Application Sheet for Fibrinogen with HEMOSTAT Fibrinogen

HumaClot Junior (model HC1) REF **18680**
HumaClot Duo Plus (model HC2) REF **15650**
HumaClot Quattro REF **15660**

The parameters defined in this application sheet have been developed to provide optimal product performance with the assay and instrument combination. Any modification to these parameters may affect the performance of this and other assays in use on your system and the resulting assay values. It is the responsibility of the user to validate any modifications and their impact on all assay results. The application sheet lists all combinations of controls and calibrators for use with the reagent and instrument system; other combinations are not validated or supported.

For additional information, please refer to the respective User Manual of the instrument and check current instructions for use (IFU) for reagents, controls, calibrators and tables of assigned/analytical values.

Typical performance data can be found in the Verification Report of the respective instrument, accessible via

www.human.de/data/gb/vr/18680.pdf
www.human.de/data/gb/vr/15650.pdf
www.human.de/data/gb/vr/15660.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Material Required

Material	REF	Size	On-Board Position
HEMOSTAT Fibrinogen	32002		
RGT **Fibrinogen reagent**		5 x 2 ml	Beside the analyzer
BUF **Imidazole buffered saline**		1 x 100 ml	Beside the analyzer for mandatory sample dilution
CAL **Fibrinogen reference plasma**		2 x 1 ml	-
CPN **HEMOSTAT Control Plasma Normal**	35001	6 x 1 ml	-
CPA **HEMOSTAT Control Plasma Abnormal**	35002	6 x 1 ml	
Cuvettes with prefilled mixers	15660/10	5 x 100 pcs	Pre-heated cuvette positions
Cuvette bag with separate mixer	15660/11	500 pcs	
Cuvette bag with separate mixer	15660/12	5 x 500 pcs	
Reagent container*	15800/40	50 x 5 ml	Beside the analyzer

Transfer the required BUF volume, used for pre-dilution of samples /controls/calibrator, from the original BUF bottle into secondary small bottles (REF 15800/40) and place the original BUF bottle back to the fridge ensuring BUF stability. Discard remaining BUF at the end of the day.

Pipetting Scheme

Sample Pre-dilution (1:20)	
Sample, control	10 µl
BUF (Imidazole buffered saline)	190 µl
Pipetting Scheme	
Prewarm RGT at room temperature and cuvettes at 37°C	
1. Pre-diluted sample	100 µl
Transfer cuvette with prediluted sample to a measuring channel	
Incubation time	180 s
2. Start reagent RGT Fibrinogen reagent	**50 µl**
Auto start	yes

Standard Curve Calibration

A new standard curve needs to be established when

- changing to a new HEMOSTAT Fibrinogen LOT
- after major maintenance or service
- if indicated by quality control results
- when required by laboratory control procedures and/or governmental regulations.

The following steps have to be followed:
Reconstitution of the kit calibrator with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).

a) Refer to the LOT-specific table of Fibrinogen reference plasma CAL for the analytical values in g/l or mg/dl, which can be found inside the Fibrinogen reagent kit (REF 32002).

b) Prepare dilution levels of the Fibrinogen reference plasma CAL with Imidazole buffered saline BUF according to the following table:

Example with a Fibrinogen reference plasma CAL of 2.57 g/l:

Please note: Cal 3 is diluted 1:20 – equivalent to pre-dilution of samples					
Preparation of Dilutions					
	Dilution	**Factor**	**Fib [g/l] ***	**BUF [µl]**	**CAL [µl]**
Cal 1	1:10	2	5.14	**540**	**60**
Cal 2	1:15	1.33	3.42	**560**	**40**
Cal 3	1:20	1	2.57	**570**	**30**
Cal 4	1:30	0.67	1.72	**580**	**20**
Cal 5	1:40	0.5	1.29	**585**	**15**

** The LOT-specific calibration values can be found on the table of analytical values of the HEMOSTAT Fibrinogen kit.*

c) Run the prepared calibrator levels in duplicates and write down or print the respective clotting time results [s]. Calculate the mean value [s] of each duplicate.
Please note: Ignore values for [g/l], as those are derived from a previous calibration.

d) Insert the calculated mean values into the instrument.
To do so, chose the test ***Fib. g/l*** by pressing the enter key  (the message "cuv(ette) in" appears).
Press the  -key, enter the first data point [g/l] from a) and press  .
Enter result [s] from c) and press  .
Repeat this process until all calibration points are inserted.
Please note, the 6th calibration point must be 0.

e) Add LOT number of HEMOSTAT Fibrinogen reagent and press  repeatedly to save new parameters.

On-Board Stability

Material	Time [h]
RGT **Fibrinogen reagent at room temperature**	72
BUF **Imidazole buffered saline at room temperature**	48

The stated stability data were established under controlled laboratory conditions. The above mentioned on- board stability values may deviate due to differences in laboratory environmental conditions.

Test Settings

Test Protocol_Printed automatically with every change / new start	
(Reduced Setup, User) <3> + Enter-Key = CuvIN or Pat-ID + 0-key	
Method Store	3
Fib. g/l	
Date	Will be displayed
Measuring Time	101 s
Gain_idx	0
Cuv in	ON
Reag_sens	OFF
Start Reagent	
LOT	**Please insert LOT number**
Volume	50 µl
incubation	180 s
Clotting	ON
Kin/ Dif	OFF
Calibration	
1st conversion	INTERPOLAT.
1. point: **Insert LOT-specific calibration value Cal 1 [g/l]**	**Insert mean result of calibration for Cal 1 [s]**
2. point: **Insert LOT-specific calibration value Cal 2 [g/l]**	**Insert mean result of calibration for Cal 2 [s]**
3. point: **Insert LOT-specific calibration value Cal 3 [g/l]**	**Insert mean result of calibration for Cal 3 [s]**
4. point: **Insert LOT-specific calibration value Cal 4 [g/l]**	**Insert mean result of calibration for Cal 4 [s]**
5. point: **Insert LOT-specific calibration value Cal 5 [g/l]**	**Insert mean result of calibration for Cal 5 [s]**
6. point**: Leave empty:** 0.00 [g/l]	**Leave empty:** 0.0 [s]
2nd conversion	NONE

Interference Studies

No interference up to ...					
Bilirubin	mg/dl	50	spiked normal plasma	50	spiked pathological plasma
Hemoglobin	mg/dl	1000	spiked normal plasma	1000	spiked pathological plasma
Lipids	mg/dl	1000	spiked normal plasma	570	spiked pathological plasma

Performance Characteristics

Measuring interval			
Valid Clotting	5 - 100 s	**Analytical measuring interval (displayed)**	0.7 g/l to 10.0 g/l

If results for the 1:20 dilution fall outside the analytical measuring interval and are displayed as < 0.70 g/l, prepare a lower 1:10 dilution and multiply the result with the dilution factor 0.5. If results are displayed as > 10.00 g/l, prepare a higher dilution 1:40 and multiply the result with the dilution factor 2.

Reference Interval

The following data was obtained with a specific HEMOSTAT Fibrinogen LOT using normal plasma according to EP28-A3.

HumaClot Quattro	Median	95% Reference interval	
		2.5th Percentile	97.5th Percentile
167 samples	3.43 g/l	2.16 g/l	4.70 g/l

Please note: The reference intervals vary from laboratory to laboratory depending on the population served, technique and reagent LOT used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the mentioned variables are changed.

For more information how to establish reference intervals see CLSI document C28-A3.

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