



Present and Future of Pathogen Reduction for Red Cells and Whole Blood

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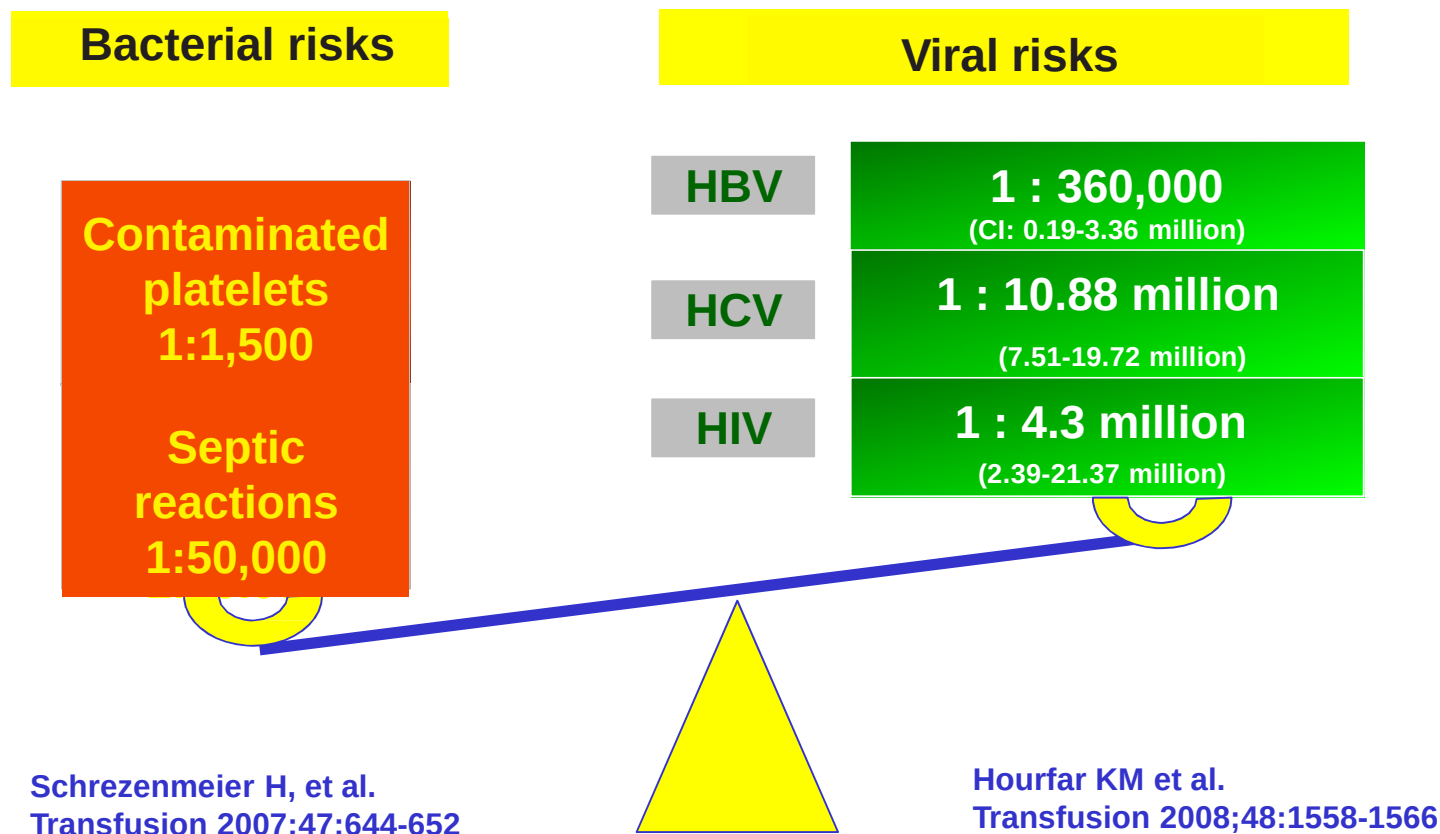
Prof. Dr. Erhard Seifried , M.D..



Disclosures

- Novartis Oncology Inc., New Jersey, USA: honorarium for consultation report
- Fresenius KABI GmbH, Bad Homburg, Germany: honorarium for lectures (satellite symposia)
- CERUS Inc., CA, USA & CERUS Europe BV, The Netherlands: support for research projects/research grant & honorarium for lectures (satellite symposium)

Residual risk for viral and bacterial transmission via transfusions

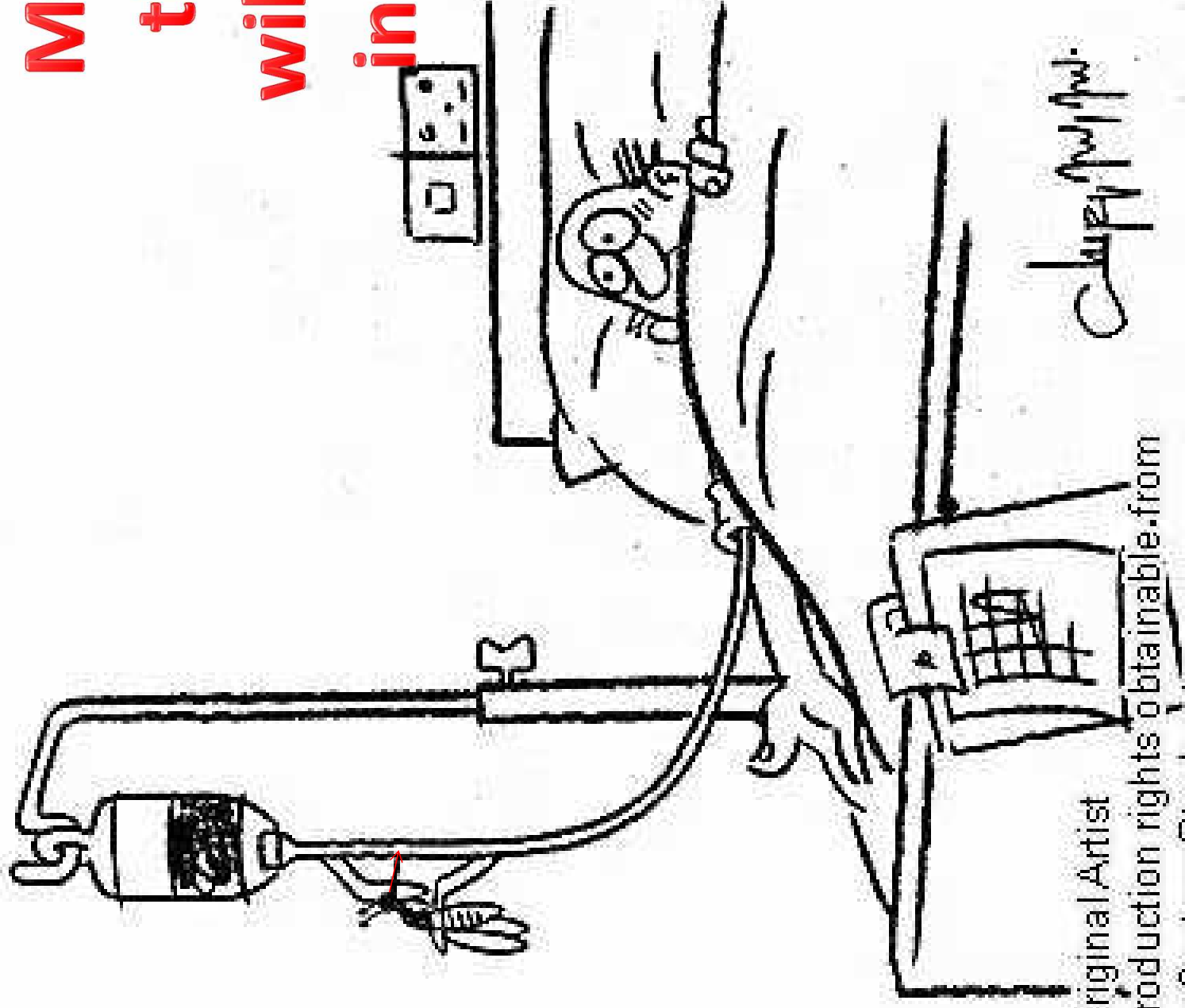


re-emerging pathogens

Year	Pathogen	Year	Pathogen
1981/'82	HTLV III (= HIV-1) / AIDS	1995	HHV 8 ¹
1986	HIV-2	1996	Variant CJD (vCJD) / Prions
1988	Hepatitis E (Caliciviridae)	1997	Avian Influenza Virus A (H5N1)
1989	Hepatitis C (Flaviviridae)	1999	West Nile Virus (WNV; Flaviviridae) in USA
1992	Vibrio O 139	2003	SARS (Coronaviridae)
1992	Bartonella hensellae	2003	Monkeypox Virus
1993	Sin Nombre Virus	2004	Metapneumo Virus
1995	Hepatitis G (Flaviviridae)	2005	Chikungunya Virus

During the last decade in Europe: Malaria, Dengue Virus, Influenza, Swine Flu, Avian Influenza, West Nile Virus, Chikungunya Virus, Hepatitis E Virus, Q Fever, Babesia, Zika Virus, etc.

**Most probably,
the situation
will not improve
in the future ...**

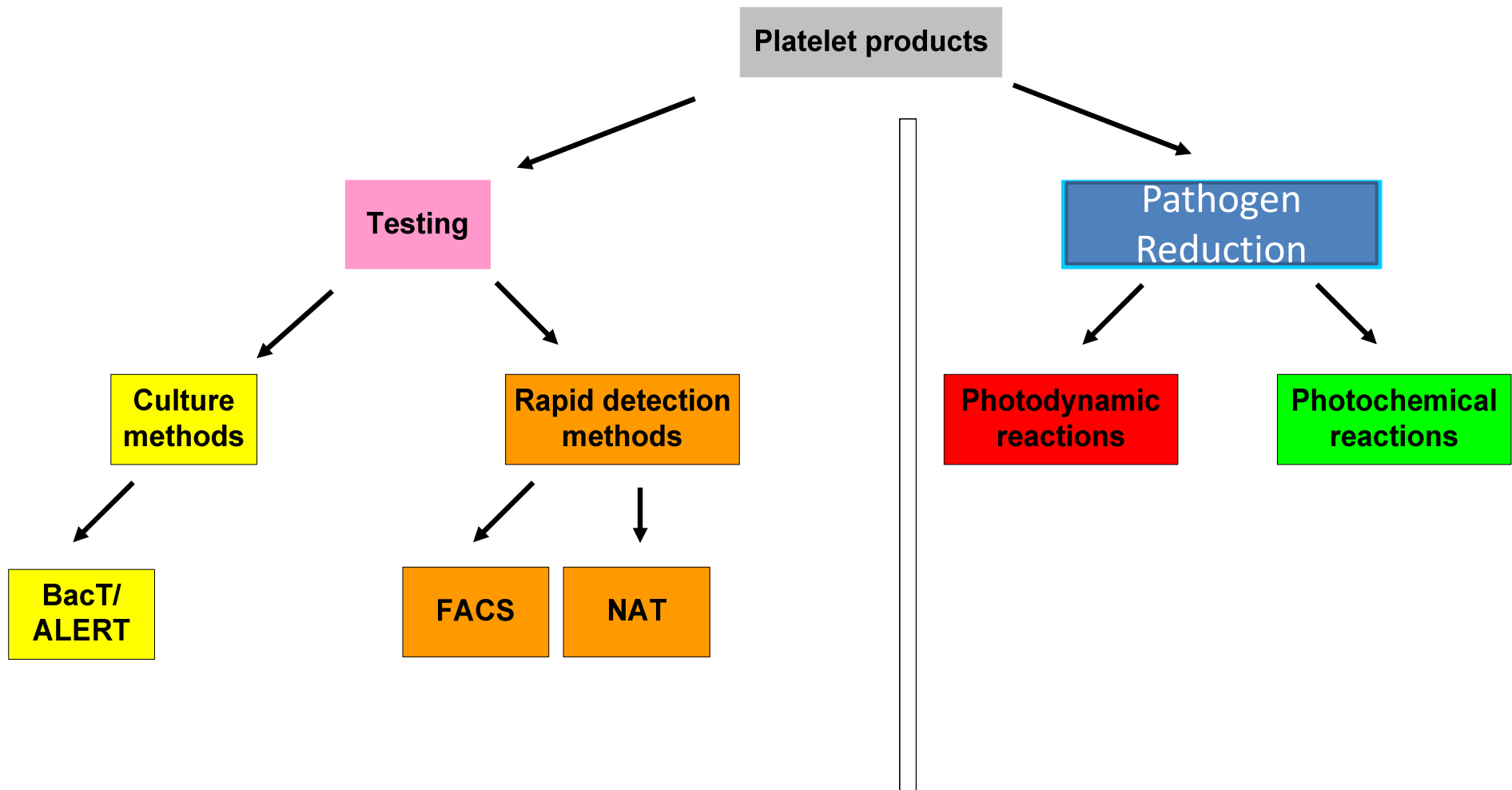


Chapman

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Possible Risk Reduction Strategies

Example: Platelet Concentrates



Testing = Reactive Strategy

Pathogen Reduction = Proactive Strategy

Testing

- What is the next bug?

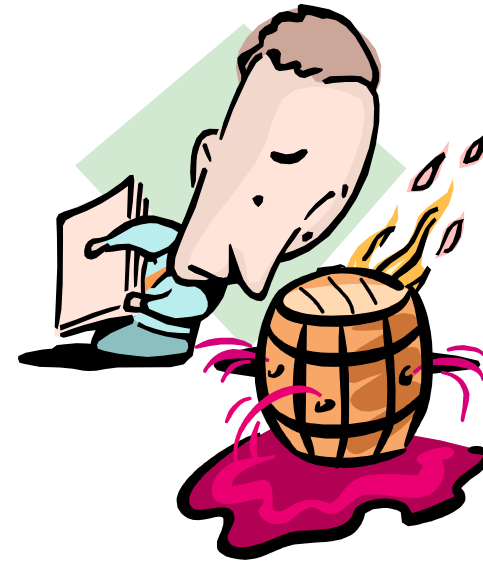


- Great achievements, but outdated already?

&

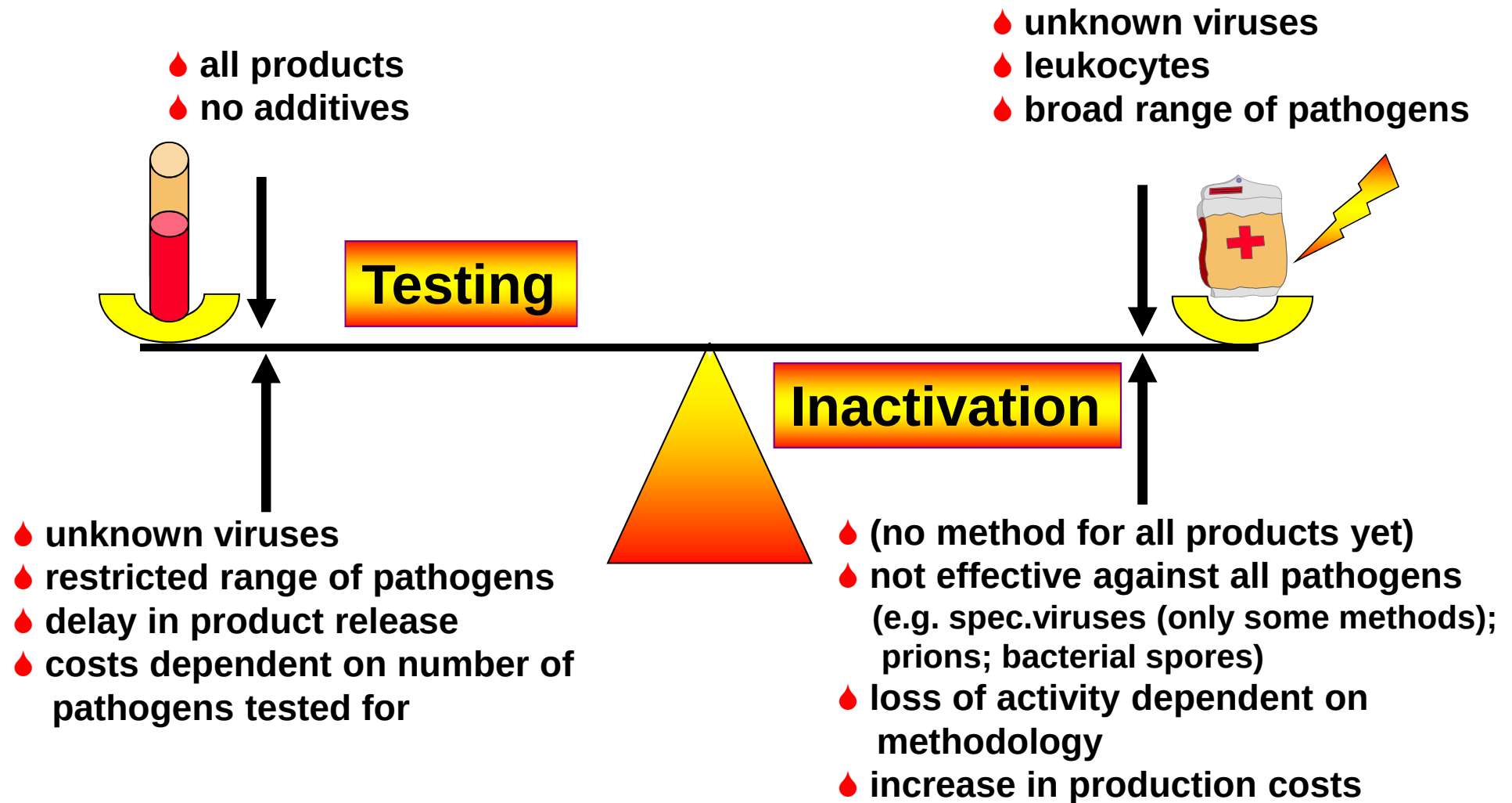
Pathogen Reduction:

- Where are the gaps?

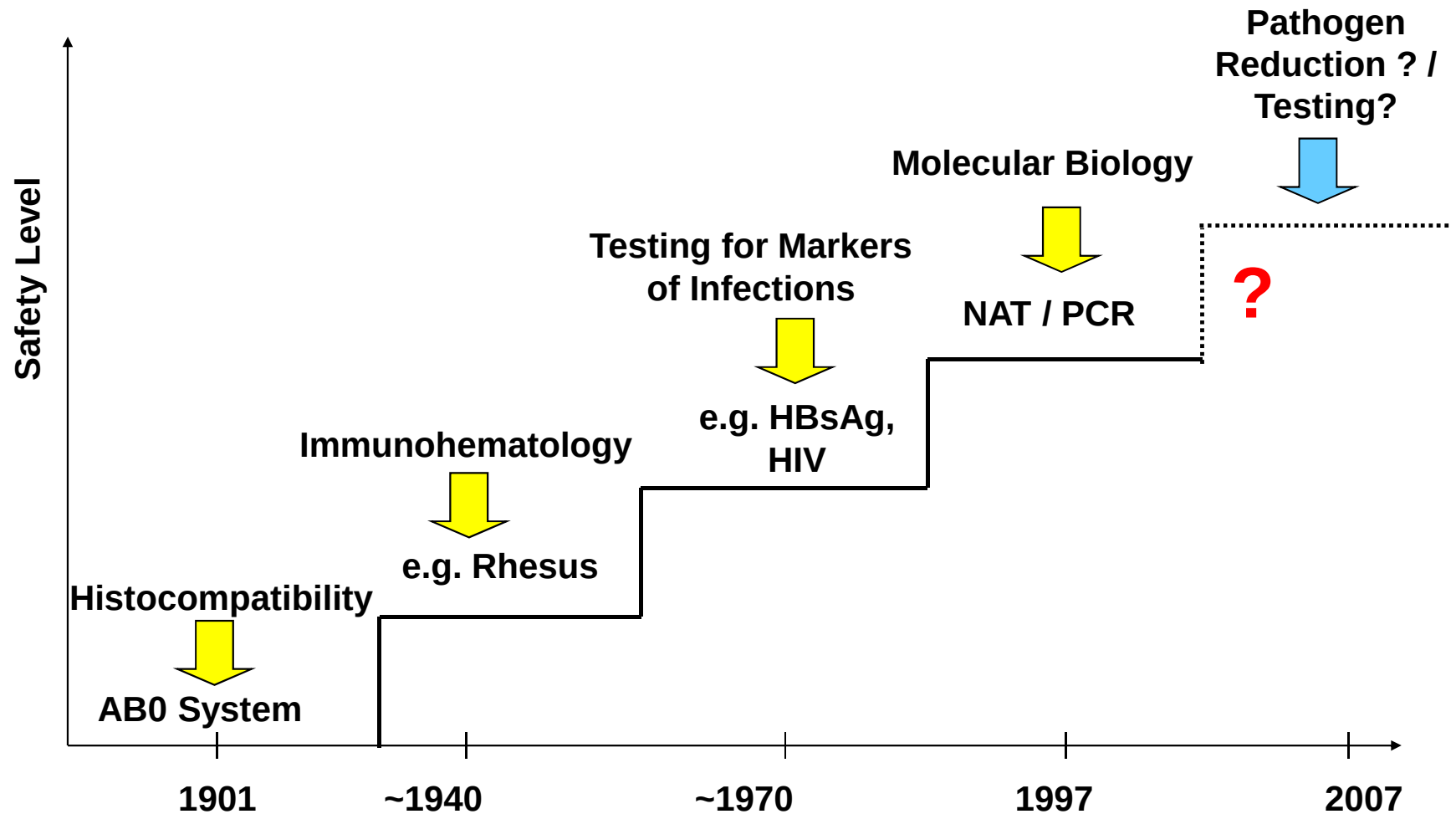


- Not all germs
- not for all blood products by one methodology

Reducing the Risks: Testing versus Inactivation

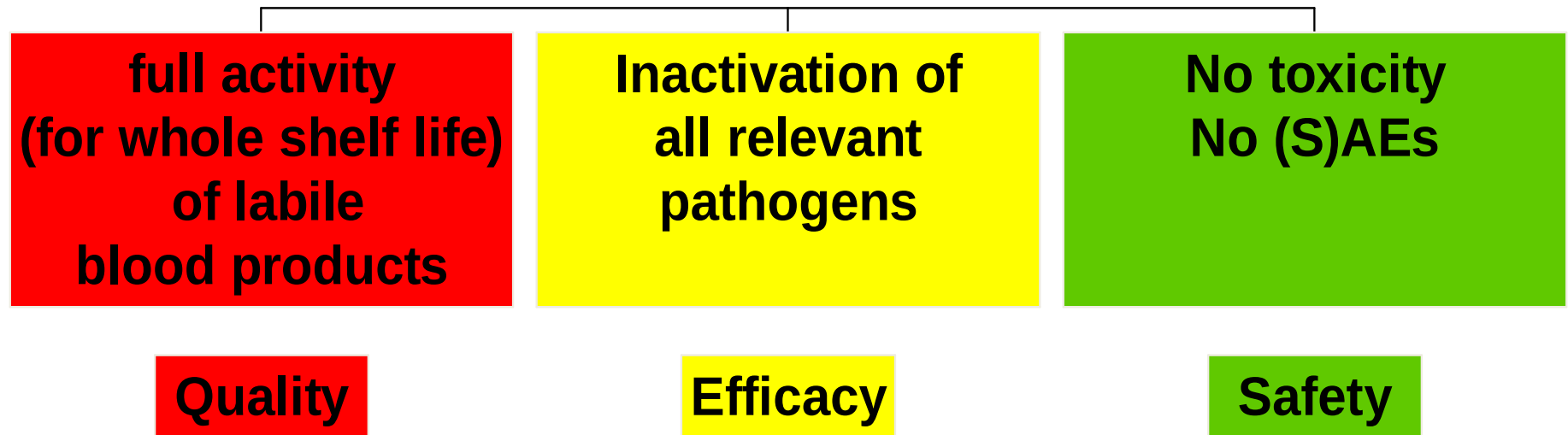


Milestones in the Development of Transfusion Safety



Adapted from Klueter 2004

Specifications for Pathogen Inactivation Methods



Problems to be solved:

- **Safety of Production and Clinical Use ?**
 - Especially in risk populations (pregnancy; intrauterine transfusion, pre-term immunocompromised babies, etc.) ?
- **Feasibility? Integration in production processes ?**
- **Costs ! Might these become prohibitive ?**

Phase 3 Evaluation of INTERCEPT RBC: In vitro characterisation and patient outcome

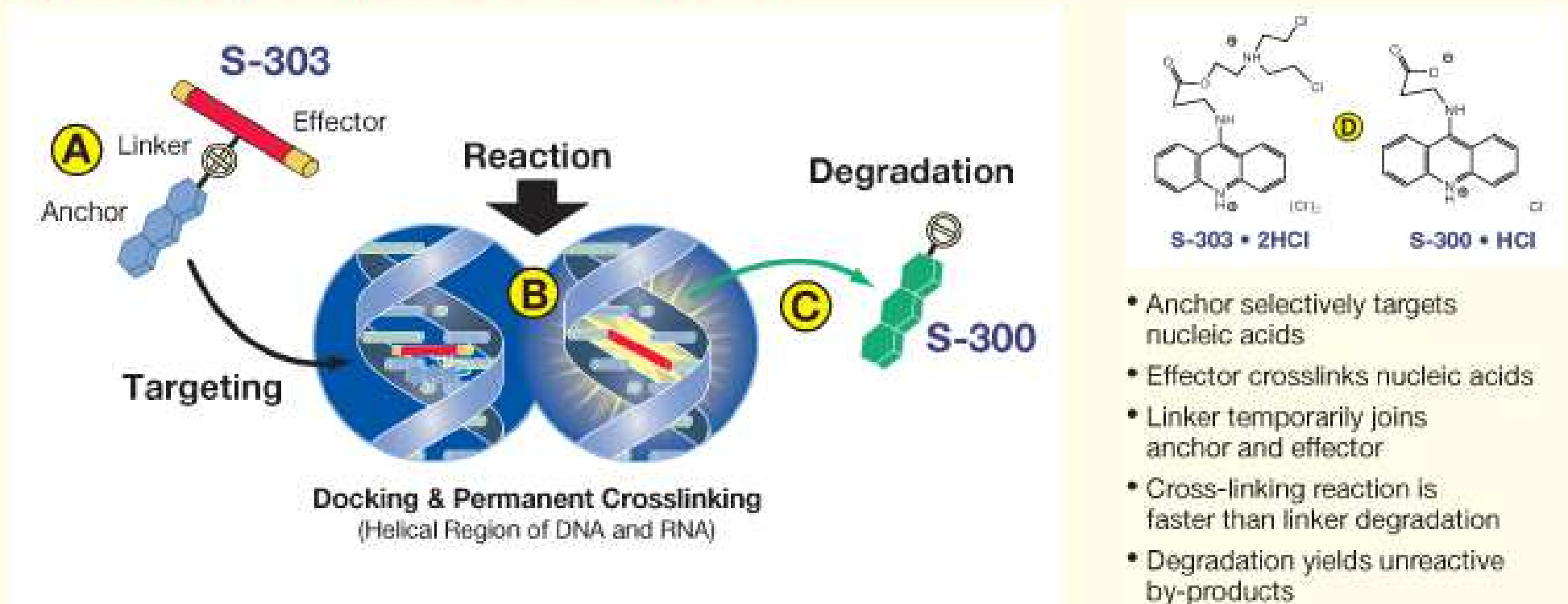
A Randomized Controlled Double-Blind Phase 3 Study to Assess Characteristics of S-303 Treated RBC Components and Evaluate Safety and Efficacy in Patients Requiring Transfusion Support for Acute Anemia

Dr. Markus M. Mueller, M.D.
German Red Cross Blood Transfusion Service
Frankfurt/Main, Germany



S-303 Mechanism of Action

Figure 1: S-303 Treatment Process Mechanism of Action



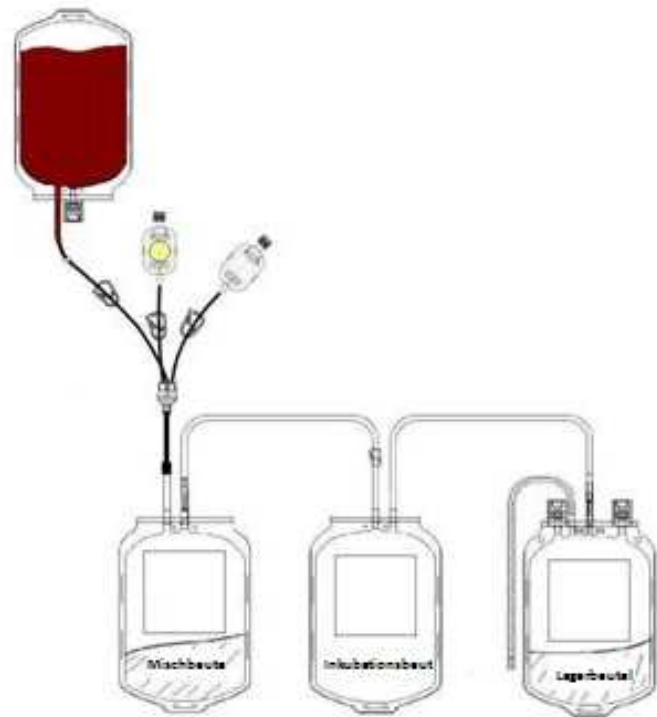
S-303 is a nucleic acid-targeted alkylator that quickly diffuses into viruses, bacteria, parasites and blood cells and is designed to react quickly and decompose

Glutathione (GSH) is used to quench side reactions of the effector with other biological materials

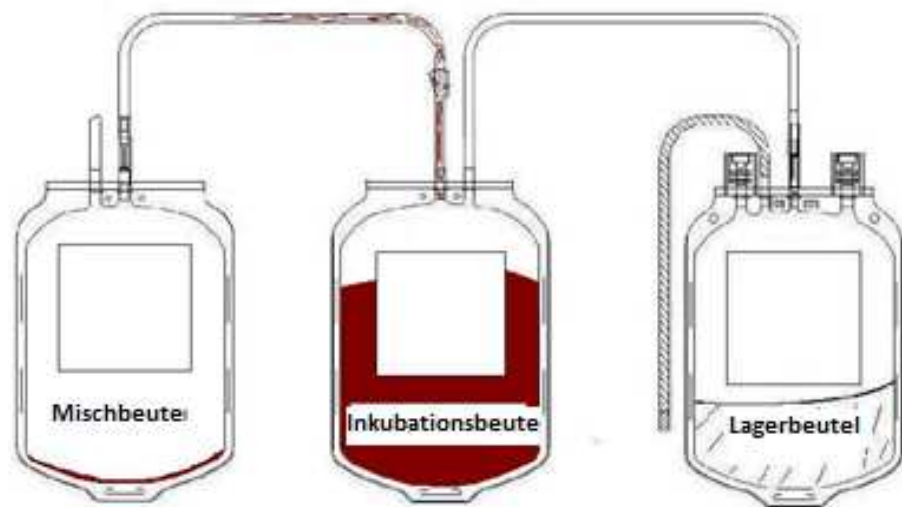
Study design

- Randomized, controlled, multicenter Phase 3 clinical trial
- Population: 50 transfused elective cardiovascular surgery patients undergoing first time CABG or valve repair or combination
- Intervention: Transfusion of S-303 treated RBC, stored up to 35 days in SAG-M, administered according to local standard clinical practice
- Comparator: Conventional RBC stored up to 35 days in SAG-M, administered according to local standard clinical practice. To prevent unblinding, the conventional RBC is transferred into the storage bag of the pathogen inactivation system
- Outcome:
 - Primary endpoint: S-303 RBCs are non-inferior to conventional RBC with respect to grams of Hb per RBC unit
 - Secondary endpoint:
 - a) Incidence of renal insufficiency as measured by creatinine
 - b) Incidence of hepatic insufficiency as measured by total bilirubin
 - full safety assessment
- Timeframe: RBC support up to 7-days (day of surgery plus 6 days post-op)

RBC, Glutathione and S-303 are mixed

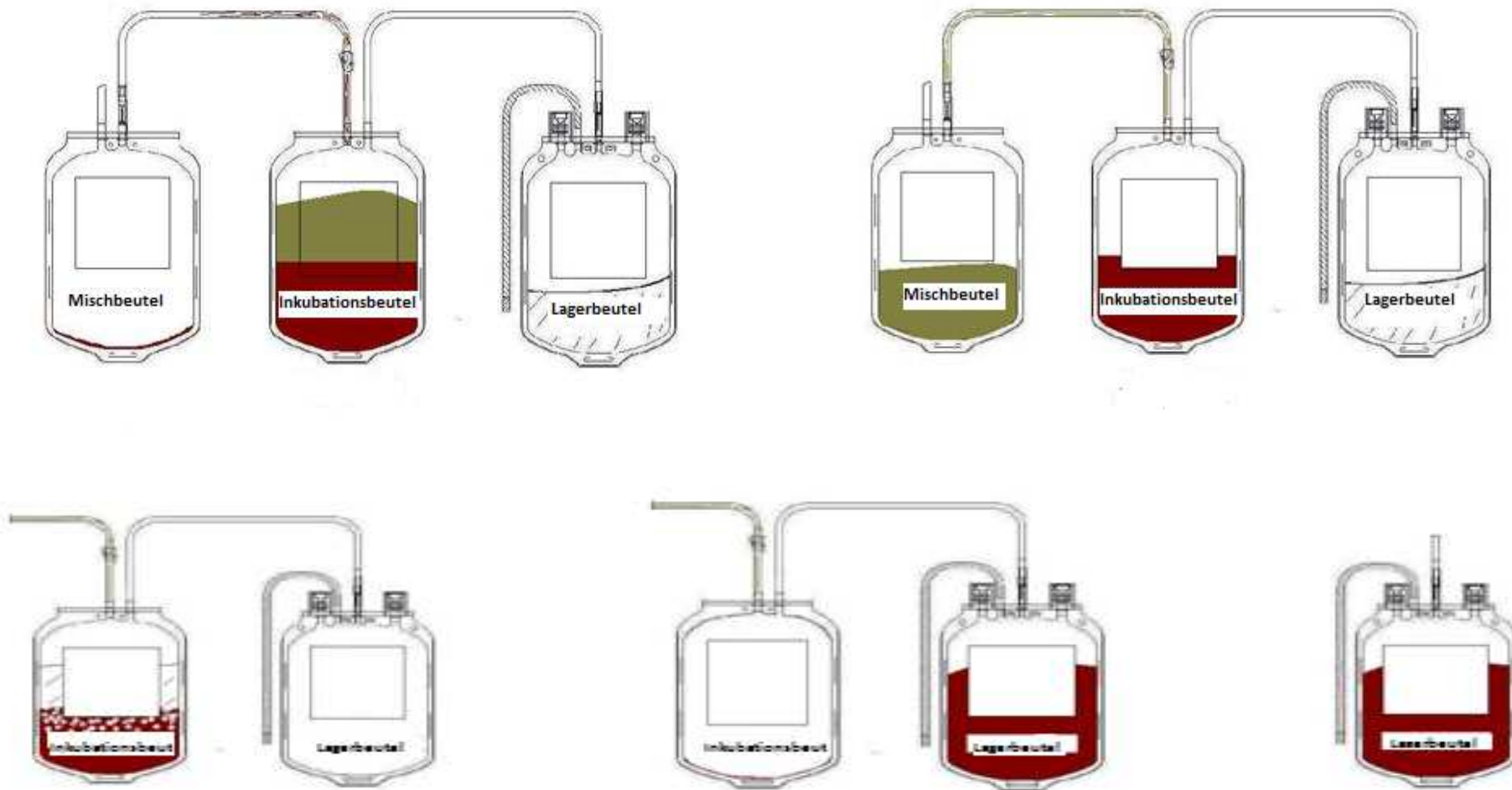


Incubation



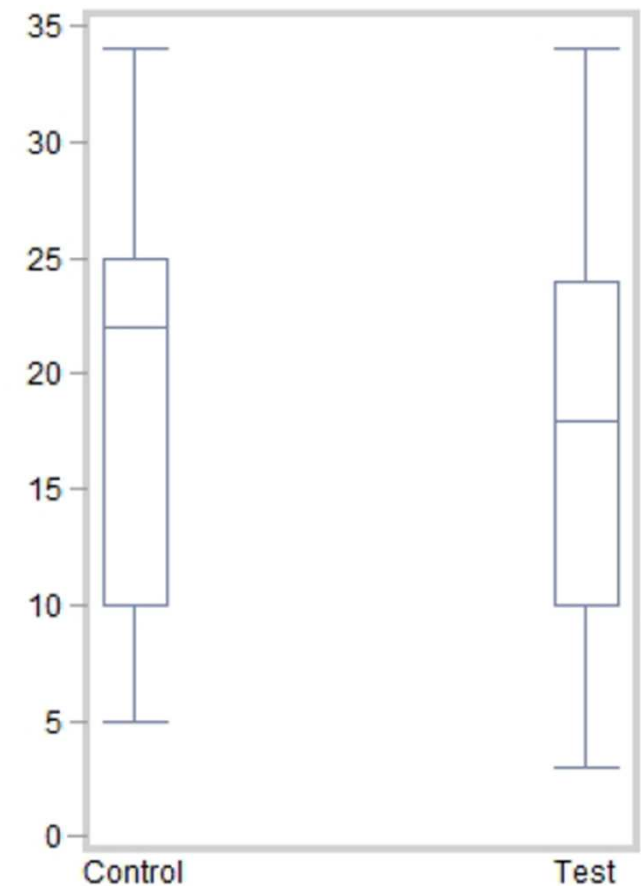
Incubation at 20°C – 25°C for 18-24h

Removal of S-303 degradation products



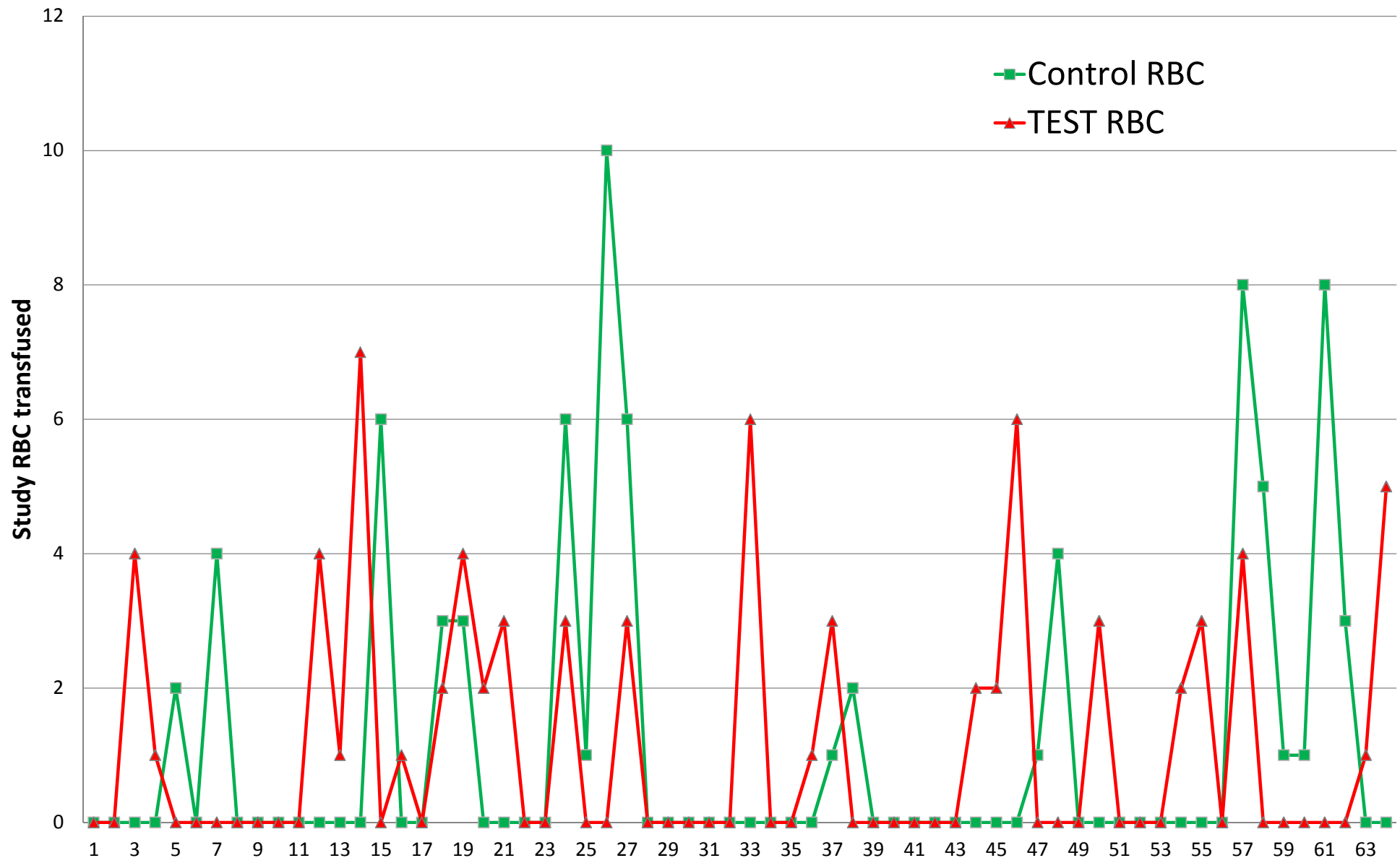
Study RBC

- Study RBC produced for the clinical trial 774
- Number of TEST RBC (S-303 treated) 402
- Number of Control RBC (untreated) 372
- RBC transfused 148
- RBC delivered (including returned RBC) 507
- Acridin Antibody Test 291
- Study RBC per patient 2.9 [1-8]
- Average RBC age at day of transfusion
TEST RBC 18.1 days
Control RBC 19.5 days



RBC age at transfusion

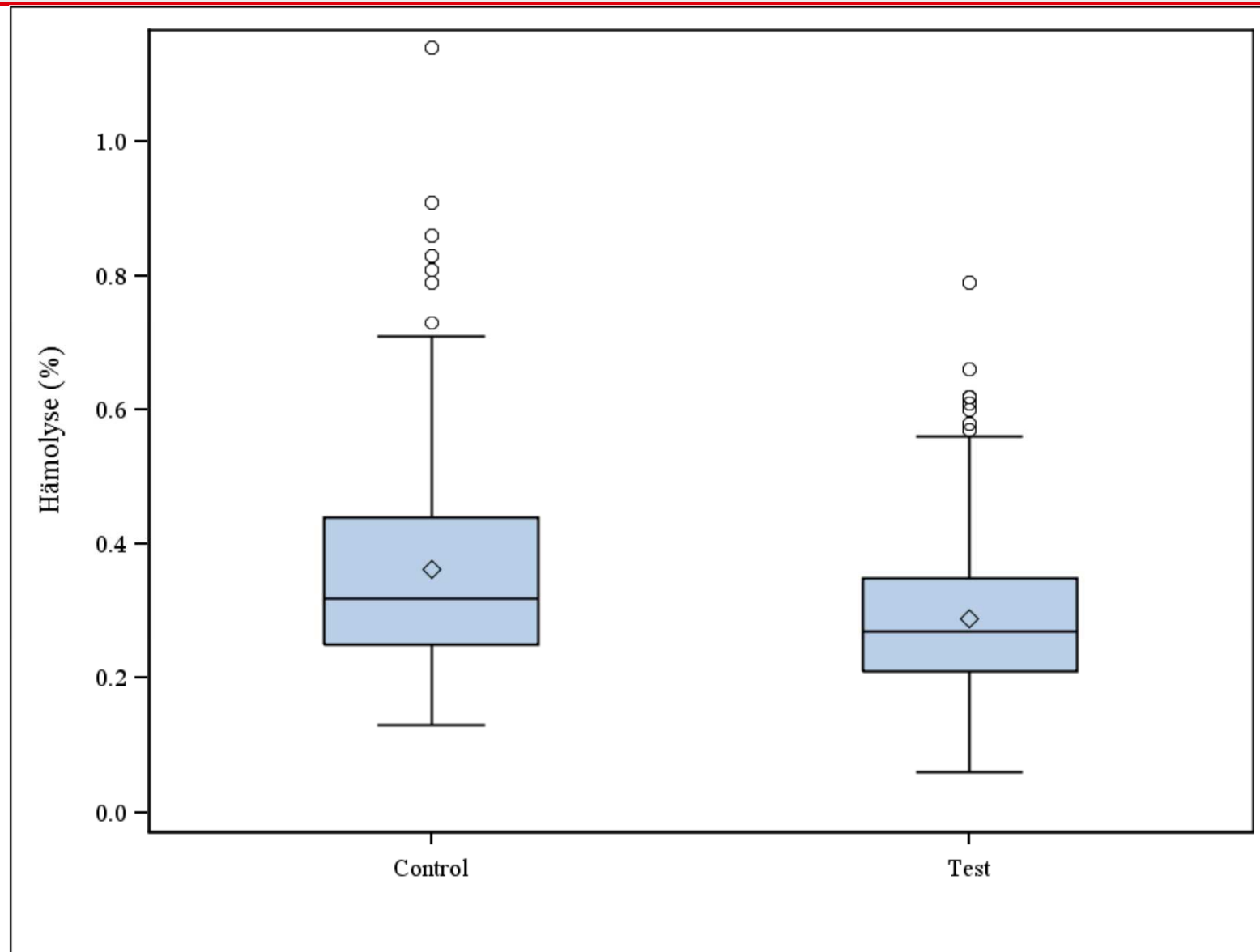
The „fever curve“ of our clinical trial – study RBC transfused per week



Study RBC QC Data 1

	Test RBC	Control RBC	P-Value
Primary Endpoint			
Post-Production Hemoglobin Content (g/unit)			
N	389	365	
Mean (SD)	53.6 (5.6)	56.3 (6.0)	0.500
Secondary Endpoints			
End-of-Storage Hemoglobin Content (g/unit)			
N	301	261	
Mean (SD)	53.1 (5.7)	55.8 (5.9)	0.691
End of Storage Hematocrit (%)			
N	301	261	
Mean (SD)	60.4 (3.2)	60.9 (3.5)	1.000
End of Storage Hemolysis (%)			
N	301	261	
Mean (SD)	0.28 (0.12)	0.35 (0.16)	0.021

Hemolysis at end of shelf life TEST versus Control RBC



Study RBC QC Data 2

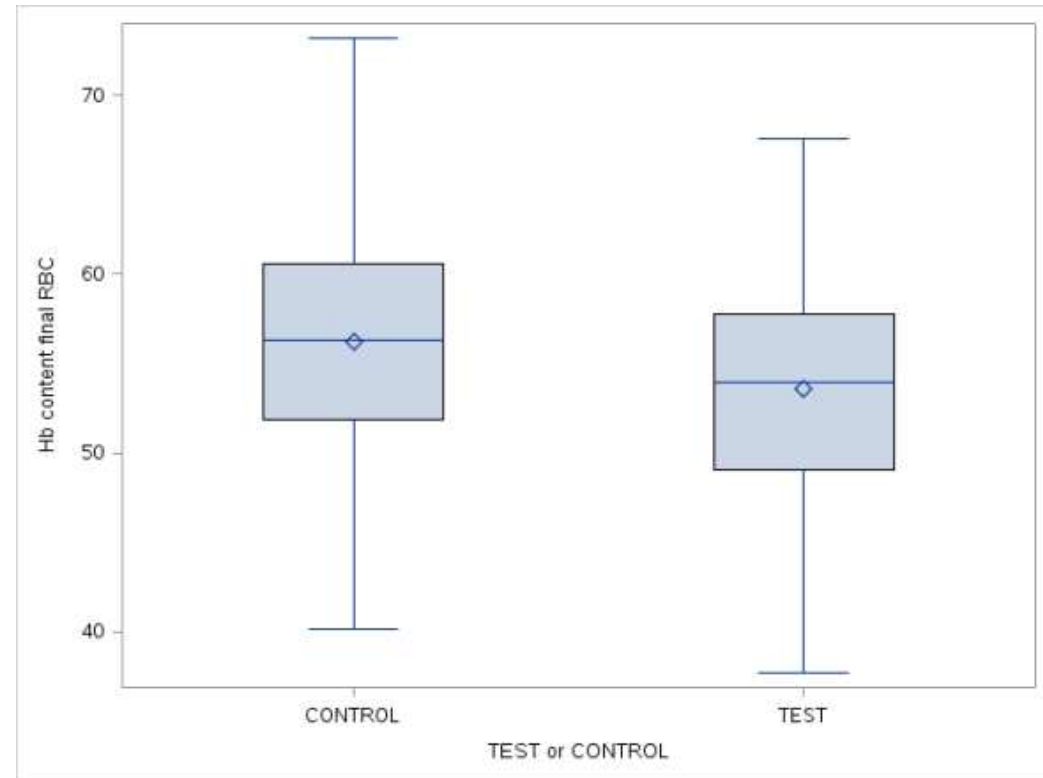
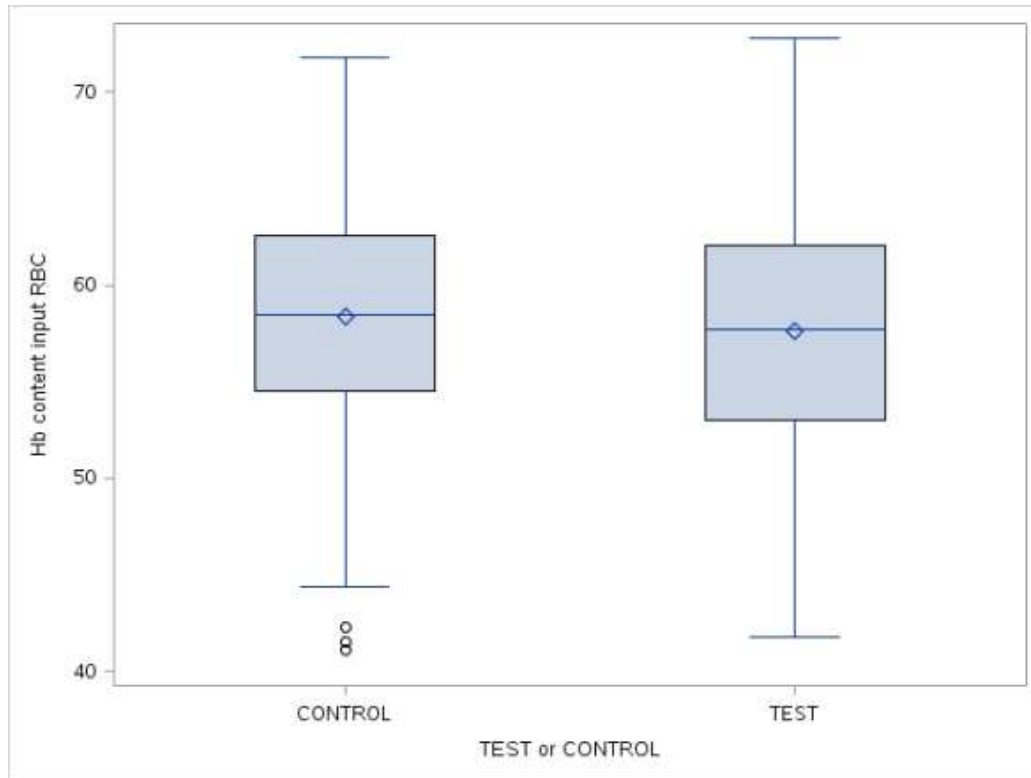
Quality Parameter	Timepoint	Control RBC	Test RBC
Hematocrit (%)	PP	57.3±2.9 (n=367)	57.4±2.0 (n=389)
MCV [fL]	PP	87.5±5.0 (n=367)	85.7±4.8 ^c (n=389)
	EOS	93.6±6.4 (n=225)	91.3±6.6 ^c (n=263)
MCHC [g/dl]	PP	33.6±1.1 (n=367)	34.2±1.2 ^c (n=389)
	EOS	31.4±1.0 (n=225)	32.3±1.4 ^c (n=263)
pH	EOS	6.4±0.1 (n=256)	6.3±0.1 ^c (n=293)
Potassium [mmol/L]	EOS	41.8±3.6 (n=261)	40.6±3.3 ^c (n=243)
Glucose [mmol/L]	EOS	308.2±33.2 (n=163)	317.4±31.0 ^c (n=259)
Lactate [mmol/L]	EOS	29.1±3.3 (n=225)	20.4±2.4 ^c (n=262)
Total Protein [mmol/L]	EOS	228.8±34.7 (n=298)	68.0±25.6 ^c (n=301)

^a PP: Post Production (Day 2)

^b EOS: End of Storage (Day 35-38)

^c p<0.05; p-values for the mean treatment difference (T-C) are based on a T-test with unequal variances.

Hemoglobin content – Input component versus final study RBC

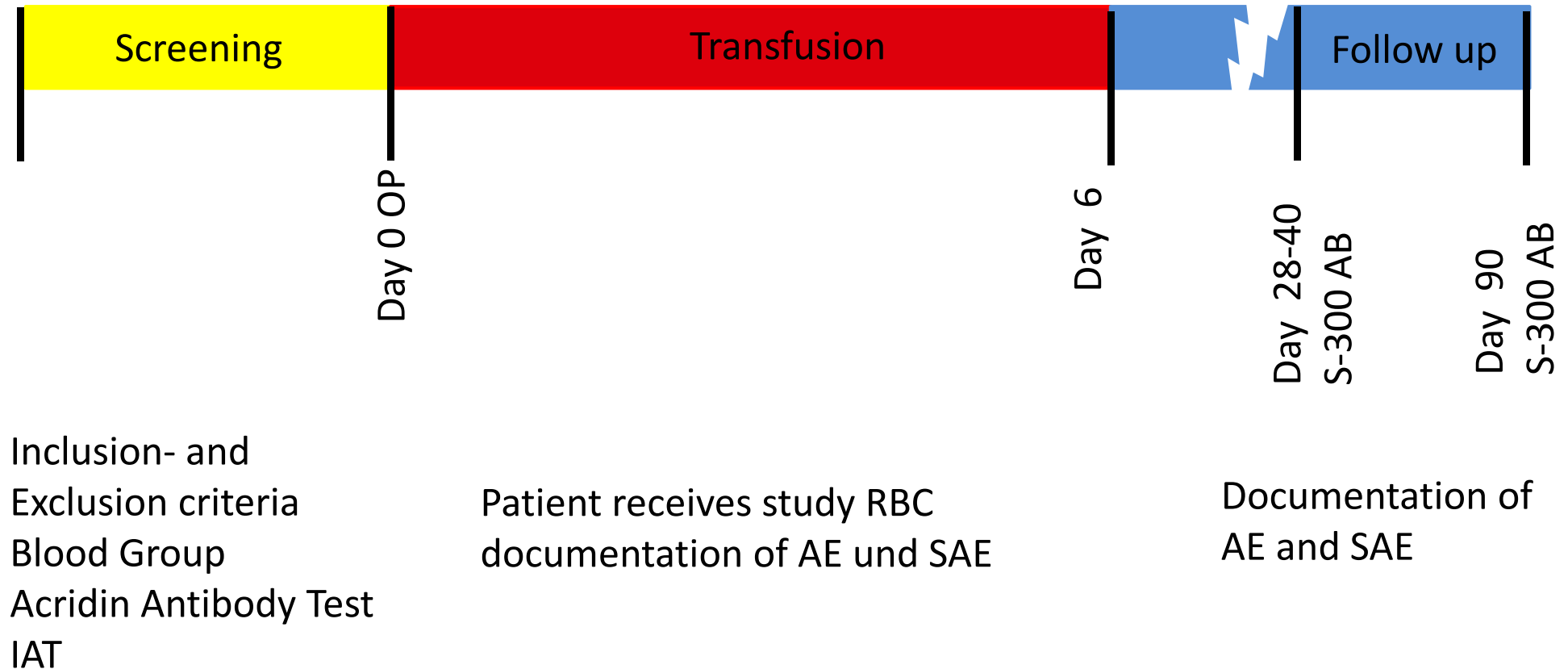


	Control	Test
Mean Hemoglobin content input RBC	58.4 (6.0)	57.6 (5.8)
Mean Hemoglobin content post production	56.3 (6.0)	53.6 (5.6)

Inclusion criteria

- **Age ≥ 18** years, of either gender.
- Must be willing to use an **acceptable form of contraceptive** while on study (as approved by the Investigator or designee)
- Must be readily available by telephone
- EC- approved informed consent
- Must have a negative cross match to S-303 RBCs at study entry (**no pre-existing Ab specific to S-303 treated RBC**)
- Must have a blood type of either **A+ or O+**
- Patients must have a **high likelihood of receiving a transfusion** as determined by the Investigator OR a TRUST Score of ≥ 3 at study entry
- Must be scheduled to receive one of the following operative procedures (exceptions possible):
 - Coronary artery bypass graft only, first procedure
 - Valve repair or replacement only, first procedure
 - A combination of first time CABG and valve repair or replacement

Study flow chart



Patients transfused

- Patients enrolled: 87
- Patients transfused: 51
- Patienten with off-protocoll transfusion: 4
- No adverse reaction due to study RBC
- No mistakes during product assignment, crossmatching or RBC delivery

Transfused Patients

	Randomized patients with any study RBC exposure (n=51)		
	Test (n=25)	Control (n=26)	P-Value]
Baseline Variables			
Age (years)	73.9 (7.7)	74.3 (6.5)	0.861
Proportion of Females	11 (44.0%)	16 (61.5%)	0.192
Body Mass Index (kg/m ²)	27.8 (5.8)	26.4 (4.2)	0.317
Baseline Hgb (g/dL)	12.7 (0.8)	12.4 (1.2)	0.217
Overall Surgical Variables			
Overall Proportion of Bypass Pump Use	22 (88.0%)	23 (88.5%)	0.912
Overall Proportion of Aortic Cross Clamp Use	22 (88.0%)	23 (88.5%)	0.912
Overall Proportion of Cell Saver Use	13 (52.0%)	15 (57.7%)	0.781
Est Vol of Surgical Bld Loss (L)	1.57 (2.13)	1.32 (0.93)	0.63
Proportion With Surgical Complications Leading to Additional Blood Usage	1 (4.0%)	2 (7.7%)	0.631
Transfusion Variables			
Number of Study RBC Units Transfused	2.9 (1.7)	2.9 (2.0)	0.87
Age of Transfused Study RBCs (days)	18.1 (8.6)	19.6 (8.1)	0.253
Est Vol of Non-Study RBCs Transfused (L)	3.17 (4.62)	1.14 (0.64)	0.625
Proportion With Platelet Exposure	7 (28.0%)	8 (30.8%)	0.91

Development of antibodies in study patients during the clinical trial

- Antibodies against acridine were not detected in any study patient at any time during the clinical trial
- A patient in the TEST treatment group [02B-0036] developed a Jk(a) antibody without clinical signs of hemolysis
- A patient in the TEST treatment group [01B-0001] developed a Lu(a) antibody without clinical significance

Adverse Events

Overall Summary of Treatment-Emergent Adverse Events (MITT Patients)

	Valve Only		CABG Only		Valve and CABG		All Patients			
	Test (N=10)	Control (N=8)	Test (N=12)	Control (N=13)	Test (N=3)	Control (N=5)	Test (N=25)	Control (N=26)	Total (N=51)	P-Value [1]
AE by Worst Severity										
No AE	1 (10.0%)	2 (25.0%)	2 (16.7%)	3 (23.1%)	0	0	3 (12.0%)	5 (19.2%)	8 (15.7%)	0.149
Grade 1	0	0	0	0	1 (33.3%)	2 (40.0%)	1 (4.0%)	2 (7.7%)	3 (5.9%)	
Grade 2	1 (10.0%)	2 (25.0%)	4 (33.3%)	7 (53.8%)	0	0	5 (20.0%)	9 (34.6%)	14 (27.5%)	
Grade 3	4 (40.0%)	1 (12.5%)	3 (25.0%)	2 (15.4%)	0	1 (20.0%)	7 (28.0%)	4 (15.4%)	11 (21.6%)	
Grade 4	3 (30.0%)	2 (25.0%)	2 (16.7%)	1 (7.7%)	1 (33.3%)	1 (20.0%)	6 (24.0%)	4 (15.4%)	10 (19.6%)	
Grade 5	1 (10.0%)	1 (12.5%)	1 (8.3%)	0	1 (33.3%)	1 (20.0%)	3 (12.0%)	2 (7.7%)	5 (9.8%)	
Total	10 (100%)	8 (100%)	12 (100%)	13 (100%)	3 (100%)	5 (100%)	25 (100%)	26 (100%)	51 (100%)	
AE by Strongest Relation										
No AE	1 (10.0%)	2 (25.0%)	2 (16.7%)	3 (23.1%)	0	0	3 (12.0%)	5 (19.2%)	8 (15.7%)	0.314
Excluded	3 (30.0%)	2 (25.0%)	4 (33.3%)	4 (30.8%)	1 (33.3%)	2 (40.0%)	8 (32.0%)	8 (30.8%)	16 (31.4%)	
Unlikely	4 (40.0%)	4 (50.0%)	5 (41.7%)	5 (38.5%)	0	1 (20.0%)	9 (36.0%)	10 (38.5%)	19 (37.3%)	
Possible	2 (20.0%)	0	1 (8.3%)	1 (7.7%)	2 (66.7%)	2 (40.0%)	5 (20.0%)	3 (11.5%)	8 (15.7%)	
Total	10 (100%)	8 (100%)	12 (100%)	13 (100%)	3 (100%)	5 (100%)	25 (100%)	26 (100%)	51 (100%)	

Summary

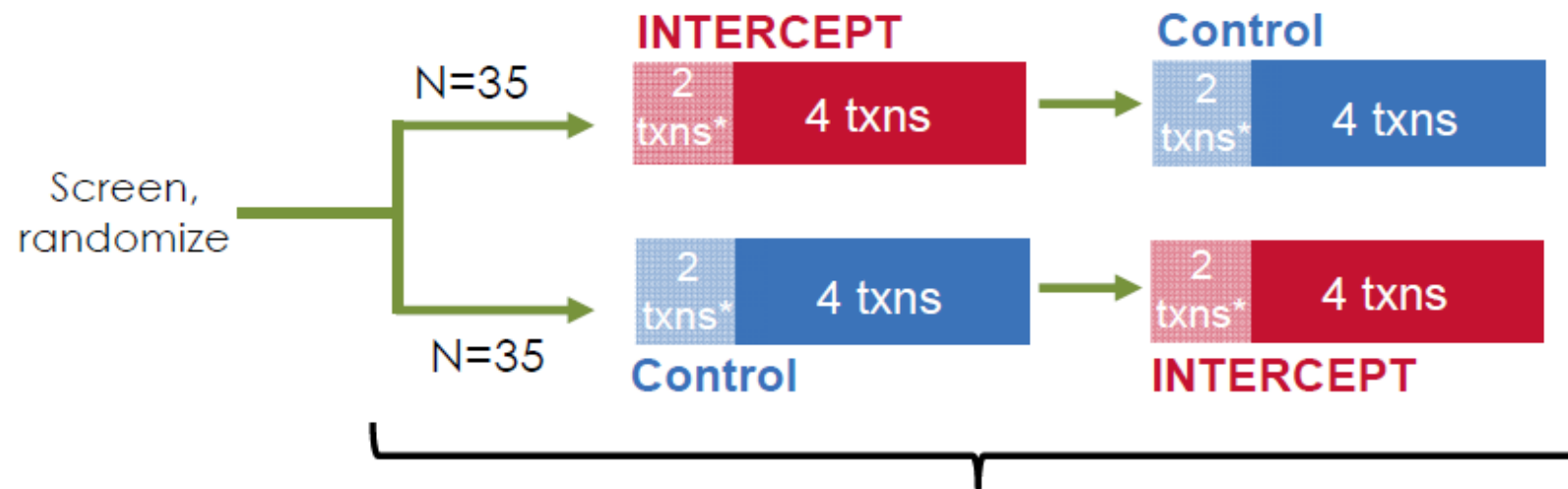
- PI RBC in this study were non-inferior compared to control RBC regarding the mean hemoglobin content post production (primary endpoint)
- S-303 treated RBC were transfused in two clinical centers without any transfusion reaction or adverse event with probable or definite relation to study medication
- Average number of study RBC transfused per patient was comparable in TEST and Control patient group (2.9 RBC per patient in both groups)
- QC data of TEST and CONTROL RBC showed promising results for S-303 treated RBC – low hemolysis rate, low total protein concentration in TEST RBC

Chronic Transfusion Study

Transfusion-dependent thalassemia major patients (n = 70)

1° efficacy endpoint = Hemoglobin usage

1° safety endpoint = Immunogenicity with repeat exposure



Whole Blood Pathogen Inactivation Safe Blood for Africa

Anaïs ALTMAYER, Ph.D
Transfusion Swiss Red Cross



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 - Dr Nina MUFTI
 - Dr Anne NORTH – Dr Tovo DAVID
- **Research Team :**
 - Dr Soraya AMAR EL DUSOUQUI
 - Dr Anaïs ALTMAYER
 - Dr Emmanuel RIGAL – Dr Andreas BUSER



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Our African partners



- Dr Claude TAYOU: University Hospital, Yaoundé – CAMEROON
- Dr Mamadou Yassongui SEKONGO: National Blood Transfusion Center, Abidjan – IVORY COAST
- Dr Ludovic ANANI: National Agency for Blood Transfusion, Cotonou – BENIN



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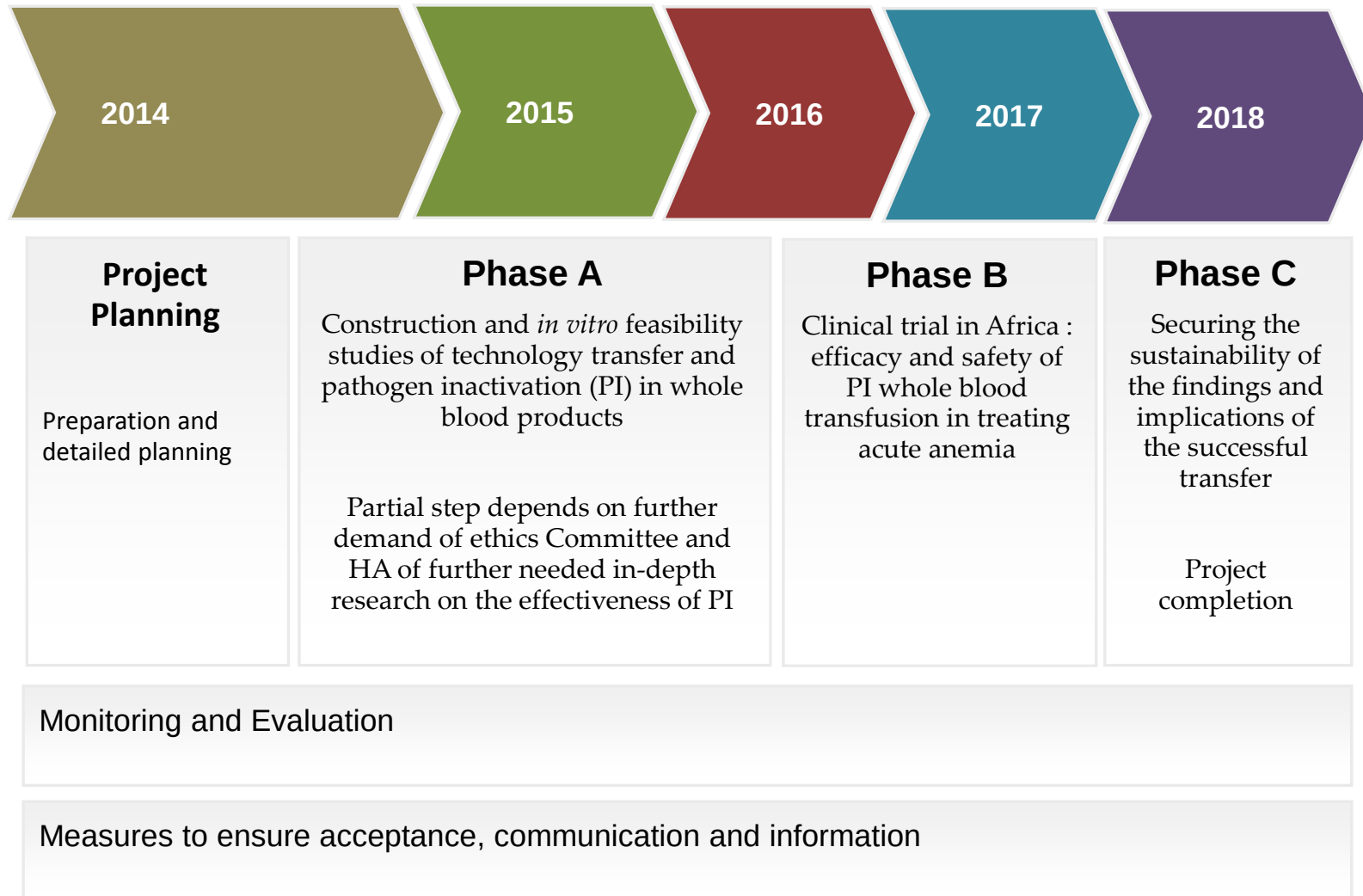
Current situation of blood bankers in low resource countries

- Availability of only 40% of blood products needed
- Zone of high prevalence of malaria, HCV, dengue fever and HIV
- No serological tests for HIV, HBV, HCV and syphilis for each donation
- Frequent inability to irradiate blood bags collected from family donors
- 75% of donations are transfused as whole blood units
- **Adaptation of CERUS' INTERCEPT Technology for Red Blood Cells**



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Phases of the WB PI project



Current phase: Phase A



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RESULTS : Coagulation factors

- **Primary outcome of our study:** preserve erythrocytes functions to treat acute anaemia but not coagulation disorders
- % of coagulation factors is decreasing when increasing GSH concentration
- **The compromise:** choose a GSH concentration that preserves coagulation factors ?



Coagulation Analysis (mechanical testing) at CERUS (Geneva samples)

	No treatment	S-303 only	2 mM GSH	5 mM GSH	7.5 mM GSH	10 mM GSH	20 mM GSH	Required for Hemostasis (% of normal)
PT (sec)	12.1	15.1	18.4	21.6	26.6	30	52.7	
aPTT (sec)	33.1	46.7	54.7	70.8	116	123	no clot	
Percent of Control								
FII		54.2	58.1	62.4	60.9	59.9	61.2	40
FV		65.5	74.4	77.9	78.8	81.1	68.7	10-15
FVII		31.5	49.8	41.1	42.6	37.5	22.4	5-10
FVIII		36.1	66.9	56.5	48.2	61.8	44.1	10-40
FIX		34.9	43.9	36.0	26.0	19.4	9.4	10-40
FX		41.1	32.4	19.2	11.9	9.2	3.9	10-15
FXI		65.3	62.6	48.8	36.5	31.9	11.2	20-30
FIB		81.1	82.2	83.2	77.3	76.4	74.3	30

Conclusion: Individual coagulation factor activity showed similar results as seen before, reduced factor activity with increased GSH concentration. Some individual factors are more affected than others.

Conclusion of the second SAB meeting

Conservation of RBC general characteristics confirmed across all GSH concentrations

Effect of GSH on coagulation factor activity confirmed with mechanical assay of coagulation activity; some attributes of the ROTEM assay also effected

**Little preservation of platelet function across all GSH concentrations
Recommend SAB to consider GSH concentration less than 20 mM and less than 10 mM**



Effect of *Plasmodium* inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial

Lancet 2016; 387: 1753-61

Jean-Pierre Allain, Alex K Owusu-Ofori, Sonny Michael Assennato, Susanne Marschner, Raymond P Goodrich, Shirley Owusu-Ofori

Summary

Background Transfusion-transmitted malaria is a frequent but neglected adverse event in Ghana. We did a randomised controlled clinical trial to assess the efficacy and safety of a whole blood pathogen reduction technology at preventing transfusion transmission of *Plasmodium* spp parasites.

Findings Between March 12, 2014, and Nov 7, 2014, 227 patients were enrolled into the study, one of whom was subsequently excluded because she did not meet the inclusion criteria. Of the 226 randomised patients, 113 were allocated to receive treated whole blood and 113 to receive standard untreated whole blood. 223 patients (111 treated and 112 untreated) received study-related transfusions, whereas three patients (two treated and one untreated) did not. 214 patients (107 treated and 107 untreated) completed the protocol as planned and comprised the per-protocol population. Overall, 65 non-parasitaemic patients (28 treated and 37 untreated) were exposed to parasitaemic blood. The incidence of transfusion-transmitted malaria was significantly lower for the pathogen-reduced (treated) patients (1 [4%] of 28 patients) than the untreated group (8 [22%] of 37 patients) in this population ($p=0.039$). Overall, 92 (41%) of 223 patients reported 145 treatment-related emergent adverse events during the conduct of the study, with a similar incidence of adverse events between groups receiving untreated or treated whole blood. No transfusion-related deaths occurred in the trial.

Interpretation Treatment of whole blood with the Mirasol pathogen reduction system for whole blood reduced the incidence of transfusion-transmitted malaria. The primary endpoint of the study was achieved in the population of non-parasitaemic patients receiving parasitaemic whole blood. The safety profile and clinical outcomes were similar across the two treatment groups.

A SLIDE KINDLY PROVIDED BY WIM DE KORT:

THE FUTURE OF PATHOGEN REDUCTION

*“Prediction is very difficult, especially,
when it concerns the future.”*

Danish Proverb



Special thanks to ...

Dr. Veronika Brixner

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Dr. Larissa Wohlgemuth

Gülten Baran

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Prof. Erhard Seifried

... and to all of you for your kind attention!