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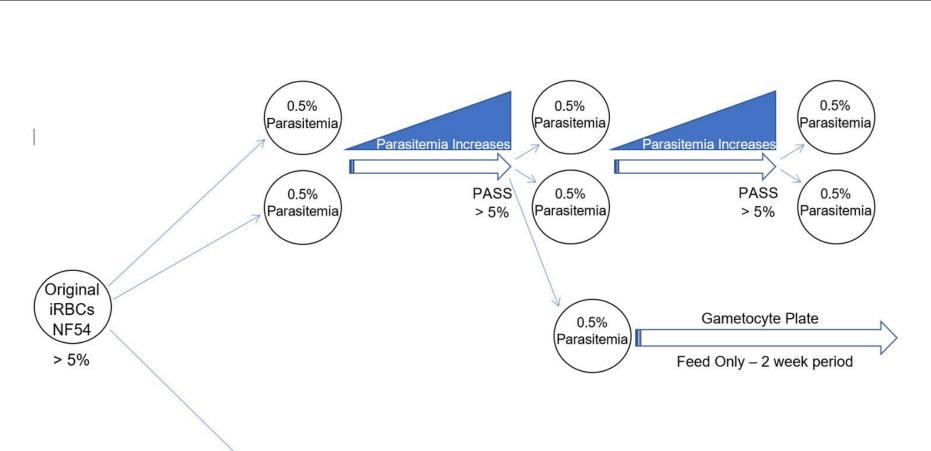
Shelf life study of human blood cryopreserved for in vitro cultivation of Plasmodium falciparum Michael Bellerose, Mina Diep, Matt O'Neil, Annalise Armstrong, Edras Méndez-Hernández, Madison Scialanca, Samuel Stambaugh, Nicole Clemente, Elyse Anderson and Lawrence Mylin

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Abstract

Malaria is caused by multiple species of the parasite Plasmodium and disproportionately affects people living in the developing world who often do not have access or funds to allow for effective control of our protection from the parasite. This study is intended to support ongoing research at the Macha Research Trust (MRT) [also known as the Malaria Institute at Macha (MIAM)], which is located in Macha, a rural area in the Southern Province of Zambia where the virulent species, *Plasmodium falciparum* is prevalent. Our goal is to support the capacity of the laboratory at MRT to culture (propagate and preserve) locally-isolated or laboratory strains of *Plasmodium*. Laboratory cultivation of *P. falciparum* requires fresh human blood which is of limited supply in Macha. To solve this problem, we have cryopreserved fresh, leukocyte-depleted erythrocyte suspensions containing either hydroxy ethyl starch (HES) and polyvinyl alcohol (PVA) or glycerol and that Our results reveal duration of serum. cryopreservation or temporary storage in liquid nitrogen no significant effect on HES/PVA frozen had However, unlike unfrozen blood or blood blood. preserved using glycerol and serum, the HES/PVA blood did not support serial asexual propagation of P. falciparum. The shelf life study on glycerol blood showed that blood stored up to 6 months could sustain asexual culture comparably to fresh blood.





0.5%

Gametocyte Plate

Feed Only – 2 week period

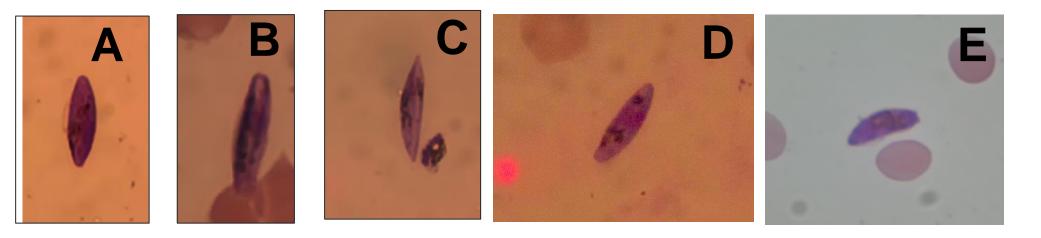
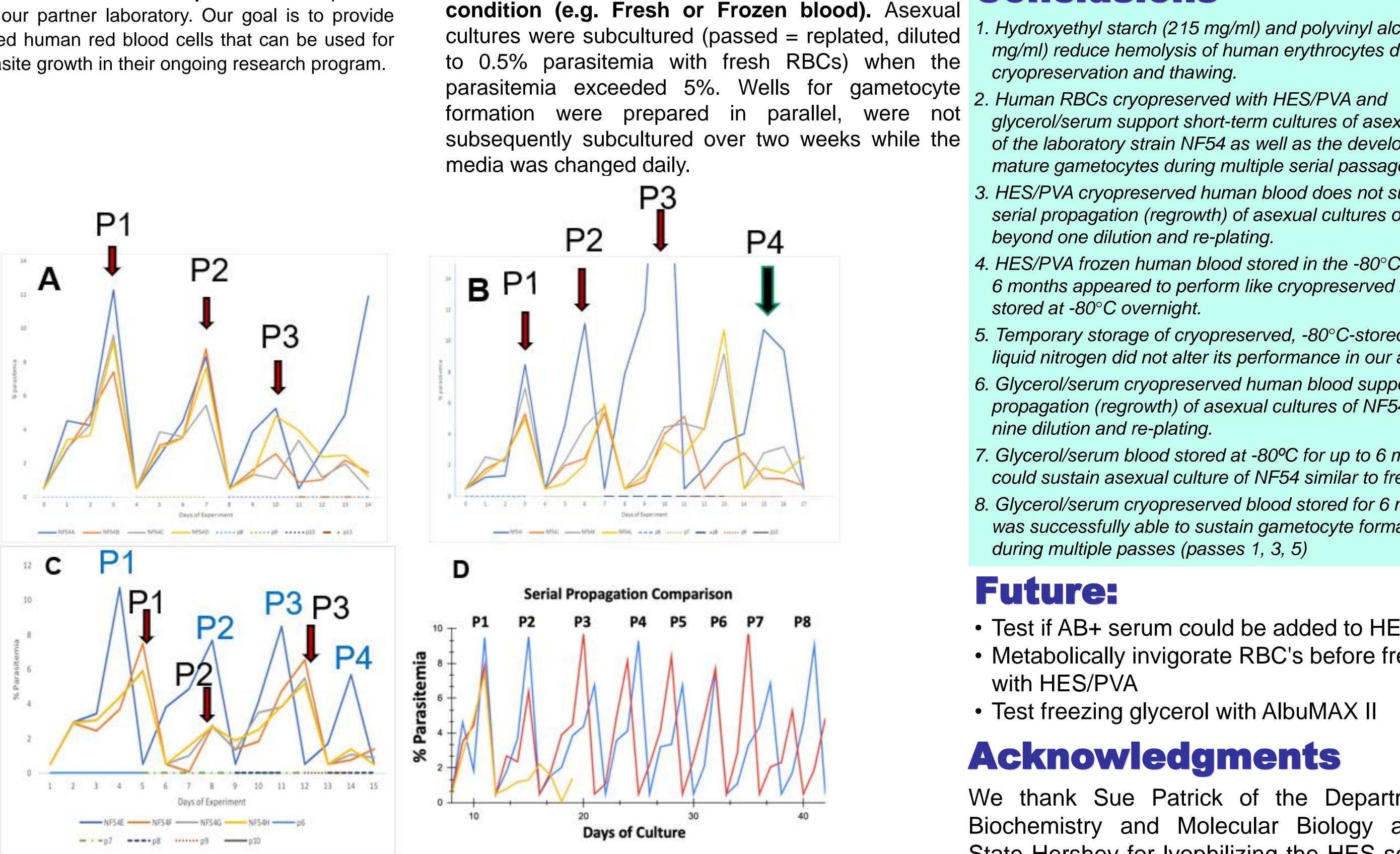


Figure 8. Gametocytes in parallel cultures. Mature gametocytes present at 2 weeks in cultures prepared at p1 using fresh or differentially stored, frozen RBCs. A. Fresh, non-

4°C; **B.** 6 month frozen -80°C pass 1; **C & D.** Glycerol/serum-

Figure 3. Macha Mission Hospital. The hospital is adjacent to our partner laboratory. Our goal is to provide cryopreserved human red blood cells that can be used for malaria parasite growth in their ongoing research program.



frozen blood stored short term at -80°C pass 1.; E Glycerol/serum-frozen short term at pass 5.

Figure 4. Serial Culture Strategy for one culture **Conclusions**

. Hydroxyethyl starch (215 mg/ml) and polyvinyl alcohol (1 mg/ml) reduce hemolysis of human erythrocytes during rapid cryopreservation and thawing.

- glycerol/serum support short-term cultures of asexual forms of the laboratory strain NF54 as well as the development of mature gametocytes during multiple serial passages.
- 3. HES/PVA cryopreserved human blood does not support the serial propagation (regrowth) of asexual cultures of NF54 beyond one dilution and re-plating.
- 4. HES/PVA frozen human blood stored in the -80°C freezer for 6 months appeared to perform like cryopreserved blood stored at -80°C overnight.
- 5. Temporary storage of cryopreserved, -80°C-stored blood in liquid nitrogen did not alter its performance in our assays.
- 6. Glycerol/serum cryopreserved human blood supported serial propagation (regrowth) of asexual cultures of NF54 beyond a nine dilution and re-plating.
- 7. Glycerol/serum blood stored at -80°C for up to 6 months could sustain asexual culture of NF54 similar to fresh blood.
- 8. Glycerol/serum cryopreserved blood stored for 6 months

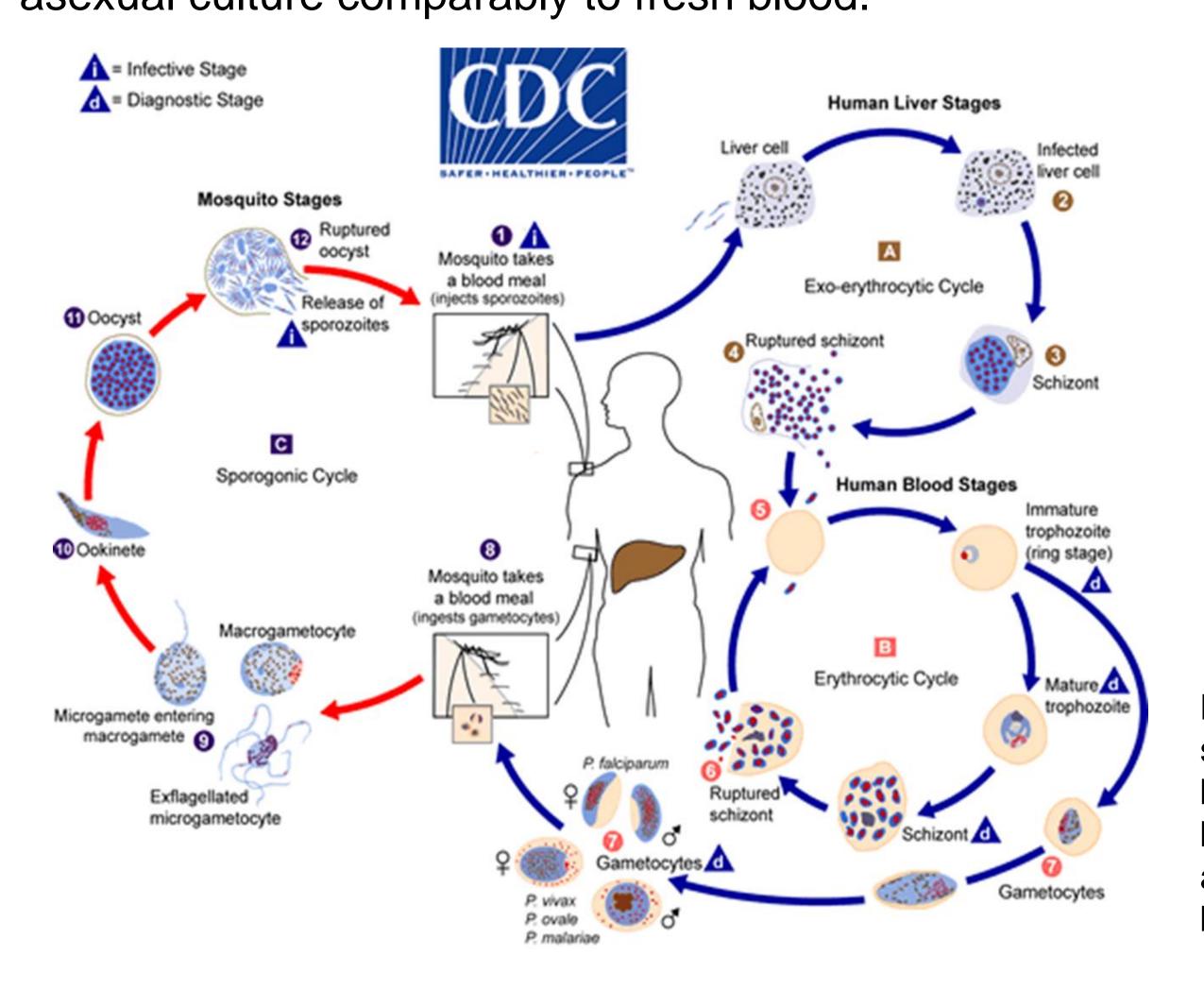
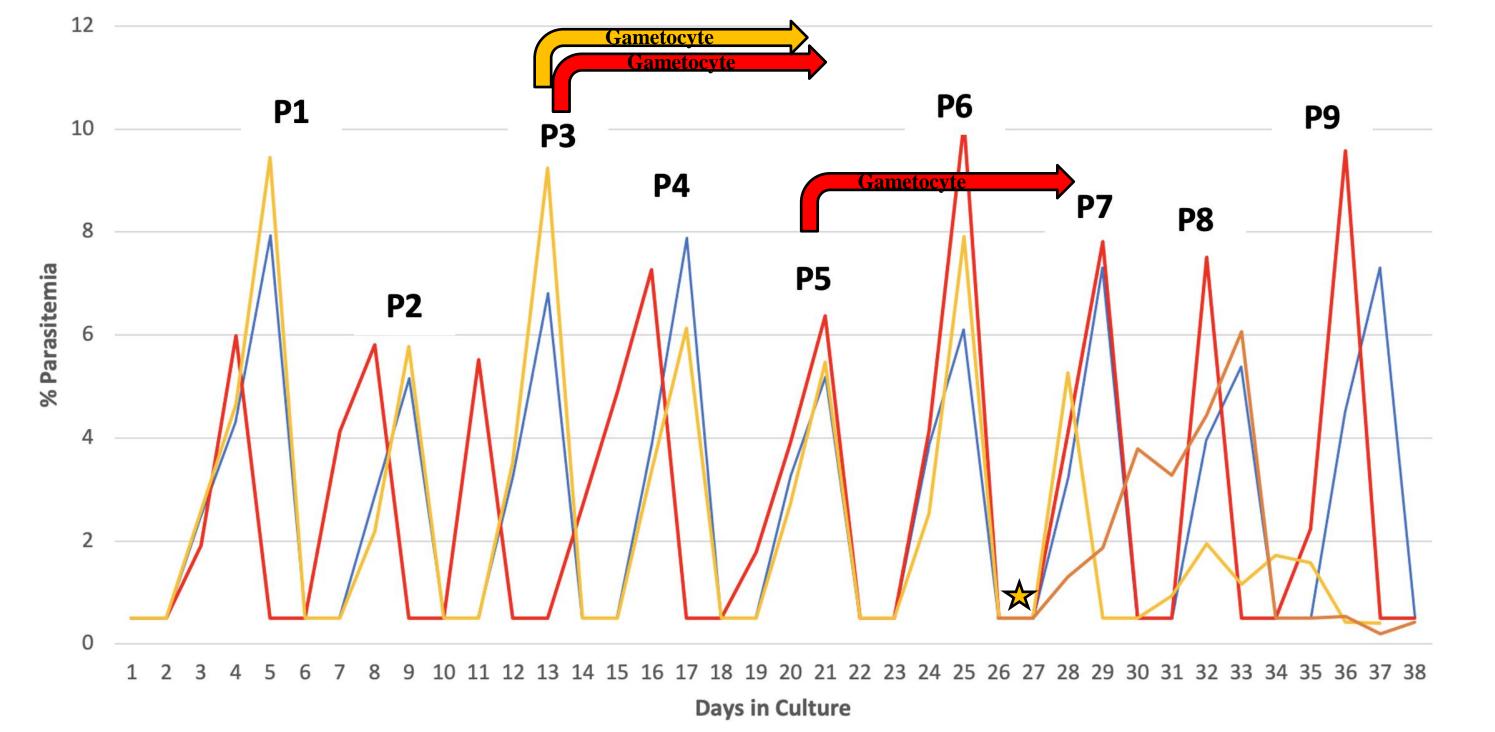


Figure 1. Malaria Life Cycle. The complex life-cycle of Plasmodium falciparum has multiple stages and two different hosts. The parasite, in its sporozoite form, is injected into its Figure 5. Parallel Cultures (Asexual). Parallel serial propagation of asexual cultures using HES/PVA and glycerol/serum short-term cryopreserved blood (stored in a -80°C overnight), or stored at -80°C for 6 months, or for 6 months interrupted by temporary storage in liquid nitrogen, or fresh, non-frozen blood. A. In both O+ experiments, none of the cryopreserved blood was able to support the regrowth of the asexual forms beyond pass 3. B & C. A+ cryopreserved blood supported an additional pass (P4); cultures prepared with different types of frozen blood progressed at different rates. **D.** Glycerol-frozen blood supported serial propagation through at least P8.



was successfully able to sustain gametocyte formation during multiple passes (passes 1, 3, 5)

Future:

- Test if AB+ serum could be added to HES/PVA.
- Metabolically invigorate RBC's before freezing with HES/PVA
- Test freezing glycerol with AlbuMAX II

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References

human host as an infected Anopheles mosquito takes a blood meal.

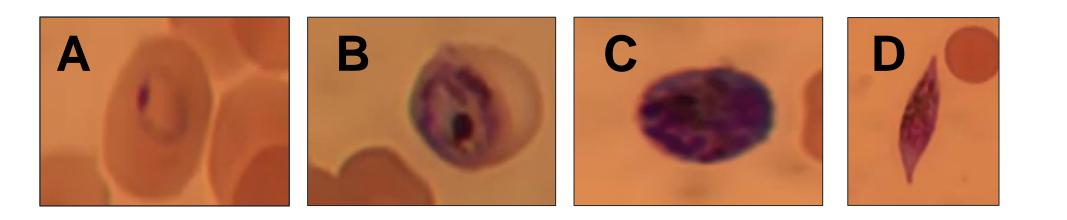


Figure 2. Parasite development: (A) Immature ring trophozoite; (B) Maturing trophozoite; (C) Schizont; (D) Gametocyte.

> ——6 Month Glycerol 2 Week Glycerol —— HES/PVA

Figure 6. Glycerol frozen Blood Shelf-Life Study. This experiment compared glycerol frozen blood stored for two weeks and six months at -80°C. An HES/PVA culture was started at Pass 6, denoted by the star on the graph, to control for artificially selected strains of NF54 that could survive in culture. This HES/PVA culture expectedly stalled after its first serial passage. The two-week stored glycerol frozen blood sustained culture continuously through the 9th serial passage, same as fresh blood. The six-month stored glycerol frozen blood was able to sustain the culture through the 7th serial passage. Arrows on the graph denote subcultures of the different conditions where gametocyte generation was tested and successful.

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