

ACTICHROME® Heparin (Anti-FXa) – REF 832

Instrument Application for the HORIBA ABX SAS Yumizen G800/G1500/G1550 Analysers

The following instrument application has been validated on the HORIBA Yumizen G800 coagulation analyser. The programming instructions suggested are based on our knowledge of the analyser and our reagents. These instructions should be used as guidelines in conjunction with your Quality Control Program for validation in accordance with local, state and/or federal regulation or accreditation requirements. If you require assistance or have any questions, please contact your local HORIBA Medical representative.

Materials Required

Item	BioMedica Diagnostics Catalogue No.	Horiba Medical Catalogue No.	Packaging
ACTICHROME® Heparin (Anti-FXa)	832	1300081562	4 vials R1 (lyophilized)* 4 vials R2 (lyophilized)* 4 vials R3 (lyophilized)*
ACTICHROME® Heparin LMWH Calibrator Set	832CALLMWH	1300081566	2 x 5 x 1 mL
ACTICHROME® Heparin UFH Calibrator Set	832CALUFH	1300081565	2 x 5 x 1 mL
ACTICHROME® Heparin LMWH Control Set	832CONLMWH	1300081564	5 x 2 x 1 mL
ACTICHROME® Heparin UFH Control Set	832CONUFH	1300081563	5 x 2 x 1 mL
Eppendorf Cups			

*** Note:** The reconstitution volumes for the Bovine Factor Xa (R1), Human Antithrombin III (R2) and the SPECTROZYME Substrate (R3) reagents for running the ACTICHROME Heparin (Anti-FXa) assay on the Yumizen analysers differ from the volumes stated in the Instructions For Use.

Reconstitute each reagent vial with 3 mL of deionized water for this Yumizen application, not 5 mL as stated in the Instructions For Use.

Yumizen G800/G1500/G1550 Programming Instructions

To begin, log in as “service”

A. To Create a Low Molecular Weight Heparin (LMWH) Application

1. Select “Test Setup” and press the “+Add” icon in the footer of the G800/G1500/G1550 screen to add a new program. Enter LMWH as the “Short Name”, enter Anti-FXa as the “Name.”
2. There are seven (7) screens for the application: Analysis, Reaction, Calibration, Control, Output, Repeat and Postcheck.
3. When finished, press the “Save” icon in the screen footer to save the program in the test menu.

Screen 1 (Analysis)

1. Select Test Method as Kinetic.
2. Select Wavelength as 405.
3. Select Algorithm as Chromogenic.
4. Select Warm reagent as Start.
5. Enter First Time as 10, End time as 50.

Screen 2 (Reaction)

1. Enter under “Sample”: Volume as 35, Incubation as 15 and Rate as 3/7.
2. Enter under “Diluent Name”: Hep R2.
3. Enter under “Reag nr”: 2
4. On the “1” line enter “Hep R1” as Name and set the following values:

Vol	70
Incubation:	15
Mixing:	Cuv: 2, Pip: 0
Before Wash:	No
After Wash:	Normal
Spec cleaner:	leave blank
Wash after spec:	leave blank
Calib:	√

5. On the “2” line enter “Hep R3” as Name and set the following values:

Vol	60
Incubation:	leave blank
Mixing:	Cuv: 5, Pip: 0
Before Wash:	No
After Wash:	Normal
Spec cleaner:	leave blank
Wash after spec:	leave blank
Calib:	√

6. Enter the following values:

Wait Time:	60
Nr of meas:	1
Max diff:	10

Screen 3 (Calibration)

1. Select Unit as IU/mL
2. Select Mode as Point-to-Point Linear regression
3. Select Fit as Lin-Lin
4. Select Level nr. as 5
5. Enter the following values:

Dilution:	DO NOT CHECK
Calibrator:	1. LMWH 1
	2. LMWH 2
	3. LMWH 3
	4. LMWH 4
	5. LMWH 5

Screen 4 (Control)

1. Select Level nr. as 2
2. On the "1" line enter "LMWH High" as Name
3. On the "2" line enter "LMWH Low" as Name
4. In the Time Period (h) enter "24"

Screen 5 (Output)

1. Enter under "Unit nr.": 2
2. On the "1" Line set the following values:

Name:	IU/mL
Screen:	√
Print:	√
Online:	√
QC:	√

3. On the "2" Line set the following values:

Name:	OD/min
Print:	√
Online:	√
QC:	√

Screen 6 (Repeat)

No selection/entries made.

Screen 7 (Postcheck)

Enter the following values:

Linearity: 0.98

B. Create an Unfractionated Heparin (UFH) Application:

1. Select "Test Setup", select "LMWH" in the list of test then press the **"Copy"** icon in the footer of the G800/G1500/G1550 screen to copy the LMWH program. Enter UFH as the "Short Name", enter Anti-FXa as the "Name."
2. There are seven (7) screens for the application: Analysis, Reaction, Calibration, Control, Output, Repeat and Postcheck.
3. When finished, press the "Save" icon in the screen footer to save the program in the test menu.

Screen 1 (Analysis)

The information is the same as for the LMWH application

Screen 2 (Reaction)

The information is the same as for the LMWH application

Screen 3 (Calibration)

1. Leave Unit as IU/mL
2. Leave Mode as Point-to-Point Linear regression
3. Leave Fit as Lin-Lin
4. Leave Level nr. as 5
5. Enter the following values:

Dilution:	DO NOT CHECK
Calibrator:	1. UFH 1
	2. UFH 2
	3. UFH 3
	4. UFH 4
	5. UFH 5

Screen 4 (Control)

1. Leave Level nr. as 2
2. On the "1" line enter "UFH High" as Name
3. On the "2" line enter "UFH Low" as Name
4. In the Time Period (h) enter "24"

Screen 5 (Output)

The information is the same as for the LMWH application

Screen 6 (Repeat)

No selection/entries made.

Screen 7 (Postcheck)

The information is the same as for the LMWH application

Catalogue Configuration:

1.1. Antithrombin III

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new Reagent.

Select Type “Other”

Enter “Hep R2” as the “Name”

Enter the reagent lot number printed on the vial as “Lot”

Enter 832AT3 as “Ref Number”

Enter the expiration date printed on the vial as “ExpDate”

Enter “8” as “Onboard Time”

Enter “3” as “Nominal Volume”

Select “Vial glass” as “Vial Type”

Press “OK” icon to confirm the Anti-Thrombin configuration.

Press the “Save” icon in the reagent screen footer to save the reagent configuration.

1.2. Factor Xa

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new Reagent.

Select Type “Reagent”

Enter “Hep R1” as the ‘Name’

Enter the reagent lot number printed on the vial as “Lot”

Enter “832FXa” as ‘Ref Number’

Enter the expiration date printed on the vial as “ExpDate”

Enter “8” as “Onboard Time”

Enter “3” as “Nominal Volume”

Select “Vial glass” as “Vial Type”

Press “OK” icon to confirm the Factor Xa configuration.

Press the “Save” icon in the reagent screen footer to save the reagent configuration.

1.3. SPECTROZYME® FXa Substrate

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new Reagent.

Select Type “Reagent”

Enter “Hep R3” as the “Name”

Enter the reagent lot number printed on the vial as “Lot”

Enter “832SUB” as “Ref Number”

Enter the expiration date printed on the vial as “ExpDate”

Enter “8” as “Onboard Time”

Enter “3” as “Nominal Volume”

Select “Vial glass” as “Vial Type”

Press “OK” icon to confirm the Substrate configuration.

Press the “Save” icon in the reagent screen footer to save the reagent configuration.

1.4. Calibrators – LMWH or UFH

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new calibrator.

Select Type “Calibrator”

Enter “LMWH 1” as the “Name”

Enter the Calibrator lot number printed on the vial as “Lot”

Enter “832LMWHCAL1” as “Ref Number”

Enter the expiration date printed on the vial as “ExpDate”

Enter “8” as “Onboard Time”

Enter “1” as “Nominal Volume”

Select “Eppendorf Cup” as “Vial Type”

In Calibrator panel, select:

Line 1: “LMWH” as “Test”, “IU/mL” as “Unit”, “XX” as “Value”

Press “OK” icon to confirm the Calibrator configuration.

Press the “Save” icon in the reagent screen footer to save the calibrator configuration.

Repeat Step 1.4 for each of the four (4) additional Calibrators. For the 2nd Calibrator, use “LMWH 2” as the “Name” and “832LMWCAL2” as the “Ref Number”. For the 3rd Calibrator, use “LMWH 3” as the “Name” and “832LMWCAL3” as the “Ref Number”. For the 4th Calibrator, use “LMWH 4” as the “Name” and “832LMWCAL4” as the “Ref Number”. For the 5th Calibrator, use “LMWH 5” as the “Name” and to “832LMWCAL5” as the “Ref Number”.

Repeat Step 1.4 for each of the five Calibrators for UFH. For the 1st Calibrator use “UFH 1” as the “Name” and “832UFHCAL1” as the “Ref Number”, for 2nd Calibrator, use “UFH 2” as the “Name” and to “832UFHCAL2” as the “Ref Number”. For the 3rd Calibrator, use “UFH 3” as the “Name” and “832UFHCAL3” as the “Ref Number”. For the 4th Calibrator, use “UFH 4” as the “Name” and “832UFHCAL4” as the “Ref Number”. For the 5th Calibrator, use “UFH 5” as the “Name” and to “832UFHCAL5” as the “Ref Number”.

1.5. LMWH or UFH High Control

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new control.

Select Type “Control”

Enter “LMWH High” as the “Name”

Enter the control lot number printed on the vial as “Lot”

Enter “832CONLMWHH” as “Ref Number”

Enter the expiration date printed on the vial as “ExpDate”

Enter “8” as “Onboard Time”

Enter “1” as “Nominal Volume”

Select “Eppendorf Cup” as “Vial Type”

In Control panel, select:

Line 1: “LMWH” as “Test”, “IU/mL” as “Unit”, “XX” as “Min”, “XXX” as “Max”

Press “OK” icon to confirm the LMWH High Control configuration.

Press the “Save” icon in the reagent screen footer to save the control configuration.

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new control.

Select Type “Control”

Enter “UFH High” as the “Name”

Enter the control lot number printed on the vial as “Lot”

Enter “832CONUFHH” as “Ref Number”

Enter the expiration date printed on the vial as “ExpDate”

Enter “8” as “Onboard Time”

Enter “1” as “Nominal Volume”

Select “Eppendorf Cup” as “Vial Type”

In Control panel, select:

Line 1: “UFH” as “Test”, “IU/mL” as “Unit”, “XX” as “Min”, “XXX” as “Max”

Press “OK” icon to confirm the UFH High Control configuration.

Press the “Save” icon in the reagent screen footer to save the control configuration.

1.6. LMWH/UFH Low Control

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new control.

Select Type “Control”

Enter “LMWH Low” as the “Name”

Enter the control lot number printed on the vial as “Lot”

Enter “832CONLMWHL” as “Ref Number”

Enter the expiration date printed on the vial as “ExpDate”
Enter “8” as “Onboard Time”
Enter “1” as “Nominal Volume”

Select “Eppendorf Cup” as “Vial Type”

In Control panel, select:

Line 1: “LMWH” as “Test”, “IU.mL” as “Unit”, “XX” as “Min”, “XX” as “Max”

Press “OK” icon to confirm the LMWH Low Control configuration.
Press the “Save” icon in the reagent screen footer to save the control configuration.

Press the “+Add” icon in the footer of the G800/G1500/G1550 reagent screen to add a new control.

Select Type “Control”
Enter “UFH Low” as the “Name”
Enter the control lot number printed on the vial as “Lot”
Enter “832CONUFHL” as “Ref Number”
Enter the expiration date printed on the vial as “ExpDate”
Enter “8” as “Onboard Time”
Enter “1” as “Nominal Volume”

Select “Eppendorf Cup” as “Vial Type”

In Control panel, select:

Line 1: “UFH” as “Test”, “IU.mL” as “Unit”, “XX” as “Min”, “XX” as “Max”

Press “OK” icon to confirm the UFH Low Control configuration.
Press the “Save” icon in the reagent screen footer to save the control configuration.

Data Summary

Linearity

The linearity of ACTICHROME Heparin (Anti-FXa) was determined by assaying plasma samples at eleven (11) different levels, prepared as ten (10) dilutions of a normal plasma spiked with 2 IU/mL of heparin, within the stated working range of the assay. Each sample was prepared by direct dilution of the original high sample rather than by serial dilutions. Triplicate measurements were made on each sample and the mean of the triplicates calculated.

ACTICHROME Heparin (Anti-FXa) is linear from 0.06 IU/mL – 1.60 IU/mL with LMWH and from 0.05 IU/mL – 1.50 IU/mL with UFH.

Limit of Detection

The Limit of Detection, LoD, for ACTICHROME Heparin (Anti-FXa) at a 95% Confidence Interval was found to be 0.12 IU/mL.

Precision

Repeatability for ACTICHROME Heparin (Anti-FXa) was determined by testing the UFH Control High plasma, UFH Control Low plasma, LMWH Control High plasma and the LMWH Control Low plasma for an n=30 each. Reproducibility for ACTICHROME Heparin (Anti-FXa) was determined by testing the UFH Control High plasma, UFH Control Low plasma, LMWH Control High plasma and the LMWH Control Low plasma over five (5) testing sessions, two (2) runs per session, and two (2) replicates per run for each sample on a single (1) instrument. The Coefficient of Variation, CV, was calculated following CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition, Section 4.6. The following Coefficient of Variations were determined.

For the G800

Test Sample	Repeatability		Reproducibility	
	Mean Heparin Level	CV	Mean Heparin Level	CV
LMWH High Control	0.68 IU/mL	3.8%	0.69 IU/mL	4.6%
LMWH Low Control	0.34 IU/mL	7.1%	0.37 IU/mL	8.4%

Test Sample	Repeatability		Reproducibility	
	Mean Heparin Level	CV	Mean Heparin Level	CV
UFH High Control	0.61 IU/mL	4.0%	0.62 IU/mL	6.4%
UFH Low Control	0.28 IU/mL	13.2%	0.32 IU/mL	10.4%

For the G1550

Test Sample	Repeatability		Reproducibility	
	Mean Heparin Level	CV	Mean Heparin Level	CV
LMWH High Control	0.68 IU/mL	3.7%	0.68 IU/mL	4.2%
LMWH Low Control	0.34 IU/mL	6.7%	0.36 IU/mL	5.0%

Test Sample	Repeatability		Reproducibility	
	Mean Heparin Level	CV	Mean Heparin Level	CV
UFH High Control	0.67 IU/mL	3.0%	0.66 IU/mL	3.7%
UFH Low Control	0.24 IU/mL	17.1%	0.34 IU/mL	10.8%

On-Board Reconstituted Reagent Stability

The stability of reconstituted reagents stored on board the Yumizen G800/G1500/G1550 analyser was confirmed as follows:

Reagent	Storage Temperature	Stability
Antithrombin III	17°C, on-board open vial	8 hours
Factor Xa	17°C, on-board open vial	8 hours
SPECTROZYME® FXa Substrate	17°C, on-board open vial	8 hours
ACTICHROME® Heparin LMWH Calibrator Set	Ambient, on-board open vial	8 hours
ACTICHROME® Heparin LMWH High Control	Ambient, on-board open vial	8 hours
ACTICHROME® Heparin LMWH Low Control	Ambient, on-board open vial	8 hours
ACTICHROME® Heparin UFH Calibrator Set	Ambient, on-board open vial	8 hours
ACTICHROME® Heparin UFH High Control	Ambient, on-board open vial	8 hours
ACTICHROME® Heparin UFH Low Control	Ambient, on-board open vial	8 hours

The stability data presented above have been established under controlled laboratory conditions. Due to possible differences in “ambient conditions” between each laboratory and reagent, the on-board stability may vary from the values stated above.

Interference Studies

The results demonstrate no significant interference in the performance of ACTICHROME Heparin (Anti-FXa) by the presence of the following substances up to the noted concentrations.

Substance	Concentration with LMWH	Concentration with UFH
Hemoglobin	170 mg/dL	50 mg/dL
Bilirubin, Conjugated	19 mg/dL	11 mg/dL
Bilirubin, Unconjugated	20 mg/dL	8.5 mg/dL
Triglycerides	450 mg/dL	550 mg/dL

Note – For an explanation on how to set up or change a test protocol on the G800/G1500/G1550, please refer to the respective HORIBA ABX SAS Operator's Manual System Set-Up.

Reference

1. ACTICHROME and SPECTROZYME are registered trademarks of BioMedica Diagnostics Inc., Windsor, NS, Canada
2. Yumizen G800, Yumizen G1500, Yumizen G1550 are marks of HORIBA ABX SAS, Montpellier, France

Changes from the previous revision are noted by red dotted lines in the left margin.