Multi-Compartment Model of Malaria Pathophysiology

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BME 260, Spring 2020

April 6, 2020

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Executive Summary

Malaria is a disease that infects over 200 million people each year on average, killing about 400,000. It is caused by the *Plasmodium falciparum* protozoan parasite, most commonly transmitted to humans through mosquito bites.These parasites replicate in the human host, first reproducing in liver cells and then moving into red blood cells. Considering the continued prevalence of malaria, the goal of this project was to develop a mathematical model that tracks infected and healthy red blood cells (iRBCs and healthy RBCs, respectively), immune cells, and relevant diagnostic markers to model the key mechanisms behind malaria symptoms. In addition, the model incorporates tunable parameters to ensure it can accurately represent a wide range of scenarios while still producing accurate and relevant results.

A three compartment mathematical model was developed to follow how the parasite replicates and creates chemical and physical changes in the human body during a malarial infection. The first compartment of the model is the liver, where the mosquito injected parasite stays hidden from immune response and replicates. The parasites then move into the next compartment, the bloodstream. The bloodstream has two subcompartments (the plasma and red blood cells) which the parasites move between through a reproduction-lysing cycle. Lastly, the model follows the immune response to the infection that takes place in the spleen. In each of these compartments, the model tracks the parasite itself, red blood cell counts, immune cells and biomarkers in the blood used to test for malaria.

Results of the model included clear anemic effects due to red blood cell lysing, consistent with the observed disease progression, and heightened immune response in individuals with subsequent malarial infections. The model also provided insight into common diagnostic testing procedures and how to potentially improve them by highlighting key regions of

false negative results. A robust set of equations incorporating concepts such as blood filtration by the spleen and vascular sequestration of infected red blood cells yield excellent modularity as many adjustable parameters exist in the model. This modularity was leveraged to simulate vaccine boosted immune response to malaria, with the model predicting a condition where a vaccine may lead to a completely asymptomatic patient. Future work includes applying the model to different clinical scenarios in hopes of gaining information for a possible prognosis of disease progression.

Overview of Plasmodium Falciparum and its Effect on the Body

Earth's deadliest predator isn't the great white shark or a tiger. A much smaller animal is responsible for the deaths of millions: the mosquito. Through blood feeding, the *Anopheles mosquito* transmits a protozoan parasite, *Plasmodium falciparum*, to human hosts, causing malaria [3]. Despite rapid advancements in modern medicine over the past decades, the malaria parasite remains a pressing problem in many countries, particularly in Africa. Some estimates put the global malaria infection rate at over 3% annually, causing over 200 million cases and 400,000 deaths globally each year [20].

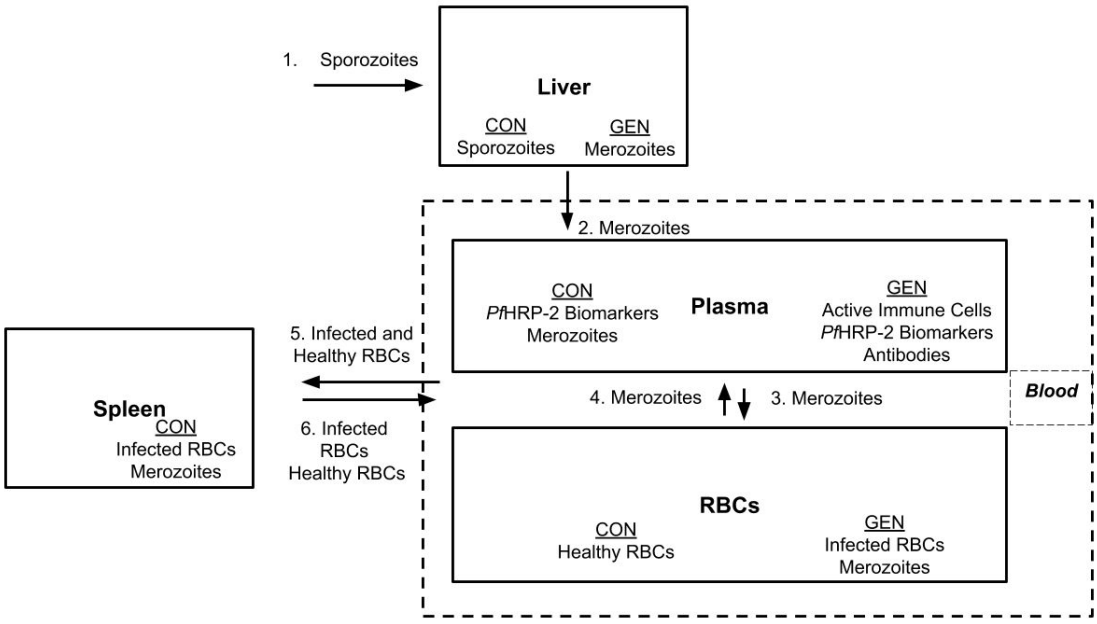
Malaria is characteristically a cyclic disease. First, the initial form of the malarial parasite called a sporozoite enters the pre-erythrocytic cycle, infecting cells in the host's liver and laying hidden within the cells while rapidly multiplying. The multiplied nuclei, called merozoites, are released into the bloodstream once the liver cell ruptures. Next, these merozoites enter the erythrocytic cycle, which occurs in the blood and entails asexual multiplication of the parasite inside of erythrocytes (red blood cells, RBCs). Finally, some of the merozoites undergo sexual reproduction to form gametocytes, which are reuptaken by mosquitoes during blood meals, restarting the malaria cycle. [3]

The erythrocytic phase is where malaria becomes symptomatic, as toxic waste builds up in infected erythrocytes and enters the bloodstream when the cells burst, resulting in metabolic acidosis [5]. As the red blood cell count drops, the body begins to lose its ability to transport oxygen and carbon dioxide around the body, resulting in potentially fatal anemia. Infected red blood cells will often adhere to endothelial cells in blood vessels, in a process known as sequestration, which creates problems with the circulation of blood. When this occurs in the brain, the host can suffer from cerebral malaria, which can result in coma, seizures, and other neurological symptoms [10].

Infected red blood cells take on a slightly different shape than healthy cells, which allows the spleen to remove the infected red blood cells from the bloodstream, subsequently removing and destroying merozoites. However, sequestration removes a portion of infected cells from the circulation in the bloodstream and effectively bypasses the spleen's filtering mechanism [13].

Considering the wealth of information about malaria and its continued presence, a simple, modular mathematical model could help turn that information into useful and relevant results in clinical settings. Although models do exist, many rely on more complex equations that are computationally expensive [15]. A simple model that captures the majority of the disease mechanics will suffice to visualize key trends. Therefore, a three compartment model was created to track major compounds related to blood cell levels, immune responses, and diagnostic testing, with the hope of establishing a simple yet effective representation of malaria that is capable of being implemented in a wide range of possible scenarios.

Model Development



Selected Compounds

Although malaria has many complex mechanisms of action, its effects on the body can be encapsulated by a short list of compounds. These compounds include merozoites, sporozoites, red blood cells (infected, sequestered, and normal), histidine-rich protein 2 (HRP2), immune cells, and antibodies. Choice of compounds and equation design for red blood cell count and immune cell tracking were loosely based on literature sources but adapted for this specific model [15].

Sporozoites

Sporozoites are a form of the parasite organism that is injected into a human by a mosquito during a blood meal [3]. On average, 16 sporozoites are introduced into the bloodstream with each mosquito bite [7]. The parasite makes its way to the liver where each

sporozoite will invade a hepatocyte cell, allowing the sporozoite to avoid the host organism's immune response [7]. Once nested inside of a hepatocyte cell, which is represented by the Liver compartment in our diagram, sporozoites replicate and mature into schizonts, which are packages of replicated sporozoites. Schizonts replicate up to 30,000 nuclei before they release the newly formed single-nucleus merozoites into the bloodstream [11].

Merozoites

Merozoites are the primary actor in a malaria infection, as this form of the parasite organism attacks the host's bloodstream. Once they are released from the schizonts, merozoites attack the host's circulatory system in two ways. First, they directly infect erythrocytes in order to continue replicating, eventually causing their lysis. The replication-lysis cycle occurs in 2 day intervals [19]. Merozoites also cause infected erythrocytes to stick to endothelial cells (sequestration), decreasing the amount of circulating red blood cells and sometimes clogging important capillaries and venules [6]. In our model, the movement of merozoites is tracked through all three major compartments: liver, blood, and spleen.

Red Blood Cells

Red blood cells, or erythrocytes, are special disk-shaped cells that transport oxygen and carbon dioxide throughout the body. These cells are the primary target of the malaria parasite, as merozoites sequester themselves inside of erythrocytes to continue replicating and eventually rupture the cells. As erythrocyte counts lower, anemia becomes a serious symptom [9]. The model tracks both infected and healthy erythrocytes present in the blood and spleen compartments.

HRP2

HRP2 is a histidine-rich protein produced by the merozoite form of the malaria parasite throughout its lifetime, and is a very common diagnostic marker for the disease considering its

slow decay and uniqueness to the parasite [8]. Tracking this compound in the blood compartment helps give the model real-world importance considering its diagnostic relevance.

Immune Cells

With any infection, the body's immune response is a vital component of the disease's ability to progress. In this model, "Active Immune Cells" within the blood compartment refer to any type of immune cell recruited to combat the parasite.

Antibodies

Antibodies encapsulate another significant feature of immune response within the blood compartment. Immune cells produce antibodies specific to the antigens produced by the parasite, which mark infected red blood cells (iRBCs) as immune system targets and help the immune system recognize the merozoites.

Selected Compartments

Liver

The liver is the main target of the initial infection. Sporozoites from the mosquito bite (*stream 1→ liver*) invade hepatocytes and begin to replicate. The replication of sporozoites into schizonts of merozoites happens exponentially over two days, with each infected liver cell eventually releasing 30,000 merozoites into the blood (*liver → stream 2*). This component of the model makes a key assumption that all 16 sporozoites from the initial infection enter unique hepatocytes at the same time, follow the same replication pathway, and burst to release merozoites into the blood at the same time. This compartment is largely modeled by Eq. 7 in Appendix B, with the process represented as a simple exponential. Note that all assumptions are included in Appendix A and all equations are included in Appendix B.

Blood

The merozoites enter initially the blood from bursting liver cells (*stream 2 → blood*), flowing directly into the plasma and circulating the body. During this time, merozoites begin producing HRP2, a *P. falciparum*-specific protein that is a common diagnostic marker for malaria; this compound spikes when merozoites are released, but simultaneously decays with a half life of around 3 days [8]. See Eq. 9 in Appendix B for the mathematical representation of this process. In addition, in response to these new parasites in the blood, antibodies are generated to fight the incoming foreign bodies and immune cells enter the plasma to help as well; both these activities are captured in the generation terms in the plasma (see Eqs. 3 and 4 for mathematical implementation).

The merozoite form of the parasite survives in the blood, entering red blood cells to reproduce and eventually lyse the cell [7]. Due to this phenomenon, the blood compartment has two sub-compartments: red blood cells and plasma. This distinction allows the model to track numbers of infected red blood cells and more accurately models the true disease state; streams 3 and 4 capture this activity, with merozoites entering red blood cells (and exiting the plasma) then exiting cells as they lyse. See Eqs. 1, 2, and 5 for how these processes were implemented and RBCs, iRBCs, and merozoites were tracked. This lysing cycle has a time period that is dependent on biological mechanisms far beyond the scope of this project, so the phenomenon was captured by a simple sinusoid, whose footprint is visible in many of the graphs above. Having subcompartments also allows analysis of the blood as a whole, a feature that was used to track numbers of infected, sequestered, and normal RBCs.

Sequestration

As infected red blood cells age throughout their 2 day cycle, they develop cytoadherence; proteins appear on their surface which allow them to stick to the endothelial

walls of blood vessels and effectively exit circulation. Most often, these iRBCs sequester in the microcirculation of organs such as the lungs, brain, and liver [21]. Using this information from [16] and [21], a model was developed which can estimate the percent of iRBCs that are sequestered and the percent that remain in circulation; the equation for this process is Eq. 8 in Appendix B. In biological scenarios, this percentage is determined by the overall maturity of the iRBC population, making this relationship only dependent on the age of iRBCs in their cycle. This vascular distribution is important in modeling, as it allows the model to separate the total iRBC population into those circulating and those sequestered, so that they can be treated differently.

Spleen

Under normal conditions, the spleen acts as a filtering device for damaged blood cells. Blood flowing into the spleen passes through the red pulp, a meshwork of tightly configured tissue and thin slits; as a red blood cell must be very deformable in order to work its way through this, old red blood cells which have become hardened or distorted in some way are filtered out [13]. Similar to old RBCs, the age of an iRBC determines some of its properties. Ring-iRBC forms which are still young in the age cycle are quite deformable, causing them to be filtered less than the Schizont-iRBCs, which are very rigid. Using literature values from [13], the filterability of iRBCs depending on their age during the 2 day cycle was determined. The model uses this variable, as well as constants such as cardiac output, blood flow rates to different parts of the spleen, and circulating iRBC concentration to determine the filtration rate at which iRBCs are removed from circulation and destroyed. That equation representing the filtration rate is included as Eq. 6 in Appendix B.

Selected Accounting Equation

Eq.1 in Appendix B is reproduced below. Although there are many other equations involved in the model that are explained in detail in Appendix B, this is the primary equation the model is based off.

$$\frac{d(iRBC)}{dt} = \left[\frac{k_{infection}}{1 + k_{efficiency} * antibodies(t)} * merezoites(t)\right] \qquad Eqn.[1a]$$

$$-[modulation(t) * iRBC(t)] \qquad Eqn.[1b]$$

$$-[k_{death} * iRBC(t)] \qquad Eqn.[1c]$$

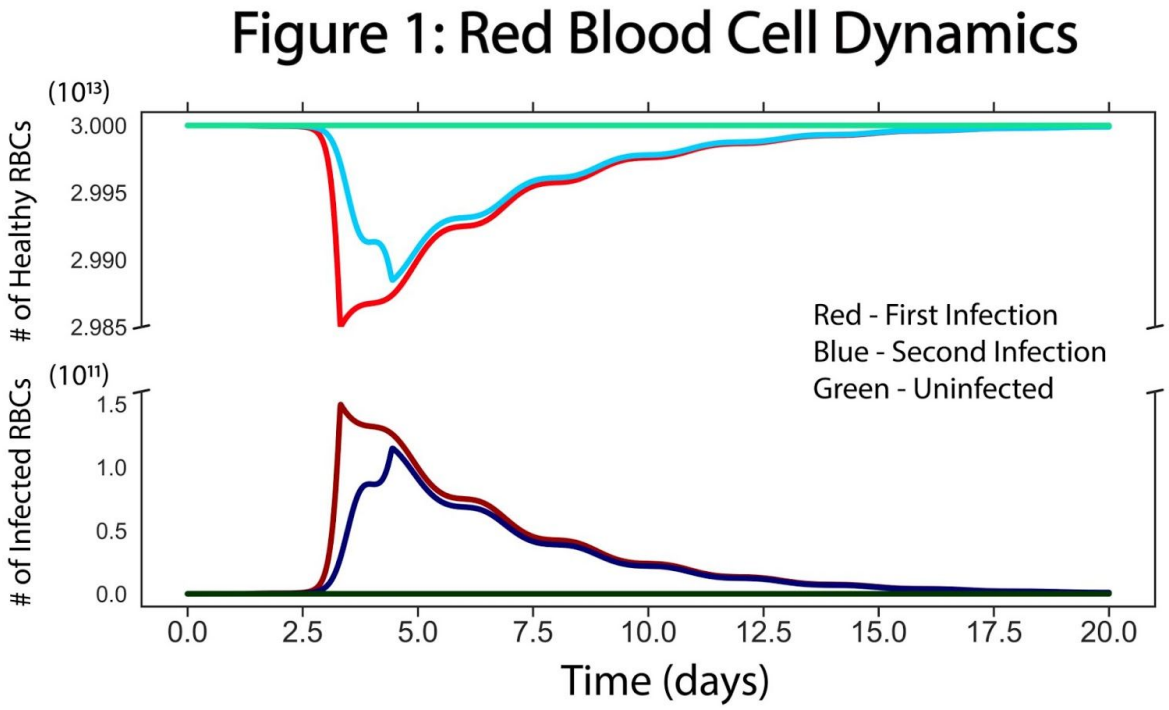
$$-[k_{immonogenicity} * immune(t) * iRBC(t)] \qquad Eqn.[1d]$$

$$-[spleen(t)] \qquad Eqn.[1e]$$

Equation 1, in general, tracks the rate of change of iRBCs. Eq.1a represents the generation of iRBCs due to merozoite infection, where $k_{infection}$ is a constant representing how susceptible RBCs are to merozoite infection. For simplicity, the model assumes that the proportion of merozoites that actually infect RBCs is directly related to the efficiency of antibodies fighting the merozoites. For example, if $k_{efficiency}$ were 0, representing completely ineffective antibodies, the merozoites would simply infect at an unfettered rate. Eq.1b is a consumption term which represents the 2-day cycling (modulation) of iRBC rupture due to merozoites. For computational feasibility, the model assumes this modulation term is a simple sine wave with a 2 day period. Eq. 1c represents the consumption of iRBCs due to the natural rate of RBC death. Eq. 1d encapsulates the consumption of iRBCs by immune cells, primarily macrophages, where $k_{immunogenicity}$ is the susceptibility of iRBCs to immune response. Finally, Eq. 1e represents consumption of iRBCs via spleen filtration.

Key Results and Implications

Key Result 1: *Modelled RBC dynamics show anemic effects caused by malaria that lead to several secondary symptoms.*



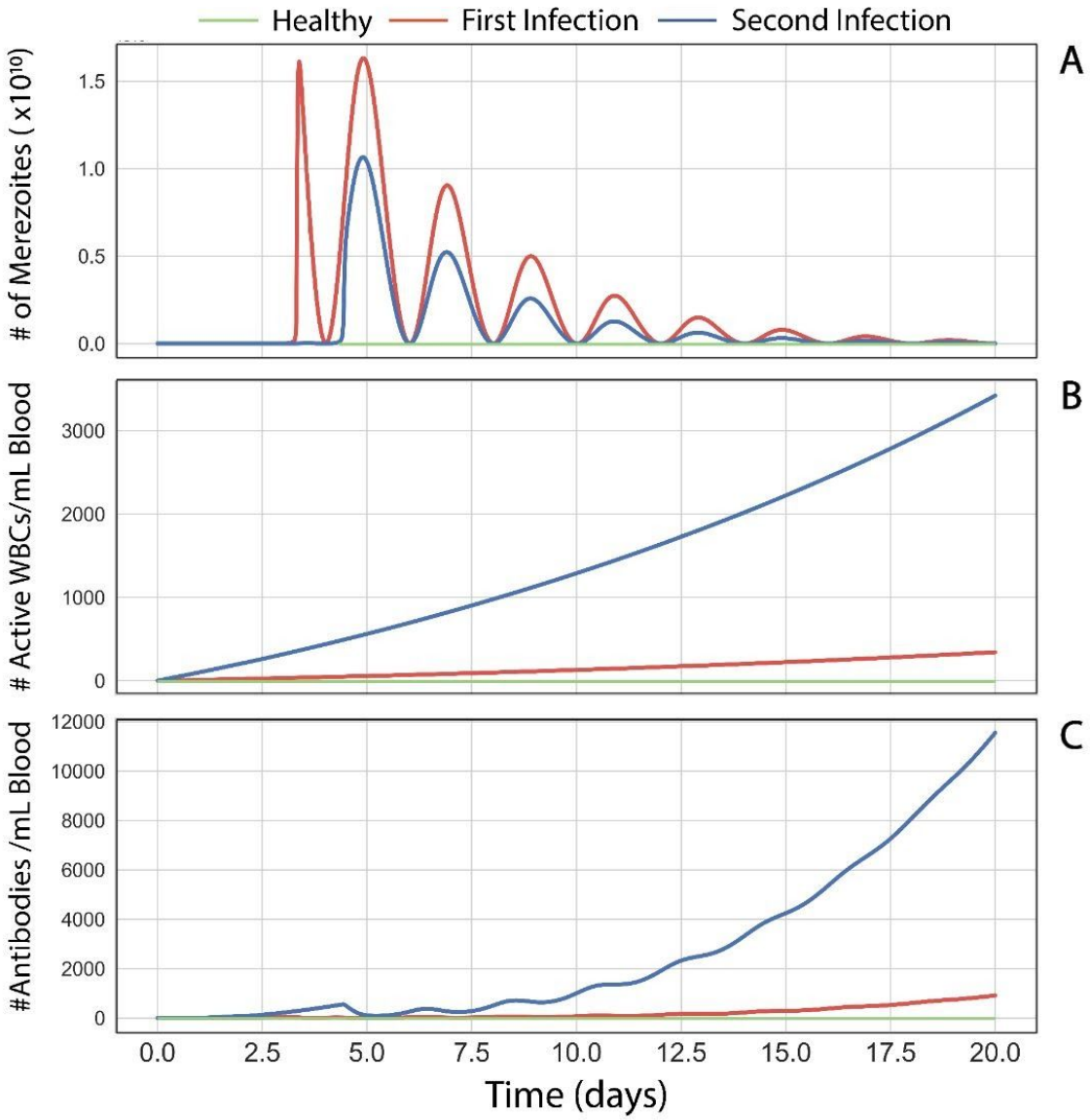
As malaria is a disease of the blood, it follows that it wreaks the majority of its havoc on the red blood cells themselves. Circulating merozoites in the plasma enter red blood cells and reproduce until they burst, killing cells and releasing more parasites. The parasite survives asexually in this manner, using and discarding red blood cells as it grows in number; therefore, one of the most important effects of malaria is anemia, or a shortage of red blood cells. As is evident in Figure 1, there are a significant number of normally circulating red blood cells that become infected and lyse, leading a decrease in the number of active, normal cells. This spike represents approximately 0.5% of all red blood cells, occurring around three days post-infection and taking approximately 2-3 weeks to return to a normal value; this implies that the individual

modelled would survive, which was assumed initially. To model more severe malaria cases, parameters regarding the immune response and initial levels of parasites could be adjusted.

Anemia is typically diagnosed through a low hemoglobin level, which is directly related to the number of red blood cells, and is even exacerbated as the parasite can affect the normal function of healthy cells. Although not considered in this model, the parasite secretes toxins that affect red blood cells and the blood vessel endothelium; combined, these affected groups cannot effectively deliver oxygen and other important nutrients, leading to anemia and decreased function overall [7]. Anemia has larger effects throughout the body, such as fatigue and weakness, considering the decreased oxygen-carrying capacity of the blood. Although the number of infected cells returns to zero within 20 days, the total number of cells may not recover so fast and may take considerably more to return to normal, extending many of the symptoms of the disease. A poorly understood mechanism exists that accelerates red blood cell production in the presence of infected blood cells [2]. However, this phenomena appears to take multiple weeks to begin and occurs outside the scope of this model; nevertheless, this process offers an idea of the total recovery time of the disease and how it extends beyond the parasitic life span in the body.

Key Result 2: *Subsequent infections and the resulting immune responses have significant effects on the disease progression.*

Figure 2: Merezoite Fluctuations and Immune Response Over Time

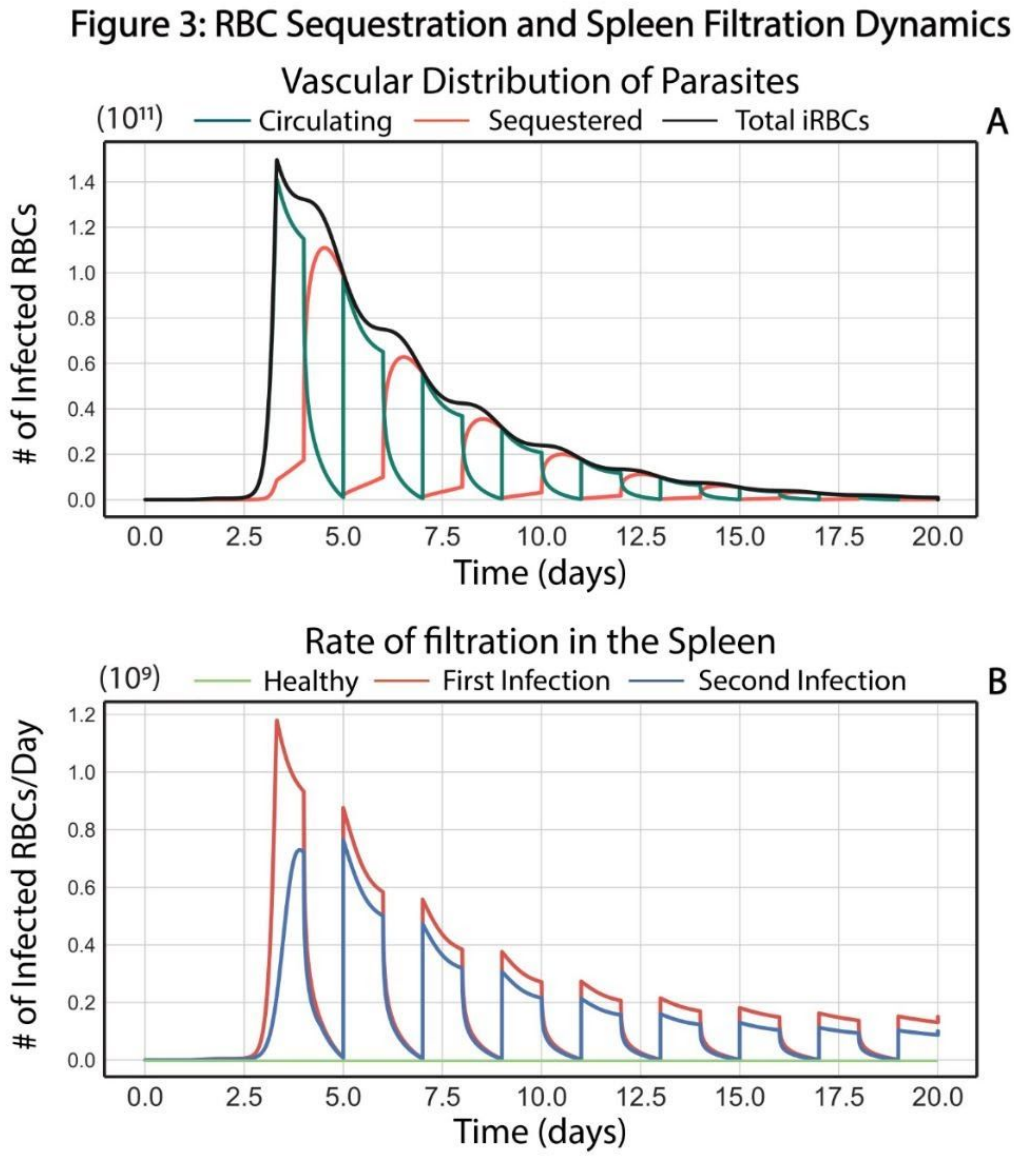


In the symptomatic phase of a malarial infection, circulating merozoites infect, multiply within, and lyse red blood cells in two-day cycles. Figure 2, part A characterizes this oscillating behavior by showing the number of merozoites circulating *in the bloodstream* for a first and second infection as functions of time over a 20 day period. The decaying trend of these

functions are influenced by the immune response shown with Figure 2, parts B and C, and the filtration rate of the spleen in Figure 3. As immune response strengthens over the course of an infection, there is a corresponding decrease in the number of parasites. A significant clinical impact of the cyclical nature of malarial pathogenesis is in the onset of paroxysms in which victims suffer from fevers recurring every 36-48 hours. This has severe implications for the health outcomes of patients who may initially be misdiagnosed if the onset of fever is less severe than subsequent attacks.

Once an individual has been exposed to a malarial infection, immunological memory allows for the rapid production of antibodies in subsequent exposures. Figure 2 compares the immune response of a first and second infection and indicates a significantly faster response and higher rate of increase of antibodies and activated white blood cells for the second infection. During the primary response, in which malarial antigens first come in contact with the immune system, memory lymphocytes are produced and eventually allow for the faster production of antibodies for later infections.

Key Result 3: *Sequestration of infected RBCs, and its effect on immune response and pathogenesis.*



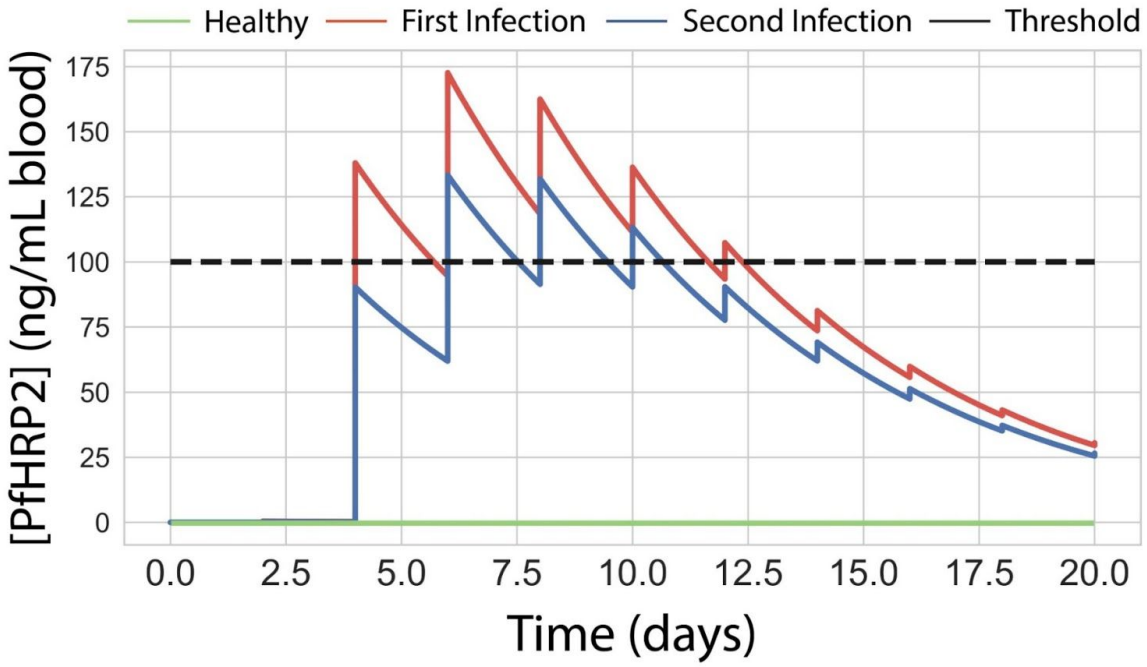
Along with the immune response, the spleen plays an important role in consuming iRBCs and fighting malaria. As blood passes through it, a fraction of the infected red blood cells are removed, helping to fight the disease. The cyclical nature of malaria, along with influencing the infection, growth, and rupture of iRBCs, also determines the nature in which they sequester.

Throughout each 2 day cycle, as iRBCs develop, they sprout proteins which allow them to sequester in the microcirculation of the circulatory system. Sequestration influences much of the pathogenesis of severe cases of malaria,in our model, its influence can best be seen by the impact on the spleen's filtration rate. In the first 24 hours of the cycle, the rate at which the cells sequester is low. The result is a large iRBC population in circulation, and a corresponding large spleen filtration rate, as can be seen in figures 3A and 3B. From 24 to 48 hours, this process is very rapid, as can be seen by the sharp downward spikes in the circulating populations: by the end of the cycle there are almost no circulating iRBCs. As the spleen can only filter what is in circulation, the filtration rate quickly drops to almost 0 RBCs per day. This behavior is only widespread in the Falcipareum strain of malaria, part of the reason why it is more dangerous: the spleen is only effective in fighting malaria for about half of the time. Sequestration was even speculated to have evolved as a mechanism for iRBCs to avoid filtration by the spleen.

Sequestration has other, more difficult to model, effects. The physical accumulation of iRBCs decreases the flow of in the microcirculations throughout the body. This becomes especially significant if the disease progresses to become cerebral malaria, where blocked capillaries are often the cause of death [1]. In severe cases of malaria, the sequestration can be enough to where the blood flow to an organ can decrease, and the tissue must perform anaerobic respiration. This results in hyperlactatemia, a well known indicator for fatal outcome in malaria diagnosis (source above).

Key Result 4: *Diagnostic testing for malaria is possible through measuring the concentration of PfHRP-2 in the blood, although not infallible.*

Figure 4: Malaria Diagnostic Testing

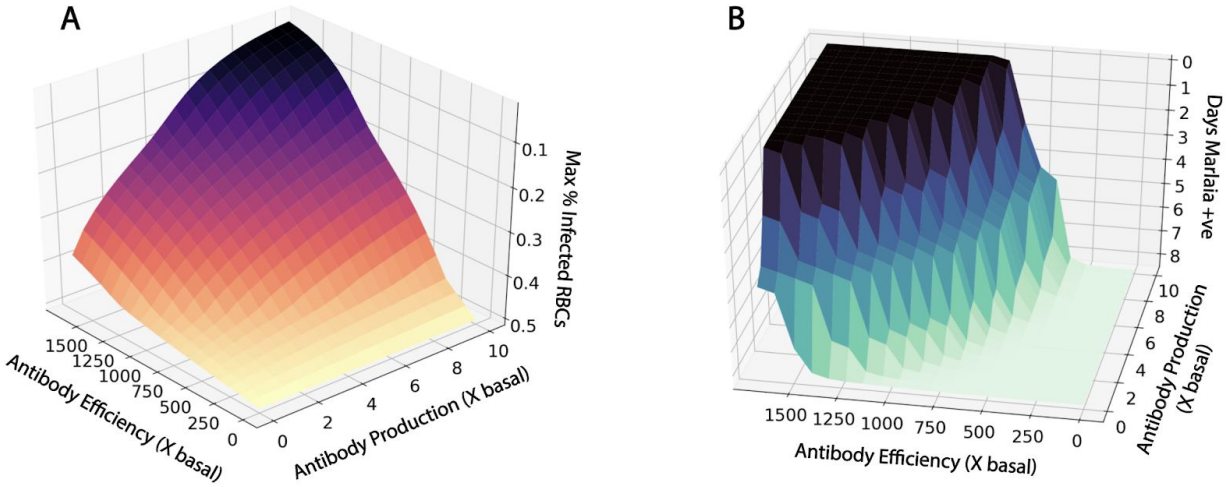


Due to the cyclic nature in the movement of the parasite, concentrations of certain constituents in the plasma become cyclic as well. Figure 4 shows the concentration of HRP2, a chemical used in malaria testing mechanisms, over the time span of infection. *Pf*HRP2*,* specifically, is a protein produced entirely by the malaria parasite, making it an ideal diagnostic marker for the disease. From Figure 4, it is clear that, from about 4 to 12 days after infection, malaria can be detected through HRP2-based diagnostic tests. As this model assumes the body successfully fights the malaria parasite, the HRP2 decreases as the number of parasites does, falling below the assumed threshold around 12 days; however, in more serious cases, the number of parasites may decrease slower or not at all, yielding a larger window for accurate diagnostic testing. This is especially beneficial, as those individuals will be at much higher risk for serious consequences.

Given the threshold shown in Figure 2, for an individual with subsequent malaria infections, there are points during the infection when HRP2 levels in the plasma fall below the threshold level of 100 ng/mL of blood used in some tests. This increases the chance of false negatives while testing someone for malaria if they have had malaria previously. Note that the threshold referenced above, in reality, varies between tests, and lowering the threshold could reduce the rate of false negatives; too low, however, and false positives begin to arise. Although other compounds were not included in this model given the time constraints, there are numerous other diagnostic markers used in malaria testing that can help combat false negatives and false positives. Testing for one of these compounds in addition to HRP2 could help eliminate false negatives, as other compounds may obey different kinetic rate laws and counteract the potential faulty test results by offering a second, different level of testing.

Key Result 5: *Variations in immune system strength and preparedness can dramatically impact the disease severity.*

Figure 5: Simulation of Vaccine Boosted Immune System



As evident in Figure 5, decreasing antibody efficiency or production can significantly affect the length of the infection and the severity, as measured by the peak number of healthy red blood cells lost. Intuitively, the relationships make sense antibody efficiency has a greater effect on the length of infection, while antibody production has a greater effect on the number of healthy cells. However, some level of antibody efficiency is required, else antibodies will not effectively fight the parasite. In Figure 5A, the number of healthy cells after one infection is given as a function of antibody efficiency and production, with production appearing to have a greater effect after some level of antibody efficiency is reached. In 5B, antibody efficiency can greatly decrease the infection while production needs a higher level of efficiency to achieve the same effect. As antibody production rates increase, even if the efficiency is somewhat low, they are still able to fight the parasite and keep the number of cells lost low; however, increased antibody efficiency is needed to shorten the infection, as the efficiency is the rate-determining factor.

Clinically, these conclusions have a few applications: first, immune therapies like immunoglobulin therapy, which helps provide antibodies to individuals with deficient immune systems, can be modeled. Immunoglobulin therapy, specifically, effectively increases the rate of antibody production by providing a source of antibodies and antibody materials for the body [4]. In addition, vaccinations against disease like malaria can be modeled by increasing one of the two parameters in the above graphs. Currently, there appears not to be a consistently effective malaria vaccine, but using the model and parameters above, the need for one is clearly demonstrated.

Reflections

Although the model was successful in modeling basic malaria pathophysiology, limitations still exist considering the complex biological mechanisms underlying the simplifications and assumptions made for this model. A necessary simplification was the removal of age dependence from several equations. The literature sources serving as the foundation of the equations in the model often including age dependence in the equations regarding healthy and infected red blood cells; although this dynamic is likely important, the equations could not be implemented if they contained partial differential equations (PDE) due to the lack of knowledge and appropriate computational tools. To work around this limitation, a single age was assumed for all red blood cells, and the basal creation and destruction rates of red blood cells were assumed to be equal. Other phenomena, like red blood cell lysing and HRP2 production, included simplifications that were deemed necessary given the scope of the project. Merozoites enter red blood cells, reproduce, and cause them to lyse approximately 2 days after their entry. This time period is dependent on biological mechanisms far beyond the scope of this project, so the phenomenon was captured by a simple sinusoid, whose footprint is visible in many of the graphs above. Although this simplification was certainly necessary for the model, it could be further refined. A similar simplification was the discretization of HRP2 production; although HRP2 diffuses into the plasma after red blood cells lyse, this entire process was modelled as a single impulse, repeating every period of the sinusoid with an amplitude dependent on the number of infected red blood cells.

Despite these limiting factors, the model was strong in capturing the essence of a complex biological process and producing feasible, diagnostically relevant values. Literature values for kinetic rate constants, as described in Appendix B, were used for all major

constituents of the model and yielded the results shown above. As the model incorporates so many specific parameters, it is very modular, so a number of scenarios could be modelled simply by changing constants in line with a given scenario. This modularity was showcased in Key Results 2 and 5, as parameters were shifted to assume a stronger and more prepared immune response for a second infection. In addition, the values produced by the model appear, at the very least, feasible, given their order of magnitude, with many values similar to their literature counterparts. This accuracy demonstrated by the model makes it even stronger, increasing the real relevance of its results.

Conclusions

Mathematical models are powerful tools that can provide insight into how a disease such as malaria affects a biological system. Results from the model can not only give a better understanding of how the disease progresses, but also explain why certain clinical phenomena occur. The model helps explain the underpinnings of malaria's mechanism of action, from merozoite asexual reproduction and red blood cell lysing to sequestration and the immune response. Up to 0.5% of the healthy red blood cells become infected according to the model, causing anemia which can lead to numerous secondary symptoms. In addition, due to the cyclic nature of the infection caused by the reproduction-lyse cycle, there are points at which the infection appears milder than it is, posing a potential problem for diagnostic testing and clinicians. This model can assist by helping track the relevant compounds over time, giving clinicians an inside look into how the disease is progressing.

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Appendix A: Assumptions and Accepted Values

Liver

- One mosquito bite results in sixteen (16) sporozoites entering the host's bloodstream. These sporozoites instantaneously enter unique liver cells and begin replicating.
- No sporozoites lay dormant.
- The time of exponential reproduction is two (2) days.
- The infected liver cells burst simultaneously from the presence of merozoites.
- Burst liver cells instantaneously move the accumulated merozoites into the bloodstream.

Blood

- One merozoite can enter one red blood cell at a time. This changes that red blood cell from "healthy" to "infected."
- Infected red blood cells have a life span of two days. During this time, merozoites within the cell replicate asexually.
- *Pf*HRP-2 has a half-life of 3.67 days [8].
- Cardiac Output = 4.7 L/min

Spleen

- 5% of the Cardiac Output goes to the spleen.
- Some % of the cells in the spleen are filtered according to the model equations, and all infected cells that get filtered are killed.

Appendix C: Model Code

```
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns
from scipy.integrate import odeint
from mpl_toolkits.mplot3d import Axes3D
X0 = 0 #initial infected RBC count
I0 = 1e-2 #initial immune cell conc
A0 = 0 #initial antibody conc
M0 = 3e13
T0 = 0
F0 = 0
tspan = np.linspace(0,20,100001)
k0 = 9e-5
k1 = 2e-9
k2 = 6e-4
ax = 0.025
k3 = 10e-8
r = 16
k4 = 8.5e-4
ay = 48
k5 = 10e-8
lamI = 10
lamx = 0.05
lamy = 0.05
ai = 0.05
k6 = 2000
k7 = 1500
nu = 0.6
aa = 5e-10
k8 = 1500
c = -(2.5)
s2 = 2
f = 5.2e-15

tspan2 = np.linspace(0,48,10000)
for i in range (4):
    tspan2 = np.append(tspan2,tspan2)
tspan2 = tspan2[:85000]
tspan2 = np.append(np.zeros(15000),tspan2)

#%% Liver
spori = 16
tspanliv = np.linspace(0,2,10000)
mern = spori*np.exp(4.95174377627*tspanliv)
Y0 = np.max(mern) # initial merozoite #
y0 = [X0,Y0,I0,A0,M0,F0]

#%% main function
def s(t):
  return 0.5*(np.sin(np.pi*t/2)**2)
def s2(t):
    return(t%2)
def s4(t):
    if(s2(t)*24<24.058):
        s3=.0046*s2(t)*24+.0215
    else:
        s3=.1422*np.log(s2(t)*24-24)+.5382
    return s3
def iRBCcount(y0,t):
  ######################### Establishing local var names
  x = y0[0]
  y = y0[1]
  i = y0[2]
  a = y0[3]
```

```
    a = y0[3]
    m = y0[4]
    f = y0[5]
    ######################### all kinetic rate constants, meanings are defined in the paper. Taken directly from the paper.
    dXdt = (k1/(1+k2*a))*y*m - s(t)*x-ax*x-k3*i*x-f
    dYdt = (r/(1+k4*i))*s(t)*x-ay*y-k5*i*y - (k1/(1+k2*a))*y*m
    dIdt = lamI+(((lamx*x)/(k6 + x)) + ((lamy*y)/(k7 + y)))*i - ai*i
    dAdt = nu*i*(y/(k8 + y)) - aa*a*y
    dMdt = (k0*x-.0083*m-k1/(1+k2*a)*y*m)*50#assuming 50 days age to avoid PDEs listed in paper
    dFdt = (0.16*s2(t)**.72)*(dXdt)*.047
    return [dXdt,dYdt,dIdt,dAdt,dMdt,dFdt]
vals = odeint(iRBCcount, y0, tspan)
lamI = 100
X0 = 1e6 #https://microbiologynotes.com/differences-between-primary-and-secondary-immune-response/
vals2 = odeint(iRBCcount, y0, tspan)
#%% sequestration
seq = np.zeros(100001)
circ = np.zeros(100001)
s3 = np.zeros(100001)
x = vals[:,0]

for i in range (0,100000):
    if(tspan2[i]<24.058):
        s3[i]=.0046*tspan2[i]+.0215
    else:
        s3[i]=.1422*np.log(tspan2[i]-24)+.5382
    seq[i] = s3[i]*x[i]
    circ[i] = (1-s3[i])*x[i]

# %% HRP2
def hrp2(x):
    hrp2 = np.zeros(len(tspan))
    nT = 0
    nmax = 0
    for i in range(len(tspan)):
        if tspan[i]%2 == 0 and nT != 0:#assuming cycle period of 2 days
            nT+=1
            hrp2[i] += hrp2[i-1]+f*x[i]
            nmax = i
        else:
            hrp2[i] = hrp2[nmax]*np.exp(-.18873*(tspan[i-nmax]))
        if nT == 0:
            nT = 1

    return hrp2/5 #per 5L blood
#%% Simulation
"""
nums = np.linspace(6e-4,1,20) #k2 range
nums2 = np.linspace(0,100,20) #lamI range
X,Y=np.meshgrid(nums/6e-4,nums2/10)

rows, cols = (20, 20)
Z1 = [[0 for i in range(cols)] for j in range(rows)]
Z2 = [[0 for i in range(cols)] for j in range(rows)]
Z3 = [[0 for i in range(cols)] for j in range(rows)]

for i in range(0,20):
    k2 = nums[i]
    for j in range(0,20):
        lamI = nums2[j]
        vals3 = odeint(iRBCcount, y0, tspan)
        #Z1[i][j] = 100-((3e13-max(vals3[:,0]))/3e13)*100
        #Z2[i][j] = tspan[np.argmax(vals3[:,0])]
    \
```

```
    \
          occurences = np.where(hrp2(vals3[:,0])*1000000>= 100)
          if(len(occurences[0]) == 0):
              Z3[i][j] = 0
          else:
              d1 = occurences[0][len(occurences[0])-1]
              d2 = occurences[0][0]
              Z3[i][j] = tspan[d1]-tspan[d2]


fig = plt.figure()
#ax = fig.add_subplot(111, projection='3d')
#ax.plot_surface(X,Y,np.asarray(Z1),cmap='inferno')
#ax = fig.add_subplot(111, projection='3d')
#ax.plot_surface(X,Y,np.asarray(Z2),cmap='inferno')
ax = fig.add_subplot(111, projection='3d')
ax.plot_surface(X,Y,np.asarray(Z3),cmap='inferno')
for t in ax.xaxis.get_major_ticks(): t.label.set_fontsize(18)
for t in ax.yaxis.get_major_ticks(): t.label.set_fontsize(18)
for t in ax.zaxis.get_major_ticks(): t.label.set_fontsize(18)
"""
#%% plotting


"""
fig,axs = plt.subplots(3,1, figsize=(20,22),sharex=True)
fig.subplots_adjust(hspace=0.075)
sns.set()
sns.set_style("whitegrid")
sns.set_context("talk")
axes_style = {'linewidth':5}
axs[0].plot(tspan, vals[:,1], sns.xkcd_rgb["pale red"],label = "first infection", linewidth = 5)
axs[0].plot(tspan, vals2[:,1],  label = "second infection", linewidth = 5)
axs[1].plot(tspan, vals[:,2], sns.xkcd_rgb["pale red"], label = "first infection", linewidth = 5)
axs[1].plot(tspan, vals2[:,2],  label = "second infection", linewidth = 5)
axs[2].plot(tspan, vals[:,3], sns.xkcd_rgb["pale red"], label = "first infection", linewidth = 5)
axs[2].plot(tspan, vals2[:,3],  label = "second infection", linewidth = 5)
axs[2].tick_params(axis='x', which='major', labelsize=32)
axs[2].tick_params(axis='y', which='major', labelsize=24)
axs[1].tick_params(axis='y', which='major', labelsize=24)
axs[0].tick_params(axis='y', which='major', labelsize=24)
plt.savefig("F2PBL2.png",dpi=500)
"""


"""
sns.set()
sns.set_style("whitegrid")
sns.set_context("talk")
plt.figure(figsize=(16,8))
plt.plot(tspan, hrp2(vals[:,0])*1000000, sns.xkcd_rgb["pale red"], label = "first infection", linewidth = 5)#1000000 ng/mL per
g/L
plt.plot(tspan, hrp2(vals2[:,0])*1000000,  label = "second infection", linewidth = 5)
plt.plot(tspan, np.full(len(tspan),100),'k--', label = "Detection Threshold",linewidth = 5)
plt.xticks(fontsize=32)
plt.yticks(fontsize=24)
plt.savefig("F4PBL2.png",dpi=500)
"""


"""
sns.set()
sns.set_style("white")
```

```
    sns.set_style("white")
    sns.set_context("talk")
    f, (ax, ax2) = plt.subplots(2, 1, sharex=True,figsize=(16,8))
    f.subplots_adjust(hspace=0.3)
    ax.plot(tspan, vals[:,0]/1e11, sns.xkcd_rgb["deep red"], label = "Infected - first infection", linewidth = 5)
    ax.plot(tspan, vals2[:,0]/1e11, sns.xkcd_rgb["deep blue"], label = "Infected - second infection", linewidth = 5)
    ax.plot(tspan, (3e13 - vals[:,0])/1e13, sns.xkcd_rgb["bright red"], label = "Healthy - first infection", linewidth = 5)
    ax.plot(tspan, (3e13 - vals2[:,0])/1e13,sns.xkcd_rgb["bright sky blue"],   label = "Healthy - second infection", linewidth =
    5)
    ax.plot(tspan, np.full(np.array(100001),3.0),sns.xkcd_rgb["aqua green"],   label = "Healthy Person", linewidth = 5)

    ax2.plot(tspan, vals[:,0]/1e11, sns.xkcd_rgb["bright red"],linestyle = "solid", label = "Infected - first infection",
    linewidth = 5)
    ax2.plot(tspan, vals2[:,0]/1e11, sns.xkcd_rgb["bright sky blue"], linestyle = "solid",label = "Infected - second infection",
    linewidth = 5)
    ax2.plot(tspan, (3e13 - vals[:,0])/1e13, sns.xkcd_rgb["bright red"], linestyle = "solid", label = "Healthy - first infection",
    linewidth = 5)
    ax2.plot(tspan, (3e13 - vals2[:,0])/1e13,sns.xkcd_rgb["bright sky blue"], linestyle = "solid",   label = "Healthy - second
    infection", linewidth = 5)
    ax2.plot(tspan, np.zeros(100001),sns.xkcd_rgb["aqua green"], linestyle = "solid",  label = "Healthy Person", linewidth = 5)

    ax.set_ylim(2.985, 3.001)  # outliers only
    ax2.set_ylim(-0.1, 1.6)  # most of the data

    ax.spines['bottom'].set_visible(False)
    ax2.spines['top'].set_visible(False)
    ax.spines['left'].set_visible(True)
    ax2.spines['left'].set_visible(True)
    ax.xaxis.tick_top()
    ax.tick_params(labeltop=False)  # don't put tick labels at the top
    ax2.xaxis.tick_bottom()

    d = .0075  # how big to make the diagonal lines in axes coordinates
    # arguments to pass to plot, just so we don't keep repeating them
    kwargs = dict(transform=ax.transAxes, color='k', clip_on=False)
    ax.plot((-d, +d), (-d, +d), **kwargs)        # top-left diagonal
    ax.plot((1 - d, 1 + d), (-d, +d), **kwargs)  # top-right diagonal

    kwargs.update(transform=ax2.transAxes)  # switch to the bottom axes
    ax2.plot((-d, +d), (1 - d, 1 + d), **kwargs)  # bottom-left diagonal
    ax2.plot((1 - d, 1 + d), (1 - d, 1 + d), **kwargs)  # bottom-right diagonal

    ax2.tick_params(axis='x', which='major', labelsize=26)
    ax2.tick_params(axis='y', which='major', labelsize=20)
    ax.tick_params(axis='y', which='major', labelsize=20)
    plt.savefig("F1PBL2.png",dpi=300)
    """



    fig,axs = plt.subplots(2,1)
    fig.subplots_adjust(hspace=0.5)
    sns.set()
    sns.set_style("white")
    sns.set_context("talk")
    axes_style = {'linewidth':5}

    axs[0].plot(tspan, seq, sns.xkcd_rgb["pale red"], label = "Sequestered", linewidth = 5,color=sns.xkcd_rgb["coral"])
    axs[0].plot(tspan, circ,  label = "Circulating", linewidth = 5,color=sns.xkcd_rgb["blue green"])
    axs[0].plot(tspan, circ+seq,  label = "Total iRBCs",color='k', linewidth = 5)
    axs[1].plot(tspan, vals[:,5]*(1-s3), sns.xkcd_rgb["bright red"], label = "first infection", linewidth = 5)
    axs[1].plot(tspan, vals2[:,5]*(1-s3),  label = "second infection", linewidth = 5,color=sns.xkcd_rgb["bright sky blue"] )

```