Title: APPARATUS AND METHODS FOR THE PRODUCTION OF HAEMATOPHAGOUS ORGANISMS AND PARASITES SUITABLE FOR VACCINE PRODUCTION

Abstract: Disclosed are apparatuses and methods for the production of parasites in haematophagous insects generally, and production of Plasmodium species sporozoites in Anopheles species mosquitoes, specifically; apparatuses and methods for the production of strains of haematophagous insects with desired properties such as hypoallergenicity or hyperinfectivity; methods of producing a parasite strain that is capable of withstanding cryopreservation at temperatures close to freezing; apparatuses and methods for the injection of an attenuated parasite vaccine; production of parasites and haematophagous insects that are free from contamination by unwanted biological agents; apparatuses for the reconstruction of complex parasitic life cycles aseptically to avoid the contamination of the parasite or the insect vector host with unwanted biological agents.
APPARATUSES AND METHODS FOR THE PRODUCTION OF HAEMATOPHAGOUS ORGANISMS AND PARASITES SUITABLE FOR VACCINE PRODUCTION

CLAIM OF PRIORITY

[1] This application claims the benefit under 35 U.S.C. 119(e) of U.S. Provisional Application No. 60/370,581 filed April 5, 2002.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[2] The present invention relates to apparatuses and methods for the production of parasites in haematophagous insects generally, and to the production of Plasmodium species sporozoites in Anopheles species mosquitoes, specifically. The present invention further relates to apparatuses and methods for the production of strains of insects (e.g., Anopheles mosquitoes) that have desired properties such as hypoallergenicity or hyperinfectivity. The present invention also relates to methods of producing a strain of a parasite that is capable of withstanding cyropreservation at temperatures close to freezing. The present invention further relates to apparatuses and methods for the
injection of an attenuated parasite vaccine. The present invention allows for the production of parasites and haematophagous insects that are free from contamination by unwanted biological agents such as bacteria and other microorganisms. The apparatuses of the present invention provide for the reconstruction of complex parasitic life cycles aseptically, so as to avoid the contamination of either the parasite or the insect vector host with unwanted biological agents. The present invention also provides for methods for the production of an attenuated Plasmodium sporozoite vaccine that is stable at relatively shallow cryogenic temperatures. The present invention further provides for apparatuses for the delivery of micro-bolus amounts of vaccine.

DESCRIPTION OF THE BACKGROUND

[3] Malaria is the most devastating parasitic disease and, as such, represents one of the most important public health problems worldwide. According to experts in the field, malaria infects 300 million people and kills up to 3 million people per year. A vaccine for malaria would drastically reduce the impact of this dangerous disease.

[4] The causative agents in malaria are various species of the eukaryotic genus Plasmodium, including Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and
*Plasmodium malariae*. These parasites have a very complex life cycle that involves both vertebrate and invertebrate hosts. The vertebrate infective form of the parasite (sporozoites) is present in the salivary glands of mosquitoes (typically of the genus *Anopheles*) and the sporozoites are transferred to humans during feeding by the mosquitoes. In the human host, the sporozoites initially infect the cells of the liver and eventually red blood cells. This infection results in an illness which is potentially fatal to those infected.

Current prophylactic approaches to malaria include the use of drugs, including chloroquine, mefloquine, and atovaquone/proguanil. However, multiple drug-resistant strains of *Plasmodium* have recently been observed. In addition, the occurrence of drug-resistant strains of malaria is thought to be promoted by the use of these prophylactic antimalarial drugs. Accordingly, significant efforts have been undertaken to develop a vaccine for malaria.

There have been some indications in the scientific literature that a vaccine for malaria could be effective. In regards to a metabolically active non-replicating (attenuated) whole sporozoite vaccine, Nussenzweig and coworkers (*Nature* 216: 160-162; 1967) reported that immunizing mice with radiation

The following 3 points summarize the most important findings: 1) Thirteen of 14 volunteers immunized by the bites of greater than 1000 infected, irradiated mosquitoes were protected against developing blood stage *P. falciparum* infection when challenged within 10 weeks of their last primary immunization; 2) Five of 6 of the 14 volunteers in (1) above when challenged from 23 to 42 weeks (23, 36, 39, 41, and 42 weeks).
after their last primary or secondary immunization were protected against experimental challenge; and.

3) Seven of seven heterologous challenges (immunized with one strain of *P. falciparum* and challenged with another strain of *P. falciparum*) in four individuals were associated with complete protection.

From this, it was demonstrated that protection was achieved in greater than 90% of immunized subjects, lasted for at least 10 months, and demonstrated cross strain (heterologous) protection. For the first time, the true efficacy of this experimental vaccine approach was demonstrated. While this study demonstrated the feasibility of an attenuated malaria vaccine, it was considered for many reasons to be impractical to immunize large numbers of susceptible individuals by employing the bites of irradiated infected mosquitoes.

One technical hurdle to the development of a clinically relevant vaccine is the production of aseptic sporozoites that are free of contamination by unwanted biological agents. Currently, it is not possible to produce *Plasmodium falciparum* sporozoites using an in vitro process. Therefore, *Plasmodium falciparum* sporozoites must be obtained from the tissues of infected female *Anopheles* mosquitoes. However, it is well known that wild and insectary
reared mosquitoes are highly contaminated with unwanted biological agents including bacteria, molds, and fungi. This contamination largely prevents the use of mosquito derived parasites in a clinically relevant vaccine suitable for regulatory licensure. An apparatus and method to produce aseptic Anopheles mosquitoes for the in vivo production of Plasmodium falciparum sporozoites is a critical step in the development of an acceptable attenuated sporozoites vaccine from both a clinical and regulatory perspective.

[10] Contamination of mosquitoes with unwanted biological agents may arise from several sources in the mosquito’s life cycle. The surface of mosquito eggs may become contaminated during oviposition from the female mosquito’s genital tract and ovipositors. The larvae may retain microbes in their gastrointestinal tract and peritrophic membrane during metamorphosis of larvae to pupae and adult mosquitoes. In addition, multiple environmental factors, including the aquatic habitat of the larvae, the external environment of the adult mosquito, and contaminated skin of an animal upon which the mosquito fed, may contribute to contamination of mosquitoes and thus the Plasmodium parasite.
For decades, non-aseptic sporozoites have been routinely obtained from infected Anopheles mosquitoes for research purposes using labor intensive techniques. There are multiple drawbacks to this standard approach. Since the entire process is conducted under non-sterile conditions, the sporozoite preparation is usually contaminated with microbes. Though sporozoites can be partially purified by a variety of techniques, contamination of the resulting product makes it unsuitable for use in developing a vaccine for human use. Microbiologically contaminated vaccines can cause an iatrogenic infection of a serious nature in both humans and animals. In addition, the processes of the prior art for rearing non-aseptic mosquitoes are labor intensive and require multiple direct manipulations of the mosquitoes during their life cycle.

Accordingly, one of the limitations in the production of a vaccine for malaria is the ability to obtain a large number of aseptic sporozoites of *Plasmodium* species. As stated above, sporozoites that are obtained from *Anopheles* species of mosquitoes using standard techniques results in sporozoites that are not useful in the development of an attenuated sporozoite vaccine. Aseptic sporozoites could be used as a vaccine to generate protective immunological responses safely and efficiently. In addition, the
production of such aseptic sporozoites will be a regulatory requirement for the commercial production of a malaria vaccine.

[13] Thus, there has been a long standing need in the medical field for the production of aseptic *Plasmodium* species, sporozoites and aseptic *Anopheles* mosquitoes for use in the development of a vaccine for malaria. Apparatuses and methods for the aseptic rearing of *Anopheles* mosquitoes and *Plasmodium* parasites can also be used for the aseptic production of other haematophagous insect species and parasites for other critically needed vaccines against parasitic diseases of humans and animals.

[14] In order to develop strains of insects that possess certain desired properties (*e.g.*, hyperinfectivity or hypoallergenicity), it would be useful to employ a device that would allow experiments selectively to evaluate the biting behavior and properties of individual insects. The apparatus would also be useful in the selection of insects that possessed desired properties.

[15] An additional hurdle for the efficient and economical development of an attenuated vaccine for malaria is the deleterious effect that the *Plasmodium* parasite has on the mosquito host. *Anopheles* mosquitoes are capable of transmitting *Plasmodium* sporozoites to a
host animal on which they feed. Research indicates that *Plasmodium* infections of *Anopheles* female mosquitoes are deleterious to the survival of mosquitoes in both the laboratory and wild-type environment. Thus, the ability to extract large numbers of mosquito phase parasites from female mosquitoes is currently limited by the inability of the mosquitoes to tolerate a heavy *Plasmodium* parasite burden.

[16] A unique strain of *Anopheles* mosquito that is tolerant to massive infection with the *Plasmodium* parasite would make the production and extraction of sporozoites from mosquitoes more efficient. The development of an attenuated *Plasmodium* sporozoite vaccine derived from this unique strain of *Anopheles* mosquitoes would thereby be more efficient and economical.

[17] An additional possible difficulty in producing live, attenuated, or killed pathogen vaccines extracted from mosquito tissue is the potential of mosquito antigens to cause hypersensitivity, Arthus, or delayed type hypersensitivity reactions in the inoculated human or animal. Many hypersensitivity salivary antigens in several mosquito species have been identified. These salivary antigens probably confer a survival advantage to wild-type mosquitoes for numerous reasons.
However, in laboratory maintained mosquito populations, such antigens are vestiges of their wild ancestors and are probably no longer necessary for selective survival advantage. By developing a technique to create hypoallergenic mosquitoes, it would be possible to create several hypoallergenic mosquito species (Anopheles, Aedes, Culex, etc.) which are specific to a wide variety of mosquito-borne infections. These hypoallergenic strains of mosquitoes would have significant utility in the production of safe and effective attenuated parasite vaccines.

Once an attenuated vaccine has been developed, the vaccine should be delivered to an individual in need effectively. However, the standard manner in which vaccines are delivered – bolus injection via a syringe – is not effective in the case of an attenuated malarial vaccine. Vaccines syringes are commonly available in a variety of sizes with an industry standard locking port to which needles of various gauges can be attached. Usually, this system is adequate for typical vaccines, as the required volume is in the range of 500 to 1000 microliters. Small volume variations from immunization to immunization have no effect on the immunogenicity of the typical vaccine.
However, in some vaccines, such as an attenuated Plasmodium sporozoite vaccine, the inoculum may be to be on the order of a microliter or less – similar to the injectate of a probing mosquito. Larger volumes may cause the carrying liquid of the vaccine to disturb tissue integrity and cause the liquid to follow tissue planes. In mice, virtually all sporozoite challenge studies and attenuated sporozoite immunization studies are accomplished by intravenous injection of sporozoites, because non-intravenous administration of sporozoites in skin, subcutaneous tissue, and muscle has been associated with much lower infection rates and protection rates. When injected into the skin, sporozoites likely expire within the spaces between tissue planes created by the fluid in which the sporozoites are suspended, providing no opportunity to move throughout the host’s cellular structures and into a capillary.

The standard procedure for current vaccines is therefore unsuitable for an ultra-low volume vaccine. To overcome this limitation, one could design a single use microneedle and syringe assembly. While this approach would likely be effective, it would also be impractical as millions of vaccines are to be given in extremely poor countries. Preferably, a malarial vaccine delivery system would be able to both deliver
ultra-low volume boluses and also employ standard syringe assemblies as a cost saving measure.

Hurdles also exist for the delivery of frozen attenuated *Plasmodium* vaccine to individuals worldwide. *Plasmodium* sporozoites have the ability to survive freezing temperatures in various preservation solutions. The best results, measured after re-warming and estimating the percentage of motile sporozoites and the ability to cause a patent blood-stage infection after injection into a study animal, demonstrate that extremely low temperatures (-196 to -70 degrees Celsius) can preserve the sporozoites for many years. However, as preservation temperatures approach zero degrees Celsius, the percentage of viable sporozoites as a function of time drops precipitously in a temperature dependent fashion. This drop in viability limits the utility of malaria vaccines derived from attenuated sporozoites that must retain a high degree of potency during storage and shipment.

The worldwide cold storage and shipment infrastructure is robust and almost all countries have the capability to store relatively large volumes of materials at temperatures approaching zero degrees Celsius. At close to zero degrees Celsius, existing equipment and infrastructure can be adopted to transport an
attenuated sporozoite vaccine to even the most remote locations on Earth. A storage and shipment infrastructure that requires temperatures in the range of minus seventy degrees Celsius, though technically feasible, is likely to be routinely possible in only the more technologically advanced nations. However, such extreme cold temperatures require special equipment, care, and will probably entail a large capital expenditure to accommodate the logistics of delivering an attenuated sporozoite vaccine.

[23] The cryopreservation studies of sporozoites have used Plasmodium strains and clones developed for purposes other than developing a freeze tolerant strain of Plasmodium sporozoites. All organisms, to some degree, have the capability to survive temperature variations by utilizing stabilizing proteins, heat shock proteins, sugars, and carbohydrates which prevent the denaturing of critical enzymes, proteins, or limit the damage to cellular substrates caused by ice crystal formation. The malaria parasite, whose life cycle includes passage through the Anopheles mosquito, must have the ability to withstand natural variations in temperature - sometimes quite side as many temperate/tropical climates can vary by thirty to fifty degrees Fahrenheit in a day-night cycle. It can be assumed that genetic variation from Plasmodium organism to organism in nature has led to a wide
variation in the ability of sporozoite to withstand temperature extremes.

[24] The key to developing a *Plasmodium* species better able to survive high temperature cryopreservation conditions is to breed selectively those sporozoites shown to have that capacity. By selecting those sporozoites that survive increasingly high cryopreservation temperatures for longer periods of time while still able to complete a natural life cycle, one can develop a *Plasmodium* strain that has much greater utility in an attenuated whole-parasite vaccine for worldwide use.

**SUMMARY OF THE INVENTION**

[25] In accordance with the present invention, there are provided systems and methods for the aseptic production of haematophagous insect vectors and parasites, generally, and production of *Anopheles* species mosquitoes and *Plasmodium* species sporozoites, specifically.

[26] The present invention preferably allows for the aseptic production of parasites through the use of a sterile environment that is capable of supporting all stages of the parasite-insect life cycle. Insects are preferably grown from surface-sterilized eggs and the growing insects are provided with filtered air,
sterile water, and sterile developmental-stage appropriate nutrition during metamorphosis from egg to adult. After reaching maturity, the insects are provided with a sterile, infectious blood meal that contains the infectious form of the parasite that is to be produced.

[27] The present invention also provides for stunning the infectious insects, allowing for efficient collection of the immobile infected insects in a safe fashion, which may then be processed for collection of parasites.

[28] The present invention may be used to produce a wide variety of aseptic parasites of vertebrate animals that have an obligate propagative, cyclo-development or cyclo-propogative life cycle phase in a haematophagous insect. Examples of suitable parasites and haematophagous insect and parasite pairs include, but are not limited to Plasmodium falciparum-Anopheles stephensi, Trypanosoma cruzi-Triatoma infestans, and Aedes quadrimaculatus-Wuchereria bancrofti.

[29] It is thus an object of the present invention to produce aseptic parasites of vertebrate animals sterilely, safely, and economically for research purposes. The aseptic parasites produced by the apparatuses and methods of the present invention are
preferably suitable for development of human and animal vaccines.

The present invention relates to apparatuses and methods for the production of parasites in haematophagous insects generally, and to the production of *Plasmodium* species sporozoites in *Anopheles* species mosquitoes, specifically. The present invention further relates to apparatuses and methods for the production of strains of insects (e.g., *Anopheles* mosquitoes) that have desired properties such as hypoallergenicity or hyperinfectivity. The present invention also relates to methods of producing a strain of a parasite that is capable of withstanding cryopreservation at temperatures close to freezing. The present invention further relates to apparatuses and methods for the injection of an attenuated parasite vaccine. The present invention allows for the production of parasites and haematophagous insects that are free from contamination by unwanted biological agents such as bacteria and other microorganisms. The apparatuses of the present invention provide for the reconstruction of complex parasitic life cycles aseptically, so as to avoid the contamination of either the parasite or the insect vector host with unwanted biological agents. The present invention also provides for methods for the production of an
attenuated *Plasmodium* sporozoite vaccine that is stable at relatively shallow cryogenic temperatures. The present invention further provides for apparatuses for the delivery of micro-bolus amounts of vaccine.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[30] For the present invention to be clearly understood and readily practiced, the present invention will be described in conjunction with the following figures, wherein like reference characters designate the same or similar elements, which figures are incorporated into and constitute a part of the specification, wherein:

[31] **Figure 1** is an external view of a presently-preferred embodiment of a parasite production chamber;

[32] **Figure 2** is an overhead view of the interior of a presently-preferred embodiment of a parasite production chamber showing the inlet and outlet tubes;

[33] **Figure 3** is an overhead view of the interior of a presently-preferred embodiment of a parasite production chamber showing the ports and points of mechanical access;

[34] **Figure 4** is an overhead view of the interior of the insect rearing portion of a presently-preferred embodiment of a parasite production chamber;
Figure 5 is an overhead view of the interior of the blood meal portion of a presently-preferred embodiment of a parasite production chamber;

Figure 6A is a view of a presently-preferred blood feeding station of the present invention;

Figure 6B is a view of a presently-preferred blood feeding station of the present invention;

Figure 7A is a view of a presently-preferred wick-based sugar feeder of the present invention;

Figure 7B is a view of a presently-preferred trough-based sugar feeder of the present invention;

Figure 8A is a view of a presently-preferred haematophagous insect bite chamber array of the present invention;

Figure 8B is a bottom view of a presently-preferred haematophagous insect bite chamber array of the present invention;

Figure 9A is view of a presently-preferred micro-bolus vaccine assembly of the present invention; and

Figure 9B is a view of a presently-preferred micro-bolus vaccine assembly of the present invention.
It is to be understood that the figures and descriptions of the present invention have been simplified to illustrate elements that are relevant for a clear understanding of the invention. The detailed description will be provided hereinbelow with reference to the attached drawings.

Generally, the present invention provides apparatuses and methods for the production of parasites in haematophagous insects. The present invention preferably supports this production at multiple steps - from selection of species of insects and parasites with certain desired properties, to growth of aseptic parasites for use in the development of an attenuated vaccine, to the delivery systems for the injection of the attenuated vaccine. The various aspects and embodiments of the present invention may be utilized together or as individual components in the production and delivery of an attenuated vaccine. While the various aspects and embodiments of the present invention may be described with specific reference to the production of Plasmodium species sporozoites, it will be appreciated by those of skill in the art that the teachings herein are applicable to other insect stage infectious parasites. In addition, the descriptions found herein may make specific reference to Anopheles mosquitoes, but one of skill in the art
will recognize that the teachings found herein are applicable to other haematophagous insects.

One aspect of the present invention provides apparatuses and methods for the production of insect stage parasites of vertebrate animals in haematophagous insects produced under aseptic conditions in vivo. These aseptic parasites and haematophagous insects are contemplated to be useful in the production of protective vaccines and in experimental research. While the aseptic production of numerous parasite-haematophagous insect pairs is contemplated as being within the scope of the present invention, the Plasmodium species parasite and the Anopheles mosquito will be used as an illustrative example.

The present invention preferably provides for a parasite production chamber that is designed to prevent microbial contamination of the internal environment where the haematophagous insects and parasites are being grown. This allows the parasite production chamber to be located in a non-sterile external environment during insect and parasite maturation such as a heated room with diurnal light. Operators are not required to wear protective clothing, masks, gloves, or shoe covers, which significantly improves comfort, efficiency, and
operating expense. Additionally, the parasite production chambers of the present invention preferably physically separate infectious Anopheles mosquitoes from the operator preventing an accidental bite that could cause a potentially dangerous malaria infection.

The preferred embodiments of the present invention provide for the aseptic production of haematophagous insects and parasites through establishing a sterile environment in which the insects and parasites are grown. The sterility of this environment is preferably maintained for the duration of the insect-parasite pair life cycle. The chambers and internal components of the parasite production chambers of the present invention are preferably sterile at the beginning of the growth procedure. Surface sterilized insect eggs are preferably employed when initially growing the insect colony. In addition, the blood on which the adult haematophagous insects feed, and which preferably provides the parasite to the insects, is also free from microbial contamination. In addition, the water, larva growth broth, feeding solutions, and all other solutions and materials used in the parasite production chambers of the present invention are also preferably sterile. Any standard means for sterilization may be employed including, but not limited to, autoclaving, chemical sterilization,
irradiation, and micro-filtration. Additional sterilization techniques will be well known to one of skill in the art.

[49] In a presently-preferred embodiment of the present invention, the parasite production chamber is constructed from high temperature-resistant materials such as metal, glass, or plastic compounds. The general shape may be rectangular or a cube of variable dimensions. The interior of a presently-preferred parasite production chambers of the present invention houses various components that support the parasite-insect host stage life cycle requirements and can be physically divided into sections as is dictated by the parasite-insect host couple to be produced.

[50] The various inlet and outlet tubes, mechanical ports, and reservoirs of parasite production chamber of the present invention will be described, followed by a description of the methods and operation of the apparatus of the present invention.

[51] A front view of a presently-preferred embodiment of the present invention 100 is displayed in FIG. 1. Three side walls 104 are made with a provision for glass or clear plastic view ports 108 with airtight seals. The final side 112 is preferably outfitted with a hatch allowing access to the interior of the chamber for cleaning and parasite production
preparation. The inner rim of the hatch preferably has a continuous rubber gasket to provide for an airtight seal to the sidewall of the apparatus. Compression latches 116 arranged on the periphery of the hatch allow for the rubber gasket to be squeezed tightly against the sidewall. Non-volatile, non-toxic lubricants such as glycerol can be coated on the rubber gasket to improve the airtight seal of the hatch when the previously mentioned hatches are engaged. The top of the parasite production chamber 120 is preferably a clear material such as glass or plexiglas that can be reinforced on its inner side by a metal lattice. The clear top allows for viewing of the inner chamber and allows light to penetrate for diurnal variation of light, which may be necessary for the development of many insects.

[52] A plurality of metal tubes 124 128 extend from the interior to the exterior of the parasite production chamber. Where the tube passes through the wall of the parasite production chamber, a metal or epoxy weld secures the tube and creates an airtight seal. The tubes allow for the sterile transfer of gasses and liquids into and out of the apparatus. The liquids may be introduced either into reservoirs or into feed apparatuses as described hereinbelow. Each tube has a different purpose depending upon the parasite species and insect species being produced. In addition, each
tube preferably has a micro-pore filter 132 attached to its exterior end to prevent contamination of the interior of the parasite production chamber. The parasite production chamber of the present invention also preferably includes operable access ports 136 that allow physical access to the interior of the parasite production chamber.

[53] **FIG. 2** is a cross-sectional view of the interior of a presently-preferred embodiment of the present invention specifically showing the tubes that extend to the interior of the parasite production chamber. The interior of the parasite production chamber may be divided into two portions by a wall 205 that preferably contains a closable door 206 between the two portions. The two portions of the parasite production chamber may be conceived of as an insect rearing portion 200 and an blood meal portion 202. An air inlet tube 204 allows humidified air to be forced into the parasite production chamber thus providing oxygen to the system as well as creating a positive pressure gradient from the internal to external environment that inhibits microbes from contaminating the system. The air inlet tube 204 may also deliver anoxic gas to the system to sacrifice the parasite-infected insects for easy collection as will be discussed hereinbelow. An air outlet tube 208 allows forced air to vent from the interior of the parasite
production chamber. Both the air inlet tube 204 and the air outlet tube 208 preferably have a micro-pore filter 205 209 attached to their exterior end to prevent air borne microbial contamination to pass from the external environment to the interior of the parasite production chamber.

[54] The presently-preferred embodiment of the present invention displayed in FIG. 2 further discloses a tube 216 useful for introduction of larva rearing broth into the insect rearing portion 200 of the parasite production chamber. The broth introduction tube 216 also may contain a micro-pore filter 217 for the filtering of the broth. The larva rearing broth that is used within the context of the present invention may be synthetic or semi-synthetic broth, as is dictated by the particular insect that is being grown. The broth introduction tube 216 allows larva rearing broth to be introduced into the larva rearing reservoir as described hereinbelow.

[55] FIG. 2 further displays a water introduction tube 220 for the introduction of water into a egg retaining reservoir as described hereinbelow. The water introduction tube 220 preferably contains a micro-pore filter 221 which allows water to be steriley filtered prior to introduction into the egg retaining reservoir.
FIG. 2 also discloses a blood warming tube 224. Both ends of the blood warming tube 224 preferably extend from the exterior of the parasite production chamber and both ends are open to the external environment. The blood warming tube 224 serves as a warm water heating coil to attract adult female mosquitoes into the blood meal portion 202 of the parasite production chamber and to warm the infectious blood meal as discussed hereinbelow. FIG. 2 further discloses a blood introduction tube 228 which acts as a conduit for infectious blood to travel into the blood meal portion 202 of the apparatus for consumption by the insects. The exterior end of the blood introduction tube 228 is preferably closed by an air-tight latex or rubber plug 232. The latex or rubber plug 232 on the exterior end of the blood introduction tube 228 allows an infectious blood meal to be aseptically injected through the plug 232 by a needle attached to a syringe.

The outer walls of a presently-preferred parasite production chamber of the present invention include a series of ports and physical access points as shown FIG. 3. Each port is preferably closed by a tightly fitting hatch, gasket, and latch to provide an airtight seal to the interior of the parasite production chamber. In other preferred embodiments, ports could be designed with a tightly fitting rubber or latex
plug as previously described. To improve the air tight seal, a non-volatile and non-toxic lubricant such as glycerol can be coated on the gasket. Each port is designed and located to serve a specific purpose in parasite production and hence its diameter can vary according to the demands of the insect being grown. An egg transfer port 304 preferably opens into the insect rearing portion 302 of the parasite production chamber. The egg transfer port 304 preferably allows surface sterilized eggs to be aseptically transferred by sterile pipet onto a semi-submersible float in the larva rearing reservoir as described hereinbelow. An insect removal port 308 preferably opens to the blood meal portion 300 of the parasite production chamber. The insect removal port 308 preferably allows infected insects to be collected after they have been sacrificed at the end of the production run.

[58] Internally, the parasite production chamber of the present invention is preferably sub-divided into an insect rearing portion 302 and an blood meal portion 300 by a partition 312, which may be a solid wall or a mesh screen. The partition 312 preferably has a door 316 between the insect rearing portion 302 and the blood meal portion 300 that can be opened or closed via a mechanical linkage rod 320. This device may also be a screw turn or other mechanical device used
to open and close the door 316. The mechanical linkage rod 320 is preferably operated by manipulating a lever 324 on the exterior surface of the parasite production chamber. The mechanical linkage rod 320 is preferably enclosed in a tight fitting rubber or metal grommet 328 lubricated with a non-volatile and non-toxic lubricant such as glycerol where the mechanical linkage rod 320 passes through the exterior wall into the interior of the apparatus. This grommet 328 helps to maintain sterility inside the parasite production chamber.

[59] As displayed in FIG. 4, the insect rearing portion 402 of the parasite production chamber contains multiple specialized components that are capable of supporting the aseptic aquatic life stages of the insect's eggs, larvae, pupae and adult. A larva rearing reservoir 406 rests on the bottom of the apparatus and is designed to hold the sterile larvae rearing broth delivered by the broth introduction tube 410 as described hereinabove. A semi-submersible float 414 preferably rests on the bottom of the larva rearing reservoir 406. When larva rearing broth partially fills the larva rearing reservoir 406, the semi-submersible float 414 rests just below the fluid surface. Surface sterilized eggs of Anopheles mosquitoes no longer float and will perish if allowed to sink too deeply into the larvae rearing broth. The
semi-submersible float 414 preferably supports the surface sterilized eggs just below the surface of the fluid insuring viability. As the float 414 is semi-submersible, the L1 larvae are not prevented from swimming to other locations within the larva rearing reservoir 406. The insect rearing portion 402 of the apparatus also contains a sugar feeding reservoir 418. The sugar feeding reservoir 418 is preferably a small sugar trough with a landing pad of mesh screen that provides the adult insects with a nutritive substance after hatching from pupae. Alternatively, the sugar feeding reservoir may be a wick-based sugar feeding system as described hereinbelow. Since sugar is highly hydrophilic and is partially dissolved in conditions of high humidity and warm temperatures, the landing pad allows the adult insects to land and feed on the sugar through the mesh without becoming stuck.

[60] The blood meal portion 502 of the parasite production chamber is displayed in FIG. 5. Within the blood meal portion 502, specialized components support the production of aseptic parasites and insect hosts, including Plasmodium species sporozoites within the adult female mosquito. A blood feeding station 506 is comprised of a blood reservoir 510 covered by thin membrane or fine mesh screen as described in greater detail below. The blood reservoir 510 can be a Rutledge type feeder or any other type of blood meal
device. The blood reservoir 510 is connected to the blood introduction tube 514 that extends to the exterior of the parasite production chamber. As previously described, the blood heating tube 522 is a heating coil that circulates warm water into and out of the apparatus. The coil of the blood heating tube 522 preferably encircles the blood reservoir 510 or circulates warm water within a Rutledge type feeder to heat the blood. When the present invention is utilized to produce Plasmodium sporozoites and Anopheles mosquitoes, the operation of the blood reservoir 510 can function to segregate female from male mosquitoes and also provides an infectious blood meal to the female mosquitoes for the cyclo-propagative development of aseptic Plasmodium species parasites.

[61] FIG. 5 further discloses a sugar feeding reservoir 526 that is similar to the sugar feeding reservoir found in the insect rearing portion of the parasite production chamber. While this aspect of the present invention is described using sugar as an example, any nutrition source may be used. The sugar feeding reservoir 526 is preferably a small sugar trough with a landing pad of mesh screen that provides the adult insects with a nutritive substance. The sugar feeding reservoir 526 may be replaced by a wick-based feeding system as described hereinbelow.
Following a blood meal, female Anopheles mosquitoes will develop their eggs in approximately 48 hours. To allow ovipositing of these eggs, an egg oviposition reservoir 530 rests on the bottom of the blood meal portion of the parasite production chamber. The egg oviposition reservoir 530 is designed to be partially filled with sterile water as delivered by the water introduction tube 534.

**FIG. 6** displays two preferred embodiments of a blood feeding station. **FIG. 6A** displays the blood warming tube 602 that allows warm water to be transferred from the exterior to the interior of the parasite production chamber for warming of the infectious blood meal. The warm water is then transferred back out of the parasite production chamber by another tube 601. The blood warming tube 602 expands into a chamber 603 that surrounds a blood feeding chamber 606. The blood introduction tube 610 extends to the exterior of the apparatus. The exterior end of the blood introduction tube 610 contains a latex or rubber plug 614. During operation of the blood feeding station, infectious blood is injected through the plug 614 into the blood introduction tube 610. The blood travels down the blood introduction tube 610 into the blood feeding chamber 606 where it spreads over the membrane 622 that lines the bottom of the blood feeding chamber 606. The membrane 622 is such that it is able to be
pierced by the proboscis of the adult female mosquito, thus providing an infectious blood meal to the mosquito. During heating of the blood, the volume of gas within the blood feeding chamber 606 expands. During heat sterilization of the blood feeding chamber 606 the volume of gas within the blood feeding chamber 606 expands. In order to not damage either the membrane 622 or the plug 614, a vent 618 is included on the superior aspect of the blood introduction tube 610 to allow for relief of any built up pressure.

Another embodiment of a blood feeding station is disclosed in FIG. 6B. Blood warming tubes 626 extend from the exterior to the interior of the parasite production chamber. In this embodiment, the blood warming tubes 626 contact a blood reservoir 634 such that when warm water is pumped through the blood warming tubes 626, the blood within the blood reservoir 634 is heated. There are multiple ways of achieving this heating, including the blood heating tubes 626 encircling the blood reservoir 634, the blood heating tubes 626 establishing contact underneath the blood reservoir 634, and the blood heating tubes 626 forming a heating reservoir 630 juxtaposed to the blood reservoir 634. One of skill in the art would recognize many such ways of heating the blood meal. The blood has a membrane or mesh 638 over top of it to provide adult insects (e.g., adult
female *Anopheles* mosquitoes) support when they take an infectious blood meal. Infectious blood may be injected into the blood introduction tube 642 through a rubber or latex plug 646 located in the exterior end of the blood introduction tube 642. The blood introduction tube 642 may also contain a vent 650 that may be used to relieve pressure that may build up during autoclaving sterilization.

**FIG. 7A** discloses a presently-preferred wick-based sugar feeder 700 to be used within the apparatuses of the present invention. The sugar feeder preferably rests on the top of the apparatus of the present invention 704. The lower portion of the sugar feeder 700 preferably extends into the interior of the apparatus of the present invention with an air tight seal. The upper portion of the sugar feeder 700 preferably contains a rubber or latex plug 708 through which sugar solution 716 be injected into a reservoir 712 in the sugar feeder 700. The sugar solution 716 drains to the bottom of the reservoir 712 into a wick 720 that extends to the interior of apparatus. Insects may then land on the wick 720 and to consume the sugar solution 716 that saturates the wick 720.

**FIG. 7B** discloses a presently-preferred trough-based sugar feeder 724 to be used within the apparatuses of the present invention. Sugar 728 is preferably placed
in the base of the trough 732 with a mesh membrane 736 over top which acts as a landing pad. Since the sugar 728 is highly hydrophilic and is partially dissolved in conditions of high humidity and warm temperature, the mesh membrane 736 allows the adult insects to land and feed on the sugar 728 through the mesh without becoming stuck.

[67] Though not specifically described, additional tubes, reservoirs, ports, and subsections may be added to adapt the parasite production chamber of the present invention to the production of various parasite/insect pairs. If desired, reservoirs can be drained with additional tubes after the function of the reservoir has been completed.

[68] The operation of the above-described parasite production chamber for the aseptic production of Plasmodium sporozoites and Anopheles mosquitoes will now be described. Initially, the entire parasite production chamber is sterilized either by irradiation, chemical treatment, autoclaving, or other sterilization process known in the art and sterility is maintained hereafter. The various sugar feeding reservoirs are filled with sugar. The door between the insect rearing portion of the parasite production chamber and the blood meal portion of the parasite
production chamber is closed so as to limit the
initial movement of the immature mosquitoes.

[69] The larva rearing reservoir is partially filled with
sterile larva rearing broth through the larva broth
introduction tube and the semi-submersible float will
come to rest just below the surface of the broth.
Surface-sterilized Anopheles eggs are then placed onto
the semi-submersible float through the egg access
port. The temperature and humidity of the interior of
the parasite production chamber is maintained at
conditions that promote the development of the
Anopheles eggs. As the eggs develop into larvae, the
larvae swim off of the float and swim around in the
larva rearing reservoir.

[1] After the larvae develop into pupae, the adults hatch
from the pupae and are free to fly around the interior
of the insect rearing portion of the apparatus feeding
on the sugar that is located in the sugar feeding
reservoir. After the mosquitoes have grown into
adults, the female mosquitoes are then ready to be fed
an infectious gametocyte blood meal in the blood meal
portion of the parasite production chamber.

[70] To begin the blood meal feeding procedure, warm water
is forced through the inlet of the blood warming tube
that coils around or through the blood feeding
reservoir. The door in the partition separating the
insect rearing portion and the blood meal portion of the parasite production chamber is opened by means of a lever. The female Anopheles mosquitoes are selectively attracted to the warm source and fly through the hatch, thus effectively segregating female and male mosquitoes. After a period of time, the hatch is then closed. Infectious blood that contains gametocytes of the Plasmodium parasite is injected through the exterior latex or rubber port of the blood introduction tube. The blood runs down the blood introduction tube and spreads over the membrane or under the mesh screen - depending on the blood feeding system that is being employed. The female Anopheles mosquitoes then feed on infectious gametocyte blood.

[71] After a sufficient period of time for sporozoites to develop, anoxic steriley filtered gas is blown into the apparatus from the air inlet tube. This stuns the mosquitoes and they fall to the bottom of the apparatus. The insect removal port is opened and a sterile vacuum tube is inserted and the female mosquitoes are aseptically removed from the blood meal portion of the parasite production chamber. These female mosquitoes are then a source of aseptic Plasmodium sporozoites.
The operation of the parasite production chamber of the present invention may be better understood with the description of the following example.

EXAMPLE

The sugar feeding reservoirs are charged with a small amount of sucrose. Several milliliters of a thick suspension of fine particulate solution suitable for ingestion by *Anopheles* larvae is mixed with 300 milliliters of water and is placed into the larva rearing reservoir of the system. The door between the insect rearing portion and the blood meal portion is closed and the container is autoclaved at 120 degrees Celsius at 30 pounds per square inch pressure for 30 minutes. The device is allowed to slowly cool to room temperature. Semi-synthetic larva rearing broth is then steriley filtered into the larva rearing reservoir to replace or replenish any heat labile nutrients.

Approximately 100 *Anopheles stephensi* eggs which have been surface sterilized by agitating for 10 minutes in 70 percent ethanol and 1 percent benzalkonium chloride and washed are transferred into the system with a pipette using sterile technique via the egg transfer port. The surface sterilized eggs, no longer buoyant, are discharged onto a semi-submersible float. The apparatus is transferred to a room with a temperature
of 28 degrees Celsius and a diurnal light phase of light:dark 14/10. Low volume air is steriley filtered into the apparatus to maintain a positive pressure gradient inside the system.

[75] The eggs hatch and larva and pupae form in the larva rearing reservoir with adult mosquitoes appearing in approximately 9 days. The adult mosquitoes derive nutrition from a sterile sugar source(s) within the device.

[76] The door between the insect rearing portion and the blood meal portion of the apparatus is then opened approximately three days after emerging from the pupae. A warmed feeding station in the blood meal portion of the apparatus induces adult female mosquitoes to self-segregate from males by flying through the opened door to seek an obligate blood meal. The males are not attracted to the heat source and do not seek a blood meal. After an amount of time sufficient for separation of males and females, the hatch is closed.

[77] After feeding on the infectious *Plasmodium* gametocyte blood meal, the infected gravid female mosquitoes are provided sterile water for egg laying and derive nutrition from a sterile sugar source. The *Plasmodium* sporozoites fully develop in the female *Anopheles* mosquito approximately 14 days after the infectious
blood meal. At this time, micro-filtered anoxic gas is discharged into the apparatus, thus stunning the mosquitoes. The stunned female mosquitoes are then removed via the insect removal port and are processed to obtain the aseptic Plasmodium sporozoites, which may be used in the development of, and the immunogen of, an attenuated whole sporozoite vaccine.

[78] An additional aspect of the present invention includes a haematophagous insect biting chamber array (FIG. 8A, 8B). This apparatus is useful for the study of the biting behavior and reactogenic/allergic potential of individual haematophagous insects. These studies are useful in the selective breeding and genetic manipulation of haematophagous insect populations.

[79] The haematophagous insect biting chamber array is preferably a three dimensional rectangular structure with two large parallel surface planes of rectangular shape. The interior of the haematophagous insect biting chamber array 800 preferably is subdivided into multiple sub-chambers 804 of similar dimensions. Each sub-chamber extends the height of the array and is capable of housing an individual insect.

[80] On one side of the array, the surface is a metal mesh 808 that prevents the escape of the insect species
under study. A plastic cover is arranged so that the mesh 808 can be covered to prevent the insects from inadvertently biting the operator or having wind drafts alter the internal environment of the sub-chambers 804. The mesh 808 thus allows insects to probe, bite, or feed on the skin of a human or animal when the haematophagous insect biting chamber array 800 is juxtaposed to a patch of skin. The mesh 808 is preferably attached and supported by the solid outside perimeter of the haematophagous insect biting chamber array and the solid interior perimeter of the sub-chambers.

[81] On the side of the array opposite that of the mesh 808, individual hatches 812 are attached to the outside perimeter of each sub-chamber 804 so as to allow access to each sub-chamber 804 individually. The sub-chambers 804 thus allow individual insects to be housed separately. In such an arrangement, each insect is not subjected to interference by other insects, and variables in the environment such as light exposure, color of the environment, chemical environment, etc. may be manipulated independently for each insect.

[82] To operate the haematophagous insect biting chamber array 800, individual insects are placed into the sub-chambers 804 and the hatches 812 are secured. The
plastic covering that is over the mesh 808 is removed and the mesh 808 side of the array is placed in contact with the skin of a human or other animal. Using a protocol that may be particularly developed for each insect species that is to be tested, the insects are allowed to probe and feed for variable lengths of time. The structure of the haematophagous insect biting chamber array 800 is such that it allows the same patch of skin to be exposed to the same insect multiple times.

[83] An additional aspect of the present invention is a strain of insects, such as mosquitoes, that is hypoallergenic to humans or other animals.

[84] The production of hypoallergenic insects may be accomplished by the following procedure. A heterogeneous, genetically diverse population of a particular mosquito species is allowed to breed freely in an enclosed insectary. Gravid females are isolated at the appropriate time in their life cycle when a blood meal is necessary to complete egg development.

[85] A number of such female mosquitoes are then preferably placed into a subdivided rectangular container that is adapted to allow the mosquitoes access to the skin of a human or other animal while at the same time not allowing them to leave the chamber. One example of this apparatus is the haematophagous insect bite
chamber array described hereinabove. Preferably, a single female mosquito occupies each chamber of the haematophagous insect bite chamber array.

[86] The haematophagous insect bite chamber array is preferably kept under strict environmental controls such that there is limited variability in temperature, humidity, and ambient light.

[87] A haematophagous insect bite chamber array that is loaded with female mosquitoes is then placed onto a previously prepared animal or human skin surface. Preferably, each female mosquito will be allowed access to a single, unique section of skin. Preparation of the skin may include shaving the hair from the skin of the test location and the restraint from exposure of the skin surface to soaps, creams, or other artificial substances for approximately 24-48 hours. The skin surface is preferably marked so that an experimenter can identify which female mosquito bites each section of skin.

[88] Test subjects and animals preferably include both individual who react normally to mosquito bites as well as those who have a history of hypersensitivity to mosquito bites.

[89] The haematophagous insect bite chamber array is preferably placed onto the skin for a short period of
time to allow the mosquitoes to probe the skin in an effort to obtain a blood meal. The haematophagous insect bite chamber array is then lifted from the skin two to three times and then returned to the same spot on the skin. This preferably allows the mosquitoes to take additional probes and thereby inject more antigens from the same patch of skin. Those female mosquitoes that are not probing or engorging are identified and discarded. Each haematophagous insect bite chamber array challenge may last for approximately two sequential feeding periods of three minutes by a one minute break.

After the haematophagous insect bite chamber array challenge is completed, the skin reaction of the test human or animal is assessed at fifteen minutes, thirty minutes, one hour, two hours, twenty-four hours, and four days. Such timer periods allow for the assessment of hypersensitivity, Arthus, and delayed type hypersensitivity reactions. The assessment will include careful objective measurement of erythema, induration, and size of lesion for each mosquito at the time in question. Subjective measurements are also preferably made for pruritus at the bite sites.

Each female mosquito in a haematophagous insect bite chamber array challenge cohort that also has been shown to have probed the skin and engorged in a blood
meal is rated on the development of hypersensitivity, Arthus, and delayed-type hypersensitivity reactions in the test subject. Those females with least reactigenicity are returned to a breeding chamber and allowed to lay eggs. Those shown to have high reactigenicity are discarded.

The eggs from low reactigenicity female mosquitoes are allowed to hatch and develop. Portions of these progeny are back bred to each other or allowed to breed randomly with other low reactigenicity female progeny. Certain eggs or larvae are cryopreserved for use at a later time.

At times that are determined by the experimenter, females with low reactigenicity and their progeny are preferably infected with insect borne pathogens including, but not limited to, Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale to insure that they retain the capacity to propagate the pathogen of interest.

The above-described procedure is preferably repeated multiple times. The result of this procedure is the development of a hypoallergenic strain of mosquitoes shown to have low or no reactigenicity in animals and humans, while retaining the ability to propagate the pathogen of interest. This mosquito strain will preferably be useful in the development of vaccines
derived from the pathogens extracted from the whole-body or saliva of the mosquitoes of that strain.

While this aspect of the present invention has been described using the example of mosquitoes, the same technique may be used to produce other species of hypoallergenic haematophagous insects including, but not limited to, gnats, biting flies, fleas, lice, and ticks.

An additional aspect of the present invention includes methods for the breeding of a strain of insects capable of enhanced ability to support infectious stage parasites. While this aspect of the present invention will be described with respect to the example of Anopheles species mosquitoes and Plasmodium species sporozoites, it is to be understood that the same approach would be useful for producing strains of any insects that are capable of supporting enhanced levels of infectious stage parasites.

The production of a strain of Anopheles mosquitoes that is capable of supporting higher burdens of Plasmodium sporozoites may be accomplished by the following procedure. Heterogeneous strains or a homogenous strain of Anopheles mosquitoes are divided into cohorts of single females and some multiple of males. Each cohort is preferably placed in an enclosed container where environmental conditions are...
controlled and monitored. Preferably, the conditions among the containers are consistent.

[98] The mosquitoes are then allowed to breed. At the appropriate time in the life cycle, the females are offered a blood meal from a pool of Plasmodium infected blood containing either gametocytes or processed blood-containing ookinetes. The duration of feeding and other variables are controlled to insure uniformity in the environment during the blood meal process. The females mosquitoes that are not observed to be feeding and engorging on the infected blood are preferably discarded.

[99] At the appropriate time in the mosquito life cycle, a nutrient water broth is added to the bottom of each container. The female mosquitoes are monitored for egg laying. Those females that are not observed laying eggs are preferably discarded.

[100] Some eggs or developing larvae are sub-divided and cryopreserved for later use in the instances where the individual characteristics in their mother’s physical and/or physiological profile be deemed beneficial. By preserving some proportion of progeny in cold storage, genetic robustness and potential is maintained.

[101] All or some portion of the eggs and larvae in each container are preferably allowed to develop. If
abnormalities in some proportion of the eggs or larvae are observed, that female and her progeny are preferably discarded.

[102] At the appropriate time in the sporozoite life cycle (approximately fourteen to sixteen days), the salivary glands from the mosquito are dissected from the head of the mosquito. The salivary glands are gently crushed and mixed into a suspension. An exact amount of the suspension is added to an exact volume of solution. [Could some detail regarding the solutions be provided?] The diluted suspension is transferred to a glass slide and examined under a microscope. The level of infection is graded initially as Grade 1, 2, 3, or 4 using standard methodology. As populations of mosquitoes are progressively selected, the number of sporozoites will preferably be counted.

[103] Other factors relating to morphology and genetics are assessed to correlate sporozoite yield with a particular attribute(s). These factors include, but are not limited to, salivary gland size, length and size of the midgut, specific proteins and genetic markers. Such factors will be used in the progressive development of the strain of mosquito.

[104] A composite scoring system preferably used to rank each female mosquito on her overall ability to sustain a high level of Plasmodium infection while
satisfactorily completing those actions necessary to sustain a progressive mosquito and parasite life cycle. Those females with a high composite score will have their progeny retained for further cycles of this process. Selected progeny will be in-bred and crossbred to steadily select for high sporozoite infection tolerance.

[105] Through continuous cycles, a highly refined, genetically distinct Anopheles strain capable of extremely high levels of Plasmodium infection and sporozoite production is created. Such an Anopheles strain preferably leads to more efficient production of viable whole cell sporozoites for use in an attenuated live or killed parasite vaccine.

[106] Although the invention has been described in terms of particular embodiments in an application, one of ordinary skill in the art, in light of the teachings herein, can generate additional embodiments and modifications without departing from the spirit of, or exceeding the scope of, the claimed invention. Accordingly, it is understood that the drawings and the descriptions herein are proffered only to facilitate comprehension of the invention and should not be construed to limit the scope thereof.
An additional aspect of the present invention includes an apparatus for injecting ultra-low volumes of vaccine suitable for attachment to standard syringes.

An aspect of the present invention is an apparatus which will be referred to as a micro-bolus vaccine assembly (FIG. 9A 9B). The micro-bolus vaccine assembly preferably uses micro-needles 904 with multiple micro-pores 908. The micro-pores 908 deliver the total volume of the vaccine into hundreds or thousands of mini-boluses. The needles 904 are attached to a plastic reservoir 912 that holds a precise volume of the sporozoite vaccine. The necessary vaccine volume is calculated by multiplying the number of needles 904 by the desired volume of the mini-boluses plus the volume of the internal micro-needles. Covering the reservoir 912 on the opposite side of the needles 904 is an elastic plate 916 that completely seals the reservoir 912. A plastic structure 920 with the same outside dimensions as the vaccine reservoir 912 is then attached. This plastic structure 920 has a standard female docking port 924 that attaches to the industry standard locking port of common syringes 928.

The micro-bolus vaccine assembly 900 is operated in the following manner. One to two milliliters of atmospheric gas is drawn into a syringe 932 and the
micro-bolus vaccine assembly 900 is attached to the syringe 932. The needles 904 are placed into the patient’s skin and the plunger 936 on the syringe is forcefully depressed. The gas inside the syringe 932 is compressed and travels into the micro-bolus vaccine assembly 900. The gas then deforms the elastic membrane 916. The membrane 916 pushes on the vaccine solution in the micro-bolus vaccine assembly reservoir 912. The vaccine is extruded in micro-boluses through the micro-pores 908 into the cutaneous tissues. The elastic membrane 916 prevents the gas in the syringe 932 from passing into the vaccine reservoir 912 of the micro-bolus vaccine assembly 900.

[110] An additional aspect of the present invention is a method for the development of cryopreservation/freeze resistant Plasmodium species sporozoites.

[111] A high temperature cryopreservable Plasmodium species may be developed by employing the following procedure. Heterogeneous strains or a homogenous strain of Plasmodium species gametocytes/ookinotes are mixed in a blood culture and fed to Anopheles mosquitoes. Ookinotes randomly assort and form zygotes within the midgut of the female mosquitoes.

[112] The Plasmodium parasites are then allowed to develop into sporozoites. Seventy-two hours before the extraction of the sporozoites from the mosquitoes, the
mosquitoes are subject to four to six hour intervals of slowly decreasing temperatures that plateau above their survival tolerance level and then are allowed to rise back to baseline temperatures. This activates cellular mechanisms that produce stabilizing proteins, enzymes, and sugar complexes that prime the sporozoites to withstand cryopreservation.

[113] The sporozoites are then preferably extracted and purified from the whole body mosquito extract or salivary glands according to techniques that are well known among those of skill in the art.

[114] Sporozoites are then preferably cryopreserved at temperatures from minus seventy degrees to zero degrees Celsius in ten to twenty degree increments in selected media suitable for direct immunization in humans. Portions of these sporozoites are thawed in vitro and assessed for motility and other markers of viability.

[115] Sporozoites are then preferably thawed and injected into humans or animals. Once the human/animal becomes parasitemic, a blood sample is extracted and placed into a blood culture. The cycle of culturing, infection, and freezing is repeated several times.

[116] Sporozoites that successfully survive cryopreservation and demonstrate the capacity to complete their full
life cycle in both the mosquito and human or animal host will be sequentially selected and in-bred with themselves and back-bred to other successful strains to ultimately produce a highly infectious, high cryopreservation temperature tolerant strain of the Plasmodium parasite.
CLAIMS

WHAT IS CLAIMED IS:

1. An apparatus for the production of aseptic insect stage parasites, comprising:

   a walled chamber;

   a reservoir within the chamber for supporting growth of adult insects from surface sterilized insect eggs; and

   a blood feeding station within the chamber for allowing adult insects to consume blood that is infected with the parasites.

2. The apparatus of Claim 1, further comprising a plurality of tubes that extend intending through the wall of the chamber for introducing fluids into the chamber.

3. The apparatus of Claim 2, wherein one of the tubes is adapted for introducing sterile larva rearing broth into the reservoir.

4. The apparatus of Claim 3, further comprising a submersible float in the reservoir for supporting the surface sterilized insect eggs.
5. The apparatus of Claim 1, having a sugar feeding reservoir in the chamber for providing to the adult insects.

6. The apparatus of Claim 5, wherein the sugar feeding reservoir is wick-based or trough-based.

7. The apparatus of Claim 2, wherein one of the tubes has means for warming blood.

8. The apparatus of Claim 1, wherein the chamber comprises a first sub-chamber and a second sub-chamber, wherein the first sub-chamber is separated from the second sub-chamber by an operable door.

9. A method for the production of aseptic insect stage parasites, comprising the steps of:

   - sterilizing the interior of an enclosed chamber;

   - introducing sterile larva rearing broth into a reservoir in the enclosed chamber;

   - placing surface sterilized insect eggs into the sterile larva rearing broth;

   - allowing the surface sterilized insect eggs to develop into adult insects within the enclosed chamber; and
providing a sterile blood meal infected with the parasite to the adult insects.

10. The method of Claim 9, wherein the providing step further includes warming the sterile blood meal.

11. The method of Claim 10, wherein the blood meal comprises a gametocyte stage parasite.

12. The method of Claim 11, further comprising stunning the adult insects, after the providing step.

13. The method of Claim 12, wherein the adult insects are Anopheles mosquitoes.

14. The method of Claim 13, wherein the parasite is selected from the group consisting of Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae.

15. An apparatus for assessing the biting behavior of insects, comprising:

   an array of rectangular compartments, wherein the rectangular compartments are capable of housing a single insect;

   a plurality of hatches on a top portion of the rectangular compartments, wherein the hatches are adapted to allow access to an interior of the rectangular compartments; and
a metal mesh on a bottom portion of the array of rectangular compartments, wherein the mesh is adapted to allow an insect to bite a surface on the opposite side of the metal mesh.

16. A method for the cultivation of a hypoallergenic haematophagous insect, comprising the steps of:

exposing sections of skin to haematophagous insects;

allowing the haematophagous insects to inflict bites on the sections of skin;

assigning a set of allergenic responses of sections of skin to the bites; and

selecting a set of insects that cause a benign set of allergenic responses based on the set of allergenic responses.

17. The method of Claim 16, further comprising the step of cross breeding the set of insects that cause the benign set of allergenic responses, after the selecting step.

18. The method of Claim 17, wherein the haematophagous insects are mosquitoes.

19. A method of cultivating a strain of haematophagous insects capable of an enhanced ability
to form insect stage infectious parasites, comprising the steps of:

providing a blood meal to a group of haematophagous insects, wherein the blood meal contains infectious parasites;

allowing the group of haematophagous insects to lay eggs;

sacrificing the group of haematophagous insects;

measuring a number of infectious parasites that was being supported by each member of the group of haematophagous insects;

selecting a subset of the group of haematophagous insects that are capable of supporting higher numbers of infectious parasites, based on the measuring step; and

cross breeding the subset of the group of haematophagous insects that are capable of supporting higher numbers of infectious parasites.

20. The method of Claim 19, wherein the haematophagous insects are mosquitoes.

21. The method of Claim 20, wherein the infectious parasite is selected from the group consisting of
Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae.

22. A method of producing cryopreservation resistant Plasmodium species sporozoites, comprising the steps of:

feeding a blood meal to Anopheles mosquitoes, wherein the blood meal is infected with Plasmodium species gametocytes;

allowing the Plasmodium species gametocytes to develop into sporozoites;

extracting the Plasmodium species sporozoites from the Anopheles mosquitoes;

freezing the Plasmodium species sporozoites;

thawing the Plasmodium species sporozoites;

and

breeding the Plasmodium species sporozoites that successfully survive.

23. The method of Claim 22, wherein the freezing step further comprises holding the Plasmodium species sporozoites at a temperature close to zero degrees Celsius.
24. The method of Claim 23, where the temperature close to zero degrees Celsius is from about -5 degrees Celsius to about 0 degrees Celsius.

25. An apparatus for the injection of ultra-low volumes of vaccine, comprising:

   a plurality of micro-needles;

   a reservoir operably connected to the plurality of micro-needles, wherein the reservoir is capable of holding a vaccine solution;

   an elastic plate, where the elastic plate covers the reservoir; and

   a plastic cap, wherein the plastic cap attaches to the reservoir over the elastic plate, further wherein the plastic cap includes a female docking port capable of attaching to a common syringe.

26. The apparatus of Claim 25, wherein the micro-needles include a plurality of micro-pores adapted to deliver a small volume of vaccine.
Figure 3