the submission and its amendments:

- 3.2.S.2.3. Control of materials
- 3.2.P.4 Excipients
- 3.2.P.5.2 Analytical Procedures (Fibrinogen and Thrombin)
- 3.2.P.5.3 Validation of Analytical Procedures (Fibrinogen and Thrombin)
- 3.2.P.6 Reference Standards or Materials (Fibrinogen and Thrombin)
- 5.3.5.5.1 Subsection *Inter Laboratory Standardization Methods Quality Assurance* (Cursory review of analytical methods used in clinical trials)

In the *Analytical procedures* sections, I reviewed the key functional tests. Most of the Pharmacopeial methods were reviewed by the OCBQ/DBSQC review team; the microbiological tests were reviewed by the DMPQ reviewer, therefore, I conducted cursory review of these methods.

Several information requests (IRs) were sent to Grifols regarding control of materials, analytical tests, excipients and reference standards or materials. The summary of the IRs and responses from Grifols is provided in the memorandum, and the IRs full text is provided in the appendix of this document.

Grifols adequately addressed all identified issues during the review. Therefore, I recommend the approval of the Fibrin Sealant (Human) based on the data in the sections I reviewed.

2. Background

FS is a two-component biologics/device combinational product, which is composed of two syringes containing frozen sterile solutions of Human Fibrinogen (Component 1) and Human Thrombin with calcium chloride (Component 2) assembled on a syringe holder. FS is indicated as an adjunct to hemostasis for mild to moderate bleeding in adults undergoing surgery when control of bleeding by standard surgical techniques (such as suture, ligature, and cautery) is ineffective or impractical. FS is effective in heparinized patients. FS is designed to be applied by dripping or spraying with cannula or spray applicator.


3. Control of materials

In section 3.2.S.2.3, I reviewed the raw materials, which are used in manufacturing of FS. The detailed evaluation of the adventitious agents' removal was reviewed by Dr. Ze Peng.

Source Plasma

Human Fibrinogen and Human Thrombin are isolated from pooled human Source Plasma following a fractionation process based on Cohn's method. All Source Plasma used in manufacture of Human fibrinogen and Human Thrombin is obtained from FDA licensed U.S. based plasmapheresis centers and complies with the requirements of 21 CFR §640, subpart G.

(b) (4)



Albumin (Human)

Albumin (human) Grifols (b) (4), licensed in the U.S. under BLA 103352, is used in the formulation of Human Thrombin. Albumin (human) Grifols is approved for intravenous use for multiple indications.

(b) (4)

SP Sepharose XL

Strong cationic exchanger chromatography resin SP Sepharose XL is supplied from (b) (4). SP Sepharose was certified by the manufacturer for function (b) (4).

Grifols performed additional tests with the resin, which included (b) (4).

Grifols provided the certificate of analysis for SP Sepharose XL from the manufacturer, and the results of the tests performed in-house.

Other reagents used in the manufacturing process:

- (b) (4)
- (b) (4)
- Calcium chloride (b) (4)
- (b) (4)
- Glutamic acid monosodium
- Glycine: meets (b) (4)
- (b) (4)
- L-Arginine: meets (b) (4)
- L-Isoleucine: meets (b) (4)
- Polysorbate 80: meets (b) (4). Polysorbate 80 used at Instituto Grifols, S.A. is prepared from (b) (4)
- (b) (4)
- (b) (4)
- Sodium chloride: meets (b) (4)
- Sodium citrate, (b) (4) : meets (b) (4)
- (b) (4)
- (b) (4)
- (b) (4)
- Tri-n-butyl phosphate: meets (b) (4)
- (b) (4)
- Water for injection (WFI): meets (b) (4)

Grifols has the defined approval and monitoring process of raw materials supplies depending on risk assessment and based on the use of the material. All materials, which are directly involved in the manufacturing process (e.g. excipients, primary packaging and devices in direct contact with the product), are considered as high risk. The qualification of suppliers of materials with high risk includes in-house analysis of different samples of the materials and execution of an audit. Grifols performed in-house the critical tests for each lot for high risk materials, according the requirements of USP or/and Ph. Eur. Grifols provided the certificate of analysis for selected lots of the materials based on the acquired in-house data. All submitted results meet the requirements of USP or/and Ph. Eur. Grifols implements periodic revision of the suppliers. For the materials of high risk, the revision is performed every (b) (4).

Additionally, I reviewed and verified the information for the raw materials in the CBER Biologics Information Tracking System (BITS), Animal, Biologics, and Chemical (BITS-ABC) database. The components information from BITS-ABC database is attached as a Table 6 to the Appendix of this document.

4. Excipients

The following excipients are added to the Fibrinogen component: Sodium citrate (b) (4), Sodium chloride, Arginine, L-Isoleucine, and L-Glutamic acid monosodium. The following excipients are added to the Thrombin component: Sodium chloride, Calcium chloride dihydrate, Human albumin, and Glycine. Control of all excipients is discussed in section 3 of this document. The justification of the formulation of the Final Drug Product (FDP) was included to the section 3.2.P.2 *Pharmaceutical development* and was reviewed by Dr. Natalya Ananyeva.

5. Analytical procedures

The analytical procedures, used as in-process controls, control of the FDP and methods' validation, are described in sections 3.2.P.5.2 and 3.2.P.5.3 and are presented in Table 1. All Pharmacopeial and commercially available tests systems were validated to demonstrate their ability to control intermediates or FDP of FS.

Table 1. Analytical procedures used as in-process controls and control of the FDP

| Parameter | Method | Part of specification or In-process control test for* |
|---------------------------------------|----------------------------------|---|
| Fibrin Sealant identification | Coagulation method (in house) | FDP specification |
| Verification of functionality | Coagulation method (in house) | FDP specification |
| Fibrinogen (clottable protein) | (b) (4) determination by (b) (4) | FDP specification FB |
| Total protein | Assay using (b) (4) reagent | FDP specification FB |
| Stability of solution | Visual stability | FDP specification FB |
| (b) (4) | (b) (4) assay | FDP specification FB |
| Thrombin | Coagulation using (b) (4) | FDP specification TH |
| Albumin | (b) (4) | FDP specification TH |
| Appearance of frozen product | Visual inspection | FDP specification FB, TH |
| Appearance of solution | Visual inspection | FDP specification FB, TH |
| pH | pH meter | FDP specification FB, TH |
| Arginine, L-Isoleucine, glutamic acid | (b) (4) | FDP specification FB |
| Glycine | (b) (4) | FDP specification FB, TH |
| Chloride | (b) (4) | FDP specification FB, TH |
| Sodium | (b) (4) | FDP specification FB, TH |
| Calcium | (b) (4) | FDP specification TH |
| Citrate | (b) (4) method | FDP specification FB |
| (b) (4) | (b) (4) method | FDP specification TH |
| Tri-n-butyl phosphate | (b) (4) | FDP specification FB, TH; (b) (4) |
| Polysorbate 80 | (b) (4) assay | FDP specification FB, TH; (b) (4) |
| (b) (4) | (b) (4) | FDP specification FB |
| Volume | | FDP specification FB, TH |
| Sterility | (b) (4) | FDP specification FB, TH |

| | | |
|------------|---------|--------------------------|
| Endotoxins | (b) (4) | FDP specification FB, TH |
| (b) (4) | | |
| (b) (4) | (b) (4) | (b) (4) |
| (b) (4) | | (b) (4) |

*FB – Fibrinogen component; TH- thrombin component

** Test was removed from specifications, amendment 125640/0.33, report IG ITEC-002990_ING in section 3.2.P.5.6. The *Specifications* were reviewed by Dr. Natalya Ananyeva.

Review of the selected analytical methods

Fibrin Sealant identification (test of performance of the combinational product)

Procedure: 3.2.P. Human Fibrinogen/ 3.2.P.5.2./ IG_MA-000664_ING v6.0

Validation: 3.2.P. Human Fibrinogen / 3.2.P.5.3./ IG ITEC-002930_ING

The test is designed to check the performance of the whole combinational product. The test consists of two procedures: *Fibrin Sealant Identification* and *Verification of functionality*. Both procedures are included in the specifications.

Fibrin Sealant identification procedure involves (b) (4)

. The results of the test are reported as “(b) (4)” or “(b) (4)”.

For *Verification of functionality procedure*,^{(b) (4)}

. The reportable value is “(b) (4)”, if the (b) (4). To test the performance of devices of different sizes, for product volume that are larger than (b) (4), the assay is repeated as many times as the syringe volume allows.

Reviewer’s comments

In the original version of the test description (IG_MA-000664_ING v4.0), the second procedure was named as “(b) (4)”, which may indicate that the test is quantitative. In response to our IR (2017/05/01), Grifols named the procedure “*Verification of functionality*” and clarified the reportable value (“(b) (4)”, if the (b) (4)).

We asked Grifols to perform the validation of the Fibrin sealant identification test. In the response, they only demonstrated the Specificity of the test by substituting (b) (4) components with the buffer. The results of such tests were negative (no clotting), as expected. In the following IR, we asked Grifols to demonstrate applicability of the test when both components are at the lower limits of their specification ranges. Grifols provided the data in amendment 125640/0.33, which justify that the proposed acceptance criterion for the (b) (4) can be met under worst-case conditions.

In summary, the description of the test and validation of the Specificity are adequate for intended purposes. In addition, the validity of the test is confirmed by Grifols experience with the product.

Fibrinogen (clottable protein) determination by (b) (4)

Procedure: 3.2.P.Human Fibrinogen/ 3.2.P.5.2./ IG_MA-000888_ING v4.0

Validation: 3.2.P.Human Fibrinogen / 3.2.P.5.3./ IG_IVMA-000408_ING v2.0

(b) (4)

(b) (4)

Reviewer's comments

I raised a concern that the Study for Accuracy was performed with (b) (4) Standard for Fibrinogen concentrate (b) (4)

I accepted their response, because they performed the Precision study using Fibrinogen component of the DP over the proposed range of the assay.

In response to an IR from DBSQC team, Grifols performed additional studies for Linearity and Accuracy to extend the range from (b) (4) of fibrinogen (clottable protein amendment 125640/0.28).

In response to IR (sent on 2017/05/01) Grifols provided the results of the Robustness study to evaluate the effect of concentrations variation of (b) (4). The influence of the variation for all the evaluated parameters was found to be statistically insignificant.

In the initial submission, the standard has been expired in the report *IG_IEST-000441_ING Preparation and standardization of the Fibrinogen secondary standard*. In response to FDA IRs, in Amendments 125640/0.28 and 125640/0.33 Grifols provided stability data of the Fibrinogen secondary standard. Grifols also clarified that value of the standard is verified every time, when a standard used as a control, due the nature of the assay.

Table 2. Summary of Fibrinogen (clottable protein) method validation

| Parameter | Validation design | Acceptance criteria | Result |
|---------------------------|-------------------|---------------------|--------|
| Accuracy | (b) (4) | (4) | |
| Precision (Repeatability) | | | |
| Precision (Intermediate) | | | |
| Specificity | | | |
| Linearity | | | |

| | |
|-------|---------|
| | (b) (4) |
| Range | |

* The information is based on the applicant's response - to IR in amendment 125640/0.28.

Thrombin determination by coagulation using (b) (4)

Procedure: 3.2.P. Human Thrombin/ 3.2.P.5.2./ IG_MA-000457A_ING v11.0
Validation: 3.2.P. Human Thrombin / 3.2.P.5.3./ IG_IVMA-000298_ING v2.0

The test is based on (b) (4)

The method was validated for Specificity, Linearity, Accuracy and Precision.

Grifols prepared Thrombin secondary standards from the DS material. (b) (4)

Reviewer's comments

In the original BLA submission, Grifols assigned the expiration date for the Thrombin secondary standards as (b) (4) after manufacturing. Then the expiration date was extended for (b) (4). However, the stability assignment of Thrombin secondary standards was not justified by stability studies. In response on our IR, Grifols provided the results of the stability study for the Thrombin secondary standards (Amendment 125640/0.28, 2017/07/05). However, in this study the internal secondary standards were calibrated against each other without standardization relative to (b) (4) standard. In the consequent communication (Amendment 125640/0.33, 2017/07/05), Grifols acknowledged the deficiency; however, they explained that (b) (4). They performed the assay with the current (b) (4) lots of the secondary standard against the (b) (4) Standard for Thrombin (b) (4) in June 2017. The reported data support that the standards are stable for (b) (4) after manufacturing (Amendment 125640/0.33, section 1.12.11, P. 11). (b) (4)

Table 3. Summary of Thrombin determination by coagulation method validation

| Parameter | Set-up | Acceptance criteria | Result |
|---------------------------|---------|---------------------|---------|
| Accuracy | (b) (4) | (b) (4) | (b) (4) |
| Precision (Repeatability) | | | |
| Precision (Intermediate) | | | |

| | |
|-------------|---------|
| | (b) (4) |
| Specificity | |
| Linearity | |
| Range | |

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Albumin concentration determination by (b) (4)

Procedure: 3.2.P. Human Thrombin/ 3.2.P.5.2./ IG_MA-000425A_ING v8.0

Validation: 3.2.P. Human Thrombin / 3.2.P.5.3./ IG_IVMA-000413_ING v1.0

For this method, Grifols uses commercially available instruments, reagents, standards and controls from (b) (4). This test is used for several other Grifols' products. Grifols provided the validation data for use of the method in Thrombin component.

Total protein concentration using (b) (4) in Fibrinogen component

Procedure: 3.2.P. Human Fibrinogen/ 3.2.P.5.2./ IG_MA-000382A_ING v12.0

Validation: 3.2.P. Human Fibrinogen / 3.2.P.5.3./ IG_IVMA-FGDI382A_ING v3.0

The procedure was developed for several Grifols' products. For total protein concentration in the Fibrinogen component, Grifols used in-house Fibrinogen standard as a calibrator. The standard was prepared from Fibrinogen DS and standardized by (b) (4) method. The samples of Fibrinogen DP require dilutions (b) (4) and (b) (4) for the assay. Method was validated to determine the protein concentration in Fibrinogen component. Method was validated for Specificity, Linearity, Accuracy, Precision, and Range.

(b) (4)

(b) (4)

6. Reference standards or materials

Table 4. Standards or reference materials used in key functional and safety tests.

| Working standard or reference material | Calibration | To be used in method | Notes |
|---|--|---|----------------------|
| (b) (4) | (b) (4) | (b) (4) | (b) (4) |
| Standard Thrombin, Grifols | (b) (4) Standard for Thrombin (b) (4) | Thrombin | Used as a calibrator |
| Fibrinogen standard for total protein determination | Value determined by (b) (4) method | Total protein determination using (b) (4) in Fibrinogen component | Used as a calibrator |
| Endotoxin, (b) (4) | According to manufacturer: Calibrated against the (b) (4) Standard Endotoxin | Endotoxins for fibrinogen and thrombin | Used as a calibrator |
| Fibrinogen secondary standard | Value determined by (b) (4) method | Fibrinogen (clottable protein) determination by (b) (4) | Used as a control |
| Albumin secondary standard | Value determined by (b) (4) method | Fibrinogen (clottable protein) determination by (b) (4) | Used as a control |
| (b) (4) standard | Not required | Fibrinogen (clottable protein) determination by (b) (4) | Used as a control |

In addition to the standards and reference materials listed in the Table 4, Grifols are using additional standards and reference materials for other analytical methods, which were not reviewed in this memo.

7. Methods used in clinical trials

In response on FDA information request, Grifols provided the list of laboratories where laboratory testing of clinical samples was performed, as well as the description of the methods and method's validation reports in the Amendment 125640/0.15, 2017/03/09.

Coagulation parameters

Grifols tested blood samples from subjects for activated partial thromboplastin time (aPTT) and for prothrombin time international normalized ratio (INR) at baseline and at several time points after treatment.

Virus Safety Testing

Blood samples were collected from adult subjects at baseline and several times at certain points after surgery. The blood samples were tested for hepatitis A virus, hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and B19V by viral nucleic acid testing and viral serology test methods. Tests were performed using commercially available or in-house methods in several clinical laboratories. Grifols provided the description of the methods and validation reports in the section 5.3.5.1.

Immunogenicity testing

According to the clinical trial protocol, the plasma samples from the subjects were tested for the presence of antibodies against several coagulation factors if one or more their post-exposure samples had inexplicable prolonged coagulation times (INR ≥ 2.0 or aPTT ratio ≥ 1.5). The plasma samples from the subjects were tested for antibodies against human coagulation factor V, human thrombin, and human fibrinogen. The method uses a tiered approach which included the initial screening, confirmation and titer by (b) (4) assay methods. These tests were performed by (b) (4)

(b) (4). Each laboratory used their in-house methods. All tests were validated and Grifols provided the description of the methods and validation reports in the section 5.3.5.1.

8. Recommendation

Upon the review of the information in the original submission and Grifols' responses to IRs, I conclude that the analytical methods used for FS are described in sufficient detail, adequately validated, and suitable for their intended use for control of intermediates and FDP. Grifols has established a defined approval and monitoring process for control of raw materials. I recommend approval of the BLA 125640/0 for Fibrin Sealant (Human) from the analytical perspective.

9. Appendix

Information requests sent to Grifols

Table 5. List of IRs which were conveyed to Grifols and related to the issues in this review

| IR date | Response from Grifols: Amendment STN and date | Issue |
|------------|--|--|
| 2017/01/02 | 125640/0.15 (2017/03/09) | Analytical methods in clinical trials |
| 2017/03/16 | 125640/0.18 (2017/03/30) 125640/0.22 (2017/04/10) | <i>Certificate of analysis</i> of (b) (4) Fibrinogen Clottable protein method |
| 2017/03/23 | 125640/0.21 (2017/04/07) | Thrombin activity and (b) (4) determination (IR was prepared by DBSQC team) |
| 2017/05/01 | 125640/0.28 (2017/05/23) | Fibrin Sealant identification test Fibrinogen (Clottable protein) method Thrombin and Fibrinogen secondary standards |
| 2017/06/15 | 125640/0.33 (2017/07/05) | Fibrin Sealant identification test Thrombin and Fibrinogen secondary standards |
| 2017/10/19 | 125640/0.60 (2017/10/23) | Total protein determination test |

IR 2017/01/02

Please submit the following information: Description and validation of the analytical methods which were used for viral safety and immunogenicity testing in clinical studies. Please include information on the laboratories where clinical laboratory tests were performed.

IR 2017/03/16

1. Please provide additional documents related to materials, which are used in the manufacture of Human Thrombin.
 - a) *Certificate of Analysis* from the supplier for (b) (4). Please provide the protocol and results, including the examples of (b) (4).
 - b) *Certificate of Analysis* from the supplier for SP Sepharose XL. Please provide the protocol and results, including the examples of spectra, for IR identification test for analysis of SP Sepharose XL.
2. In regard to the analytical procedure FIBRINOGEN (CLOTTABLE PROTEIN) DETERMINATION BY (b) (4) (IG_MA-000888_ING) and VALIDATION FOR FIBRINOGEN (SEALANT) OF FIBRINOGEN (CLOTTABLE PROTEIN) DETERMINATION BY (b) (4) (IG_IVMA-000408_ING, version 2.0):

- c) Please provide the description of the Secondary standard for Fibrinogen control, which is used in the protocol IG_MA-000888_ING. The additional data related to the Secondary standard for fibrinogen control should include the following information:
Preparation, storage and stability of the standard;
Calibration against the current (b) (4) standard.
 - d) The system suitability acceptance criteria in the protocol IG_MA-000888_ING include the criterion for CV ((b) (4)) for the samples and Fibrinogen control. However, please note that the CV is reflecting only the precision, but not the accuracy of the assay. Please add to the protocol an additional criterion for the range of the Fibrinogen control.
 - e) Please provide the description and clarify the usage of Secondary standard as proteins control (IG_MA-000888_ING, p.3).
 - f) Please provide the description of the (b) (4) standard (IG_MA-000888_ING, p.3).
 - g) Please provide the justification for the correction factor (b) (4) used for the calculation of Fibrinogen (Clottable Protein).
 - h) Based on the provided validation report (IG_IVMA-000408_ING), the Range of the assay is (b) (4) of Fibrinogen (Clottable Protein). Please add the criterion for each sample after the dilution step to be within the Range of the assay in the protocol IG_MA-000888_ING. This acceptance criterion is currently not included.
 - i) Specificity
The description of the procedure for Specificity is unclear. Please clarify did you treated the specificity solution (formulation buffer) exactly the same way as samples?
Please include to the study the results obtained for clot formation with the formulation buffer (without fibrinogen).
 - j) Linearity
In the current version of the validation report IG_IVMA-000408_ING, the parameters in X and Y axes in Tables 2 and 3 are identical and defined as % (w/V) Fibrinogen (clottable protein). Please clarify the definitions of "Concentration" and "Response" and adjust the labelling of axes in the results for Linearity.
 - k) Accuracy
In the validation report IG_IVMA-000408_ING, the Accuracy study was performed with (b) (4) Standard for Fibrinogen at (b) (4). Also, the study did not address the effect of the excipients on the assay.
Please provide the experimental data to assess the potential effect of excipients in the Final Drug Product formulation on the Accuracy of the assay.
Please assess the Accuracy at minimum three concentration levels covered by the Range of the procedure. Ideally, such tests should be performed in the presence of appropriate concentration of the excipients. Please follow the recommendations in the ICH Q2(R1) guidance.
 - l) Precision
Please clarify how you choose the acceptance criterion for Precision" (b) (4)".
 - m) Robustness
Please provide data for evaluation of the robustness of the assay.
3. Please make updates to the relevant documents in the eCTD file after addressing the above requests.
 4. Please update the Section 3.2.P.6. Reference standards or Materials with description of all standards, which are in use for Control of Drug Substance and Drug Product.

Please address the following deficiencies identified with analytical procedures:

1. The document IG_MA-000664_ING *FIBRIN SEALANT IDENTIFICATION* includes the description of two analytical methods: Fibrin Sealant Identification and (b) (4). Because these parameters represent the functionality of the final drug product (FDP) and are included in the release specifications, each analytical method requires validation. In addition, the procedure of the method Fibrin Sealant Identification does not include sufficient details on how to perform the method.
 - a) Please provide a detailed description of the procedure Fibrin Sealant Identification. Please clearly define the acceptance criteria for the test.
 - b) Please validate the analytical procedure for Fibrin Sealant Identification, including an assessment of *Specificity*, and submit the results in a validation report. Please include data to support the suitability of the test for its intended purpose (assessment of functionality), as well as the sensitivity of the test to detect changes in the quality attributes of the FDP components (Fibrinogen and Thrombin), e.g., (b) (4)
 - c) In regard to the (b) (4) test, please clarify how the results will be reported. We note inconsistency in reporting by actual (b) (4) (3.2.P.5.4 Batch Analysis) or as “Pass test” (3.2.P.8.3. Stability Data). If you plan to report the actual (b) (4) (which appears to be implied from the test definition), the test will be considered as a quantitative method requiring validation of *Accuracy*, *Precision*, *Specificity*, *Linearity*, *Range* and *Robustness*. If this method is considered as an identification test, then the validation should include the assessment of *Specificity* and *Robustness*; in this case, please explain the difference between the Fibrin Sealant Identification and (b) (4) tests. Please submit the validation report. Please also specify the acceptance criteria for this test in the respective SOP.
 - d) In the description of the analytical procedure for (b) (4), please clarify for what “specific studies” the test can be performed with a total product volume of (b) (4). Please provide a detailed description of the procedure, and the validation data for low-volume testing.
2. We have the following questions/comments regarding the method validation report for the determination of Fibrinogen (Clottable protein) by (b) (4) Method, Document IG_IVMA-000408_ING:
 - a) You have indicated in section 4.2 that *Linearity* was assessed using Fibrinogen in the range of (b) (4). This range is equivalent to a range of (b) (4) after considering the dilution specified in the test method SOP. Thus, your linearity data do not cover the lower specification limit of (b) (4) for the Fibrinogen component of your product. Please provide additional linearity data to cover the proposed specification range.
 - b) You have evaluated *Accuracy* using only the International Standard but not your fibrinogen product. Please provide data using the Fibrinogen FDP to assess *Accuracy* over the proposed range of the assay. Alternatively, please provide data to support the suitability of the use of the standard in the study, e.g., recovery data from the *Precision* study in the proposed range.
 - c) We acknowledge your response to the March 16, 2017 Information Request regarding the evaluation of *Robustness* of the assay. However, it only includes variations in the equipment used, and lots of reagents and standards. Please provide additional data for your method by evaluating the effect of variation of operating conditions and parameters, specifically, concentration of calcium and Thrombin, and temperature at the extremes of the operational ranges.
3. In regard to your Thrombin secondary standards: in the report IG_IEST-000295_ING and communications EV160930/1 and EV170331/1 (section 3.2.P.6 *Human Thrombin Reference Standards or Materials*), you reported the extension of the expiry date for Thrombin secondary standard batch (b) (4). Similarly, you reported the extension of the expiry date for Thrombin secondary standard batch (b) (4) (report IG_IEST-000296_ING and communication EV170227/1). However, you have not

provided data to support these extensions. Please submit stability data for the Thrombin secondary standards to justify the extension of their expiry dates.

4. In regard to the Secondary standard for Fibrinogen, you provided the report IG_IEST-000441_ING, *Preparation and standardization of the Fibrinogen secondary standard lot (b) (4)*, and the communication EV151215/16, *Extension of expiry date for (b) (4) secondary standard batch (b) (4)*, you extended the shelf-life of the standard to (b) (4), which is beyond the shelf-life of the Fibrinogen component of the FDP (24 months). The extension of the shelf-life of the standard was not justified by real-time stability data. Please provide stability data to justify the extension of the shelf-life of the Fibrinogen secondary standard.

IR 2017/06/15

1. We acknowledge your May 23, 2017, response related to the validation of the Identification test and Verification of Functionality test (Question 1). You demonstrated the specificity of these tests by replacing one or both components with the specificity solutions to confirm absence of clotting. However, you did not provide data to demonstrate test applicability (i.e., sensitivity) within the entire ranges of Thrombin Activity and Fibrinogen (Clottable Protein), which are wide (Question 3). Please perform additional studies with concentrations of both components at the lower limits of their specification ranges to justify that the proposed acceptance criterion for the (b) (4) can be met under worst-case conditions.
2. We acknowledge your May 23, 2017, response related to the extension of the shelf-life of your Thrombin secondary standards (Question 3). Please clarify if you used the (b) (4) Standard for Thrombin (b) (4) as the reference standard in the stability study for your Thrombin secondary standards.
3. We acknowledge your May 23, 2017, response related to the extension of the shelf-life of your Fibrinogen secondary standard (Question 4). The stability study for your Fibrinogen secondary standard batch (b) (4): communication EV151215/16 included the period from June to November 2015, whereas the shelf-life was extended to July 2017. Please provide the latest Fibrinogen (Clottable Protein) test result for this lot. Please include a requirement of periodic (e.g., (b) (4)) testing of your Fibrinogen secondary standard in protocol IG_IEST- 000441_ING for standard qualification.

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Please provide the description of the Fibrinogen standard for the method Total protein determination by (b) (4) method in Fibrinogen component (IG_MA-000382A_ING v4.0), and specify which Fibrinogen standard is currently used.

Table 6. Components Information in CBER Biologics Information Tracking System (BITS), Animal, Biologics, and Chemical (BITS-ABC) module for Fibrin Sealant (Human) Grifols

| Ingredient | Category | Priority Type | Final Product | Impurity | Manufacturer/Supplier | Country of Origin | Stage of Manufacture | Primary Address | Source Organism | Subsource Organism | Source Organism Countries | Reviewer's comments |
|-------------------------------------|-----------|---------------|---------------|----------|-------------------------|-------------------|----------------------|---|-----------------|--------------------|---------------------------|---------------------|
| (b) (4) | (b) (4) | (b) (4) | (b) (4) | | | | (b) (4) | | | | | |
| HUMAN PLASMA | Animal | Priority | N | | Undefined | USA | | | Human | Blood | USA | |
| SODIUM CHLORIDE | Chemical | Basic | Y | | | | | | | | | |
| ARGININE | Undefined | Unknown | Y | | (b) (4) | Undefined | | | Undefined | Undefined | Undefined | |
| L-ISOLEUCINE | Undefined | Unknown | Y | | (b) (4) | Undefined | | | Undefined | Undefined | Undefined | |
| HUMAN ALBUMIN | Animal | Priority | Y | | Instituto Grifols, S.A. | Spain | | | Human | Plasma | USA | |
| GLYCINE | Undefined | Priority-1 | Y | | (b) (4) | Undefined | | | Undefined | Undefined | Undefined | |
| HUMAN FIBRINOGEN | Animal | Priority | Y | | Instituto Grifols, S.A. | Spain | | C/ Can Guasch, 2, Poligono Industrial Levante Parets del Valles, Barcelona Spain 08150 | Human | Plasma | USA | |
| SODIUM CITRATE, (b) (4) | Chemical | Basic | Y | | | | | | | | | |
| L-GLUTAMIC ACID, MONOSODIUM SALT | Undefined | Unknown | Y | | (b) (4) | Undefined | | | Undefined | Undefined | Undefined | |
| WATER FOR INJECTION | Chemical | Basic | Y | | | | | | | | | |

| | | | | | | | | | | | | |
|-----------------|-----------|---------|---|--|---------|-----------|--------------|--|-----------|-----------|-----------|--|
| (b) (4) | | | | | | | | | | | | |
| SP SEPHAROSE XL | Undefined | Unknown | N | | (b) (4) | Undefined | Purification | | Undefined | Undefined | Undefined | |
| (b) (4) | | | | | | | | | | | | |
| (b) (4) | | | | | | | | | | | | |
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(b) (4)