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MILITARY UNIVERSITY HOSPITAL
PRAGUE

Department of Hematology and Blood Transfusion



Frozen Platelets in Urgent Care

Col.Milos Bohonek, MD, PhD et all

2018 RDCR Symposium
June 17-20, 2018



Disclosure

No relevant financial or nonfinancial relationships to disclose.



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Platelets for transfusion



- from apheresis
- from buffy coat (pooled / single units)
- from PRP



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Clinical use of platelets

- **hematology**
- **oncology**
- **special (i.e. before liver transplantation, cardiosurgery)**
- **polytrauma, massive bleeding**



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Early and aggressive use of blood products in these patients may correct coagulopathy, control bleeding, and improve outcomes – „1st. golden hour rule“.

Majority is preventable if red cells, plasma and platelets are available as soon as possible.

- Transfusion in trauma =



Platelets

Due to very short shelf life, having a daily stockpile of fresh platelets is not possible for many hospital blood banks.



The alternative solution is a **stock of frozen platelets** which are successfully used in military medicine.



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Methods of PLTs cryopreservation - background

1. Platelets can be cryopreserved in four different cryoprotectives:

- intracellular (DMSO, glycerol)
- extracellular (HES, dextran)
- **5% DMSO is the most suitable**

HES and dextran were found to be poor cryoprotectives, PLTs cryopreserved in glycerol gave lower yields and poor in-vitro viability compared with those cryopreserved in DMSO

Taylor M., Cryopreservation of platelets: an in-vitro comparison of four methods, J.Clin.Pathol, 1981; 34:71-75

2. Platelets can be frozen with 6 % DMSO at -80°C for up to 2 years. This method has been modified by concentrating the PLTs and removing the supernatant before freezing.

Valeri C.R., Rando G., Khuri S., Freezing human platelets using 6% DMSO with removal of the supernatant solution prior to freezing and storage of at -80°C without post-thaw processing, Transfusion 2005; 45:1890-8

3. Experiences with frozen PLT in the Netherlands military

Lelkens C.C.M., Koning J.G., de Kort B., Floot I.B.G., Noorman F, Experiences with frozen blood products in the Netherlands military, Transfus.Apheresis Sci, 2006 Jun; 34(3):289-98

Norman F., Strelitski R., Badloe J.F., -80°C Frozen platelets are activated compared to 24 hour liquid stored platelets and quality of frozen platelets is unaffected by a quick preparation method (15 min) which can be used to prepare platelets for the early treatment of trauma patients in military theatre, AABB meeting, Oct 2012, SP23; Poster presentation



Frozen PLT (DPF) - Methods

PLT for freezing:

- Apheresis (Haemonetics MCS+), group O
- $> 280 \times 10^9$ PLT/unit
- 280 - 330 ml
- Leucodepleted ($< 1 \times 10^6$ WBC/unit)
- $< 5 \times 10^6$ RBC/unit

PLT - freezing:

- ad 6% DMSO (75 ml 25% solution in 0,9%NaCl)
- transfer to freezing bag
- centrifugation
- remove of supernatant
- deep freeze (-80°C)

DFP - thawing and reconstitution:

- thawing ($+36^{\circ}\text{C}$)
- reconstituted in thawed plasma, group AB,
- shelf life 6 hrs in $20-24^{\circ}\text{C}$.



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Preparation and freezing



Apheresis



Ad DMSO



Sterile connetion with cryo bag



Centrifugation



Removing of supernatant



PLT concentrate ready to freeze



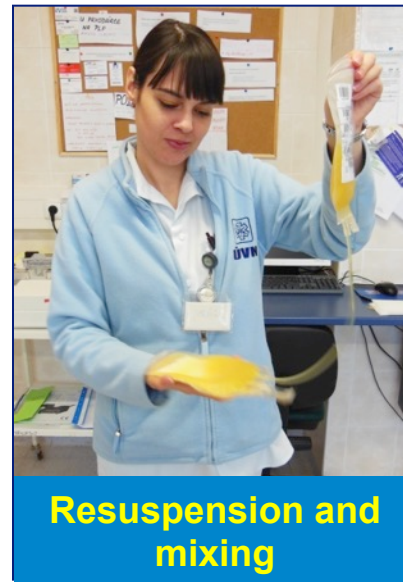
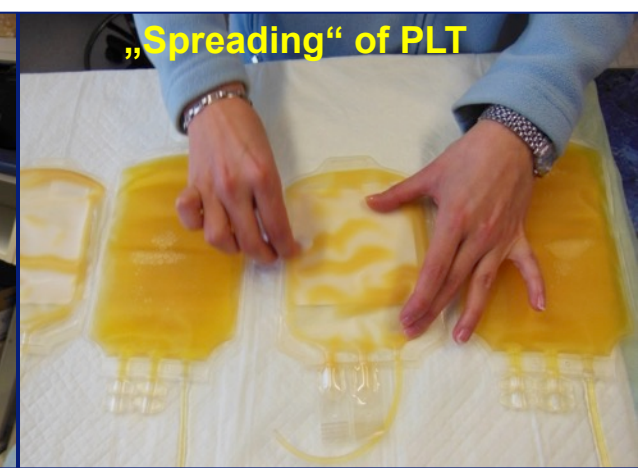
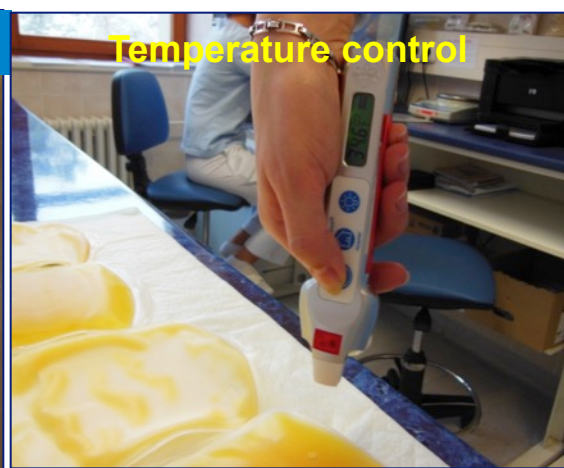
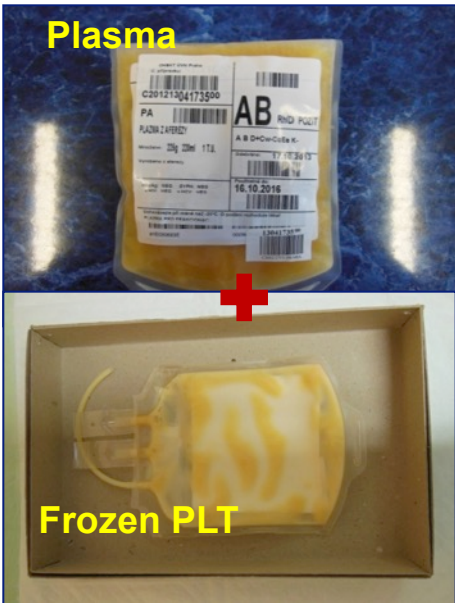
Final product



Deep freezing box



Thawing and reconstitution



Clinical experience with DFP

05/2014 - 05/2018 **375** units PLTs were frozen
09/2014 beginning of clinical use
(bleeding patients, preferably polytrauma)

Transfused total (09/2014 - 05/2018):
90 patients / 281 units DFP

Comparative study:

Classified patients:
25 patients / 81 units DFP

Control group
19 patients / 61 units FAP



Comparative study (09/2014 – 08/2016)

	PTS with DFP	PTS with FAP
Polytrauma (T068)	16	10
Intrakranial bleeding (S0650)	2	3
Hemoperitoneum (T 810)	2	5
Other (hemorrhagic anemia of other origin)	5	1
Total	25	19



Comparative study - parameters

Products:

- TEG

Patients (T0 – before transfusion / T1 – after transfusion):

- Vital signs
 - T
 - P
 - BP
 - GSC
- Laboratory parameters - Blood count (Hbg / Htc, Plt)
 - aPTT
 - PT
 - Fbg
 - ROTEM
- ISS
- Survival
- Adverse posttransfusion effects
- No of transfused blood products (RBC, Plasma, PLT)
- Dosage of Fbg and TXA

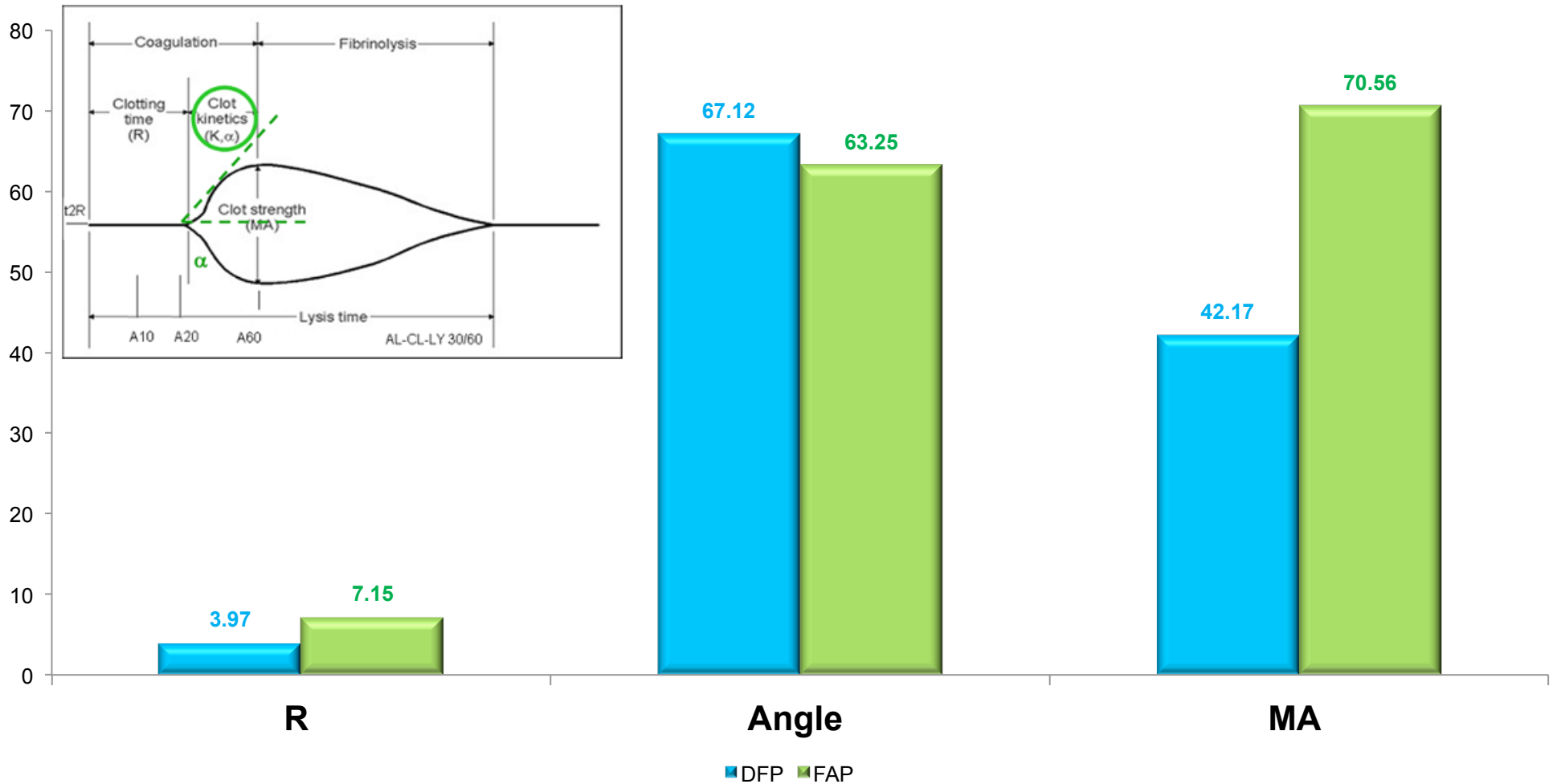


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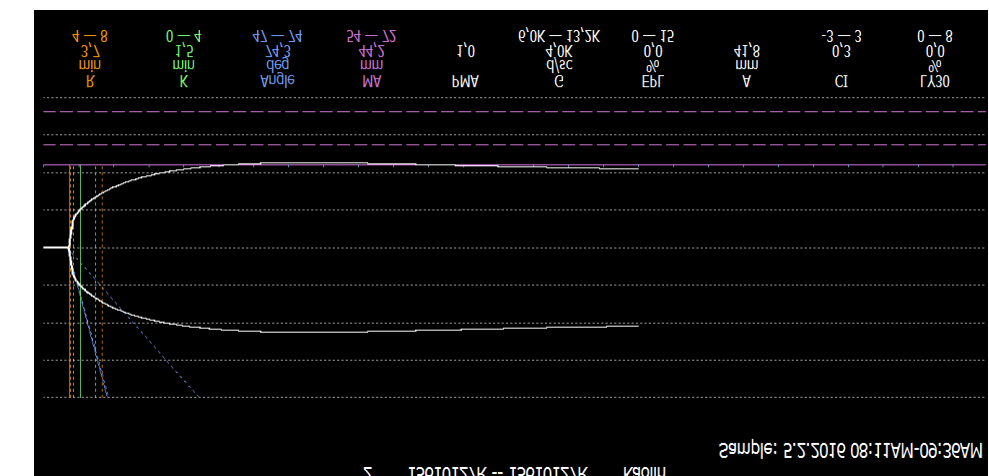
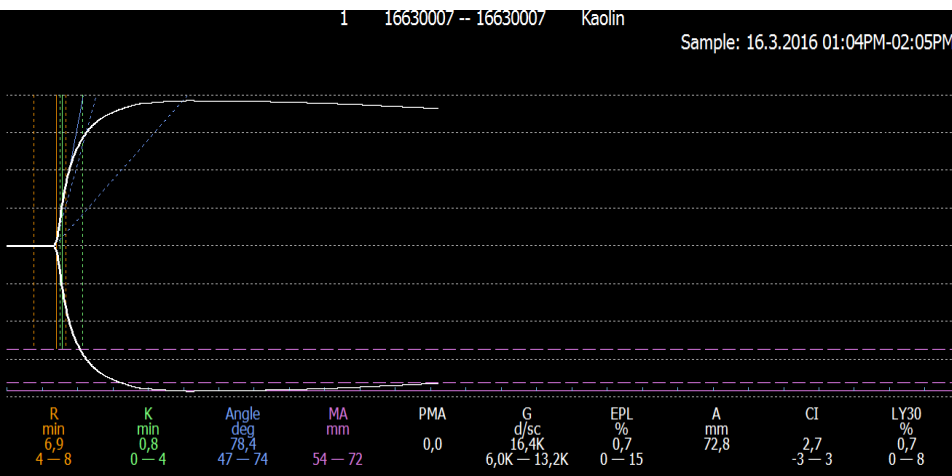
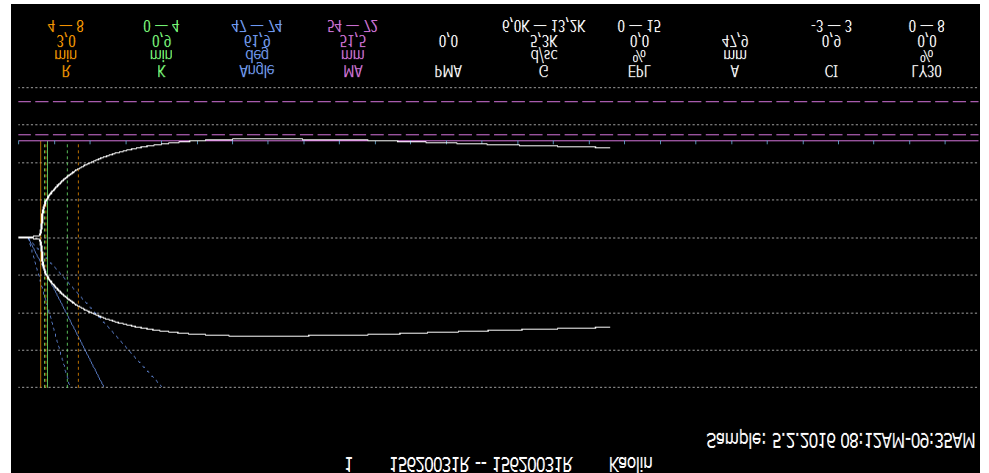
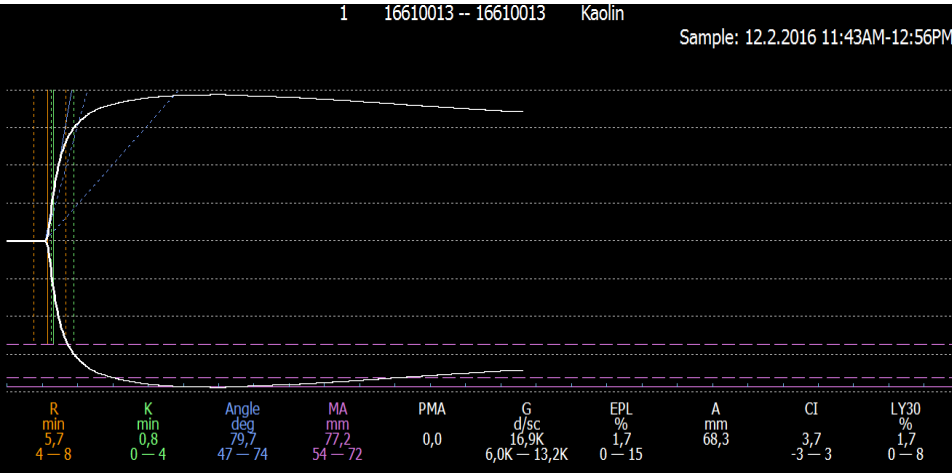


Comparative study: TEG



FAP

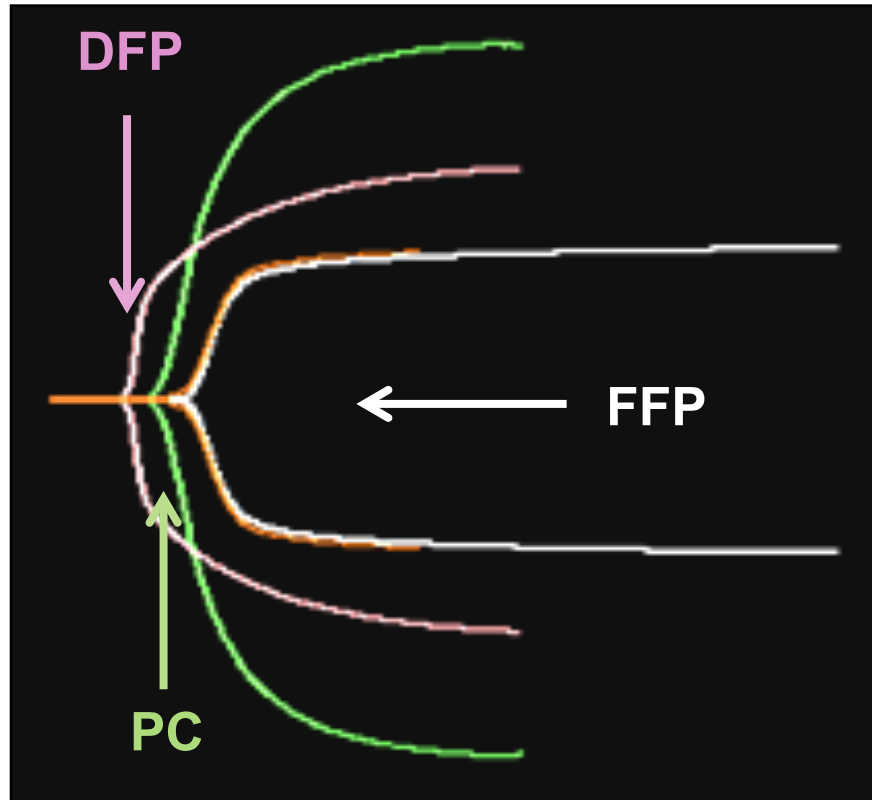
DFP



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... we confirmed what is known:



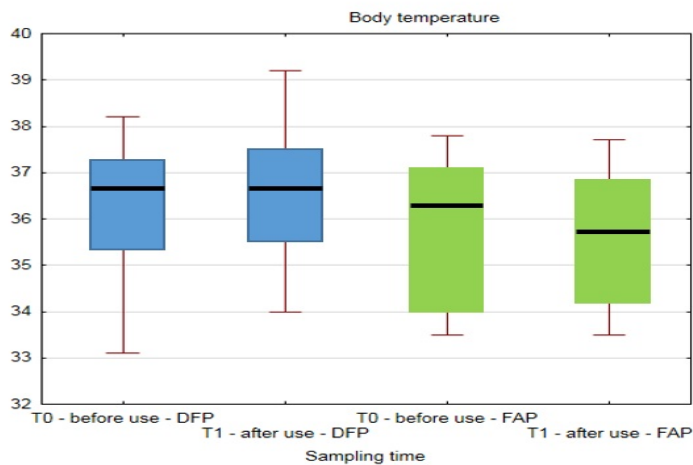
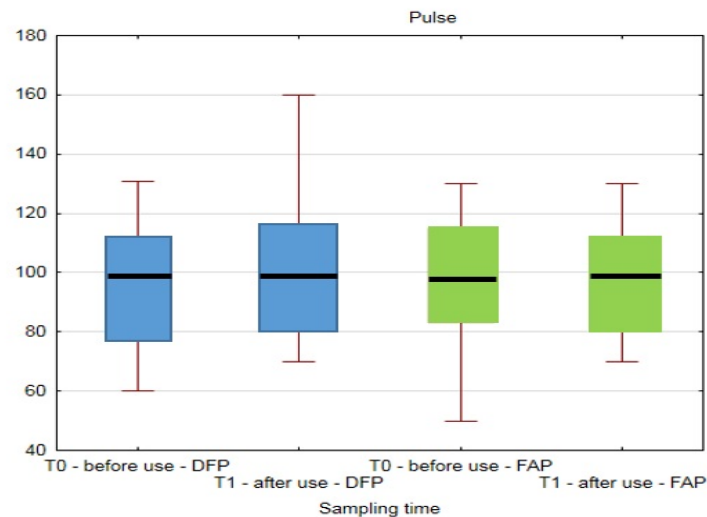
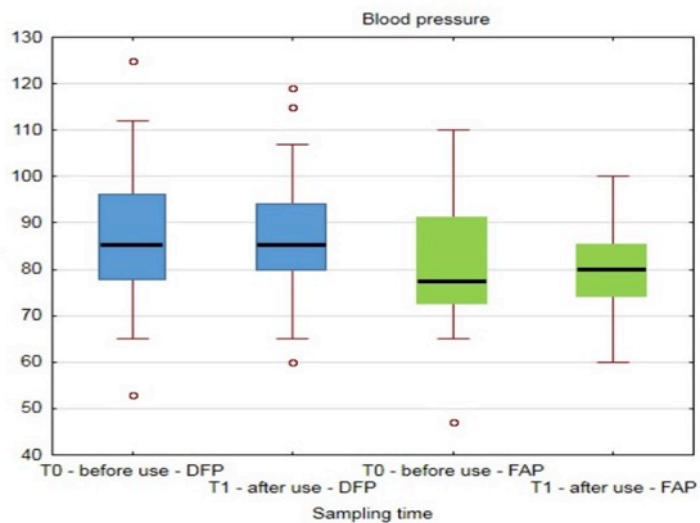
DFP:

- Clotting time is shorter (higher procoagulation activity)
- Clot kinetics is higher (angel α is wider)
- Cloth strenght is reduced (but effective ehough)

Noorman F., -80°C Frozen platelets, plasma and red blood cells, Efficient logistics, Available, Compatible, Safe and Effective, AABB anual meeting, 2012



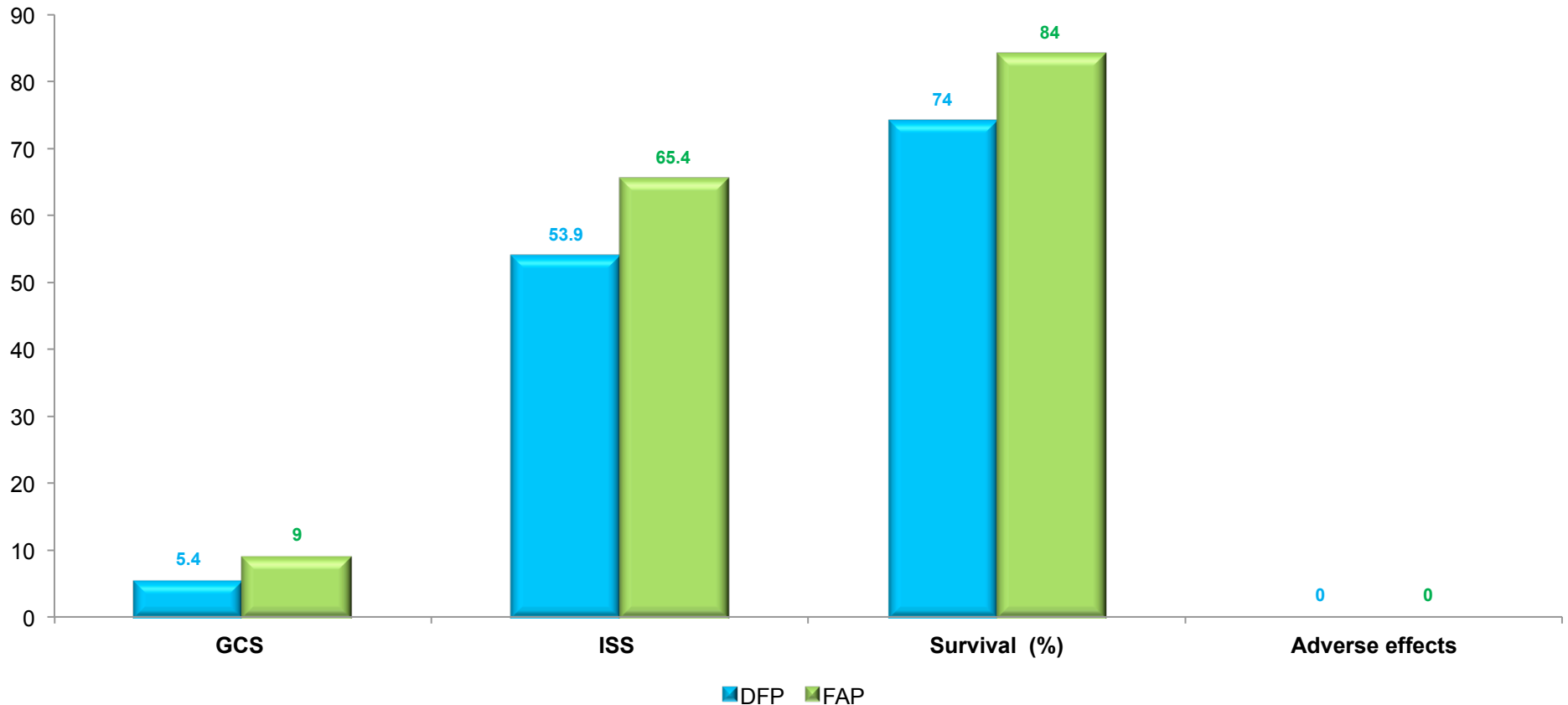
Comparative study – vital signs: Temperature / Pulse / Blood pressure



Student t-test $p < 0.05$



Comparative study: GCS / ISS / Survival / Adverse effects

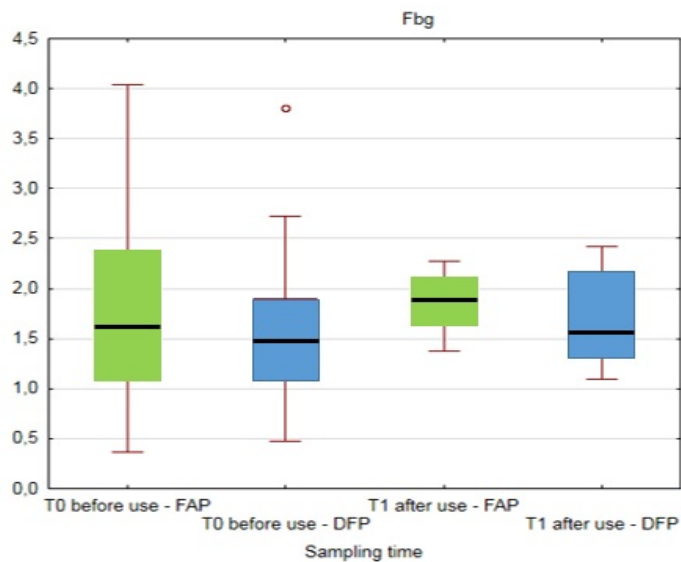
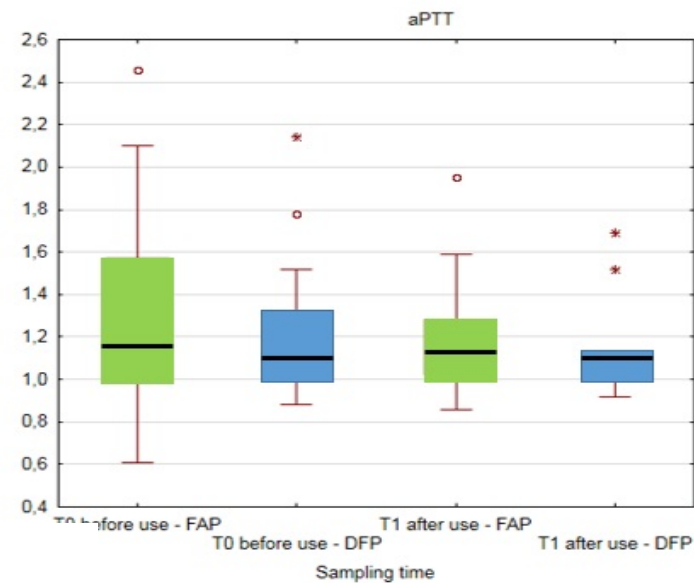
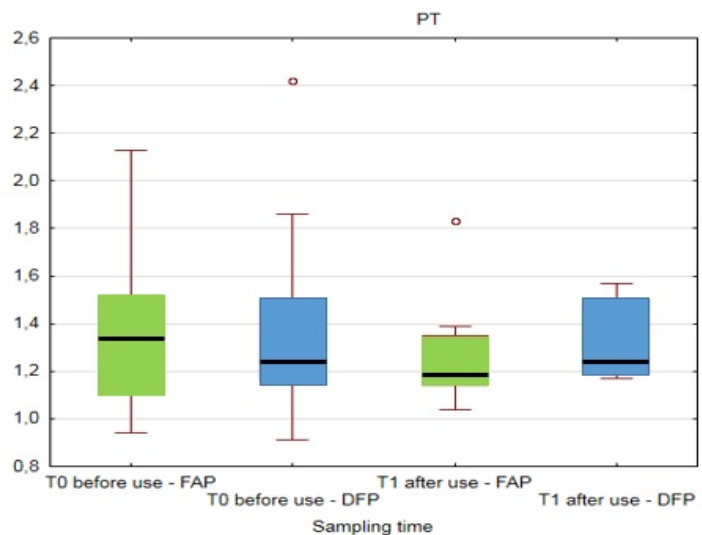


Comparative study
Vital functions / clinical effect:
Conclusion

Patients transfused with DFP did not display any significant differences compared to the group transfused with FAP.



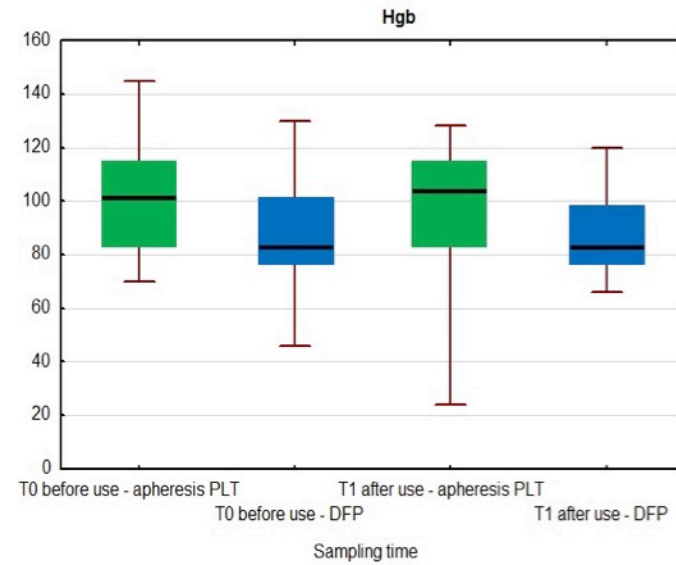
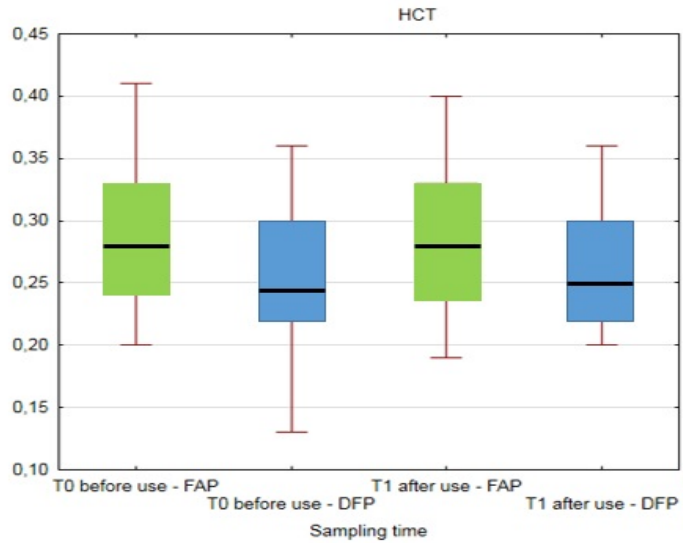
Comparative study: PT / aPTT / Fbg



Student t-test $p < 0.05$



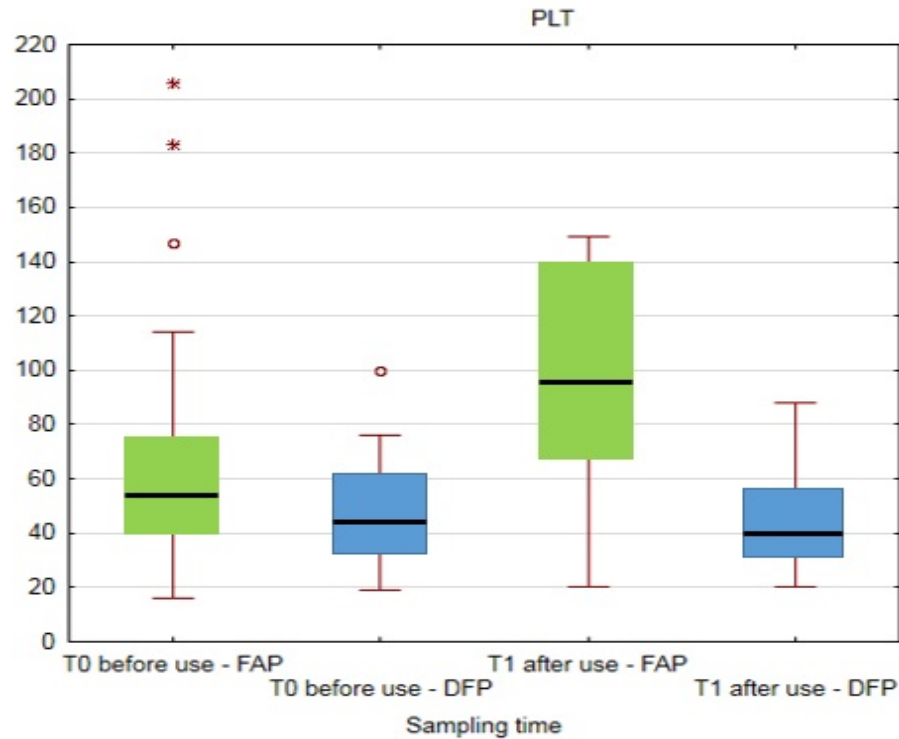
Comparative study: Hgb / Htc



Student t-test $p < 0.05$



Comparative study: Plt



Student t-test $p < 0.05$



Comparative study

Coagulation parameters and blood count:

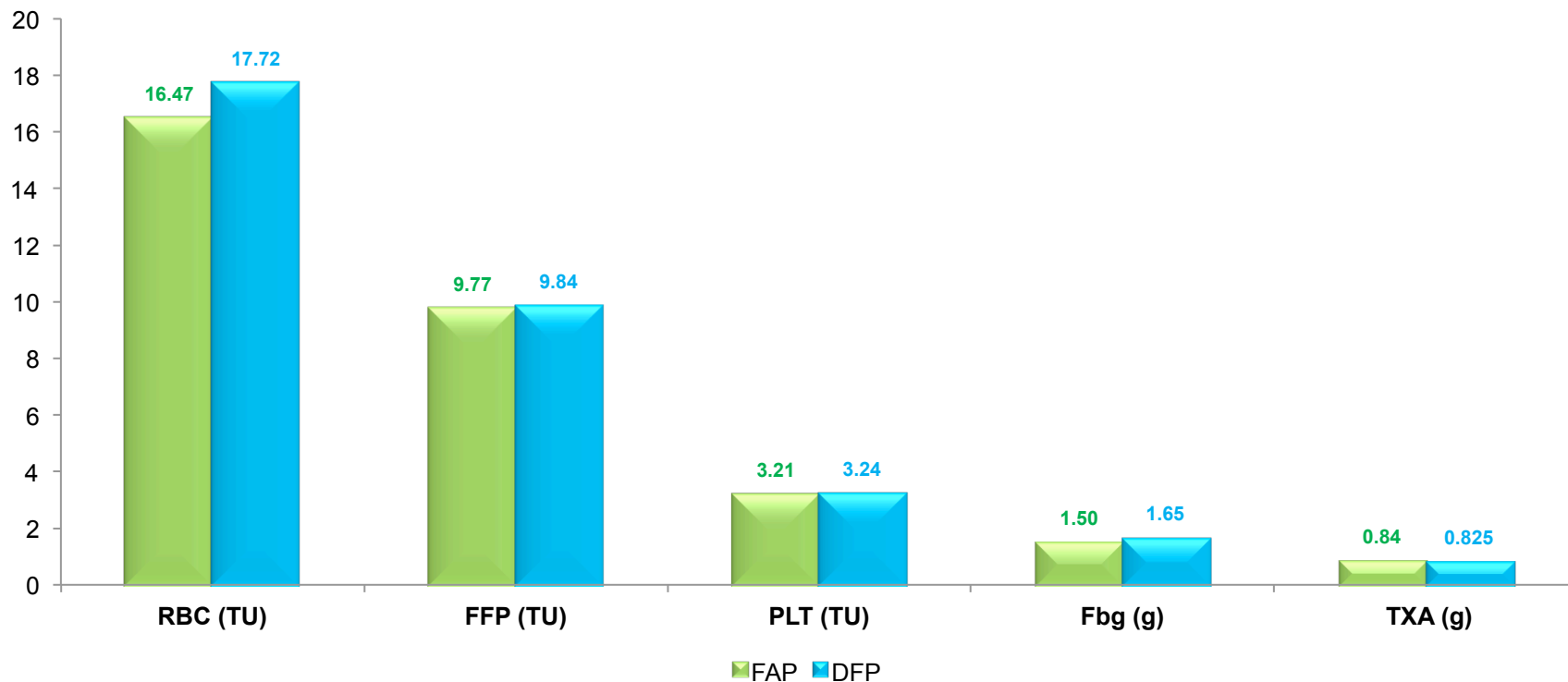
Conclusion

- **No significant differences in PT, aPTT, Fbg, Hb.**
- **PLT count was significantly higher for the group transfused with FAP ($p < 0,05$)**



Comparative study

Use of blood products, Fbg and TXA:



Comparative study

Use of blood products, Fbg and TXA:

Conclusion

No significant differences between the two groups in the mean amount of given **RBC, PLT, FFP, Fbg** and **TXA**



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General conclusion

- 1. Frozen platelets are a beneficial alternative** not only for military blood banks, but also for civilian blood banks which do not have a permanent, or sufficient stock of fresh platelets available.
- 2. Due to a relatively easy preparation, the cost of frozen platelets is not high** and their storing in small portable deep freezers does not bring any significant additional expenses (at most 10% + plasma for resuspension)
- 3. Frozen platelets are safe and effective**
- 4. Procedure of thawing and reconstitution of frozen platelets is very simple and fast**, and it allows for having quality platelets products when dealing with massive bleedings and other urgent situations.



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Department of Hematology and Blood Transfusion



Blood Typing in Pre-hospital Setting
Comparison of Available Tests for Walking Blood Bank

Col.Milos Bohonek, M.D., Ph.D.



Introduction

Integral part of RDCR is the accelerated hemostatic resuscitation, “Blood Far Forward”, where trained medical personnel applies blood products or blood derivatives, including fresh whole blood, collected on-site – “Walking Blood Bank”



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Aim

For safe blood collection and transfusion by the Walking Blood Bank, it is necessary to have a robust, usable assays for determining and verifying the blood group, at least in the ABO system.

With the participation of members of the special forces of the Czech Army, we conducted a check of selected blood group bed-side tests to assess their usability for pre-hospital care during combat deployment.



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Methods

Three CLS specialists from 601st Special Forces Group (601 SFG) were selected. After training, they performed a blood group typing using all the evaluated test-kits.

Each examiner examined 5 blood samples and individually evaluated the parameters:

- test preparation time (sec)
- test time (sec), total test time (sec)
- readability (1-5 points, 1 = worst, 5 = best)
- ease of testing (1-5)
- practicality of the test from the point of view of its field use (1-5)
- influence of the environment on the performance and reading of the test (1-5)
- robustness (1-5)
- durability of the result (1-5)



Examinations were conducted on the ground.

Of all the values, the arithmetic average was used to compare the individual experiments.



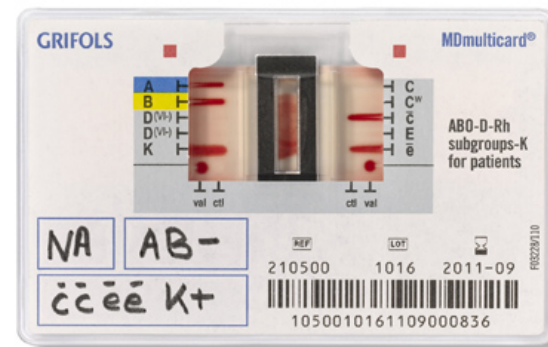
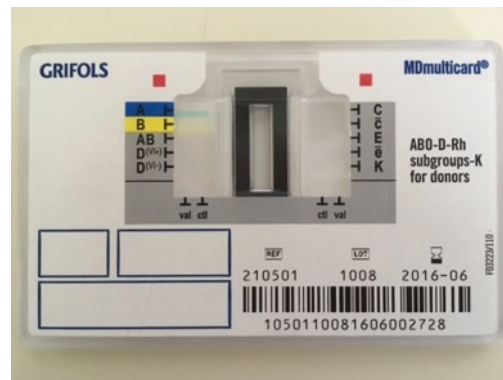
MDmulticard® ABO-D

Manufacturer: Grifols, Spain

Principle: Lateral flow technology is based on the principle of hemagglutination. Monoclonal antibodies anti-A (blue), anti-B (yellow), anti-DVI+ and anti-DVI- are impregnated on the lateral zone membrane. The result is visually readable in 5 minutes.

Positive result: If the erythrocytes carry the given antigen, agglutination and formation of the red strip occur.

Negative result: If the erythrocytes do not carry the given antigen, the agglutination does not occur and the lateral zone is not colored in red.



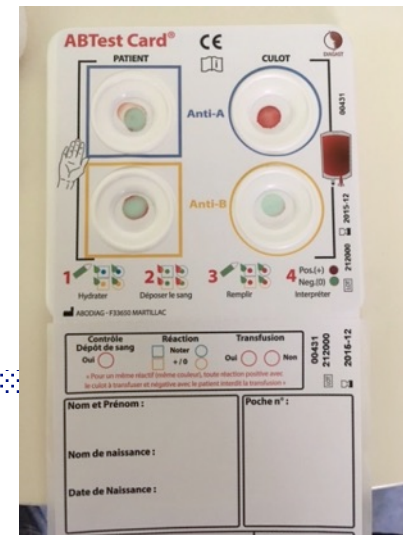
ABTest card®

Manufacturer: DIAGAST, France

Principle: The test uses technology of **covalently bounded antibodies** (anti-A, anti-B) **on the surface of a special membrane in the collection zones - wells**. The individual zones are separated and there is no cross-contamination. The result is read based on the **color change** of the membrane in the well.

Positive result: If the erythrocytes carry the antigen corresponding to the antibody on the membrane, they will be bound and captured and **the membrane in the well turns red**.

Negative result: If the erythrocytes do not carry the antigen corresponding to the antibody on the membrane, these red blood cells pass through and **the membrane in the well turns green**.



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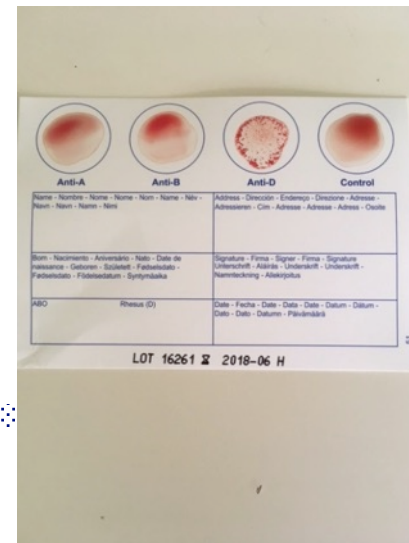
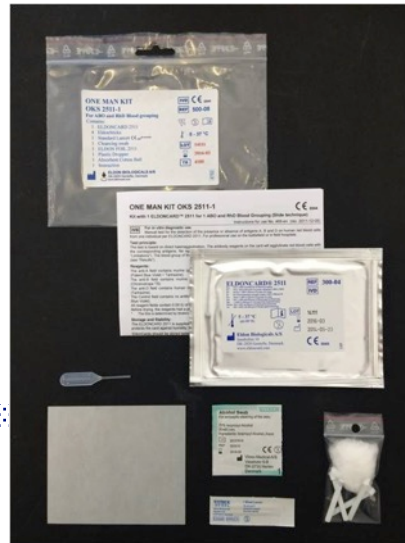
One man kit Eldon Card

Manufacturer: ELDON, Denmark

Principle: Each Eldon card has **four panels**. The first **three panels are impregnated with dried monoclonal antibody sera** anti-A, anti-B, and anti-D; the fourth, **control panel does not contain antibodies**. Adding a few drops of water to each panel activates the antibody sera. Samples of blood can then be added spread across each test area. The results can be read in within a couple of minutes – **agglutination +/-**.

Positive result: **Agglutination** indicates the presence of the antigen on the erythrocyte.

Negative result: **Absence of agglutination** indicates absence of the given antigen on the surface of red blood cells.



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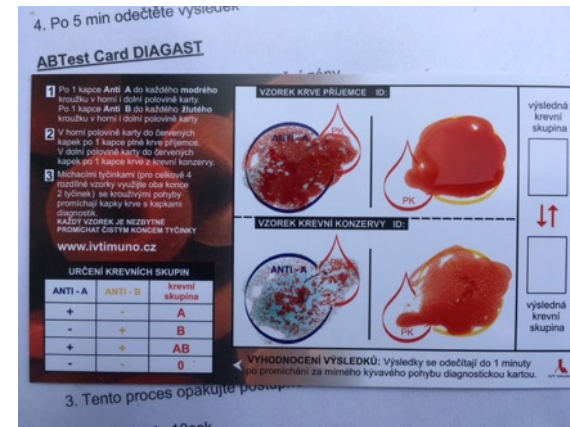
ABO set MP

Manufacturer: EXBIO, Czech Republic

Principle: The test is based on the principle of **hemagglutination**. On the diagnostic card, the ABO blood group of the red blood cells is determined by **mixing a drop of the monoclonal antibody** anti-A (blue vial) and anti-B (yellow vial) **with a drop of whole blood**. The result is ready in 1 minute, with gently swinging of the diagnostic card.

Positive result: **Agglutination** indicates the presence of the antigen on the erythrocyte.

Negative result: **Absence of agglutination** indicates absence of the given antigen on the surface of red blood cells.



Results and Conclusion

Parameter	MD Multicard GRIFOLS	ABTest Card Diagast	One Man Kit Eldoncard	AB0 set MP Exbio
Test preparation tim (s)	24,33	27,4	62,73	31,8
Time of typing (s)	451,6	62,66	119,93	88,7
Total time (s)	475,4	90,2	190,33	110,2
Readability (1-5)	3,26	4,8	4,33	4,66
Ease of testing (1-5)	4,4	4,4	3,13	4,26
Practicality (1-5)	3,46	4,53	2,66	3
Influence of the environment (1-5)	3,8	5	2,26	2,2
Robustness (1-5)	4,33	4,66	5	3,33
Durability of results (1-5)	5	5	1,86	1

ABTest card® Diagast looks to be the most appropriate test for prehospital and field blood typing. The test has the best rating in almost all of the parameters.

The examiners have appreciated its user-friendly features, robustness, and its rapid and easy readability.

Surprisingly, the Eldon Card diagnostic kit, which is currently the most widespread field test for blood typing, did not succeed in the evaluation.



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Thank You !



Questions?

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ANNOUNCEMENT

November 15th 2018

Orea Hotel Pyramida
Bělohorská 24, Prague
Czech Republic



12th



**Střešovice Blood
Transfusion Day**

**Whole Blood
is Comming Back !**

Registration (to November 1st 2018)
and conferece website

www.uvn.cz/transfuzniden



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12th Střešovice Blood Transfusion Day



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The event has the character of a postgraduate education and will be guaranteed by The Czech Medical Chamber.

Date of event: **November 15th, 2018**

Congress Hall - Orea Hotel Pyramida, Bělohorská 24, Prague
The event will be held under the auspices of the Director of the Military Health Department, Ministry of Defense, Colonel Petr Král, MD



The use of **whole blood** has become a valid option in a number countries, be it for the treatment of massive bleeding in cases of polytrauma at emergency rooms, for pre-hospital care in remote areas, or for meeting the needs of air ambulances and military medical care. As such, the use of whole blood, other blood products and blood derivatives in pre-hospital care is being widely discussed in the field of emergency medicine and military health care. A world-renowned experts on this current topic has agreed to participate.

Expert Guarantor:

Col. Miloš Bohoněk, MD, PhD



12th



**Střešovice Blood
Transfusion Day**

**Whole Blood
is Comming Back !**

