

1 Title:

2 **Heparin release kinetics in blood gas syringes**

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11 Running title:

12 ***Is heparinization fast enough in blood gas syringes?***

13 **Keywords:**

14 Point of care testing, Rotation Thrombelastometry, Anticoagulation, Heparin, Syringe

15

16 **Abstract**

17 **Background:** In view of the urgency of results quality and operational availability of point of care
18 analyzers is of high importance in ICUs and operational theatres.

19 **Methods:** We investigated the influence of time on release and total amount of Heparin in various
20 types of syringes used for blood gas analysis (BGA). Heparin activity in three types of liquids
21 (electrolyte solution, fresh frozen plasma and whole blood) was quantified in its time dependency.
22 The ability of clot formation was measured with rotation thrombelastography in whole blood. All
23 tests were done with three types of preanalytical procedures (“resting”, “rolling” and “rotating”).

24 **Results:** We found different time dependencies of heparin activities in syringes releasing dried
25 heparin from pads, the inner syringe wall or liquid heparin solutions during the first 20 minutes
26 after liquid aspiration. The heparin activities lie in a wide range below or above their nominal
27 content in 200µl aliquots withdrawn from the BGA-samples. Preanalytical treatment has influence
28 particularly on the range of heparin activity during the first 10 minutes.

29 **Conclusion:** The risk of blockages by clots in analyzers is lower when syringes with liquid heparin
30 or heparin dried at the inner syringe wall are used.

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32

33

34 ***Introduction***

35 During the last decades many efforts have been done to upgrade reliability, speed, accuracy and
36 operational availability of blood gas analyzers [1]. Oximetry and sensor stability allow extremely
37 long durability in analyzers up to several years. The sensors with the shortest lifetime are biosensors
38 with immobilized enzyme systems for the identification and quantification of glucose and lactate. It
39 is obvious that quality of the measurement is strongly dependent on the quality of the sample matrix
40 [2] and its homogeneity [3]. Sensor membranes, valves and gaskets are not built to e.g. resist
41 stomach acid and there is no permission for testing e.g. intraperitoneal liquids to estimate pH or
42 hemoglobin in it. It is also obvious, not to aspire unheparinized blood samples routinely into a
43 blood gas analyzer, because clotting and blockage is the inevitable consequence. Every disturbance
44 of the operational availability of point of care analyzers is associated with higher costs and a
45 missing basis for urgent therapeutic interventions.

46 Manufacturers of syringes for blood gas analysis sell their products loaded with a wide range of
47 heparin activities. The way of application heparin into blood by the syringes is also variable (both
48 shown for the syringes used in our study in table 1). All of these products should safely avoid
49 coagulation in the syringe and in consequence in blood gas analyzers. The general order for
50 preanalytical handling samples is to rotate them without air bubbles and to transfer blood as soon as
51 possible into an analyzer. None of the users would expect that measurement of a syringe resting at
52 room temperature for 20 min or more would lead to reliable, unattended results [4, 5]. In clinical
53 routine most of the samples reach the analyzer within 5 minutes as speed in return of results is
54 general purpose of any point of care system.

55 Information which level of heparin activity in syringes is sufficient for ongoing anticoagulation in
56 artificial vessels as BGA syringes and the analyzers themselves is given by WHO or IFCC (ISO
57 DIS 6710) [6]. We quantified anticoagulation activity in blood (or other liquid) samples drawn with
58 different systems handled on different levels of clinical practice to fix the current, time
59 corresponding potency of anticoagulation.

60 Depending on the number of parameters modern analyzers only consume about 35 μ l up to 200 μ l
61 blood volume. Total syringe volumes between 1 and 3.5 ml always imply the risk of
62 inhomogeneous release of heparin and therefore an insufficient heparinized aliquot of blood might
63 be aspired.

64 ***Materials and Methods***

65 **Determination of Heparin activity:**

66 Berichrom Heparin test (Dade Behring Marburg GmbH, Marburg, Germany) was modified to fit
67 96-well-plates despite the provided 1 cm cuvettes. Total perfusate/FFP/blood volume needed for
68 each test could be reduced to 10 μ l. Standard curves were prepared with Heparin-Natrium-25.000-
69 ratiopharm (Ratiopharm GmbH, Ulm, Germany) on every 96-well-plate and every test liquid.

70 **Clot formation:**

71 To identify potency of clot formation in analyzers we measured blood samples with rotation
72 thrombelastography (ROTEM, Pentapharm, Munic, Germany. Three tests are performed: InTEM,
73 ExTEM with every blood sample and HepTEM additionally with the last sample of every series.

74 The funding corporate sponsor Sarstedt AG&Co had no influence on any tasks of the manuscript.

75 **Quality control**

76 ***Heparin activity test:***

77 Every 96-well-plate was loaded in the same manner. The first 20 wells were used for calibration
78 (Blank and 0.1 i.U./ml to 1 i.U./ml). Depending on the investigated matrix calibrators were
79 prepared in Perfusate or FFP. The next wells were 4 spiked samples (Perfusate (pH 7.4; Na⁺ 149
80 mmol/l; K⁺ 5.5 mmol/l; Mg⁺⁺ 0.5 mmol/l; Ca⁺⁺ 1.15 mmol/l, Cl⁻ 130 mmol/l, HCO₃⁻ 25 mmol/l;
81 PO₄⁻⁻⁻ 2.8 mmol/l, Glucose 5.5 mmol/l) or FFP) on two different activity levels (twice 0.17 i.U./ml
82 and 0.75 i.U./ml) followed by two commercially available probes (Dade Ci-Trol Heparin Control
83 Low, Dade Behring). The last 6 well were spiked just the same. In between all unknowns were
84 quantified after adequate dilution with Perfusate or FFP (Dilution factors between 10 and 100) not
85 to extend the calibration curve. Whole blood samples were also diluted with FFP.

86 ***Rotem:***

87 Rotrol N (Pentapharm, Munich, Germany) normal control for thrombelastometry was used to verify
88 the functionality of the Rotem on every working day.

89 ***Setup part 1: Testing of heparin activity in 8 syringe types with non –***
90 ***blood liquids.***

91 We simulated the preanalytical situation in clinics defining three attending types and quantified the
92 heparin activity at 0.25; 0.5; 0.45; 1; 2; 3; 4; 5; 10; 15 and 20 min after contact between the test
93 liquids and heparin.

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94 “Resting”: Worst case situation: Sample liquid is aspirated into a heparinized syringe, shaken once,
95 rested on the table until it is taken again, once shaken and 200µl volume are sucked with a needle
96 into a plastic syringe out of the middle of the sample.

97 “Rolling”: Normal case situation: Sample liquid is aspirated into a heparinized syringe and rolled by
98 hand until removal of 200µl volume.

99 “Rotating”: Superior case situation: After aspiration the heparinized syringe is rotated mechanical
100 with 10 turns per minute along its long axis of rotation.

101 We used two different test liquids (perfusate* and pooled FFP) to overcome variability in e.g.
102 protein content and two lot numbers (table 1) per syringe type. Every syringe was tested in two
103 series per lot. Every heparin test was done in duplicate.

104 **Table 1 Tested Blood Gas Samplers. Types of heparin formylation and activity per ml**

Purchaser/ Lot Numbers	Syringe type / nominal volume	Formylation	Heparin Amount [i.U./ml] / characterization
Beckton Dickinson 7046333 / 7073875	A-Line / 3 ml	Liquid	27 / Ca balanced Lithium Heparin
Radiometer RY05 2008-09 / TA 03 2008-11	PICO 50 / 2 ml	Dry on a mobile pad	40 / electrolyte balanced Heparin
Westmed, Tucson, AZ 34710 / 34988	PULSSET / 3 ml	Dry	8.3 / Balanced Heparin
Radiometer TJ-01 2009-01 / TR-01 2009-04	PICO 70 / 1.5 ml	Dry on a mobile pad	40 / compensated Heparin
Sarstedt 7091002 / 7092202	BG-Monovette /2 ml	Liquid	50 / Ca balanced Lithium Heparin
Smith-Medical 1085040 / 1129201	Pro-Vent / 3 ml	Dry	23 / dried Lithium Heparin
Bayer 1051566 / 1116183	Rapidlite / 3ml	Spray dried at the inner wall	23 / Ca balanced Lithium Heparin
Roche 702001 / 708002	BS2 / 2 ml	Liquid	15 / Sodium-Heparin

105 **Setup part 2: Testing of heparin activity and clot firmness in 2 types of**
106 **syringes with whole blood.**

107 Citric blood from 18 healthy volunteers was taken with written consent by the local ethics
108 committee and heparin activity tested under the same conditions as mentioned above. 1 ml blood
109 was aspirated out of the BGA syringes at the specified time points. The higher blood volume was
110 needed to process thrombelastometric measurements. The syringe with the highest liquid heparin
111 content and one with heparin on a pad were chosen because in these two syringe types the biggest
112 distinctions are to be expected from the results of part one investigations. With this attempt not only
113 variations in heparin activity could be identified, but also a magnitude of real clot formation in
114 Analyzers could be quantified.

115 **Statistics:**

116 To create a meaningful parameter for comparison of each syringe type heparin activities of the
117 beginning 10 min a Simple E-max model (Software WinNonLin, Pharsight Corporation, Mountain
118 View, CA) was used to calculate a fitting curve. The areas under these curves were compared with
119 SPSS 15.0.01 for Windows (SPSSinc, Chicago, Illinois).

120 **Results**

121 Details of quality control results for the determination of heparin activity are given in table 2.

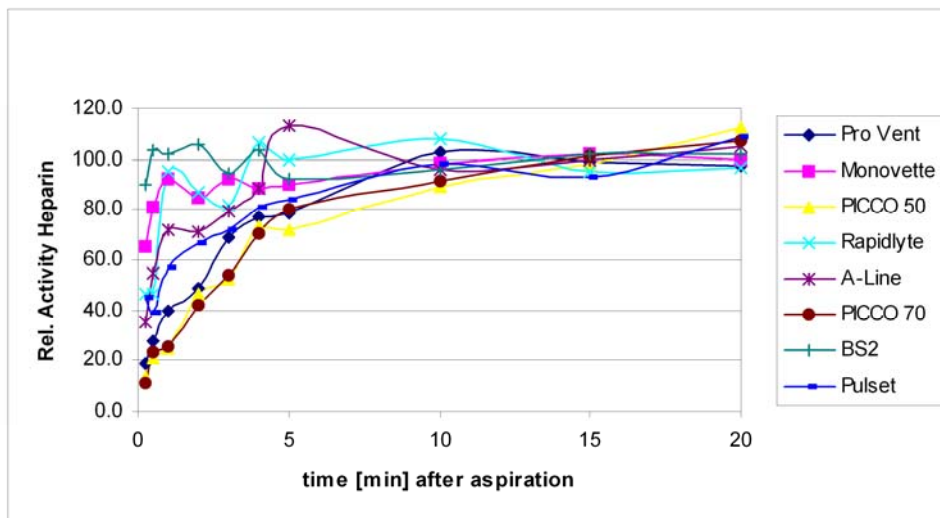
122 **Table 2: Results of quality controls for heparin activity.**

Target value [i.U./ml]	Mean [i.U./ml]	N	Bias [%]	SD [i.U./ml]	Number of plates	Inter – plate SD [i.U./ml]
0.17	0.148	292	-13	0.0421	66	0.0288
0.75	0.722	268	-3.8	0.0919	66	0.0638
Ci-Trol-low	0.274	264	-	0.0936	66	0.0892

123

124 To compare the results of maximum heparin activity identified by the BeriChrom-test with the
 125 activity given from the purchaser we averaged the activity in the tests at 10, 15 and 20 min after
 126 application of the liquids into the blood-gas-syringes. This implies the assumption that a contact
 127 time of 10 min is nearly sufficient for the total release of heparin into the test solutions. This can be
 128 proven e.g. by a plot of mean relative heparin activities in FFP. (figure 1)

129 Figure 1: Time dependency of relative heparin activities in BGA-syringes measured in FFP. The
 130 three types of clinical preanalytical treatment are averaged. Each data point results from 36
 131 analyzed syringes.



132

133 Absolute heparin activity data have to be accepted under reserve, because we calibrated the
 134 BeriChrom test with one type of heparin. Results are given in table 3 wherein the high bias for
 135 measured maximum mean values is probably due to the single type calibration.

136 **Table 3 Relative Areas under the curve of heparin activities released during the first 10 minutes after aspiration.**

Formylation	AUC[%]	[%]	Number	Post Hoc Bonferroni significance niveau p		
	Mean	SD	N	liquid	Pad	Dry
Liquid	89.3	21.8	72		0.00001	0.48
Pad	66.3	24.9	48	0.00001		0.001
Dry	83.4	28.3	72	0.48	0.001	

137
 138 Despite the lack of missing calibration material for all syringe types we found the nominal heparin
 139 activity quite precisely in three of the syringe types (Monovette, Rapidlite and A-Line) in Perfusate
 140 (Bias less than $\pm 5\%$).

141 Deviations between -66% and +80% were found in the other syringes (table 3)

142 As marker for differences in heparin release during the observed time period we chose the relative
 143 AUC (WinNonLin, Pharsight, Mountain View, CA) calculated from 0 to 10 minutes versus heparin
 144 activity standardized to averaged values from 10, 15 and 20 minutes after aspiration. The
 145 standardization was done separately for every liquid, modus and syringe type. We found strong
 146 significant differences in relative AUCs between the liquid and the other two types of heparin
 147 formulation. (table 4) Gaussian distribution was tested with a q-q Plot and p values were calculated
 148 with one way anova and Bonferroni Post Hoc analysis.

149 **Table 4: Mean heparin activities in the BGA-syringes 10 to 20 min after aspiration. Each mean value is**
 150 **quantified with the same type of calibrator (heparin-sodium; ratiopharm). The denoted bias is in relation to the**
 151 **desired values given by the purchaser.**

Activity	Pro Vent	Mono- vette	PICO 50	Rapid- lyte	A- Line	PICO 70	BS2	Puls- set	mean
Mean perfusat i.U./ml	27.0	49.0	28.9	24.0	26.9	32.7	6.5	15.0	
SD perfusat	11.1	7.1	8.9	9.9	8.6	10.2	1.9	3.6	
Bias perfusat %	17.5	-2.0	-27.8	4.5	-0.2	-18.4	-56.6	79.8	-0.4
mean FFP i.U./ml	21.5	43.9	23.0	16.3	21.7	25.0	5.0	13.9	
SD FFP	9.6	7.2	10.3	9.9	9.1	10.0	1.1	4.6	
Bias FFP %	-6.4	-12.2	-42.5	-29.2	-19.7	-37.6	-66.4	66.4	-18.4
Desired value i.U./ml	23.0	50.0	40.0	23.0	27.0	40.0	15.0	8.3	

152
 153 By employing a pharmacodynamic Simple Emax – model on the normalized heparin activities a
 154 table of time points corresponding to the release of 8 i.U./ml Heparin (minimum required amount)
 155 was calculated (figure 2).

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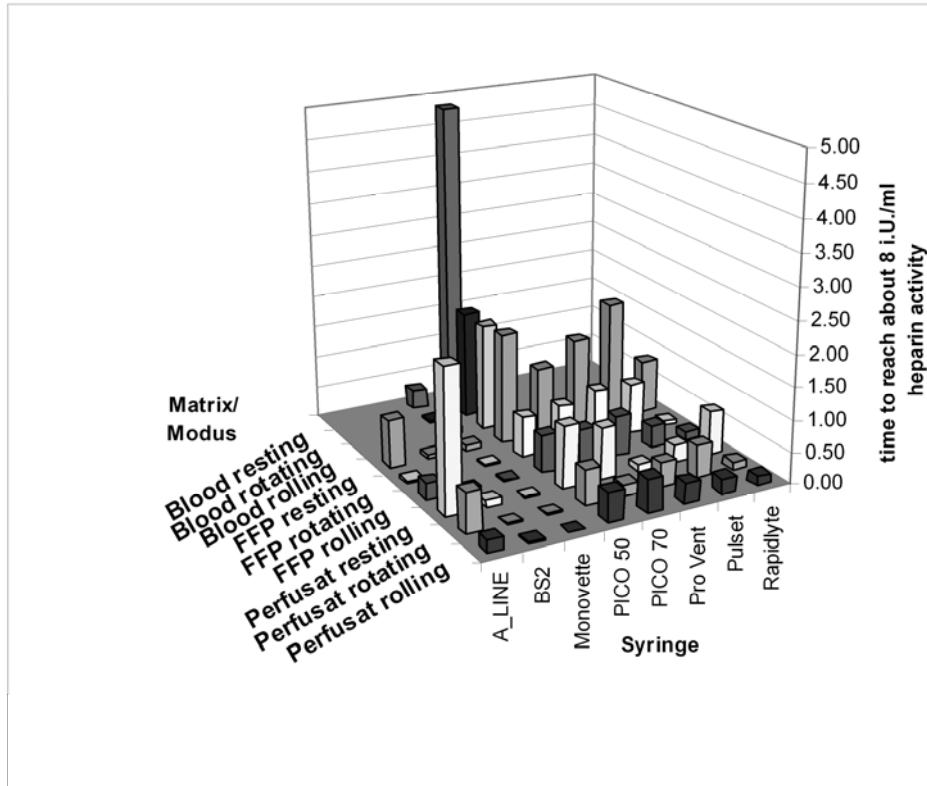
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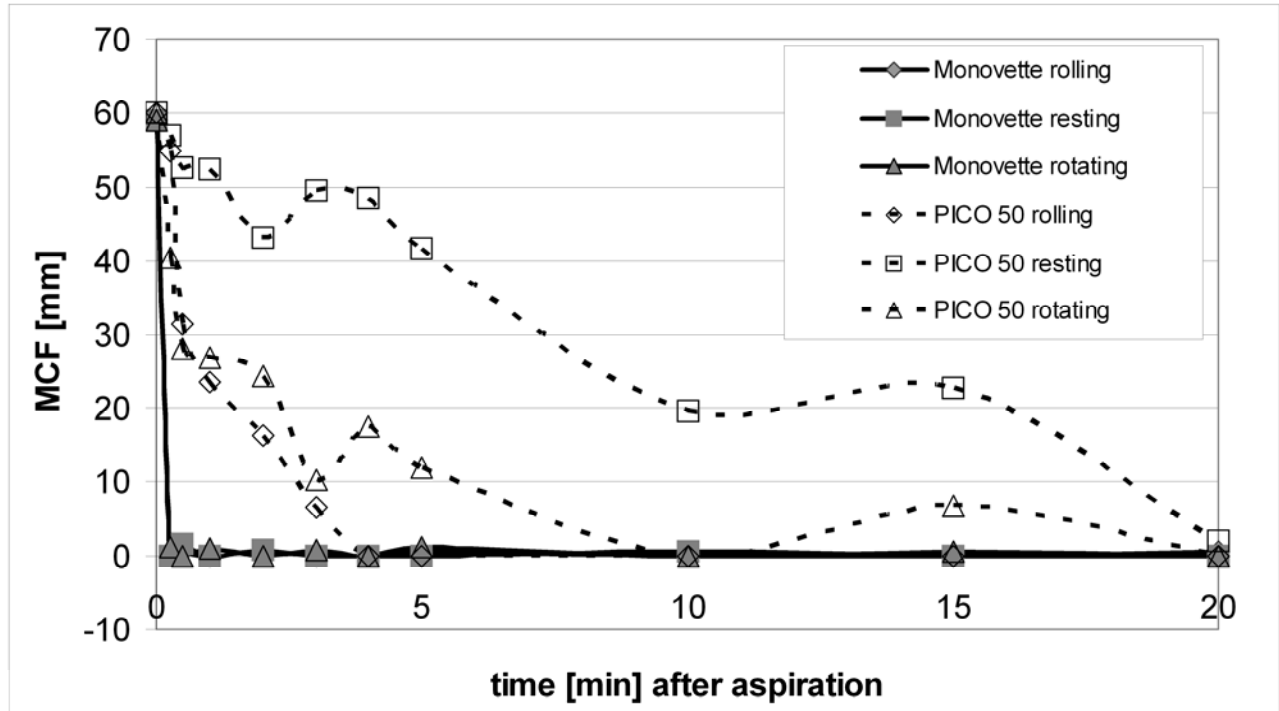
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162 Figure 2: Calculated time [min] to release about 8 i.U./ml heparin activity in BGA syringes in
163 perfusate, FFP or in blood (two syringes only) under different preanalytical conditions.



164
165 Using the curves and defining the last calculated value as 100% time points could be identified until
166 defined values of relative heparin activities are to be expected.
167 With liquid heparin from Monovettes in 10% of the syringes with the resting preanalytic treatment a
168 clot firmness of more than 50 % could be detected (table 5). With PICO 50 between 30 and 100%
169 of all syringes had a MCF of more than 50%. Time dependency of all mean INTEM – MCF results
170 are shown in figure 3.

171 Figure 3: Results from ROTEM measurements of maximum clot formation [MCF] along the
 172 intrinsic pathway (mean of nine measurements per point).



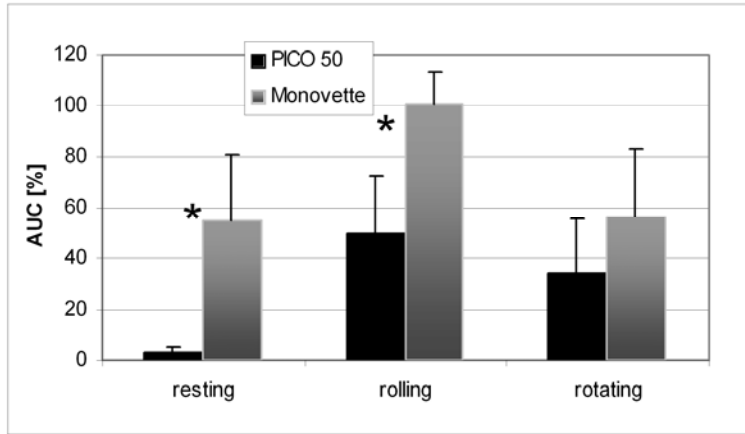
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 174 A direct linear correlation between MCF and relative heparin activities could be found (EXTEM:
 175 $r^2 = 0.91$ and INTEM: $r^2 = 0.66$ for PICO 50 syringes; EXTEM: $r^2 = 0.32$ and INTEM: no
 176 correlation, because MCF was always below 5 mm for Monovettes

177 **Discussion**

178 The sum of data we obtained from quantifying heparin activities in Perfusate and fresh frozen
 179 plasma indicates that liquid formulation of heparin in BGA syringes leads to the fastest heparin
 180 detachment in solutions. Other formulations release their stock of heparin slower. We tried to
 181 answer the question whether even smaller amounts of heparin, released within the first few minutes
 182 lead to a sufficient heparinization by combining the quantification of heparin activity with the

183 determination of maximum clot firmness out of identical samples of whole blood. (figure 4 and
 184 table 5).

185 Figure 4: Relative AUCs during the first 10 min after aspiration of whole blood in two different
 186 syringes depending on the preanalytic modus. Data are corrected by the calibration bias in perfusat.



187

188 **Table 5: Portions of Syringes (PICO 50 and Monovette) producing no reduction in clot firmness (MCF more**
 189 **than 50 mm) and 50% or less reduction (MCF more than 25mm) independently of contact time within 20**
 190 **minutes.**

Treatment	MCF more than 50 mm				MCF more than 25 mm			
	in-TEM %		ex-TEM %		in-TEM %		ex-TEM %	
	PICO	Monovette	PICO	Monovette	PICO	Monovette	PICO	Monovette
Rolling	10	0	30	0	20	0	50	0
Resting	30	0	90	0	70	0	100	10
Rotating	0	0	40	0	30	0	90	0

191

192 The high significant differences in relative AUC (0-10min) correspond to the higher portions of
 193 syringes with no or insufficient reduction in clot firmness (MCF = normal or more than 25 mm).

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194 The considered amount of required heparin activity varied during the last 25 years from 20 i.U./ml
195 [7] to 4-6 i.U./ml [8] for plastic syringes. WHO guidelines from 2002 assume distinctive levels of
196 unfractionated heparin (lithium, sodium or ammonium heparin from 12 to 30 i.U./ml) to get
197 standardized heparinized plasma. Dry calcium titrated heparin from 40 to 60 i.U./ml and in liquid
198 formulation 8 to 12 i.U./ml should be used to determine ionized calcium.

199 Assuming that the heparin release in syringes is completed after 20 min and that the calculated
200 Simple E-Max model reflects the specified heparin activities we calculated the necessary time
201 period to achieve the contemplated 8 i.U./ml. (figure 2)

202 To existing discussions about syringe types [9-12], preanalytical errors [4, 13] or influences of
203 Heparin formulation on quantitation results [8, 14-23] e.g. due to dilution [24, 25], changes in time
204 depending contents [11, 26-28], carryover [29], heparin amounts (less than 15 iU/ml lithium or
205 sodium heparinate and less than 50 iU/ml calcium heparinate) [18, 30, 31] or necessity of heparin in
206 single use cuvettes [32] we have to add a time resolved clotting discussion.

207 With this investigation we could proof the qualitative different heparin release namely liquid > dry
208 at the syringe inner wall > dry on pads in BGA syringes. We also showed that preanalytical
209 treatment rolling (by hand) is superior to rotating (mechanical 10/min) and resting.

210 Resting however (shaking once at aspiration of blood and once again before removal of the 200 µl
211 aliquot) is responsible for a prolongation of release particularly with regard to the heparin
212 containing pads. The ROTEM analysis showed that the heparin activity correlates with clot
213 firmness. In worst case situation no reduction in clot firmness activated along the extrinsic pathway
214 was found over 15 min (data not shown) in resting syringes with heparin on pads. The usual clinical
215 preanalytical treatment can be assumed as somewhere in between rolling and resting. Therefore a

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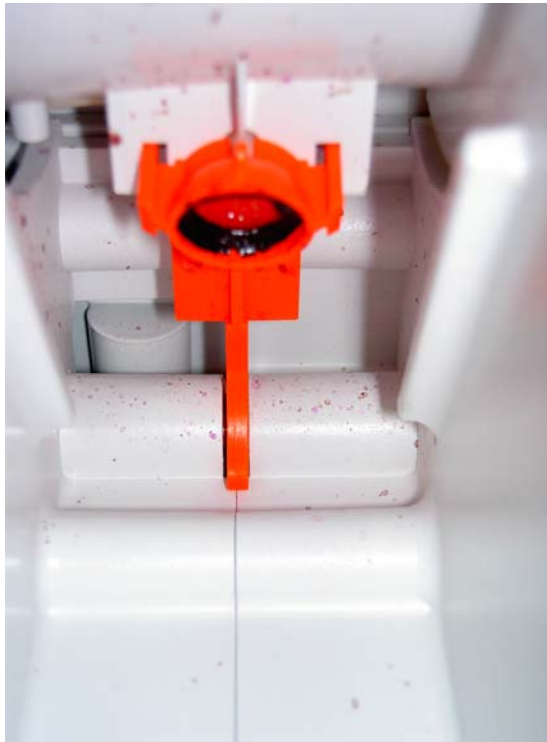
216 perfect anticoagulation from the first moment of aspiration is not achievable for any syringe type.
217 Anyhow the use of liquid heparin formulation is superior to spray dried and in particular to dried
218 heparin on pads.
219 Relative AUCs of heparin activity from beginning of aspiration to 10 minutes fit to ROTEM results.
220 The relative AUC (0-10min) lies for PICCO 50 syringes between 3 and 49 % and for the
221 Monovettes between 55 and 101% of the maximum possible theoretical value (when heparin
222 activity results are corrected with the factor found in Perfusate calibrations) (figure 4). With these
223 results we could classify the firmness of clot formation in different types of BGA syringes.
224 To demonstrate the real effect on the functionality of a blood gas analyzer three pictures of the
225 waste outlets of a Siemens (former Bayer Diagnostics) 1265 blood gas analyzer are shown (figure 5
226 a-c). The instrument was in use for about one week (about 400 measurements) in our neurosurgical
227 – anesthesiological ICU with every of the three syringe types. a) Bayer Rapidlite b) Radiometer
228 PICO 50 and c) Sarstedt Monovette

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229 Figure 5a-c: Photographies of waste outlets of a Siemens 1265 BGA after charging with about 400

230 blood samples for each syringe type a) Rapidlyte b) PICO 50 and c) Monovette

231 a) Rapidlyte



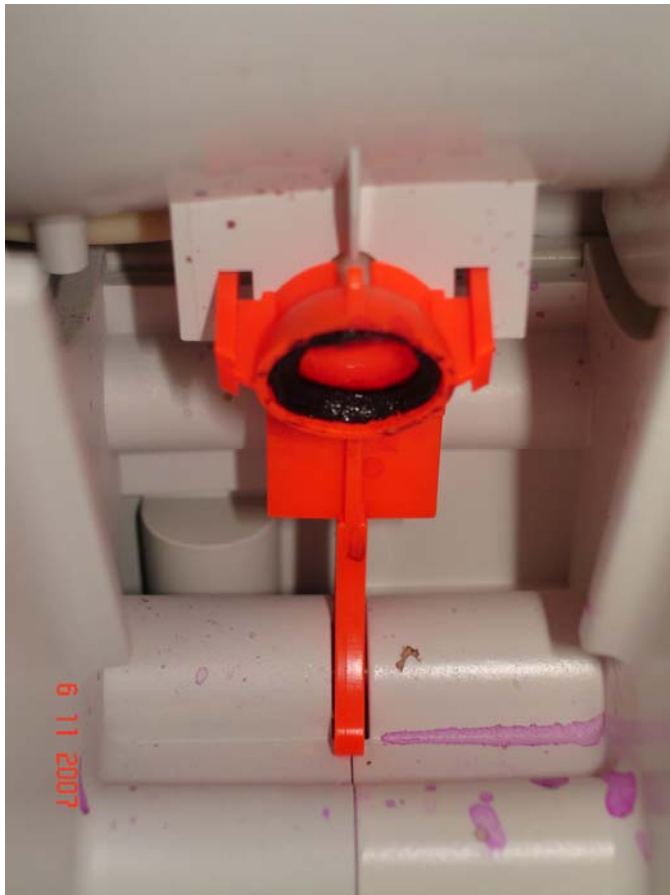
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233 b) PICO 50



234

235 c) Monovette



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240 Anesthesiology of the University of Regensburg.

241 Perfusate composition was chosen according to Dr. Michael Dittmar, Department of
242 Anesthesiology, Clinic of the University of Regensburg, Germany)

243

244 **Abbreviations:**

245 Blood Gas Analysis = BGA, Rotationthrombelastometry = ROTEM, Fresh Frozen Plasma = FFP,

246 Area under the Curve = AUC; Maximum Clot Firmness = MCF

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248

249 **References**

250 [1.] Gilbert HC, Szokol JW. Point of care technologies. *Int Anesthesiol Clin* 2004;42:73-94.

251 [2.] Narayanan S. The preanalytic phase. An important component of laboratory medicine. *Am J*
252 *Clin Pathol* 2000;113:429-52.

253 [3.] Grenache DG, Parker C. Integrated and automatic mixing of whole blood: an evaluation of a
254 novel blood gas analyzer. *Clin Chim Acta* 2007;375:153-7.

255 [4.] Wuillemin WA, Gerber AU. [Sources of error in the pre-analytical phase of blood gas
256 analysis]. *Schweiz Rundsch Med Prax* 1995;84:200-3.

257 [5.] Harsten A, Berg B, Inerot S, Muth L. Importance of correct handling of samples for the
258 results of blood gas analysis. *Acta Anaesthesiol Scand* 1988;32:365-8.

259 [6.] World_Health_Organization. Use of anticoagulants in diagnostic laboratory investigations.
260 WHO/DIL/LAB/991 Rev2, Vol., 2002.

261 [7.] Hutchison AS, Ralston SH, Dryburgh FJ, Small M, Fogelman I. Too much heparin: possible
262 source of error in blood gas analysis. *Br Med J (Clin Res Ed)* 1983;287:1131-2.

263 [8.] Muller-Plathe O, Schreiber R. [An electrolyte-adapted heparin solution for the determination
264 of blood gases and electrolytes in whole blood]. *Anesthesiol Intensivmed Notfallmed Schmerzther*
265 1991;26:161-4.

- 266 [9.] Smeenk FW, Janssen JD, Arends BJ, Harff GA, van den Bosch JA, Schonberger JP,
267 Postmus PE. Effects of four different methods of sampling arterial blood and storage time on gas
268 tensions and shunt calculation in the 100% oxygen test. *Eur Respir J* 1997;10:910-3.
- 269 [10.] Knowles TP, Mullin RA, Hunter JA, Douce FH. Effects of syringe material, sample storage
270 time, and temperature on blood gases and oxygen saturation in arterialized human blood samples.
271 *Respir Care* 2006;51:732-6.
- 272 [11.] Picandet V, Jeanneret S, Lavoie JP. Effects of syringe type and storage temperature on
273 results of blood gas analysis in arterial blood of horses. *J Vet Intern Med* 2007;21:476-81.
- 274 [12.] Sachs C, Rabouine P, Dautzenberg MD. Evaluation of syringes for ionized calcium
275 measurements. *Scand J Clin Lab Invest Suppl* 1996;224:193-201.
- 276 [13.] Biswas CK, Ramos JM, Kerr DN. Heparin effect on ionised calcium concentration. *Clin*
277 *Chim Acta* 1981;116:343-7.
- 278 [14.] Hopper K, Rezende ML, Haskins SC. Assessment of the effect of dilution of blood samples
279 with sodium heparin on blood gas, electrolyte, and lactate measurements in dogs. *Am J Vet Res*
280 2005;66:656-60.
- 281 [15.] Kinoshita H, Fukuda S, Ataka T, Watanabe I, Shimoji K. [Problems of commercially
282 available heparinized syringes in measurements of blood gases and electrolytes]. *Masui*
283 1995;44:1563-7.
- 284 [16.] Carter BG, Tibballs J, Hochmann M, Osborne A, Chiriano A, Murray G. A comparison of
285 syringes to collect blood for analysis of gases, electrolytes and glucose. *Anaesth Intensive Care*
286 1994;22:698-702.

- 287 [17.] Swanson JR, Heeter C, Limbocker M, Sullivan M. Bias of ionized calcium results from
288 blood gas syringes. *Clin Chem* 1994;40:669-70.
- 289 [18.] Boink AB, Buckley BM, Christiansen TF, Covington AK, Maas AH, Muller-Plathe O, et al.
290 International Federation of Clinical Chemistry (IFCC) scientific division IFCC recommendation.
291 Recommendation on sampling, transport and storage for the determination of the concentration of
292 ionized calcium in whole blood, plasma and serum. *Ann Biol Clin (Paris)* 1991;49:434-8.
- 293 [19.] Gayed AM, Marino ME, Dolanski EA. Comparison of the effects of dry and liquid heparin
294 on neonatal arterial blood gases. *Am J Perinatol* 1992;9:159-61.
- 295 [20.] Shin CS, Chang CH, Kim JH. Liquid heparin anticoagulant produces more negative bias in
296 the determination of ionized magnesium than ionized calcium. *Yonsei Med J* 2006;47:191-5.
- 297 [21.] Madiedo G, Sciacca R, Hause L, Sasse E. Use of syringes containing dry (lyophilized)
298 heparin in sampling blood for pH measurement and blood-gas analysis. *Clin Chem* 1982;28:1727-9.
- 299 [22.] Calaf N, Giner J, Codina E, Feixas T, Gonzalez M, Casan P. [Comparison of arterial blood
300 sample kits]. *Arch Bronconeumol* 2004;40:378-80.
- 301 [23.] Toffaletti JG, Wildermann RF. The effects of heparin anticoagulants and fill volume in
302 blood gas syringes on ionized calcium and magnesium measurements. *Clin Chim Acta*
303 2001;304:147-51.
- 304 [24.] Jiang HX. [The effect of dilution and heparin on the blood gas analysis]. *Zhonghua Jie He*
305 *He Hu Xi Za Zhi* 1992;15:225-7, 55-6.
- 306 [25.] Borner U, Muller H, Hoge R, Hempelmann G. The influence of anticoagulation on acid-base
307 status and blood-gas analysis. *Acta Anaesthesiol Scand* 1984;28:277-9.

- 308 [26.] Calatayud O, Tenias JM. Effects of time, temperature and blood cell counts on levels of
309 lactate in heparinized whole blood gas samples. *Scand J Clin Lab Invest* 2003;63:311-4.
- 310 [27.] Beaulieu M, Lapointe Y, Vinet B. Stability of PO₂, PCO₂, and pH in fresh blood samples
311 stored in a plastic syringe with low heparin in relation to various blood-gas and hematological
312 parameters. *Clin Biochem* 1999;32:101-7.
- 313 [28.] Muller-Plathe O, Heyduck S. Stability of blood gases, electrolytes and haemoglobin in
314 heparinized whole blood samples: influence of the type of syringe. *Eur J Clin Chem Clin Biochem*
315 1992;30:349-55.
- 316 [29.] Brown JM, Dimeski G. Contamination of coagulation tests with heparin from blood gas
317 samples. *Br J Anaesth* 2001;87:628-9.
- 318 [30.] Boink AB, Buckley BM, Christiansen TF, Covington AK, Maas AH, Muller-Plathe O, et al.
319 Recommendation on sampling, transport, and storage for the determination of the concentration of
320 ionized calcium in whole blood, plasma, and serum. IFC Scientific Division, Working Group on
321 Ion-Selective Electrodes (WGSE). *J Int Fed Clin Chem* 1992;4:147-52.
- 322 [31.] Narayanan S. [Effects of anticoagulants used at blood specimen collection on clinical test
323 results]. *Rinsho Byori* 1996;Suppl 103:73-91.
- 324 [32.] Bailey SR, Russell EL, Martinez A. Evaluation of the AVOXimeter: precision, long-term
325 stability, linearity, and use without heparin. *J Clin Monit* 1997;13:191-8.

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