Application Sheet for D-dimer with HEMOSTAT D-Dimer

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 D-Dimer	36002		
RGT **D-Dimer latex reagent**		2 x 1 ml	Heated reagent position (with reducer ring on HumaClot Duo Plus / Quattro)
BUF **Reaction buffer**		2 x 2.5 ml	Beside the analyzer
CAL **Calibrator**		1 x 1 ml	-
DIL **Diluent**		1 x 6 ml	Beside the analyzer for calibration and potential sample dilution
HIGH **HEMOSTAT D-Dimer Control High**	36012	2 x 1 ml	-
LOW **HEMOSTAT D-Dimer Control Low**		2 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	
Reducer Ring	15660/52	2 pcs	(Standard accessory HumaClot Duo Plus / Quattro)

Pipetting Scheme

Pipetting Scheme	
Pre-warm RGT *D-Dimer latex reagent and cuvettes at 37°C*	
1. Sample	50 µl
Incubation time	0 sec
2. BUF Reaction buffer	80 µl
Transfer cuvette with sample and BUF *into the measuring channel*	
Incubation time	150 sec
3. Start reagent RGT D-Dimer latex reagent	**40 µl**
Auto start	yes

Standard Curve Calibration

A new standard curve needs to be established when

- changing to a new HEMOSTAT D-Dimer 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 HEMOSTAT D-Dimer's CAL Calibrator with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).
performed.

a) For HEMOSTAT D-Dimer a 5-point calibration with fixed calibrator points needs to be performed:
D-dimer values 2600 ng/ml, 1600 ng/ml, 800 ng/ml, 400 ng/ml, 220 ng/ml.
Since the original lot-specific concentration of the CAL Calibrator (printed on the vial label) is always higher than 2600 ng/ml, the calibrator needs to be diluted with HEMOSTAT D-Dimer's DIL Diluent. Thus, the dilution needs to be adapted accordingly to prepare the right concentration for each calibrator point.
Please note: 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 with a pre-filled excel-calculator on www.human.de/aps-coag.

b) Prepare the dilution levels with the calculated volumes of calibrator and diluent (see example in the table below).

c) Measure the prepared calibrator levels in duplicates and write down or print the respective kinetics [mE/min] . Calculate the mean value [mE/min] of the duplicate results.
Please note: Ignore values for [ng/ml], as those are derived from a previous calibration.

d) Insert the calculated mean values into the instrument by the following steps:
Choose the test ***D-Dimer 405*** by pressing the enter key  (the message "cuv(ette) in" appears).
Press the  -key, enter the first data point from c) [mE/min] starting with the lowest calibrator level (Cal 5) and press  .
Repeat this process until all calibration points are inserted and add LOT number of HEMOSTAT D-Dimer.

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

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

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

Example with a CAL D-Dimer calibrator of 3207 ng/ml DDU:

Preparation of Dilutions					
	Cal 1	**Cal 2**	**Cal 3**	**Cal 4**	**Cal 5**
[ng/ml] DDU	2600	1600	800	400	220
Volume CAL [µl]	243.2	149.7	74.8	37.4	20.6
Volume DIL [µl]	56.8	150.3	225.2	262.6	279.4

* *The LOT-specific calibration value can be found on the vial of HEMOSTAT D-Dimer CAL Calibrator.*

On-Board Stability

Material	Time [h]
RGT **D-Dimer latex reagent at 37°C**	4
BUF **Reaction buffer**	72
DIL **Diluent**	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.

Test Settings

Test Protocol Printed automatically with every change / new start	
(Reduced Setup, User) <7> +Enter-Key=CuvIN or Pat-ID + 0-Key	
Method store	7
D-Dimer 405	
Date	Will be displayed
Meas.time	151 s
Gain_idx	0
Cuv in	ON
Reag_sens	OFF
Start Reagent	
LOT	**Please insert LOT number**
Volume	40 µl
Incubation	150 s
Clotting	OFF
Kin/Dif	ON
Calibration	
3rd conversion	INTERPOLATION
1. point: 220 ng/ml	**Insert mean from measurement of Cal 5 [mE/mn]**
2. point: 400 ng/ml	**Insert mean from measurement of Cal 4 [mE/mn]**
3. point: 800 ng/ml	**Insert mean from measurement of Cal 3 [mE/mn]**
4. point: 1 600 ng/ml	**Insert mean from measurement of Cal 2 [mE/mn]**
5. point: 2 600 ng/ml	**Insert mean from measurement of Cal 1 [mE/mn]**
no more points	Max value reached

Interference Studies

No interference up to ...					
Bilirubin	mg/dl	5.2	spiked low positive plasma	12.1	spiked high positive plasma
Hemoglobin	mg/dl	250	spiked low positive plasma	250	spiked high positive plasma
Lipids	mg/dl	41.5	spiked low positive plasma	32.8	spiked high positive plasma

Interfering substances like hemoglobin, bilirubin and lipids (HIL) can influence the test result. It is recommended to centrifuge lipemic patient samples at 15 000 x g for 10 minutes, prior to analysis. Use the lipid-free lower phase.

Also, the auto start function might be impaired due to elevated levels of HIL and it is recommended to collect new blood samples from the patient. If this is not applicable or the auto start function still is not initiating, it is possible to start the measurement manually by pressing the respective channel button. *Please note: a manual start may lead to reduced D-dimer levels. Therefore, each result of a HIL-sample should be reported with restrictions and marked with notes.*

Performance Characteristics

Measuring interval	
Analytical measuring interval (displayed)	150 ng/ml to 2 600 ng/ml DDU
Reportable interval	150 ng/ml to 100 000 ng/ml DDU

The Analytical measuring interval, which is displayed on the instrument, is 150 ng/ml to 2600 ng/ml DDU. For sample results displaying "> 2600 ng/ml" DDU, a manual dilution of the sample with HEMOSTAT D-Dimer DIL Diluent needs to be done and re-measured. To obtain the true result of the diluted sample, the displayed result needs to be multiplied by the dilution factor.

Samples with values below 150 ng/ml are displayed as <150 ng/ml.

Note: Sample Results = "ERR lin"

For samples with very low or very high D-dimer values the "ERR lin" can appear or when interfering substances like lipids, bilirubin and hemoglobin are present. In this case, please follow instructions below.

- **Re-measurement of the sample**
  1a) If result is "< 150 ng/ml" or within the analytical measuring range, this result can be reported.
  1b) If result is again "ERR lin"
  - **Perform a 1:6 dilution**
    2a) If result is "<150 ng/ml"
    - Repeat measurement with new sample without interfering substances.

    2b) If result is within analytical measuring interval
    - Multiply result by 6 to report the true value

    2c) If result is "> 2600 ng/ml" or "ERR lin" again
    - The diluted sample needs to be **diluted 1:8** again to exclude very high D-dimer levels.

      3a) If result is within analytical measuring interval
      - Multiply the result by 48 to report the true value.

      3b) If result is "<150 ng/ml" or „ERR lin" again
      - Repeat measurement with new sample without interfering substances.

Example 1: If the true D-dimer concentration of an undiluted patient sample is at 3 600 ng/ml DDU, the result is shown as ">2600 ng/ml". The sample needs to be diluted 1:6 and re-measured. The displayed result needs to be multiplied by 6 to obtain the true D-dimer result of this diluted sample.

Example 2: If the correct sample result of an undiluted patient sample is at approx. 17 000 ng/ml, 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 is within the analytical measuring interval. The displayed result needs to be multiplied by 48 to obtain the true D-dimer result of this diluted sample.

Example 3: If the correct sample result of an undiluted patient sample is at approx. 220 ng/ml, the result might be shown as "ERR lin". After re-measurement, in rare cases the result could be "ERR lin" again. Consequently, a 1:6 dilution of the sample is mandatory. If the result, again, will be displayed as "ERR lin", the 1:6 diluted sample, subsequently, needs to be diluted 1:8. If the result, will be displayed as "ERR lin" or "<150 ng/ml", a very high D-dimer level is excluded and it is recommended to repeat measurement with new sample without interfering substances.

Reference Interval

Normal D-dimer level	
Adults	< 200 ng/ml DDU (equivalent to 500 ng/ml FEU)

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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