Measurement of Direct Anti-Xa Activity of Rivaroxaban (Reference — 2013.03.007)
Notice of Assessment

April 2014
1 GENERAL INFORMATION
1.1 Requestor
CHUM

1.2 Application for Review Submitted to MSSS
December 17, 2012

1.3 Application Received by INESSS
November 1, 2013

1.4 Notice Issued
February 28, 2014

Note:
This notice is based on the scientific and commercial information submitted by the requestor and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY, AND LICENCE(S)
2.1 Name of the Technology
The requestor uses the commercially available kit Biophen DiXaI (Direct Xa Inhibitors) standardized for calibration and measurement.

2.2 Brief Description of the Technology, and Clinical and Technical Specifications
Rivaroxaban is a highly selective oral factor Xa (FXa) inhibitor anticoagulant [CPhA, 2013]. FXa plays a central role in the coagulation cascade, directly converting prothrombin to thrombin, which precedes fibrin formation and platelet activation [CPhA, 2013]. Rivaroxaban is indicated for prevention of venous thromboembolism after total hip- or knee-joint replacement surgery, treatment of venous thromboembolism, prevention of recurrent thromboembolism, and prevention of stroke and systemic embolism in patients with atrial fibrillation [CPhA, 2013]. Unlike warfarin, rivaroxaban does not require regular biological monitoring or patient diet restrictions.

The Biophen DiXaI kit is used for in vitro quantitative measurement of direct factor Xa inhibitors (DiXals) using a chromogenic anti-Xa assay that can be automated [HYPHEN BioMed, 2013a]. The assay with this kit has two stages. The first is inhibition of a constant and excess amount of exogenous FXa by the tested DiXaI. Second, hydrolysis is triggered by adding an FXa-specific chromogenic substrate to the residual FXa. This step releases the chromophore (para-Nitro-anilin or pNa) from the substrate (oligopeptides of 3 to 4 amino acids), dying the tested sample yellow. The amount of pNa released (measured by absorbance at 405 nm) is directly related to the residual FXa activity. It is inversely proportional to the concentration of DiXaI in the tested sample.

Calibration plasma samples with increasing concentrations of rivaroxaban must be used to calibrate the assay method (Biophen Rivaroxaban® Plasma Calibrator kit) [HYPHEN BioMed, 2013b]. Adapted control plasma samples, titrated for the assayed DiXaI, can be used to validate the calibration curve and the homogeneous reactivity of the assay in different series for the same lot of reagents (Biophen Rivaroxaban Control Plasma kit) [HYPHEN BioMed, 2013c].
2.3 Company or Developer
HYPHEN BioMed, Neuville-sur-Oise, France.

2.4 Licence(s)
Not applicable.

2.5 Patent, If Any
Not applicable.

2.6 Approval Status (Health Canada, FDA)
The Biophen DiXal kit is approved for in vitro diagnostic use in Europe. The kit is not yet approved for in vitro diagnostic use in Canada or the United States. In these two countries it is indicated “for research use only.”

However, the Biophen Rivaroxaban® Plasma Calibrator kit (licence number 88035) and the Biophen Rivaroxaban Control Plasma kit (licence number 88034) have been licensed in Canada since January 12, 2012.

2.7 Weighted Value
18.0.

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group
Patients with severe bleeding, such as intracerebral hemorrhage, or who must undergo emergency surgery, while on anticoagulant therapy with rivaroxaban.

3.2 Targeted Disease(s)
The test under consideration does not target a specific disease but rather the therapeutic monitoring of patients treated with rivaroxaban who meet the criteria set out in 3.1. The predictable pharmacokinetic and pharmacodynamic profiles of rivaroxaban make it an alternative to warfarin. However, as there is very little evidence on the preferred method of intervention to manage major or clinically significant bleeding in patients taking rivaroxaban, and as there is no specific antidote to reverse its anticoagulant effect, many physicians are reluctant to prescribe it [Fawole et al., 2013; Miyares and Davis, 2012].

3.3 Number of Patients Targeted
In December 2012, the requestor anticipated a volume of approximately 200 tests in Québec over the next three years. This number could increase with the projected increase in rivaroxaban prescriptions. Based on analyses by INESSS, the number of individuals taking rivaroxaban and covered by the Public Drug Insurance Plan was 3,160 and 6,714 in 2011 and 2012, respectively (results not published). This number reached 17,515 in the first 10 months of 2013. The number of tests carried out could be higher than anticipated by the requestor owing to the increase in rivaroxaban prescriptions and the fact that its usage might not be limited to specific situations such as severe bleeding or emergency surgery.
3.4 Medical Specialties and Other Professions Involved
The main medical specialties involved are hematology, surgery, emergency medicine, and family medicine. In all likelihood, dental surgeons and pharmacists will also prescribe the quantitative measurement of rivaroxaban.

3.5 Testing Procedure
Blood samples (9 volumes) must be collected into a plastic or siliconized glass tube containing 0.109 mol/L trisodium citrate (1 volume) by clear venipuncture, avoiding any activation [HYPHEN BioMed, 2013a]. The first drops of blood must be discarded. Within four hours, the sample must be centrifuged at 2,500 g for 15 minutes,\(^{33}\) at room temperature (18°C to 22°C). The citrated plasma must be decanted into a plastic tube. The plasma samples may be stored up to four hours at room temperature (18°C to 25°C), or up to one month frozen at –20°C or colder (before use, thaw plasma for 15 minutes in a water bath at 37°C).

The Biophen DiXaI kit is used with automated kinetics methods, and can also be used for end-point manual methods. Adaptations to other automated analyzers are available upon request. The assay is performed at 37°C, and the color developed is measured at 405 nm. Calibration must be done during the following hour for optimal assay performance. Calibration is acceptable if the coefficient of determination \((r^2)\) is ≥ 0.98 and if measured values for controls are in the acceptable range. A new calibration curve must be carried out for every new lot of reagents, after any significant maintenance on the analyser, and when quality control results are outside the acceptable range for the method. Each laboratory can define its own acceptable range based on the protocols and instruments used.

4 TECHNOLOGY BACKGROUND
4.1 Nature of the Diagnostic Technology
Unique test.

4.2 Brief Description of the Current Technological Context
The predictable pharmacokinetics and pharmacodynamics of rivaroxaban generally make routine biological monitoring of this anticoagulant unnecessary [Garcia et al., 2013; Mueck et al., 2008b]. It may, however, be useful to assess the anticoagulant activity of rivaroxaban in patients with severe bleeding or in need of emergency surgery [Garcia et al., 2013; Samama et al., 2013; Tripodi, 2013]. Rivaroxaban modifies current clotting assays such as prothrombin time (PT), cephalin time (ACT) and the HepTest [CPhA, 2013; Garcia et al., 2013; Helin et al., 2013; Samama et al., 2013; Tripodi, 2013; Douxfils et al., 2012; Samama et al., 2010c]. None of these assays is sufficiently accurate (valid and precise) for quantitative determination of rivaroxaban’s anticoagulant activity under usual conditions. Nevertheless, PT can be used to determine whether the anticoagulant activity is excessive (qualitative estimate) because it varies in proportion to the rivaroxaban dose if Neoplastin is used as reagent [CPhA, 2013]. High-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) is the preferred method for detecting and quantifying rivaroxaban, but its availability is limited. The chromogenic anti-Xa assay, using rivaroxaban calibrators and controls, is an option for indirectly and accurately estimating the concentration of rivaroxaban [Douxfils et al., 2013b; Douxfils et al., 2013c; Rathbun et al., 2013; Asmis et al., 2012]. Various kits using the chromogenic technique, with rivaroxaban calibrations and controls, are currently available (STA®-Liquid anti-Xa, Stago; TechnoChrom®, Technoclone, Vienna, Austria; 3

\(^{33}\)The resulting acceleration, written as g, is a function of the rate of rotation and the distance from the axis of rotation. It is expressed by the following formula: \(g = w^2 r = 1.119 \times 10^5 \times r \times n^2\), where \(w\) = angular velocity (rad/s); \(r\) = distance from the axis of rotation; \(n\) = number of revolutions per minute (rpm).
Biophen DiXal, HYPHEN BioMed, Neuville-sur-Oise, France) [Garcia et al., 2013]. Nonetheless, calibrated PT assays are less expensive than chromogenic assays and are suggested for screening for concentrations that exceed a specified cutoff [Douxfils et al., 2012]. Biophen DiXal, coupled with plasma calibrators and control plasmas, would then be reserved for the previously screened cases [Douxfils et al., 2012].

4.3 Brief Description of the Advantages Cited for the New Technology

The new test serves to measure rivaroxaban in emergency situations—such as emergency surgery or severe bleeding—and determine the residual quantity of rivaroxaban. The time interval between the administration of rivaroxaban and blood sampling for the assay is important, because the onset/offset actions of the new oral anticoagulants are relatively fast [Garcia et al., 2013; Tripodi, 2013]. Consequently, clinicians must be cautious when interpreting laboratory results because this data may not truly represent the in vivo situation [Garcia et al., 2013; Tripodi, 2013].

Nevertheless, emergency situations such as bleeding or thrombosis leave little choice in terms of the timing of blood sampling and rivaroxaban measurement [Tripodi, 2013]. Clinicians will require education about when the rivaroxaban assay should be done and how to interpret the results of these laboratory tests correctly [Garcia et al., 2013].

4.4 Cost of Technology and Options

Not assessed.

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced

The chromogenic anti-Xa technique is used in currently available, standard heparin assays. These tests are not appropriate for rivaroxaban assays because they are designed for indirect Factor Xa inhibitors requiring antithrombin for their activity. Furthermore, the kinetics and mechanisms of direct factor Xa inhibitors are completely different, requiring the use of specific rivaroxaban calibrator and control plasmas [HYPHEN BioMed, 2013d; Samama et al., 2010a; Samama et al., 2010b].

5.1.2 Therapeutic Value

Assaying rivaroxaban in an asymptomatic, stable patient on chronic therapy could be hazardous if a clinician is prompted to deviate from the recognized effective dosage [Garcia et al., 2013]. Indeed, clinicians may overestimate the significance of an assay measurement that slightly exceeds (or falls below) the laboratory-defined normal range. Although pharmacokinetic studies have identified an expected range of rivaroxaban concentration, this range does not necessarily define the limits beyond which the bleeding or thrombosis risk would increase significantly for an individual. In contrast to warfarin, for which the relation between the international normalized ratio (INR) and concentrations outside the therapeutic range is better understood, the clinical significance of coagulation assay results that fall outside the therapeutic range is not known for rivaroxaban. In addition, the interpretation of laboratory results for rivaroxaban must take into account when the sample was collected, which is not an issue for the INR in warfarin-treated patients. Thus, even if an evidence-based dosage adjustment strategy were available, the potential to misinterpret (or act improperly upon) a single measurement would be significant [Garcia et al., 2013].
5.2 Clinical Validity
No studies of the clinical validity of the rivaroxaban assay were identified.

5.3 Analytical (or Technical) Validity

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<th>COMPONENT</th>
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<td>Correlation between test and comparator</td>
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5.3.1 Precision
In a assay working range of 0 to 500 ng/mL of rivaroxaban, the manufacturer of the Biophen DiXaI kit estimates the intra-assay variability at less than 3% and the inter-assay variability at less than 10% to 15%, depending on the calculation of the coefficient of variation (CV = standard deviation/mean * 100) [HYPHEN BioMed, 2013a]. The intra- and inter-assay CV was respectively 3.4% and 3.9% based on 10 measurements of 5 concentrations (0, 22, 110, 218, and 436 ng/mL) [Douxfils et al., 2012]. The mean CV represented the sum of the CV for the concentrations divided by 5. For the inter-assay variability, measures were performed once a day during 10 days with the same lot of reagents.

5.3.2 Coefficient of variation (CV)
Douxfils et al. [2012] estimated the reproducibility of Biophen DiXaI kit results by performing triple assays of seven different concentrations of plasma spiked with rivaroxaban at concentrations from 11 to 1,090 ng/mL. Reproducibility, expressed as CV, averaged 1.3% for the seven concentrations [Douxfils et al., 2012].

The precision of the chromogenic anti-Xa assay with rivaroxaban calibrators has also been evaluated [Asmis et al., 2012]. Plasma samples from 20 healthy volunteers were taken two to three hours after ingesting 10 mg of rivaroxaban. The samples were assayed by nine laboratories, of which seven were double-blinded. The mean rivaroxaban concentration obtained using the chromogenic technique was 114 ± 43 ng/mL. The mean result using HPLC-MS/MS, regarded as the most accurate measurement possible, was 100 ± 26 ng/mL. The validity and precision of the assays of spiked plasma samples were 7.0% and 8.8%, respectively. Validity was calculated by dividing the difference between the mean of chromogenic assay results and the calculated mean for the HPLC-MS/MS assays by the latter. The quotient was then multiplied by 100. Inter-laboratory precision of in vitro measurements was estimated by dividing the standard deviation of the seven double-blinded laboratories by the mean of their measurements and multiplying the quotient by 100.

5.3.3 Analytical sensitivity
Sensitivity of the rivaroxaban quantitative assay using Biophen DiXaI is 9 ng/mL [Douxfils et al., 2013c; 2012]. This is less than that obtained with HPLC-MS/MS (3 ng/mL), but greater than that obtained by PT (66 to 258 ng/mL) or by ACT (208 to 420 ng/mL) [Douxfils et al., 2013a; 2012]. The detection threshold is about 50 ng/mL for rivaroxaban, according to the manufacturer of
Biophen DiXaI, in an assay working range of 0 to 500 ng/mL in plasma [HYPHEN BioMed, 2013a]. Douxfils and Biophen DiXaI, in its monograph, define sensitivity and detection threshold differently,\(^{34}\) hence the observed disparity between the two values. The plasma concentrations obtained with the two main rivaroxaban dosages explain the sensitivities observed depending on the assay technique used. Thus, at the dosage of 10 mg per day of rivaroxaban prescribed to prevent venous thromboembolism after total hip- or knee-joint replacement surgery, the median maximum concentration of this anticoagulant reaches 125 ng/mL (5th to 95th percentile: 91 to 196 ng/mL) and the median minimum concentration \(C_{\text{trough}}\) just before the following dose was 9 ng/mL (5th to 95th percentile: 1 to 38 ng/mL) [Douxfils et al., 2012; Mueck et al., 2008a]. At the dosage of 20 mg/day used in prevention of stroke and systemic embolism in patients with atrial fibrillation, the maximum concentration is approximately 290 ng/mL (5th to 95th percentile: approximately 177 to 409 ng/mL) [Douxfils et al., 2012]. The concentration measured just before the following dose \(C_{\text{trough}}\) is approximately 32 ng/mL (5th to 95th percentile: approximately 5 to 155 ng/mL) [Douxfils et al., 2012; Mueck et al., 2011].

5.3.4 Interference
When used in accordance with the manufacturer’s instructions, Biophen DiXaI does not interfere with indirect FXa inhibitors such as fondaparinux, heparin, and low-molecular weight-heparins [Douxfils et al., 2012; HYPHEN BioMed, 2012].

5.3.5 Bland-Altman method
The Bland-Altman analysis of the two values obtained for the same assays showed a mean difference of -16 ng/mL with a standard deviation of 25 ng/mL and a 95% confidence interval from -65 to 32 ng/mL. The Biophen DiXaI kit is little influenced by inter-individual variability [Douxfils et al., 2013c].

5.3.6 Correlation between test and comparator
Biophen DiXaI measurements were compared with the HPLC-MS/MS reference measurement [Douxfils et al., 2013c]. The plasma concentration of 52 samples from rivaroxaban-treated patients, measured by HPLC-MS/MS, ranged from 0 to 485 ng/mL. The Biophen DiXaI and HPLC-MS/MS were highly correlated, with a Pearson correlation coefficient \(r\) of 0.95 (95% CI from 0.91 to 0.97).

5.3.7 Linear regression
The relation between the rivaroxaban plasma concentration and the measurement of absorbance reported by minute using the Biophen DiXaI assay (amount of pNA released, measured by absorbance at 405 nm) is linear up to a concentration of 545 ng/mL, but decreases in sensitivity [Douxfils et al., 2012]. The coefficient of determination \(r^2\) of the regression line is 0.99 between the 5th and 95th percentiles of concentrations in a simulated population receiving 20 mg rivaroxaban per day [Douxfils et al., 2012]. The relation between the two variables is no longer linear at concentrations higher than 545 ng/mL [HYPHEN BioMed, 2013a; Douxfils et al., 2012]. A higher dilution of the samples (initially diluted to 1/50).

\(^{34}\)Douxfils defines sensitivity as follows: “The final concentration in rivaroxaban needed to double (or halve) the analytical parameter; \(2\times CT\) \([\text{CT} = \text{Clotting Time}]\); \(C_{\text{IC}50}\) [The inhibitor concentration reducing the \(C_{\text{max}}\) of 50%] and \(2 \times \text{OD/min}\)” [The concentration needed to halve the change in the optical density reported by minute)]. According to the Biophen DiXaI monography, the detection threshold is evaluated on the calibration curve by measuring the “apparent” DiXaI concentration, which corresponds to the mean absorbance value obtained for a DiXaI-free reagent concentration (buffer only) less than three standard deviations.
is needed for assaying samples with concentrations higher than 545 ng/mL [Douxfils et al., 2012].

5.4 Recommendations from Other Organizations
In February 2012, the American College of Chest Physicians published the 9th edition of its oral anticoagulation therapy guidelines [Ageno et al., 2012]. The authors concluded that there are no validated biological assays for monitoring the anticoagulant activity of rivaroxaban, and there are no recommendations for dosage adjustments based on laboratory test results [Ageno et al., 2012]. The Australian government published a report assessing rivaroxaban in 2012. In the section on the effect of rivaroxaban on laboratory tests, the authors of the Australian report indicate that, if measurement of rivaroxaban is required, for example in an overdose or emergency situation, both PT and chromogenic anti-Xa assays standardized and calibrated for rivaroxaban have the potential to assess rivaroxaban plasma concentrations [TGA, 2012]. The most recent recommendations on measuring the anticoagulant activity of rivaroxaban endorsed by the International Society on Thrombosis and Haemostasis indicate that a PT assay can be used to determine the intensity of anticoagulation caused by rivaroxaban (with a rivaroxaban-specific reagent) in emergency situations only [Baglin et al., 2013]. The same recommendations state that each laboratory should be aware of the sensitivity of its PT assay to rivaroxaban, which can be achieved by the use of commercially available rivaroxaban plasma calibrants. Lastly, correctly standardized chromogenic anti-Xa assays (without exogenous antithrombin) can be used with specific PT assays to determine rivaroxaban concentration [Baglin et al., 2013].

6  ANTICIPATED OUTCOMES OF INTRODUCING THE TEST
6.1 Impact on Material and Human Resources
Not assessed.

6.2 Economic Consequences of Introducing Test Into Quebec’s Health Care and Social Services System
Not assessed.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues
Not assessed.
7 IN BRIEF

7.1 Clinical Relevance
The test became valid and precise with the commercial availability of plasma calibrators and control plasmas standardized for rivaroxaban. This test is designed for the quantitative measurement of rivaroxaban in a clinical setting.

7.2 Clinical Validity
No study available, to our knowledge.

7.3 Analytical Validity
Very good.

7.4 Recommendations from Other Organizations
The Australian government and the International Society on Thrombosis and Haemostasis prefer the use of PT and chromogenic anti-Xa assays coupled with a rivaroxaban-specific standard and control for estimating rivaroxaban plasma concentrations when an emergency situation justifies it.
Measurement of Direct Anti-Xa Activity of Rivaroxaban

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<th>Status of the Diagnostic Technology</th>
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<tr>
<td>□ Established</td>
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<td>✔ Innovative</td>
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<td>□ Experimental (for research purposes only)</td>
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<td>□ Replacement for technology________________________, which becomes obsolete</td>
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**INESSS Recommendation**
- □ Include test in the Index
- ✔ The assumption of clinical utility and the relevance of this test are significant; it concerns patients with active bleeding or a risk of bleeding because of emergency surgery.
- ✔ The production of clinical evidence will take time. Controlling the serious side effects of rivaroxaban is imperative.
- ✔ Including the test is recommended despite the lack of data on its clinical validity, in view of the seriousness of the problem and the risks associated with the use of the drug.
- □ Do not include test in the Index
- □ Reassess test

**Additional Recommendation**
- □ Draw connection with listing of drugs, if companion test
- □ Produce an optimal use manual
- ✔ Develop indicators for monitoring outcomes, and produce (or publish) use protocols
REFERENCES


