A computer-based model to assess costs associated with the use of factor VIII and factor IX one-stage and chromogenic activity assays

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Essentials
- Chromogenic factor VIII and factor IX assays have not been widely adopted partly due to perceived cost.
- Chromogenic assays may be needed for monitoring therapy with some extended half-life concentrates.
- Efficient use of reagents makes the costs of chromogenic and one-stage factor IX assays similar.
- A similar approach for factor VIII delivers lower costs for chromogenic compared with one-stage assays.

Summary. Background: Measurement of coagulation factor VIII (FVIII) and factor IX (FIX) activity can be associated with a high level of variability using one-stage assays based on activated partial thromboplastin time (APTT). Chromogenic assays show less variability, but are less commonly used in clinical laboratories. In addition, one-stage assay accuracy using certain reagent and instrument combinations is compromised by some modified recombinant factor concentrates. Reluctance among some in the hematology laboratory community to adopt the use of chromogenic assays may be partly attributable to lack of familiarity and perceived higher associated costs. Objectives: To identify and characterize key cost parameters associated with one-stage APTT and chromogenic assays for FVIII and FIX activity using a computer-based cost analysis model. Methods: A cost model for FVIII and FIX chromogenic assays relative to APTT assays was generated using assumptions derived from interviews with hematologists and laboratory scientists, common clinical laboratory practise, manufacturer list prices and assay kit configurations. Results: Key factors that contribute to costs are factor-deficient plasma and kit reagents for one-stage and chromogenic assays, respectively. The stability of chromogenic assay kit reagents also limits the cost efficiency compared with APTT testing. Costs for chromogenic assays might be reduced by 50–75% using batch testing, aliquoting and freezing of kit reagents. Conclusions: Both batch testing and aliquoting of chromogenic kit reagents might improve cost efficiency for FVIII and FIX chromogenic assays, but would require validation. Laboratory validation and regulatory approval as well as education and training in the use of chromogenic assays might facilitate wider adoption by clinical laboratories.

Keywords: blood coagulation disorders; blood coagulation tests; factor IX; factor VIII; hemophilia.

Introduction
Hemophilia A and B are rare bleeding disorders associated with episodes of bleeding into joints and muscle tissue that can occur both spontaneously and secondary to trauma [1,2]. Both hemophilia types are characterized by low levels of plasma coagulation factor and factor VIII (FVIII) in hemophilia A or factor IX (FIX) in patients with hemophilia B, and have an incidence of 1 in 5000 and 1 in 30 000 male births, respectively [2]. Administration of replacement coagulation factor concentrates during acute bleeding episodes or as prophylaxis forms the standard treatment and reduces morbidity and mortality.
Coagulation factor activity levels are measured for initial diagnosis and as needed for treatment-monitoring purposes, surgery or surveillance and diagnosis of inhibitor formation [4,5]. Different assay methods are available for measuring clotting factor activity; the one-stage activated partial thromboplastin time assay (APTT) is most commonly used in clinical practice [1,6]. Two-stage chromogenic assays are predominantly used by manufacturers of replacement factor concentrates, but are not yet in widespread use in clinical laboratories [1].

The large number of commercially available aPTT assay reagents and analyzers complicates the standardized measurement of coagulation factor activity and is often associated with a high level of variability that arises from the use of different combinations of reagents, instruments, calibration standards and factor-deficient plasmas [6–11]. Assay discrepancies of up to 40% or underestimation of activity depending on APTT reagents used have been reported [8,9]. This variability is of particular concern in the lower factor activity range and in the diagnosis of mild FVIII deficiencies, in which some genetic mutations lead to discrepant results between one-stage and chromogenic assays [12–15]. Some newer modified FVIII and FIX molecules have been shown to interfere with measurement of factor activity using APTT assays and have drawn attention to the limitations of APTT assays [11,16–21]. In contrast, chromogenic assays show less variability in the measurement of FVIII and FIX activity levels, possibly due to a smaller number of available assay kits and reagents and the more stringent assay conditions, but are currently less commonly used in clinical laboratories [6,19,22]. In 2014, the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation (NHF) recommended that laboratories that routinely perform coagulation factor activity assays using samples from patients with hemophilia A or B consider the addition of chromogenic assays where available and once approved by regulatory authorities [16].

Reluctance among the hematology laboratory community to universally adopt use of chromogenic assays for FVIII and FIX monitoring may be attributable in part to a lack of familiarity and standardized support with implementation from manufacturers, a perception of higher associated costs for these assays, and lack of Food and Drug Administration (FDA) approval in the USA for FIX kits. This study used interviews with hematologists and laboratory scientists to identify, characterize and compare the key cost parameters associated with the use of one-stage APTT and chromogenic assays. Based on the interview findings, a computer-based cost model was generated (Cambridge Consultants Limited, Cambridge, UK) to assess the impact of price-sensitive variables on both assays. Batch testing of patient samples, increased laboratory throughput, improved kit reagent stability and aliquoting of kit reagents were tested as possible means by which to optimize assay efficiency and reduce associated costs.

Materials and methods

Reference assay kits and additional reagents

The reference chromogenic and one-stage APTT assay kits and additional reagents (Table 1) were mapped to the assay workflow (Fig. 1A and B) in order to quantify relevant parameters. Commercial list prices (current as of September 2014) of base-case and alternative reference assay kits and additional reagents were used as a basis for the cost model. The chromogenic assay kits assessed in this study carry a Conformité Européenne (CE) mark in the

| Table 1 Chromogenic and one-stage activated partial thromboplastin time (APTT) reference assay kits and additional reagents used in the cost model |
|-----------------------------------------------|
| **Chromogenic assay kits** | **Base-case** | **Alternative** |
| **FVIII** | | |
| Name | BIOPHEN® Chromogenic Factor VIII:C | Coamatic® Factor VIII |
| Manufacturer | Hyphen-Biomed (Neuville sur Oise, France) | Chromogenix/Instrumentation Laboratory (Bedford, MA, USA) |
| List price | £195.89 (equivalent from list price in Germany) | £170.05 |
| Configuration | 2 × 100 tests | 2 × 50 tests |
| FIX | Base-case | Alternative |
| Name | Rox Factor IX | BIOPHEN® Chromogenic Factor IX:C |
| Manufacturer | Rossix (Mölndal, Sweden) | Hyphen-Biomed (Neuville sur Oise, France) |
| List price | £183.33 | £217.33 |
| Configuration | 2 × 100 tests | 2 × 100 tests |
| **One-stage APTT assay kits** | **Base-case** | **Alternative** |
| **FVIII and FIX** | | |
| Name | HemosIL® APTT-SP | Dade® Actin® FS |
| Manufacturer | Instrumentation Laboratory | Siemens Healthcare Diagnostics Inc. (Barrytown, NY, USA) |
| List price | £55.50 | £183.33 |
| Configuration | ~900 tests (depending on analyzer protocol) | 2000 tests |
| Additional reagents | FVIII-deficient plasma, FIX-deficient plasma, HemosIL® Normal Control Plasma, HemosIL® Abnormal Control Plasma and HemosIL® Calibration Plasma (all Instrumentation Laboratory) | |
UK and the European Union (EU), while some are approved for research only in the USA; therefore, list prices in this study are quoted in pounds sterling (GBP, £).

Cost model

Interviews with members of the author group, comprising hematologists and laboratory scientists, as well as chromogenic assay kit manufacturers (Rossix [Mölndal, Sweden] and Hyphen-Biomed [Neuville sur Oise, France]), informed core assumptions for the model and the underlying workflow (Fig. 1A and B).

A detailed list of assumptions underlying the cost model is included in the Supplementary Information (Tables S1, S2, S3). Core assumptions, derived from the analysis of initial stakeholder interviews, were applied to both one-stage APTT and chromogenic factor assay cost models and include the following. (i) Factor assays are performed on an automated basis using a reference bench-top analyzer. The Instrumentation Laboratory (Bedford, MA, USA) ‘ACL TOP’ was chosen as the reference instrument based on the high frequency of use reported in a market research survey among practicing hematologists in the UK, Spain, Germany, France, Italy, Japan and the USA (Haemophilia Assay Survey, PSL Research, March 2012, Novo Nordisk, data on file). (ii) The majority of hemostasis laboratories use a single analyzer that performs multiple coagulation assays in addition to FVIII and FIX assays. (iii) Most factor assays performed in clinical laboratories are for patient diagnosis and monitoring purposes, requiring a single assay protocol that uses the reference kit and additional reagents. (iv) Once kit reagents have expired, fresh reagent is not added to the analyzer until a new patient sample requires testing on the analyzer. (v) Room temperature is used as a basis for estimating reagent shelf-life on analyzers.

Other assumptions include the costs of assay kits and reagents, and costs per patient test, which are given in GBP (£) unless otherwise stated, and with list prices assumed, without accounting for discounts for bulk purchase. The costs associated with capital expenditure items, such as analyzer instrumentation, centrifuges and pipette tips, have not been included. Costs associated with ‘hands-on’ laboratory scientist time have not been included because the results of interviews with hematologists indicated that a similar amount of hands-on laboratory scientist time is required to conduct APTT and chromogenic assays. Similarly, the cost impact of assay turnaround time has not been assessed.

The base-case assumptions for chromogenic factor assays were outlined as follows. The number of patient tests that can be performed using a set of reagent vials is the nominal number possible under ideal conditions. The maximum stability of the reagent on the analyzer is 24 h for FVIII and 8 h for FIX chromogenic kit reagents [23,24]. Duplicate samples are assayed. The monthly throughput of assays for FVIII and FIX was set at 100 and 20 assays, respectively.

Base-case assumptions made for one-stage APTT assays are as follows. Automated aliquoting of factor-deficient plasma results in approximately 10% loss of reagent. The maximum stability of the reagent on the analyzer is 24 h for FVIII-deficient and 8 h for FIX-deficient plasma. Duplicate samples are assayed using multiple dilutions (three per sample). The monthly throughput of assays for FVIII and FIX was set at 100 and 20 assays, respectively.

These general and specific sets of assumptions were used to generate base-case cost models for FVIII and FIX APTT and chromogenic assays.

Results

Base-case cost modelling analysis

Interviews with hematologists and laboratory scientists indicated that in general, low laboratory-level factor activity assay throughput rates in both Europe and the USA are associated with poor efficiency of chromogenic testing, particularly for FIX assays. In agreement with this, the range of reported laboratory assays performed showed a clear difference between the coagulation factor types, with annual testing rates reported to be between 700 and 1600 (FVIII) and 70 and 700 (FIX). In addition, chromogenic assays are perceived as technically complex compared with other coagulation factor assays.

Results from the base-case model analysis for FVIII one-stage APTT and chromogenic assays yielded similar costs per patient test of £20.32 (APTT) and £21.70 (chromogenic). These broadly comparable costs per patient test.
for both FVIII assay types reflect the optimization of APTT kit configuration (and additional reagents required) for low-throughput rates and FVIII assay throughput rates that permit multiple chromogenic tests to be carried out within the window of the kit reagent shelf-life.

The FIX assay base-case analysis predicted higher costs for chromogenic FIX testing, resulting in costs per patient test of £98.46 (chromogenic) compared with £21.68 (APTT). Costs are influenced by two principal confounding factors: shorter stability of the reagent on the analyzer compared with the FVIII chromogenic reagent (8 h vs. 24 h) and lower FIX assay throughput rates compared with FVIII (approximately 2.5-fold lower). Shortened stability of the reagent reduces the number of assays that can be performed within the window of reagent stability, and lower throughput rates have a deleterious impact on assay efficiency, and in turn increase the costs per test.

The cost analysis identified factor-deficient plasma as the primary cost factor for both the FVIII and FIX APTT assays (Fig. 2A). Plasma used for calibration of factor assays contributes approximately 9% of total APTT assay costs (Fig. 2A). The primary cost factor for both FVIII and FIX chromogenic assays is kit reagent, while calibration costs for chromogenic assays are negligible in relative terms (Fig. 2B).

Alternative-case cost modelling analysis

Additional cost modelling using alternative APTT and chromogenic assay kits for both FVIII and FIX was performed to account for variations in list prices between assay manufacturers (see Table S2). The resulting kit costs for FVIII APTT vs. chromogenic assay kits were £20.86 and £26.39, respectively. Predicted costs for FIX APTT vs. chromogenic assay kits were £22.22 and £109.18, respectively. Although the additional modelling showed a degree of variation in individual costs per test, the relative cost differences remained in line with the assay kits used in the base-case analysis described above.

Adjustment of select model parameters

The model was adjusted to determine the impact of batch testing, an increased laboratory factor assay throughput rate, reagent stability and the possibility of using frozen aliquots of kit reagents. Modelled batch testing of up to four patient samples to improve testing efficiency reduced the cost of performing both APTT and chromogenic factor assays (Fig. 3A and B). Batch testing patient samples significantly reduced the cost per patient test for one-stage APTT assays in part because the cost of control and calibration materials can be distributed across multiple patient tests, and although the APTT kit reagent is used for each assay, the aliquot cost is negligible in relative terms (Fig. 3A). For chromogenic factor assays batch testing...
testing showed the strongest impact on cost reduction for
FIX assays by increasing the number of tests carried out
within the limited kit-reagent shelf-life, with costs being
reduced by close to 50–75% for batches of two to four
patient samples, respectively (Fig. 3B). The cost of addi-
tional reagents may be distributed across multiple patient
tests; however, this cost is low and therefore exerts little
influence on overall cost savings.

Increasing the laboratory factor assay throughput rate
yielded a cost reduction for chromogenic FVIII assays,
but had no impact on the difference between one-stage
APTT and chromogenic FIX assays (Fig. 4A and B). An
increase in laboratory assay throughput enables more
assays to be performed within the window of kit reagent
stability and optimizes the cost per patient relative to kit
configuration (Fig. 4A). At the highest expected labora-
tory FIX assay rate (80 assays per month), chromogenic
testing operates at less than a single test within the win-
dow of kit reagent stability, maintaining the cost per
patient at the price per set of kit vials (Fig. 4B). Although
chromogenic FIX assay kits are optimized for high
throughput by design, the associated costs in this model
remain unaffected because the potential capacity exceeds
clinical laboratory requirements and the general frequency
of FIX assay use in clinical practise.

Limited reagent stability was identified as a confound-
ing factor for chromogenic FIX assay costs in the base-
case analysis compared with FVIII assay costs. With a
shelf-life of 24 h on the analyzer, doubling kit reagent
stability should yield an approximately 50% reduction in
chromogenic FVIII assay costs (Fig. 5A). In contrast, in
order to achieve the same level of efficiency with a shelf-
life of 8 h on the analyzer, FIX reagent stability would
need to increase by 6-fold before a small improvement in
cost per patient could be expected (Fig. 5B). The limited
effect of an extended shelf-life on chromogenic FIX
assays is partly due to the relatively low frequency with
which this assay is carried out.

Fig. 4. Impact of laboratory throughput on the cost of factor (F)
VIII (A) and FIX (B) one-stage activated partial thromboplastin
time (APTT) and chromogenic assays. The cost model was adjusted
to include an increased frequency of assay procedures and the effect
on cost per patient test compared with the base-case analysis was
determined.

Fig. 5. Impact of kit reagent stability on the cost of activated partial
thromboplastin time (APTT) and chromogenic factor (F) VIII (A)
and FIX (B) assays. An extension to the reagent shelf-life was
included in the cost model to determine the effect on cost per patient
test compared with base-case. N/A, not applicable.

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A model that included the preparation of aliquots of chromogenic kit vial reagents yielded the most significant reductions in cost per patient test for both FVIII and FIX assays, both in absolute terms and relative to APTT assay costs. By aliquoting and freezing the most labile kit reagents, assay efficiency might be improved by minimizing wastage of reagent due to shelf-life expiration despite a low overall testing frequency (Fig. 6A and 6B). The preparation of four instead of two chromogenic kit reagent aliquots permits a reduction in cost per patient test close to 50% for both FVIII and FIX assays. A further doubling of aliquots showed only a limited reduction in cost per patient test for FVIII (Fig. 6A), but resulted in an additional reduction in cost of close to 75% compared with the base-case for FIX chromogenic assays (Fig. 6B).

Discussion

The hematologist and laboratory scientist interviews identified assay throughput, chromogenic kit configuration and reagent stability as key cost parameters that were tested using the model. Low throughput of laboratory-level factor assays in Europe and the USA contributes to the poor cost efficiency of chromogenic testing, particularly for FIX assays because large sample batch sizes are rare due to the low incidence of hemophilia B. Currently available chromogenic kit configurations are not optimized for median laboratory factor assay rates, and smaller kit sizes might be more cost-effective for clinical laboratory use. In addition, the narrow window of chromogenic kit reagent on-analyzer stability limits the cost efficiency of chromogenic assays relative to APTT testing. While manufacturer list prices for APTT and chromogenic assay kits vary, additional analyses using alternative kits showed that the overall cost difference between assay types remained in line with the base-case reference kits used for cost modelling.

Due to demand, the majority of chromogenic kits are designed for pharmaceutical industry needs rather than clinical laboratory requirements. This stems from the fact that chromogenic kits were originally developed for use by pharmaceutical companies in testing the potency of FVIII replacement factor therapy as per European Pharmacopoeia guidelines [25,26]. Potency testing requires high-throughput testing; therefore, kit configuration (i.e. the number of tests per kit) is aligned with this. The typical chromogenic kit configuration is 100 to 240 tests, which is not optimally aligned with the requirements of clinical laboratories. While feasible, the reconfiguration of chromogenic kits would involve significant technical, commercial and regulatory challenges for manufacturers.

Compared with APTT assays, the narrow window of chromogenic kit reagent on-analyzer stability has a negative impact on the chromogenic assay’s cost efficiency. Common hemostasis laboratory practise involves loading the analyzer with factor assay reagents at the start of a shift. The reconstituted chromogenic kit reagents remain stable for up to 28 days at 2–8 °C, but their on-analyzer shelf-life at room temperature is limited to 8–24 h. This narrow window of on-analyzer shelf-life for the reagent limits the number of assays that can be performed with a single kit. While the impact on cost per patient of improvement to the ‘on-board’ analyzer kit reagent’s stability can be modelled, this permutation is theoretical and might face feasibility challenges in that extensions to shelf-life would require significant reformulation of kit reagents.

Chromogenic assay kits typically require additional reagents, notably calibration and control plasma. Provided these additional reagents are used in a range of coagulation factor assays in clinical laboratories, as was often the case amongst survey participants whose
responses were used to fix assumptions, these do not represent a cost constraint. The cost modeling analyses showed that the primary factors contributing to assay costs are factor-deficient plasma for APTT assays and kit reagents for chromogenic assays. In the case of APTT assays, the cost of the calibrator plasma in factor assays contributes approximately 9% to assay costs, whereas this is negligible for chromogenic assays. This is due to the low cost of the factor-deficient plasma compared with calibrator plasma, resulting in the cost of the calibrator plasma having a greater impact on total APTT assay costs. In contrast, the high cost of chromogenic reagent compared with calibrator plasma, results in the calibrator plasma contributing only ≤1% of total assay costs. The model also assumes that a calibration is carried out for each run for both assays. In both cases local practise such as live assay calibration will have an important influence on the contribution of calibrator costs to overall costs. The cost model, however, acknowledged that calibration practise varies between centers by enabling users to choose whether calibration plasma was used for each assay run to generate a calibration curve or not. It also allowed users to change parameters used in the cost per patient test, such as calibration plasma diluent factor, number of calibration plasma aliquots required per assay and volume of diluted plasma per assay. Furthermore, the cost contribution of calibration plasma was divided by the number of patient samples being analyzed per assay run.

Use of batch testing and aliquoting of chromogenic kit reagents may represent means with which to improve assay cost efficiency. Chromogenic kits are typically supplied as a set of reagent vials to be reconstituted and stored at 2–8 °C. Aliquoting and freezing of kit reagents, particularly the more labile kit reagents, may provide an option that could improve cost efficiency for both FVIII and FIX chromogenic assays, but would require validation and verification. Empirical testing and validation studies are needed to demonstrate that freeze-thawed reagent aliquots provide assay performance equivalent to freshly prepared reagent. The feasibility of batch testing in real-life clinical practise would also need to be evaluated locally.

Findings from the hematologist and laboratory scientist interviews suggest that the chromogenic assay is perceived as a technically complex assay, compared with other coagulation factor assays. The reported lack of familiarity with chromogenic assay testing may be attributable in part to the current research-only status of some chromogenic assays in the USA; a change in regulatory status might improve this situation. The majority of laboratories have the instrumentation to run chromogenic assays. However, a lack of standardized test protocols for analyzers, diminishing assay sensitivity at low factor levels and the impression of many laboratory scientists that they do not have the necessary expertise may also contribute to this perception. Furthermore, most manufacturers do not provide validated protocols for their assays for the various available automated coagulation assay platforms. Therefore, APTT-based factor assays are perceived as the incumbent assay format, regardless of its documented performance limitations [14,18,20,27,28]. The cost of training is also perceived as an obstacle; particularly given the relatively large number of ‘off-hours’ staff that may be required to perform factor assays. In addition, accreditation standards and regulations that laboratories in many countries must meet are associated with additional tasks before introducing a new test [29]. However, the improved standards may ensure validated consistency in assay performance in the future. In conclusion, regulatory approval of chromogenic assays in countries in which they are at present for research use only, and laboratory verification once approved, as well as education and training with respect to the use of chromogenic assays, might facilitate wider adoption by mainstream clinical laboratories. Collaborative efforts between clinical laboratories and assay kit manufacturers will be necessary to facilitate the implementation of coagulation assays.

Addendum

S. Kitchen, S. Platton, D. Tan-Castillo and R. Luddington contributed to the hematologist interview program to define factor assay materials, methods and key cost parameters. J. Blakemore carried out the hematologist and scientist interview program and development of the costing model and analysis. S. Kitchen, J. Blakemore, K. Friedman, D. Hart, R. H. Ko, D. Perry, S. Platton, D. Tan-Castillo, G. Young and R. J. Luddington participated in data analysis and revision of model core assumptions and provided insight into clinical laboratory practise. All authors contributed to the writing, critical revision and approval of the manuscript.

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Disclosure of Conflict of Interests

S. Kitchen has received consultancy and speaker fees from Novo Nordisk. J. Blakemore has received personal fees from Novo Nordisk. K. Friedman has received consultancy fees from Biogen Idec, CSL Behring, Instrumentation Laboratories and Novo Nordisk, and has received speaker fees from Alexion. D. Hart has served on advisory boards for Baxalta, Bayer, Novo Nordisk and

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Octapharma; has received investigator-initiated grants from Baxter and Octapharma; has received consultancy and speaker fees from Biogen, Octapharma and Pfizer; was involved in organizing meetings for Biogen and Pfizer; and has received a travel bursary from CSL Behring. R. Ko has received consultancy fees from Novo Nordisk. Young has received personal fees from Novo Nordisk, outside the submitted work. The other authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Core assumptions included in the cost models.

Table S2. Specific assumptions and sources for the one-stage APTT assay base-case and alternative assay kits. Prices were current as of September 2014.

Table S3. Specific assumptions and sources for the chromogenic assay base-cases and alternative assay kits. Prices were current as of September 2014.

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