ORIGINAL ARTICLE Laboratory science

BAY 81-8973, a full-length recombinant factor VIII: results from an International comparative laboratory field study

S. KITCHEN,* H. BECKMANN,† Y. KATTERLE,‡ S. BRUNS,§ D. TSENEKLIDOU-STOETER† and M. MAAS ENRIQUEZ†
*Royal Hallamshire Hospital, Sheffield, UK; †Bayer Pharma AG, Wuppertal; ‡Bayer Pharma AG, Berlin; and §Winicker Norimed GmbH, Nuremberg, Germany

Introduction: BAY 81-8973 is a full-length, unmodified, recombinant human factor VIII (FVIII) with the same primary amino acid sequence as sucrose-formulated recombinant FVIII but produced with certain more advanced manufacturing technologies. Aim: This global laboratory study evaluated variability in measurement of BAY 81-8973 using one-stage and chromogenic assays compared with antihaemophilic factor (recombinant) plasma/albumin-free method (rAHF-PFM; Advate®) under assay conditions routinely used in clinical laboratories. Methods: BAY 81-8973 or rAHF-PFM was spiked into FVIII-deficient plasma at 0.043 (low), 0.375 (medium) and 0.865 (normal) IU mL⁻¹. Participating laboratories analysed blinded samples and normal plasma in triplicate using their routine assay, reagents and standards. Results were analysed for intra- and interlaboratory variability. Results: Forty-one laboratories in 11 countries participated in the study. One-stage assay and chromogenic assays were used by 40 and 10 laboratories, respectively; 9 laboratories used both assays. Intralaboratory variability was <11% for both assays and both products at all concentrations. Interlaboratory variability was highest at the low concentration in the chromogenic and one-stage assay for BAY 81-8973 (60.0% and 33.7%, respectively) and rAHF-PFM (51.0% and 30.8%) and was lowest at the normal concentration (BAY 81-8973, 5.4% and 14.0%; rAHF-PFM, 5.8% and 12.4%), which was similar to the plasma control (6.6% and 10.3%). The chromogenic:one-stage assay ratio ranged from 0.95 (low concentration) to 1.10 (normal concentration) for BAY 81-8973 and 0.96–1.18 for rAHF-PFM. Conclusions: BAY 81-8973 can be accurately measured in plasma using the one-stage and chromogenic assays routinely used in clinical laboratories without a product-specific standard.

Keywords: chromogenic assay, factor VIII, field study, one-stage assay, postinfusion monitoring, recombinant factor VIII

Introduction

Factor VIII (FVIII) replacement therapy is the standard of care for patients with haemophilia A [1]. Precise measurement of FVIII in patients after FVIII infusion is critical to monitor FVIII activity and guide treatment. Different assays are available to measure FVIII activity [2]. The one-stage assay is used in most laboratories worldwide for postinfusion FVIII:C monitoring, whereas the chromogenic assay has only limited use [3,4]. Results of the one-stage assay are highly variable because of their dependence on reagents, including the composition and concentration of phospholipids [5], contact pathway activator [6] and FVIII-deficient plasma [7]. Wide interlaboratory variability in calibration practices and in the plasmas and buffers used for dilutions and predilutions has also been reported [3]. The chromogenic assay, which is less influenced by different reagents compared with the one-stage assay, is more reliable than the one-stage assay [8] and is the reference method for the European Pharmacopoeia [9,10]. The chromogenic assay has also been recommended by the National Hemophilia Foundation for postinfusion monitoring in the United States provided kits, which have been approved by the Food and Drug Administration [11]. Despite these recommendations, the chromogenic assay is not frequently used in clinical laboratories for postinfusion monitoring [3], in part, because of perceived higher costs compared with the one-stage assay [8].

Correspondence: Steve Kitchen, Coagulation Laboratory, Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Glossop Road, S10 2JF Sheffield, UK.
Tel.: +44 0 114 2712955; fax: +44 0 114 2712149;
email: steve.kitchen@sth.nhs.uk
Accepted after revision 21 January 2016

© 2016 The Authors. Haemophilia Published by John Wiley & Sons Ltd.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
Discrepancies in results obtained with the one-stage and chromogenic assays have been reported, the extent of which is product dependent. For full-length products, approximately 10% to 30% higher activity has been reported with the chromogenic assay compared with the one-stage assay [7,12–14]. Higher discrepancy (up to 50%) has been observed for a B-domain deleted product [8,15,16]. The use of a product-specific standard, as opposed to a plasma standard, has shown to substantially reduce assay discrepancy [17–20]. Because of the product-dependent nature of assay discrepancy, a thorough understanding of the unique biochemical properties of each FVIII product in different assay conditions is needed to identify the appropriate conditions to ensure accurate FVIII measurements [21].

BAY 81-8973 is a full-length, unmodified, recombinant human FVIII with the same primary amino acid sequence as Bayer’s sucrose- formulated recombinant FVIII (rFVIII-FS; Kogenate FS, Bayer, Berkeley, CA, USA) but is produced with certain more advanced manufacturing technologies [22]. The safety and efficacy of BAY 81-8973 has been demonstrated in the Long-Term Efficacy Open-Label Program in Severe Hemophilia A Disease (LEOPOLD) clinical trial programme [23–25]. The ability to measure FVIII activity in postinfusion samples of patients treated with BAY 81-8973 using the one-stage and chromogenic assays was also assessed in LEOPOLD I and II. FVIII recovery was similar when measured with both assays, suggesting that either assay may be used for monitoring patients treated with BAY 81-8973 [26]. Because of the potential for high interlaboratory variability in FVIII measurements, particularly with the one-stage assay, a global field study was conducted to evaluate the ability to accurately measure BAY 81-8973 in spiked plasma samples with FVIII assays used routinely in clinical laboratories. Plasma samples spiked with antihaemophilic factor (recombinant) plasma/albumin-free method (rAHF-PFM; Advate®, Baxter, Westlake Village, CA, USA) and a normal plasma control were used as comparators. This article presents the results of this field study with BAY 81-8973.

Methods

Study design

Participating laboratories used their in-house assays, reagents and standards to determine FVIII activity in the spiked test samples; if two different assays were routinely used, testing was performed using both assays. Laboratories were blinded with respect to the identity of the analyte (i.e. BAY 81-8973, rAHF-PFM or plasma control) and to the exact FVIII target level. FVIII activity results of the analysed samples, which were calculated by each laboratory using their in-house standard curves, as well as information regarding assay type, instrument, reagents and calibrators were documented on case report forms, which were verified for completeness and plausibility.

Field study kits

Varying volumes of BAY 81-8973 (117.8 IU mL⁻¹) and rAHF-PFM (Advate; 50 IU mL⁻¹) concentrates were added to pooled plasma from severe haemophilia A patients with normal levels of von Willebrand factor (VWF; HRF Inc, Raleigh, NC, USA) to generate pools of the different FVIII concentrations for test sample preparation. Each participating laboratory received 21 test samples (three aliquots [0.5 mL each] of seven samples). Low (<0.10 IU mL⁻¹), medium (0.10–0.50 IU mL⁻¹) and normal (0.50–1.00 IU mL⁻¹) levels of FVIII (BAY 81-8973 or rAHF-PFM) as well as a control of normal human plasma (Siemens Healthcare Diagnostic Products GmbH, Marburg, Germany) with normal levels of FVIII (0.50–1.00 IU mL⁻¹) were supplied. Samples were identified by unique numbers and target FVIII ranges (i.e. low, medium and normal). Nominal spiked target levels were 0.043 IU mL⁻¹ (low), 0.375 IU mL⁻¹ (medium) and 0.865 IU mL⁻¹ (normal) for BAY 81-8973 and rAHF-PFM and 0.960 IU mL⁻¹ for the control plasma based on the labelled potency. Frozen samples were shipped to participating laboratories and analysed within 4 weeks of receipt.

Assessment of sample preparation accuracy

Spiked samples of both analytes and the plasma control were measured in Bayer’s bioanalytics laboratory using the one-stage (APTT-SP kit and ACL Advance System; Instrumentation Laboratory, Bedford, MA, USA) and chromogenic (BIOPHEN FVIII:C; Aniara Diagnostica, West Chester, OH, USA) assays against the same calibration curve.

Statistical analysis

All laboratories that provided data for ≥1 sample were included in the full analysis set. Laboratories that measured samples using both the one-stage and chromogenic assays were also included in a separate selected full analysis set. Statistical analyses were performed using SAS software 9.2 (SAS Institute Inc., Cary, NC, USA) and were descriptive. Results were analysed for intralaboratory and interlaboratory variability. Sample statistics (mean, SD, median, quartiles and ranges) were calculated for numerical data, and frequencies were calculated for categorical data. One-stage and chromogenic results were analysed separately.
Results

Participating laboratories

Eighty-two laboratories were contacted, and 41 from 11 countries participated in the study; most laboratories were in North America (n = 20) or Europe (n = 19). One laboratory used only the chromogenic assay, 31 laboratories used only the one-stage assay and nine laboratories used both assays.

The most common one-stage assay reagents/kits used included HemosIL® SynthASil (Instrumentation Laboratory, n = 12 [30%]), Actin® FS (Siemens, Marburg, Germany, n = 9 [22.5%]), PTT-A (Stago, Asnières sur Seine, France, n = 4 [10%]), HemosIL® APTT-SP (Instrumentation Laboratory, n = 3 [7.5%]) and Pathromtin® (Siemens, n = 3 [7.5%]). Of the 40 laboratories that used the one-stage assay, 34 used FVIII-depleted plasma as deficient plasma and six used congenital FVIII-deficient plasma. The VWF content varied in the deficient plasmas used by the laboratories (>40 IU dL⁻¹ VWF, n = 10; <10 IU dL⁻¹ VWF, n = 5; not specified, n = 25). Daily calibration was done in 11 laboratories and at the discretion of each laboratory in the remaining 29 laboratories. Freshly prepared calibrators were used in 33 laboratories, and four laboratories used frozen calibrators (not specified, n = 4). Coagulant FVIII (FVIII:C) in the calibrator had been assigned against the WHO standard. Eight laboratories used frozen calibrators (not specified, n = 4). Daily calibration was done in 11 laboratories and at the discretion of each laboratory in the remaining 29 laboratories. Freshly prepared calibrators were used in 33 laboratories, and four laboratories used frozen calibrators (not specified, n = 4). Coagulant FVIII (FVIII:C) in the calibrator had been assigned against the WHO standard. Eight laboratories used freshly prepared calibrators (not specified, n = 2). Calibration was performed daily in three laboratories.

One-stage assay results

Mean FVIII:C values for BAY 81-8973 and rAHF-PFM were slightly higher than the target value at low concentrations (0.046 and 0.051 IU mL⁻¹, respectively, vs. 0.043 [target]) and slightly lower than the target value at normal concentrations (0.763 and 0.835 IU mL⁻¹, respectively, vs. 0.865 IU mL⁻¹ [target]); in samples of the medium concentration, mean values were lower than the target for BAY 81-8973 and higher than the target for rAHF-PFM (0.346 and 0.391 IU mL⁻¹, respectively, vs. 0.375 IU mL⁻¹ [target]; Table 1 and Fig. 1). Similar results were obtained from the nine laboratories that reported data using both the one-stage and chromogenic assays (data not included in Table 1 and Fig. 1).
shown). Data for all 41 laboratories are presented as percentages of the nominal target levels by individual laboratory in Fig. 2.

Intralaboratory variability (percent coefficient of variation [CV]) was <10% at all concentrations and was similar for BAY 81-8973, rAHF-PFM and the plasma control (Table 2). Interlaboratory variability (percent CV) was highest at low concentrations and decreased at higher concentrations for BAY 81-8973 (14.0%) and rAHF-PFM (12.4%) and was similar to the plasma control (10.3%).

Chromogenic assay results

With the chromogenic assay, deviations from the nominal target levels in BAY 81-8973 samples were qualitatively similar compared with the one-stage assay but were quantitatively closer to the target value at all concentrations with <3% deviation for mean values (Table 1 and Fig. 1). In contrast, mean values in rAHF-PFM samples were approximately 12% to 14% above the target value at all concentrations. Data for all 10 laboratories are presented as percentages of the nominal target levels by individual laboratory in Fig. 3.

Chromogenic assay interlaboratory variability was highest at low concentrations of BAY 81-8973 (60.0%) and rAHF-PFM (51.0%) but was substantially reduced at normal concentrations (5.4% and 5.8%, respectively [plasma control, 6.6%]; Table 2). Intralaboratory variability was <11% at all concentrations for BAY 81-8973, rAHF-PFM and the plasma control.

Chromogenic:one-stage assay ratios

The ratio of the mean values determined by the chromogenic and one-stage assay in all 41 laboratories ranged from 0.95 at the low concentration to 1.10 at the normal concentration for BAY 81-8973 and from 0.96 (low concentration) to 1.18 (normal concentration) for rAHF-PFM; the ratio for the plasma control was 1.00 (Table 1). Similar ratios were obtained when only the nine laboratories that reported data for both assays were analysed (1.04 and 1.14 for low and normal concentrations of BAY 81-8973, respectively; 1.02 and 1.21 for low and normal concentrations of rAHF-PFM, respectively). In a further analysis, spiked samples and the plasma control were measured in Bayer’s bioanalytics laboratory using the one-stage and chromogenic assay. Chromogenic:one-stage assay ratios (1.14, 1.10 and 1.06 for BAY 81-8973 at low, medium and normal concentrations, respectively; 1.12, 1.17 and 1.17 for rAHF-PFM at low, medium and normal concentrations, respectively; 1.00 for plasma control) were in the range of those reported by the external laboratories. In an internal control measurement using the chromogenic assay, a differ-
Fig. 2. Mean percentage of nominal target FVIII:C levels for individual laboratories measured by one-stage assay. Mean values are from three analysed aliquots for each laboratory. FVIII:C, coagulant factor VIII; rAHF-PFM, antihaemophilic factor (recombinant) plasma/albumin-free method.

Table 2. Interlaboratory and intralaboratory variability.

| Assay          | BAY 81-8973 |       |       |       |       |       | rAHF-PFM |       |       |       | Plasma control |
|----------------|-------------|-------|-------|-------|-------|-------|----------|-------|-------|-------|----------------|
|                | 0.043 IU mL\(^{-1}\) | 0.375 IU mL\(^{-1}\) | 0.865 IU mL\(^{-1}\) | 0.043 IU mL\(^{-1}\) | 0.375 IU mL\(^{-1}\) | 0.865 IU mL\(^{-1}\) | 0.960 IU mL\(^{-1}\) |
| One-stage CV, %| 33.7        | 20.0  | 14.0  | 30.8  | 16.3  | 12.4  | 16.3     | 16.3  | 12.4  | 10.3  | 16.3          |
| Intralaboratory CV, %| 9.8        | 6.3   | 4.7   | 6.9   | 6.2   | 5.4   | 6.2      | 6.2   | 5.4   | 5.2   | 6.2          |
| Chromogenic    |             |       |       |       |       |       |          |       |       |       |                |
| Interlaboratory CV, %| 60.0       | 10.9  | 5.4   | 51.0  | 12.8  | 5.8   | 6.6      | 6.6   | 5.8   | 6.6   | 6.6          |
| Intralaboratory CV, %| 10.1       | 5.4   | 6.0   | 6.3   | 2.1   | 4.2   | 4.2      | 4.2   | 4.2   | 4.2   | 4.2          |

CV, coefficient of variation; rAHF-PFM, antihaemophilic factor (recombinant) plasma/albumin-free method.
*Nominal target levels.
ence of approximately 15% between the spiked levels of BAY 81-8973 and rAHF-PFM was observed for all concentrations in every run. The same difference between products was reproducibly found in the results by the external laboratories that used the chromogenic assay, indicating that this difference is intrinsic to the samples because of unavoidable differences in the spiking procedure.

Discussion
This study evaluated the ability of clinical laboratories around the world to accurately measure various concentrations of BAY 81-8973 spiked in FVIII-deficient plasma using their own in-house assays and reagents. Samples spiked with rAHF-PFM at the same concentrations were used as comparators. Overall, measured FVIII:C levels were similar between BAY 81-8973 and rAHF-PFM at all concentrations. Although there were a wide range of methods and reagents for FVIII measurements used by the participating laboratories in this study, mean FVIII:C levels were within 12% of the nominal target level at low (0.043 IU mL$^{-1}$), medium (0.375 IU mL$^{-1}$) and normal (0.865 IU mL$^{-1}$) concentrations of BAY 81-8973. Using the chromogenic assay, mean deviations
from the target level were <3% for all concentrations of BAY 81-8973; for rAHF-PFM, mean FVIII:C levels were from 12% to 14% above nominal target levels for all concentrations. This difference in deviation from target values between products may be partially explained by the spiking procedure.

Assay variability was similar for BAY 81-8973 and rAHF-PFM. Intralaboratory variability for both assays was <11% at all concentrations with both products. Interlaboratory variability was highest at low concentrations of both products in the chromogenic assay and decreased with increased concentration; variability at normal concentrations was similar to the plasma control. There were a small number of outliers for both the one-stage and chromogenic assay results. These outliers were reported from different laboratories and the reasons were unknown. The relatively small number of laboratories that used the chromogenic assay (n = 10) may have contributed to the higher interlaboratory variability at low concentrations reported for both products with the chromogenic assay. An inverse relationship between variability and concentration has been reported in field studies with turoctocog alfa (NovoEight®; Novo Nordisk, Plainsboro, NJ, USA), rFVIII-Fc fusion (rFVIIIFc; Eloctate™; Biogen Idec, Cambridge, MA, USA) and rAHF-PFM, which was used as a comparator in both studies [27,28].

The chromogenic:one-stage assay ratio ranged from 0.95 (low concentration) to 1.10 (normal concentration) for BAY 81-8973 using data from all participating laboratories. The ratios were similar to data from laboratories that reported data using both assays. Although a direct comparison is not possible because of methodology differences, the range of chromogenic:one-stage assay ratios (i.e. assay discrepancy) across the various concentrations used in this study for BAY 81-8973 appears to be smaller compared with some other FVIII products reported in field studies [27,28]. Overestimation of FVIII:C levels at low concentrations using the one-stage assay and at high concentrations using the chromogenic assay has been previously reported and may contribute to assay discrepancy [2,27,28]. In our study, these trends were observed for rAHF-PFM (mean, 119% and 114% of the nominal target level for low concentrations in the one-stage assay and normal concentrations in the chromogenic assay, respectively). In contrast, overestimation at low concentrations of BAY 81-8973 using the one-stage assay was relatively low (mean, 107% of the nominal target level) and was within the mean intralaboratory variability. Furthermore, FVIII:C levels were slightly underestimated at normal concentration in the chromogenic assay (mean, 97% of the nominal target level). Thus, the extent of assay discrepancy for BAY 81-8973 may be considered clinically irrelevant.

Conclusion
This global field study showed no relevant difference in the ability to accurately measure BAY 81-8973 in spiked plasma samples using the one-stage and chromogenic assays. The ratio of the chromogenic assay to the one-stage assay for BAY 81-8973 ranged from 0.95 at the low concentration to 1.10 at the normal concentration. Results were similar to samples spiked with rAHF-PFM. BAY 81-8973 can be reliably measured in plasma with the one-stage and chromogenic assays routinely used in clinical laboratories, and BAY 81-8973 activity measurement does not require a product-specific standard.

Acknowledgements
M. Maas Enriquez, H. Beckmann, D. Tseneklidou-Stoeter and S. Kitchen contributed to study design, data analysis and data interpretation. Y. Katerle participated in sample preparation and analysis, data analysis and data interpretation. S. Bruns participated in data management and data analysis. All authors contributed to the development of the manuscript, reviewed and commented on each draft, and approved the final draft.

This study was funded by Bayer HealthCare AG (Leverkusen, Germany). Medical writing assistance was provided by Ken Wannemacher, PhD, from Complete Healthcare Communications, LLC (Chadds Ford, PA, USA) and was funded by Bayer.

We thank the participating laboratories: Pennsylvania State University (Hershey, PA, USA); Emory Medical Laboratory (Atlanta, GA, USA); Florida Hospital Center for Thrombosis Research (Winter Park, FL, USA); Cincinnati Children’s Hospital Medical Center Special Hemostasis Laboratory (Cincinnati, OH, USA); University of California Davis Health System (Sacramento, CA, USA); Columbia University College of Physicians and Surgeons (New York NY, USA); Boston Children’s Hospital (Boston, MA, USA); Children’s Hospital of Michigan (Detroit, MI, USA); University of Tennessee (Knoxville, TN, USA); University of North Carolina Hospitals (Chapel Hill, NC, USA); University of Texas Health Science Center (Houston, TX, USA); All Children’s Research Institute Inc. (St. Petersburg, FL, USA); University of Rochester (Rochester, NY, USA); University of Minnesota Medical School (Minneapolis, MN, USA); Bloodworks Seattle (Seattle, WA, USA); University of Kentucky (Lexington, KY, USA); University of North Carolina Hospitals (Sacramento, CA, USA); BloodCenter of Wisconsin (Milwaukee, WI, USA); Children’s Medical Center of Dallas (Dallas, TX, USA); Bloodworks (Seattle, WA, USA); MVZ Berlin Leipzig Dr. Reising-Ackermann und Kollegen (Leipzig, Germany); Institut für Exp. Hämostaseologie/Gerinnungslabor (Bonn, Germany); Institute für Thrombophilie und Hämostaseologie (Münster, Germany); Klinikum der Universität München Hämostasierzentrum (München, Germany); CRC-Coagulation Research Center GmbH (Duisburg, Germany); Newcastle upon Tyne Hospitals (Newcastle upon Tyne, UK); St. Thomas Hospital (London, UK); Royal Hallamshire Hospital (Sheffield, UK); Manchester Royal Infirmary (Manchester, UK); Hospital Universitario La Paz (Madrid, Spain); Hospital Universitari Vall D’Hebron (Barcelona, Spain); Hospital Universitari i Politècnic La Fe (Valencia, Spain); Hospital Teresa Herrera Materno Infantil (Coruña, Spain); Calgary Laboratory Services (Calgary, AB, Canada); St. Michael’s Hospital Laboratory (Toronto, ON, Canada); CHU Sainte-Justine Laboratoire d’Hémostase (Montréal, QC, Canada); D.A.I. di Medicina di Laboratorio A.O.U. Federico II (Napoli, Italy); Palazzina Bertarelli Solteraneo Laboratorio Emocoagulazione (Milan, Italy); Klinik Biokemisk Afdeling Aarhus Universitetshospital (Aarhus, Denmark); Rigshospitalet (Copenhagen, Denmark); sheba Medical Center at Tel Hashomer (Ramat-gan, Israel); University of Pretoria Faculty of Health Sciences (Pretoria, South Africa); Specialised Hospital for Active Treatment of Hematology Diseases-Hemostasis Sector (Sofia, Bulgaria); Institutul Clinic Fundeni Central de Hematologie si Transplant Medular (Bucharest, Romania).
Disclosures

M. Maas Enriquez, H. Beckmann, Y. Katterle and D. Tseneklidou-Stoeter are employees of Bayer Pharma AG. S. Bruns has acted as a paid consultant.

References

1 Srivastava A, Brewer AK, Mauser-Bunschoten EP et al. Guidelines for the management of haemophilia. Haemophilia 2013; 19: e1–47.
2 Dodt J, Hubbard AR, Wicks SJ et al. Potency determination of factor VIII and factor IX for new product labelling and postinfusion testing: challenges for caregivers and regulators. Haemophilia 2015; 21: 543–9.
3 Kitchen S, Signer-Romero K, Key NS. Current laboratory practices in the diagnosis and management of haemophilia: a global assessment. Haemophilia 2015; 21: 550–7.
4 Maki M, Peyandi F. Assaying FVIII activity: one method is not enough, and never was. Haemophilia 2014; 20: 301–3.
5 Mikaëlsson M, Oswaldsson U, Sandberg H. Influence of phospholipids on the assessment of factor VIII activity. Haemophilia 1998; 4: 646–50.
6 Gu JM, Ramsey P, Evans V et al. Evaluation of the activated partial thromboplastin time assay for clinical monitoring of PEGylated recombinant factor VIII (BAY 94–9027) for haemophilia A. Haemophilia 2014; 20: 593–600.
7 Barrowcliffe TW, Raut S, Sands D, Hubbard AR. Coagulation and chromogenic assays of factor VIII activity: general aspects, standardization, and recommendations. Semin Thromb Hemost 2002; 28: 247–56.
8 Mikaëlsson M, Oswaldsson U, Jankowski MA. Measurement of factor VIII activity of B-domain deleted recombinant factor VIII. Semin Hematol 2001; 38: 13–23.
9 Hubbard AR. Potency labeling of novel factor VIII and factor IX concentrates: past experience and current strategy. Semin Thromb Hemost 2015; 41: 849–54.
10 Assay of human coagulation factor VIII (2.7.4). European Pharmacopoeia, 5th edn. Strasbourg, France: Council of Europe, 2005: 194–5.
11 National Hemophilia Foundation. Medical and Scientific Advisory Council (MASAC) statement regarding use of various clotting factor assays to monitor factor replacement therapy. Document #228. Available at http://www.hemophilia.org/sites/default/files/document/files/masac-228.pdf. Accessed October 5, 2015.
12 Lusher JM, Hillman-Wiseman C, Hurst D. In vivo recovery with products of very high purity-assay discrepancies. Haemophilia 1998; 4: 641–5.
13 Barrowcliffe TW, Hubbard AR, Kitchen S. Standards and monitoring treatment. Haemophilia 2012; 18: 61–5.
14 Jennings K, Kitchen DP, Woods TA, Kitchen S, Walker ID. Emerging technologies and quality assurance: the United Kingdom National External Quality Assessment Scheme perspective. Semin Thromb Hemost 2007; 33: 243–9.
15 Ingerslev J, Jankowski MA, Weston SB, Charles LA, ReFacto Field Study Participants. Collaborative field study on the utility of a BDD factor VIII concentrate standard in the estimation of BDDr factor VIII: C activity in hemophilic plasma using one-stage clotting assays. J Thromb Haemost 2004; 2: 623–8.
16 Pouplard C, Caron G, Allaud MF et al. The use of the new ReFacto AF Laboratory Standard allows reliable measurement of FVIII: C levels in ReFacto AF mock plasma samples by a one-stage clotting assay. Haemophilia 2011; 17: e538–42.
17 Hubbard AR, Bevan SA, Weller LJ. Potency estimation of recombinant factor VIII: effect of assay method and standard. Br J Haematol 2001; 113: 533–6.
18 Lee CA, Owens D, Bray G et al. Pharmacokinetics of recombinant factor VIII (recombine) using one-stage clotting and chromogenic factor VIII assay. Haemophilia 2011; 17: e958–62.
19 Hubbard AR, Bevan SA, Weller LJ. Potency estimation of recombinant factor VIII: effect of assay method and standard. Br J Haematol 2001; 113: 533–6.
20 Cauchie M, Toelen J, Peerlinck K, Jacquemin M. Practical and cost-effective measurement of B-domain deleted and full-length recombinant FVIII in the routine haemostasis laboratory. Haemophilia 2013; 19: e133–8.
21 Kitchen S, Gray E, Mertens K. Monitoring of modified factor VIII and IX products. Haemophilia 2014; 20: 36–42.
22 Vogel JH, Huesslein A, Goudar C et al. BAY 81–8973 – a new full-length recombinant FVIII product using novel manufacturing technologies. Haemophilia 2010; 16: 40.
23 Saxena K, Lailezari S, Oldenburg J et al. Pharmacokinetics, efficacy, and safety of BAY 81–8973, a full-length plasma-protein-free recombinant factor VIII product: results from the LEOPOLD I trial. J Thromb Haemost 2013; 11: 928.
24 Kavakli K, Yang R, Rusen L, Beckmann H, Tseneklidou-Stoeter D, Maas Enriquez M. Prophylaxis versus on-demand treatment with BAY 81–8973, a full-length plasma-protein-free rFVIII product: results from a randomized trial (LEOPOLD II). J Thromb Haemost 2015; 13: 360–9.
25 Ljung R, Kenet G, Mancuso ME et al. BAY 81–8973 safety and efficacy for prophylaxis and treatment of bleeds in previously treated patients with severe haemophilia A: results of the LEOPOLD Kids trial. Haemophilia 2015. doi:10.1111/hae.12866.
26 Oldenburg J, Lailezari S, Rusen L et al. Efficacy and safety of BAY 81–8973, Bayer’s new full-length recombinant factor VIII: results from the LEOPOLD I and II trials. Presented at: German, Austrian, and Swiss Society of Thrombosis and Haemostasis Research; February 24–27, 2015; Düsseldorf, Germany.
27 Viuff D, Barrowcliffe T, Saugstrup T, Ezban M, Lilliac DI. International comparative field study of N8 evaluating factor VIII assay performance. Haemophilia 2011; 17: 695–702.
28 Sommer JM, Moore N, McGuiffie-Valentine B et al. Comparative field study evaluating the activity of recombinant factor VIII Fc fusion protein in plasma samples at clinical haemostasis laboratories. Haemophilia 2014; 20: 294–300.