



Application Sheet for Measurement of Emicizumab using a Modified One-Stage Factor VIII assay on the Siemens BCS XP.

Refer to the current Instructions for Use for reagents, calibrator and controls for additional information, limitations, interferences and assigned values. This application sheet lists the reagents, calibrator and controls to be used on the Siemens BCS XP; other combinations have not been validated.

The parameters defined in this application sheet have been developed by r² Diagnostics to provide optimal performance with the prescribed reagents and instrument combination. Any modification of these parameters may affect the performance of this assay, other assays on the system and the resulting assay values. It is the responsibility of the user to validate any modifications on all assay results. Results of the test should always be interpreted in conjunction with the patient's clinical presentation.

Performance of the Modified One-Stage Factor VIII assay for Measurement of Emicizumab:

Materials Required			
Material	Catalog #	Size	On-Board Position
r ² Emicizumab Calibrator	151-201	5 x 1mL	Lanes 5-14
r ² Emicizumab Controls	152-401	5 x 1mL Level 1, 5 x 1mL Level 2	Lanes 5-14
Siemens Actin FSL	B4219	8 x 2 mL or 8 x 15 mL	Lanes 1-4
Siemens Calcium Chloride	ORHO37	8 x 15 mL	Lanes 1-4
Siemens FVIII Deficient Plasma	OTXW17	8 x 1 mL	Lanes 5-14
Sodium Chloride Solution (0.9%)	N/A	N/A	Lanes 3 or 4

Emicizumab Calibrator and Controls are a product of r² Diagnostics and r² Diagnostics is not a supplier of Siemens reagents.

Reconstituted Stability		
Material	Room Temperature	2-8 °C
r ² Emicizumab Calibrator	4 hours	8 hours
r ² Emicizumab Controls	8 hours	8 hours

The stability data presented here were established under controlled laboratory conditions. Due to differences in laboratory environmental conditions performance may vary. Room temperature stability was evaluated with the calibrator and controls stored in reagent rack lanes 5-16 and 2-8 °C stability was determined as closed vial storage in-between tests.

Precision (% CV)					
Material	Mean (µg/mL)	Within-run	Between-run	Between-day	Total
r ² Emicizumab Control Level 1	24.3	3.9%	2.2%	2.0%	4.9%
r ² Emicizumab Control Level 2	75.1	2.6%	6.1%	0.0%	6.6%

Precision was evaluated over 5 days from a single calibration with 2 runs per day and 4 replicates per run.



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Assay Limits	
Limit of Blank	1.1 µg/mL
Limit of Detection	2.1 µg/mL
Limit of Quantification	10.0 µg/mL

Assay limits were evaluated over 3 days with 5 samples for limit of blank and 7 samples for limit of detection.

Measurement Range
10 – 100 µg/mL without dilution (Emicizumab test setup)
100 – 250 µg/mL with dilution (Emicizumab.High test setup)

Measurement range was evaluated using pooled severe hemophilia patient plasma spiked with Emicizumab at known concentrations.

Interferences		
Factor VIII replacement	Concentration	Bias (µg/mL)
BDDrFVIII (ReFacto, Pfizer)	0.74 IU/mL	+20 µg/mL
FLrFVII (Advate, Baxter)	0.74 IU/mL	+20 µg/mL
Bypassing agents		
Anti-Inhibitor Coagulant Complex (FEIBA, Shire)	5.9 U/mL	No observed bias
rFVIIa (NovoSeven, Novo Nordisk)	5.3 mcg/mL	No observed bias

Interference was determined by spiking different interferants into Emicizumab Control Level 1 and Control Level 2.



Instructions for Programming the Modified One-Stage Factor VIII Assay for Measurement of Emicizumab on the Siemens BCS:

All reagent definitions, assay procedures, assay definitions, software configuration, lot specific information, and reflex testing should only be entered by trained personnel, since incorrect information may affect assay performance, assay results, reagent stability, and work flow. This application sheet does not include information on laboratory information systems (LIS). Additional information may be found in the Siemens BCS XP Instruction Manual.

Note: Copies of all Reagent Definitions, Assay Procedures, Assay Definitions, and Auto Assistant instructions are available in the appendices of this Application Sheet. Information to be entered by the user is in colored cells.

Reagent Definitions

1. Exit the Siemens BCS XP Application to get to the desktop.
2. From the computer desktop open the *Data Definition Tool* and follow prompts to log in.
3. After the utility opens, select *Definitions* from the menu and then choose either *Standards / Calibrators* for adding Emicizumab calibrator or *Controls* for adding Emicizumab Control Level 1 and Level 2.
4. To add products, click on the button that says *New* and enter information as shown for Emicizumab Calibrator and Controls in **Appendix A**.

Assay Procedure

1. Exit the Siemens BCS XP Application to get to the desktop.
2. From the computer desktop open the *Data Definition Tool* and follow prompts to log in.
3. After the utility opens, select *Definitions* from the menu and then choose *Assay procedures*.
4. To add a new assay procedure, click on the button that says *New*. This will open a blank assay procedure.
5. Use the assay procedures located in either **Appendix B** (Emicizumab) or **Appendix C** (Emicizumab.High) to fill in all parameters.
 - For the **Evaluation and checking methods** it is necessary to enter the parameters individually once each method is selected from the scroll box. Do not enter parameters for any method not listed in this application sheet.
 - To enter **Pipetting cycles** and **Pipetting media** click on the button at the bottom of the entry window labeled *Pipetting*. Once the pipetting entry window pops up it is necessary to add Pipetting cycles and their associated media one cycle at a time. (e.g.: click the button that says *Add new cycle*, enter the parameters for the first pipetting cycle and then click the button that says *Add new row* for each pipetting media step. Once all media steps have been added for the corresponding pipetting cycle add the next pipetting cycle. Once all steps have been entered, it is okay to close the pipetting window by clicking on the close button.
6. Once all parameters have been successfully added, click the *save* button on the bottom of the screen.



Assay Definition

1. Exit the Siemens BCS XP Application to get to the desktop.
2. From the computer desktop open the *Data Definition Tool* and follow prompts to log in.
3. After the utility opens, select *Definitions* from the menu and then choose *Assays*.
4. To add a new assay definition, click on the button that says *New*. This will open a blank assay definition.
5. Use the assay definitions located in **Appendix B** (for Emicizumab) or **Appendix C** (for Emicizumab.High) to fill in all parameters.
 - Under **Selection of assay procedures, evaluation and checking methods** it is necessary to click the [+] button located on the left, followed by selection of the Emicizumab Assay Procedure that was previously entered. Once the assay has been selected, it is necessary to click the [+] button located on the right until all evaluation and checking methods have been added.
 - To add the calibrator, click on the *add calibrator* button for each calibration point and enter each calibration point individually (100, 75, 50, 25, and 10 µg/mL).
6. Once all parameters have been successfully added, click the *save* button on the bottom of the screen.
7. Additionally: Add control assignment of Emicizumab Control Level 1 and Control Level 2 to the Emicizumab assay by clicking on *control assignment*. From within this menu select Emicizumab Control Level 1 and Level 2 from the scroll list and fill out parameters in accordance with your organizations practices. Click the *save* button to exit the control assignment window and click the *save* button once more to save the assay definition.

Software Configuration

1. From the desktop, select the BCS XP application to launch the instrument software.
2. Once the software launches and initializes, select *Definitions* from the menu and then choose *Configuration*.
3. A window will open and in the left column select *Assays*.
4. In the list on the left, scroll down to the Emicizumab assay that was previously programmed and click to select it. Once the correct assay is highlighted, click on the  transfer the assay to the right column and activate the assay. The assay may also be reorganized with the  &  buttons on the right-hand side of the open window.

Lot Specific Information

1. From the desktop, select the BCS XP application to launch the instrument software.
2. Once the software launches and initializes, select *Definitions* from the menu and then choose *Lot Info*.
3. Scroll through the list on the left to enter new product codes and value assignments when necessary. Lot assignment information can be found for each product on their respective assignment sheet which is included within each kit.



Reflex Testing for samples greater than 100 µg/mL

1. Exit the Siemens BCS XP Application to get to the desktop.
2. From the computer desktop open the *Auto Assistant Utility* and follow prompts to log in.
3. After the utility opens, select *Definitions* from the menu and then choose *New Instruction*. This will open the window shown in **Appendix D**.
4. Under *Name*, *Instruction*, and *Text* as shown in **Appendix D**.
Note: Refer to the assay numbers and names as previously programmed on the instrument.
5. After all information is entered click the button that reads check. If OK appears below the text area, then the instruction was entered correctly. If any part of the instruction is highlighted, fix the error per the corresponding message and check again.
6. Click the *OK* button to confirm entries.



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Appendix A: Reagent Definitions for Emicizumab Calibrator and Controls:

Emicizumab Calibrator:

Name:	Emicizumab Calibrator		
Abbreviation:	Emi Calibrator		
Version number:	1		
Manufacturer:	r2 Diagnostics		
Control ID:			
Wash when reagent is changed:	normal		
☐ Recalibrate when lot no. is changed			
☐ Control measurement with bottle change			
☐ Enzyme blank value measurement with bottle change			
Permitted lanes			
☒ within reach of the sample arm			
Stability			
☐ cooled ☒ uncooled ☐ irrelevant			
< 15°C ≥ 15°C 4 hours			
Stirring parameters			
Duration: 0 sec ☐ Continuous			
Interval: 0 min ☐ stirring			
Reference numbers			
Number: [*]			
Add Delete Replace			
Save			

* Enter 4-digit reference number assigned by the user and then click add.

Emicizumab Controls:

Name:	Emicizumab Control Level 1		
Abbreviation:	Emi Level 1		
Version number:	1		
Manufacturer:	r2 Diagnostics		
Control ID:			
Wash when reagent is changed:	normal		
☐ Recalibrate when lot no. is changed			
☐ Control measurement with bottle change			
☐ Enzyme blank value measurement with bottle change			
Permitted lanes			
☒ within reach of the sample arm			
Stability			
☐ cooled ☒ uncooled ☐ irrelevant			
< 15°C ≥ 15°C 8 hours			
Stirring parameters			
Duration: 0 sec ☐ Continuous			
Interval: 0 min ☐ stirring			
Reference numbers			
Number: [*]			
Add Delete Replace			
Save			

Name:	Emicizumab Control Level 2		
Abbreviation:	Emi Level 2		
Version number:	1		
Manufacturer:	r2 Diagnostics		
Control ID:			
Wash when reagent is changed:	normal		
☐ Recalibrate when lot no. is changed			
☐ Control measurement with bottle change			
☐ Enzyme blank value measurement with bottle change			
Permitted lanes			
☒ within reach of the sample arm			
Stability			
☐ cooled ☒ uncooled ☐ irrelevant			
< 15°C ≥ 15°C 8 hours			
Stirring parameters			
Duration: 0 sec ☐ Continuous			
Interval: 0 min ☐ stirring			
Reference numbers			
Number: [*]			
Add Delete Replace			
Save			



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Appendix B: Emicizumab Assay Procedure and Definition for measuring samples below 100 µg/mL:

Assay Procedure:

General Information	
Date of last change	
Name	Emicizumab
Number	[*]
Version number	1
Measure enzyme blank value every	0 hours
Finish measurement after	200 sec or 0 mA
Measurement speed	Normal
Mixing intensity	Normal
Wavelength	405 nm
Primary absorbance range	201 - 240 mA
n-replicate(s)	
Samples and controls	2 * 10 %
Calibration / EBV:	2 * 10 %
Raw value merging	Arithmetic mean
Dilution factor	1: 8
Dilution medium	NaCl
Evaluation and checking methods:	
Fixed Absorbance	
Lag phase:	7 sec
Threshold:	350 mA
Dynamic change of threshold (0=off, 1=on):	1
AMaxMin	
Lag phase:	7 sec
Limit for minimum absorbance:	5000 mA
Angle	
Lag phase:	7 sec
Threshold:	100 mA
Dynamic change of threshold (0=off, 1=on):	1
Window width:	3 sec
Minimum angle:	15 Degree
Drop	
Lab phase:	7 sec
Max permitted drop in absorbance:	10 %
New Wave	
Base threshold:	100 mA
Dynamic change of threshold (0=off, 1=on):	1
Lag phase:	7 sec
Threshold factor:	2
Number of steps:	10
Limit value:	20
Minimum amplitude:	100 mA
Pipetting	Save

* Enter 4-digit assay number assigned by the user.



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Pipetting cycles		Add new cycle		Delete last cycle	
Cycle	Transfer arm	Final washing	Rotor / Transfer action	Time window [sec]	
				min	max
1	sample arm	normal	Prioritize undiluted	0	0
2	sample arm	normal	Prioritize undiluted	0	0
3	reagent arm	normal	Incubation	175	185
4	reagent arm	normal	No mixing / Start measurement	0	0

Pipetting media		Add new row		Delete last row	
Cycle No.		Pipetting medium / Target	Reagent	Speed	Volumes [μL]
1	1	↑ Air		slow	20
	2	↑ Reagent	NaCl	slow	45
	3	↑ Sample		slow	5
	4	↓ Outer cuvette		slow	50
2	1	↑ Air		slow	20
	2	↑ Reagent	FVIII.DP	slow	50
	3	↓ Center cuvette		slow	50
3	1	↑ Air		slow	20
	2	↑ Reagent	Actin FSL	slow	50
	3	↓ Inner cuvette		slow	50
4	1	↑ Air		slow	20
	2	↑ Reagent	CaCl2	slow	50
	3	↓ Inner cuvette		slow	50



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Assay Definition:

General Identification			
Date of Last Change			
Assay Name	Emicizumab		
Assay Number	[*]	Host ID	
		Host result ID	
Version	1		
Screen display			
Short code in lists	Emicizumab		
Assay priority	0		
Internal result unit	µg/mL		
User-defined result unit and number format	µg/mL	100.0	
One internal unit is	1.0	User-defined units	
Host unit	µg/mL		
One internal unit is	1.0	Host units	
Result Factor	1.0		
Selection of assay procedures, evaluation and checking methods			
Emicizumab	Fixed Absorbance		
[+] [-]	Angle		
	New Wave		
	AMaxMin		
	Drop		
	[+] [-]		
Formula to calculate the raw value			
Arithmetic mean	Own Formula		
Unit of raw value			
	sec		
Result determination			
Reference range / Cut-off	☐ 0	to	☐ 0 µg/mL
☐ without reference curve			
Evaluation method	Raw Value = Result		
☐ Calculate result based on ref. curve of another assay			
Assay Selection			
☒ Calculate result based on own reference curve			
Calibration procedure	Regression	x	log y log
Minimum raw value	0.0		
Extrapolation limits:			
(0.01-1.0)*lowest concentration	1.0		
(1.0-2.0)*highest concentration	1.0		
Recording Curve			
☐ Calculate using master curve			
☒ Calculate using measured reference curve			
☐ Tube curve validation activated			
Permitted deviation from the tube curve			
No. of automatic repeats of invalid reference curve points			
☐ Calibration with calibrator set			
☒ Calibration with automatic dilution of a standard			
Calibrator			
Desired concentrations:			
Emicizumab Calibrator	100	µg/mL	
Emicizumab Calibrator	75	µg/mL	
Emicizumab Calibrator	50	µg/mL	
Emicizumab Calibrator	25	µg/mL	
Emicizumab Calibrator	10	µg/mL	
Add calibrator			
Remove calibrator			
Control Assignment	Save		

* Enter 4-digit assay number assigned by the user.



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Appendix C: Emicizumab Assay Procedure and Definition for measuring samples **above 100 µg/mL**:

Assay Procedure:

General Information			
Date of last change			
Name	Emicizumab.High		
Number	[*]		
Version number	1		
Measure enzyme blank value every	0	hours	
Finish measurement after	200	sec or	0 mA
Measurement speed	Normal		
Mixing intensity	Normal		
Wavelength	405 nm		
Primary absorbance range	201 - 240 mA		
n-replicate(s)			
Samples and controls	2	*	10 %
Calibration / EBV:	2	*	10 %
Raw value merging	Arithmetic mean		
Dilution factor	1:	20	
Dilution medium	NaCl		
Evaluation and checking methods:			
Fixed Absorbance			
	Lag phase:	7	sec
	Threshold:	350	mA
	Dynamic change of threshold (0=off, 1=on):	1	
AMaxMin			
	Lag phase:	7	sec
	Limit for minimum absorbance:	5000	mA
Angle			
	Lag phase:	7	sec
	Threshold:	100	mA
	Dynamic change of threshold (0=off, 1=on):	1	
	Window width:	3	sec
	Minimum angle:	15	Degree
Drop			
	Lab phase:	7	sec
	Max permitted drop in absorbance:	10	%
New Wave			
	Base threshold:	100	mA
	Dynamic change of threshold (0=off, 1=on):	1	
	Lag phase:	7	sec
	Threshold factor:	2	
	Number of steps:	10	
	Limit value:	20	
	Minimum amplitude:	100	mA
		Pipetting	Save

* Enter 4-digit assay number assigned by the user.



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Pipetting cycles		Add new cycle		Delete last cycle	
Cycle	Transfer arm	Final washing	Rotor / Transfer action	Time window [sec]	
				min	max
1	sample arm	normal	Prioritize undiluted	0	0
2	sample arm	normal	Prioritize undiluted	0	0
3	reagent arm	normal	Incubation	175	185
4	reagent arm	normal	No mixing / Start measurement	0	0

Pipetting media		Add new row		Delete last row	
Cycle No.		Pipetting medium / Target	Reagent	Speed	Volumes [μL]
1	1	↑ Air		slow	20
	2	↑ Reagent	NaCl	slow	45
	3	↑ Sample		slow	5
	4	↓ Outer cuvette		slow	50
2	1	↑ Air		slow	20
	2	↑ Reagent	FVIII.DP	slow	50
	3	↓ Center cuvette		slow	50
3	1	↑ Air		slow	20
	2	↑ Reagent	Actin FSL	slow	50
	3	↓ Inner cuvette		slow	50
4	1	↑ Air		slow	20
	2	↑ Reagent	CaCl2	slow	50
	3	↓ Inner cuvette		slow	50



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Assay Definition:

General Identification			
Date of Last Change			
Assay Name		Emicizumab.High	
Assay Number		[*]	Host ID
Version		1	Host result ID
Screen display			
Short code in lists		Emicizumab.High	
Assay priority		0	
Internal result unit		µg/mL	
User-defined result unit and number format		µg/mL	100.0
One internal unit is		1.0	User-defined units
Host unit		µg/mL	
One internal unit is		1.0	Host units
Result Factor		2.5	
Selection of assay procedures, evaluation and checking methods			
Emicizumab		Fixed Absorbance	
[+] [-]		Angle	
		New Wave	
		AMaxMin	
		Drop	
		[+] [-]	
Formula to calculate the raw value			
Arithmetic mean		Own Formula	
Unit of raw value			
		sec	
Result determination			
Reference range / Cut-off		☐ 0	to ☐ 0 µg/mL
☐ without reference curve			
Evaluation method		Raw Value = Result	
☒ Calculate result based on ref. curve of another assay			
Assay Selection		Emicizumab	
☐ Calculate result based on own reference curve			
Calibration procedure		Regression	x log y log
Minimum raw value		0.0	
Extrapolation limits:			
(0.01-1.0)*lowest concentration		1.0	
(1.0-2.0)*highest concentration		1.0	
Recording Curve			
☐ Calculate using master curve			
☐ Calculate using measured reference curve			
☐ Tube curve validation activated			
Permitted deviation from the tube curve			
No. of automatic repeats of invalid reference curve points		0	
☐ Calibration with calibrator set			
☐ Calibration with automatic dilution of a standard			
Calibrator		Desired concentrations:	
Control Assignment		Add calibrator	Remove calibrator
		Save	

*Enter 4-digit assay number assigned by the user.



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Appendix D: Auto Assistant instruction for reflex testing of Emicizumab samples **greater than 100 µg/mL:**

New Instruction		X
Name	Instruction Set	
<Emicizumab/Emicizumab.High	Emicizumab	
if result for * {Emicizumab} is "over measurement range" then measure ** {Emicizumab.High}		
OK	Check	
☒ Active		
Help	Cancel	OK

* Enter user selected assay number as used in the assay definition for the Emicizumab Assay.

**Enter user selected assay number as used in the assay definition for the Emicizumab.High Assay.