Transformation of UK Transplantation Centres to Provide Routine ATMP Services

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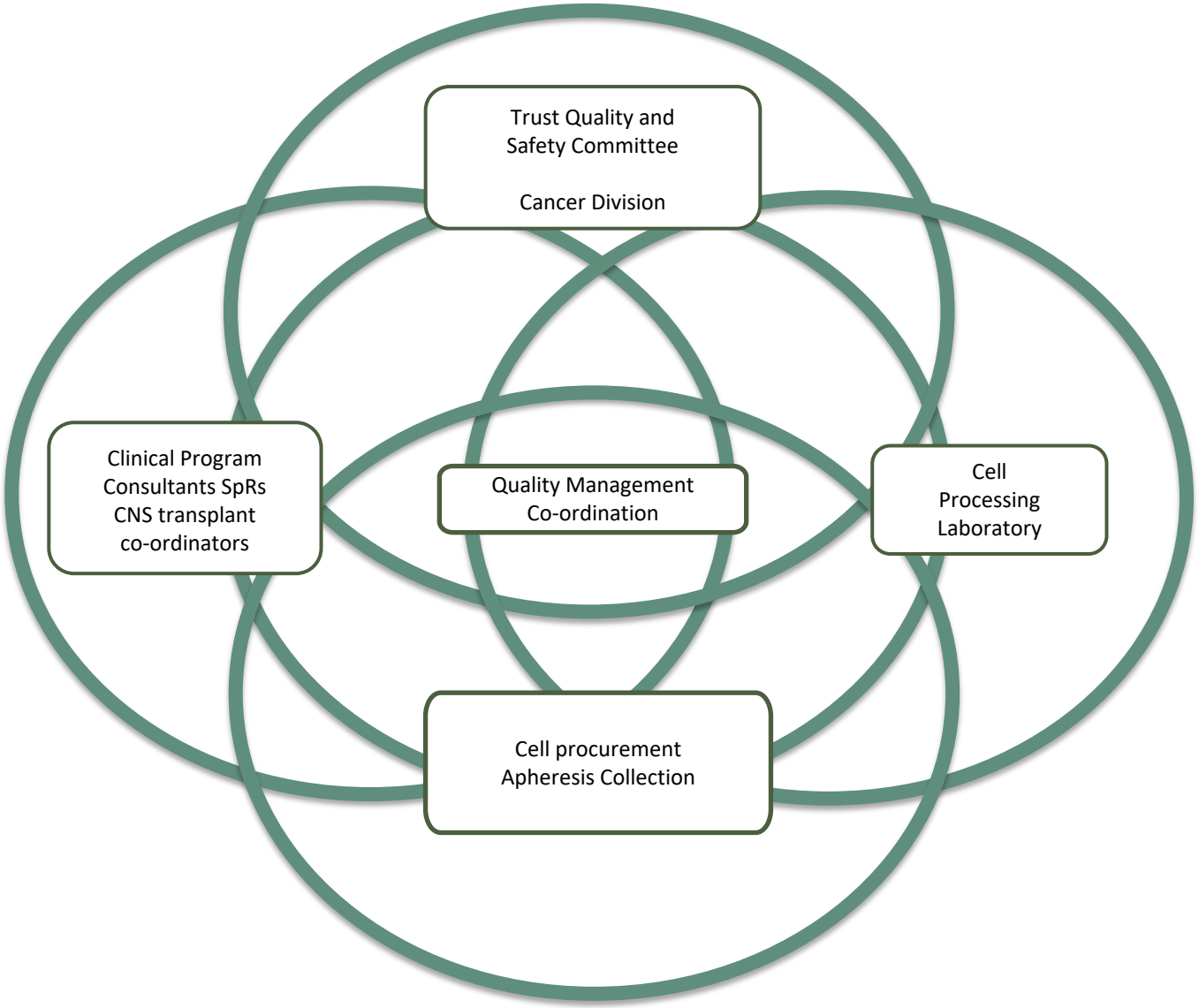
Disclaimer:
No conflicts of interest

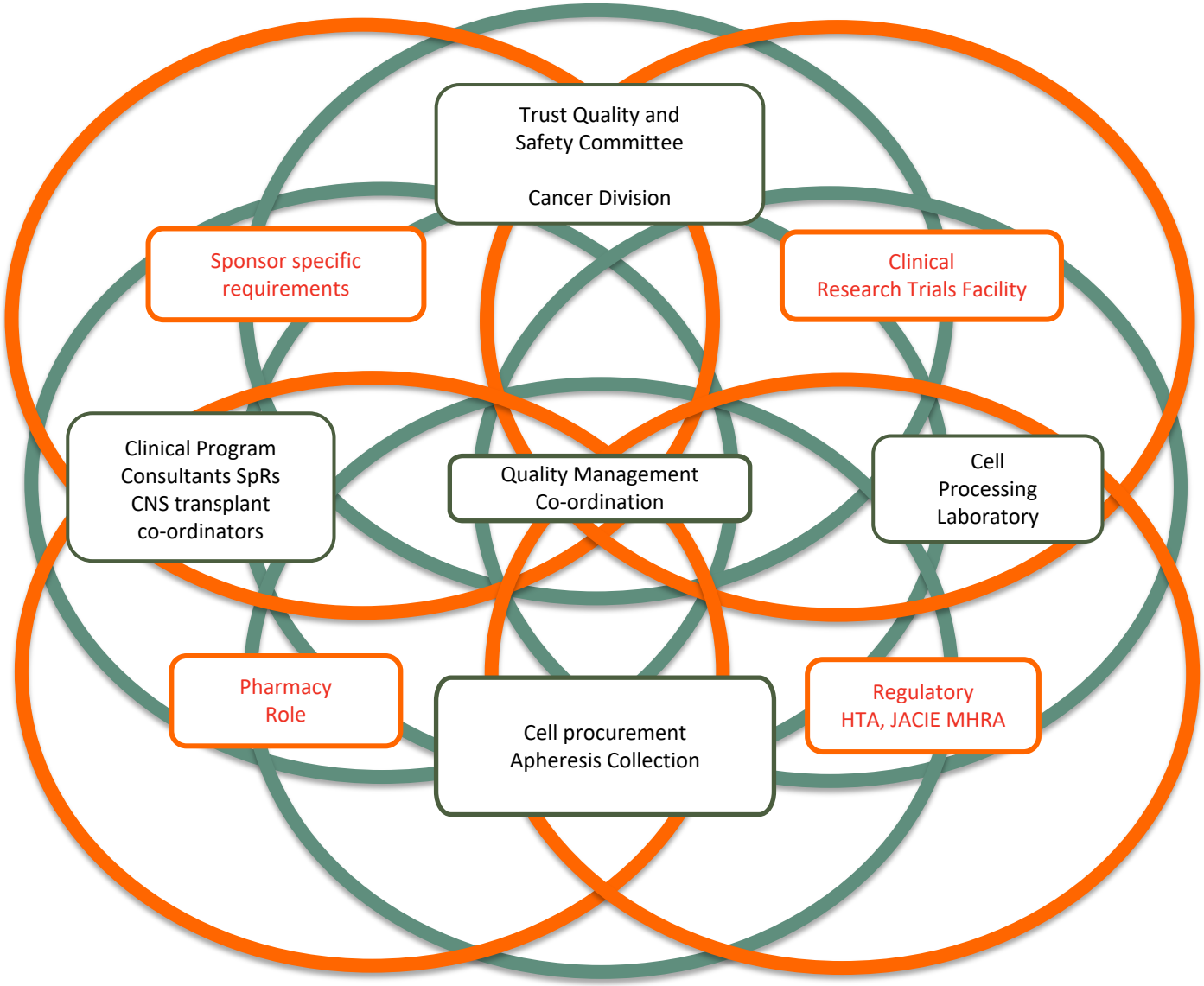
Transformation of UK Transplantation Centres to Provide Routine ATMP Services

1. Impact of incorporating routine ATMP on transplantation service capacity
2. HPC Apheresis Work up for β-thalassaemia Gene Therapy Trial example
3. Prior experience and published data
4. Optimising collection to meet sponsor apheresis targets and a pre-apheresis blood test

GOVERNANCE OF A TRANSPLANTATION PROGRAM



GOVERNANCE OF A TRANSPLANTATION PROGRAM – ADDITION OF ATMP PROGRAM



Sponsor ATMP Feasibility Assessment: Capacity, Costing and Impact Assessment

- Clinical trials office, Haematology Governance & Consultants, Trust risk assessment, funding. Mandatory role of Pharmacy for MHRA licenced products. Review of Clinical infra-structure, dedicated specialist medical/nursing staff, bed capacity (including ITU) neurology and pharmacy support

- **Implementation: Quality Management co-ordination of HSCT team with sponsor and ATMP services**

- Protocol review, team brief, initial visit by the sponsor QA team/audit of facilities, preparation for Site Initiation Visit

- Dedicated trial staff assignments, trial e-mails. Adapt trial-specific deviation from routine practice with new SOPs, policies, procedures, labels/barcodes, staff training (inc. GCP), risk assessments, validation of new procedures/equipment, **e.g. work up of trial-specific Apheresis, cell processing/cryopreservation, ATMP infusion.**

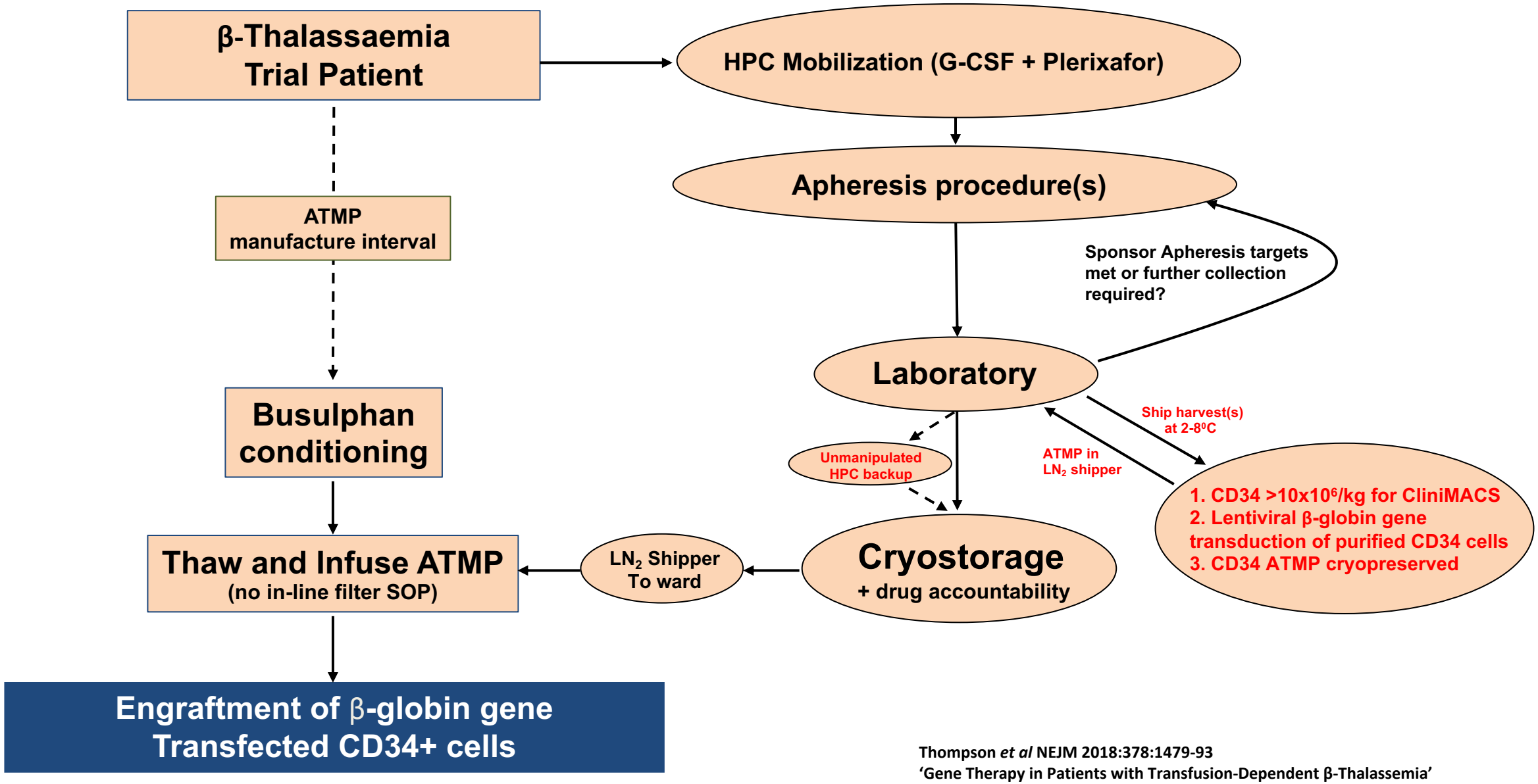
- Trial related Third Part Agreements may be required eg for ATMP storage, starting MNC cryopreservation

- Tenfold or more staff time compared to a routine patient allowance for extended communications, couriers, two person checks, ATMP documentation, Site Licence Files, monitor visits, drug accountability etc. Often long delays in trial recruitment then urgent review of trial details/update check – trial research nurse/practitioner essential to assist

- Regulatory : e.g. HTA Import/Export capability, Preparation Process Dossiers (PPD), JACIE immunotherapy module

HPC Apheresis Work up for β-thalassaemia Gene Therapy Trial

Autograft Pathway for Thalassaemia β-globin Gene Therapy



Thompson *et al* NEJM 2018:378:1479-93
'Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia'

Prior experience 1 - HPC Apheresis and β-Thalassaemia Trait

Case 1. Sibling donor β-thalassemia trait HPC,A (Allogeneic) collection (2004)

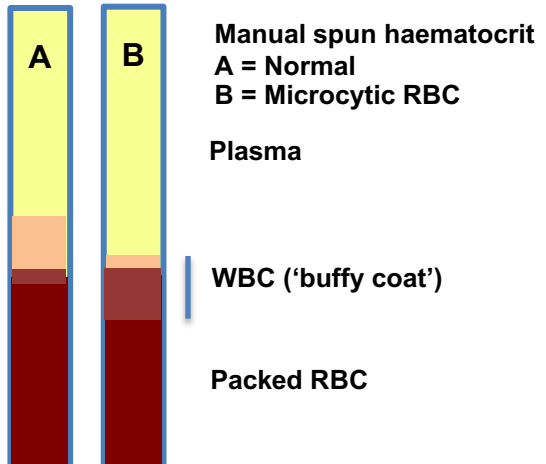
Pre mobilisation blood count:

WBC = 4.8 x10^9/l
HB = 101 g/l
RBC = 5.13 x10^{12}/l
MCV = 61 fl
HBA2 = 5.7%

Apheresis

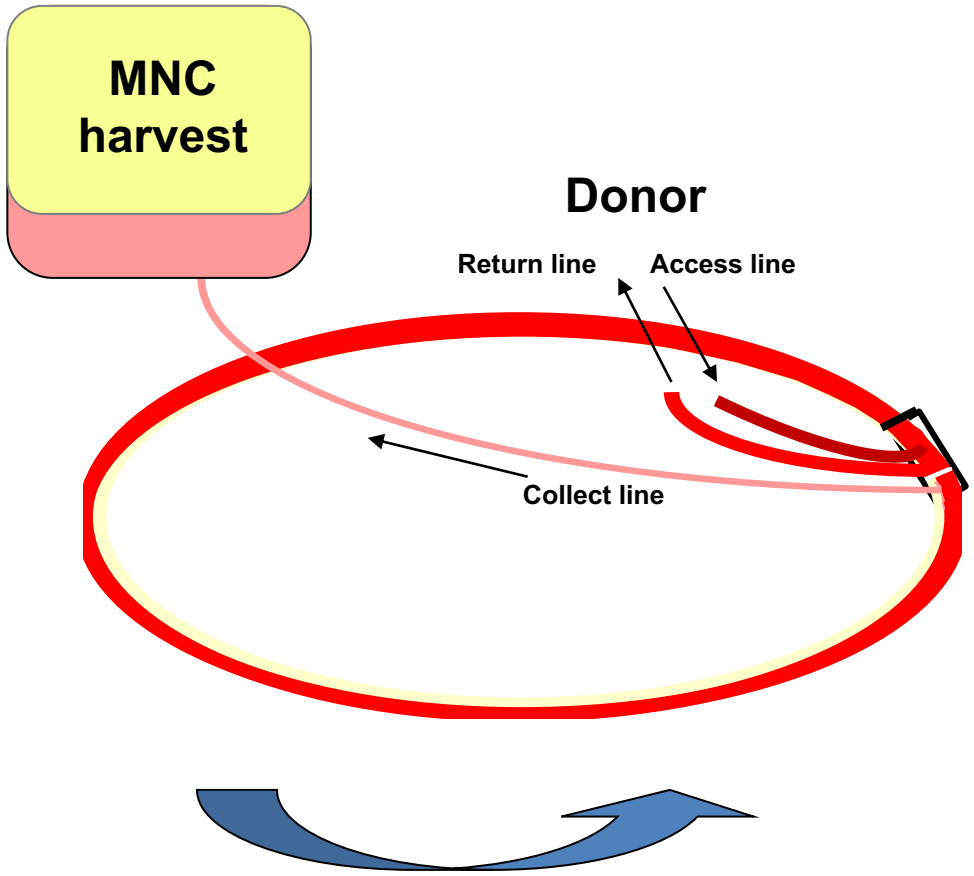
Colorgram	pbWBC x 10^9/l	Pb CD34%	Pb CD34/ul	hWBC x 10^9/l	hVol (ml)	TNC x10^{10}	hCD34 %	CD34 x10^6/kg	HCT%	RBC ml
Aph1= 1-3%	26.6	NA	NA	26.4	170	0.45	0.53%	0.3	0.5	0.9

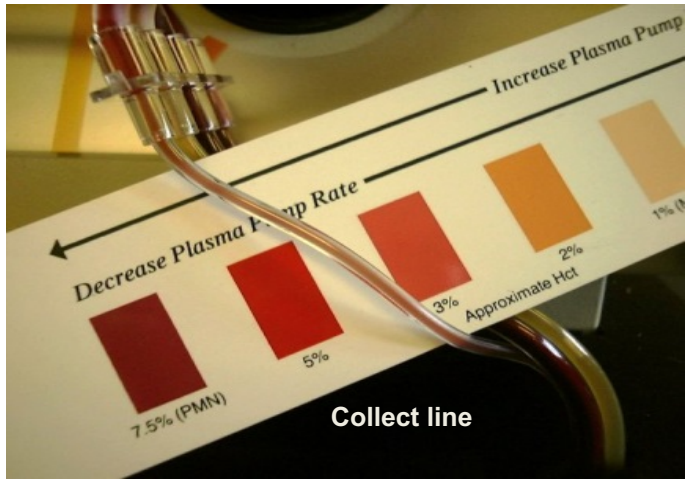
- Very poor harvest CD34 dose of 0.3 x10^6/kg
- Apheresis harvest WBC the same as peripheral blood!
- Microcytic RBC known not to 'pack' as expected in spun haematocrit. WBC 'buffy coat' can be embedded lower in red cell interface. Normal apheresis MNC 1-3% colorgram increase required?



SPECTRA MNC Apheresis

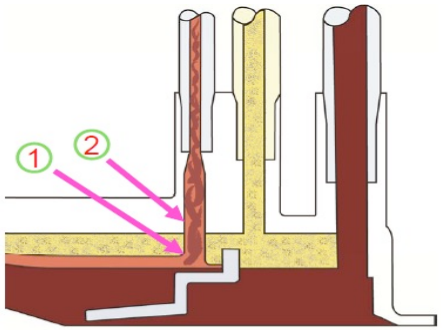
Target cell collection selected by colorgram



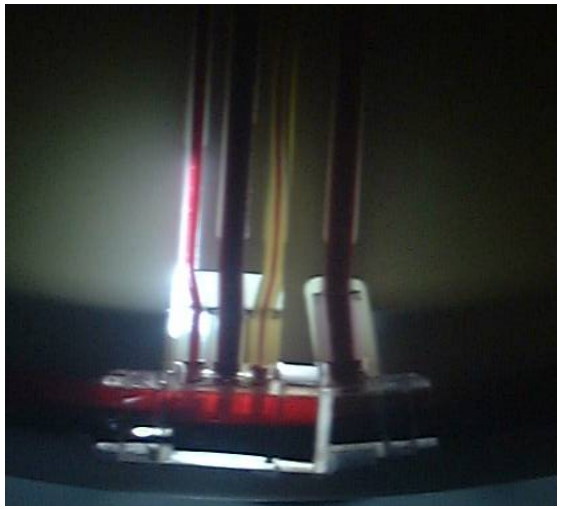


SPECTRA Colorgram (approximate HCT)

'Skimmer dam' MNC separation



(Also applies to Optia CMNC Optia WBC depletion kit)



'Skimmer dam' during run (Strobe light)

Prior experience 1 - HPC Apheresis and β-Thalassaemia Trait

Case 1. Sibling donor β-thalassemia trait for allogeneic HPC,A collection (2004)

Pre mobilisation blood count:

WBC = 4.8 $x10^9/l$
HB = 101 g/l
RBC = 5.13 $x10^{12}/l$
MCV = 61 fl
HBA2 = 5.7%

Apheresis

Colorgram	pbWBC x $10^9/l$	Pb CD34%	Pb CD34/ul	hWBC x $10^9/l$	hVol (ml)	TNC $x10^{10}$	hCD34 %	CD34 $x10^6/kg$	HCT%	RBC ml
Aph1= 1-3%	26.6	NA	NA	26.4	170	0.45	0.53%	0.3	0.5	0.9
Aph2 = 3-5%	34.0	NA	NA	203.0	130	2.64	0.75%	2.7	1.6	2.1

Increasing colorgram to 3-5% captures MNC and CD34 cells but some increase in RBC contamination

Prior experience 2 - HPC Apheresis and α-Thalassaemia Trait

Case 2. α-thalassemia trait patient for HPC,A (Autologous) collection (2015)

Pre mobilisation blood count
WBC = 7.8 x10^9/l
HB = 139 g/l
RBC = 6.44 x10^{12}/l
MCV = 72 fl
Normal HBA2 and serum iron

Apheresis

Colorgram	pbWBC x 10^9/l	Pb CD34%	Pb CD34/ul	hWBC x 10^9/l	hVol (ml)	TNC x10^{10}	hCD34%	CD34 x10^6/kg	HCT%	RBC ml
Aph1 = 3-5%	24.0	0.11%	26.4	47.0	112	5.1	1.09%	0.8	5	5.6
Aph2 = 3-5%	53.0	0.09%	47.7	37.0	187	6.6	1.05%	1.0	3	5.6
Aph3 = 3-5%	63.9	0.07%	44.7	39.0	191	7.3	1.28%	1.3	3	5.7

Same 3-5% colorgram as for β-thalassaemia trait. Adequate but relatively poor CD34 yields considering pbCD34/ul counts

Low harvest WBC compared to peripheral blood suggests higher colorgram may have improved CD34 yields but could increase RBC contamination

Apheresis Harvest Targets for Thalassaemia Intermedia β-globin Gene Therapy Trial

(G-CSF/Plerixafor mobilisation) – Targets set for single CliniMACS column capacity*

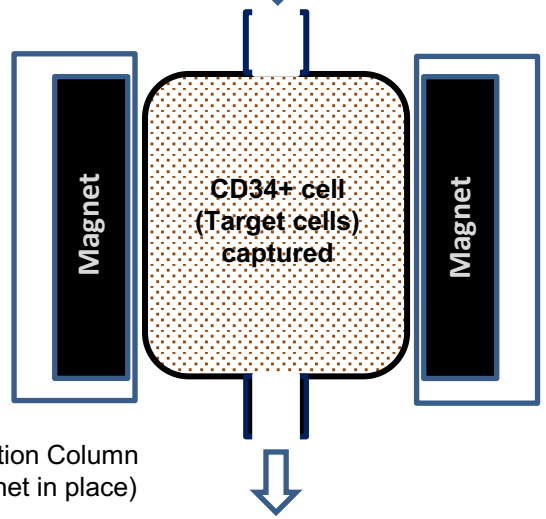
HPC Apheresis Targets	Acceptable Values (Total up to 2 bags) Set for single CliniMACS column capacity
$CD34^+$ cells	≥10.0 x10^6 CD34+ cells/kg Total < 12^8 CD34+ *
Total Nucleated Cells (TNC)	<12^{10} TNC *
Total RBC volume	< 50ml (not a CliniMACS defined limit)
Total Harvest volume	< 450 ml (single harvest) < 1000 ml (two harvests pooled)

CliniMACS CD34+ Cell Magnetic Capture

1. CD34 antibody-magnetic bead reagent added to HPC harvest

Incubate
PBS wash

Pass cells through magnetic capture column

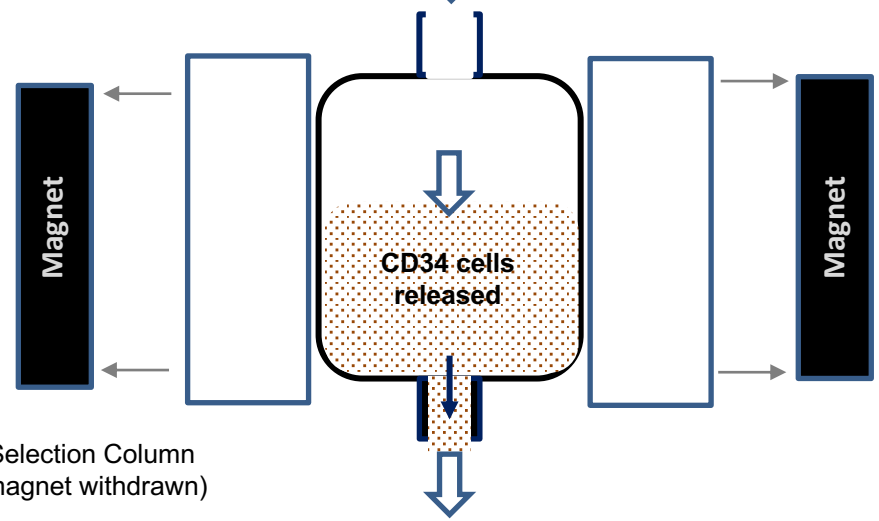


Selection Column
(magnet in place)

**2. Non-target CD34-ve cells run to 'waste'
(WBC+RBC)**

3. Magnet Withdrawn to release captured CD34

4. PBS rinse of CD34 cells



Selection Column
(magnet withdrawn)

**5. CD34+ purified cell fraction
For lentiviral gene therapy**

Mobilised Healthy Donor Counts (1st aph x 2.5BV)	TNC $x10^{10}$	CD34 $x10^{8}$
HPC,A (Allo) n=1058 Mean ± 1SD	5.4 ± 1.9	5.2 ± 3.0
CliniMACS capacity	12.0	12.0

Potential HPC Apheresis Problems for β-Thalassaemia Intermedia/Major Patients?

1. **G-CSF/plerixafor mobilisation (especially if asplenic) = high blood counts/harvest counts, harvest volume**
2. **To achieve CD34 > 10x10^6/kg, Large Volume Leukapheresis (I.V. line, increase flow rate x4 BV) - High TNC if poor mobiliser**
3. **Patients red cell transfused - will residual microcytic RBC affect colorgram?**
4. **How much RBC contamination?** [1]
5. **WBC correction for nucleated RBC present, NRBC in harvest? (nb. NRBC CD45-ve, Flow CD34 counts unaffected)**

1. Sanford *et al* 2014 TRANSFUSION 54:1881-1886

- **Beta-thalassemia Intermedia HPC apheresis for tandem autograft for myeloma**
- **Patient asplenic, failed initial mobilisation, G-CSF and plerixafor at second attempt**

Pre-apheresis blood :second mobilisation
WBC = 62.0 x10^9/l
HB = 109 g/l
RBC = 3.91 x10^{12}/l
MCV = 87.6 fl
RDW = 20.5 (11.5-15.0%)
NRBC = 29 %
PLT = 269 x10^9/l
First Apheresis CD34 dose = 0.04 x10^6/kg
Colorgram increased from 4% to 7%

- **Pooled CD34 dose of all four large volume leukapheresis harvests (x5.5 BV) = 4.4x10^6/kg but HCT 17%!!**

‘Snapshot’ CD34 Extraction Efficiency During Apheresis

Lapierre et al EBMT abstract 2016

CD34 count from blood inlet line (access line from patient to apheresis machine) is reduced in the return (or outlet) line from machine to patient, according to the number of CD34 cells extracted into the collection bag

Target: > 50% extraction efficiency

CD34 extraction efficiency (%) = 100 – (Inlet line CD34 count / Return line CD34 count x 100)

NB: Requires Optia CMNC or WBC depletion kit (used off label) to overide automated interface detection of Optia MNC

HPC Apheresis for Thalassaemia pt1 :
CD34 Extraction Efficiency During Apheresis

Line Samples	MNC Collection Colorgram	Inlet line blood CD34/ul	Return line blood CD34/ul	Calculation	CD34 blood extraction efficiency
Aph 1 day 11:04 am	5%	25	9	100 - (9/25 x100)	64%
Aph 2 day 11:45 am	5%	35	4	100 - (4/35 x100)	89%

HPC Apheresis for Thalassaemia Intermedia pt1

Peripheral blood counts (nb. asplenic)

Pre G-CSF/plerix	pbAph1	pbAph2	
WBC = 4.6	107.1	94.8	$x10^9/l$
HB = 125	139	123	g/l
RBC = 4.60	5.05	4.21	$x10^{12}/l$
MCV = 83.5	84.0	83.6	fl
RDW = 16.6	16.4	16.2	(11.5-15.0%)
NRBC = 54.4%	9.3%	9.0%	%
Retic = 3.54%	3.69%	2.06%	(0.38-2.64%)
PLT = 344	246	88	$x10^9/l$

Apheresis (LVL x 4 BV)

Colorgram	pbWBC x 10^9/l	Pb CD34%	Pb CD34/ul	hWBC x 10^9/l	hVol (ml)	TNC $x10^{10}$	hCD34 %	CD34 $x10^6$/kg	HCT %	RBC ml
Aph 1 = 5%	107.1	0.07	70.3	264	507	13.4	0.35	6.9	10	50.7
Aph 2 = 5%	94.8	0.08	80.2	211	482	10.2	0.35	5.3	7	33.7

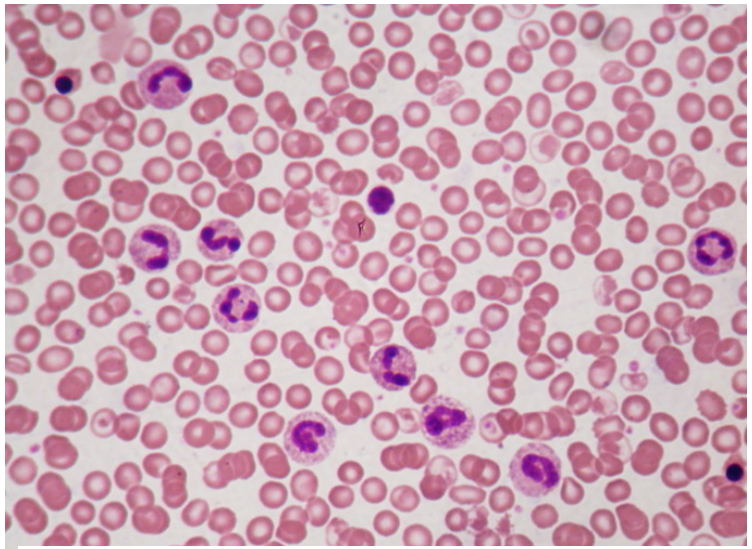
Four to five-fold concentration of pbCD34% - but low harvest CD34% means higher TNC + RBC collection to meet CD34 target dose.

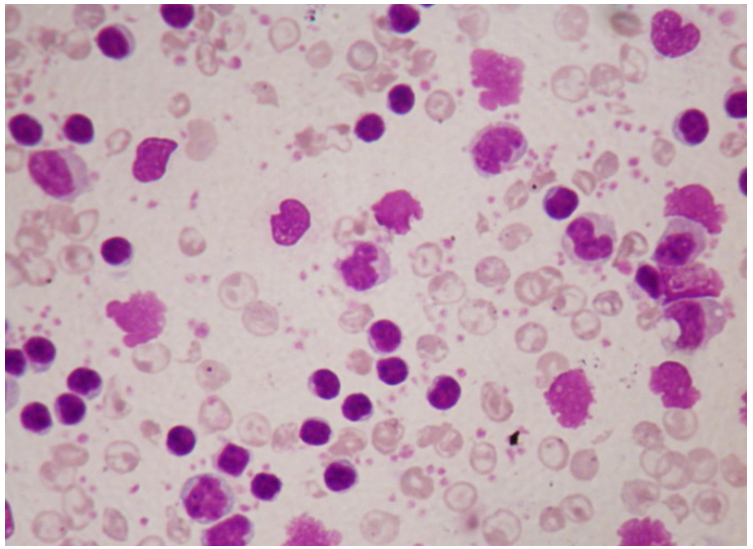
Major degree of red cell contamination

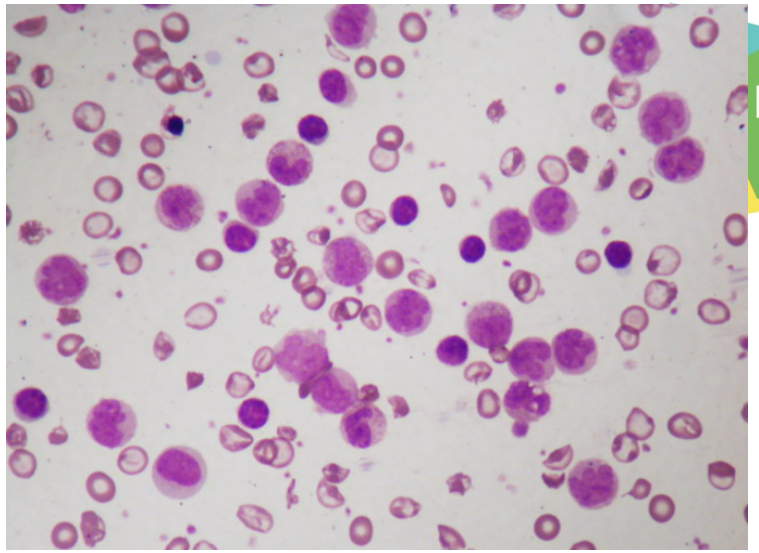
Autologous HPC Targets for CliniMACS CD34 purification and β-globin gene therapy for Thalassaemia (G-CSF/Plerixafor mobilisation) Pt. 1

HPC Apheresis Targets	Acceptable Values (Total up to 2 bags) Set for single CliniMACS column capacity	Day 1	Day2	Total
$CD34^+$ cells	≥10.0 x 10^6 CD34+ cells/kg Total < 12^8 CD34+	6.9 x10^6/kg 4.6 x10^8	5.3 x10^6/kg 3.6 x10^8	12.2 x10^6/kg 8.2 x10^8
Total Nucleated Cells (TNC)	<12^{10} TNC	13.4 x10^{10}	10.2 x10^{10}	23.6 x10^{10} FAIL
Total RBC volume	< 50ml	50.7ml	33.7ml	84.4ml FAIL
Total Harvest Volume	< 450 ml (single harvest) < 1000 ml (two harvests pooled)	507ml	482ml	989ml

β-Thalassaemia Intermedia Post Transfusion Mobilised Blood and Apheresis Product Cells







	Day 1 Blood	Day 1 Apheresis product	Day 2 Apheresis product
WBC x10^9/l*	107.7	264.0	362.0
Neutrophils	72.9%	<1%	2%
Lymphocytes	14.5%	75%	33%
Monocytes	9.7%	24%	46%
Myelo/meta	5.0%	1%	18%
NRBC	9.3%	<1%	1%

*WBC corrected for NRBC

Observations

- **Blood film < 5% microcytic/hypochromic RBC**
- **Aph 1: Microcytic RBC increase significantly in Aph1 HPC product but no mature neutrophils (correct MNC fraction)**
- **Aph 2: Microcytic RBC, fewer lymphocytes, more myelo-monocytic cells (2% segmented PMN)**
- **NRBC 1% or less in both HPC products**

HPC Apheresis for Thalassaemia Intermedia 2 : CD34 Extraction Efficiency During Apheresis

Line Samples	MNC Collection Colorgram	Access line blood CD34/ul	Return line blood CD34/ul	Calculation	CD34 blood extraction efficiency
Aph day 1 09:38	3%	198	40	100 - (40/198 x100)	80%
Aph day 1 12:22	3%	209	111	100 - (111/209 x100)	47%
Aph day 1 12:40	5%	174	46	100 - (46/174x100)	74%

Colorgran set at 3% to reduce RBC contamination
Fall in CD34 collection efficiency - increased to 5%

Specific Gravity (Relative Density) of Blood Components

Water at 4^0C = 1.000

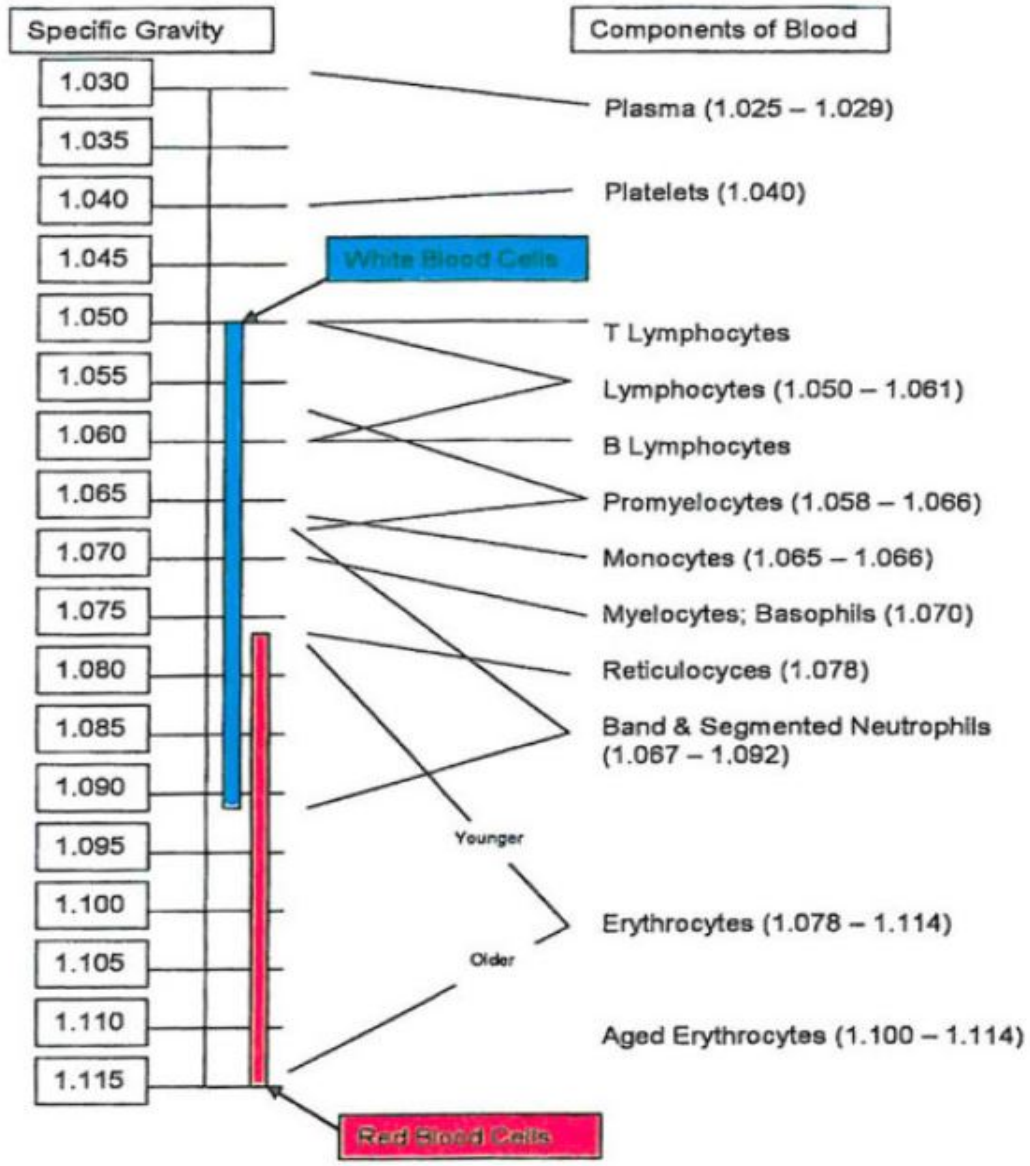


Fig. 1. Specific gravity of cellular elements of whole blood. Courtesy of Therakos, Inc. Excerpted from the THERAKOS UVAR XTS Photopheresis System Basic Training Manual, Volume II, Section 4, Appendices for Basic Training Guidelines. Johnson & Johnson, Inc., New Brunswick, NJ.

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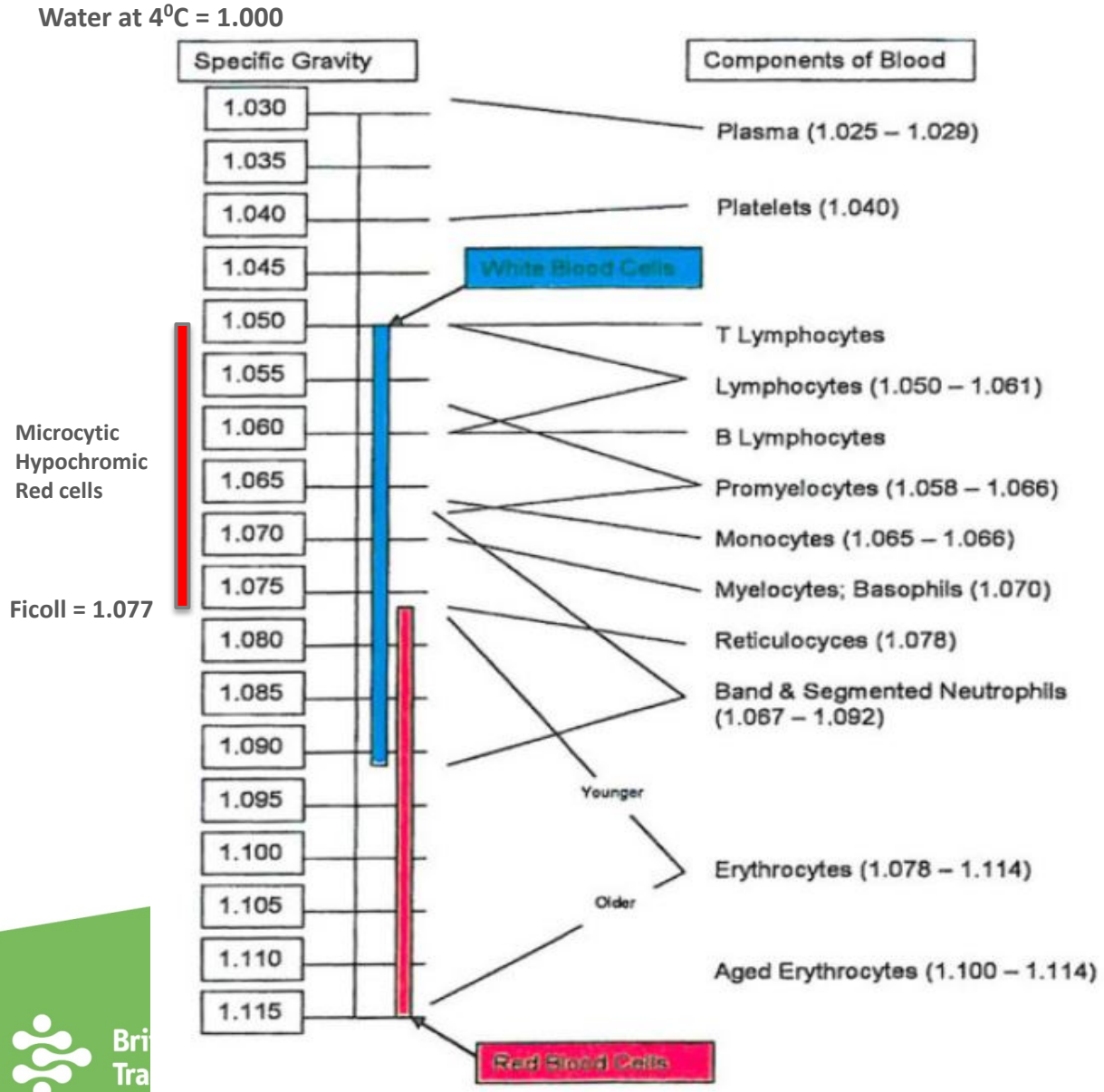

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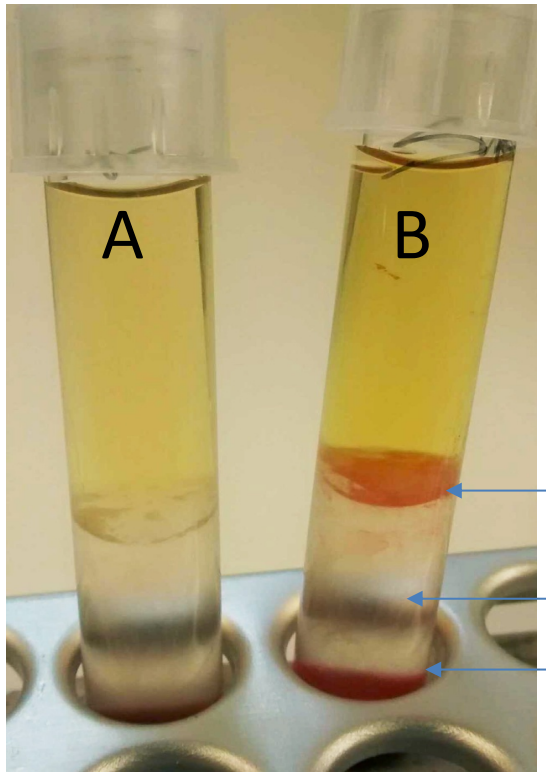
Ficoll Separation of Pre-apheresis Thalassaemia Blood Sample pt.2



Ficoll separation of
Pre-apheresis mobilised blood

A = control mobilised blood
B = β-thalassaemia pt 2

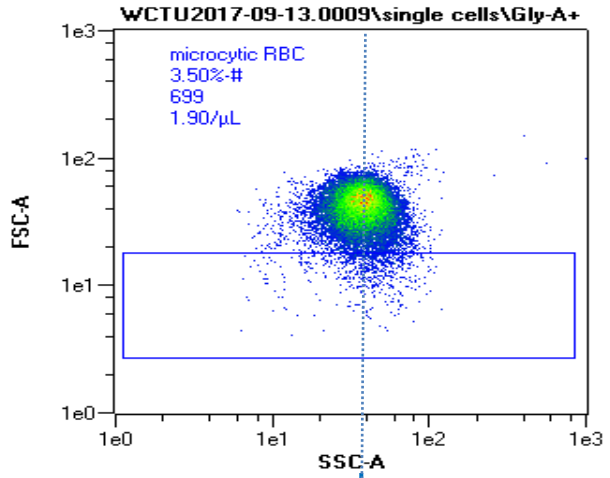
Light density MNC layer
+ Microcytic, hypochromic RBC

Ficoll (SG = 1.077)

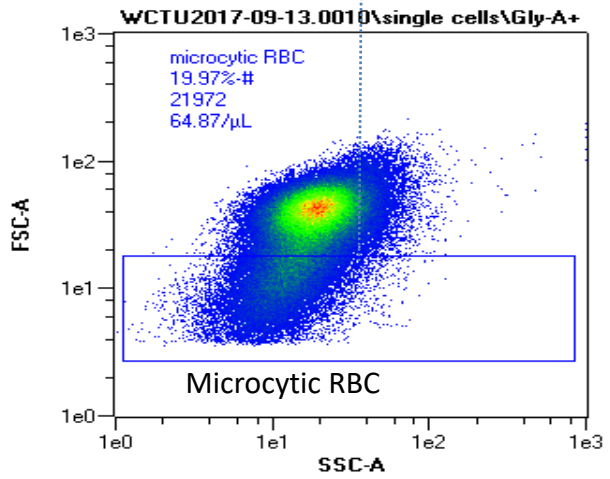
Granulocytes
Red Cells

Red Cell Flow cytometry
Glycophorin A monclonal

1. Pre-apheresis whole blood pt 1



2. Apheresis harvest pt 1



HPC Apheresis for Thalassaemia Intermedia Pt 2

Case 2 beta-thalassemia intermedia blood counts (intact spleen)

Pre G-CSF/plerix	Aph 1	Aph 2	Units
WBC = 7.4	60.9	68.5	$x10^9/l$
HB = 119	102	100	g/l
RBC = 4.41	3.86	3.76	$x10^{12}/l$
MCV = 79.4	78.0	78.5	fl
RDW = 14.9	16.4	16.4	(11.5-15.0%)
NRBC = 3.6%	2.0%	0.8%	%
Retic = 0.57%	0.93%	0.97%	(0.38-2.64%)
PLT = 146	158	88	$x10^9/l$

Colorgram	pbWBC x 10^9/l	Pb CD34%	Pb CD34/ul	hWBC x 10^9/l	hVol ml	TNC x10^{10}	hCD34%	CD34 x10^6/kg	HCT %	RBC ml
Aph 1 = 3-5%	60.9	0.40	258.8	168	459	7.7	1.33	19.4	6	27.5
Aph 2 = 5% (used as backup)	68.5	0.37	276.8	346	152	5.2	1.58	15.7	15	22.8

5% colorgram superior to 3% for CD34 extraction efficiency.
Higher CD34 mobilisation reduces TNC number and red cell contamination to achieve the target CD34 dose of 10 $x10^6$/kg

Autologous HPC Targets for CliniMACS CD34 purification and β-globin gene therapy for Thalassaemia (G-CSF/Plerixafor mobilisation) Pt.2

HPC Apheresis Targets	Acceptable Values (Total up to 2 bags) Set for single CliniMACS column capacity	Day 1	Day 2 (used for Backup)	Total (Day 1 only shipped)
$CD34^+$ cells	≥10.0 x 10^6 CD34+ cells/kg Total < 12^8 CD34+	19.4 x10^6/kg 10.3 x10^8	15.7 x10^6/kg 8.3 x10^8	19.4 x10^6/kg 10.3 x10^8
Total Nucleated Cells (TNC)	<12^{10} TNC capacity	7.7 x10^{10}	5.3 x10^{10}	7.7 x10^{10}
Total RBC volume	< 50ml	27.5 ml	22.8ml	27.5 ml
Total Harvest Volume	< 450 ml (single harvest) < 1000 ml (two harvests pooled)	459 ml	152ml	459 ml

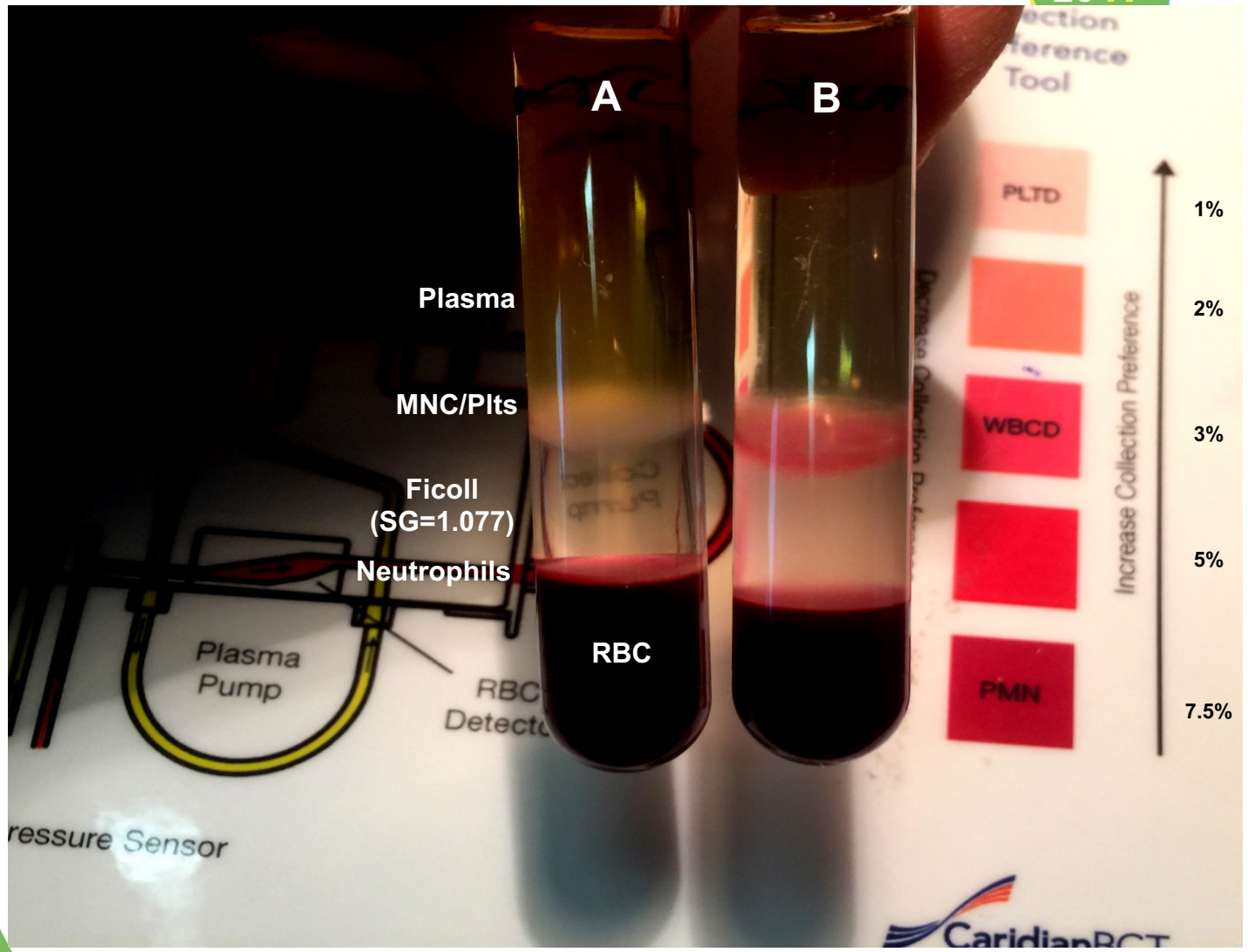
Pre-Apheresis Ficoll Separation to determine Optia CMNC Colorgram Pt.3

Pre-Apheresis blood sample test

Control sample = A
WBC= 31.8 x10^9/l
pbCD34 = 47.7/ul
MCV=89fl
RDW = 17.6%

Transfused β-thal pt.3 = B
WBC = 37.8 x10^9/l
pbCD34= 18.9/ul
MCV=85fl (normal 80-99fl)
RDW=13.6% (normal 11.5-15%)
Retics 0.23% (0.45-2.42%)

NB: MCV/RDW insensitive to residual microcytic RBC



Autologous HPC Targets for CliniMACS CD34 purification and β-globin gene therapy for Thalassaemia (G-CSF/Plerixafor mobilisation) Pt.3

HPC Apheresis Targets	Acceptable Values (Total up to 2 bags) Set for single CliniMACS column capacity	Day 1	Day2 (Backup CD34 of 2 x10^6/kg removed)	Total (Backup CD34 subtracted)
$CD34^+$ cells	≥10.0 x 10^6 CD34+ cells/kg Total < 12^8 CD34+	17.7 x10^6/kg 7.9 x10^8	11.6 x10^6/kg 6.1 x10^8	29.3 x10^6/kg 13.2 x10^8
Total Nucleated Cells (TNC)	<12^{10} TNC capacity	7.3 x10^{10}	5.7 x10^{10}	12.0 x10^{10}
Total RBC volume	< 50ml	26.9ml	20.2ml	47.1ml
Total Harvest Volume	< 450 ml (single harvest) < 1000 ml (two harvests pooled)	449ml	403-60ml	792ml

Autologous HPC Targets for CliniMACS CD34 purification and β-globin gene therapy for Thalassaemia (G-CSF/Plerixafor mobilisation) Pt.1(rpt)

HPC Apheresis Targets	Acceptable Values (Total up to 2 bags) Set for single CliniMACS column capacity	Day 1	Day2	Total
$CD34^+$ cells	≥10.0 x 10^6 CD34+ cells/kg Total < 24^8 CD34+	4.7 x10^6/kg 3.2 x10^8	8.1 x10^6/kg 5.6 x10^8	12.8 x10^6/kg 8.7 x10^8
Total Nucleated Cells (TNC)	<24^{10} TNC capacity	5.7 x10^{10}	6.7 x10^{10}	12.4 x10^{10}
Total RBC volume	< 100ml	42.0ml	55.0ml	97.0ml
Total Harvest Volume	< 450 ml (single harvest) < 1000 ml (two harvests pooled)	507ml	482ml	989ml

Conclusions

1. **CD34 collection efficiency ‘in-process’ control of apheresis useful where location of the target cell collection fraction is uncertain**

2. **Microcytic/hypochromic red cells in thalassaemia fall within the same light density fraction as MNC/CD34 cells. Potential interference of other red cell anomalies unknown**

3. **Ficoll separation of a pre-apheresis blood sample may be a useful lab test to demonstrate potential red cell interference and suggest a modified MNC/CD34 collection colour**

4. **Optia MNC machines introduce greater automation and standardisation of apheresis but manual control of harvest volume and interface collection adjustment minimal. Operator over-ride possible with Optia CMNC**

Questions?