Application Sheet for Manual Testing of HEMOSTAT Reagents

The parameters defined in this application sheet have been developed to provide optimal product performance with manual testing or at least on a suitable manual coagulation instrument. It is in the responsibility of the user to validate all assay results. A quality control routine should be established according to governmental regulations or laboratory standards. Controls should be run in conjunction with patient samples to ensure proper function of reagents.

The parameters defined in this application sheet can be applied to the following manual HumaClot instruments:

HumaClot Junior	HumaClot DuoPlus
	

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

1 Manual Testing HEMOSTAT Thromboplastin

Material Required

Material	REF	Size
HEMOSTAT Thromboplastin-SI	31002	
RGT Thromboplastin reagent		6 x 2 ml
BUF Reconstitution medium	or	6 x 2 ml
HEMOSTAT Thromboplastin-SI	31003	
RGT Thromboplastin reagent		6 x 10 ml
BUF Reconstitution medium	or	6 x 10 ml
HEMOSTAT Thromboplastin^{liquid}	31012	
RGT Thromboplastin reagent		6 x 2 ml
CAL HEMOSTAT Calibrator (optional)	35500	4 x 1 ml
0.9% Sodium chloride	-	
CPN HEMOSTAT Control Plasma Normal	35001	6 x 1 ml
CPA HEMOSTAT Control Plasma Abnormal	35002	6 x 1 ml

Pipetting Scheme

Pipetting Scheme*	
Pre-warm RGT Thromboplastin reagent and cuvettes or clear reaction tubes at 37° C	
1. Sample or Control Plasma	100 µl*
Transfer cuvette with sample to a measuring position and activate optics	
Incubation time	1 - 3 min at 37° C
2. Start reagent RGT Thromboplastin reagent	**200 µl***
Start timer upon addition of RGT Thromboplastin reagent. Record time [s] required for clot formation	

*depending on the used cuvettes or reaction tube, a proportional variation can be applied

Results
The results can be reported in seconds, in Prothrombin ratio, in % of activity or as INR.

Seconds [s]:
Calculate the mean time of duplicate PT determination for each plasma and report to the nearest 0.1 second.

Prothrombin Ratio $PR = \frac{PT_{Patient\,[s]}}{MNPT\,[s]}$

To obtain the Prothrombin ratio, the clotting time of the sample is divided by the geometric mean of the prothrombin time from at least 20 normal patients (MNPT).

Please note: The MNPT value can be derived from the calibration with the HEMOSTAT Calibrator.
The HEMOSTAT CALIBRATOR can be used as a normal plasma pool to represent the MNPT (please refer to the table "Examples of Reconstitution" below). Alternatively, a normal plasma pool of apparently "healthy" donors can be prepared.

International Normalized Ratio $INR = PR^{ISI}$

The interdependency of the Prothrombin time of the used Thromboplastin and instrument can be corrected through the determination of the INR. For this purpose, the Prothrombin ratio can be converted into internationally comparable values by means of the "International Sensitivity Index (ISI)". The LOT- and instrument-specific ISI and MNPT can be obtained using an commercially available INR-Calibrator (Refer to following sheet: *PT ISI and MNPT determination*) or the international reference plasma.

[%] of activity
To report results in % of activity a calibration curve is required. Generate a calibration curve using HEMOSTAT CALIBRATOR (please refer to the table "Examples of Reconstitution" below). Alternatively, the calibration curve can be generated with a normal plasma pool prepared from apparently "healthy" donors, declaring the Prothrombin time as 100%.

Standard Curve Calibration

A new standard curve needs to be established when

- changing to a new HEMOSTAT Thromboplastin LOT
- if indicated by quality control results
- when required by laboratory control procedures and/or governmental regulations.

The following steps must be followed:

a) Reconstitution of the HEMOSTAT Calibrator (REF 35500) with variable amounts of distilled water, depending on the analytical value (in the table of analytical values of the calibrator kit) of the HEMOSTAT Calibrator CAL (REF 35500). Keep it at room temperature (18-25 °C) for 30 minutes. Swirl the vial gently before use and do not shake. Avoid the contact of fluid with the stopper.

Examples for reconstitution of calibrator for Prothrombin % (PT [%]):

Examples of analytical values of CAL as indicated in the Analytical Value Sheet	Required reconstitution volume (distilled or deionized water)
102 %	1020 µl
100 %	1000 µl
97 %	970 µl
95 %	950 µl

b) Prepare a serial dilution of the HEMOSTAT Calibrator CAL (Cal 1) using 0.9 % Sodium chloride to obtain the calibrator levels Cal 2, Cal 3 and Cal 4.

Pipetting Scheme of HEMOSTAT Calibrator CAL with 0.9 % Sodium chloride:

Preparation of Calibrator Dilutions				
	Cal 1	**Cal 2**	**Cal 3**	**Cal 4**
% PT* (% Quick)	Cal 1 [%]	Cal 1 [%] / 2	Cal 1 [%] / 4	Cal 1 [%] / 8
[%] PT	**100.0 %**	**50.0 %**	**25.0 %**	**12.5 %**
HEMOSTAT Calibrator	400 µl	400 µl of Cal 1	400 µl of Cal 2	400 µl of Cal 3
0.9 % NaCl	**0 µl**	400 µl	400 µl	400 µl

c) Measure the prepared calibrator levels including Cal 1 in duplicates and write down or print the respective clotting time results [s]. Calculate the mean value [s] of each duplicate and round to the nearest 0.1 s.

d) Insert the calculated mean values into the instrument like HumaClot Junior or HumaClot Duo^{Plus} (old models) according to the instrument's User Manual or document results on log-log-graph-paper (Appendix I).

e) To obtain INR values it is necessary to determine a LOT and instrument specific ISI. This can be done using a commercially available INR-Calibrator (e.g. Siemens PT-Multi Calibrator, Technoclone AK Calibrant: Refer to excel sheet: *PT_ISI and MNPT determination.xlsx*) or the international reference plasma.

2 Manual Testing HEMOSTAT Fibrinogen

Material Required

Material	REF	Size
HEMOSTAT Fibrinogen	32002	
RGT Fibrinogen reagent		5 x 2 ml
BUF Imidazole buffered saline		100 ml
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

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 or clear reaction tubes at 37° C	
1. Pre-diluted sample	100 µl*
Transfer cuvette with prediluted sample to a measuring position and activate optics	
Incubation time	3 min
2. Start reagent RGT Fibrinogen reagent	**50 µl***
Start timer upon addition of RGT Fibrinogen reagent. Record time [s] required for clot formation	

*depending on the used cuvettes or reaction tube, a proportional variation can be applied

Results

The measurements should be conducted in duplicates and their mean value in [s] must be converted in [g/l] or [mg/dl] by comparing the results of patient with the calibration curve.

Standard Curve Calibration

A new standard curve needs to be established when

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

The following steps must be followed:

a) Reconstitution of the kit calibrator with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).
b) 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).
c) 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:

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.*

*** Cal 3 is diluted 1:20 – equivalent to pre-dilution of samples*

d) *Measure 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 and round to the nearest 0.1 [s].*
e) *Insert the calculated mean values into the instrument like HumaClot Junior or HumaClot Duo^{Plus} (old model) according* to the instrument's User Manual or document results on log-log-graph-paper (Appendix I).

Please note: If results for the default 1:20 dilution fall outside the measuring interval and are displayed/calculated as < 1.0 g/l, prepare a 1:10 dilution and multiply the result by 0.5. If results are displayed or calculated as > 5.00 g/l, prepare a higher dilution 1:40 and multiply the result with the dilution factor 2.

Please note: If clotting times for the default 1:20 dilution seem too long, a default 1:10 dilution might be useful. Lower dilutions for the calibration curve should be prepared accordingly and the change of the dilution factors should be kept in mind.

3 Manual Testing HEMOSTAT Thrombin Time

For the measurement of HEMOSTAT Thrombin Time at least a manual coagulation instrument is required.

Material Required

Material	REF	Size
HEMOSTAT Thrombin Time		
RGT **Thrombin reagent**	34002	3 x 3 ml
CPN **HEMOSTAT Control Plasma Normal**	35001	6 x 1 ml

Pipetting Scheme

Pipetting Scheme*	
Pre-warm cuvettes or clear reaction tubes at 37 °C	
1. Sample or Control Plasma	75 µl*
Transfer cuvette with sample to a measuring position and activate optics	
Incubation time	3 min at 37 °C
2. Start reagent RGT Thrombin reagent	**75 µl***
Start timer upon addition of RGT Thrombin reagent. Record time [s] required for clot formation	

*depending on the used cuvettes or reaction tube, a proportional variation can be applied

4 Manual Testing HEMOSTAT aPTT-EL

Material Required

Material	REF	Size
HEMOSTAT aPTT-EL	33002	6 + 6 x 4 ml
RGT1 aPTT-EL		6 x 4 ml
RGT2 $CaCl_2$		6 x 4 ml
HEMOSTAT aPTT-EL	or	
RGT1 aPTT-EL	33012	6 x 4 ml
+ RGT2 $CaCl_2$	33022	4 x 30 ml
HEMOSTAT aPTT-EL	or	
RGT1 aPTT-EL	33013	6 x 10 ml
+ RGT2 $CaCl_2$	33022	4 x 30 ml
CPN HEMOSTAT Control Plasma Normal	35001	6 x 1 ml
CPA HEMOSTAT Control Plasma Abnormal	35002	6 x 1 ml
Reagent container	15800/40	50 x 5 ml

Additional Notes

If REF 33022 is used as RGT2 it is necessary to transfer the required volume into appropriate vials for example REF 15800/40 or cuvettes to prewarm the reagent. Discard remaining RGT2 at the end of the day.

Pipetting Scheme

Pipetting Scheme*	
Pre-warm RGT2 $CaCl_2$ and cuvettes or clear reaction tubes at 37 °C	
1. Sample or Control Plasma	50 µl*
2. RGT1 aPTT-EL	50 µl*
Transfer cuvette with sample and RGT1 to a measuring position and activate optics	
Incubation time	3 min at 37 °C
3. Start reagent RGT2 $CaCl_2$	**50 µl***
Start timer upon addition of RGT2 $CaCl_2$ reagent. Record time [s] required for clot formation	

*depending on the used cuvettes or reaction tube, a proportional variation can be applied

5 Manual Testing HEMOSTAT D-Dimer

For the measurement of HEMOSTAT D-Dimer at least a manual coagulation instrument is required to record a kinetic in ΔOD over a definite time interval at a wavelength of 400 -600 nm. The quality of the kinetic should be verified by the correlation coefficient because correlation values R < 0.95 can indicate the presence interfering substances or high dose hook effects.

Material Required

Material	REF	Size
HEMOSTAT D-Dimer	36002	
RGT **D-Dimer latex**		2 x 1 ml
BUF **Reaction buffer**		2 x 2.5 ml
CAL **Calibrator**		1 x 1 ml
DIL **Diluent**		1 x 6 ml
HIGH **HEMOSTAT D-Dimer Control High**	36012	2 x 1 ml +
LOW **HEMOSTAT D-Dimer Control Low**		2 x 1 ml

Pipetting Scheme

Pipetting Scheme for small cuvettes like for HumaClot Junior or HumaClot Duo^{Plus} (old models)	
Prewarm RGT *and D-Dimer latex reagent and cuvettes at 37 °C*	
1. Sample or Control	25 µl*
2. Add BUF Reaction buffer	100 µl*
Transfer cuvette with sample and BUF *to a measuring position and activate optics*	
Incubation time	2 - 10 min
3. Start reagent RGT D-Dimer reagent	**50 µl***
Mix well by pumping with pipette (15 repeats). Avoid air bubbles.	
Record kinetic [ΔOD/min] or [E] from T1 = 40 s to T2 = 160 s	

If values appear to be too low the alternative pipetting scheme can be applied:

Pipetting Scheme for cuvettes ~ 0.4 - 0.5 mm	
Prewarm RGT *and D-Dimer latex reagent and cuvettes at 37 °C*	
1. Sample or Control	50 µl*
2. Add BUF Reaction buffer	80 µl*
Transfer cuvette with sample and BUF *to a measuring position and activate optics*	
Incubation time	2 – 10 min
3. Start reagent RGT D-Dimer reagent	**40 µl***
Mix well by pumping with pipette (15 repeats). Avoid air bubbles.	
Record kinetic [ΔOD/min] or [E] from T1 = 10 s to T2 = 150 s	

*depending on the used cuvettes, a proportional variation can be applied

Please note: If double cuvettes are used, do not touch the cuvette during measurement. Using single cuvette may be reasonable.

Results

The measurements should be conducted in duplicates and their mean value in ΔOD/time [E] or [mE/min] must be converted in [ng/ml] DDU by comparing the results of patient with calibration curve.

Standard Curve Calibration

A new standard curve needs to be established when

- changing to a new HEMOSTAT D-Dimer LOT
- if indicated by quality control results
- when required by laboratory control procedures and/or governmental regulations.

The following steps must be followed:

a) Reconstitution of the kit calibrator with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).

b) Refer to the LOT-specific D-Dimer Calibrator CAL for the analytical values in ng/ml DDU, which can be found on the vial label of the kit calibrator (REF 36002).

c) Prepare at least 2 dilution levels of the D-Dimer Calibrator CAL with D-Dimer Diluent DIL with serial dilution according to the following table:

Example with a CAL D-Dimer Calibrator of 3320 ng/ml DDU:

Preparation of Dilutions					
	Dilution	**Factor**	**D-dimer [ng/ml] ***	**DIL [µl]**	**CAL [µl]**
Cal 1	1:1	1	3320	0	200
Cal 2	1:2	2	1660	200	200 of Cal 1
Cal 3	1:4	4	840	200	200 of Cal 2
Cal 4	1:8	8	420	200	200 of Cal 3
Cal 5	1:16	16	210	200	200 of Cal 4

** The LOT-specific analytical value is printed on the vial label of CAL.*

d) Measure the prepared calibrator levels in duplicates and write down or print the respective kinetic ΔOD/time [E]. Calculate the mean value [E] of each duplicate.

e) Insert the calculated mean values into the instrument like HumaClot Junior or HumaClot Duo^{Plus} (old models) according to the instrument's User Manual. The last calibration point should be entered as concentration 1 ng/ml and with 0.001 E. If only 2 calibration points (Cal 1 and Cal 2) plus the last one (1 ng/ml; 0.001 E) are inserted, a linear regression can be applied to convert the raw data ΔOD/time [E] or [mE/min] into ng/ml DDU. Otherwise, a point-to-point interpolation is recommended.

f) Check sample kinetics (examples in Figure 1):

(1) below measuring interval < 150 ng/ml DDU generally, mathematically $R^2 << 0.900$

(2) within measuring interval 150 ng/ml – Cal 1 $R^2 > 0.950$
! interfering substances might decrease signal and cause noisy kinetics (R^2 decreases, too)

(3) extended measuring interval > Cal 1 frequently $R^2 > 0.900$
! mind high dose hook effect

Please note: If results are > Cal 1 the sample needs to be diluted 1:5 and re-measured. The displayed result needs to be multiplied by 5 to obtain the true D-dimer result of this diluted sample. If the result is still out of the measuring interval the 1:5 diluted sample must be diluted again 1:10 and the result needs to be multiplied by 50 to report the true value.

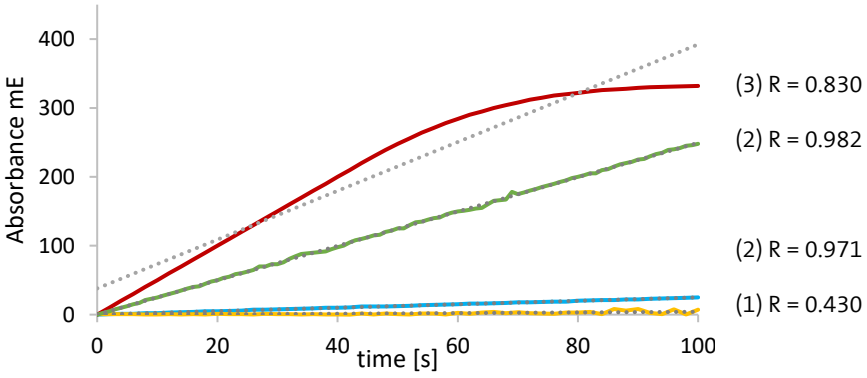


Figure 1: relationship of light absorbance and concentration D-dimer. The slope of the kinetic is the raw data [mE/min]

6 Manual Testing HEMOSTAT Antithrombin liquid

For the measurement of HEMOSTAT Antithrombin liquid at least a manual coagulation instrument is required to record a kinetic in ΔOD over a definite time interval at a wavelength of 405 nm.

Material Required

Material	REF	Size
HEMOSTAT Antithrombin liquid	36102	
RGT Antithrombin reagent		4 x 3 ml
SUB Substrate		2 x 3 ml
CAL HEMOSTAT Calibrator	35500	4 x 1 ml
0.9% Sodium chloride	-	
CPN HEMOSTAT Control Plasma Normal	35001	6 x 1 ml
CPA HEMOSTAT Control Plasma Abnormal	35002	6 x 1 ml

Pipetting Scheme

Sample Pre-dilution (1:11)	
Sample or Control	10 µl
0.9 % NaCl Sodium chloride (0.154 mol/l)	100 µl
Pipetting Scheme	
Prewarm RGT Antithrombin reagent and cuvettes at 37 °C	
1. pre-diluted Sample or Control	40 µl*
2. Add RGT Antithrombin reagent	140 µl*
Transfer cuvette with diluted sample and RGT to a measuring position and activate optics	
Incubation time	2 min
3. Start reagent SUB Substrate	**40 µl***
Mix well shortly by pumping with pipette. Avoid air bubbles.	
Record kinetic [ΔOD/min] or [E] from T1 = 10 s to T2 = 70 s	

*depending on the used cuvettes and instrument specification, a proportional variation can be applied

Standard Curve Calibration

A new standard curve must be established when

- changing to a new HEMOSTAT of Antithrombin^{liquid} LOT
- if indicated by quality control results
- when required by laboratory control procedures and/or governmental regulations.

The following steps must be followed:

a) Reconstitution of the HEMOSTAT Calibrator (REF 35500) with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).

Find the LOT-specific Antithrombin %-value (AT [%]) in the analytical value sheet of the HEMOSTAT Calibrator CAL.

b) Calculate the respective AT [%] -value for Cal 2, Cal 3 and Cal 4 based on a serial dilution (1:2, 1:4, 1:8) of Cal 1 (= analytical value of HEMOSTAT Calibrator CAL on the analytical value sheet).

c) Prepare a serial dilution of the 1:11 diluted HEMOSTAT Calibrator CAL (Cal 1) using 0.9 % Sodium chloride to obtain the calibrator levels Cal 2, Cal 3 and Cal 4.

Example with a HEMOSTAT Calibrator CAL showing an analytical value of 88 % of norm:

Preparation of Calibrator Dilutions				
	Cal 1**	**Cal 2**	**Cal 3**	**Cal 4**
AT* % of norm	Cal 1 [%]	Cal 1 [%] / 2	Cal 1 [%] / 4	Cal 1 [%] / 8
Example AT % of norm	88.0 %	44.0 %	22.0 %	11.0 %
HEMOSTAT Calibrator	20 µl of CAL	100 µl of **Cal 1**	100 µl of **Cal 2**	100 µl of **Cal 3**
0.9 % NaCl	200 µl	100 µl	100 µl	100 µl

**LOT-specific analytical value of the calibrator. It can be found on the table of analytical values in the calibrator kit HEMOSTAT Calibrator (REF 35500).*

***Please note: Cal 1 level is diluted 1:11 – equivalent to pre-dilution of samples*

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

e) Insert the calculated mean values into the instrument like HumaClot Junior or HumaClot Duo^{Plus} (old models) according to the instrument's User Manual. A linear point-to-point interpolation is recommended.

Please note: If results for the default 1:11 dilution fall outside the measuring interval and are displayed/calculated as < 11.0 %, prepare a 1:5.5 dilution and multiply the result by 0.5. If results are displayed or calculated as > 120 %, prepare a higher dilution 1:22 and multiply the result with the dilution factor 2.

7 Manual Testing HEMOSTAT free Protein S

For the measurement of HEMOSTAT free Protein S at least a manual coagulation instrument is required to record a kinetic in ΔOD over a definite time interval at a wavelength of 400 -600 nm.

Material Required

Material	REF	Size
HEMOSTAT free Protein S	36201	
RGT free Protein S latex reagent		2 x 2.5 ml
BUF Buffer		2 x 4 ml
DIL Diluent		2 x 6.5 ml
CAL HEMOSTAT Calibrator	35500	4 x 1 ml
0.9% Sodium chloride	-	
CPN HEMOSTAT Control Plasma Normal	35001	6 x 1 ml
CPA HEMOSTAT Control Plasma Abnormal	35002	6 x 1 ml

Pipetting Scheme

Sample Pre-dilution (1:6)	
Sample or Control	25 µl
DIL free Protein S Diluent	125 µl
Pipetting Scheme	
Prewarm cuvettes at 37 °C	
1. BUF Buffer	55 µl*
2. Add pre-diluted Sample or Control	25 µl*
Transfer cuvette with diluted sample and BUF to a measuring position and activate optics	
Incubation time	2 min
3. Start reagent RGT free Protein S latex reagent	**70 µl***
Mix well shortly by pumping with pipette. Avoid air bubbles.	
Record kinetic [ΔOD/min] or [E] from T1 = 40 s to T2 = 100 s	

*depending on the used cuvettes and instrument specification, a proportional variation can be applied

Standard Curve Calibration

A new standard curve must be established when

- changing to a new HEMOSTAT free Protein S LOT
- if indicated by quality control results
- when required by laboratory control procedures and/or governmental regulations.

The following steps must be followed:

a) Reconstitution of the HEMOSTAT Calibrator (REF 35500) with 1 ml of distilled or deionized water without preservatives, as mentioned in the instruction for use (IFU).

Find the LOT-specific free Protein S %-value (fPS [%]) in the analytical value sheet of the HEMOSTAT Calibrator CAL.

b) Calculate the respective fPS [%]-value for Cal 2, Cal 3 and Cal 4 based on a serial dilution (1:2, 1:4, 1:8) of Cal 1 (= analytical value of HEMOSTAT Calibrator CAL on the analytical value sheet).

c) Prepare a serial dilution of the 1:11 diluted HEMOSTAT Calibrator CAL (Cal 1) using 0.9 % Sodium chloride to obtain the calibrator levels Cal 2, Cal 3 and Cal 4.

Example with a HEMOSTAT Calibrator CAL showing an analytical value (**AV**) of 92% activity:

Preparation of dilutions			
	fPS [%]	**Volume Diluent DIL [µl]**	**Volume Calibrator CAL [µl]**
Cal 1	10	= AV*6 – 10 µl = 92·6 – **10** = 542	**10**
Cal 2	30	= AV*6 – 30 µl = 92·6 – **30** = 522	**30**
Cal 3	60	= AV*6 – 60 µl = 92·6 – **60** = 492	**60**
Cal 4	90	= AV*6 – 90 µl = 92·6 – **90** = 462	**90**
Cal 5	120	= AV*6 – 120 µl = 92·6 – **120** = 432	**120**

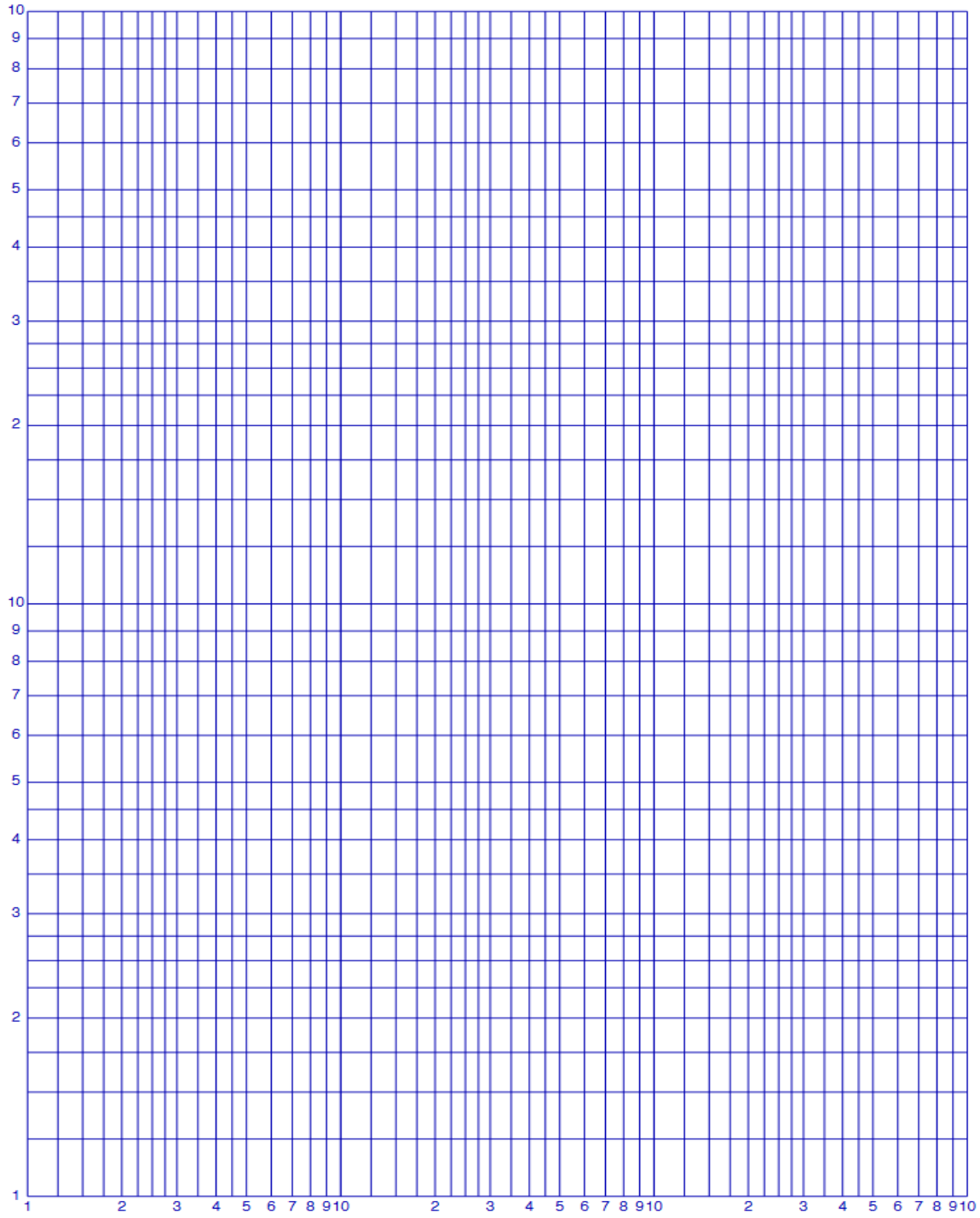
**LOT-specific analytical value of the calibrator. It can be found on the table of analytical values in the calibrator kit HEMOSTAT Calibrator (REF 35500).*

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

e) Insert the calculated mean values into the instrument like HumaClot Junior or HumaClot Duo^{Plus} (old models) according to the instrument's User Manual. A linear point-to-point interpolation is recommended.

Please note: If results for the default 1:6 dilution fall outside the measuring interval and are displayed/calculated as < 10 %, measure again with undiluted sample and divide the result by 6. If results are displayed or calculated as > 120 %, prepare a higher pre-dilution 1:12 (further 1:2 dilution) and multiply the result with the dilution factor 2 to report the true value.

Appendix I: log-log graph paper



	HEMOSTAT Reagent	Calibrator CAL
REF		
LOT		
Expiry		
Analytical value	-	
Additional Information		