EDQM Blood Conference
Innovation in Blood Establishment Processes

14-15 January 2025
Strasbourg, France

Session B2:
Risk-based approach for implementing process changes
(10:45 – 12:15)

Moderators: **Stephen Vardy**, NHS Blood and Transplant, England
Mirela Bušic, Head of SoHO Standards Section, EDQM

Speakers: **Simonetta Pupella**, Italian National Blood Centre, Italy
Dinara Samarkanova, Banc de Sang i Teixits & Transfusional medicine study group, Vall d'Hebron Research Institute, Spain
Marco Amato, Tirol Kliniken, Central Institute for Blood Transfusion and Immunology, Austria
Anita Siller, Tirol Kliniken, Central Institute for Blood Transfusion and Immunology, Austria

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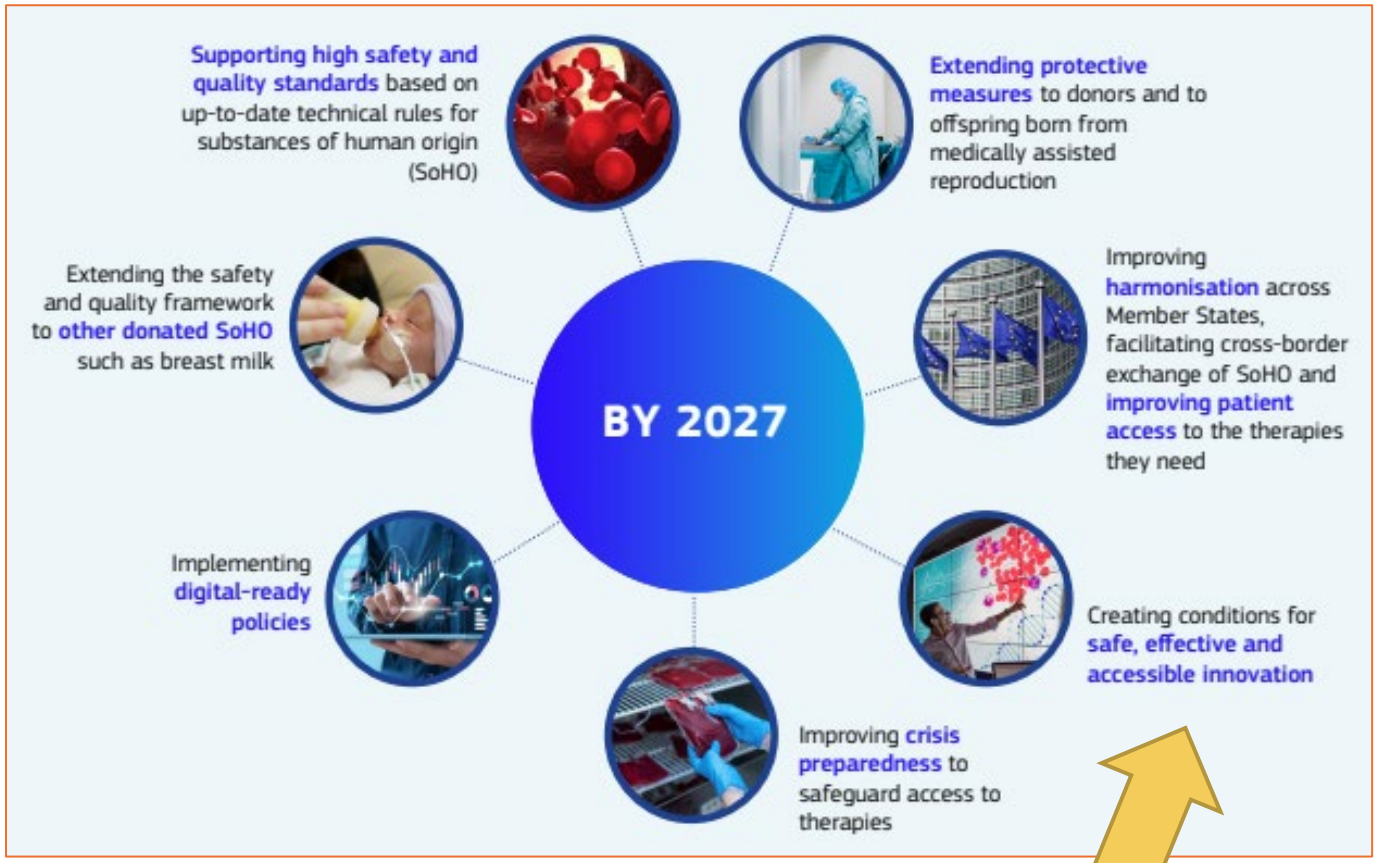
Toward a common approach to authorization of a novel blood component: GAPP-PRO experience

Simonetta Pupella
Italian national Blood Centre – CNS
On behalf of the GAPP-PRO Consortium

Disclosure

I, Simonetta Pupella, hereby declare that I have neither financial nor nonfinancial relationships related to any of the products or services described, reviewed, evaluated or compared in this presentation.

The pathway toward a EU common approach



An early access for patients to new Blood Tissues and Cells (BTC) products addressing unmet clinical needs, and/or providing potentially improved safety and efficacy, requires **adapted regulatory tools** and concepts using **risk-based approaches** to evaluate quality, safety, and effectiveness/efficacy of BTC products.

The roadmap toward a common approach for authorizing novel BTC

1. VISTART Joint Action (2015-2018)

"Principles for Competent Authorities for the **evaluation and approval of clinical follow-up protocols** for blood, tissues and cells prepared with newly developed and validated processing methodologies"

2. GAPP Joint Action (2018-2021)

"Facilitating the authorization of preparation process for blood, tissues and cells"

3. GAPP-PRO Joint Action (2024-2027)

Piloting GAPP model approach for assessing and authorizing novel substances of human origin preparation PROcesses


Official Journal of the European Union

EN
L series

2024/1938

17.7.2024

REGULATION (EU) 2024/1938 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 13 June 2024

Definitions (Regulation EU 2024_1938)

(39) **‘effectiveness of SoHO’** means the extent to which the human application of SoHO achieves the intended biological or clinical outcome in the SoHO recipient;

(40) **‘SoHO clinical study’** means an experimental evaluation of a SoHO preparation, with the objective of drawing conclusions regarding its safety and effectiveness;

(41) **‘SoHO compendium’** means a list kept up-to-date by the SoHO Coordination Board (SCB) of decisions, taken at Member State level, and opinions, issued by SoHO competent authorities and by the SCB, on the regulatory status of specific substances, products or activities, and published on the EU SoHO Platform;

Definitions (Regulation EU 2024_1938)

(37) **‘SoHO preparation**’ means a type of SoHO that:

(a) has been subjected to processing and, where relevant, one or more other SoHO activities referred to in Article 2(1), point (c);

(b) has a specific clinical indication; and

(c) is intended for human application to a SoHO recipient or is intended for distribution

(38) **‘SoHO preparation authorisation’** means the formal approval by a SoHO competent authority of a SoHO preparation;

Preparation Process Authorization (PPA)

On the side of BEs

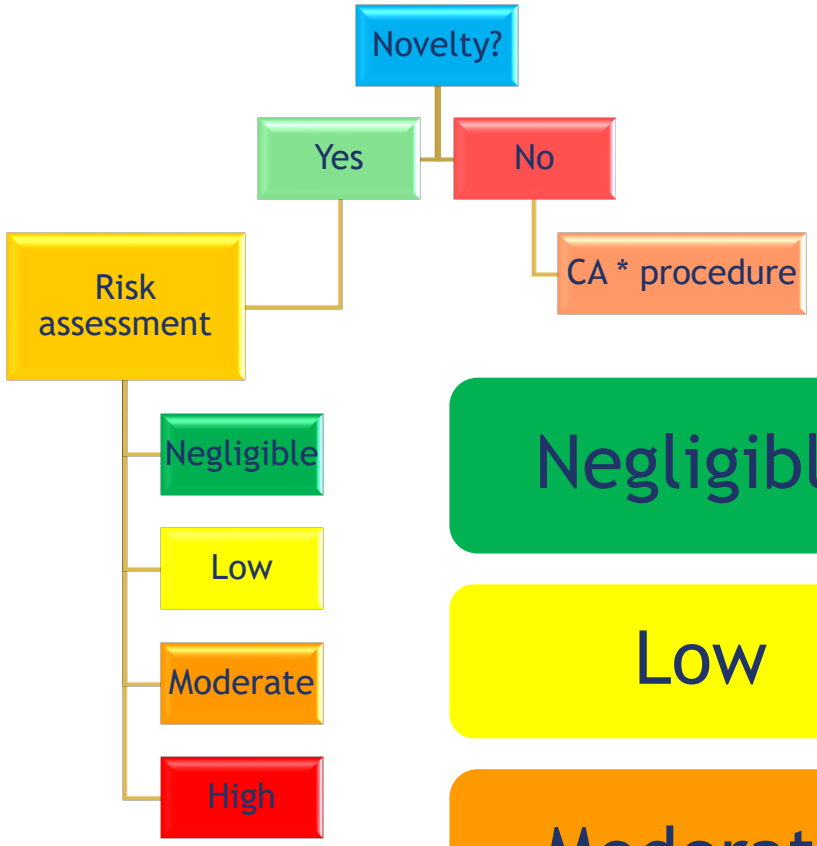
1. Systematic Benefit/Risk Assessment to determine the evidence available on safety, quality and effectiveness

Taking into account any relevant EDQM monograph

2. Submission of an application, including laboratory validation and other safety, quality and effectiveness data and, **where relevant**, a **clinical outcome monitoring plan** proportionate to risk

GAPP

Preparation Process Authorization (PPA)



Negligible

- Novelty questions, risk assessment, PPD (only the information regarding the novelty).

Low

- Novelty questions, risk assessment, PPD (only the information regarding the novelty) and the proposal of follow up.

Moderate

- Novelty questions, risk assessment, PPD (only the information regarding the novelty) the proposal of follow up and the clinical investigational plan.

High

- Novelty questions, risk assessment, PPD (only the information regarding the novelty) the proposal of follow up, the clinical investigational plan and the comparison to the standard therapy.

Preparation Process Authorization (PPA)

On the side of CA

3 Assessment of the application by the competent authority

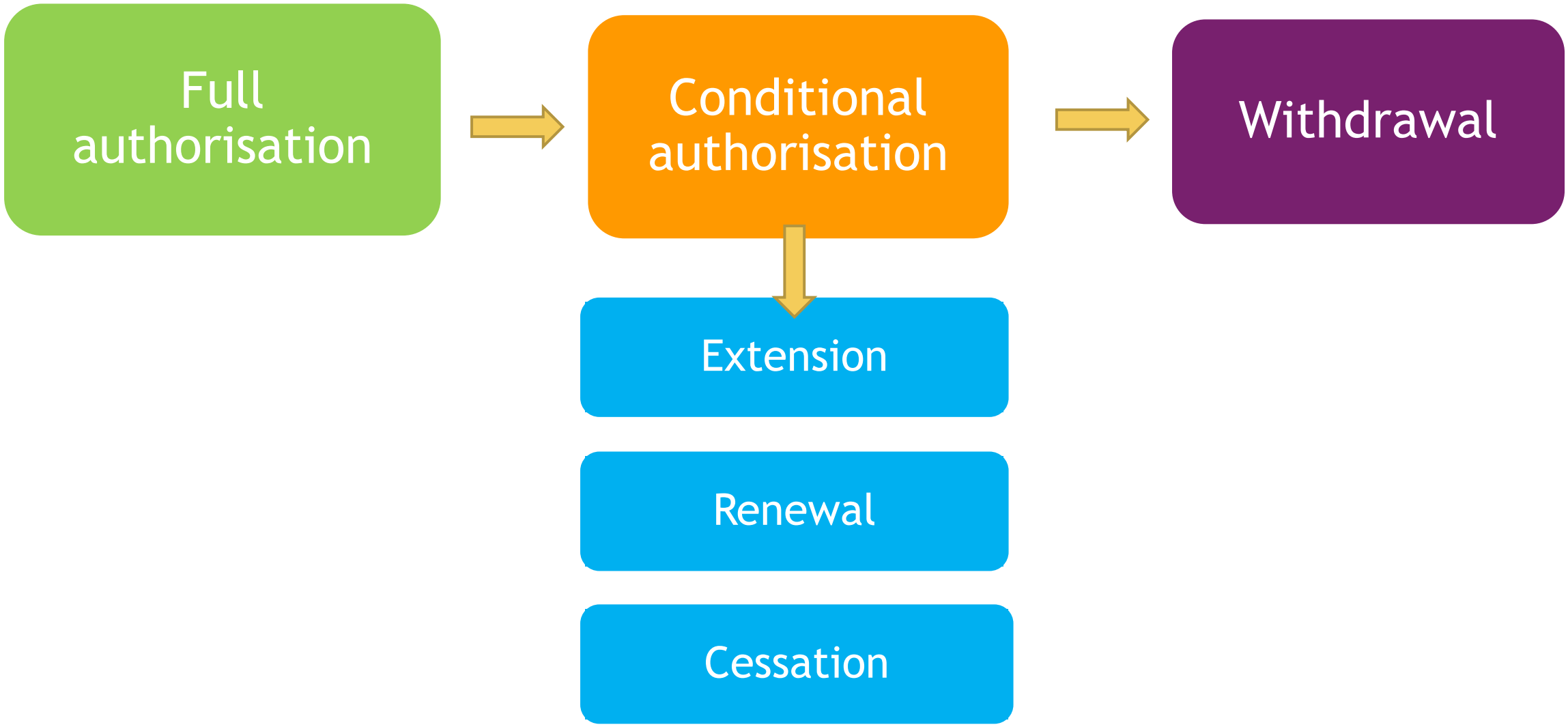
Grant authorisation for the SoHO preparation

OR

Grant of an approval of the Clinical Outcome Monitoring plan

4 Assessment by the competent authority of evidence of safety, quality and effectiveness data gathered in clinical outcome monitoring

Preparation Process Authorization (PPA)



GAPP methodology: application process

Applicant information	BTCE information and data of person responsible for the dossier.
BTC novelty	Information of the BTC where the novelty will be applied as well as the description of the novelty.
Risk Assessment	Using EUROGTP II tool.
Quality	Information of the new related SOPs, quality control procedure, validation, stability and evaluation.
Preclinical studies	Information of non-clinical (in vitro or/and in vivo) studies.
Clinical information	To support the implementation of the novelty.

GAPP methodology: technical annexes

- **Deliverable 6.1**: Technical Annex on authorisation changes in donation, procurement and collection, processing, preservation, storage and distribution of BTC.
- **Deliverable 7.1**: Technical annex on assessing the quality and safety of donor testing, microbial inactivation and sterilisation steps as part of PPA.
- **Deliverable 8.1**: Catalogue of existing clinical data appropriate to provide information on the quality and safety of BTC once applied to patients, under the conditions of current state-of-the-art manufacturing and testing protocols.
- **Deliverable 8.2**: Catalogue of risk-based set of criteria, appropriate to evaluate the established catalogue of clinical data for completeness and suitability in case of introduction of innovation to the current manufacturing and testing protocols for human BTC.
- **Deliverable 8.3**: Methodological framework to evaluate quality and safety of BTC based on clinical outcome data requested for authorisation processes upon introduction of innovation to the current manufacturing and testing protocols for BTC.
- **Deliverable 8.4**: Data model of information on clinical outcome of application of BTC.

FACILITATING THE AUTHORISATION OF

GAPP

PREPARATION PROCESS FOR BLOOD, TISSUES AND CELLS

GOOD PRACTICE GUIDELINE TO AUTHORISATION ON PREPARATION PROCESSES IN BLOOD, TISSUES AND CELLS ESTABLISHMENTS

GAPP methodology: preparation process dossier (PPD)

Module 1: Applicant information

- BE/TE data.
- Data of the responsible person for the PPD.

Modules 2 and 3: Novelty and risk assessment

- Description of BTC.
- Novelty Questions.
- Activity information.
- Risk Assessment.

Module 4: Quality

- Updated SOPs.
- Validation.

Module 5: Preclinical studies

- *In-vitro/In-vivo* studies
- Performed studies.
- Bibliography.

Module 6: Clinical information

- General clinical information.
- Clinical indication.
- CIP.
- CFUpP

GAPP-PRO will pilot and roll-out approach by 2027

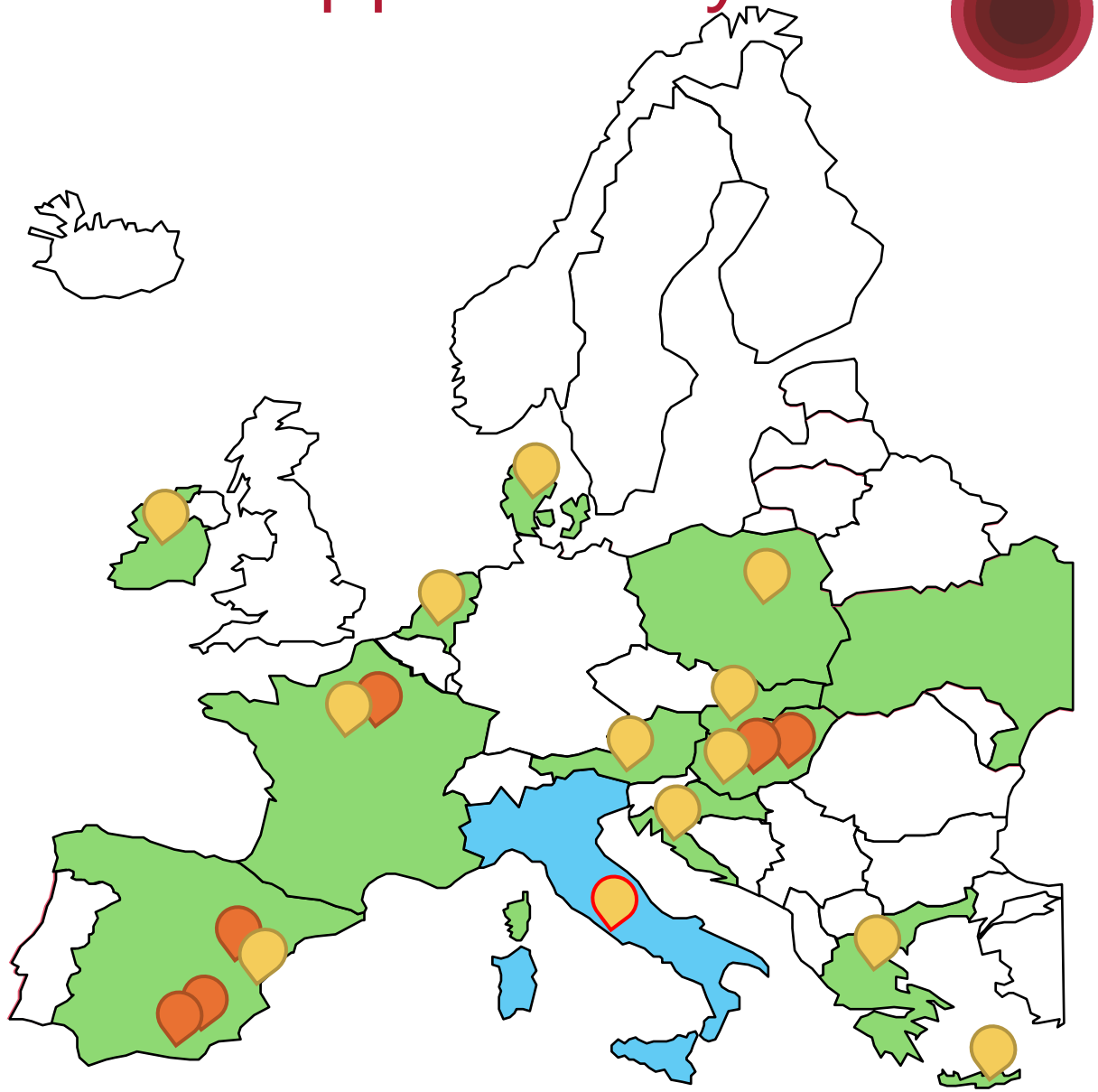
14 Main beneficiaries

7 Affiliated entities

from 13 EU countries and 1 non-EU country

Project start date: 15/02/2024

Project duration: 40 months (14/06/2027)



GAPP-PRO: main objectives

- Map current status of authorised SoHO preparations and inherent risks
- Pilot GAPP methodology: test, assess and improve
- Test cross-entity/country applications and assessments
- Test cross-sector collaboration for SoHO preparations entailing medical devices
- Refine and update the methodology

GAPP-PRO: technical WP objectives

WP4 **Snapshot of SOHO preparation processes in Europe grouped by different risk level, including bed-side preparations**	To gain clear insight into the current European authorization of SoHO preparation processes, including bed-side preparations, grouped by different risk level. In particular it will: • investigate the presence of ongoing evaluation of new SoHO preparation processes; • investigate the presence of **already authorised SoHO preparation processes in relation to identified risk level**
WP5 **Pilot-test of GAPP methodology on SoHO**	To **assess the GAPP methodology applicability** on selected SoHO (including at least 2 autologous bedside preparations), from application to final assessment in order to: • Test the evaluation of different levels of risk (negligible, low, medium, high); • Detect strengths and weaknesses of GAPP methodology through the performance of a SWOT analysis.
WP6 **Pilot-test of GAPP methodology for cross country and joint country assessments**	To organise and perform **cross-country applications and joint-country assessments** involving a group of Member States and experts (inspectors and assessors) in order to test and prove its feasibility and added value.
WP7 **Analysis of pilot tests results**	To perform a thorough **analysis of pilot outcomes**, including interactions in the assessments and authorisation process with those of other regulatory frameworks, for example, where a new SOHO preparation process relies on the use of a new medical device.
WP8 **Refine of GAPP Guideline**	To **refine/update the GAPP Guidelines** on the basis of the pilot-tests results. Moreover, within this WP, the existing EUROGTP II platform will be extended to other SoHO (i.e. breast milk and faecal microbiota) so to provide European professionals with the opportunity to perform risk assessment also for other products.

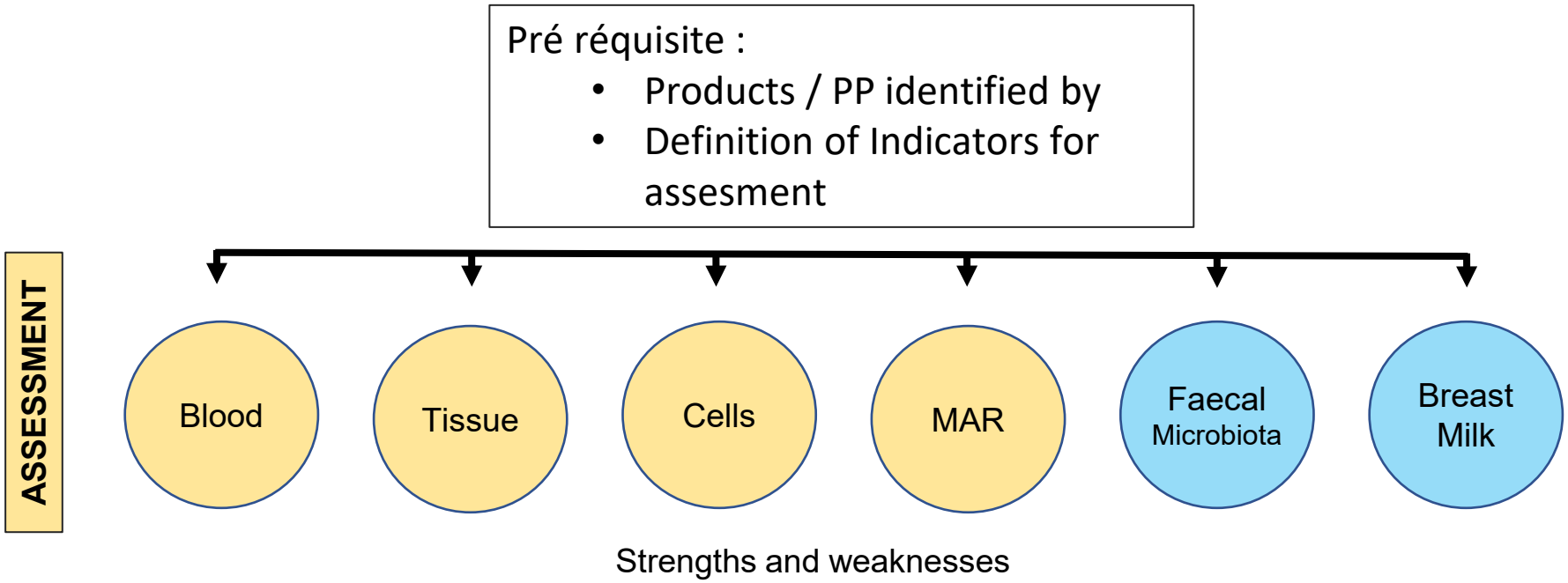
GAPP-PRO: technical work packages

WP5 **Assessment of the GAPP methodology applicability on selected SoHO, from application to final assessment.**

The WP5 objective is to assess the GAPP methodology for different risk levels. The desired aim is the improvement of the method that will be standardized for all EU members.

Objectives are :

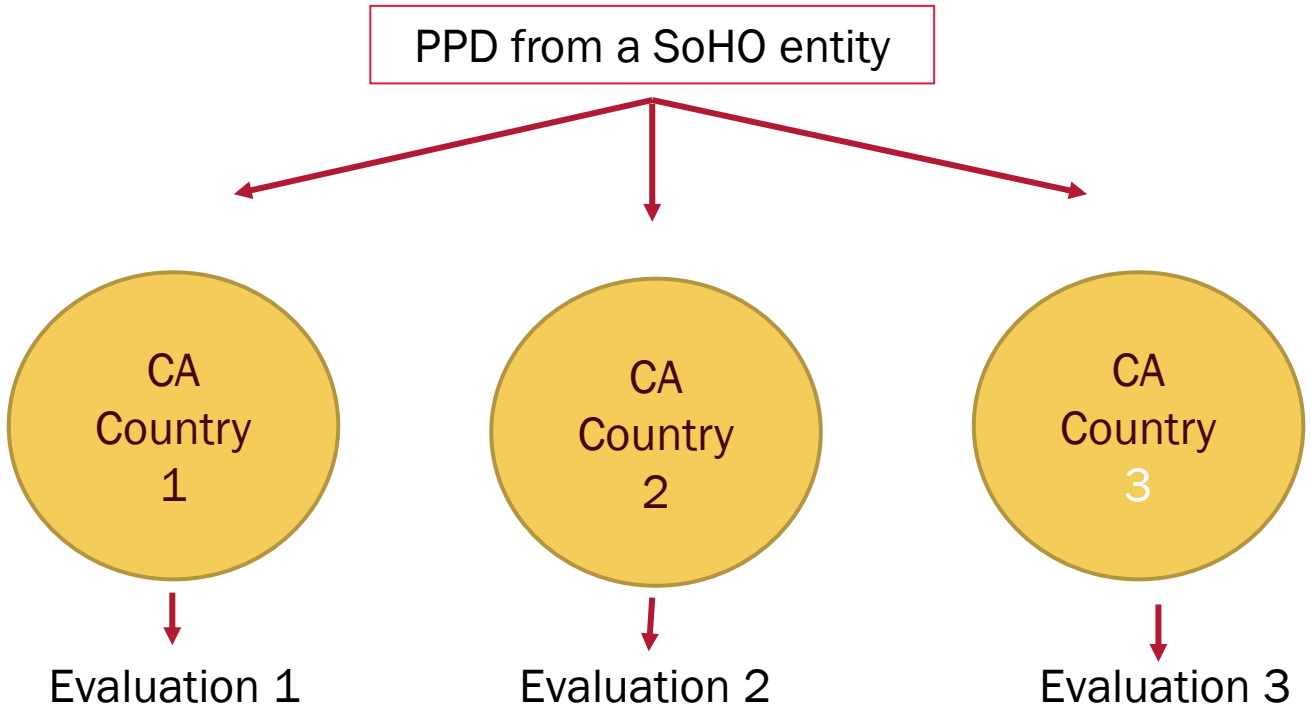
- Test the evaluation of different levels of risk (negligible, low, medium, high);
- Detect strengths and weaknesses of GAPP methodology through the performance of a SWOT analysis.



WP6

Constitution of WGs for cross country evaluation

- Each PPD will be evaluated by at least 3 CAs using the GAPP Methodology. Evaluations will be done separately.

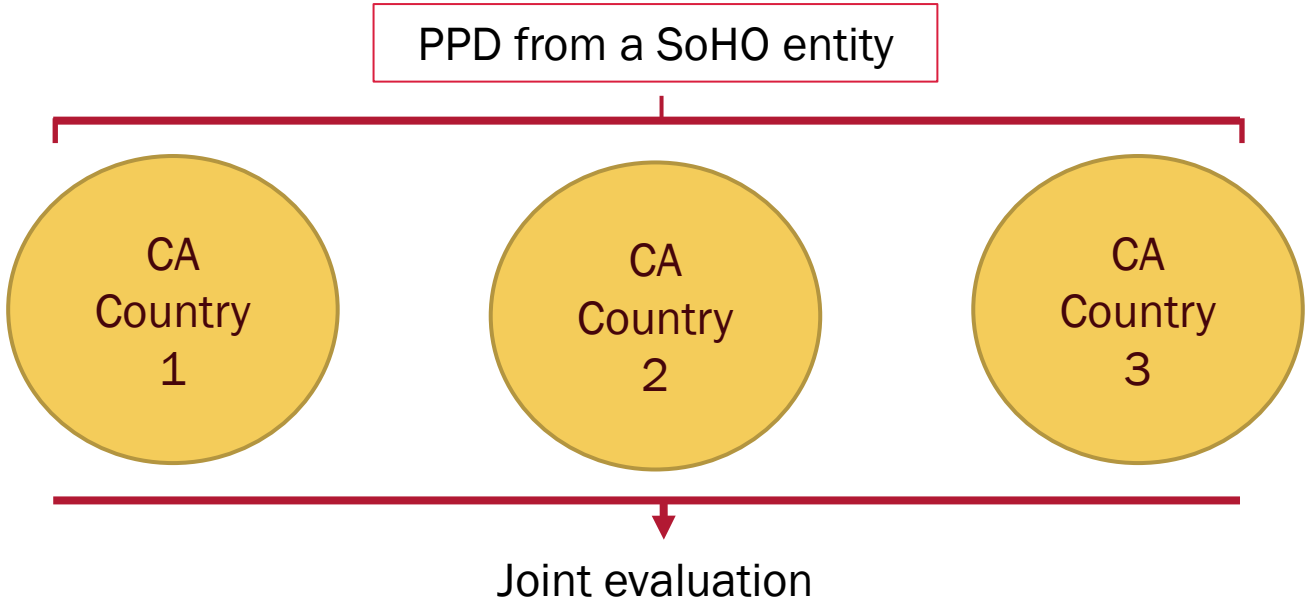


Identification of potential obstacles in the interpretation of GAPP methodology.

WP6

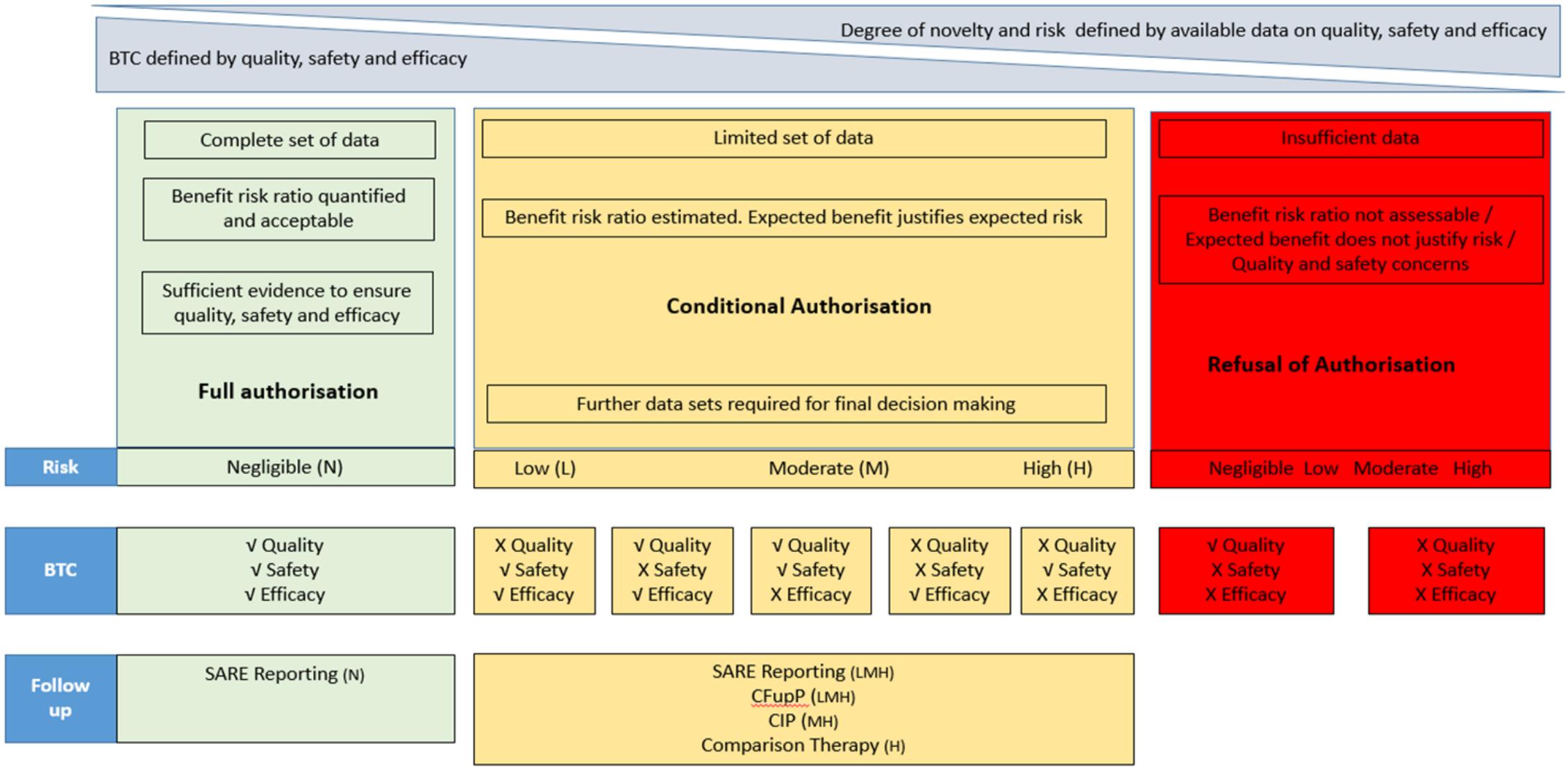
Constitution of WGs for cross country evaluation

- 2 or more CAs of different countries will jointly perform the application and authorization (on same product/process) covering blood, T&C and MAR.



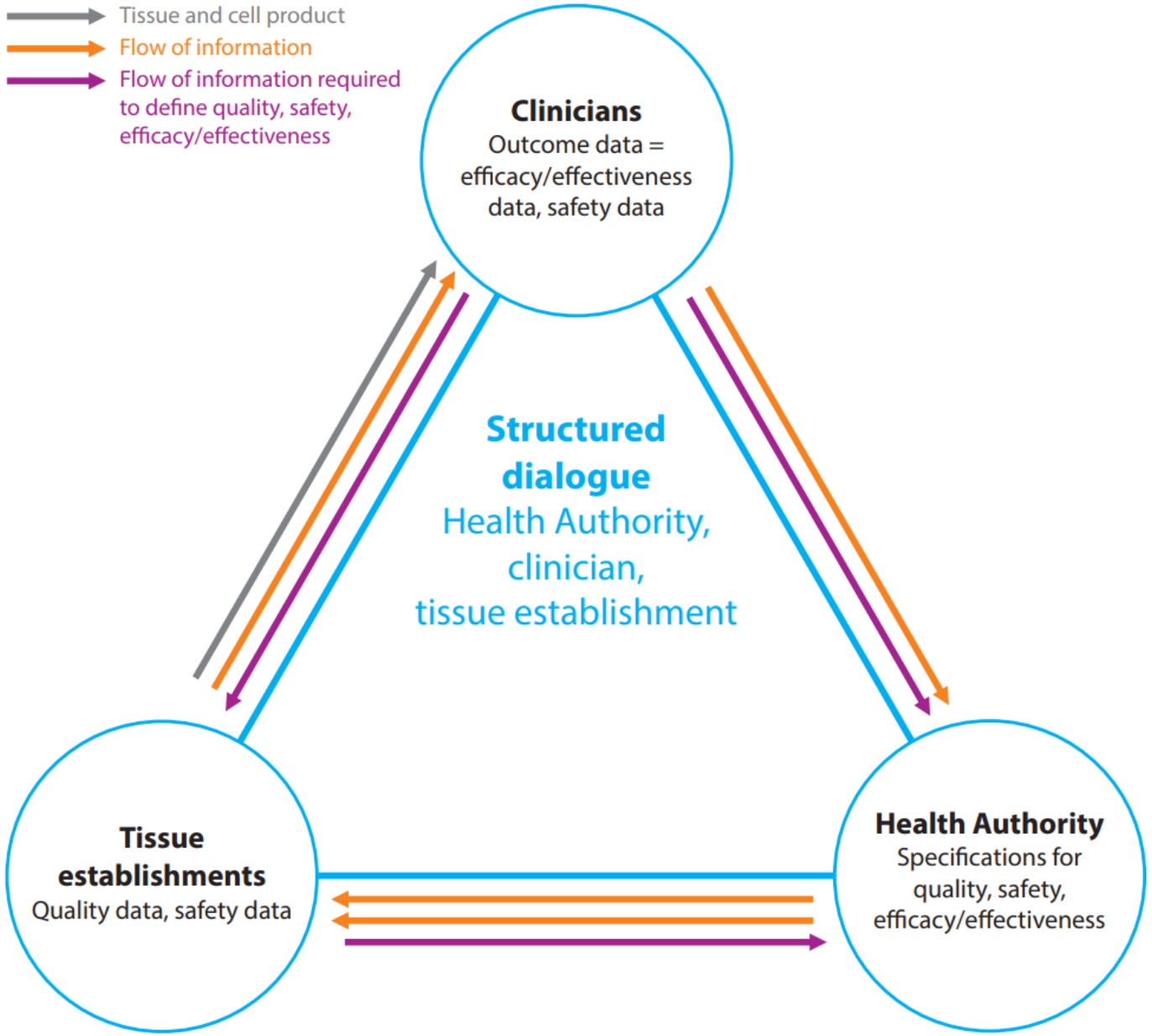
Identification of potential obstacles in the interpretation of GAPP methodology.

Risk/benefit balance



EDQM – Guide
T&C 2022 5° Ed
Ch 18
Introduction of novel processes and clinical applications

Figure 18.1. **Flow of information between tissue establishments, clinicians and Health Authorities**



Expectations from GAPP-PRO

- Member States know how to manage SoHO preparation authorisations (SPA)
  - Awareness building, preparation, training
  - Organisation of national pathway for SPA
  - Leverage cross-country collaboration, bringing all MS to high/similar level of SPA
  - Trust building with other sector authorities (in particular medical devices)

- Link to SoHO digital platform:
  - Compendium
  - (application/authorisation module)
  - EuroGTP-II tool

Thanks to the Consortium

WP	GAPP-PRO
WP1 and WP2	ISS/CNS-CNT (Italy): L. Cannata, A. Palmieri, I. Denaro, P. Di Ciaccio, C. Carella, S. Pisanu, M. Mareri, B. Mazzanti, E. Pianigiani, S. Pupella, A. Vassanelli, M.C. De Stefano, U. La Rocca, F. Bariani
WP3	PUMS (Poland): K. Wszołek, K. Żukowska, B. Raducha, M. Nowicki
WP4	PUMS (Poland): K. Wszołek, B. Raducha, M. Nowicki, J. Rogalinski, S. Tomczak SZU (Slovakia): S. Bopegamage, M. Borsányiová
WP5	ABM (France): S. Arrabal, B. Derycke
WP6	ABM (France): S. Arrabal, B. Derycke AGES (Austria): V. Plattner
WP7	OCATT (Spain): R. Barrio, J. Tort
WP8	OCATT (Spain): R. Barrio, J. Tort ISS/CNS-CNT (Italy): L. Cannata, A. Palmieri, I. Denaro, P. Di Ciaccio, C. Carella, S. Pisanu, M. Mareri, B. Mazzanti, E. Pianigiani, S. Pupella, A. Vassanelli, M.C. De Stefano, U. La Rocca, F. Bariani

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Thanks for your attention

Expanding the use of cord blood units for manufacturing platelet derived products:

assessment of clinical-grade products from *low volume (<75 mL)* units and/or *48-80 hours of* storage time

within multicomponent Cord Blood Bank at Blood and Tissue Bank of Barcelona

Dinara Samarkanova[1,2], *Nina A.M. Houben* [3,4], *Margarita Codinach*[1,2], *Elisenda Farssac*[1,2], *Carmen Azqueta*[1], *Elena Valdivia*[1,2], *Lluis Martorell*[1,2], *Nuria Rubio*[1], *Nerea Castillo-Flores*[1], *Sergi Querol*[5], *Jesus Fernandez-Sojo*[1,2]

1. Banc de Sang i Teixits, Barcelona, Spain
2. Transfusional medicine study group, Vall d´Hebron Research Institute, Barcelona, Spain
3. Sanquin Research, Sanquin Blood Supply Foundation, Amsterdam, the Netherlands
4. Leiden University Medical Center, Leiden, the Netherlands
5. Josep Carreras International Foundation, Barcelona, Spain

Disclosure

- No conflicts of interest to declare

Index

Introduction

- Biology of cord blood and cord blood components in public cord blood banks (CBB)

Methods

- Processing in a multicomponent CBB
- Key topics for risk assessment: one donor-one dose; expiration time; use of units above 100 grams
- Quality control, rapid microbiology testing; and *in vitro* validation

Results

- Clinical applications of red blood cells, platelet gel and eye drops
- Risk assessment

Discussion

- Biovigilance: main adverse events and follow up
- Improving storage: inactivation prior lyophilisation

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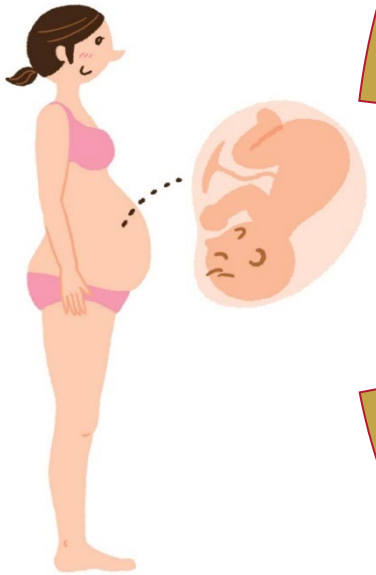
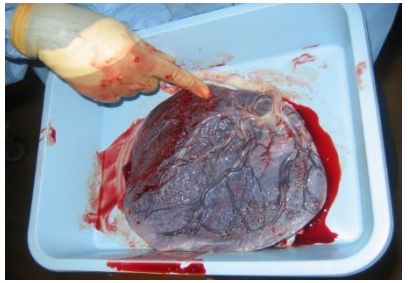
Introduction – biology of cord blood and cord blood components in public CBB

DELIVERY ROOM



CONCORDIA DONOR PROGRAM MATERNITIES

MEDICAL HISTORY
INFORMED CONSENT



TRANSPLANT

CORD BLOOD BANK

BLOOD COMPONENTS

PLATELET RICH PLASMA

HPC,CB

RBC CONCENTRATE

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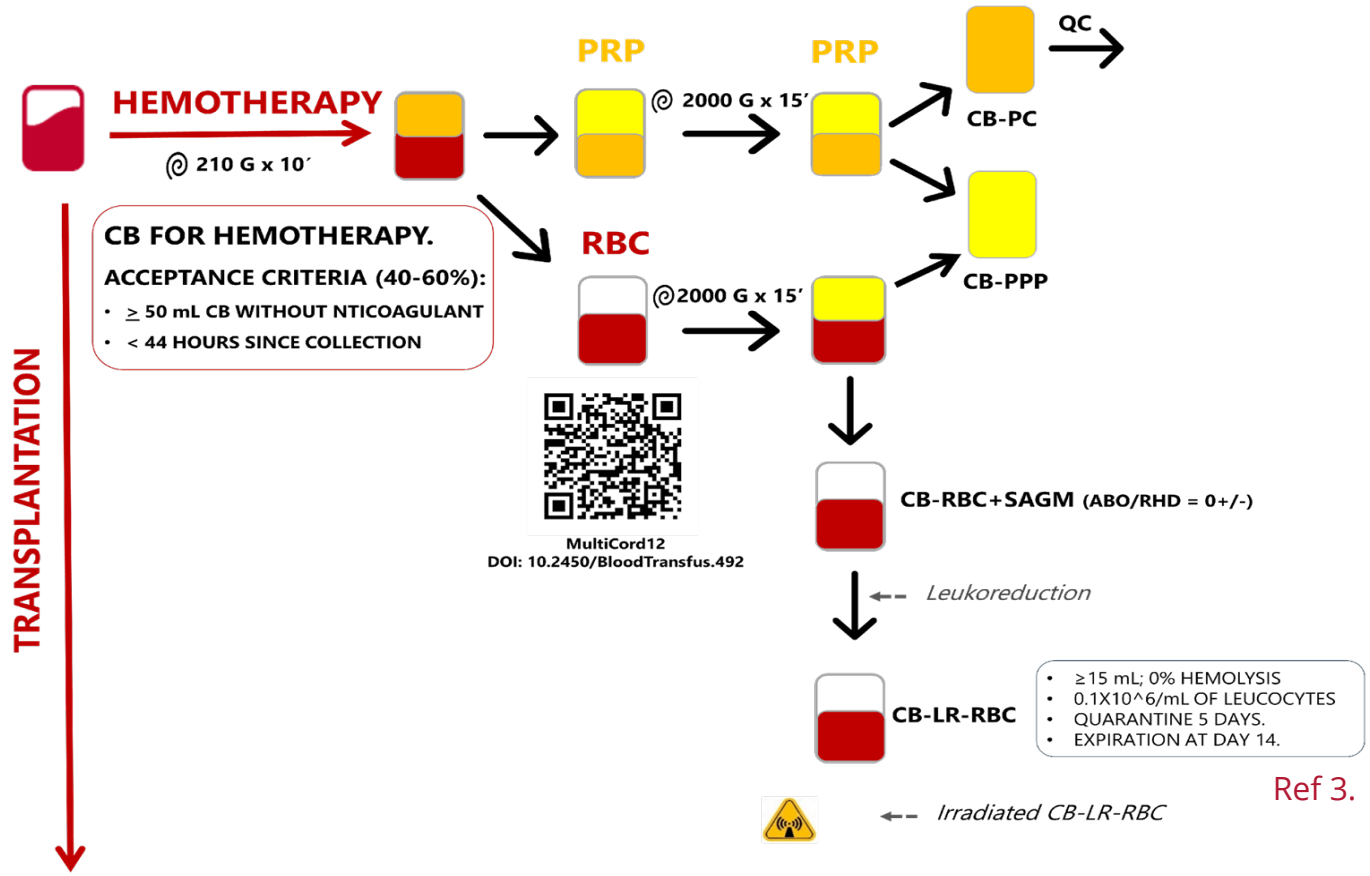
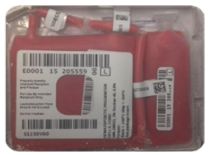
Methods



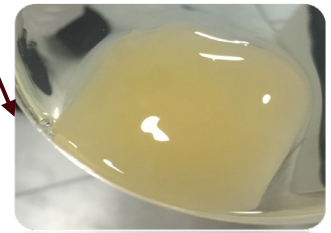
Ref 3.



CB FOR TRANSPLANTATION
ACCEPTANCE CRITERIA:(10-15%):
- ≥1500E6 CN
- ≥4E6 CD34

- 1000±200 x10^6 platelets / mL
- 10±5 mL
- Sero/Micro negative

Ref 1.



Ref 2.

1. Samarkanova D et al. Cord blood and amniotic membrane extract eye drop preparations display immune-suppressive and regenerative properties. Sci Rep. 2021, 2;11(1):13754. doi: 10.1038/s41598-021-93150-7.
2. Samarkanova D et al. Cord blood-derived platelet concentrates as starting material for new therapeutic blood components prepared in a public cord blood bank: from product development to clinical application. Blood Transfus. 2020;18(3):208-216. doi: 10.2450/2020.0305-19.
3. Samarkanova D et al. Quality and stability studies of red blood cell concentrates from umbilical cord blood compared to their adult counterparts. Blood Transfus. 2024 Aug 2. doi: 10.2450/BloodTransfus.761.

Methods – validation of expiration time to start the process of CBPC retrieval/preparation

Comparison of whole CB units to obtain **platelets**

- < 100 grams
- > 48 hours
- vs control > 100g and < 48h

Parameters

- Process success rate (>5mL; 800-1200x10^6/L of platelets; Free haemoglobin = 0 g/dL)
- Functionality
  - GFs (EGF, bFGF, VEGF, PDGF AA/BB)
  - Pro-inflammatory cytokines (IL-6, TNF-alpha)
  - MSCs growth curve (toxicity on cell viability) and biological activity)

Objective

- To validate an extension of expiration time before starting the process
- To modify acceptance criteria of multicomponent cord blood bank, beyond transplantation
- To discuss risk assessment for implementation

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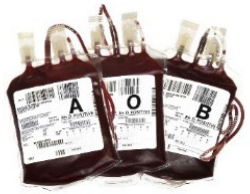
Results – clinical applications of red blood cells

PRODUCT VALIDATION

PROCESS VALIDATION



CLINICAL TRIAL

BANC DE SANG I TEIXITS

CORD BLOOD BANK

PRETERM TRANFUSION WITH ALLOGENEIC CORD BLOOD RED CELL CONCENTRATES

CLÍNIC BARCELONA Hospital Universitari

NEONATOLOGY WARD

QC1: Biochemistry and RBC function

QC2: Rapid "Molecular" sterility testing

QC3: Neurotoxicans in RBC concentrates

VIRTUAL STOCK MANAGEMENT

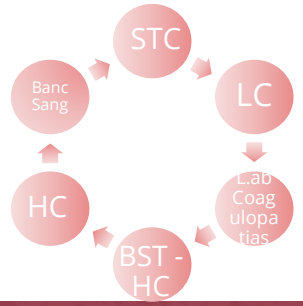
Pilot study on 10 preterm babies < 28 weeks of gestational age

2023

2024

2025

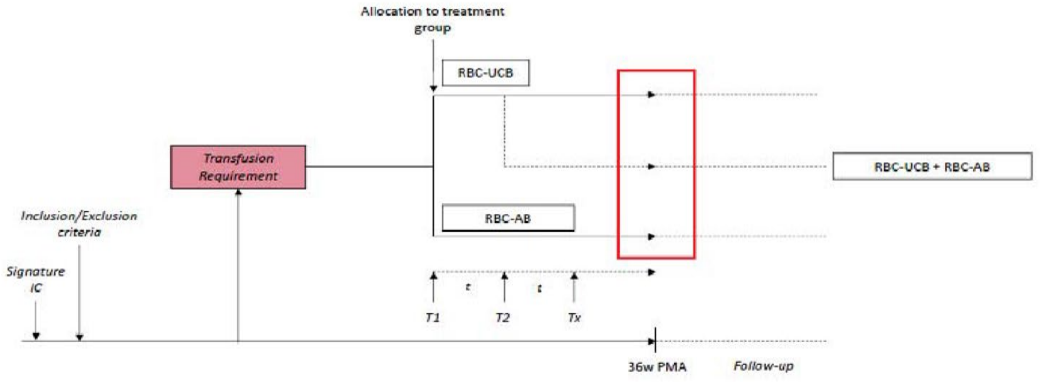
Validation UCB-RBC vs AB -RBC
"*in vitro*" (12 month)

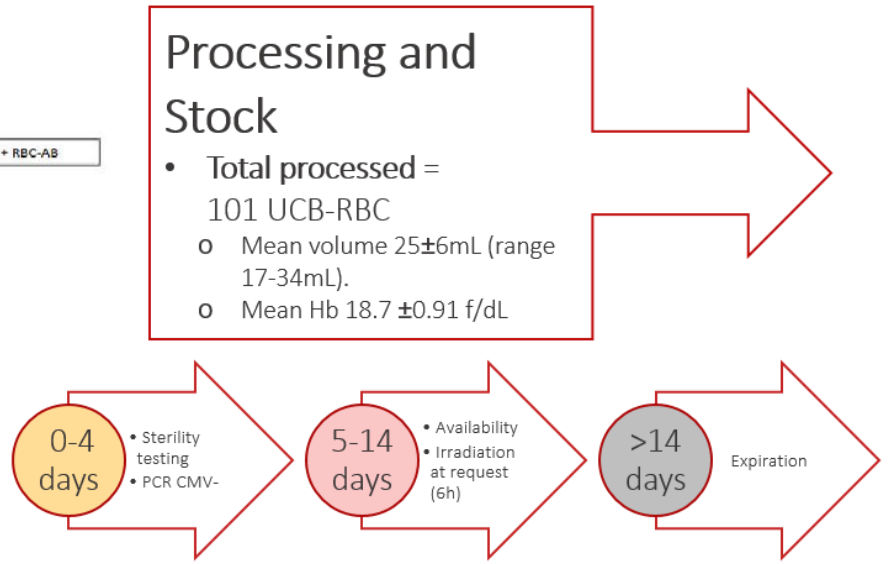


Validation UCB-RBC
"*in vivo*" Pilot Clinical Study
(22 month + 2 month report)

Results – clinical applications of red blood cells

CB-RBC Pilot study





Requests

- **Requests** for UCB-RBC transfusions: N=20.
- UCB-RBC **unavailable** at request: N= 4.
  - 2 for 1st transfusion
  - 1 for 2nd
  - 1 for 3rd
- **Availability** of UCB: **80%** (16/20).

	UCB-transfusion (N of transfusions=16)	Adult-RBC transfusion (N of transfusions=6)
HbF before RBC transfusion (%)	93.8 (90.9 - 94.6)	88.3 (84.9 - 94.5)
HbF 24 hours after transfusion (%)	89.8 (88.3 - 90.9)	57.7 (37.3 - 64.5)

Results – clinical applications of cord blood platelet gel

CBPG applied in:

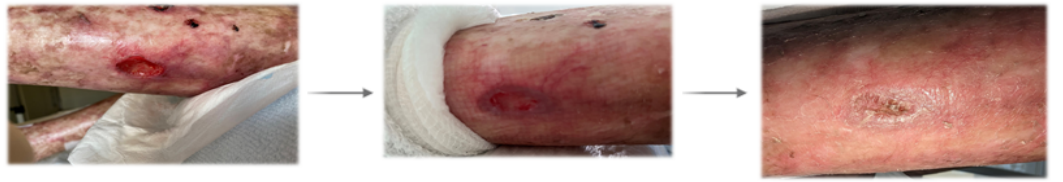
- Clinical trail on diabetic foot ulcers, with a total of 11 patients recruited
- Compassionate treatment on 9 patients with following indications:
  - Diabetic foot ulcers (n=2)
  - Epidermolysis Bullosa (n=1)
  - Pressure ulcers (n=1)
  - Oral ulcers on GvHD patients (n=3)
  - Cutaneous ulcers on GvHD patient (n=1)
  - Malleolar ulcer on sickle cell disease patient (n=1)

Left Lower Limb Exterior TIBIA

Treatment start: 26-09-2023
Treatment end: 23-10-2023

Concomitant treatment: ruxolitinib and corticoids

Number of applications	BL	1	2	3	4	5	6	After the last CBPG application, wound was treated with alginate with silver. Lesion was fully closed on 13-11-2023
Wound size in mm (length)	25	25	18	16	14	8	5	



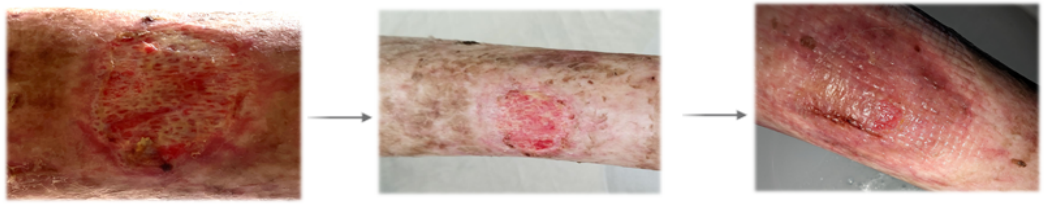
Baseline (BL): 25 mm | 1 month of treatment: 7 mm | Follow-up visit at 1.5 month: 5 mm

Left Lower Limb Anterior TIBIA

Treatment start: 06-11-2023
Treatment end: 25-01-2024

Concomitant treatment: ruxolitinib and corticoids

Number of applications	BL	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Wound size in mm (length)	48	45	NR	37	NR	38	NR	30	NR	15	15	NR	8	4	3	2	3	NR



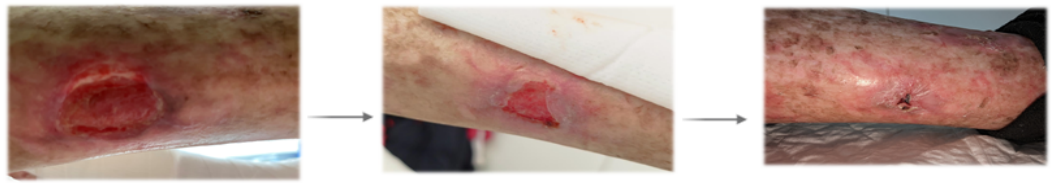
Baseline (BL): 48 mm | 1 month of control: 45 mm | 2 month of treatment (16 applications): 3 mm

Right Lower Limb Exterior TIBIA

Treatment start: 06-11-2023
Treatment end: 11-12-2023

Concomitant treatment: ruxolitinib and corticoids

Number of applications	BL	1	2	3	4	5	6	7	8	9	10	Lesion was fully closed after the last application of CBPG
Wound size in mm (length)	20	38	NR	21	NR	25	NR	10	NR	6	0	



Baseline (BL): 20 mm | 1 month of control: 38 mm | 1 month of treatment (8 applications): 0 mm

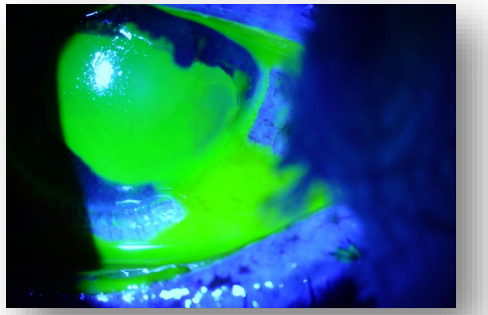
Results – clinical applications of cord blood platelet lysate eye drops

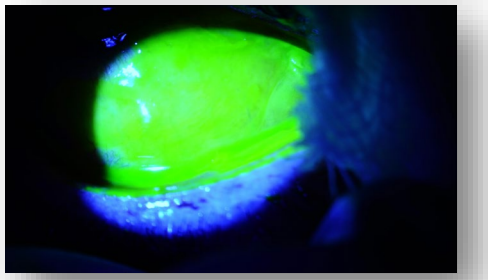
INDICATION (data 2016-2023):	Total n patients	Min. age	Max. age	Response to treatment	
I. Neurotrophic ulcers	32	7	91	31	97%
II. Ulcers (others ethiology)	22	0	85	21	95%
III. Ocular GvHD	9	22	74	6	67%
IV. Severe dry eye	29	4	92	22	76%
V. Ocular burn	20	3	62	20	100%
VI. Toxic epidermal necrolysis	10	6	71	10	100%
Total number of cases	**122**	**0**	**92**	**110**	**90%**

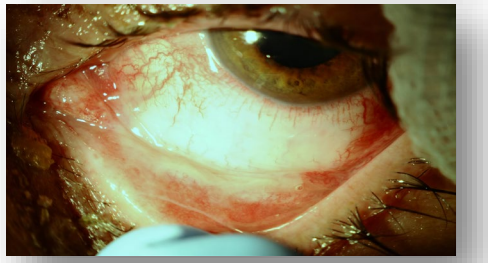
Results – clinical applications of cord blood platelet lysate eye drops

53 y.o. ocular burn, bilateral (hydrochloric acid)

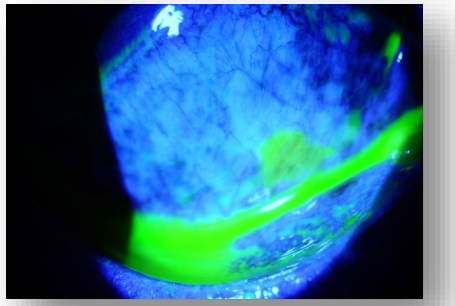
Pre treatment

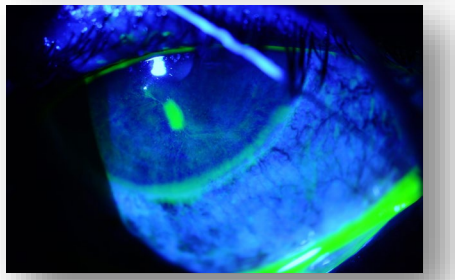




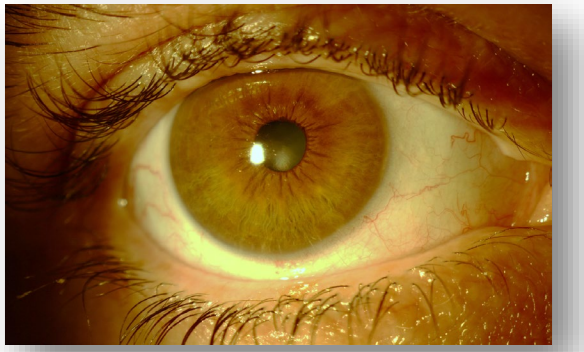


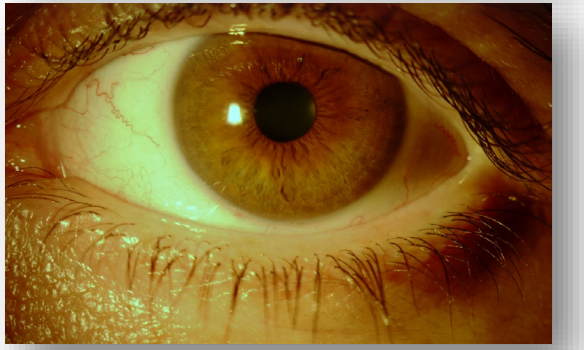
4 days of treatment





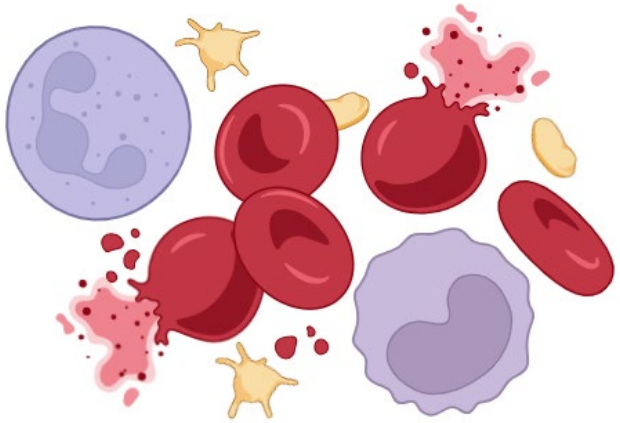
1 month of treatment





Results – validation and risk assessment of extending expiration time

The objective: Validate whole CB units at reception >48h or <100g to obtain platelets



- **Haemolysis:** all validated units presented 0 g/dL of free Hb
- Decrease in **functionality** of active ingredients (growth factors/MSCs): see Tables 1 & 2
- Increase in **pro-inflammatory** cytokines: see Tables 1 & 2
- Possible **toxicity**: viability of MSCs

Results – validation and risk assessment of extending usage of low weight units

Table 1. Comparison of initial UCB collection bag weights: minimum 85 vs. 100 grams

	Collections 85-100 grams (validation)	Collections ≥100 grams (standard)	Comparison 85-100g vs ≥100
Characteristics at initial collection (median, range)	(n=10)	(n=3)	
Weight collection bag, grams	93 (85-97)	122 (113-131)	ND*
Volume PRP, mL	6 (5-9)	10 (5-11)	ND
Platelet count, x10⁹/L	853 (748-992)	887 (848-937)	ND
Functionality assay results, per pool (acceptance criteria)	(1 pool, n=10)	(1 pool, n=3)	
Growth rate, x 1/days (≥0.33)	*ND***	0.64	ND
Duplication time, days (≤2.1)	*Not determined**	1.08	ND
Exponential phase duration, days (≥5)	3	5	ND
Growth factor levels (mean ± SD), (pg/mL)	(n=10)	(n=3)	
Epithelial growth factor (EGF)	3574 (2566-3851)	3531±114	0.5
Platelet derived growth factor (PDGF)	12242 (8236-14462)	11955±1603	0.8
Vascular endothelial growth factor (VEGF)	3594 (2535-6586)	3828±1136	0.2
Basic fibroblast growth factor (bFGF)	1542 (560-1956)	1479±392	0.3
Interleukin-6 (IL-6)	1582 (1133-1873)	1525±204	0.9
Conformity for accomplishing required platelet count (800-1200x10⁹/L)	(n=51)	(n=3)	
Accepted:	85-89 g (n=10) 1/10 (10%) 90-99 g (n=41) 14/51 (34%)	3/3 (100%)	ND

*ND – not determined

***It is not possible to determine growth rate and duplication time if the exponential phase is less than 5 days.*

Results – validation and risk assessment of extending expiration time

Table 2. Comparison initial UCB collection expiry: minimum 48 vs. 80 hours

	Collections processed within 49-80 hours (validation)	**Collections processed within <48 hours** (standard)	**Comparison 49-80 vs <48h**
Characteristics at initial collection (median, range)	(n=9)	(n=3)	P=
Time since collection, hours	70 (55-80)	32 (16-32)	ND*
Volume PRP, mL	6 (4-10)	10 (5-11)	ND
Platelet count, x10^9/L	1074 (703-1173)	887 (848-937)	ND
Functionality assay results, per pool	(1 pool, n=8)*	(1 pool, n=3)	
Growth rate, x 1/days	0.49	0.64	ND
Duplication time, days	1.41	1.08	ND
Exponential phase duration, days	10	>5	ND
Growth factor levels (mean ± SD), (pg/mL)	(n=8)**	(n=3)	
Epithelial growth factor (EFG)	4456±731	3531±114	0.1
Platelet derived growth factor (PDGF)	11862±1809	11677±1084	1.0
Vascular endothelial growth factor (VEGF)	4105±1112	4349±813	0.8
Basic fibroblast growth factor (bFGF)	1304±2176	1700±115	0.6
Interleukin-6 (IL-6)	1254±1769	1605±110	0.5
Conformity	(n=9)	(n=3)	
Accepted	8/9 (88.8%)	3/3 (100%)	ND

**ND – not determined*

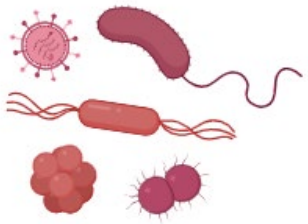
***One validation unit (1/9, 11.1%) in the expiry comparison did not accomplish acceptance criteria due to low platelet count (703 x10^9/L).*

Index

Discussion – biovigilance: main adverse events and follow-up

Risk of donor pooling:

- 1 donor = 1 batch ≠ pool
- Double serology: CB + mother blood
- Microbiology:
  - 14 days of BacTAlert for PRP
  - 4 days of molecular testing (PCR) for RBC fast release



Discussion – biovigilance: main adverse events and follow-up

CBED

- 1st 33 cases described:
  Samarkanova D, et al. Clinical evaluation of allogeneic eye drops from cord blood platelet lysate. Blood Transfus. 2021;19(4):347-356. doi: 10.2450/2020.0130-20.

- Untill November 2024: a total of 170 compassionate cases were included

- "Expired" units: clinical application (since July 2024):

Total # batches	Total # patients	New cases
24	18	3 (16%)

✓ No SAEs registered

Discussion – storage

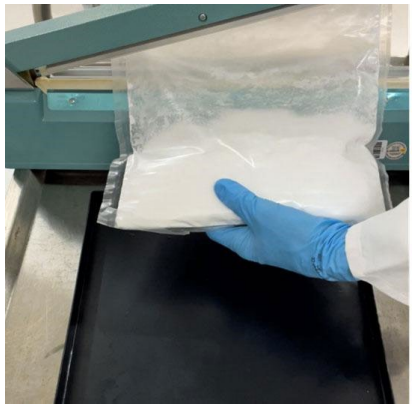
- RBC 14 days at 2-6ºC
- CBPC/CBED = 3 years at <-65ºC
- Technological development – freeze drying (FD)
- For FD: in case pooling is used, the risk asociated with pathogens can be solved by mirasol or similar inactivation methods

Discussion – technological development – freeze drying

1 mL

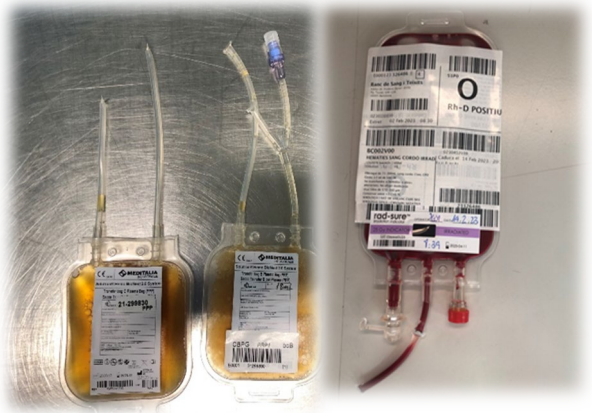
10 mL

10-1000 mL



Conclusions

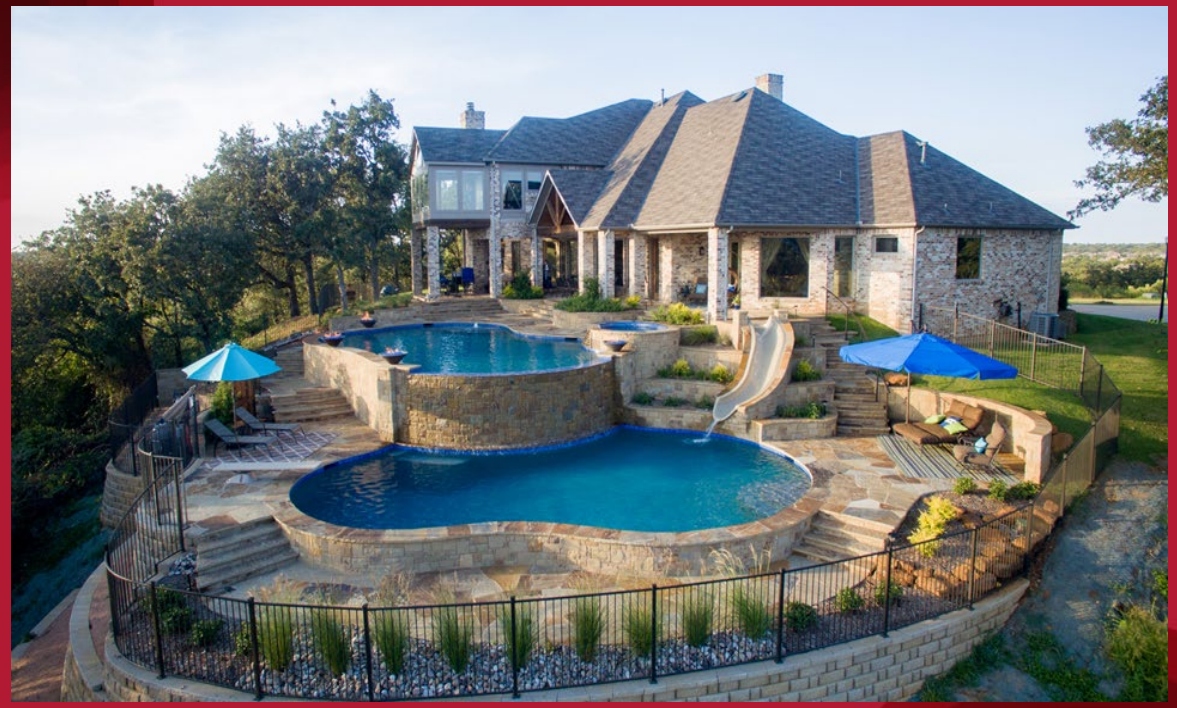
- Multicomponent fractionation is feasible, reproducible and implemented under GMP conditions
- Extension of expiration time (<80 hours from collection to processing) of collected preparation is validated, which increases sample availability
- Small volume (85-99 g) derived CB is not suitable to obtain standardized platelet concentrates
- CB donations can be used for new therapies extending their application beyond transplantation in:
  - Specialized blood therapy like transfusion in neonatology
  - Wound healing of eye and skin lesions



Acknowledgements

- Donors and maternities staff
- BST staff
- Hospital Vall de Hebron Ophthalmologists
- Hospital Clínic Neonatologists, especially Dr Miquel Alsina

One pool isn’t enough! Production of double dose PLT units with 6 pooled buffy coats



Dr. Amato Marco, LL.M.

Central Institute of Blood Transfusion and Immunology, Tirol Kliniken, Innsbruck, Austria

Conflicts of interest

Conflict of interest statement: None declared.

Overview Slide

We talk about a strategy to enhance pooled platelet production by:

- Optimizing pooled platelet processes
- Improving pooling protocol by a stepwise optimization
- Maximizing yield and efficiency through double-dose units
- Keeping costs and workload stable
- Improved resource management to adapt to increasing demand and demographic changes

Our old method

Before our optimization we used 5 buffy coats (BCs) and we were able to produce pooled platelet concentrates with a yield of 2.83 (mean) - after pathogen inactivation (PI) with Intercept

Imagine that one buffy coat (BC) has a yield of about 1.00 (1x 10^{11} platelets), by pooling 5 BCs we should have a yield of 5.00 in the intermediary pool

The Loss of PI is ~10%, so we should find ~4.5 (yield) after PI (5.00 – 10% = 4.5)

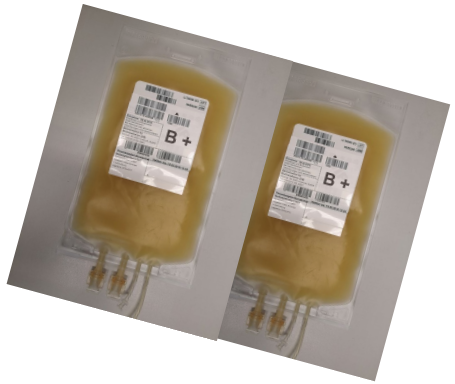
Conclusion:

We loose an additionally yield of 1.67 (4.5 – 2.83 = 1.67) through the production process

This is an additional loss of ~37%

Optimization Step 1

5 + 1 = 2 ?



Challenge:

- Optimize the pooling protocol, so that we get a product with a yield > 4.4 after PI (border to divide the unit)

1. Step:

Switch from 5 BCs to 6 BCs

We see an (expected) increase

Method	n	Measurement date, range	PLT Yield after PI, mean (SD)	Volume, median (IQR)	PLT x 10^3/µl, mean (SD)	BC PLT volume in mL, mean (SD)
BC5	107	03.01. - 03.02.2023	2.83 (0.39)	308.0 (302.5-318.0)	916.46 (128.38)	-
BC6	110	03.05.- 06.06.2023	3.12 (0.38)	301.0 (292.0-309.8)	1039.03 (138.43)	-

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Optimization Step 2

- Switching to a new hematology analyzer
- Sysmex XN-1000 with blood bank mode
  - Approved for platelet samples with high concentration
  - A control (platelet check) which corresponds in its concentration to a double product
- The yield has increased again

BC6	110	03.05.-06.06.2023	3.12 (0.38)	301.0 (292.0-309.8)	1039.03 (138.43)	-
BC6+XN-1000	106	03.08-06.09.2023	4.03 (0.49)	301.0 (293.2-308.0)	1343.46 (162.89)	-

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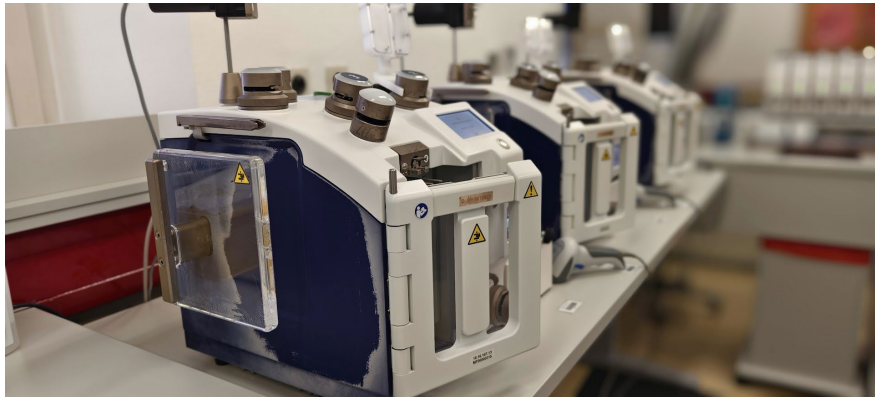
Optimization Step 3

- For a double dose unit, the volume should be higher:
  - Inbound volume from a DS Set is between 300 – 420 mL
  - Plasma ratio must be between 32-47%
  - But the Pooling bag has a volume of 600 mL maximum
- Step 3:
- Adapting the cell separator program (Macopress smarter)
- Plasma increased the BC volume from 47 mL to 55 mL
  - We extended the time for extracting the air from the plasma bag to the BC bag. With this setting we get:
    - More Plasma in the BC bag and
    - the platelet rich plasma in the tube between BC and plasma bag rinses back to the BC bag
- Switch from PAS 300 mL to 280 mL
- Plasma Ratio after this step = 40%



Method	n	Measurement date, range	PLT Yield after PI, mean (SD)	Volume, median (IQR)	PLT x 10^3/µl, mean (SD)	BC PLT volume in mL, mean (SD)
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BC6	110	03.05.- 06.06.2023	3.12 (0.38)	301.0 (292.0-309.8)	1039.03 (138.43)	-
BC6+XN-1000	106	03.08- 06.09.2023	4.03 (0.49)	301.0 (293.2-308.0)	1343.46 (162.89)	-
BC6+XN1000+CM	107	28.09.- 08.11.2023	4.30 (0.53)	343.0 (332.0-352.0)	1256.80 (163.04)	-

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Optimization Step 4

- Step 4:
- With the second centrifugation (soft spin) we produce a platelet rich supernatant
- But we found lots of platelets in the residual cell bag, so we changed the duration of the centrifugation and the deceleration to increase the yield
- This last optimization step increased the yield again and we found less platelets in the residual cell bag.

Method	n	Measurement date, range	PLT Yield after PI, mean (SD)	Volume, median (IQR)	PLT x 10^3/µl, mean (SD)	BC PLT volume in mL, mean (SD)
BC5	107	03.01. - 03.02.2023	2.83 (0.39)	308.0 (302.5-318.0)	916.46 (128.38)	-
BC6	110	03.05.- 06.06.2023	3.12 (0.38)	301.0 (292.0-309.8)	1039.03 (138.43)	-
BC6+XN-1000	106	03.08- 06.09.2023	4.03 (0.49)	301.0 (293.2-308.0)	1343.46 (162.89)	-
BC6+XN1000+CM	107	28.09.- 08.11.2023	4.30 (0.53)	343.0 (332.0-352.0)	1256.80 (163.04)	-
BC6+XN1000+CM+CF	197	04.12.2023- 15.02.2024	4.81 (0.58)	358.0 (349.0-368.0)	1344.85 (165.16)	-

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Why this way?

- 60 BCs / 5 BCs = 12 single dose units
- 60 BCs / 6 BCs = 10 single dose units

after our adapting steps

- 60 BCs / 6 BCs = 10 (potentially) double dose units

(divisible are 76% ~ 18 units)

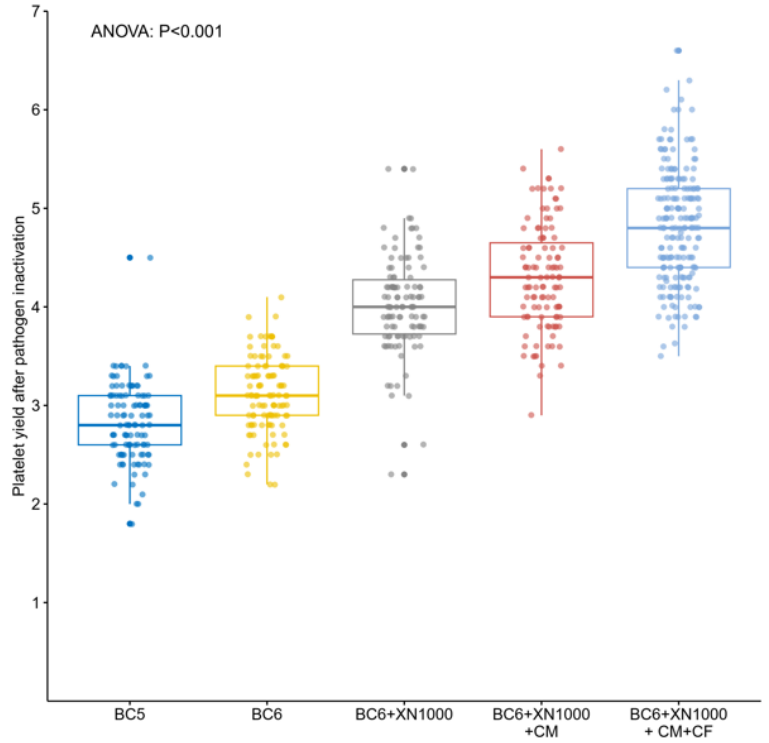
Considerations:

- Costs are equal: 12 single sets = 10 double sets
- More platelet units with the same quantity of BCs

(~ 1000 BCs per month)

- No need to change the pooling set
- The process doesn't need to be changed, the workload for the employees stays the same

Figure 2 Comparing buffy coat pooled platelet contents produced by five different methods.



Abbreviations: BC, buffy coat; CF, centrifugation; CM, compomat.

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Flexibility

Now we have more flexibility:

With 12.000 BCs we are now able to produce more products:

12.000 / 5 = 2.400 single dose units

12.000 / 6 = 2000 units (76% are double dose)
480 single dose units
1520 double dose units
3520 platelet units

↗ 46.6% more units

alternative

If the annual number of platelet units should remain the same:

8400 BCs / 6 = 1400 units (76% are double dose)
336 single dose units
1064 double dose units
2464 platelet units

↘ 30% less BCs

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Option 1 – 12.000 BCs

With 1120 (46.6%) more units we are able to reduce:

- the workload of apheresis
- the workload of productior
- the costs of production

and

- We are prepared for increased consumption due to demographic change
- We are able to quickly scale-up our production
- We are prepared for declining apheresis donations

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Option 2 – 8400 BCs

With less BCs we are able to reduce:

- the workload of production again
- we save the volume of 3600 BCs

3600 BCs means that we save

- 400 RBC concentrates / year
- 400 FFP concentrates / year

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Summary

- We have achieved a higher work efficiency by producing double-dose units
- We managed to reduce our loss of yield from ~37% to ~11% through the production process. Now we loose only a yield of 0.59 (5.4 – 4.81 = 0.59) after our optimizations.
- We increased platelet unit production by 46.6% (with 12.000 BCs)
- We reduced costs
- We reduced workload

With these steps we enhanced efficiency which supports sustainability and the requirements for the future.

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Thanks for your attention

Using a hematology analyzer to count residual cells in blood components instead of flow cytometry

Anita Siller, PhD

Central Institute of Blood Transfusion and Immunology, Tirol Kliniken, Innsbruck, Austria

Conflicts of Interest statement

No conflict of interest to declare.

Relevance and Background

- Depletion of white blood cells (WBC) is particularly important to e.g. reduce the risk of transmitting intracellular pathogens, transfusion-associated GvHD and WBC antigen alloimmunization

- Residual cells need to be enumerated in blood products as part of routine quality control testing

- These tests are performed in a random subset of produced units as determined by statistic process control

- Relevant specifications/limits of residual cells in different blood products are given in the chapter "Blood component monographs" of the *EDQM Blood Components Guide*

Specifications / Limits according to the EDQM guide

- Erythrocyte concentrate, leucocyte-depleted (RBC)
  - Residual WBC (rWBC): < $1 * 10^6$ per unit

- Fresh Frozen Plasma (FFP)
  - rWBC: < $0.1 * 10^9$ per Litre (L)
    - If leucocyte-depleted: < $1 * 10^6$ per unit
  - Residual platelets (rPLT): < $50 * 10^9$ per L
  - Residual erythrocytes (rRBC): < $6.0 * 10^9$ per L

- Platelet concentrate, pooled or from apheresis, leucocyte-depleted (PLT)
  - rWBC: < $1 * 10^6$ per unit

At least 90% of the units tested should meet the given values.

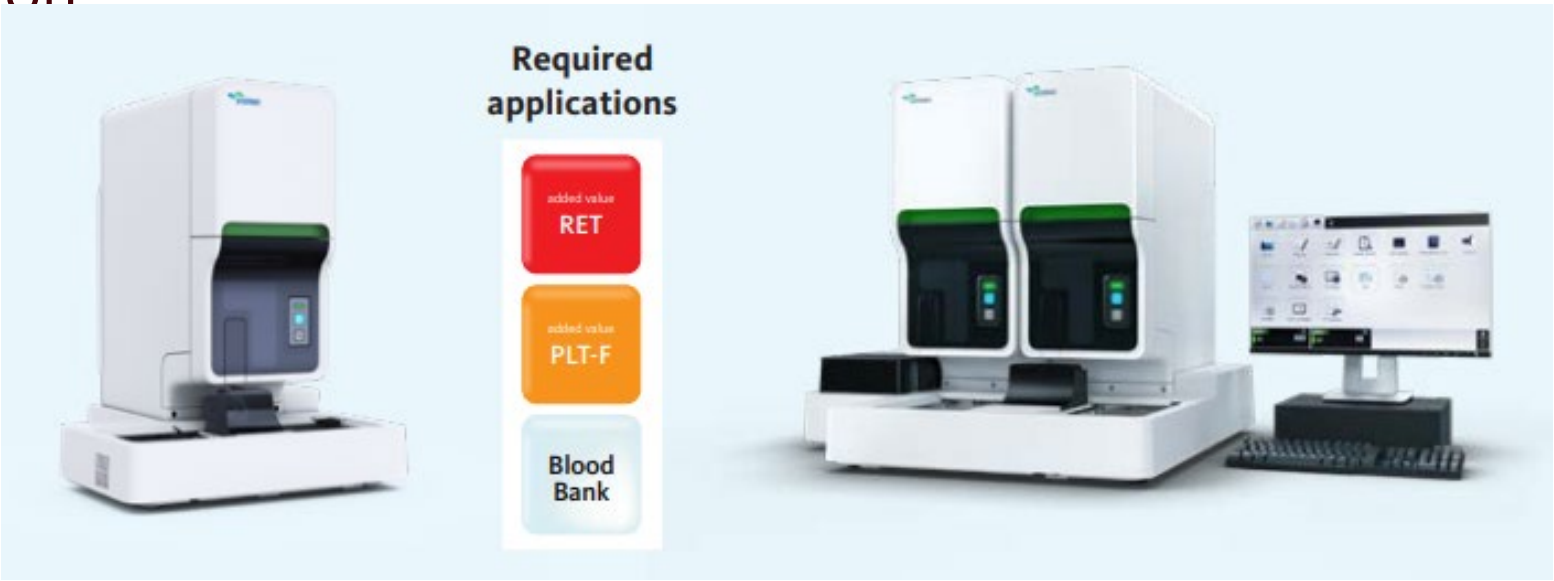
Methods for residual cell measurements

- Flow cytometry
  - Currently most widely used
  - Time consuming for staff
  - Manual handling steps → less standardized
  - Staff needs to be skilled and specifically trained
  - Relatively expensive

- Haematology Analyzer Sysmex XN-1000 (or XN-2000) equipped with Blood Bank Mode (BBM)
  - First possibility to measure residual cells in a simple haematology analyzer
  - No manual steps required → higher standardization for more consistent results
  - Little staff commitment required (tubes only need to be swiveled and placed on autosampler)
  - Comparatively cheap

Technical Requirements

- XN-1000 or XN-2000 haematology analyzers can be equipped with BBM which is a software licence
- Further required applications are the so-called RET and the PLT-F application



Taken from: Sysmex Europe; https://www.sysmex-europe.com/products/diagnostics/haematology/xn-series/xn-blood-bank-mode/

Measuring profiles

- BBM offers the analysis of blood components with 4 different profiles (2 for RBC and 2 for PLT; Plasma can be measured in the PLT pack + residual cells profile)

Taken from: Sysmex Europe; https://www.sysmex-europe.com/products/diagnostics/haematology/xn-series/xn-blood-bank-mode/

Further specifications

- Manual and automated sampler mode
- Aspirated volume:
  - RBC pack mode: 150 µL
  - PLT pack mode: 205 µL
- Hourly throughput of a standalone XN-1000 analyser in BBM is fast

Blood pack	Residual cell counts	Throughput	Time per single measurement
RBC pack	No	79 samples/h	Less than 1 minute
	Yes	33 samples/h	Less than 2 minutes
PLT pack	No	62 samples/h	Approx. 1 minute
	Yes	19 samples/h	Approx. 3 minutes

Taken from: Sysmex Europe; https://www.sysmex-europe.com/products/diagnostics/haematology/xn-series/xn-blood-bank-mode/

Relevant Literature

- Key messages from these Publications
  - Reliable Performance of XN-Series analyzer with BBM for rWBC and rRBC enumeration
  - Very good correlation with flow cytometer data
  - Acceptable limits of quantification

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Validation of the Sysmex XN analyser and Blood Bank mode for the quality and safety of donor blood and transfusion products

Ondrej Valina, Ida Vankova, Konstantinos Mintzas, Klara Knappova, Zdenka Gasova

Results of our study

- rWBC in **RBC** concentrates measured by Flow cytometry vs. Sysmex XN-1000 BBM

  - Whole-blood inline-filtrated RBC concentrates (P for difference = 0.427)
    - FACS: 0.4 cells/µL (IQR 0.2 – 0.7 cells/µL)
    - XN-1000: 0.4 cells/µL (0.2 – 0.9 cells/µL)

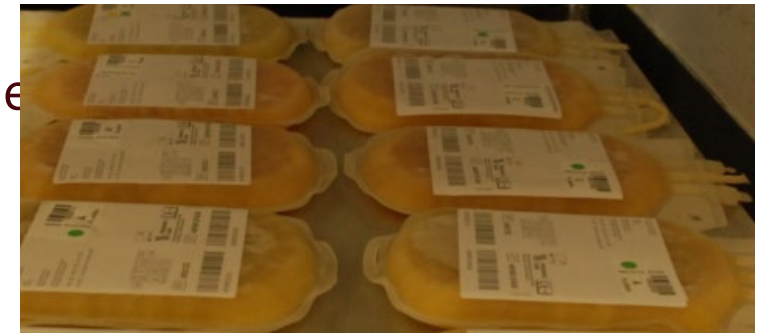
  - Pass rates based on cut-off values specified in the EDQM guide
    - FACS: 100.0% (92.9, 100.0%) passed (n=61)
    - XN-1000: 98.4% (90.4, 100.0%) passed (n=60; n=1 did not pass)

  - FACS and XN-1000: > 90% of units/volumes analyzed passed the cut-off values

Data taken from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

- rWBC in **FFP** measured by Flow cytometry vs. Sysmex XN-1000 BBM
  - FFP derived from whole-blood inline-filtrated bags (P for difference < 0.001)
    - FACS: 0.0 cells/µL (0.0 – 0.0 cells/µL)
    - XN-1000: 0.0 cells/µL (0.0 – 0.1 cells/µL)
  - Pass rates based on cut-off values specified in the EDQM guide
    - FACS: 100.0% (92.2, 100.0%) passed (n=55)
    - XN-1000: 100.0% (92.2, 100.0%) passed (n=55)



Data taken from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

- rWBC in **pooled PLT** concentrates measured by Flow cytometry vs. Sysmex XN-1000 BBM (after pathogen inactivation)

  - Pooled PLT, leucocyte-depleted in additive solution (P for difference < 0.001
    - FACS: 1.1 cells/µL (0.4 – 1.7 cells/µL)
    - XN-1000: 2.3 cells/µL (1.4 – 3.0 cells/µL)

  - Pass rates based on cut-off values specified in the EDQM guide
    - FACS: 91.2% (76.3, 97.7%) passed (n=31, n=3 did not pass)
    - XN-1000: 70.6% (53.7, 83.3%) passed (n=24, n=10 did not pass)

    - FACS : > 90% of units/volumes analyzed passed the cut-off values
    - XN-1000: < 90% of units/volumes analyzed passed the cut-off values → *not acceptable*

Data taken from: Siller et al., Ann Lab Med, preliminary accepted manuscript

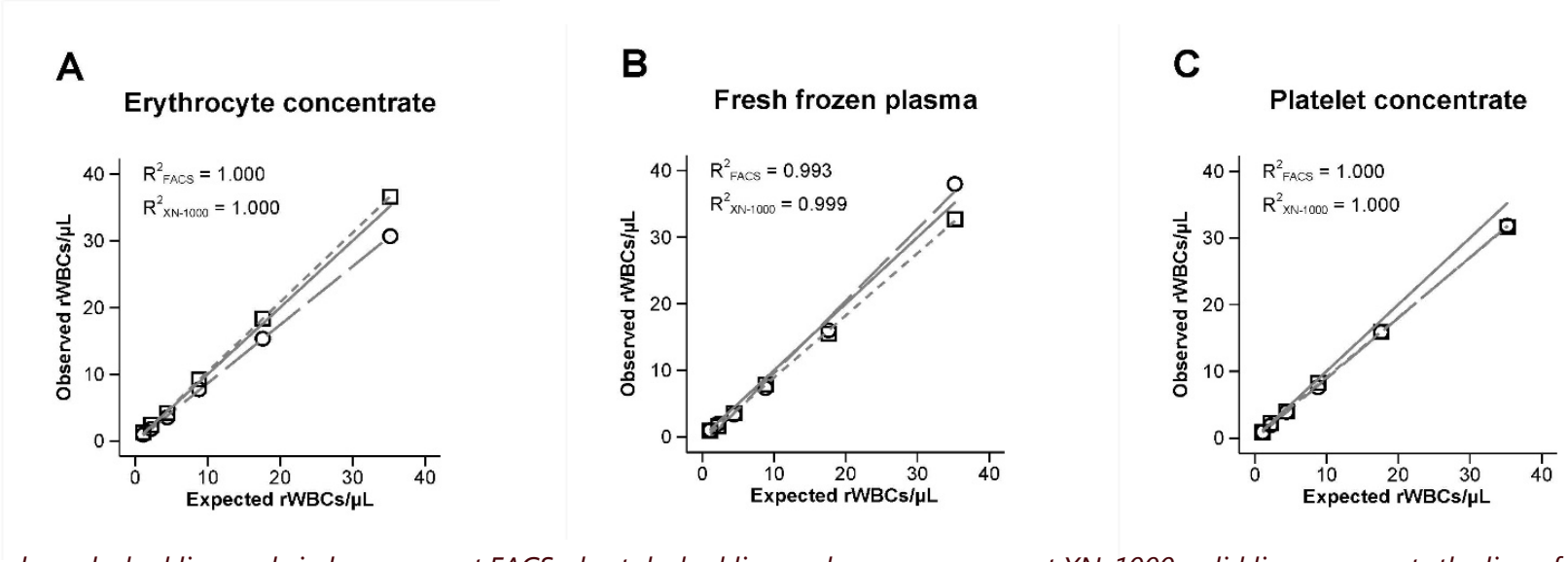
Results of our study

- rWBC in **PLT** concentrates derived from **apheresis** measured by Flow cytometry vs. Sysmex XN-1000 BBM (after pathogen inactivation)
  - PLT, derived from apheresis, in additive solution (P for difference < 0.001)
    - FACS: 0.0 cells/µL (0.0 – 0.1 cells/µL)
    - XN-1000: 0.8 cells/µL (0.6 – 1.1 cells/µL)
  - Pass rates based on cut-off values specified in the EDQM guide
    - FACS: 100.0% (82.5, 100.0%) passed (n=22)
    - XN-1000: 95.5% (76.5, 100.0%) passed (n=21, n=1 did not pass)
  - FACS and XN-1000: > 90% of units/volumes analyzed passed the cut-off values

Data taken from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

- rWBC in **RBC, FFP and PLT** measured by Flow cytometry vs. Sysmex XN-1000 BBM – Spiking experiments with known rWBC counts
  - revealed a high linear correlation between expected and observed WBCs

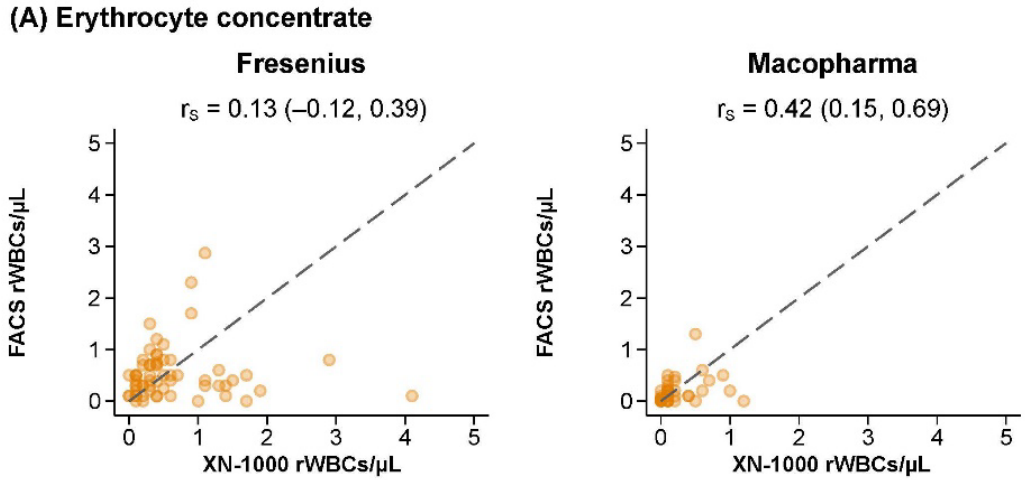


long dashed line and circles represent FACS, short dashed line and squares represent XN-1000, solid line represents the line of equivalence

Adapted from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

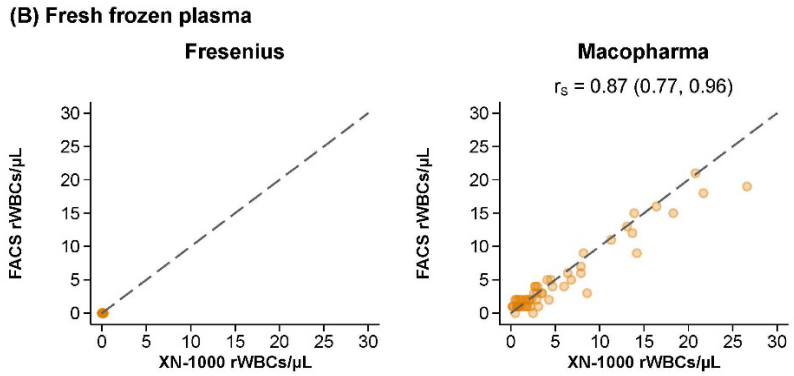
- rWBC in **RBC** measured by Flow cytometry vs. Sysmex XN-1000 BBM - Correlations

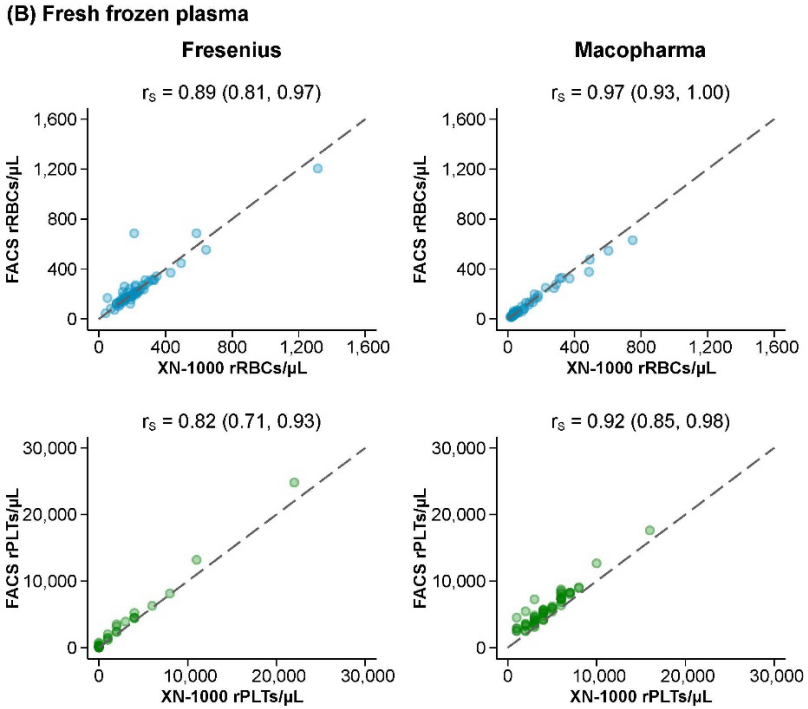


Adapted from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

- Residual cells in **FFP** measured by Flow cytometry vs. Sysmex XN-1000 BBM - Correlations

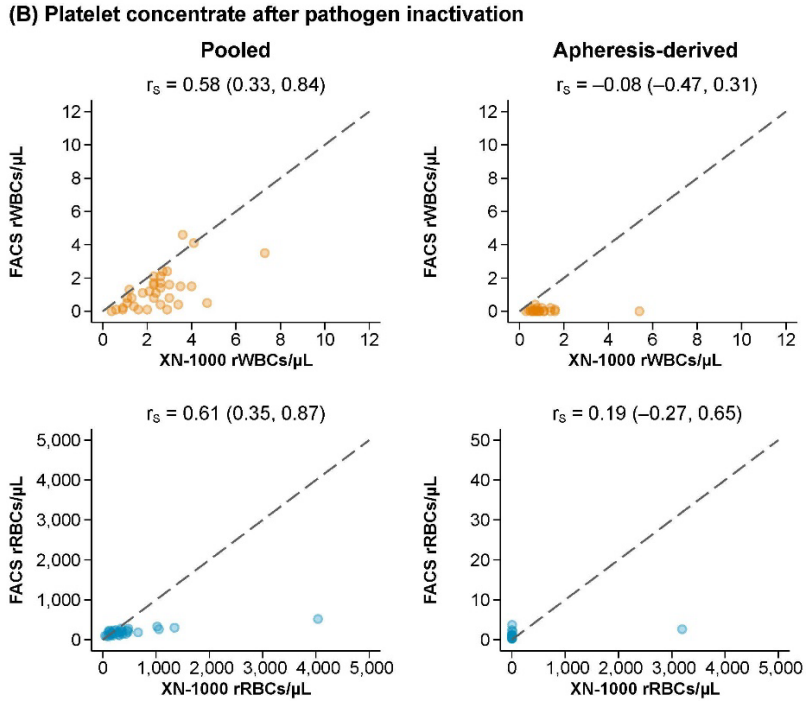




Adapted from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

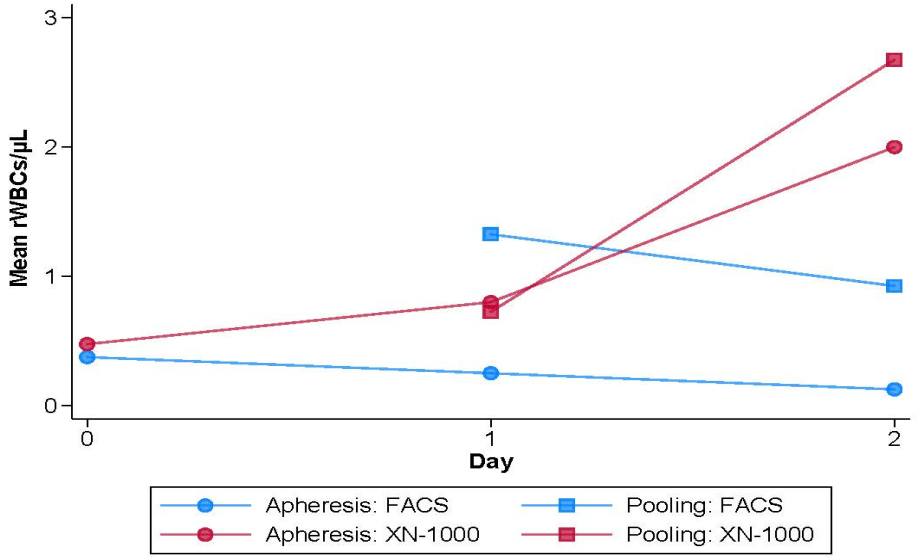
- rWBC and rRBC in **PLT** (after pathogen inactivation) measured by Flow cytometry vs. Sysmex XN-1000 BBM - Correlations



Adapted from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Results of our study

- Why do we encounter problems when measuring pathogen inactivated PLT products?
  - One potential explanation is the time-point when the measurement is conducted



Taken from: Siller et al., Ann Lab Med, preliminary accepted manuscript

Summary and Conclusion

- Sysmex XN-1000 equipped with BBM is a **suitable alternative method to enumerate residual cells in RBC and FFP units**
  - Although exact values can vary, a correct discrimination if the product is below or above the cut-off value is possible

- Especially when performing pathogen inactivation, there are some **limitations for PLT units**, especially for pooled products
  - Values measured with Sysmex XN-1000 BBM get higher if the sample is measured at a later time point (e.g. day 2 after donation, which is necessary for quality control of pooled, pathogen-inactivated PLT products)
  - A correct discrimination if the product is below or above the cut-off value is not always possible

THANK YOU!

tirol kliniken
universitätskliniken
innsbruck

Central Institute for Blood Transfusion and Immunology

Prim. Univ.-Doz. Dr. Schennach Harald
Dr. Dr. Amato Marco, LL.M.
BMA Schmidt Daniela

MEDIZINISCHE UNIVERSITÄT
INNSBRUCK

Institute of Clinical Epidemiology, Public Health, Health Economics, Medical Statistics and Informatics

Univ.-Prof. Dr. Willeit Peter, MPhil PhD
Lisa Seekircher, PhD
Lena Tschiderer, PhD