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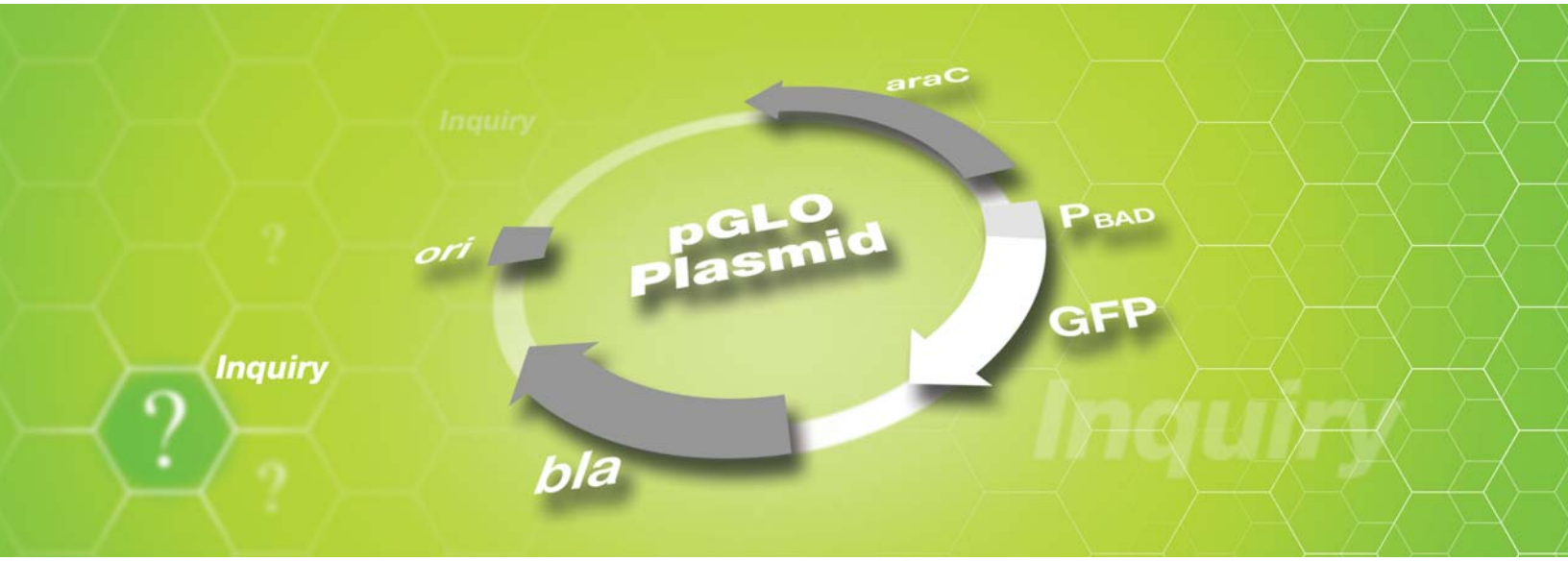


pGLO™ Transformation and Inquiry Kit

A ThINQ!™ Investigation

Science Case Study

BIO-RAD

Contents

“Can Bacterial Transformation Stop the Spread of Malaria?”

I. The Global Impact of Malaria

Lerato sits in the clinic, watching her son Baruti, age 4, writhe with fever. He is sleeping for the first time in days, and Lerato is anxious for signs that the medication the doctor had given her son is working. She worries she may have waited too long to bring Baruti to the clinic.

Baruti had fallen ill a week before with fever, chills, and body aches, all vague flu-like symptoms that Lerato had assumed would clear in a few days, as so many illnesses had in the past. Lerato and her family live an hour’s walk away from the medical clinic in Seronga, a remote village in Okavango, Botswana. Making that long trip with a sick child is difficult, but it was a trip she had to make when her son’s symptoms grew more severe. He was now in a clinic bed, suffering from extreme anemia secondary to (brought on by) malaria.

Malaria is spread by mosquitoes, and like so many others in the Okavanago region, Lerato’s family had been issued mosquito nets for their beds. The nets are covered with insecticidal chemicals and are an effective and relatively inexpensive method for controlling mosquitoes. The nets, though, do not allow much air circulation and so are very hot to sleep under, and little Baruti tends to kick them away while he sleeps, exposing his limbs to the bites of mosquitoes.

Questions

1. Malaria is the third leading cause of infectious disease death in the world, after tuberculosis and AIDS. According to the World Health Organization, 3.4 billion people — nearly half the global population — are currently at risk for malaria. Most prevalent in African or tropical Asian countries, malaria is often considered a “disease of the developing world.” Though vaccines are not yet available, it can be cured if diagnosed and treated promptly.

   Given this information, what might the biggest hurdles be in fighting malaria? Consider the regions the disease affects and the challenges faced by the people living there.

2. Malaria can be spread only through the bite of a mosquito, and it was nearly eliminated in the U.S. back in the 1950s. Despite this, as many as 1,500–2,000 new cases of malaria are reported in the U.S. annually. How can this be? How might malaria be coming into the country?

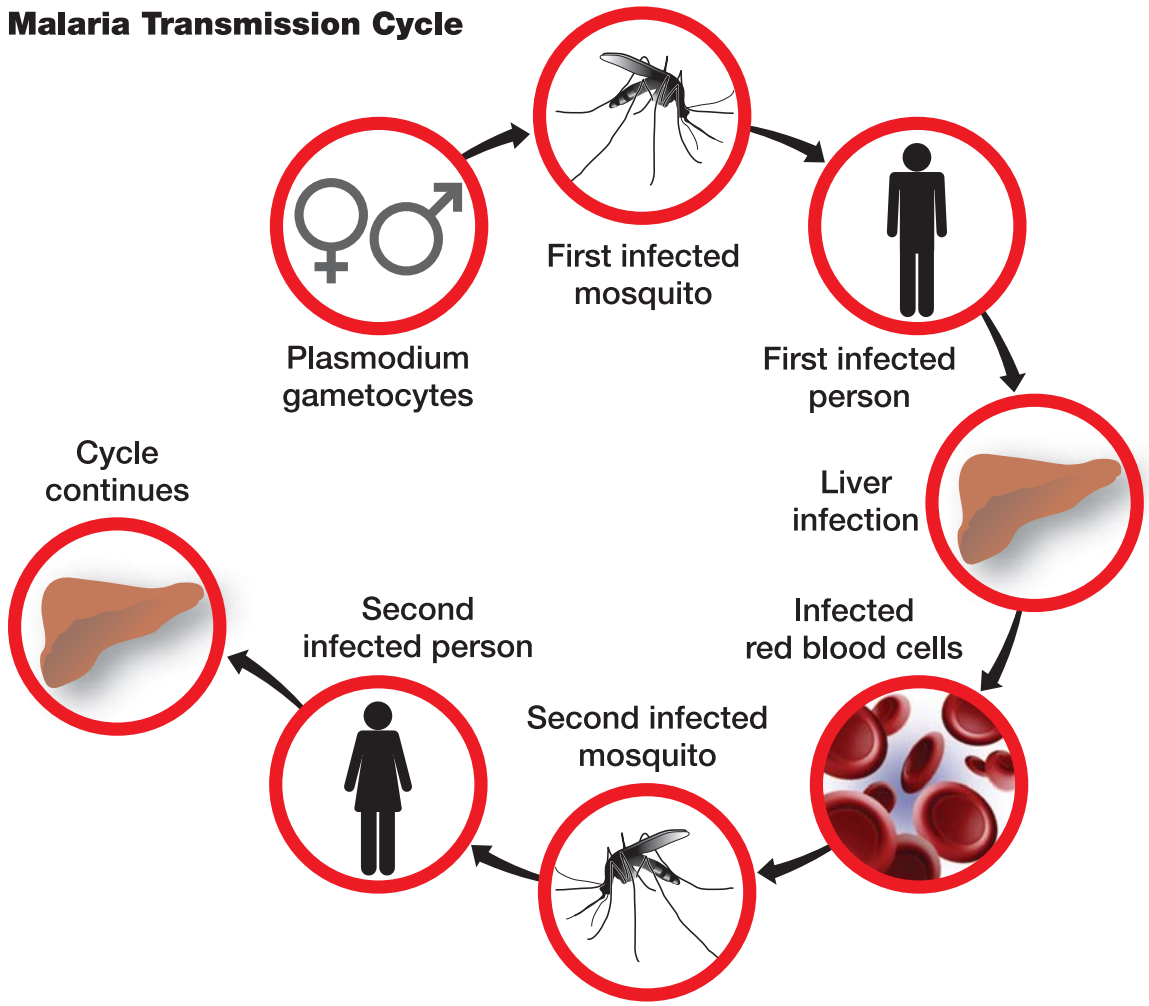
3. Considering that malaria can be spread only from infected blood and through mosquito bites, how might malaria eventually be eradicated in a particular region, such as the U.S.?

II. Mosquitoes — Flying Factories of Malaria

In order to expand the discussion of malaria and possible methods for its treatment, control, and eradication, it is important to understand the biology behind the disease.

Plasmodium — the parasitic protist behind malaria

Malaria is a parasitic infection caused by single-celled protists in the genus *Plasmodium*. Of the more than 100 species of *Plasmodium*, only four infect humans and cause disease. *Plasmodium* is a member of the phylum *Apicomplexa*, a fascinating group of protists believed to have evolved from photosynthetic dinoflagellates (forms of plankton). It has a complex life cycle that will not be discussed here except to say that to complete that life cycle, *Plasmodium* requires two hosts: (1) humans and (2) the female *Anopheles* mosquito.

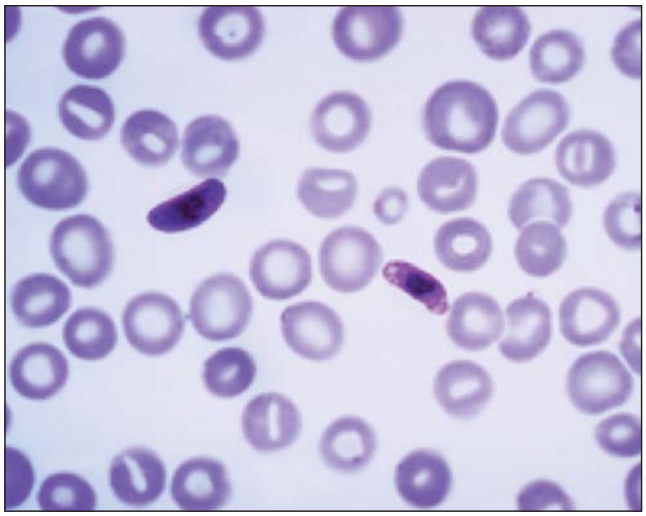


The* Plasmodium *life cycle depends on two hosts: humans and mosquitoes.

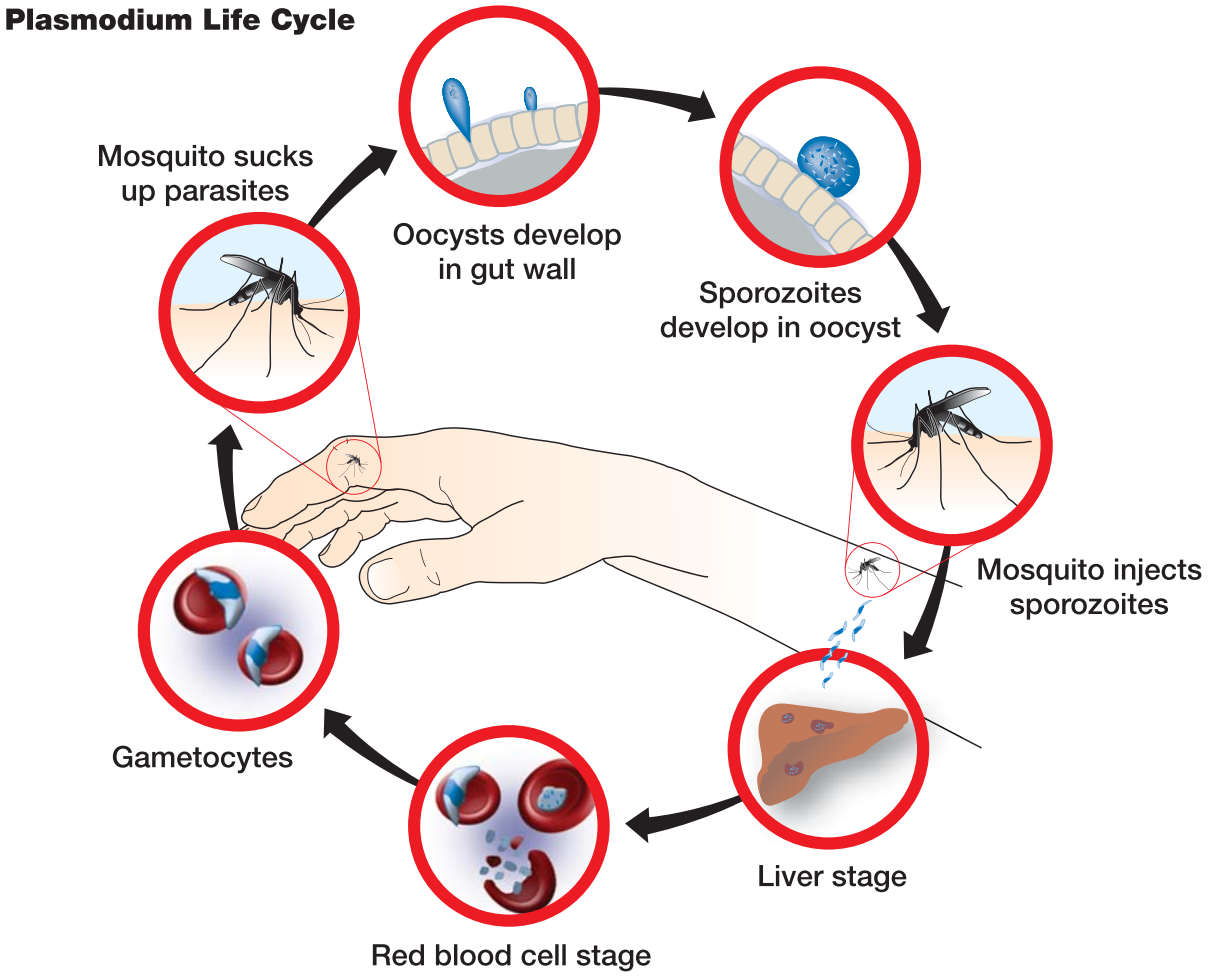
In humans, the *Plasmodium* parasite invades the cells of the liver, lymph nodes, and red blood cells (erythrocytes) in the bloodstream, where it replicates and eventually causes cells to rupture. The human immune system also responds to the invasion, producing the high fevers, nausea, diarrhea, and other flu-like symptoms characteristic of malaria. In mosquitoes, *Plasmodium* lives in the gut and salivary glands; it has no known negative effects on the mosquito host.

As mentioned, *Plasmodium* is mainly transmitted between infected humans by mosquitoes. Specifically, it is spread only by female mosquitoes of the genus *Anopheles*. This is because only female mosquitoes bite humans. They ingest human blood to obtain the proteins necessary for egg development. Most female *Anopheles* mosquitoes are nocturnal feeders (they bite only at night).

When a female mosquito bites and takes blood from a person infected with *Plasmodium*, the microscopic parasite moves along with the human's red blood cells to the mosquito's gut, where it continues through its life cycle. It then moves to the mosquito's salivary gland; when the mosquito takes a bite from another human, *Plasmodium* is injected along with the mosquito's saliva. (It is the proteins in mosquito saliva that trigger an immune response from your body, causing bites to itch.) The parasites can then be ingested by another mosquito, completing the life cycle and transmitting the disease from human to human to human.



Blood smear showing the presence of the* Plasmodium *parasite (crescent shapes). *In the absence of more sophisticated tests, microscopic analysis of blood samples is a common diagnostic approach for malaria in clinics.*



Malaria is passed from person to person through mosquito bites.

Questions

1. Malaria is most often transmitted by mosquitoes, but considering *Plasmodium* lives in erythrocytes, what are other ways in which the disease might spread from human to human?

2. Understanding the biology of malaria, it is not surprising that the most common methods of reducing outbreaks involve mosquito control. Relatively inexpensive and simple to perform, indoor spraying with insecticides kills mosquitoes for 3–6 months, and insecticidal bed nets provide additional protection from bites, with the insecticidal qualities of the nets lasting 3–5 years. What are the main benefits and drawbacks of both these approaches?

Indoor spraying

Benefits:

Drawbacks:

Bed nets

Benefits:

Drawbacks:

3. Malaria is only one of many parasite-mediated, mosquito-borne illnesses affecting the world's population. For this reason, some researchers advocate mosquito elimination — completely killing off mosquito populations in affected regions. The benefits of elimination are obvious (many of us would love to see mosquito-free lakes, streams, and ponds). However, what might some of the concerns be in eliminating a species from a habitat? Do we know what roles mosquitoes play in the environment? Does it make a difference if we know or don't know?

4. Many regions in Africa are reporting increasing populations of *Anopheles* mosquito species that show resistance to insecticides. Even more troubling are findings that climate change is expanding the habitat of the mosquitoes into regions where malaria had not been a health concern. How does your answer to the previous question change if the mosquito in question is an invasive species (for example, in the spread of dengue fever, the mosquito vector in question is often an invasive species new to habitats in North America)?

III. A Novel Approach Involves Bacterial Transformation

Biotechnology and genetic engineering methods are also being investigated as mechanisms for eliminating malaria. Theoretically, any of the species involved — the *Plasmodium* parasite or the human or mosquito host — can be the target of genetic modifications that disrupt either the life cycle or transmission of the parasite. Practically, however, these systems have their drawbacks in terms of their ability to be either cultured or manipulated genetically. Therefore, researchers have turned their attention to a more familiar subject: bacteria.

How can DNA "transform" bacteria? A background to bacterial transformation

In the 1920s, scientists demonstrated how to turn a harmless strain of bacteria into a virulent strain, just by mixing the two strains together (Griffith 1928). What is truly incredible about this experiment is that the virulent strain had been killed prior to mixing, so something in the dead bacteria could "transform" the harmless bacteria, making them virulent.

It wasn't until the 1940s that scientists understood the chemical basis for this transformation. A team of scientists led by Oswald Avery at the Rockefeller Institute found that an extract of the bacteria was unaffected by treatment with protein-digesting enzymes, but was destroyed by a DNA-digesting enzyme. This showed that the agent that transformed the harmless bacteria was DNA (Avery et al. 1944).

Today, we understand that genes within DNA encode proteins that give rise to certain traits. We also know how to exploit the fact that many bacteria can acquire new genes by taking up DNA molecules encoding those genes (for instance, a plasmid) from their surroundings. The process is optimized by adding salts to the transformation medium and using a heat shock step, steps we use deliberately to transform bacteria and other microorganisms. The ability to transform the bacterium *E. coli*, for example, has made possible the cloning of genes, the cornerstone of many modern advances in sciences and of the biotechnology industry.

So how does bacterial transformation relate to our battle against malaria?

Mosquitoes also have gut microbiota?

It is surprising to many that, like humans, mosquitoes harbor a number of symbiotic bacteria within their gut. These symbiotic bacteria can be engineered, using procedures like those you used to transform *E. coli* bacteria, to produce proteins. However, in this case the symbiotic bacteria can be engineered to produce and secrete proteins that interfere with the life cycle of the *Plasmodium* parasite.

In one experiment (Wang et al. 2012), researchers used a bacterium called *Pantoea agglomerans*, which grows abundantly inside *Anopheles* mosquitoes. *P. agglomerans* can be grown and transformed using the same culturing and transformation techniques used with other more common bacteria, like *E. coli*. Researchers used these techniques to engineer *P. agglomerans* to express the genes of the hemolysin (hly) A system of *E. coli* bacteria, three proteins that cause red blood cells to lyse. The transformed bacteria were fed to mosquitoes through sugar solutions. The idea was that when the transformed bacteria colonized the mosquito gut, they would produce the toxic proteins. If that host mosquito then fed upon a human infected with malaria, the toxins produced by the transformed bacteria would cause the red blood cells (from the human blood) to burst. This would halt the life cycle of the *Plasmodium* parasite and stop the spread of malaria.

However, the researchers also hypothesized that transformation and expression of foreign genes might affect the ability of the transformed *P. agglomerans* bacteria to grow or colonize the mosquito gut (in other words, their fitness for that environment might be reduced). This could jeopardize the effectiveness of this strategy for fighting malaria in the wild. So they carried out another experiment: they transformed the bacteria with a plasmid that contains a green fluorescent protein (GFP), derived from the jellyfish *Aequorea victoria*, and fed the transformed bacteria to mosquitoes. The researchers then monitored how much fluorescence came from the mosquito gut. They found that after the host mosquitoes were given a blood meal, the GFP fluorescence in their guts increased, indicating the number of transformed bacteria there had rapidly increased. This demonstrated that transformed *P. agglomerans* could grow in the mosquito gut and, more importantly, replicate quickly when the mosquito ingested a blood meal. This meant the bacteria would also likely produce more hly A proteins when the host mosquito ingested potentially infected blood cells. In terms of the efficacy of the transformed bacteria against the *Plasmodium* parasite, when mosquitoes with the transformed bacteria were fed a blood meal containing the *Plasmodium* parasite, the development of the parasite was inhibited by nearly 98% (Wang et al. 2012).

Questions

1. Scientists have also demonstrated that it is possible to genetically modify the *Anopheles* mosquito to produce a substance in their gut that kills off *Plasmodium*. Why might symbiotic bacteria be a more suitable subject for transformation than the *Anopheles* mosquito?

2. As in the human gut, many different bacterial species inhabit the *Anopheles* gut. If you were the researcher, how would you pick the best species for use in this transformation experiment? What factors should you consider?

3. Why was GFP used in the bacterial transformation experiment?

4. *E. coli* was also used in this experiment for plasmid production (to grow more copies of the plasmids), and the plasmid also contained genes for antibiotic resistance. After transformation, the bacteria were grown on plates with antibiotic in them. Why do you think this is a common step in bacterial transformation?

5. The experiment demonstrated that in the lab the transformed bacteria could survive and proliferate within the mosquito gut after transformation and that they could inhibit *Plasmodium* growth and development by nearly 98%. What other experiments might be needed to demonstrate this is a viable option for malaria elimination in the wild? Consider the life cycles and roles of all the key players in the spread of disease.

6. The hly A system used in the experiment described causes lysis of red blood cells. Considering this, would you have concerns about releasing these transformed bacteria into the environment? Under what conditions might these concerns be alleviated?

7. As a final thought, what do you think the greatest hurdles will be to successfully implement this bacteria-based approach in the wild? What are the technical, ethical, or regulatory challenges, and how might they be handled?

IV: Prognosis

Baruti opens his eyes and sees his mother sitting at his side. She takes his hand and tells him she loves him. She has waited for this moment for three days. She is exhausted from the sleepless nights and constant worry.

The doctors come into the room to check on his progress. They are cautiously optimistic that the mixture of antimalarial drugs is working, and they tell Lerato to be patient. They tell her that her boy was very ill and that treatment takes time, but they are encouraged by the progress he is making. They expect him to recover.

Questions

1. Most antimalarial drugs target the red blood cell (erythrocytic) stage of malaria infection, which is the phase of infection that causes symptomatic illness. Why might it be important to research other medications targeting other stages (for example, the liver stage) of the life cycle? Refer to the figure describing the life cycle of *Plasmodium*.

2. When *Plasmodium* becomes resistant to antimalarial drugs, this results in a delayed or incomplete clearance of the parasite from the patient's blood. How might an organism develop resistance to a chemical that can otherwise kill it?

3. The problem of antimalarial drug resistance can be compounded by cross-resistance, in which resistance to one drug confers resistance to other drugs that belong to the same chemical family or have similar modes of action. How and why do you think this might happen?

4. Current practice in treating cases of malaria is based on combination therapy, in which several different classes of drugs are combined. What might some advantages of this approach be?

5. Many people take antibiotics to treat bacterially mediated illnesses like strep throat or sinus infections. When you take antibiotics, you are told you must take the entire course of the medication in order to reduce the risk of developing antibiotic resistance. Why is a full course needed?

"Can Bacterial Transformation Stop the Spread of Malaria?"

Introduction

Malaria is the third leading cause of infectious disease death in the world, and roughly half of the world's population is currently at risk for contracting it. Caused by the parasitic protist *Plasmodium*, malaria can be cured if diagnosed and treated promptly; early infections, however, are often incorrectly self-diagnosed as flu-like symptoms, and access to treatment in the most commonly affected regions of the world is often limited and expensive. Prevention is the best weapon against the disease, but current prevention methods are woefully inadequate — in Sub-Saharan Africa, a child dies of malaria every 30 seconds.

What does this have to do with bacterial transformation and the pGLO Transformation and Inquiry Kit? This case begins with a fictitious story based on actual events that likely take place daily throughout Africa and Southeast Asia. A woman is in a clinic with her young son who, despite the family's mosquito nets, has fallen ill with malaria. She waits for his recovery, anxious that she may not have brought him to the clinic in time. This story serves as an introduction to the challenges many countries face in fighting this disease, challenges brought about by poverty and the long distances people often have to travel to reach the nearest clinic. The case then moves into a discussion of the biological basis for malaria and examines the various methods in use or under investigation for malaria control and eradication. One such method, bacterial transformation, ties in with the techniques and theory that students experience firsthand with the pGLO Transformation and Inquiry Kit.

The case study guides students through a background into malaria, its causes and mechanisms of transmission, and an overview of the benefits and drawbacks of current mediation methods. Along the way, it challenges them to expand on the knowledge they gained while using the kit, asking them to apply concepts to a real-world problem. The study covers the technical challenges of genetic engineering, as well as ethical, ecological, and other considerations.

This case was designed for high school or freshman non–science major students in a biology course. However, given the scope of topics covered, it could also be used in a course on molecular biology, microbiology and therapeutics, medical ethics, or environmental or public health. It can serve as a springboard for discussing the various technical, ethical, ecological, and regulatory issues surrounding genetic modification and the ways in which we can address them. Students must have some prior knowledge of bacterial transformation methods. This case study can be completed in a 1-hour period.

Objectives

Upon completion of this case study, students will be able to:

- Describe the biological cause of malaria and its transmission
- Apply the considerations used in the pGLO Bacterial Transformation to this real-world example (from selection of bacterial species to plasmid design)
- Discuss the practical (social, economic) issues in preventing and treating malaria around the world
- Debate the ethical, ecological, and regulatory issues surrounding species eradication
- Debate the ethical, ecological, and regulatory issues surrounding genetic modification
- Deduce the implications of using live agents capable of replication as a form of mosquito abatement
- Formulate ideas for follow-up experiments that take what is possible in the lab to something that might be effective in the wild

Classroom Management

This case has four parts, which for a class of about 30 students can take 15 minutes each to complete. Students arrive in class having performed the pGLO Bacterial Transformation and had some follow-up discussion on the technical and regulatory challenges of genetic engineering.

In groups of four or five, students read the information provided and answer the questions. Answers from each group should be tracked on a central board or screen, as some groups will have answers that others do not. Instructors should be prepared to lead a guided discussion based on the students' answers.

Part I — The Global Impact of Malaria

This case begins with a story about a mother watching her son fight malarial fever and introduces some of the facts about malaria and the people who tend to suffer most from it. It challenges the students to play the role of a health worker or government agency tasked with bringing malaria under control. What would it take to truly eradicate malaria from a region?

Question 1 focuses on the people and places that suffer most from malaria. Though treatments are available, what are the practical hurdles to offering those treatments to these populations? Students should think about the effects of poverty and the inability, for example, to simply drive to a local doctor's office. Often, affected people live far from the nearest clinics, and travel there means taking valuable time from work or from tending to their land, something few can afford to do. Have students picture some of the most remote regions of the world and what life must be like for the people living there.

Questions 2 and 3 are related. They challenge students to look at the big picture: How does malaria spread through a human population, and what might it take to eradicate it? Malaria has been eliminated in the U.S., yet we still see thousands of new cases each year — this is due to travelers to malaria-affected regions bringing the disease back with them. To effectively eradicate any disease within a region, it is important to stop both (i) active disease in affected individuals (either through treatment or death) and (ii) disease transmission. In the U.S., a massive government-led mosquito elimination program was undertaken over the course of several years, during which insecticide was applied to all buildings and sprayed from planes, and ponds and other mosquito breeding sites were drained and treated.

Part II — Mosquitoes, the Flying Factories of Malaria

Part II of the case introduces students to the biological agents of malaria transmission: the parasitic protist *Plasmodium* and the female mosquito. The questions in this section expand on questions 2 and 3 of Part I.

These questions may be discussed in more detail after students proceed through Part II, though the discussion can begin here.

Question 1 raises the issue of other non–mosquito-mediated methods of malaria transmission. Since *Plasmodium* is a blood pathogen, what are other ways in which it might spread from human to human? Answers include contaminated blood transfusions or organ donations, or even *in utero*, as an infected pregnant woman can pass the infection on to her unborn child before or during delivery.

Question 2 relates back to the story told in Part I, of the child who kicked off the mosquito netting because it was hot to sleep under. Though massive spraying efforts may have been effective in the U.S. back in the late 1940s, there are drawbacks to these approaches in other regions of the world. Indeed, there are distinct benefits and drawbacks to both massive spraying and mosquito nets, which the students are asked to consider here. For example, since the female mosquito tends to bite only at night, perhaps the mosquito nets offer more "bang for the buck."

Many philanthropic efforts from all over the globe aim to provide mosquito netting to people in malaria-infected regions of the world. Nets are relatively cheap to produce, and their efficacy extends over several years. However, as noted in Part I, nets can be very hot to sleep under, people worry about the health effects of sleeping under chemical-doused netting, and some cultures even opt to use the fine mesh as clothing or as fishing nets. Clearly, bed nets are a great interim help, but are not a complete solution to the malaria problem.

Question 3 should draw some interesting and dynamic discussion from the students. Why do we even put up with mosquitoes? What are they good for? Why not get rid of them outright? *This discussion will likely need to be teacher-led.* Students should voice their personal beliefs while considering other factors:

- Does it matter if you as a person know the true "value" of a species?
- Mosquitoes are often not the primary source of food for most fish, birds, or other insect eaters. If they were eradicated, would other insects take their place in the food chain?
- What if a particular species depends on mosquito meals, a species we do not know about yet?
- Would concern for the impact of that species trump the potential elimination of all mosquito-borne disease? Why or why not?

It may be useful to point out that some ecologists have postulated that without mosquitoes, rainforests would have ceased to exist long ago. Were it not for the pesky little biters (and the diseases they carry), man may have invaded and cut down these forests long ago, possibly leading to massive environmental impact. Indeed, in a related argument, the migration routes of caribou are impacted by mosquitoes. Caribou choose routes to avoid mosquito swarms, and these migratory routes affect plant distribution, the range of predatory wolves, etc. Sometimes the effects of things we do are not directly apparent, or even known within our lifetime.

Question 4 expands on question 3, adding the following consideration: how does the answer change if the mosquito in question is actually a non-native species, an invader of a new habitat? Is it okay to kill it off then? Why or why not?

Part III — A Novel Approach Involves Bacterial Transformation

This part of the case ties malaria treatment directly to the pGLO Transformation and Inquiry Kit. It introduces a novel malaria elimination approach that involves symbiotic bacteria within the mosquito gut. The experiment mentioned also used green fluorescent protein (GFP), which is featured in the kit. The questions in this section tie directly to the students' understanding of bacterial transformation and ask them to envision themselves as the researchers designing the experiment.

Question 1 asks why would we transform bacteria instead of the mosquitoes? The answers recall the discussion of why bacteria are so amenable to genetic manipulation: easily and inexpensively grown in the laboratory, they grow and reproduce quickly, and we have established techniques for their transformation and the selection of transformants, etc. Though transformation techniques exist for mosquitoes, their life cycles are more complex. Therefore the process will take longer, cost more, and be more technically challenging.

Question 2 expands on this discussion by asking how students would choose which of the many species of bacteria colonizing the mosquito gut to transform. They should consider practical aspects, such as which are the dominant species in the gut, how easy they are to isolate, grow in culture, transform, and select, how robust the species might be following transformation. They should also think about downstream considerations, such as which of the bacteria are limited in their range: does the species live outside the mosquito gut? Outside the mosquito? Does it colonize the guts of other mammals, such as humans? Could their transformation then have an impact on species other than *Plasmodium* and mosquitoes? For this question, students could be encouraged to learn more about the bacterium used in the experiment: *Pantoea agglomerans*.

Question 3 also brings the students back to the bacterial transformation experiments they performed, in which expression of GFP was the objective and they examined conditions under which expression was induced by arabinose. In the case study, GFP was used for another purpose: to track the amount of transformed bacteria present in the mosquito gut.

Question 4 asks the students to extend the experiment beyond the lab. What would be needed to make this symbiotic GMO approach effective in the wild? Students need to consider all the steps, including:

- How the bacteria can be introduced into and survive in mosquitoes. Would they be passed on to offspring, or would baiting be a long-term process?
- Mosquitoes harboring the transformed bacteria must also exhibit some fitness for survival in the wild
- The transformants must be effective at eliminating virtually all the *Plasmodium* in the gut
- There must be minimal ill effects on any of the organisms involved (and on any others) for this procedure to be a viable alternative. What do we know about *P. agglomerans* in the wild?

Question 5 reflects on the use of the Hly A system, which relies on red blood cells (erthrocytes) for efficacy. Hly A proteins will cause lysis regardless of the source of red blood cells, so any significant transfer of transformed bacteria to any blood-containing animal may have harmful effects. The risk can be lowered, though, through use of bacterial species not known to colonize other animal species.

Question 6 asks the students to recap all their discussions and present a summary of the challenges of this novel approach.

Question 7 this question could go in many different directions. Challenge your students to think about the big picture.

Part IV — Prognosis

This final section returns students to the child's bedside as doctors discuss how to continue treatment. This section reaches back to the ampicillin and satellite colony labs, to the topic of resistance and how organisms develop resistance to drugs that otherwise kill them or retard their growth.

It is important for students to distinguish the *Plasmodium* resistance to antimalarials discussed here from the ampicillin resistance discussed in relation to *E. coli*. Though similar mechanisms of resistance may be at play in both organisms, *Plasmodium* is a eukaryote, while *E. coli* is a bacterium (prokaryote).

Question 1 raises the point that most antimalarial drugs target the stage of the *Plasmodium* life cycle that produces the most common symptoms of malaria (the red blood cell stage). Why might it be important to research other medications targeting other stages (for example, the liver stage) of the life cycle? *Plasmodium* can stay dormant in parts of the body other than red blood cells, such as the liver. This means it can survive in a body treated with those drugs, only to reemerge or continue to spread at another time. Therefore, antimalarials that could target more than one stage of the parasitic protist's development might ultimately be much more effective.

Question 2 gets at the question of how drug resistance occurs within organisms. In many organisms, insufficient selective pressure (for example, if the dosage is not quite strong enough) can be a cause of drug resistance. In bacteria, antibiotic resistance may be an inherent trait that renders it naturally resistant, or it may be acquired (for example, by mutations in its own DNA or by acquisition of resistance-conferring DNA from another source). Resistance can be brought about by an organism in several ways, including:

- Secreting enzymes or other chemicals to inactivate the drug
- Not taking up the drug anymore
- Altering/metabolizing the drug, rendering it inactive
- Altering the drug target so it no longer reacts with or responds to the drug
- Overwhelming the drug by producing more of the drug's target

Question 3 addresses cross-resistance: resistance to one drug confers resistance to other drugs that belong to the same chemical family or have similar modes of action. If resistance to one class of drugs evolves, chances are the biochemical basis of the resistance will be effective against related drugs.

Question 4 extends the discussion of drugs and cross-resistance. Combination therapy is a strategy whereby two or more drugs with different modes of action are used in combination. For example, artemisinin-based combination therapy (ACT), wherein fast acting artemisinin-based compounds are combined with drugs from different classes, is a very commonly used approach for malaria treatment around the world. Combination therapy is recommended to produce an adequate cure rate while also delaying development of resistance.

Question 5 relates this discussion back to something almost all students will have experienced: having to take a full course of antibiotics. Why is this necessary? Challenge the students to think in terms of a natural population of bacteria, their reproduction rates, growth rates, etc. Chances are the antibiotic kills off the "low-hanging fruit" (the easy-to-kill bacteria) in the first few days of treatment. The full treatment is needed to kill off the entire population, especially those that may start developing resistance.

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Suggested Reading, Listening

Kill 'Em All (NPR Radiolab podcast) http://www.radiolab.org/story/kill-em-all/

What if We Don't Kill 'Em All (NPR Radiolab podcast) http://www.radiolab.org/story/what-if-we-dont-kill-em-all/

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