Title:

Heparin release kinetics in blood gas syringes

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The Study was funded by Sarstedt AG&Co (Nuembrecht, Germany) and the Department of Anesthesiology of the University of Regensburg.

Acknowledgement: Dr. Michael Dittmar, Department of Anesthesiology, University of Regensburg, Germany

Running title:

Is heparinization fast enough in blood gas syringes?

Keywords:

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

Abstract

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

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

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 The heparin activities lie in a wide range below or above their nominal content in 200µl aliquots withdrawn from the BGA-samples.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

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

Introduction

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

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

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

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

Materials and Methods

Determination of Heparin activity:

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

Clot formation:

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

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

Quality control

Heparin activity test:

Every 96-well-plate was loaded in the same manner. The first 20 wells were used for calibration (Blank and 0.1 i.U./ml to 1 i.U./ml). Depending on the investigated matrix calibrators were prepared in Perfusate or FFP. The next wells were 4 spiked samples (Perfusate (pH 7.4; Na^{+} 149 mmol/l; K^{+} 5.5 mmol/l; Mg^{++} 0.5 mmol/l; Ca^{++} 1.15 mmol/l, Cl^{-} 130 mmol/l, HCO_3^{-} 25 mmol/l; PO_4^{---} 2.8 mmol/l, Glucose 5.5 mmol/l) or FFP) on two different activity levels (twice 0.17 i.U./ml and 0.75 i.U./ml) followed by two commercially available probes (Dade Ci-Trol Heparin Control Low, Dade Behring). The last 6 well were spiked just the same. In between all unknowns were quantified after adequate dilution with Perfusate or FFP (Dilution factors between 10 and 100) not to extend the calibration curve. Whole blood samples were also diluted with FFP.

Rotem:

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

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

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

94 “Resting”: Worst case situation: Sample liquid is aspired 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 aspired 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

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

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

Statistics:

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

Results

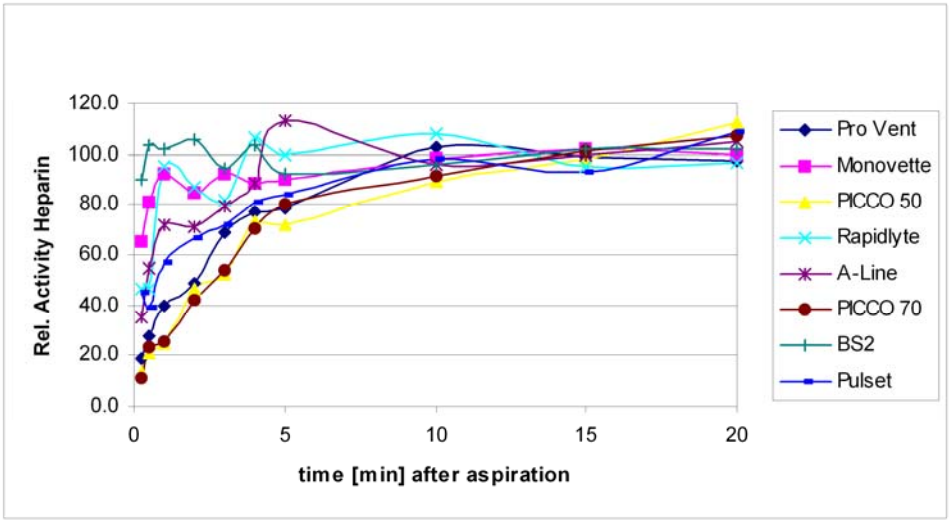
Details of quality control results for the determination of heparin activity are given in table 2.

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

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

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



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

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	

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

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

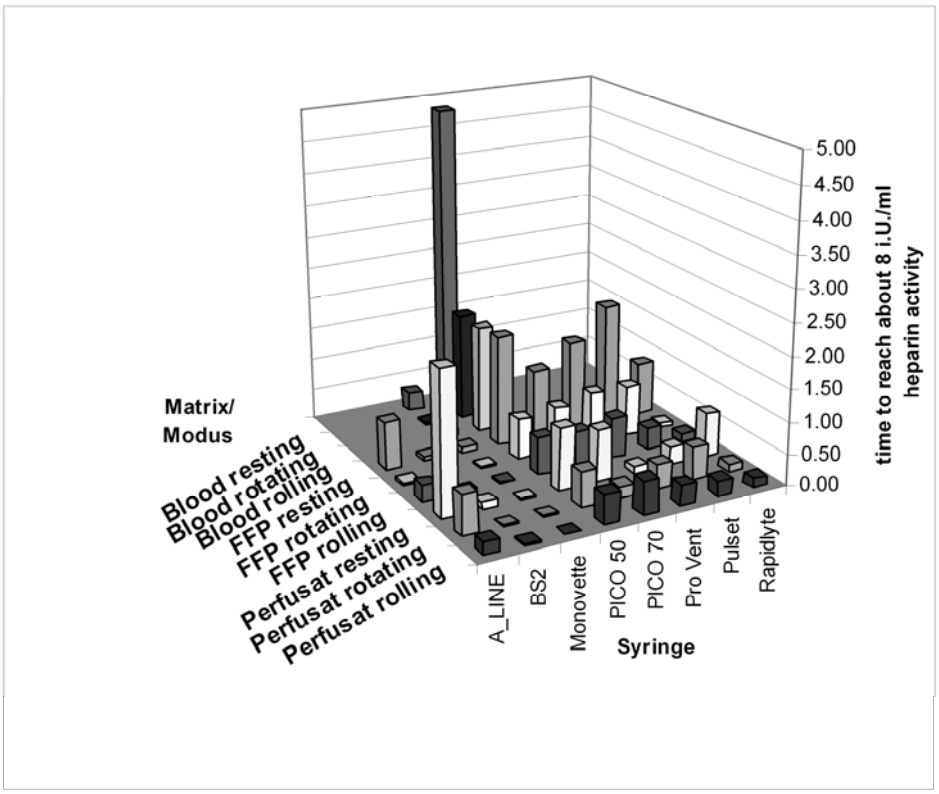
As marker for differences in heparin release during the observed time period we chose the relative AUC (WinNonLin, Pharsight, Mountain View, CA) calculated from 0 to 10 minutes versus heparin activity standardized to averaged values from 10, 15 and 20 minutes after aspiration. The standardization was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

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

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

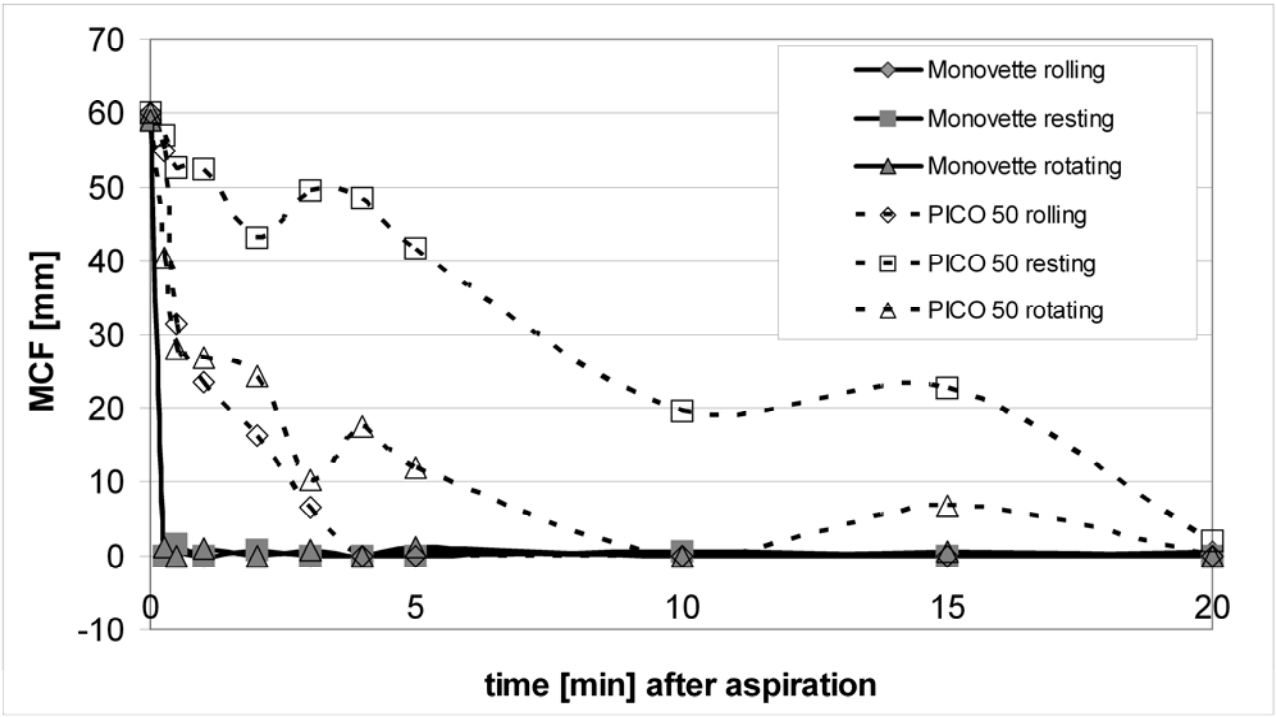
Figure 2: Calculated time [min] to release about 8 i.U./ml heparin activity in BGA syringes in perfusate, FFP or in blood (two syringes only) under different preanalytical conditions.



Using the curves and defining the last calculated value as 100% time points could be identified until defined values of relative heparin activities are to be expected.

With liquid heparin from Monovettes in 10% of the syringes with the resting preanalytic treatment a clot firmness of more than 50 % could be detected (table 5). With PICO 50 between 30 and 100% of all syringes had a MCF of more than 50%. Time dependency of all mean INTEM – MCF results are shown in figure 3.

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



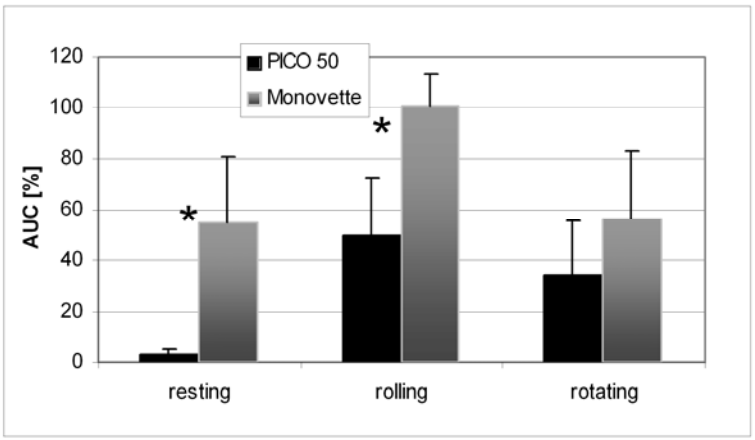
A direct linear correlation between MCF and relative heparin activities could be found (EXTEM: r^2 = 0.91 and INTEM: r^2 = 0.66 for PICO 50 syringes; EXTEM: r^2 = 0.32 and INTEM: no correlation, because MCF was always below 5 mm for Monovettes

Discussion

The sum of data we obtained from quantifying heparin activities in Perfusate and fresh frozen plasma indicates that liquid formulation of heparin in BGA syringes leads to the fastest heparin detachment in solutions. Other formulations release their stock of heparin slower. We tried to answer the question whether even smaller amounts of heparin, released within the first few minutes 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.



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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

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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).

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

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

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

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

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

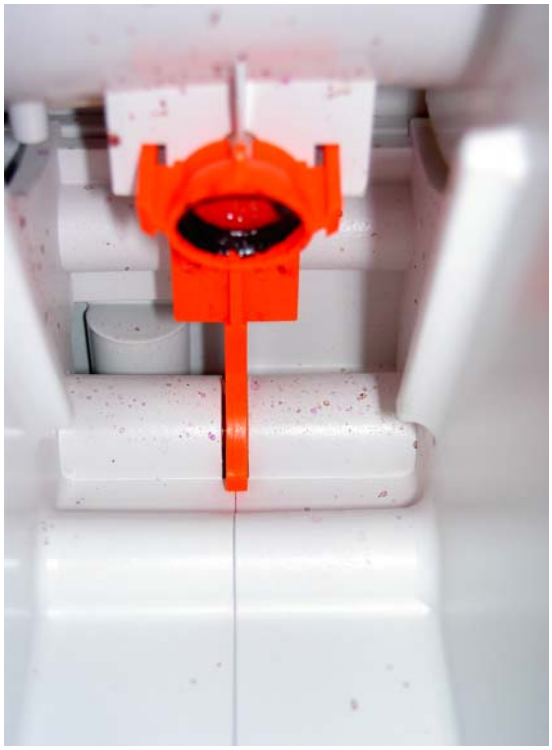
perfect anticoagulation from the first moment of aspiration is not achievable for any syringe type. Anyhow the use of liquid heparin formulation is superior to spray dried and in particular to dried heparin on pads.

Relative AUCs of heparin activity from beginning of aspiration to 10 minutes fit to ROTEM results. The relative AUC (0-10min) lies for PICCO 50 syringes between 3 and 49 % and for the Monovettes between 55 and 101% of the maximum possible theoretical value (when heparin activity results are corrected with the factor found in Perfusate calibrations) (figure 4). With these results we could classify the firmness of clot formation in different types of BGA syringes.

To demonstrate the real effect on the functionality of a blood gas analyzer three pictures of the waste outlets of a Siemens (former Bayer Diagnostics) 1265 blood gas analyzer are shown (figure 5 a-c). The instrument was in use for about one week (about 400 measurements) in our neurosurgical – anesthesiological ICU with every of the three syringe types. a) Bayer Rapidlite b) Radiometer PICO 50 and c) Sarstedt Monovette

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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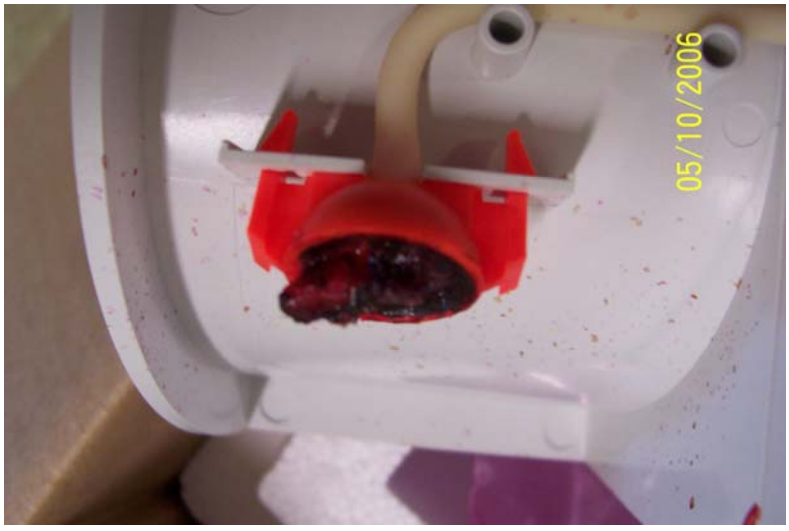
233 b) PICO 50



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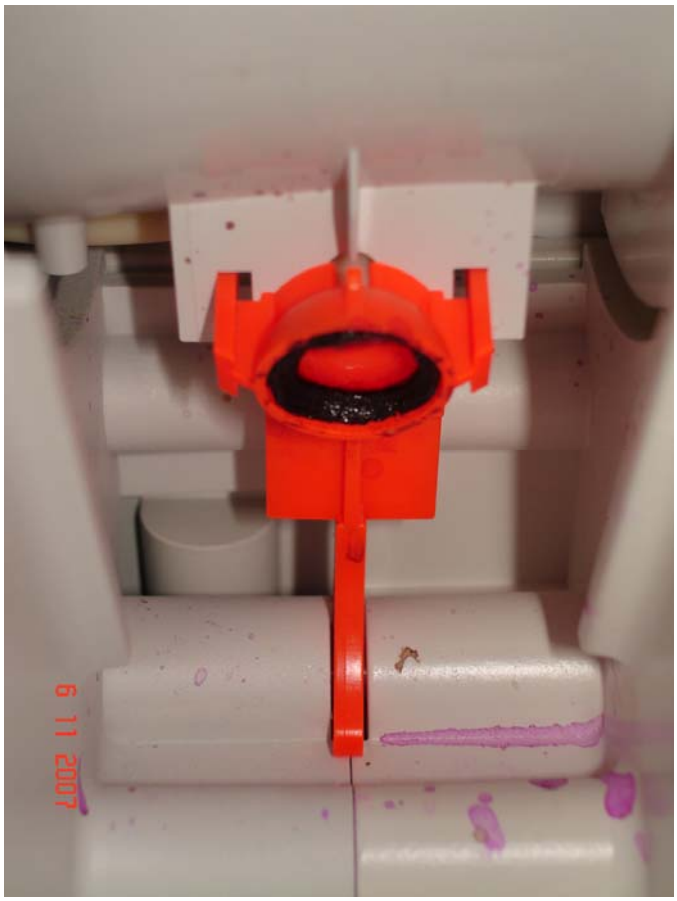
c) Monovette



Acknowledgement:

The Study was funded by Sarstedt AG&Co (Nuembrecht, Germany) and the Department of Anesthesiology of the University of Regensburg.

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

Abbreviations:

Blood Gas Analysis = BGA, Rotationthrombelastometry = ROTEM, Fresh Frozen Plasma = FFP, Area under the Curve = AUC; Maximum Clot Firmness = MCF

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