Workshop report: Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples

Workshop held on 28-29 November 2013 at the European Medicines Agency (EMA) and organised jointly by EMA (with Biologics Working Party and Blood Products Working Party support) and the European Directorate for the Quality of Medicines and HealthCare (EDQM).

Introduction

The workshop discussed potency assays used for product labelling and testing of post-infusion samples for new clotting factor VIII and IX concentrates. A number of new recombinant products are in the late stages of development and it was felt that a more harmonised approach to assigning potency to these clotting factor concentrates was required. The objectives of the workshop were to provide a point of reference when making future decisions for licensing or changes to the relevant European Pharmacopoeia texts.

The report summarises the key points from the workshop. Annex 1 of the report provides a more detailed summary and Annex 2 gives the final agenda and list of participants.

1. ISTH Recommendations on the potency labelling of factor VIII and factor IX concentrates

- Manufacturers of new products are undertaking a thorough characterisation of the performance of new products in different assay systems as recommended by ISTH. The decision tree defined in

the ISTH recommendations is useful in many cases. However, for some products, valid assays could be obtained with both chromogenic and one-stage clotting assays but these different assays give very different potency values. Furthermore, for a number of the products, valid one-stage clotting assays give very different potency values depending on the APTT reagent used, especially for some long-acting products. Therefore, correlation with biological activity from in vitro, non-clinical and clinical efficacy studies is important in selecting the appropriate assay. These aspects are included in the ISTH recommendations and emphasise the importance of not relying on the decision tree alone. Further guidance on how to select the most appropriate assay in this situation may be helpful. Comparison of biological activity with that of a licensed product has been used to support the validity of the potency assignment and maintain consistency in the value of the IU.

- It is important to recognise that there are different assay requirements depending on the use of the assay: precise methods are needed for potency labelling of products (80-120%), whereas for clinical diagnosis sensitivity is important (down to <1%) and for clinical monitoring a broad range is needed to cover dosing and trough levels (1-5%).
- For all products in development, valid assays versus the international concentrate standard were obtained and potency was expressed in IU.
- Addition of a reference to these ISTH recommendations in EMA’s guidance for clinical investigation for FVIII and FIX may be helpful.
- Future development by ISTH of a post-infusion testing decision tree could be useful to both manufacturers and clinicians.

2. Chromogenic versus one-stage clotting assays for potency labelling of FVIII products

- Discrepant potency determination between chromogenic and some one-stage clotting assays for the licensed B-domain deleted product is not seen or not seen to the same extent with the new B-domain deleted products.
- For two of the pegylated products, one-stage clotting methods result in very different potency estimates depending on the reagent used in the activated partial thromboplastin time (APTT) assay.
- If a one-stage clotting method were to be defined as a pharmacopoeial method for FVIII potency assay, the APTT reagent used would also need to be standardised because of its influence on the measured potency with some products.
- Most manufacturers of new products have chosen the chromogenic assay for potency labelling because this is the established Ph. Eur. assay method and, for some products, because of the very variable results seen with one-stage clotting assays.
- One manufacturer, developing the only pegylated full length FVIII product, has chosen a defined one-stage clotting assay for potency labelling which correlates with potency assigned by the chromogenic assay method. Deviation from the Ph. Eur. monograph chromogenic method for FVIII and approval of the 1-stage assay by regulators for potency labelling is possible provided that equivalence of results obtained with both methods is shown. According to the General notices of the Ph. Eur., ‘with the agreement of the competent authority, alternative methods may be used for control purposes, provided that the methods used enable an unequivocal decision to be made as to
whether compliance with the standards of the monographs would be achieved if the official methods were used’.

- One manufacturer of a modified product (single chain rFVIII with truncated B-domain) sees a significant discrepancy between chromogenic and one-stage potency values.

- It is important to use a FVIII deficient plasma containing sufficient von Willebrand factor (VWF) for pre-dilution for potency determination of recombinant products by either chromogenic or one-stage clotting assays. Some commercial FVIII deficient plasmas do not contain sufficient VWF and this can affect the result of the potency determination.

- For current products: the chromogenic assay has proved to be a robust, standardised method for the control of the quality of FVIII products. New products/products under development (e.g. pegylated products): it is premature to include considerations on these products in the Ph. Eur.; the need for Ph. Eur. texts will be re-evaluated at a later stage, when the licensing situation has evolved.

- Regulators will need to keep an open mind and evaluate the most appropriate assay for a particular new FVIII product when assessing the marketing authorisation dossier. If a different method is validated and there is good scientific reason/justification provided that the pharmacopoeial method is not appropriate for the specific product, the Ph. Eur. should be informed so that Ph. Eur. requirements could be adapted (e.g. the monograph revised).

3. Clinical monitoring of FVIII levels during treatment

- Clinicians wish to have one assay that could be used to assign potency for product labelling and which correlates with FVIII levels measured by the clinical laboratory. A particular concern is that using different assays could lead to discrepant high product plasma values measured post-infusion and result in underdosing of patients.

- From the data presented, the chromogenic assay would fit this purpose. However, most clinical laboratories use one-stage clotting assays versus a plasma standard for clinical monitoring of FVIII levels. The two pegylated B-domain deleted/truncated new products show highly variable results depending on the one-stage clotting assay used. Some one-stage clotting assays with silica activated APTT reagents could not detect activity for one of the products.

- Use of a chromogenic assay for clinical monitoring avoids the variability seen with the one-stage clotting assays. Concern was expressed about the ability of laboratories to make the change from the established one stage clotting assay to the chromogenic assay for clinical monitoring of patients.

- Use of a laboratory standard with a valid potency assignment is one approach to correct for assay discrepancies. This approach is already successfully used by some laboratories for the licensed B-domain deleted FVIII product. It relies on good communication between the clinician and the laboratory on the product that the patient has received in order to ensure that the laboratory standard for the product is used in the assay. S. Kitchen stated that the use of a laboratory standard in place of the plasma standard can be readily accommodated into standard operating procedures for the method. However, this view was not commonly shared. Especially, the practicality of this approach in emergency situations on a 24h/day basis was questioned by clinicians. Concern was also expressed on the ability of laboratories to cope with a number of different products with different laboratory standards.
Correction factors are another approach to deal with assay discrepancies. It was questioned whether this would be workable for clinical monitoring. Clinicians felt that correction factors could undermine confidence in the results reported by the laboratory.

When measuring FVIII plasma levels in spiked samples, one-stage clotting assays can overestimate product plasma levels at low concentrations; chromogenic assays gave results closer to the expected value. This may be of relevance to measurement of FVIII trough levels during clinical monitoring.

One company reported that results from spiking experiments could not be relied upon to reflect performance of assays during clinical monitoring.

If an assay gives discrepant results that are much lower than the labelled potency of the product, it will not have the sensitivity to measure low levels of the product in plasma.

A question was raised about how to deal with clinical monitoring in the situation where a patient may be treated with a long-acting product and then receive a non-modified product in hospital for a bleed – both products will be potentially present in the plasma. The validity of the results in such a situation would depend on the suitability of the assay for the individual products. The chromogenic assay and some one-stage assays would give valid results in this situation.

In clinical laboratories method understanding is crucial and is to be achieved by education.

Manufacturers are responsible for providing users with the necessary information for use of their products including laboratory monitoring of plasma levels. The licensed B-domain deleted product provides an example of how this can be done in a situation where a significant discrepancy between the one-stage clotting and the chromogenic assay methods exists. Alternative approaches could be possible depending on the situation e.g. information on which assays give consistent results and which discrepant or invalid results, laboratory standards, conversion factors. It is important that any discrepant potency problems are clearly worked out at an early stage so that patients have trust in the potency that is assigned to a product they are receiving.

4. One-stage clotting versus chromogenic assays for potency labelling of FIX products

A one-stage clotting assay is the established Ph. Eur. method for potency labelling.

There are 2 chromogenic assays available, but they are less established than the chromogenic assay for FVIII.

Valid potency labelling of the recombinant FIX products is possible versus the international standard for FIX (plasma or concentrate) but results are more precise versus a recombinant FIX reference material.

All manufacturers of new products have chosen a one-stage clotting assay for potency labelling because this is the established Ph. Eur. assay method.

One-stage clotting assay methods result in different potency values (for some products the values are highly different), depending on the APTT reagent used. Manufacturers have selected an APTT reagent that correlates with the biological activity for their particular product.

This dependence on a particular APTT reagent means that the method is not robust if the APTT reagent changes or the assay is discontinued. The manufacturer’s internal reference material will be very important to ensure continuity of potency assignment in this situation.
reference materials, how continuity is ensured when they are replaced, storage and stability will be of particular importance in this situation.

- One manufacturer of a modified product will investigate the possible use of a chromogenic assay in the future since this correlates with its chosen one-stage clotting assay.

5. **Clinical monitoring of FIX levels during treatment**

- A second recombinant FIX product is now licensed in the US. The manufacturer highlights in the product information that assay discrepancies of up to 40% are possible during clinical monitoring. Apparently, this is also the case for the already licensed recombinant FIX, BeneFIX, but does not appear to be widely known.

- The wide variation in results from one-stage clotting assays depending on the method / APTT reagent used will raise similar difficulties to those described above for the new FVIII products. How APTT reagent dependency of 1-stage test results can be indicated to clinical laboratories needs to be answered during the Marketing Authorisation application when data are evaluated. Some information will be included in the product information but other approaches may also be needed including educational material for training of clinical laboratories. The Risk Management Plan is an appropriate place to address the risk of discrepant monitoring of plasma levels and the measures to avoid this.
Annex 1

This annex provides notes of Session 2 and 3 of the workshop.

2. Characterisation of new FVIII and FIX concentrates with respect to potency assays and testing of post infusion material

2.1 FVIII

NovoNordisk

- **NovoEight (B-domain truncated rFVIII)** – ratio of clotting and chromogenic results close to 1. Do not know why different to ReFacto where difference is seen although only slight differences in B-domain linker.

- Field study with plasma samples spiked with NovoEight and Advate (0.03-0.9 IU/ml). Huge variations in methods used and not all follow SSC recommendations! No variation in OSCA potency assay with different APTT reagents. May not be sensitive enough situation to tease out differences in APTT reagents (S. Kitchen). Tendency of OSCA to underestimate values at high levels and to overestimate values at low levels with both NovoEight and Advate.

- **N8-GP (NovoEight glycopegylated)** - using chromogenic assay as in Ph. Eur. OSCA – some interference of pegylation with some APTT reagents (25% to 100% of target value). Confident to have chosen chromogenic – comparable activity of NovoEight and N8-GP in Thrombin generation test and similar potency in animal bleeding models. For clinical efficacy, await trial results.

- Valid assays with chromogenic and clotting assays, label in IU

Biogen-Idec – (B-domain deleted rFVIII-Fc)

- One-stage and chromogenic results show valid results for both methods. Can label in IU. Potency labelling by chromogenic method chosen for commercial product.

- Ex vivo thrombin generation and ROTEM results in early post-infusion samples were comparable for FVIII-Fc and Advate. Potency labelling of rFVIII-Fc thus produces the expected coagulation activity per IU of drug product in plasma.

- Field study with spiked plasma samples (0.05 – 0.8 IU/ml). Comparable OSCA results for rFVIII-Fc and Advate, no specific rFVIII-Fc APTT reagent discrepancies; chromogenic activity 10-20% higher for FVIII-Fc than for Advate. OSCA slightly overestimated activity at low levels (same as seen by Novo Nordisk). Conclusion: Post-infusion monitoring by local one stage clotting or chromogenic assays is possible using commercial plasma standards. Do not know why different to ReFacto despite same FVIII primary structure.

- In patient samples, OSCA not good for low levels. Chromogenic adapted for this purpose has better chance of measuring low levels (S. Kitchen).

Bayer – B-domain deleted rFVIII, pegylated (BAY 94-9027)

- Chromogenic assay used for potency labelling in IU.

- Problem with some silica activated one-stage assays (little or no activity measured). With ellagic acid activated APTT saw analyser and reagent differences. Chromogenic assay accurate and precise.

- Study with spiked plasma samples: expected results with chromogenic assay vs recombinant release standard, with 1-stage assay saw constant bias (130%) with one reagent vs plasma
standard. However, no discrepancy seen so far when analysing patient samples against plasma standard, so do not think a reference standard is necessary.

- Investigation of further 1-stage assay reagents with respect to bias in spiked samples vs plasma standard in comparison to patient samples is ongoing.
- Caution to not overinterpret spike samples– have to confirm with clinical samples (S. Kitchen).

**Baxter – pegylated (full-length rFVIII (Advate))**

- Potency – only small difference between chromogenic and one-stage clotting assay (clinical lots: ratio clot/chrom 0.9 – 1.0). Both methods give parallel assay results versus the IS, label in IU. Proposed to label with one-stage for potency using Actin FSL reagent. As one stage clotting is primarily used for clinical monitoring, it is the method of choice for the physicians. Some silica based reagents give ratio clotting/chromogenic somewhat lower.
- Seeing expected recovery in clinical study.
- EMA’s FVIII guideline suggests the use of chromogenic assay only for assignment of label potency. This requirement may present conflict with clinical practice where almost exclusively the one-stage clotting assay is used to test product recovery and pharmacokinetics.
- Does it help clinical labs to use one-stage for labelling if values are dependent on APTT reagent? Baxter response - except for 2 APTT reagents all would be in range.

**Octapharma – Human-cl rhFVIII**

- Importance of pre-dilution with VWF containing FVIII deficient plasma for OSCA for potency determination of the recombinant product. Some commercial FVIII deficient plasmas do not contain VWF. J. Dodt confirmed that PEI had also encountered this with the chromogenic assay. It is not an issue for plasma-derived FVIII products which contain VWF.
- This should not be an issue for measuring patient plasma levels as patient plasma contains VWF and there is no predilution step. (T. Hubbard)
- Octapharma BDD product - chromogenic assay used to determine potency of process validation and some clinical batches. Potency can also be assayed using one-stage assay that will be used as release assay for the US and Canada.

**CSL – (single chain rFVIII with truncated B-domain and covalent linkage between heavy and light chain)**

- Potency – discrepant between chromogenic and one-stage (more in line with ReFacto). Opted for chromogenic because Ph. Eur., aligns with biological activity and clinical efficacy, and has indications one-stage results can vary.
- How manage monitoring? Think correction factor approach may be possible. CSLB recognizes the potential of high variation in FVIII measurement in other laboratories outside of its central laboratory. A field study will be initiated to investigate clinical laboratory performance measuring CSL627 and full length rFVIII activity in predetermined blinded samples. The field study will allow to determine variability of measurements of both CSL627 and full length rFVIII across comparable assay methods as well as evaluate the utility of a product specific reference standard for use in the one-stage assay.
Workshop discussion on FVIII

Discussion will focus on key issues arising from the presentations and identify where the new products fall in the flow diagram in the ISTH/SSC recommendations.

Manufacturers of new products are undertaking a thorough characterisation of the performance of new products in different assay systems as recommended by ISTH.

Some new products can be assayed for potency by both chromogenic and one-stage clotting assays. For two of the pegylated products interference of pegylation with some APTT reagents in the OSCA resulted in low potency values. The single chain rFVIII (CSL) is showing a discrepancy between chromogenic and OSCA similar to ReFacto.

The discrepancy between chromogenic and some one-stage clotting assays for ReFacto AF is not seen with the new B-domain deleted products.

APTT reagent dependency of 1-stage assay results in choice of product-specific APTT reagent by manufacturers which gives potency results that are in best alignment with chromogenic assay results.

Will labelling in IU be feasible?

It was agreed that all products could be labelled in IU as valid assays versus the IS were obtained for all new products. It was most important to get product potency labelling correct.

Further guidance on how to select the most appropriate assay may be helpful in the situation where chromogenic and one stage clotting assays both give valid but different potency values.

Which assay best links to clinical efficacy?

Where discrepant potency values were obtained with different assay methods, the validity of the method chosen for potency labelling was supported by:

- correlation with biological activity from in vitro, non-clinical and, where already available, clinical efficacy studies
- Comparison of biological activity with that of a licensed product.

One participant raised the option of changing to the thrombin generation assay, although recognising that this would need standardisation. However, M. van den Berg felt that data has not been shown that the thrombin profile would be better than measuring FVIII activity and this would make the situation more complicated.

Is there a need for potency labelling of any of the new products using a one-stage clotting assay for FVIII?

All of the new products could be assayed by the chromogenic assay.

One manufacturer (Baxter) has chosen the one-stage clotting assay in order to align with the assays used for clinical monitoring. There was no relevant discrepancy between clotting and chromogenic assays for the Baxter product. An alternative method to a Ph. Eur. method can be used provided that equivalence and robustness of results obtained with both methods is shown.

Causes of variability in assay results for both clotting and chromogenic assays (e.g. reagents) and whether there is a need for further standardisation.

For the pegylated products, low assay values in the one-stage clotting assay were seen with some APTT reagents.
**How to manage the clinical monitoring of plasma FVIII levels for new products?**

For the pegylated B-domain deleted/truncated products and the single chain rFVIII (CSL), users will need guidance about how to manage discrepancies between the product release assay and some assays used for clinical monitoring. The need to provide guidance will depend on how large the discrepancies are. Some discrepancy in values can be tolerated in the clinical monitoring situation since the posology for the individual patient will be related to the clinical efficacy achieved. For one of the pegylated products, some assays will not work at all.

A question was raised about how to deal with clinical monitoring in the situation where a patient may be treated with a long-acting product and then receive a non-modified product in hospital for a bleed – both products will be present in the plasma. The chromogenic assay and some one-stage assays would give non-discrepant results in this situation.

Product information, risk management plans, proactive information plan for clinical laboratories, and educational material will be important for new products where significant discrepant results are seen with some of the assays used for clinical monitoring. It was noted that for ReFacto AF, this was only briefly described in the product information and other means are used for more detailed guidance. For emergency situations on a 24h basis it was important to keep things as simple as possible to avoid errors.

There were divergent views on the best way to deal with assays that give discrepant results. However, the different approaches provide alternative options to manage the individual situation. There is not one answer for all products. There was a suggestion to develop a decision tree for post-infusion testing.

- **Chromogenic assay for clinical monitoring:** Difficult to switch in daily laboratory routine from 1-stage to chromogenic assay; some in favour of chromogenic assay because of better sensitivity in low FVIII activity range and overestimation by the one stage clotting assay at low levels. This would be relevant for trough levels in 1-5% FVIII activity range (e.g. if deciding prophylactic dosing schedule on the basis of trough levels). Some manufacturers would like regulators to put pressure on the clinicians to use the chromogenic assay but regulators consider that it is not in their remit to make such recommendations.

- **Product-specific laboratory standards in clinical monitoring:** main concern feasibility in daily laboratory practice (not easy to implement especially in emergency situations, testing at night, small laboratories). At night large general laboratories perform coagulation assays needed for acute situations.

- **Conversion factor:** One company is exploring this as an option. A number of reservations about this approach were expressed. The conversion factor would need to be validated (no experience, criteria?). Would different conversion factors be needed for different APTT reagents? Would the conversion factor change if the assay kit changed? It could undermine trust of physicians in laboratory results. However, there was a comment that it would be for laboratories to make the needed correction, in the knowledge of the product tested, and then report this value to clinicians.

- **Manufacturers to provide information on which assays can and which cannot be used to monitor the product.**

  It was commented that testing of FVIII levels for diagnosis is different to the clinical monitoring situation. For diagnosis of severe haemophilia, a sensitive chromogenic assay suited to measuring very low levels can be used.
If chromogenic and one-stage clotting assay methods are now equally precise, why not change to the one-stage clotting assay method to align with clinical monitoring?

The majority of manufacturers supported the use of the chromogenic assay for product labelling because it is more robust; least variability and influenced by least factors and correlates with biological activity and to clinical efficacy. There is not “a single” one-stage clotting assay and for some new products the potency assignment depends on the APTT reagent used or even the equipment. If a one-stage clotting method were to be defined in the Ph. Eur., it would have to define the APTT reagent; how to standardise the APTT reagent? If the method was not tightly defined it would be meaningless as a standard method.

In contrast, the speaker for Baxter stated that ideally the same method should be used for potency labelling and clinical monitoring therefore a defined 1-stage clotting assay should become a monograph method.

It was noted that prior to the chromogenic assay, the pharmacopoeial method for potency assay had been a 2 stage clotting assay, which worked on a similar principle as the current chromogenic assay.

2.2. FIX products

**NIBSC collaborative study on FIX potency in preparation for the replacement of the international standard - E. Gray**

- Focus of the study is on potency label and selection/development of a new FIX IS. A similar study is planned for FVIII products.
- All assays valid; “like vs like” paradigm confirmed for plasma-derived and recombinant full length FIX products – it reduces variability and minimises assay discrepancy.
- Recombinant FIX products are better with a recombinant FIX standard (no assay discrepancies and low inter-laboratory variability). Assay discrepancies and high variability against IS (concentrate) and plasma IS (4th IS Factors II, VII, IX, X plasma). Suggestion to label potency against a recombinant standard that has a robust value assignment relative to the plasma derived concentrate IS?
- For all long-acting products, the correlation is not good across assays and there is high inter-laboratory variability using any of the reference materials. 1-stage results highly dependent on APTT reagent. Suggested options:
  - label potency relative to concentrate IS using a specific method and reagent
  - label potency against a product specific standard that has a robust value assignment relative to the plasma derived concentrate IS, using a specified method and reagent.
- There may need to be a product specific standard for long acting FIX products. (Question over who would be the guardian of this? WHO?)

**Novo-Nordisk – N9-GP (glycopegylated rFIX)**

- Decision tree was not of much help. Has chosen one-stage clotting assay with SynthAFax – correlates with chromogenic assays. Intend to label in IU. Interested researching more with chromogenic.
- Pegylation affects activity in 1-stage assay, APTT reagent dependent, valid assays can result in very different activities.
• Comparable activity of N9-GP and rFIX on a molar or protein amount basis by thromboelastography (TEG) in human haemophilia B blood, by haemostatic effect in haemophilia B mice bleeding model and in haemophilia B dogs. Therefore, APTT reagent chosen that gives comparable 1-stage clotting assay results for N9-GP and Benefix for the same protein level; comparable results with chromogenic assays; labelling with 1-stage assay.

• Phase 3 clinical data shows that the dose to stop most bleeds is comparable to the dose used for BeneFIX and Mononine, supporting the potency assignment.

• Are continuing to investigate the chromogenic assay in clinical trials. Chromogenic potency correlates with non-clinical/clinical efficacy

• Will talk with manufacturer re ongoing availability of SynthAFax

Biogen Idec – rFIX-Fc

• One stage clotting assay with Siemens Actin reagent (ellagic acid) chosen and lined up with studies of pharmacological effect in animal models. In addition, ex vivo thrombin generation and whole blood ROTEM results were comparable for rFIXFc and BeneFIX in the Phase 3 clinical study.

• Depending on type of APTT reagent used, a different relative potency may be obtained for rFIXFc vs WHO standard (60-120% of potency value with Siemens Actin).

• See variability in field study of post-infusion testing of spiked samples (0.05 – 0.8 IU/ml) – also seen with BeneFIX (CV 31% at lowest concentration) but variability larger than with BeneFIX as getting reagent differences with rFIXFc (CV 45% at lowest concentration).

• Field study showed over-estimation of BeneFIX vs plasma standard in most laboratories (67% overestimate at 0.05 IU/ml). Non-linearity vs plasma standard was observed to a similar extent for both products. Over-estimation of BeneFIX was seen with all types of APTT reagents. For rFIXFc see similar over-estimation by ellagic acid, underestimation with silica and kaolin reagents.

• General issue – If do individualised treatments some laboratories may get very discrepant results.

• Is discrepancy of 10% due to concentrate standard and plasma standard – get bump up due to this in clinical monitoring.

• Chromogenic kits see lot to lot differences in one of kits so may not be that reliable yet. (Novo Nordisk has not seen this difference.) In discussion it was mentioned that the chromogenic kit of one manufacturer changed and that differences seen may be due to the use of an older version.

• How much variability can be tolerated in clinical monitoring? Experience implies quite a lot (discussion).

CSL – rFIX – Albumin (rIX-FP)

• One-stage clotting assay (Siemens APTT reagent) used that lines up with BeneFIX activity (in-vitro and non-clinical) and the clinical programme to date supports the potency labelling approach. Clinical monitoring – one outlier laboratory changed its method.

• Use of same APTT reagent for potency labelling, non-clinical and clinical studies; choice of APTT reagent should take also into account how widespread is use of APTT reagent in clinical labs (some rarely used, others are market leaders) (discussion).

Baxter – full length rFIX (RIXUBIS)

• The one-stage clotting activity of a rFIX product is dependent on the APTT reagent when the 4th IS for FIX concentrates is used as the reference. Chose DAPTTIN (silica type) as developed in Immuno
lab (Baxter history); DAPTTIN gives relatively low FIX activity values. The type of aPTT reagent used prevents overestimation of FIX activity. Clinical efficacy was demonstrated with the dosing regimen based on values obtained by one-stage clotting test with DAPTTIN.

- Clinical results showing clinical efficacy – Have recently submitted the MAA to EMA. FDA authorised.
- Rixubis shows good agreement between one-stage and the two chromogenic assays in a study performed during product development. Getting consistently lower results with chromogenic for the commercial rFIX comparator product. (NIBSC study showed a discrepancy between chromogenic assays vs the concentrate IS, this may be because reagents have changed between the 2008 company study and the NIBSC study.)
- FIXa content influencing clotting activity (NIBSC). Seeing FIXa in commercial rFIX comparator product and lower amounts in Rixubis. According NIBSC FIXa levels of all rFIX in study below a limit which would influence the clotting assay result.
- Only recently approved by the FDA with the following product information statement ‘Factor IX potency results can be affected by the type of APTT reagent and reference standard used in the assay; differences of up to 40% have been observed’. However, the observation is not limited to RIXUBIS and has been confirmed with other commercial rFIX products.

Workshop discussion on FIX

*Discussion will focus on key issues arising from the presentations and identify where the new products fall in the flow diagram in the ISTH/SSC recommendations.*

All manufacturers of new products have chosen a one-stage clotting assay with a specific APTT reagent for potency labelling because this is the established Ph. Eur. assay method. (There are 2 chromogenic assays available (from Rossix and Hyphen-Biomed) but they are less established than the chromogenic assay for FVIII.)

**Will labelling in IU be feasible?**

Yes. For all rFIX molecules valid assays, unit can be traced back to IS

**Do assay results link to clinical efficacy?**

Yes to non-clinical and to clinical that is available so far.

**Causes of variability in assay results (e.g. reagents) and whether there is a need for further standardisation.**

Variability is seen with the different APTT reagents and also is affected by the reference material.

**How to manage the clinical monitoring of plasma FIX levels for new products?**

As seen with the new FVIII products, very variable results can be expected in clinical monitoring. This depends on the assay used and the comparator. A product specific laboratory standard could reduce discrepancies but if a particular assay gives discrepant very low assay values, it will not have the sensitivity to measure low plasma levels. This could be a problem for measuring trough plasma levels.

Clinically, a particular concern for both FVIII and FIX is that discrepant high values measured post-infusion could lead to underdosing.
The over-estimation and variability of assays of BeneFIX reported at the workshop do not appear to be widely known. It is unclear whether this has any clinical implications.

3. Summary and close of meeting by J. Dodt, Chairperson

- For the new FVIII and FIX products valid assays versus the IS are obtained and potency can be expressed in IU.
- Difficulty to translate potency label to clinical laboratory results.
- Product specific standard is one possibility but will not overcome limited sensitivity of an assay.
- Could correction factor be used? It would need to be followed-up/monitored as reagent might change.
- It is unclear if clinical laboratories are following best laboratory practices, educational work is needed.
- Spiking samples into plasma may not behave the same as post-infusion samples.
- FVIII chromogenic assay more sensitive in low activity range, preferred for patient monitoring, however, would need change of routine practice in clinical laboratories.
- All FVIII products can reliably be measured with chromogenic assay.
- Physicians prefer use of single method, difficult to solve because is not just clotting vs chromogenic, reagents play critical role for individual products, unclear how could solve in near future.
- Some physicians prefer chromogenic assay because do not want to be dependent on a single APTT reagent (and OS assay results, for a number of products, are dependent on the APTT reagent).
- Better harmonisation for potency labelling is needed in licensing; regulators to exchange information. Preference for one-stage clotting assay in some regions. There is not a problem where chromogenic and one-stage clotting assays give the same result.
- Regulatory decisions will be based on scientific data to be submitted for individual product.
- May be value in further ISTH recommendations on how to translate valid assays to the clinic.
Annex 2

Workshop agenda

EMA/135928/2014
12 November 2013

Workshop on “Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples”

1. Introduction

Group 6B (Blood Products) of the European Pharmacopoeia started a review of the FVIII potency assays in October 2010. The current Ph. Eur. assay of FVIII (2.7.4) was introduced in 1995 based on recommendations of the ISTH SSC FVIII/FIX. In the Ph. Eur. the chromogenic FVIII assay is described whereas in clinical laboratories and regulatory areas outside of Europe predominantly the one-stage clotting assay is used.

In the EU the labelled potency of ReFacto AF, a BDD-rFVIII, is based on the European Pharmacopoeia chromogenic substrate assay, in which the manufacturing potency standard has been calibrated to the WHO International Standard using the chromogenic substrate assay. The same product (XYNTHA) approved for use outside Europe has a different potency assigned using a manufacturing potency standard that has been calibrated to the WHO International Standard using a one-stage clotting assay. Due to the difference in methods used to assign product potency 1 IU of the XYNTHA product (one-stage assay calibrated) is approximately equivalent to 1.38 IU of the ReFacto AF product (chromogenic assay calibrated). This situation has an impact for the safe treatment of patients travelling between regulatory areas. Many experimental details were identified which could contribute to the performance of the respective assays and influence the resulting potency assignment. The complexity of the issue is large and novel recombinant and/or modified products (fusion proteins with albumin, Fc portions, pegylation or sialylation as well as site-directed mutagenesis of clotting factors) may challenge even more the labelling of clotting factor concentrates.

Group 6B, therefore, is concerned about the best way of labelling of potency of factor VIII products (or other clotting factor concentrates). A working group of the ISTH (International Society on Thrombosis and Haemostasis) has recently published “Recommendations on the potency labelling of factor VIII and factor IX concentrates”2. These included advice for the characterisation of products with respect to potency assays, calibration of manufacturers’ product reference, pharmacokinetic studies and testing of post-infusion samples.

---

During their meeting in April 2013 experts of group 6B expressed their strong wish to be informed about the current knowledge of characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays and testing of post infusion material. Therefore it was proposed to find possibilities to have a workshop on “Characterisation of new clotting factor concentrates (FVIII, FIX) with respect to potency assays used for labelling and testing of post infusion samples”.

At the EMA, this proposal received positive support from the Blood Product Working Party (BPWP) and Biologics Working Party (BWP). It will be very helpful to have an overview of this topic so that discussion of possible regulatory consequences can be initiated in advance of individual applications for Marketing Authorisation.

2. **Purpose of the workshop**

The purpose of this workshop is to provide an overview of the current knowledge of the characterisation of new FVIII and FIX concentrates with respect to potency assays and testing of post infusion material. This overview will provide the basis for further consideration of the following related issues:

- Regulatory authorities discussion on the most appropriate potency assay for the individual products
- European Pharmacopoeia Group 6B discussion on whether to propose revision of the European Pharmacopoeia monographs in the light of information on new FVIII and FIX concentrates.

3. **Time and location of the workshop**

28 November (start time 1pm) to 29 November 2013 (approx. finish time 4pm open session, 5pm for those participating in closed session), Room 4A at the European Medicines Agency

4. **Organisation of the meeting**

Organised jointly by EMA (with BWP/BPWP support) and EDQM. (Correspondence to BPWPSecretariat@ema.europa.eu)

5. **Steering committee**

Nanna Aaby Kruse, BWP
Johannes Dodt, Chair of Group 6B (blood products) European Pharmacopoeia
Anneliese Hilger, Chair of Blood Products Working Party (BPWP)
Anthony Hubbard, NIBSC
Brigitte Neugebauer, EMA
Glenda Silvester, EMA
Cathie Vielle, EDQM
6. Participants

Please see Annex I.

7. Structure of workshop

Meeting with Industry followed by closed session (approx. 1 hour) with regulators (European, US and Canadian)/OMCLs/EDQM

Closed Session

In the light of the information presented in the workshop, the closed session will identify key points relevant to the issues identified under the purpose of the workshop (point 2 above).
# Agenda

**28 – 29 November 2013, European Medicines Agency, London**

**Chairperson: J. Dodt**

28 November 2013, 13:00 – 18:15, meeting room 4A  
29 November 2013, 09:00 – 17:00, meeting room 4A  
*NB. The workshop will be recorded.*

## Day 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Topic</th>
<th>Speaker</th>
<th>Timings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Introductory session</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Background and expectations for the workshop</td>
<td>J. Dodt, PEI, Chairperson</td>
<td>13:00 - 13:10</td>
</tr>
<tr>
<td></td>
<td>Presentation on assay methods, standards, and ISTH/SSC recommendations</td>
<td>A. Hubbard, NIBSC</td>
<td>13:10 - 13:45 (30’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>Presentation on current Ph. Eur. monographs and on how such</td>
<td>S. Wicks, EDQM</td>
<td>13:45 - 14:10 (20’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>monographs are updated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UK NEQAS studies of UK haemophilia centre assays of FVIII and FIX for clinical monitoring</td>
<td>S. Kitchen, UK NEQAS</td>
<td>14:10 - 14:50 (30’ presentation + 10’ questions)</td>
</tr>
<tr>
<td></td>
<td>Current approach on labelling of new products with respect to potency assays in US and Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FDA</td>
<td>G. Michaud - (via Adobe)</td>
<td>14:50 - 15:05 (15’ presentation)</td>
</tr>
<tr>
<td></td>
<td>Health Canada</td>
<td>W. Stevens</td>
<td>15:05 - 15:20 (15’ presentation)</td>
</tr>
<tr>
<td></td>
<td>Questions to FDA and Health Canada</td>
<td></td>
<td>15:20 – 15:30</td>
</tr>
</tbody>
</table>

**Coffee break 15:30 – 15:50**

|     | Refacto AF –in-use experience with a product-specific laboratory standard | M. Westfeld, European Medical Team Lead Haematology/Transplant, | 15:50 – 16:15 (20’ presentation + 5’ questions) |

---

*EMA/135928/2014*
<table>
<thead>
<tr>
<th>No.</th>
<th>Topic</th>
<th>Speaker</th>
<th>Timings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall discussion on introductory talks:</td>
<td></td>
<td>16:15 – 16:55</td>
</tr>
<tr>
<td></td>
<td>As part of this discussion: M. van den Berg to bring in perspective</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>from a haemophilia treater on the importance placed on clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>laboratory results, and issues encountered. EHC and EAHAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>representatives to also contribute.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Characterisation of new FVIII and FIX concentrates with respect to</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>potency assays and testing of post infusion material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td><strong>FVIII products</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Presentations from manufacturers with new products in development.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manufacturers to address within their presentations the workshop</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>discussion questions (see below) for their particular product(s).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific questions will be taken after individual presentations.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NovoEight® (rFVIII) and N8-GP (glycoPEGylated rFVIII) potency assays</td>
<td>M. Ezban, Senior Principal Scientist,</td>
<td>16:55 – 17:25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Haemophilia, NovoNordisk</td>
<td>(25’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>rFVIIIFc – Integrated approach for potency assignment and clinical</td>
<td>E. Belitsky, Bioanalytical Development, and</td>
<td>17:25 – 17:55</td>
</tr>
<tr>
<td></td>
<td>monitoring</td>
<td>J. Sommer, Bioanalytical Development, Biogen</td>
<td>(25’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>Determination of BAY 94-9027 in final container and patient plasma</td>
<td>Yvonne Katterle, Bioanalytics Berlin, Bayer</td>
<td>17:55 – 18:15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Global Early Development, Bayer</td>
<td>(15’ presentation + 5’ questions)</td>
</tr>
</tbody>
</table>

**Day 1 to close at approx. 18:15**

**Day 2**

<table>
<thead>
<tr>
<th>No.</th>
<th>Topic</th>
<th>Speaker</th>
<th>Timings</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Characterisation of new FVIII and FIX concentrates with respect to</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>potency assays and testing of post infusion material (continued)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td><strong>FVIII products (continued)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Presentations from manufacturers with new products in development.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manufacturers to address within their presentations the workshop</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>discussion questions (see below) for their particular product(s).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific questions will be taken after individual presentations.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Activity measurement of Baxter’s BAX</td>
<td>P. Turecek, Senior Director</td>
<td>09:00 - 09:30</td>
</tr>
<tr>
<td>No.</td>
<td>Topic</td>
<td>Speaker</td>
<td>Timings</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>855, a PEGylated recombinant Factor VIII, for labeling potency and testing of post-infusion samples</td>
<td>R&amp;D, Baxter</td>
<td>(25’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>Impact of pre-dilution medium on potency assessment of FVIII concentrates using FVIII:C one stage assay</td>
<td>M. Stadler, Octapharma</td>
<td>09:30 – 10:00 (25’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>Characterisation of CSL627 (rVIII-SingleChain) with respect to potency assays used for labeling and testing of post infusion samples</td>
<td>U. Kalina, Director, Research &amp; Development, CSL Behring</td>
<td>10:00 – 10:30 (25’ presentation + 5’ questions)</td>
</tr>
</tbody>
</table>

**Coffee break 10:30 – 10:50**

**Workshop discussion on FVIII**

Discussion will focus on key issues arising from the presentations and identify where the new products fall in the flow diagram in the ISTH/SSC recommendations.

- Will labelling in IU be feasible?
- Which assay best links to clinical efficacy?
- Is there a need for potency labelling of any of the new products using a one-stage clotting assay for FVIII?
- Causes of variability in assay results for both clotting and chromogenic assays (e.g. reagents) and whether there is a need for further standardisation.

**10:50 – 11:50**

**2.2 FIX products**

Summary of outcome of NIBSC collaborative study on FIX potency in preparation for the replacement of the international standard (including October 2012 meeting) | E. Gray, NIBSC | 11:50 – 12:30 (30’ presentation + 10’ questions) |

**Lunch break: 12:30 – 13:15**

Presentations from manufacturers with new products in development. (Overlap with E. Gray presentation should be minimised.) Manufacturers to address within their presentations the workshop discussion questions (see below) for their particular product(s).

- N9-GP (GlycoPEGylated rFIX) potency assignment | M. Ezban, Senior Principal Scientist, Haemophilia, | 13:15 – 13:40 (20’ presentation) |
<table>
<thead>
<tr>
<th>No.</th>
<th>Topic</th>
<th>Speaker</th>
<th>Timings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rFIXFc - Integrated approach for potency assignment and clinical monitoring</td>
<td>J. Sommer, Bioanalytical Development, Biogen Idec Haemophilia</td>
<td>13:40 – 14:05 (20’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>Characterisation of CSL654 (rIX-FP) with respect to potency assays used for labeling and testing of post infusion samples</td>
<td>U. Kalina, Director, Research &amp; Development, CSL Behring</td>
<td>14:05 – 14:30 (20’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td>Activity measurement of Baxter’s RIXUBIS, a novel recombinant Factor IX product, for labeling potency and testing of post-infusion samples</td>
<td>P. Turecek, Senior Director R&amp;D, Baxter</td>
<td>14:30 – 14:55 (20’ presentation + 5’ questions)</td>
</tr>
<tr>
<td></td>
<td><strong>Workshop discussion on FIX</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Discussion will focus on key issues arising from the presentations and identify where the new products fall in the flow diagram in the ISTH/SSC recommendations.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Will labelling in IU be feasible?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Do assay results link to clinical efficacy?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Causes of variability in assay results (e.g. reagents) and whether there is a need for further standardisation.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><strong>Summary and close of open session</strong></td>
<td>J. Dodt, PEI, Chairperson</td>
<td>15:35 – 15:45</td>
</tr>
</tbody>
</table>

**Coffee break 15:45 – 16:00**

| 4.  | **Closed Session** with regulators (European, US and Canadian)/OMCLs/EDQM |                                                                        |                              |
|     | In the light of the information presented in the workshop, the closed session will identify key points relevant to the issues identified under the purpose of the workshop |                                                                        | 16:00 – 17:00                |

**End of meeting 17:00**
Annex I – List of participants

**Biologics Working Party (BWP)** - Jean-Hugues Trouvin (Chair BWP), Nanna Aaby Kruse, Sirkku Saarela, András Holczinger

**Blood Products Working Party (BPWP)** – Anneliese Hilger (Chair of BPWP), Bengt Ljungberg (Vice-Chair of BPWP), Marijke van den Berg, Karri Penttila, Ida Walsh, Angelo Silva

**EDQM** - Emmanuelle Charton (Deputy Head, European Pharmacopoeia Department), Stephen Wicks (Secretary to Group 6B)

**NIBSC** - Elaine Gray, Anthony Hubbard

**UK NEQAS** - Steve Kitchen, Coagulation Department, Royal Hallamshire Hospital, Sheffield

**EMA** - Glenda Silvester (Scientific secretary to BPWP), Brigitte Neugebauer

**Royal Free Hospital London** - Anne Riddell, Haemophilia Laboratory Manager

**EDQM Group 6B experts** - Johannes Dodt (Chair of Group 6B (blood products) European Pharmacopoeia), Eva Sandberg (Danish Health and Medicines Authority), Juan I. Jorquera (Grifols)

**Industry Associations** - Françoise Rossi (IPFA), Ilka von Hoegen (PPTA)

**Representatives from FDA and Health Canada** (Adobe Connect link for 28 November pm and 29 November pm)

Virtual link with FDA, Ginette Michaud and Paul Mintz

Will Stevens, Chief Blood Products Division, Biologic and Genetic Therapies Directorate, Health Canada

**Industry representatives**

* Baxter* - Peter Turecek, Mehrshid Alai, Maria Loeflund; *Bayer* – Georg Lemm, Yvonne Katterl; *Biogen Idec* - Elena Belitsky, Jurg Sommer, Eleanor Davies, Sofi Fexby; *CSL Behring* - Hartmut Landgrebe, Tony Stowers, Debbie Bensen-Kennedy, Uwe Kalina; *Novo Nordisk* - Karin Knobe, Mirella Ezban, Alice Troy, Pernille Svane; *Pfizer* - Martina Westfeld, Brian T. Colvin, Michael Jankowski – via Adobe connect; *SOBI* - Margareta Wikén; *Octapharma* - Monika Stadler, Malin Edblad, Markus Krieger, Ann-Charlotte Hinz

**Representatives from the European Haemophilia Consortium (patients’ association)**

Michael Makris and Flora Peyvandi

**Representative from European Association for Haemophilia and Allied Disorders**

Michael Makris (representing also EHC, see above)

**Other BWP, BPWP and Group 6B members and other experts from European regulatory authorities and OMCLs joining via Adobe Connect to listen to the workshop**

**Declarations of interest**

Anne Riddell: Received honoraria from Novo Nordisk for participation in an advisory board - international assay expert meeting.

Anthony Hubbard: Grant from various institutions - In some instances, it is appropriate for NIBSC to charge commercial organisations for its products and services in line with guidance issued from HM Treasury (‘Fees & Charges Guide’ and ‘Selling into Wider Markets’). NIBSC endeavours to make the same products and services equally available to commercial organisations, without prejudice. Where there is a significant risk of a conflict of interest, this is managed using a transparent, auditable framework. This approach ensures that NIBSC does not undermine its reputation for independence, impede its research programme or otherwise reduce its ability to deliver its core mission.

Elaine Gray: Consultancy - GTC Biotherapeutics - Antithrombin concentrate (Atryn) and Novo Nordisk - Long-Acting Recombinant FIX N9-GP

Flora Peyvandi: Fees/honoraria pharmaceutical companies. Principal investigator for companies in the coagulation factor field. Grant/funding to institution: Biotest, NovoNordisk. Further details in DoI.

Marijke van den Berg: Bayer - unrestricted funding for clinical research PedNet registry, Baxter - unrestricted funding for clinical research PedNet and RODIN study.

Mike Makris: Consultancy - Novo Nordisk – N8; CSL Behring – Beriplex; principal investigator – BPL - Factor X concentrate, Investigator – Inspiration - Factor IX concentrate

Nanna Aaby Kruse: Family member works at NDA Regulatory Service

Steve Kitchen: Consultancy – Novo Nordisk - N8-GP and N9-GP; Strategic advisory – Pfizer - Reafcto AF, Benefix