Application Sheet for D-DIMER with Hemostat D-DIMER (REF 36002)

HumaCLOT Junior (model HC1) REF 18680
HumaCLOT Duo Plus (model HC2) REF 15650
HumaCLOT Quattro (model HC4) REF 15660

For additional information, please refer to the respective User Manual of the instrument and check current instructions for use for reagents, controls, calibrators and tables of assigned/analytical values. Typical performance data can be found in the Verification Report of the instrument, accessible via

www.human.de/data/gb/vr/18680.pdf
www.human-de.com/data/gb/vr/18680.pdf

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

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 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.

Material Required

Material	REF	Size	On-Board Position
Hemostat D-DIMER	36002		
RGT **D-Dimer Latex reagent**		1ml	heated reagent position with reducer ring (HC Duo Plus, Quattro)
BUF **Reaction buffer**		2,5 ml	-
CAL **Calibrator**		1 ml	-
DIL **Diluent**		6 ml	-
HIGH **Hemostat D-DIMER Control High**	36012	2 x 1 ml	-
LOW **Hemostat D-DIMER Control Low**	36012	2 x 1 ml	
Cuvettes with prefilled mixers	15660/10	5 x 100 pcs	-
Cuvette bag with separate mixer	15660/11	500 pcs	
Cuvette bag with separate mixer	15660/12	5 x 500 pcs	
Reducer Ring	15660/52	2pcs	Standard accessory HumaCLOT Duo Plus/ Quattro

Additional Notes

If reagents, rinse solutions or buffers are not supplied in exactly fitting vials it is necessary to transfer them into appropriate vials. The required controls have to be transferred into appropriate sample cups.

On-Board Stability

Material	Time [h]
RGT **D-Dimer Latex Reagent at 37°C**	4h
BUF **Reaction buffer at RT**	72
DIL **Diluent**	8
CAL **Calibrator**	8
Hemostat D-DIMER Control High	8
Hemostat D-DIMER Control Low	8

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

Measuring Range

Measuring Range	
Valid Range	150 ng/ml to 99999 ng/ml DDU

Reference Interval
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.

The total measuring range with Hemostat D-DIMER covers 150 ng/ml DDU to 99999 ng/ml DDU. This total measuring range can be obtained through manual sample dilution. A dilution is neccessary when the maximum value of > 2600 ng/ml DDU) is exceeded.

Example 1: If the correct sample result of an undiluted patient sample is at e.g. 3600 ng/mL DDU , then the result is shown as “>2600 ng/ml”. The sample needs to be re-run after manual 1:6 dilution. The obtained value needs to be multiplied by 6 to obtain the correct value.

Example 2: If the correct sample result of an undiluted patient sample is at e.g. approx. 17000 ng/mL, then the result is shown as “>2600 ng/ml” or “Err lin”. After 1:6 dilution of the sample, the result, again, will be displayed as “>2600 ng/ml” or “Err lin”. The 1:6 diluted sample, subsequently, needs to be diluted 1:8 in order to obtain a value that lies within the calibration curve. This value needs to be multiplied by 48.

HEMOSTAT D-DIMER DILUENT needs to be used for all manual sample dilutions.

Please note: also for samples with very low D-dimer values the “Err lin” can appear. In this case please follow instructions below.

Note: Sample Results below Cut-Off
Samples with results well below the cut-off (200 ng/mL), may be notified as „Err lin“. In this case, the negative D-Dimer result must be verified, as follows: • Re-measurement of the sample • 1:6 dilution to exclude samples >2600ng/ml • The 1:6 diluted sample, subsequently, needs to be diluted 1:8 again in order to exclude very high D-DIMER samples • The low value D-dimer is verified if the diluted sample still causes the “Err lin”. Then very high-D-DIMER result is excluded

Pipetting Scheme	
Pre-warm RGT D-Dimer Latex reagent and cuvettes at 37°C	
Sample	50 µl
Incubation time	0 sec
Reaction Buffer BUF	80 µl
Transfer cuvette with sample and BUF into the measuring channel	
Incubation time	150 sec
D-Dimer latex reagent RGT	40 µl
Autostart	yes

Reagent Settings

Test Protocol	
(Full Setup, User) <D-Dimer 405>+Enter-Key=CuvIN or Pat-ID + 0-Key	
Method store	7
'D-Dimer 405'	
Date	Will be displayed
Meas.time	**150** s
Gain_idx	0
Cuv in	ON
Reag_sens	OFF
Start Reagent	
LOT	**Please insert LOT**
Volume	40 µl
incu	150 s
clotting	OFF
Kin/Dif	ON
1st convers	INTERPOLAT.
220 ng/ml	Insert mE/min
400 ng/ml	Insert mE/min
800 ng/ml	Insert mE/min
1600 ng/ml	Insert mE/min
2600 ng/ml	Insert mE/min

Standard Curve Calibration

A new standard curve must be established when changing to a new D-Dimer kit LOT, after major maintenance or service, if indicated by quality control results and when required by laboratory control procedures and/or governmental regulations.

Calibration Settings

Calibration Steps	
	ng/ml D-Dimer
Cal 1	2600
Cal 2	1600
Cal 3	800
Cal 4	400
Cal 5	220

The following steps have to be followed:

a) Reconstitution of the calibrator with 1ml of distilled or deionized water without preservatives.
b) For D-Dimer a 5-point calibration with fixed calibrator points needs to be performed: 2600 ng/ml, 1600 ng/ml, 800 ng/ml, 400 ng/ml, 220 ng/ml. Because the original concentration of the calibrator CAL is always higher than 2600 ng/ml, the calibrator needs to be diluted with DIL. The D-Dimer concentration of the calibrator is LOT-specific, as printed on the vial label. Thus the dilution needs to be adapted accordingly to prepare the right concentration for each calibrator point.
c) The respective dilution levels for Cal 1, Cal 2, Cal 3, Cal 4 and Cal 5 can be calculated by the user with the help of the formula below or a pre-filled excel-calculator on www.human.de/aps-coag can be used
d) Prepare the dilution levels with the calculated volumes of calibrator and diluent (see example in the table below).
e) Run the prepared dilution levels in duplicates and calculate the mean value of the duplicate results. Insert the calculated mean values into the instrument. To do so, choose the test *D-Dimer* by pressing  (the prompt "cuv(ette) in" appears). Press the  -key, enter the first data point starting with the lowest calibrator level (Cal5) and press  . Repeat this process until all calibration points are inserted.

The following formula can be applied for preparing the dilution levels 1-5:

$$\text{Volume ([CAL] Calibrator µl)} = \frac{Target\ concentration\ calibration\ point}{Lot\ specific\ concenctration\ calibrator}\ x\ 300\ \mu l$$

$$\text{Volume ([DIL]Diluent µl)} = 300\ \mu l - \text{Volume ([CAL]Calibrator µl)}$$

Performance Characteristics

Method comparison			
Test Device	**Predicate Device**	**Regression Equation**	**r**
Hemostat DD / Junior	Hemostat DD /HC Pro	y=0.8709x-8.5427	0.9963
Hemostat DD / Duo Plus		y=0.8933x-11.315	0.9969
Hemostat DD / Quattro		y=0.8888x-4.7192	0.9961

Precision				
		Within Run CV (%)	**Run to Run CV (%)**	**Total CV (%)**
HumaCLOT Junior				
BioRad Lyphocheck Coagulation Control	Level 1	Max: 7.82	1.65	5.87
Hemostat D-Dimer Control	Low	Max: 4.01	0.85	3.37
	High	Max: 1.84	0.45	1.68
HumaCLOT Duo Plus				
BioRad Lyphocheck Coagulation Control	Level 1	Max: 6.78	1.35	6.94
Hemostat D-Dimer Control	Low	Max: 3.07	0.44	2.59
	High	Max: 2.44	0.34	2.03
HumaCLOT Quattro				
BioRad Lyphocheck Coagulation Control	Level 1	Max: 7.77	1.44	6.46
Hemostat D-Dimer Control	Low	Max: 3.77	0.71	8.15
	High	Max: 4.44	1.24	3.05

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