Supplementary Material

Noordin, S., Al-Amoudi, B., Mohamed, R., Aziz, M., Noor, S., & Zabidi, M. (2025). Unraveling Common Stem Cell Sources and Key Reporting Parameters in Studies Related to Stem Cell-Derived Red Blood Cells: A Review. *Biomedical Research and Therapy*, *12*(5), 7372-7385. https://doi.org/10.15419/bmrat.v12i5.976

Supplementary 1: Scoping review data extraction form

Study ID	Author (Year)	DOI	Stem Cell Types	Cell Source	Culture Duration (Days)	Culture Media & Supplements	Oxygen Tension (if stated)	Differentiation Protocols	RBCs Yield	Enucleation Rate (%)	Hemoglobin Type	Key Outcomes	Notes
S1	Chang et al. (2006)	https://doi.org/10.1182/blood-2005-11-011874	hESCs (H1 line)	Embryonic (Embryoid bodies or EBs from hESCs)	15 to 56 days	Fetal bovine serum (FBS), vascular endothelial growth factor VEGF, Flt3-L 10ng/mL, or coculture with OP-9 during erythroid differentiation, growth expansion medium (GEM), IL-6 10ng/mL. IL-3 10ng/mL, G-CSF, TPO 96U/mL, SCF 200ng/mL, EPO 3U/mL	NA	The differentiation protocol adopted from Carotta et al (2004) involves processing cells present at early and late stages of embryoid body differentiation, with a focus on the emergence of hematopoietic markers like CD45, CD34, and CD41, EPO 3U/mL stem cell factor SCF 200ng/mL *Carotta S, Pilat S, Mairhofer A, et al. Directed differentiation and mass cultivation of pure erythroid progenitors from mouse embryonic stem cells. Blood. 2004; 104:1873-1880.*	NA	NA	The erythroid cells coexpress high levels of embryonic globins (ζ and) and fetal globin, with little or no adult globin (α)	The study concludes that the high frequency of erythroid cells coexpressing embryonic and fetal globins can serve as a valuable tool for exploring molecular mechanisms of hematopoiesis. The cells resemble definitive-type erythroid cells morphologically but do not mimic either yolk sac embryonic or fetal liver counterparts	The study highlights the challenges in studying primitive erythropoiesis due to ethical concerns and the transient nature of these cells. It emphasizes the need for further exploration of the molecular mechanisms involved in hematopoietic differentiation from human embryonic stem cells
S2	Lu et al. (2008)	https://doi.org/10.1182/blood-2008-05-157198	hESCs	Embryonic	**19 -21**	The media used includes BGM	NA	Formation of embryoid	The study reports a	60%	The derived	The study demonstrates	The presence of

				The hESCs used in the study include MA01, H1, HuES-3, and MA99 lines, with MA01 yielding the highest efficiency		(Basal Growth Medium) and Stemline II, supplemented with various factors such as: Stem Cell Factor (SCF): 100 ng/mL Interleukin-3 (IL-3): 5 ng/mL Erythropoietin (Epo): 3 IU/mL,Methylcellulose (0.2% to 0.5% to prevent aggregation) Other supplements like inositol, folic acid, monothioglycerol, transferrin, insulin, ferrous nitrate, ferrous sulfate, BSA, L-glutamine, and penicillin-streptomycin		bodies (EBs) from hESCs. Expansion of blast colonies (BCs). Erythroid differentiation and amplification into RBCs. Enrichment of RBCs	yield of approximately 3.86 x 10^10 RBCs from one 6-well plate of MA01 hESCs, which is about 1.2 x 10^7 cells		cells primarily expressed fetal and embryonic globins, with the capacity to express adult β-globin upon further maturation	the feasibility of differentiating hESCs into functional RBCs on a large scale. The oxygen equilibrium curves of the hESC-derived cells were comparable to normal RBCs, indicating functional maturity	methylcellulose in the culture media was noted to enhance cell expansion by preventing aggregation
S3	Hiroyama et al. (2008)	https://doi.org/10.1371/journal.pone.0001544	Mouse Embryonic Stem Cells (ESCs)	Murie (ESC lines: E14TG2a, BRC4, BRC5)	~120 days total: Phase I: 0–10 days, Phase II: up to day 60, Phase III–IV: up to day 1201	MDM + 15% FBS, ITS (insulin 10 mg/ml, transferrin 5.5 mg/ml, selenium 5 ng/ml), 50 µg/ml ascorbic acid, 0.45 mM monothioglycerol, antibiotics; cytokines: SCF (50 ng/ml), EPO (5 U/ml), IL-3 (10 ng/ml), VEGF (20 ng/ml), IGF-II (200 ng/ml), dexamethasone (10^{-6} M)		4-phase method using OP9 feeder cells, cytokine cocktails; Method A (with IL-3), Method B (without IL-3)	Visible red cell pellet formation; improved RBC count in vivo	Enucleated RBCs observed by SYTO85 and morphology (no exact % stated)	Adult type (α- and β-globin expressed; γ, ε, ζ not expressed)	MEDEP cell lines proliferate >1 year, differentiate in vitro/in vivo into functional RBCs, and ameliorate acute anemia in mice	Cytokine dependence varied by line; IL-3 not essential for erythroid lines; RBCs produced were functional and non-tumorigenic
S4	Ma et al. (2008)	https://doi.org/10.1073/pnas.0802220105	hESCs	Embryonic **H1 line hESCs**	Up to 18 + 6	α-MEM + 15% FBS, glutamine, NEAA, β-mercaptoethanol SCF 100, IL-3 10, IL-6 100, TPO 10, EPO 4 U/ml	5% CO_2, no hypoxia	Coculture with mFLSCs, colony + suspension cultures	Up to 1×10^6 from 1×10^4	Up to 82%	Embryonic → Fetal → Adult	Progressive maturation and Hb switching	

S5	Honig et al. (2010)	https://doi.org/10.3109/03630261003676850	hESCs	Embryonic MA-01, H-1, H-7	~21	Unspecified medium; co-culture with OP9	NA	Direct differentiation to primitive erythrocytes	Not quantified	**No data**	Embryonic Hb (ζ2ε2, γ4) dominant Embryonic & fetal	Embryonic-type RBCs; resembles early yolk sac stage	
S6	Dias et al. (2011)	https://doi.org/10.1089/scd.2011.0078	hESC	Transgenic & transgene-free fibroblast iPSC	**Up to 75**	OP9 co-culture + SFEM + cytokines SCF 50–100 ng/ml, EPO 2–6 U/ml, IL-3 5 ng/ml, TPO 50 ng/ml, Dex 10^{-6} M	NA	2 methods: CD34+ expansion or reaggregation on MS5	High (up to 4,000x per hESC)	12%	Hb dominant; low β-globin	Long-term expansion, robust erythropoiesis, scalable	**Same paper as in S13**
S7	Malik et al. (1998)	https://doi.org/10.1182/blood.V91.8.2664.2664_2664_2671	$CD34^+$ HSPCs	Bone marrow, cord blood	Up to 21	IMDM + BSA + EPO + IL-3 + GM-CSF EPO 10 U/ml, IL-3 0.01 U/ml, GM-CSF 0.001 ng/ml	Reduced O_2 (hypoxic)	Single-step liquid culture	Sufficient for phys. studies; 42% enucleation	42%	Adult Hb (β-globin predominant)	Functional RBCs with physiological Hb pattern	
S8	Neildez-Nguyen et al. (2002)	https://doi.org/10.1038/nbt0502-467	CD34+ progenitors	**Cord blood**	18-21	IMDM + FBS, insulin, SCF, IL-3, IL-6, EPO transferrin, heparin	Normoxic	3-step: expansion + erythroid + terminal	~1.5×10^8 per flask	60–80%	HbF Mostly fetal, some adult	RBCs matured in vivo in NOD/SCID mice	
S9	Giarratana et al. (2005)	https://doi.org/10.1182/blood-2011-06-362038	CD34+ HSCs	**Peripheral blood (G-CSF mobilized)**	18	IMDM + transferrin, insulin, heparin, 5% inactivated plasma SCF 100 ng/ml, IL-3 5 ng/ml, EPO 3 IU/ml, hydrocortisone 10^{-6} M	5% CO_2 in air	3-step: SCF+IL-3+EPO → SCF+EPO → EPO only	61,500-fold expansion	**81%**	88% HbA, 10%	First-in-human transfusion of cRBCs; 41–63% survival at day 26	
S10	Shah et al. (2016)	https://doi.org/10.1371/journal.pone.0166657	CD34+ HSPCs	**Human cord blood**	18	IMDM/Glutamax + AB serum, FBS, transferrin, insulin, heparin	Normoxic	4-step: proliferation + maturation	18,700-fold expansion	82% after filtration	Mixed fetal & adult (25% HbF)	Oxygen delivery in vivo confirmed	
S11	Zhang et al. (2017)	https://doi.org/10.1002/sctm.17-0057	Hematopoietic Stem and Progenitor Cells (HSPCs)	Human Cord Blood $CD34^+$ cells	21 days	MDM with nutrition supplements: putrescine (100 µM), selenium (5 ng/mL), insulin (25 µg/mL), transferrin (200 µg/mL), folic acid (10 µg/mL), plus FBS (15%) in early stages Flt3L, SCF 10, IL-3 1, EPO 3 U/ml	Cultured at 37°C with 5% CO_2 in air (normoxic conditions)	4-step process: Step 1: MM1SFT (SCF 100 ng/mL, TPO 50 ng/mL) Step 2: SE31F1FL1GM(15) (SCF 100 ng/mL, IL-3 20 ng/mL, FL 100 ng/mL, GM-CSF 15 ng/mL, EPO 6 IU/mL)	2.9×10^{11} total cells from 10^6 $CD34^+$ cells (up to 2×10^8-fold expansion)	50.0% ± 5.7%	Normal hemoglobin detected; functionality confirmed by hemoglobin content and oxygen equilibrium curves	High-yield erythrocyte generation - In vivo terminal maturation (murine xenotransplantation) - Safe and functional in non-human primate model with	Used a bottle turning bioreactor system; large-scale culture feasible for clinical translation

								Step 3: SE1F1IL-31FL(50) (IL-3 10 ng/mL, FL 50 ng/mL) Step 4: SE (SCF 100 ng/mL, EPO 6 IU/mL)				hemorrhagic anemia	
S12	Lapillone et al. (2010)	https://doi.org/10.3324/haematol.2010.023556	Human iPSCs and hESCs	**IMR90 fetal fibroblasts, FD136 adult fibroblasts, hESC line H1**	45 days (20 EB + 25 maturation)	IMDM, human plasma, SCF, FLT3L, TPO, BMP4, VEGF, IL-3, IL-6, EPO SCF 100 ng/ml, TPO 100 ng/ml, FL 100 ng/ml, BMP4 10 ng/ml, VEGF 5 ng/ml, IL-3/6 5 ng/ml, EPO 3 U/ml	5% CO_2; O_2 not	**EB formation + liquid culture (3-stage cytokine addition)**	High erythroid yield, large-scale; up to 4.4 x 10^8	4 to 10%	Fetal (HbF) in vitro	Functional HbF RBCs with CO binding; large-scale RBC production possible	Erythroid commitment without co-culture or animal products
S13	Dias et al. (2011)	https://doi.org/10.1089/scd.2011.0078	hiPSC,	Transgenic & transgene-free fibroblast iPSC	**Up to 90**	OP9 co-culture + SFEM + cytokines SCF 50–100 ng/ml, EPO 2–6 U/ml, IL-3 5 ng/ml, TPO 50 ng/ml, Dex 10^{-6} M	NA	2 methods: CD34+ expansion or reaggregation on MS5	6 x 10*	2-10%	ε - and γ-globins Hb dominant; low β-globin	Long-term expansion, robust erythropoiesis, scalable	
S14	Kobari et al. (2012)	https://doi.org/10.3324/haematol.2011.055566	hiPSC (normal & SCD)	FD-136, Amniotic fluid	**25**	IMDM + human plasma + SCF, TPO, FL, BMP4, VEGF, IL-3/6, EPOSCF 100 ng/ml, TPO 100 ng/ml, FL 100 ng/ml, BMP4 10 ng/ml, VEGF 5 ng/ml, IL-3/6 5 ng/ml, EPO 3–4 U/ml	Normoxic	2-step EB + sequential cytokine culture (D0–25)	1.5 -2.8 x 10^9	Yes 20 - 26%	HbF in vitro; switch to HbA in vivo	Full terminal maturation in vivo; HbA synthesis observed in mice	
S15	Park et al. (2020)	https://doi.org/10.1186/s12967-020-02403-y	hhiPSC (rare blood types)	PB-MNCs	~31	Serum-free Stemline II with basal + SCF, EPO, IL-3, HCSCF 100 ng/ml, EPO 6 IU/ml, IL-3 10 μg/ml, HC 1 μM, others in multi-step culture	5% CO_2, 37°C	EB formation, mesoderm induction, HSC, erythroid	Moderate yield (not quantified)	Yes (to reticulocyte stage)	Not clearly reported	Proof-of-concept for autologous rare blood RBCs from iPSC	