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Biosciences Europe

Helena C-Series

User Guide

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Statement of Use

This User Guide is intended as a reference document providing application notes, example reference values, example calibration curves and data demonstrating performance claims for Helena Biosciences Europe haemostasis assays specifically when used as part of a test system with the Helena C-Series coagulometer.

Prior to using any Helena Biosciences Europe haemostasis reagents please refer to the kit specific Instructions For Use (IFU) supplied with every Helena Biosciences Europe kit. Please also refer to the Helena C-Series Family Operator Manual for additional information on operation. Always follow product labelling and appropriate manufacturer's recommendations. All unopened Helena Biosciences Europe reagents when stored according to the instructions set out on their packaging and in their IFUs are stable until the expiry date stated on each individual vial.

Helena Biosciences Europe have developed the assay and analyser specific applications defined in this User Guide to optimise product performance and meet specific product specifications. Any subsequent modifications or adjustments made to application parameters are not supported by Helena Biosciences Europe as they may affect product performance and the reported assay result.

Users should establish a quality control program. Normal and abnormal control plasma should be tested (as a minimum) prior to each batch of patient samples to ensure satisfactory instrument and operator performance. If controls do not perform as expected, patient results should be considered invalid. This document details combinations of controls and calibrators for use with the specific assay and analyser test system as supported by Helena Biosciences Europe. Assay results should always be interpreted in conjunction with the patient's medical history, clinical presentation and any other findings.

Reference data and performance claims have been provided by Helena Biosciences Europe as a guide. Each laboratory should establish its own reference and performance data as values can vary between laboratories depending on the population and the techniques and reagent lots used. All patient plasma tested in the generation the performance claims published in this User Guide have been drawn into ~3.2% Sodium Citrate.

Only the latest version of this document should be used. It is the user's responsibility to ensure they are using the most current document.

For any queries you may have, please do not hesitate to contact Helena Biosciences Europe directly.

Haemostasis Sales: sales@helena-biosciences.com

Haemostasis Technical Support: techsupport-HS@helena-biosciences.com

Revision History

Revision	Date of Issue	Amendments
Rev.1	Mar-2020	First edition

Contents

Thromboplastin L (5262L, 5265HL, 5265L)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Thromboplastin L*[1]	5262L 5265HL 5265L	10 x 2 mL 2 x 5 mL 8 x 5 mL	On Board Reagent position Ø22mm, stirred*[2]	36.5 - 37.5 ºC	Original Vial
Calibration Plasma	5504R	4 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Routine Control N	5186	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Routine Control A	5187	10 x 1 mL			
Routine Control SA	5183	10 x 1 mL			
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

*[1] Place a 12mm x 4.5mm magnetic stir bar into the Thromboplastin L vial prior to placing on-board the Helena C-2 and Helena C-4.

*[2] No stirred position available on the Helena C-1.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Clot Time	seconds	1	Also stated as 'Clot (s)'
%PT	%	1	% of Normal
INR	-	2	International Normalised Ratio

Application Settings

Setup PT (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%+INR	0.0-250.0
Incubation (s):	120	
Stop (s):	120	

On Helena C-2 and Helena C-4 analysers the setting *Mixer* must be set to *Normal*.

Testing Protocol

Step	Action
1	Add **25µL** of the **plasma sample** to a pre-warmed cuvette and start an incubation timer.
2	Before the end of the sample incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
3	Once the sample incubation is complete, add **50µL** of pre-warmed **Thromboplastin L** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report %PT and INR results with Thromboplastin L on the Helena C-Series users must follow these instructions to construct %PT and INR calibrations.

Step	Action
1	Prepare a Calibration Plasma kit (5504R) and sufficient Thromboplastin L reagent for use as per the current issue of the relevant product IFUs.
2	Use the Helena C-Series to assay the Calibration Plasma kit (5504R) PT Calibrants 1-4 as samples, in duplicate, for PT clot times using Thromboplastin L reagent. Ignore any %PT and INR results output for these samples.
3	Open the current issue of HL-2-2835P, “ISI & MNPT Data Calculator for use with 5504R” Excel workbook (Contact techsupport-HS@helena-biosciences.com for a copy). Complete sections “1. Calibration Details” and “2. Material Details”.
4	Complete section “3. Calibration Data” using the mean clot times obtained on the Helena C-Series for each PT Calibrant in Step 2 and the relevant PT% and INR reference values for each PT Calibrant stated on the Assay Insert supplied in the Calibration Plasma kit (5504R).
5	Section “4. Calculated Data” will report test system (analyser and reagent lot) specific ISI and MNPT values.
6	Ensure all data entered is correct.
7	Return to the Helena C-Series and select the PT test in channel 1, select PT [Lot 1] or [Lot 2]* and select [Setup >].
8	Enter the lot number and expiry date of the Thromboplastin L reagent used to assay the Calibration Plasma kit (5504R).
9	Select [Setup] to access the calibration input screen, all data input in this screen must be made by selecting each field in turn and using the on-screen controls.
10	Enter the %PT reference values for each PT-Calibrant 1-4 in rows 1-4 of the column labelled “%”.
11	Enter the corresponding mean PT clot times obtained in step 2 for each PT-Calibrant 1-4 in rows 1-4 of the column labelled “s”.
12	Enter the “MNPT (s)” and "ISI" values from section “4 Calculated data” of the ISI & MNPT Data Calculator in the fields labelled “Normal (s)” and "ISI" respectively. Select [OK] to save the inputted data.
13	Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
14	The curve data is test system, analyser and Thromboplastin L reagent lot, specific and cannot be transferred.

*Not available on the Helena C-1.

An example of a %PT and INR calibration constructed using Calibration Plasma (5504R) with Thromboplastin L on the Helena C-Series is stated below.

ISI:	1.26
NORMAL:	13.1

1:	115.8%=12.4s
2:	47.0%=19.4s
3:	26.4%=30.5s
4:	16.7%=49.9s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5186	Routine Control N	21488332	Clot (s)	11.7	11.8	10.0	13.6
			%PT	120.0	114.2	97.1	131.4
			INR	0.90	0.93	0.79	1.07
5187	Routine Control A	21480766	Clot (s)	21.6	22.2	18.9	25.6
			%PT	40.5	37.7	32.0	43.3
			INR	1.97	2.07	1.76	2.38
5183	Routine Control SA	21434066	Clot (s)	34.7	34.1	29.0	39.3
			%PT	22.0	22.0	18.7	25.3
			INR	3.69	3.55	3.02	4.09

In Use Stability

Helena Biosciences Europe have established the stability of Thromboplastin L on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use, stirred, on board a Helena C-2 or Helena C-4.	Thromboplastin L - 2 mL vial	4 hours
	Thromboplastin L - 5 mL vial	8 hours
Original vials prepared for use, opened, and placed into use, without stirring, on board a Helena C-1.	Thromboplastin L - 2 mL vial	4 hours
	Thromboplastin L - 5 mL vial	8 hours

Thromboplastin L is not suitable for freezing.

Comparison of Method

Helena Biosciences Europe have established a method comparison of Thromboplastin L on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens PT with Thromborel® S on a Sysmex CS-5100 system	Clot (s)	114	y = 0.874x + 3.020	0.97
	%PT	111	y = 0.877x + 8.376	0.91
	INR	123	y = 0.947x + 0.123	0.95
Siemens PT with Dade® Innovin® on a Sysmex CS-5100 system	Clot (s)	124	y = 1.146x + 1.217	0.97
	%PT	120	y = 0.910x + 9.292	0.91
	INR	124	y = 1.008x + 0.098	0.90

The following bias statistics were generated using the regression trends plotted in the method comparison evaluations.

		Bias Statisitics		
Measurand	Result	Siemens result	Helena result	Bias
Clot (s)	Thromborel® S Expected Values - 2.5th percentile	9.9	11.7	1.8
	Thromborel® S Expected Values - 97.5th percentile	12.3	13.8	1.5
	Dade® Innovin® Expected Values - 2.5th percentile	9.7	12.3	2.6
	Dade® Innovin® Expected Values - 97.5th percentile	11.8	14.7	2.9
% PT	Thromborel® S Expected Values - 2.5th percentile	76.6	75.6	-1.0
	Thromborel® S Expected Values - 97.5th percentile	116.2	110.3	-5.9
	Dade® Innovin® Expected Values - 2.5th percentile	78.1	80.4	2.3
	Dade® Innovin® Expected Values - 97.5th percentile	123.3	121.5	-1.8
INR	Thromborel® S - lower limit of example measuring interval	0.95	1.02	0.07
	Thromborel® S - upper limit of example measuring interval	5.25	5.10	-0.16
	Dade® Innovin® - lower limit of example measuring interval	0.98	1.09	0.11
	Dade® Innovin® - upper limit of example measuring interval	4.75	4.89	0.14

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for each Thromboplastin L measurand using plasma samples from healthy subjects, multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
Clot (s)	133	11.6 - 14.2
%PT	133	89.5 - 128.5
INR	133	0.87 - 1.14

Precision

Helena Biosciences Europe have established the precision performance of Thromboplastin L on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, multiple calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
Clot (s)	12.0	0.19	1.59%	0.30	2.53%
	21.9	0.77	3.52%	0.97	4.40%
	34.2	0.98	2.86%	1.80	5.26%
%PT	115.3	2.90	2.52%	5.03	4.36%
	39.4	1.87	4.76%	2.20	5.59%
	22.5	0.57	2.53%	0.85	3.78%
INR	0.93	0.02	2.09%	0.03	3.67%
	1.98	0.09	4.48%	0.10	4.82%
	3.46	0.12	3.54%	0.20	5.82%

Limitations of the Method

Helena Biosciences Europe have established the limitations of Thromboplastin L on the Helena C-Series following protocol set out in CLSI guidelines EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018 and HL47-A2 *One-Stage Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline* 2nd Ed, 2008.

The sensitivity of Thromboplastin L to extrinsic factor deficiencies was evaluated on the Helena C-Series with the upper limit of the established Thromboplastin L reference interval used as the limit of interference.

Clotting Factor	Factor concentration at the limit of interference
Factor II	44.4 % FII
Factor V	58.6 % FV
Factor VII	53.3 % FVII
Factor X	60.4 % FX

The analytical specificity of Thromboplastin L in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.6 U/mL
LMW Heparin	2.0 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	1000 mg/dL
Conjugated Bilirubin	60 mg/dL
Unconjugated Bilirubin	60 mg/dL

Linearity

Not Applicable.

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of Thromboplastin L on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring intervals for reporting INR and %PT results are defined by the reference limits of the currently assigned 5504R calibration curves. Users who have a need to report results above the calibrations may report results up to an INR of 12.00 and down to 11.0 %PT without application modification where results are obtained from a valid, non-flagged, clot time.

Users who have a need to report more pathological results may modify their Test Parameters to extend the *Stop (s)* parameter to a maximum of 300s. Once any application modification is validated by the user all results obtained from a valid, non-flagged, clot time may be reported.

APTT Si L Minus (5562SLQ, 5560SLQ, 5558SLQ, 5559SLQ)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
APTT Si L Minus	5562SLQ 5560SLQ 5558SLQ 5559SLQ	5 x 5 mL 10 x 5 mL 5 x 10 mL 10 x 10 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Calcium Chloride: 0.025M	5562SLQ 5560SLQ	5 x 5 mL 10 x 5 mL	On Board Reagent position Ø22mm	36.5 - 37.5 ºC	Original Vial
	5558SLQ 5559SLQ	5 x 10 mL 10 x 10 mL	On Board Reagent position Ø24mm		
Routine Control N Routine Control A Routine Control SA Speciality Assayed Control N Speciality Assayed Control A	5186 5187 5183 5301 5302	10 x 1 mL 10 x 1 mL 10 x 1 mL 10 x 1 mL 10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Clot Time	seconds	1	Also stated as 'Clot (s)'
Ratio	-	2	Clot (s) / MNAPTT (Local normal plasma pools)

Application Settings

Setup APTT (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	R*[1]	0.00-99.00*[1,2]
Incubation (s):	180	
Stop (s):	180	

*[1] For users only wishing to report APTT results as clot times leave the unit as "-" and the range as 0.0 – 0.0.

*[2] When reporting results as Ratios the range defaults to 0.00 – 99.00.

Testing Protocol

Step	Action
1	Add **25µL** of the **plasma sample** to a pre-warmed cuvette.
2	Immediately add **25µL** of the **APTT Si L Minus** to the reaction cuvette and start an incubation timer.
3	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
4	Once the sample incubation is complete, add **25µL** of pre-warmed **Calcium Chloride: 0.025M** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Normal Value to report Ratio results

To report Ratio results with APTT Si L Minus on the Helena C-Series users must enter a normal value into the analyser software.

Step	Action
1	On the Helena C-Series select the APTT test in channel 1, select APTT [Lot 1] or [Lot 2]* and select [Setup >].
2	Enter the lot number and expiry date of the APTT Si L Minus reagent used.
3	Select [Setup] to access the calibration input screen, all data input in this screen must be made by selecting each field in turn and using the on-screen controls.
4	Enter the appropriate normal APTT value in seconds.
5	Select [OK] to save the inputted data. Ensure the normal value entered in the analyser is correct before using it to report Ratio results.

*Not available on the Helena C-1.

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5186	Routine Control N	21488332	Clot (s)	32.4	32.6	27.7	37.5
			Ratio	1.10	1.07	0.91	1.23
5187	Routine Control A	21480766	Clot (s)	46.6	47.8	40.6	55.0
			Ratio	1.59	1.56	1.33	1.80
5183	Routine Control SA	21434066	Clot (s)	64.2	67.3	57.2	77.4
			Ratio	2.18	2.20	1.87	2.53

In Use Stability

Helena Biosciences Europe have established the stability of APTT Si L Minus and Calcium Chloride: 0.025M on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Kit	Time
Original vials prepared for use, opened, and placed into use on board the analyser (Calcium Chloride: 0.025M) and on bench top (APTT Si L Minus) for 8 hour shifts and returned, re-capped in original vials, to 2ºC - 8ºC overnight.	5562SLQ, 5560SLQ, APTT Si L Minus (5 mL vials)	5 days
	5558SLQ, 5559SLQ, APTT Si L Minus (10 mL vials)	7 days

APTT Si L Minus is not suitable for freezing.

Comparison of Method

Helena Biosciences Europe have established a method comparison of APTT Si L Minus on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens APTT with Dade® Actin® FS Activated PTT Reagent on a Sysmex CS-5100 system	Clot (s)	123	y = 1.248x + 1.198	0.86
	Ratio	121	y = 0.955x + 0.046	0.88

The following bias statistics were generated using the regression trends plotted in the method comparison evaluations.

		Bias Statisitics		
Measurand	Result	Siemens result	Helena result	Bias
Clot (s)	Dade® Actin® FS Expected Values - 2.5th percentile	21.6	28.1	6.5
	Dade® Actin® FS Expected Values - 97.5th percentile	28.7	37.0	8.3
Ratio	Dade® Actin® FS - Calculated 2.5th percentile	0.87	0.88	0.01
	Dade® Actin® FS - Calculated 97.5th percentile	1.16	1.15	-0.01

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for each APTT Si L Minus measurand using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
Clot (s)	120	25.8 to 36.0
Ratio	120	0.85 to 1.23

Precision

Helena Biosciences Europe have established the precision performance of APTT Si L Minus on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
Clot (s)	32.6	0.85	2.62%	1.68	5.16%
	48.4	1.57	3.24%	2.74	5.66%
	67.9	2.42	3.56%	3.95	5.82%
Ratio	1.08	0.03	2.63%	0.04	3.76%
	1.61	0.05	3.21%	0.06	3.86%
	2.25	0.08	3.51%	0.08	3.63%

Limitations of the Method

Helena Biosciences Europe have established the limitations of APTT Si L Minus on the Helena C-Series following protocol set out in CLSI guidelines EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018 and HL47-A2 *One-Stage Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline* 2nd Ed, 2008.

The sensitivity of APTT Si L Minus to intrinsic factor deficiencies was evaluated on the Helena C-Series with the upper limit of the established APTT Si L Minus reference interval used as the limit of interference.

Clotting Factor	Factor concentration at the limit of interference
Factor VIII	44.6 % FVIII
Factor IX	51.5 % FIX
Factor XI	60.7 % FXI
Factor XII	64.4 % FXII

The analytical specificity of APTT Si L Minus in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.1 U/mL
LMW Heparin	0.1 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	1000 mg/dL
Conjugated Bilirubin	60 mg/dL
Unconjugated Bilirubin	60 mg/dL

Linearity

Not Applicable.

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of APTT Si L Minus on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

Users may report all valid, non-flagged, Clot (s) and Ratio results.

Clauss Fibrinogen 100 (5376, 5376R, 5374, 5378)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Thrombin: 100 NIH/mL	5376 5376R 5374 5378	5 x 2 mL 5 x 2 mL 10 x 2 mL 10 x 5 mL	On Board Reagent position Ø22mm	36.5 - 37.5 ºC	Original Vial
Fibrinogen Calibrator	5376 5376R 5379	2 x 1 mL 2 x 1 mL 10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Owren's Buffer	5376 5375	2 x 25 mL 10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Imidazole Buffer	5376R 5375R	2 x 25 mL 10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Routine Control N	5186	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Routine Control A	5187	10 x 1 mL			
Routine Control SA	5183	10 x 1 mL			
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

Helena Biosciences Europe performed all evaluation testing using Imidazole Buffer, comparable performance using Owren's Buffer has been verified. Users may use either Imidazole Buffer or Owren's Buffer for Clauss Fibrinogen 100 testing but all samples must be assayed off a curve constructed using the same buffer.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Fibrinogen	g/L	2	-

Application Settings

Setup FIB (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	g/L	0.00-99.00
Incubation (s):	120	
Stop (s):	180	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/10** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **450 µL** of **buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **50µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette and start an incubation timer.
3	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
4	Once the sample incubation is complete, add **25µL** of pre-warmed **Thrombin: 100 NIH/mL** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

For samples reporting g/L results above 5.0 g/L or below 0.75 g/L users should re-analyse using the following alternative Test Protocol Step 1 pre-dilutions. Correct the reported results by multiplying by the factor stated.

Condition	Alternative Pre-Dilution		Correction Factor (reported g/L * corr. fact.)
	Ratio	Alternative Step 1	
Reported g/L > 5.00 g/L	1/20	Add **25 µL** of **plasma sample** to **475 µL** of **buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam, use pre-diluted plasma immediately.	2
Reported g/L < 0.75 g/L	1/5	Add **50 µL** of **plasma sample** to **200 µL** of **buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam, use pre-diluted plasma immediately.	0.5

Calibration Construction and Example Calibration

To report Fibrinogen (g/L) results with Clauss Fibrinogen 100 on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Fibrinogen Calibrator or Calibration Plasma vial and sufficient Thrombin: 100 NIH/mL and either Imidazole Buffer or Owren's Buffer for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling four fresh sample cups 1, 2, 3 and 4.
3	In cup 1 prepare the **200%** calibration point by diluting **200µL of the Calibrator** prepared in step 1 with **800µL Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **500µL of the 200% dilution,** prepared in cup 1, with **500µL Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **66.6%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **300µL Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **33.3%** calibration point by diluting **300µL of the 66.6% dilution,** prepared in cup 3, with **300µL Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	Use the Helena C-Series to assay the four prepared dilutions as samples, in duplicate, following the steps 2-4 of the Clauss Fibrinogen 100 Test Protocol. The calibration dilutions prepared in steps 3-6 are already pre-diluted and are ready for analysis, do not dilute further.
8	To calculate the Fibrinogen concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 200% dilution concentration using a calibrator with a fibrinogen concentration of 2.80 g/L : (200/100)*2.80 = 5.60 g/L.
9	On the Helena C-Series select the FIB test in channel 1, select FIB and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup Fib Lot #".
10	Enter the lot number and expiry of the Thrombin: 100NIH/mL reagent used to assay the calibration dilutions.
11	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
12	For each calibration dilution enter the fibrinogen concentration calculated in step 8 and the corresponding mean clot time assayed in step 7.
13	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
14	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of a Fibrinogen (g/L) calibration constructed using Calibration Plasma (5185) with Clauss Fibrinogen 100 on the Helena C-Series is stated below.

1:	5.60 g/L = 5.2s
2:	2.80 g/L = 8.0s
3:	1.87 g/L = 10.7s
4:	0.93 g/L = 21.0s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21452608	g/L	2.60	2.66	2.26	3.06
5302	Speciality Assayed Control A	21452632	g/L	1.49	1.47	1.25	1.70

In Use Stability

Helena Biosciences Europe have established the stability of Clauss Fibrinogen 100 reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on board the analyser	Thrombin: 100 NIH/mL (2 mL vial)	8 hours
	Thrombin: 100 NIH/mL (5 mL vial)	8 hours

Once opened store Imidazole Buffer and Owren's Buffer at 2 - 8ºC, discard in use aliquots of buffer daily.

Comparison of Method

Helena Biosciences Europe have established a method comparison of Clauss Fibrinogen 100 on the Helena C-Series to a market leading analyser following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Fibrinogen with Dade® Thrombin Reagent on a Sysmex CS-5100 system	Fibrinogen (g/L)	105	y = 0.980x + .0183	0.99

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
Fibrinogen (g/L)	Dade® Thrombin Expected Values - 2.5th percentile	1.70	1.85	0.15
	Dade® Thrombin Expected Values - 97.5th percentile	4.20	4.30	0.10

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for Clauss Fibrinogen 100 using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
Fibrinogen (g/L)	127	2.10 to 4.82

Precision

Helena Biosciences Europe have established the precision performance of Clauss Fibrinogen 100 on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
Fibrinogen (g/L)	2.62	0.10	3.71%	0.12	4.63%
	1.47	0.06	4.18%	0.08	5.62%
	1.09	0.06	5.96%	0.08	7.07%

Limitations of the Method

Helena Biosciences Europe have established the limitations of Clauss Fibrinogen 100 on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of Clauss Fibrinogen 100 in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	1.2 U/mL
LMW Heparin	3.0 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	236 mg/dL
Conjugated Bilirubin	40 mg/dL
Unconjugated Bilirubin	27 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of Clauss Fibrinogen 100 following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Measurand	Linear Range
Fibrinogen (g/L)	0.77 g/L to 7.92 g/L

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of Clauss Fibrinogen 100 on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of Clauss Fibrinogen 100 on the Helena C-Series is defined by the linear range.

Thrombin Time (5392, 5377)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Thrombin Time	5392 5377	10 x 2 mL 10 x 5 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Routine Control N Routine Control A Routine Control SA	5186 5187 5183	10 x 1 mL 10 x 1 mL 10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial

All reagents must be prepared following the instructions provided in individual kit IFUs.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Clot Time	seconds	1	Also stated as 'Clot (s)'.
Ratio	-	2	Clot (s) / MNTT (Local normal plasma pools)

Application Settings

Setup TT (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	R*[1]	0.00-99.00*[1,2]
Incubation (s):	180	
Stop (s):	120	

*[1] For users only wishing to report Thrombin Time results only as clot times leave the unit as "-" and the range as 0.0 – 0.0.

*[2] When reporting results as Ratios the Measuring Interval defaults to 0.00 – 99.00.

Application Note: Thrombin Time

Testing Protocol

Step	Action
1	Add **50µL** of the **plasma sample** to a pre-warmed cuvette and start an incubation timer.
2	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
3	Once the sample incubation is complete, add **25µL** of **Thrombin Time** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Normal Value to report Ratio results

To report Ratio results with Thrombin Time on the Helena C-Series users must enter a normal Thrombin Time value into the analyser software.

Step	Action
1	On the Helena C-Series select the TT test in channel 1, select TT [Lot 1] or [Lot 2]* and select [Setup >].
2	Enter the lot number and expiry date of the Thrombin Time reagent used.
3	Select [Setup] to access the calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
4	Enter the appropriate normal TT value in seconds.
5	Select [OK] to save the inputted data. Ensure the normal value entered in the analyser is correct before using it to report Ratio results.

*Not available on the Helena C-1.

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5186	Routine Control N	21541198	Clot (s)	13.2	13.0	11.0	14.9
			Ratio	1.07	1.06	0.90	1.22

Application Note: Thrombin Time

In Use Stability

Helena Biosciences Europe have established the stability of Thrombin Time on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top for an 8 hour shift per working day, for a five day working week. Vials stored at 2 - 8ºC outside of shift and mixed and acclimatised prior to use each shift.	Thrombin Time - 2 mL vial	7 days
	Thrombin Time - 5 mL vial	7 days

Comparison of Method

Helena Biosciences Europe have established a method comparison of Thrombin Time on the Helena C-Series to a market leading analyser following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Thrombin Time with Thromboclotin® on a Sysmex CS-5100 System	Clot (s)	117	y = 0.874x - 1.720	0.80
	Ratio	113	y = 1.000x - 0.025	0.81

The following bias statistics were generated using the regression trends plotted in the method comparison evaluations.

		Bias Statisitics		
Measurand	Result	Siemens result	Helena result	Bias
Clot (s)	Thromboclotin® Expected Values - 2.5th percentile	15.5	11.8	-3.7
	Thromboclotin® Expected Values - 97.5th percentile	19.4	15.2	-4.2
Ratio	Thromboclotin® - Calculated 2.5th percentile	0.90	0.88	-0.02
	Thromboclotin® - Calculated 97.5th percentile	1.12	1.10	-0.02

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for Thrombin Time using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
Clot (s)	132	10.4 to 15.0
Ratio	132	0.85 to 1.17

Application Note: Thrombin Time

Precision

Helena Biosciences Europe have established the precision performance of Thrombin Time on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
Clot (s)	12.7	0.34	2.67%	0.49	3.85%
	20.5	0.78	3.82%	1.01	4.93%
Ratio	1.07	0.03	2.65%	0.06	5.68%
	1.72	0.07	3.79%	0.11	6.35%

Limitations of the Method

Helena Biosciences Europe have established the limitations of Thrombin Time on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of Thrombin Time in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.02 U/mL
LMW Heparin	0.10 U/mL
Haemoglobin	500 mg/dL
Triglycerides	308 mg/dL
Conjugated Bilirubin	40 mg/dL

Linearity

Not Applicable.

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of Thrombin Time on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

Users may report all valid, non-flagged, Clot (s) or Ratio results.

Factor II Assay (5790)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor II Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor II Deficient Plasma (Immunodepleted)	5790	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Thromboplastin L*[1]	5262L 5265HL 5265L	10 x 2 mL 2 x 5 mL 8 x 5 mL	On Board Reagent position Ø22mm, stirred	36.5 - 37.5 ºC	Original Vial
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

*[1] Place a 12mm x 4.5mm magnetic stir bar into the Thromboplastin L vial prior to placing on-board the Helena C-2 and Helena C-4.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor II Concentration	%	1	% of Normal, also stated as '% FII'

Application Settings

Setup FII (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	120	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor II Deficient Plasma** to the reaction cuvette and start an incubation timer.
4	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
5	Once the plasma incubation is complete, add **50µL** of **Thromboplastin L** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FII results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-5 of the Factor II Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor II concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor II concentration of 96.7% : (12.5/100)*96.7 = 12.1%.
10	On the Helena C-Series select the FII test in channel 1, select FII and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FII Lot #".
11	Enter the lot number and expiry of the Factor II Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor II concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FII calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	174.1% = 15.3s
2:	96.7% = 17.9s
3:	48.4% = 22.4s
4:	12.1% = 40.1s
5:	1.5% = 127.9s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FII	116.1	116.8	93.5	140.1
5302	Speciality Assayed Control A	21590804	% FII	57.9	60.5	45.4	75.7

In Use Stability

Helena Biosciences Europe have established the stability of Factor II Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor II Deficient Plasma	5 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor II Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor II with Thromborel® S on a Sysmex CS-5100 System	% FII	137	y = 0.835x + 3.833	0.96

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FII	Siemens Coagulation Factor II - example LLMI (Lower Limit of Measuring Interval)	6.1	8.9	2.8
	Siemens Coagulation Factor II - Arbitrary value	25.0	24.7	-0.3

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor II Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FII	136	73.8 to 115.7

Precision

Helena Biosciences Europe have established the precision performance of the Factor II Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FII	108.7	4.23	3.89%	7.18	6.61%
	58.5	2.24	3.84%	4.96	8.47%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor II Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor II Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	2.0 U/mL
LMW Heparin	2.0 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	44 mg/dL
Unconjugated Bilirubin	60 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor II Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FII	2.2 % to 91.2 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor II Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor II Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor V Assay (5191, 5791)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor V Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor V Deficient Plasma (Immunodepleted)	5791	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor V Deficient Plasma (Congenital)	5191	10 x 1 mL			
Thromboplastin L*[1]	5262L 5265HL 5265L	10 x 2 mL 2 x 5 mL 8 x 5 mL	On Board Reagent position Ø22mm, stirred	36.5 - 37.5 ºC	Original Vial
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

*[1] Place a 12mm x 4.5mm magnetic stir bar into the Thromboplastin L vial prior to placing on-board the Helena C-2 and Helena C-4.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor V Concentration	%	1	% of Normal, also stated as '% FV'

Application Settings

Setup FV (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	120	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor V Deficient Plasma** to the reaction cuvette and start an incubation timer.
4	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
5	Once the plasma incubation is complete, add **50µL** of **Thromboplastin L** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FV results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-5 of the Factor V Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor V concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor V concentration of 118.2% : (12.5/100)*118.2 = 14.8%.
10	On the Helena C-Series select the FV test in channel 1, select FV and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FV Lot #".
11	Enter the lot number and expiry of the Factor V Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor V concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FV calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	212.8% = 15.3s
2:	118.2% = 18.3s
3:	59.1% = 23.3s
4:	14.8% = 32.5s
5:	1.8% = 53.0s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FV	129.1	138.6	110.8	166.3
5302	Speciality Assayed Control A	21590804	% FV	73.6	71.2	53.4	89.0

In Use Stability

Helena Biosciences Europe have established the stability of Factor V Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor V Deficient Plasma	5 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor V Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor V with Thromborel® S on a Sysmex CS-5100 System	% FV	104	y = 0.897x + 0.675	0.95

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FV	Siemens Coagulation Factor V - example LLMI (Lower Limit of Measuring Interval)	6.1	6.1	0.0
	Siemens Coagulation Factor V - Arbitrary value	25.0	23.1	-1.9

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor V Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FV	141	63.2 to 125.4

Precision

Helena Biosciences Europe have established the precision performance of the Factor V Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FV	121.3	4.47	3.68%	9.97	8.22%
	68.7	3.13	4.55%	5.69	8.28%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor V Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor V Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	1.4 U/mL
LMW Heparin	1.6 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	1477 mg/dL
Conjugated Bilirubin	20 mg/dL
Unconjugated Bilirubin	27 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor V Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FV	2.6 % to 105.8 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor V Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor V Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor VII Assay (5192, 5792)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor VII Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor VII Deficient Plasma (Immunodepleted)	5792	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor VII Deficient Plasma (Congenital)	5192	10 x 1 mL			
Thromboplastin L*[1]	5262L 5265HL 5265L	10 x 2 mL 2 x 5 mL 8 x 5 mL	On Board Reagent position Ø22mm, stirred	36.5 - 37.5 ºC	Original Vial
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

*[1] Place a 12mm x 4.5mm magnetic stir bar into the Thromboplastin L vial prior to placing on-board the Helena C-2 and Helena C-4.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor VII Concentration	%	1	% of Normal, also stated as '% FVII'

Application Settings

Setup FVII (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	120	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor VII Deficient Plasma** to the reaction cuvette and start an incubation timer.
4	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
5	Once the plasma incubation is complete, add **50µL** of **Thromboplastin L** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FVII results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-5 of the Factor VII Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor VII concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor VII concentration of 111.0% : (12.5/100)*111.0 = 13.9%.
10	On the Helena C-Series select the FVII test in channel 1, select FVII and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FVII Lot #".
11	Enter the lot number and expiry of the Factor VII Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor VII concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FVII calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	199.8% = 16.3s
2:	111.0% = 20.0s
3:	55.8% = 25.1s
4:	13.9% = 43.4s
5:	1.7% = 110.4s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FVII	132.5	127.1	101.7	152.5
5302	Speciality Assayed Control A	21590804	% FVII	61.5	62.5	46.9	78.1

In Use Stability

Helena Biosciences Europe have established the stability of Factor VII Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor VII Deficient Plasma	7 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor VII Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor VII with Thromborel® S on a Sysmex CS-5100 System	% FVII	110	y = 0.892x + 1.914	0.94

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FVII	Siemens Coagulation Factor VII - example LLMI (Lower Limit of Measuring Interval)	5.9	7.2	1.3
	Siemens Coagulation Factor VII - Arbitrary value	25.0	24.2	-0.8

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor VII Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FVII	136	66.9 to 138.1

Precision

Helena Biosciences Europe have established the precision performance of the Factor VII Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FVII	123.7	4.98	4.03%	9.27	7.50%
	60.5	3.08	5.10%	5.54	9.17%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor VII Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor VII Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	2.0 U/mL
LMW Heparin	2.0 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	60 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor VII Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FVII	1.9 % to 119.9 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor VII Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor VII Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor X Assay (5195, 5795)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor X Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor X Deficient Plasma (Immunodepleted)	5795	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor X Deficient Plasma (Congenital)	5195	10 x 1 mL			
Thromboplastin L*[1]	5262L 5265HL 5265L	10 x 2 mL 2 x 5 mL 8 x 5 mL	On Board Reagent position Ø22mm, stirred	36.5 - 37.5 ºC	Original Vial
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

*[1] Place a 12mm x 4.5mm magnetic stir bar into the Thromboplastin L vial prior to placing on-board the Helena C-2 and Helena C-4.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor X Concentration	%	1	% of Normal, also stated as '% FX'

Application Settings

Setup FX (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	120	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor X Deficient Plasma** to the reaction cuvette and start an incubation timer.
4	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
5	Once the plasma incubation is complete, add **50µL** of **Thromboplastin L** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FX results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-5 of the Factor X Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor X concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor X concentration of 110.1% : (12.5/100)*110.1 = 13.8%.
10	On the Helena C-Series select the FX test in channel 1, select FX and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FX Lot #".
11	Enter the lot number and expiry of the Factor X Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor X concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FX calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	198.2% = 16.1s
2:	110.1% = 20.0s
3:	55.1% = 25.9s
4:	13.8% = 44.7s
5:	1.7% = 118.5s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FX	125.1	124.7	99.8	149.7
5302	Speciality Assayed Control A	21590804	% FX	61.5	55.6	41.7	69.5

In Use Stability

Helena Biosciences Europe have established the stability of Factor X Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor X Deficient Plasma	6 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor X Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens® Coagulation Factor X with Thromborel® S on a Sysmex® CS-5100 System	% FX	132	y = 0.851x + 0.526	0.96

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FX	Siemens Coagulation Factor X - example LLMI (Lower Limit of Measuring Interval)	5.9	5.5	-0.4
	Siemens Coagulation Factor X - Arbitrary value	25.0	21.8	-3.2

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor X Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FX	141	66.4 to 126.5

Precision

Helena Biosciences Europe have established the precision performance of the Factor X Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FX	119.5	5.73	4.80%	9.23	7.72%
	56.1	2.63	4.69%	3.87	6.91%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor X Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor X Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	2.0 U/mL
LMW Heparin	2.0 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	60 mg/dL
Unconjugated Bilirubin	60 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor X Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FX	2.1 % to 147.5 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor X Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor X Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor VIII Assay (5193, 5793)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor VIII Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor VIII Deficient Plasma (Immunodepleted)	5793	10 x 1 mL	Bench Top	18.0 - 24.0 °C	Original Vial
Factor VIII Deficient Plasma (Congenital)	5193	10 x 1 mL			
APTT Si L Minus	5562SLQ 5560SLQ 5558SLQ 5559SLQ	5 x 5 mL 10 x 5 mL 5 x 10 mL 10 x 10 mL	Bench Top	18.0 - 24.0 °C	Original Vial
Calcium Chloride: 0.025M	5562SLQ 5560SLQ	5 x 5 mL 10 x 5 mL	On Board Reagent position Ø22mm	36.5 - 37.5 °C	Original Vial
	5558SLQ 5559SLQ	5 x 10 mL 10 x 10 mL	On Board Reagent position Ø24mm		
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 °C	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 °C	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor VIII Concentration	%	1	% of Normal, also stated as '% FVIII'

Application Note: Factor VIII Assay

Application Settings

Setup FVIII (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	300	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor VIII Deficient Plasma** to the reaction cuvette.
4	Add **50µL** of **APTT Si L Minus** to the reaction cuvette and start an incubation timer.
5	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
6	Once the reaction incubation is complete, add **50µL** of **Calcium Chloride: 0.025M** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FVIII results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-6 of the Factor VIII Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor VIII concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor VIII concentration of 98.3% : (12.5/100)*98.3 = 12.3%.
10	On the Helena C-Series select the FVIII test in channel 1, select FVIII and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FVIII Lot #".
11	Enter the lot number and expiry of the Factor VIII Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor VIII concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FVIII calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	176.9% = 44.5s
2:	98.3% = 53.6s
3:	49.2% = 63.0s
4:	12.3% = 86.1s
5:	1.5% = 112.6s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FVIII	102.4	96.0	76.8	115.2
5302	Speciality Assayed Control A	21590804	% FVIII	46.0	51.8	38.7	64.9

In Use Stability

Helena Biosciences Europe have established the stability of Factor VIII Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor VIII Deficient Plasma	5 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor VIII Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor VIII with Dade® Actin® FS Activated PTT Reagent on a Sysmex CS-5100 System	% FVIII	128	y = 0.928x + 3.985	0.97

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FVIII	Siemens Coagulation Factor VIII - Arbitrary value	10.0	13.3	3.3
	Siemens Coagulation Factor VIII - Arbitrary value	25.0	27.2	2.2

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor VIII Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FVIII	136	56.0 to 162.5

Precision

Helena Biosciences Europe have established the precision performance of the Factor VIII Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FVIII	88.0	3.98	4.52%	7.62	8.66%
	48.6	3.11	6.39%	6.30	12.95%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor VIII Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor VIII Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.1 U/mL
LMW Heparin	0.2 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	1592 mg/dL
Conjugated Bilirubin	36 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor VIII Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FVIII	6.5 % to 102.6 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor VIII Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor VIII Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor IX Assay (5194, 5794)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor IX Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor IX Deficient Plasma (Immunodepleted)	5794	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor IX Deficient Plasma (Congenital)	5194	10 x 1 mL			
APTT Si L Minus	5562SLQ 5560SLQ 5558SLQ 5559SLQ	5 x 5 mL 10 x 5 mL 5 x 10 mL 10 x 10 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Calcium Chloride: 0.025M	5562SLQ 5560SLQ	5 x 5 mL 10 x 5 mL	On Board Reagent position Ø22mm	36.5 - 37.5 ºC	Original Vial
	5558SLQ 5559SLQ	5 x 10 mL 10 x 10 mL	On Board Reagent position Ø24mm		
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor IX Concentration	%	1	% of Normal, also stated as '% FIX'

Application Settings

Setup FIX (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	300	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor IX Deficient Plasma** to the reaction cuvette.
4	Add 50µL of APTT Si L Minus to the reaction cuvette and start an incubation timer.
5	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
6	Once the reaction incubation is complete, add **50µL** of **Calcium Chloride: 0.025M** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FIX results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-6 of the Factor IX Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor IX concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor IX concentration of 116.7% : (12.5/100)*116.7 = 14.6%.
10	On the Helena C-Series select the FIX test in channel 1, select FIX and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FIX Lot #".
11	Enter the lot number and expiry of the Factor IX Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor IX concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FIX calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	210.1% = 43.8s
2:	116.7% = 52.1s
3:	58.4% = 61.9s
4:	14.6% = 86.6s
5:	1.8% = 116.6s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FIX	103.8	107.6	73.7	141.5
5302	Speciality Assayed Control A	21590804	% FIX	50.5	54.1	36.2	72.0

In Use Stability

Helena Biosciences Europe have established the stability of Factor IX Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor IX Deficient Plasma	5 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor IX Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor IX with Dade® Actin® FS Activated PTT Reagent on a Sysmex CS-5100 System	% FIX	117	y = 0.902x + 6.249	0.92

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FIX	Siemens Coagulation Factor IX - Arbitrary value	10.0	15.3	5.3
	Siemens Coagulation Factor IX - Arbitrary value	25.0	28.8	3.8

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor IX Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FIX	135	76.2 to 150.9

Precision

Helena Biosciences Europe have established the precision performance of the Factor IX Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FIX	113.3	7.42	6.55%	12.16	10.73%
	52.4	4.07	7.76%	8.06	15.37%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor IX Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor IX Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.2 U/mL
LMW Heparin	0.1 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	40 mg/dL
Unconjugated Bilirubin	59 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor IX Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FIX	2.8 % to 96.0 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor IX Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor IX Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor XI Assay (5196, 5796)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor XI Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor XI Deficient Plasma (Immunodepleted)	5796	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor XI Deficient Plasma (Congenital)	5196	10 x 1 mL			
APTT Si L Minus	5562SLQ	5 x 5 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
	5560SLQ	10 x 5 mL			
	5558SLQ	5 x 10 mL			
	5559SLQ	10 x 10 mL			
Calcium Chloride: 0.025M	5562SLQ	5 x 5 mL	On Board Reagent position Ø22mm	36.5 - 37.5 ºC	Original Vial
	5560SLQ	10 x 5 mL			
	5558SLQ	5 x 10 mL	On Board Reagent position Ø24mm		
	5559SLQ	10 x 10 mL			
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor XI Concentration	%	1	% of Normal, also stated as '% FXI'

Application Settings

Setup FXI (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	300	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor XI Deficient Plasma** to the reaction cuvette.
4	Add **50µL** of **APTT Si L Minus** to the reaction cuvette and start an incubation timer.
5	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
6	Once the reaction incubation is complete, add **50µL** of **Calcium Chloride: 0.025M** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FXI results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-6 of the Factor XI Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor XI concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor XI concentration of 99.3% : (12.5/100)*99.3 = 12.4%.
10	On the Helena C-Series select the FXI test in channel 1, select FXI and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FXI Lot #".
11	Enter the lot number and expiry of the Factor XI Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor XI concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FXI calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	178.7% = 47.9s
2:	99.3% = 57.4s
3:	49.7% = 70.6s
4:	12.4% = 92.4s
5:	1.6% = 116.1s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FXI	102.9	96.9	77.5	116.3
5302	Speciality Assayed Control A	21590804	% FXI	49.2	51.5	38.6	64.3

In Use Stability

Helena Biosciences Europe have established the stability of Factor XI Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor XI Deficient Plasma	5 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor XI Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor XI with Dade® Actin® FS Activated PTT Reagent on a Sysmex CS-5100 System	% FXI	104	y = 0.880x + 6.055	0.94

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FXI	Siemens Coagulation Factor XI - Arbitrary value	10.0	14.9	4.9
	Siemens Coagulation Factor XI - Arbitrary value	25.0	28.1	3.1

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor XI Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FXI	141	64.7 to 135.7

Precision

Helena Biosciences Europe have established the precision performance of the Factor XI Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FXI	92.2	3.99	4.33%	7.34	7.96%
	52.4	2.62	5.01%	6.16	11.76%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor XI Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor XI Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.3 U/mL
LMW Heparin	0.2 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	41 mg/dL
Unconjugated Bilirubin	60 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor XI Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FXI	2.8 % to 50.1 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor XI Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.*

The measuring interval of the Factor XI Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Factor XII Assay (5197, 5797)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that the Factor XII Assay is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor XII Deficient Plasma (Immunodepleted)	5797	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor XII Deficient Plasma (Congenital)	5197	10 x 1 mL			
APTT Si L Minus	5562SLQ 5560SLQ 5558SLQ 5559SLQ	5 x 5 mL 10 x 5 mL 5 x 10 mL 10 x 10 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Calcium Chloride: 0.025M	5562SLQ 5560SLQ	5 x 5 mL 10 x 5 mL	On Board Reagent position Ø22mm	36.5 - 37.5 ºC	Original Vial
	5558SLQ 5559SLQ	5 x 10 mL 10 x 10 mL	On Board Reagent position Ø24mm		
Imidazole Buffer	5375R	10 x 25 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N	5301	10 x 1 mL			
Speciality Assayed Control A	5302	10 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Factor XII Concentration	%	1	% of Normal, also stated as '% FXII'

Application Note: Factor XII Assay

Application Settings

Setup FXII (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	300	
Stop (s):	300	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/5** to prepare the 100% working dilution. To do this add **50 µL** of **plasma sample** to **200 µL** of **Imidazole Buffer** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.
3	Add **25µL** of **Factor XII Deficient Plasma** to the reaction cuvette.
4	Add **50µL** of **APTT Si L Minus** to the reaction cuvette and start an incubation timer.
5	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
6	Once the reaction incubation is complete, add **50µL** of **Calcium Chloride: 0.025M** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report % FXII results on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **180%** calibration point by diluting **720µL of the Calibration Plasma** with **1280µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **750µL of the 180% dilution,** prepared in cup 1, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **600µL of the 100% dilution,** prepared in cup 2, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **12.5%** calibration point by diluting **200µL of the 50% dilution,** prepared in cup 3, with **600µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **1.6%** calibration point by diluting **100µL of the 12.5% dilution,** prepared in cup 4, with **700µL Imidazole Buffer**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-6 of the Factor XII Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Factor XII concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 12.5% dilution concentration using a calibrator with a Factor XII concentration of 105.3% : (12.5/100)*105.3 = 13.2%.
10	On the Helena C-Series select the FXII test in channel 1, select FXII and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup FXII Lot #".
11	Enter the lot number and expiry of the Factor XII Deficient Plasma used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Factor XII concentration calculated in step 9 and the corresponding mean clot time assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an % FXII calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	189.5% = 45.3s
2:	105.3% = 54.9s
3:	52.7% = 65.8s
4:	13.2% = 97.4s
5:	1.6% = 124.6s

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% FXII	100.6	95.2	76.2	114.2
5302	Speciality Assayed Control A	21590804	% FXII	47.6	46.7	35.0	58.4

In Use Stability

Helena Biosciences Europe have established the stability of Factor XII Assay reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top.	Factor XII Deficient Plasma	4 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of the Factor XII Assay on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Coagulation Factor XII with Dade® Actin® FS Activated PTT Reagent on a Sysmex CS-5100 System	% FXII	101	y = 1.020x + 0.600	0.94

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% FXII	Siemens Coagulation Factor XII - Arbitrary value	10.0	10.8	0.8
	Siemens Coagulation Factor XII - Arbitrary value	25.0	26.1	1.1

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for the Factor XII Assay using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
% FXII	141	58.1 to 163.6

Precision

Helena Biosciences Europe have established the precision performance of the Factor XII Assay on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
% FXII	99.2	4.65	4.69%	2.15	4.48%
	47.9	7.61	7.67%	4.78	9.99%

Limitations of the Method

Helena Biosciences Europe have established the limitations of the Factor XII Assay on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of the Factor XII Assay in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	0.1 U/mL
LMW Heparin	0.4 U/mL
Haemoglobin	700 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	60 mg/dL
Unconjugated Bilirubin	30 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of the Factor XII Assay following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Outside of the linear range the relationship between the primary and calculated units is accurately modelled by the point to point interpolation used to construct the calibration curve.

Measurand	Linear Range
% FXII	2.3 % to 129.8 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of the Factor XII Assay on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of the Factor XII Assay on the Helena C-Series is defined by the reference limits of the current calibration.

Antithrombin Xa (5502, 5507)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that Antithrombin Xa is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Factor Xa Substrate	5502 5507	3 x 10 mL 5 x 2 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Factor Xa	5502 5507	3 x 10 mL 5 x 2 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Sample Diluent*	5502 5507	4 x 10 mL 5 x 3 mL	Bench Top	18.0 - 24.0 ºC	Original Vial or sealable container
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Routine Control N Routine Control A Routine Control SA Speciality Assayed Control N Speciality Assayed Control A	5186 5187 5183 5301 5302	10 x 1 mL 10 x 1 mL 10 x 1 mL 10 x 1 mL 10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial

*Sample Diluent must be diluted 1/5 (1 + 4) with distilled / deionised water and mixed well before use.

All reagents must be prepared following the instructions provided in individual kit IFUs.

Buffers must be allowed to acclimatise to ambient temperature before use.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Antithrombin Xa %	%	1	% of Normal, also stated as '% AT'

Application Settings

Setup AT (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	
Incubation (s):	120	0.0-250.0
Stop (s):	90	

Testing Protocol

Step	Action
1	To prepare the 100% working dilution pre-dilute the plasma sample **1/100** in two steps, first create a stock by adding **50 µL** of **plasma sample** to **450 µL** of **Sample Diluent** (diluted for use) in a fresh, disposable, plastic sample cup. Mix thoroughly but gently avoiding foam. Use diluted stock immediately.
2	In a second fresh, disposable, plastic sample cup add **50 µL** of the **stock plasma dilution** (made in step 1) to **450 µL** of **Sample Diluent** (diluted for use). Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
3	Add **50µL** of the **pre-diluted plasma sample** (made in step 2) to a pre-warmed cuvette and start an incubation timer.
4	After **60s** add **50µL** of **Factor Xa** to the reaction cuvette.
5	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
6	Once the sample incubation is complete, add **50µL** of **Factor Xa Substrate** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

For samples reporting % AT results outside of the calibration curve users should re-analyse using the following alternative Test Protocol Step 2 pre-dilutions. Prepare alternative pre-dilutions using the stock plasma dilution created in Test Protocol Step 1. Correct the reported results by multiplying by the factor stated.

	Alternative Pre-Dilution		Correction Factor (reported % AT * corr. fact.)
Condition	Ratio	Alternative Step 2	
Reported % AT > curve limits	1/200	In a fresh, disposable, plastic sample cup add **50 µL** of the **stock plasma dilution** (made in step 1) to **950 µL** of **Sample Diluent** (diluted for use). Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.	2
Reported % AT < curve limits	1/20	In a fresh, disposable, plastic sample cup add **100 µL** of the **stock plasma dilution** (made in step 1) to **100 µL** of **Sample Diluent** (diluted for use). Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.	0.2

Calibration Construction and Example Calibration

To report Antithrombin Xa % results with Antithrombin Xa on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient Antithrombin Xa reagents for use as per the current issue of the relevant product IFUs, pre-dilute sufficient Sample Diluent for use.
2	Prepare a calibration dilution set by first labelling five fresh sample cups "S", 1, 2, 3, 4 and 5.
3	Prepare the calibration stock dilution in the cup labelled "S" by diluting **50µL of the Calibrator** prepared in step 1 with **450µL Sample Diluent**. Mix thoroughly but gently avoiding foam. Use the calibration stock immediately.
4	In cup 1 prepare the **150%** calibration point by diluting **75µL of the Stock dilution** prepared in step 3 with **425µL Sample Diluent**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 2 prepare the **100%** calibration point by diluting **300µL of the 150% dilution, prepared in cup 1**, with **150µL Sample Diluent.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 3 prepare the **50%** calibration point by diluting **200µL of the 100% dilution, prepared in cup 2**, with **200µL Sample Diluent.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 4 prepare the **25%** calibration point by diluting **200µL of the 50% dilution, prepared in cup 3**, with **200µL Sample Diluent.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	In cup 5 prepare the **6.3%** calibration point by diluting **100µL of the 12.5% dilution, prepared in cup 4**, with **300µL Sample Diluent.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
9	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-6 of the Antithrombin Xa Test Protocol. The calibration dilutions prepared in steps 4-8 are already pre-diluted and are ready for analysis, do not dilute further.
10	To calculate the Antithrombin Xa concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 6.3% dilution concentration using a calibrator with an Antithrombin Xa concentration of 93.4% : (6.3/100)*93.4 = 5.8%.
11	On the Helena C-Series select the AT test in channel 1, select AT and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup AT Lot #".
12	Enter the lot number and expiry of the Antithrombin Xa kit used to assay the calibration dilutions.
13	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
14	For each calibration dilution enter the Antithrombin Xa concentration calculated in step 10 and the corresponding mean E value assayed in step 9.
15	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
16	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an Antithrombin Xa % calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	140.1% = 0.224E
2:	93.4% = 0.571E
3:	46.7% = 0.840E
4:	23.4% = 1.003E
5:	5.8% = 1.164E

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	% AT	129.1	129.8	103.8	155.7
5302	Speciality Assayed Control A	21590804	% AT	68.5	64.6	48.5	80.8

In Use Stability

Helena Biosciences Europe have established the stability of Antithrombin Xa reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top for 8 hour shifts and returned, re-capped in original vials, to 2 - 8ºC overnight.	Factor Xa Substrate and Factor Xa (5502 - 10 mL vials)	2 consecutive days
	Factor Xa Substrate and Factor Xa (5507 - 2 mL vials)	
Original vials prepared for use, opened, and placed into use on bench top.	Factor Xa Substrate and Factor Xa (5502 - 10 mL vials)	12 hours
	Factor Xa Substrate and Factor Xa (5507 - 2 mL vials)	

Replace diluted Sample Diluent in use on bench top daily.

Store diluted Sample Diluent in a tightly sealed bottle at 2 - 8°C and use within one month.

Comparison of Method

Helena Biosciences Europe have established a method comparison of Antithrombin Xa on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Antithrombin with INNOVANCE® Antithrombin on a Sysmex CS-5100 System	% AT	111	y = 0.984x - 3.009	0.98

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% AT	Siemens Antithrombin Expected Values - 2.5th percentile	79.0	74.7	-4.3
	Siemens Antithrombin Expected. Values - 97.5th percentile	119.8	114.9	-4.9

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for Antithrombin Xa using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
Antithrombin Xa %	124	86.0 to 132.2

Precision

Helena Biosciences Europe have established the precision performance of Antithrombin Xa on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
Antithrombin Xa %	121.0	6.84	5.65%	11.15	9.21%
	61.0	4.02	6.59%	6.54	10.71%

Limitations of the Method

Helena Biosciences Europe have established the limitations of Antithrombin Xa on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of Antithrombin Xa in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	2.6 U/mL
LMW Heparin	3.0 U/mL
Haemoglobin	1000 mg/dL
Triglycerides	861 mg/dL
Conjugated Bilirubin	60 mg/dL
Unconjugated Bilirubin	60 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of Antithrombin Xa following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Measurand	Linear Range
Antithrombin Xa %	0.6 % to 195.5 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of Antithrombin Xa on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of Antithrombin Xa on the Helena C-Series is defined by the linear range.

Protein C (5543)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that Protein C is only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
Protein C Substrate	5543	6 x 2 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Protein C Activator	5543	6 x 2 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Protein C Diluent	5543	3 x 5 mL	Only used to reconstitute the Protein C Substrate		
Saline (0.85% NaCl)	-	-	Bench Top	18.0 - 24.0 ºC	Original Vial
Calibration Plasma	5185	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
Speciality Assayed Control N Speciality Assayed Control A	5301 5302	10 x 1 mL 10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial

All reagents must be prepared following the instructions provided in individual kit IFUs.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
Protein C %	%	1	% of Normal, also stated as 'PC %'

Application Settings

Setup PC (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	%	0.0-250.0
Incubation (s):	180	
Stop (s):	180	

Testing Protocol

Step	Action
1	Pre-dilute the plasma sample **1/4** to prepare the 100% working dilution, to do this add **50 µL** of **plasma sample** to **150 µL** of **Saline (0.85% NaCl)** in a fresh, disposable, plastic sample cup. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
2	Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette and start an incubation timer.
3	After **60 seconds** add **25µL** of **Protein C Activator** to the reaction cuvette.
4	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
5	Once the sample incubation is complete, add **25µL** of **Protein C Substrate** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Calibration Construction and Example Calibration

To report Protein C % results with Protein C on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a Calibration Plasma vial and sufficient Protein C reagents for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 1 prepare the **150%** calibration point by diluting **300µL of Calibration Plasma** prepared in step 1 with **500µL Saline**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 2 prepare the **100%** calibration point by diluting **500µL of the 150% dilution,** prepared in cup 1, with **250µL Saline**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 3 prepare the **50%** calibration point by diluting **300µL of the 100% dilution,** prepared in cup 2, with **300µL Saline.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 4 prepare the **25%** calibration point by diluting **300µL of the 50% dilution,** prepared in cup 3, with **300µL Saline.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 5 prepare the **6.3%** calibration point by diluting **100µL of the 25% dilution,** prepared in cup 4, with **300µL Saline.** Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 2-5 of the Protein C Test Protocol. The calibration dilutions prepared in steps 3-7 are already pre-diluted and are ready for analysis, do not dilute further.
9	To calculate the Protein C concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 6.3% dilution concentration using a calibrator with a Protein C concentration of 117.1% : (6.3/100)*117.1 = 7.3%.
10	On the Helena C-Series select the PC test in channel 1, select PC and select [Lot 1] or [Lot 2]. Select [Setup >] to enter "Setup PC Lot #".
11	Enter the lot number and expiry of the Protein C kit used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the Protein C concentration calculated in step 9 and the corresponding mean E value assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

An example of an Protein C % calibration constructed using Calibration Plasma (5185) on the Helena C-Series is stated below.

1:	175.7% = 0.243E
2:	117.1% = 0.183E
3:	58.6% = 0.102E
4:	29.3% = 0.054E
5:	7.3% = 0.014E

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5301	Speciality Assayed Control N	21543174	PC %	107.5	113.9	91.1	136.6
5302	Speciality Assayed Control A	21590804	PC %	54.6	53.9	40.4	67.4

In Use Stability

Helena Biosciences Europe have established the stability of Protein C reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent(s)	Time
Original vials prepared for use, opened, and placed into use on bench top for 8 hour shifts and returned, re-capped in original vials, to 2ºC - 8ºC overnight.	Protein C Activator and Protein C Substrate	5 days

Replace Saline in use on bench top daily.

Comparison of Method

Helena Biosciences Europe have established a method comparison of Protein C on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens Protein C with Berichrom® Protein C on a Sysmex CS-5100 System	PC %	108	y = 0.974x - 2.211	0.99

The following bias statistics were generated using the regression trend plotted in the method comparison evaluation.

Measurand	Result	Bias Statisitics		
		Siemens result	Helena result	Bias
% PC	Siemens Protein C - Arbitrary Value	10.0	7.5	-2.5
	Siemens Protein C - Arbitrary Value	25.0	22.1	-2.9

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for Protein C using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition,* 2008.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	Normal Range
Protein C %	129	77.3 to 141.1

Precision

Helena Biosciences Europe have established the precision performance of Protein C on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

		Repeatability		Within-device	
Measurand	Mean	SD	CV%	SD	CV%
Protein C %	112.3	4.70	4.19%	7.90	7.03%
	57.9	2.30	3.96%	3.39	5.85%
	29.0	1.30	4.49%	2.35	8.08%

Limitations of the Method

Helena Biosciences Europe have established the limitations of Protein C on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of Protein C in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Unfractionated Heparin	3.0 U/mL
LMW Heparin	3.0 U/mL
Haemoglobin	80 mg/dL
Triglycerides	2000 mg/dL
Conjugated Bilirubin	30 mg/dL
Unconjugated Bilirubin	43 mg/dL

Linearity

Helena Biosciences Europe have established the Linearity of Protein C following the protocol set out in CLSI guideline EP06-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*, 2003.

Measurand	Linear Range
Protein C %	0.6 % to 210.6 %

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of Protein C on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval of Protein C on the Helena C-Series is defined by the linear range.

Auto Blue D-Dimer 400 (5552)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
D-Dimer Blue Latex	5552	4 x 4 mL	On Board Reagent position Ø22mm	36.5 - 37.5 ºC	Original Vial
D-Dimer Blue Buffer	5552	4 x 7 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
D-Dimer Diluent	5552	2 x 7 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
D-Dimer Calibrator	5552	2 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
D-Dimer Control L D-Dimer Control H	5509	5 x 1 mL 5 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Buffers must be allowed to acclimatise to ambient temperature before use.

Please note that D-Dimer lots contain paired Latex and Buffer reagents; as such if the remains of one vial are discarded, the remains of the paired vial should also be discarded.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
D-Dimer	µg/L DDU	0	µg/L DDU (D-Dimer Units)
	µg/L FEU	0	µg/L FEU (Fibrinogen Equivalent Units)

Results may be reported either in D-dimer units (DDU) or in fibrinogen equivalent units (FEU); an approximate conversion factor, originating from the Fibrinogen/D-dimer weight ratio of 340 kDa/195 kDa, is 1.74. Rounding errors are avoided by use of the ratio 340/195 (Edlund B, Nilsson TK (2006) *A proposed stoichiometrical calibration procedure to achieve transferability of D-Dimer measurements and to characterize the performance of different methods*, Clin Biochem, 39(2):137-142).

Application Settings

Setup DD (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	µg/L	0-27500
Incubation (s):	120	
Stop (s):	180	

Testing Protocol

Step	Action
1	Add **25µL** of the **plasma sample** to a pre-warmed cuvette.
2	Immediately add **100µL** of the **Reaction Buffer** to the cuvette and start an incubation timer.
3	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
4	Once the sample incubation is complete, add **50µL** of **Latex** to the cuvette to start the reaction.
5	Immediately after the addition of Latex, while the channel timer is still blinking green, mix the reaction by carefully drawing the cuvette contents in and out of the pipette tip five times avoiding the formation of foam. Ensure mixing is finished and the reaction is not disturbed once the channel timer starts to blink orange.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

For samples reporting results greater than the D-Dimer Calibrator reference value (the upper curve limit) users should re-analyse using the following alternative Test Protocol Step 1 pre-diluting the sample in D-Dimer Diluent. Correct the reported results by multiplying by the factor stated.

	Alternative Pre-Dilution		Correction Factor (reported µg/L * corr. fact.)
Condition	Ratio	Alternative Step 1	
Reported µg/L > curve upper limit	1/10	In a fresh, disposable, plastic sample cup add **50µL** of the **plasma sample** to **450µL** of **D-Dimer Diluent**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately. Add **25µL** of the **pre-diluted plasma sample** to a pre-warmed cuvette.	10

Calibration Construction and Example Calibration

To report D-Dimer results with Auto Blue D-Dimer 400 on the Helena C-Series users must first follow these instructions to construct a calibration curve.

Step	Action
1	Prepare a vial of D-Dimer Calibrator vial, D-Dimer Blue Latex, D-Dimer Blue Buffer and D-Dimer Diluent for use as per the current issue of the relevant product IFUs.
2	Prepare a calibration dilution set by first labelling five fresh sample cups 1, 2, 3, 4 and 5.
3	In cup 2 prepare the **50%** calibration point by diluting **250µL of the D-Dimer Calibrator** prepared in step 1 with **250µL D-Dimer Diluent**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
4	In cup 3 prepare the **25%** calibration point by diluting **250µL of the 50% dilution,** prepared in cup 2, with **250µL D-Dimer Diluent**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
5	In cup 4 prepare the **12.5%** calibration point by diluting **250µL of the 25% dilution,** prepared in cup 3, with **250µL D-Dimer Diluent**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
6	In cup 5 prepare the **6.3%** calibration point by diluting **250µL of the 12.5% dilution,** prepared in cup 4, with **250µL D-Dimer Diluent**. Use a pipette to mix thoroughly avoiding foam. Use pre-diluted plasma immediately.
7	In cup 1 prepare the **100%** calibration point by pipetting **500µL of the D-Dimer Calibrator** prepared in step 1.
8	Use the Helena C-Series to assay the five prepared dilutions as samples, in duplicate, following the steps 1-5 of the Auto Blue D-Dimer 400 Test Protocol. The calibration dilutions prepared in steps 3-7 are ready for analysis, do not dilute further.
9	To calculate the D-Dimer concentration of each calibration dilution multiply the reference value of the calibrator used by the dilution prepared. For example to calculate the 25% dilution concentration using a calibrator with a D-Dimer concentration of 3333 µg/L : (25/100) * 3333 µg/L = 833 µg/L.
10	On the Helena C-Series select the DD test in channel 1, select DD and select [Lot 1] or [Lot 2]*. Select [Setup >] to enter "Setup DD Lot #".
11	Enter the lot number and expiry of the Auto Blue D-Dimer 400 kit used to assay the calibration dilutions.
12	Select [Setup] again to access calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
13	For each calibration dilution enter the D-Dimer concentration calculated in step 9 and the corresponding mean E result assayed in step 8.
14	Select [OK] to save the inputted data. Ensure all calibration data entered in the analyser is correct before using the calibration to assay samples.
15	The curve data is test system, analyser and reagent lot, specific and cannot be transferred.

*Not available on the Helena C-1.

An example of an D-Dimer (µg/L DDU) calibration constructed using Auto Blue D-Dimer 400 on the Helena C-Series is stated below.

1:	3333 µg/L = 0.281E
2:	1667 µg/L = 0.170E
3:	833 µg/L = 0.093E
4:	417 µg/L = 0.044E
5:	208 µg/L = 0.026E

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Data		
					Value	Lower Limit	Upper Limit
5509	D-Dimer Control - L	21528441	µg/L DDU	291	305	238	372
	D-Dimer Control - H	21528441	µg/L DDU	2068	2116	1607	2626

In Use Stability

Helena Biosciences Europe have established the stability of Auto Blue D-Dimer 400 reagents on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Each working day of a five day working week, original vials prepared for use, opened, and placed into use for 1 hour of batch testing, then recapped and stored at 2 - 8ºC.	D-Dimer Blue Latex	2 weeks
	D-Dimer Blue Buffer	2 weeks
Original vials prepared for use, opened, recapped and stored at 2 - 8ºC.	D-Dimer Blue Latex	4 weeks
	D-Dimer Blue Buffer	4 weeks
	D-Dimer Diluent	4 weeks
Original vials prepared for use, opened, recapped and stored at 20ºC.	D-Dimer Blue Latex	2 weeks
	D-Dimer Blue Buffer	2 weeks
Original vials prepared for use at 4 - 25ºC.	D-Dimer Calibrator	12 hours

Comparison of Method

Helena Biosciences Europe have established a method comparison of Auto Blue D-Dimer 400 on the Helena C-Series to a market leading test system following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Siemens D-Dimer with INNOVANCE® D-Dimer on a Sysmex CS-5100 System	µg/L FEU	109	y = 0.983x - 720	0.93

Reference Cut-Off

Helena Biosciences Europe recommends a negative predictive cut-off value of 200 µg/L DDU, 349 µg/L FEU. With this cut-off the sensitivity / negative predictive value is relatively high and of good practical use for the exclusion of DVT and PE.

It is advisable for each laboratory to establish its own cut-off value.

Precision

Helena Biosciences Europe have established the precision performance of Auto Blue D-Dimer 400 on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots, four calibrations and consisted of 80 observations, over 40 runs of 2 replicates, over 20 days with 2 runs per day.

Measurand	Mean	Repeatability		Within-device	
		SD	CV%	SD	CV%
D-Dimer (µg/L DDU)	2093	109	5.22%	137	6.53%
	284	28	9.84%	31	11.05%

Limitations of the Method

Helena Biosciences Europe have established the limitations of Auto Blue D-Dimer 400 on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of Auto Blue D-Dimer 400 in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±15% drift from the normal result.

Interferent	No interference observed below
Haemoglobin	496 mg/dL
Triglycerides	86 mg/dL
Conjugated Bilirubin	52 mg/dL
Unconjugated Bilirubin	12 mg/dL

Linearity

Not Applicable

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of Auto Blue D-Dimer 400 on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The measuring interval for reporting D-Dimer is 76 - 3500 µg/L DDU, equivalent to 133 – 6103 µg/L FEU, with no prozone effect observed below 39,500 µg/L DDU, equivalent to 68,872 µg/L FEU.

DRVVT Screen & DRVVT Confirm (5484, 5485)

The application defined in this User Guide has been developed by Helena Biosciences Europe to provide optimal product performance with the assay and analyser test system. Any modification of these parameters may impact performance of this and other assays in use and the reported assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. Results of this test should be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

This application note lists all combinations of controls and calibrators for use with the assay and analyser test system; other combinations are not validated or supported by Helena Biosciences Europe.

Please note that DRVVT Screen & DRVVT Confirm are only available on the Helena C-2 and Helena C-4 analysers.

Materials

Material			Usage		
Component	REF	Kit Format	Position for Use	Temperature	Vessel for Use
DRVVT Screen	5484	10 x 2 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
DRVVT Confirm	5485	10 x 1 mL			
Routine Control N	5186	10 x 1 mL	Bench Top	18.0 - 24.0 ºC	Original Vial
LA Positive Control S	5486	1 x 1 mL			

All reagents must be prepared following the instructions provided in individual kit IFUs.

All reagents used on board the Helena C-Series analyser are pre-warmed to 37ºC, fresh reagents must be placed on board and left for 10 minutes before use to acclimatise fully to 37ºC.

Expression of Results

Measurand	Unit	Expressed to # decimal places	Notes
DRVVT Screen Clot Time	seconds	1	Also stated as 'Clot (s)'.
DRVVT Screen Normalised Ratio	-	2	Used as the numerator to calculate the Lupus Ratio
DRVVT Confirm Clot Time	seconds	1	Also stated as 'Clot (s)'.
DRVVT Confirm Normalised Ratio	-	2	Used as the denominator to calculate the Lupus Ratio
Lupus Ratio	-	2	To be calculated by the user as: Screen Norm. Ratio / Confirm Norm. Ratio

Application Settings

Setup LA-S / LA-C (LOT#):		
LOT:	[Lot number]	
Expiry:	[Expiry date]	
Units:	R	0.00-99.00*
Incubation (s):	120	
Stop (s):	300	

* When reporting results as Ratios the unit range defaults to 0.00 – 99.00.

Testing Protocol

Step	Action
1	Add **50µL** of the **plasma sample** to a pre-warmed cuvette and start an incubation timer.
2	Before the end of the incubation period move the cuvette to a measuring channel, press 'Optic', enter a PID (Patient ID) and press 'OK'. The channel is now active and ready for reagent addition.
3	Once the sample incubation is complete, add **50µL** of **DRVVT Screen or DRVVT Confirm** to the cuvette to start the reaction.

Once a channel is active the user must take care not to disturb the reaction cuvette, directly or by moving the analyser, as this may unintentionally start the reaction measurement. If a measurement is unintentionally started the reaction should be discarded and the test repeated.

If the addition of the final reagent does not automatically start the measurement the reaction should be discarded and the test repeated. Where a measurement has failed to start automatically the user may repeat the test and manually start the measurement simultaneous to the addition of the final reagent, refer to the Operator Manual for details.

Normal Value to report Ratio results

To report Ratio results with DRVVT Screen and DRVVT Confirm on the Helena C-Series users must enter an appropriate normal clot time value into the analyser software for each assay.

Step	Action
1	On the C-Series select the LA-S or LA-C test as required in channel 1, select [Lot 1] or [Lot 2] and select [Setup >].
2	Enter the lot number and expiry date of the reagent used.
3	Select [Setup] to access the calibration input screen, all data input in this screen must be made by selecting each field in turn and using the onscreen controls.
4	Enter the appropriate normal value in seconds.
5	Select [OK] to save the inputted data. Ensure the normal value entered in the analyser is correct before using it to report Ratio results.

Example Quality Control Material Reference Data

REF	Product	LOT	Measurand	Observed Mean	Reference Range	
					Lower Limit	Upper Limit
5486	LA Positive Control S	21548433	Screen - Clot (s)	78.3		
			Confirm - Clot (s)	32.9		
			Screen - Normal (s)	35.3		
			Confirm - Normal (s)	31.3		
			Screen Normalised Ratio	2.22	2.10	2.85
			Confirm Normalised Ratio	1.05	0.95	1.29
			Lupus Ratio	2.11	1.87	2.54

In Use Stability

Helena Biosciences Europe have established the stability of DRVVT Screen & DRVVT Confirm on the Helena C-Series following protocol designs set out in CLSI guideline EP25-A *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*.

Helena Biosciences Europe perform all stability evaluation in a controlled laboratory environment, local in use conditions and reagent usage may impact stability.

Stability Conditions	Reagent	Time
Original vials prepared for use, opened, and placed into use on bench top for an 8 hour shift per working day. Vials stored at 2 - 8ºC outside of shift and mixed and acclimatised prior to use each shift.	DRVVT Screen - 2 mL vial	3 days
	DRVVT Confirm - 1 mL vial	3 days

Comparison of Method

Helena Biosciences Europe have established a method comparison of DRVVT Screen & DRVVT Confirm on the Helena C-Series to a market leading analyser following the protocol set out in CLSI guideline EP09c: *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition,* 2013.

Comparative Test System (x)	Measurand	n	Regression Line	r^2
Helena DRVVT Screen & DRVVT Confirm on a Sysmex CA-1500 system	Lupus Ratio	66	y = 1.013x - 0.017	0.97

Reference Interval

Helena Biosciences Europe have established a Helena C-Series specific reference interval for DRVVT Screen & DRVVT Confirm using plasma samples from healthy subjects and multiple lots of reagent and following a nonparametric method as set out in CLSI guideline EP28-A3c: *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition, 2008*.

The following data should be considered only as a guide, with each laboratory establishing its own reference intervals.

Measurand	n	95% Confidence Reference Interval
Lupus Ratio	110	0.83 to 1.19

Precision

Helena Biosciences Europe have established the precision performance of DRVVT Screen & DRVVT Confirm on the Helena C-Series following protocol set out in CLSI guideline EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition,* 2008. The evaluation of each level of sample was performed using multiple reagent lots and consisted of 75 observations, over 3 runs of 5 repeats each with 5 replicates, over 3 days with 1 run per day.

			Repeatability		Within-device	
Sample	Measurand	Mean	SD	CV%	SD	CV%
LA Positive Control S	Screen - Clot(s)	79.6	0.89	1.12%	1.05	1.32%
	Confirm - Clot(s)	33.8	0.70	2.08%	0.89	2.63%
	Screen - ratio	2.25	0.03	1.12%	0.03	1.32%
	Confirm - ratio	1.08	0.02	2.08%	0.03	2.63%
	Lupus Ratio	2.09	0.05	2.29%	0.05	2.48%
Routine Control N	Screen - Clot(s)	32.4	0.28	0.86%	0.38	1.16%
	Confirm - Clot(s)	28.1	0.56	1.98%	0.80	2.86%
	Screen - ratio	0.92	0.01	0.86%	0.01	1.16%
	Confirm - ratio	0.90	0.02	1.98%	0.03	2.86%
	Lupus Ratio	1.02	0.02	1.98%	0.03	2.72%
Normal Pooled Plasma (double spun, frozen)	Screen - Clot(s)	32.3	0.24	0.74%	0.24	0.74%
	Confirm - Clot(s)	27.9	0.52	1.86%	0.66	2.36%
	Screen - ratio	0.96	0.01	0.74%	0.01	0.74%
	Confirm - ratio	0.95	0.02	1.86%	0.02	2.36%
	Lupus Ratio	1.01	0.02	1.88%	0.02	2.37%

Limitations of the Method

Helena Biosciences Europe have established the limitations of DRVVT Screen & DRVVT Confirm on the Helena C-Series following protocol set out in CLSI guideline EP07 *Interference Testing in Clinical Chemistry* 3rd Ed, 2018.

The analytical specificity of DRVVT Screen & DRVVT Confirm in the presence of various interferents was evaluated on the Helena C-Series. Interference was defined as the point at which the trend of observed results breached ±10% drift from the normal result.

Interferent	No interference observed below
Haemoglobin	173 mg/dL
Triglycerides	176 mg/dL
Conjugated Bilirubin	60 mg/dL

Linearity

Not Applicable.

Measuring Interval

Helena Biosciences Europe have evaluated the measuring interval of DRVVT Screen & DRVVT Confirm on the Helena C-Series with guidance from CLSI document EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition*.

The DRVVT Screen measuring interval allows users to report all valid, non-flagged, Clot (s) results up to a maximum of 160 seconds and all derived Ratio results.

The DRVVT Confirm measuring interval allows users to report all valid, non-flagged, Clot (s) results up to a maximum of 145 seconds and all derived Ratio results.

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Tel +44 (0)191 482 8440
Fax +44 (0)191 482 8442

info@helena-biosciences.com
techsupport-hs@helena-biosciences.com

Queensway South,
Team Valley Trading Estate, Gateshead,
Tyne and Wear, NE11 0SD, United Kingdom

HL-2-P-3429 Rev. 1

helena
Biosciences Europe

www.helena-biosciences.com