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ANIMAL IDENTIFICATION STATEMENTS:

1. The research is conducted in accordance with the applicable laws, regulations, and institutional animal care and use policies.
2. All procedures involving non-human animals are performed under the supervision of qualified veterinary personnel.
3. The research is conducted in a manner that minimizes animal pain and distress.
4. The research is conducted in a manner that maximizes animal welfare.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL:

[Signature]

APRIL 2023

(AUG 91)
Column E: Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 10-E-0002

2. Number 1 of animals used in this study.

3. Species (common name) Rabbit of animals used in the study.

4. Explain the procedure producing pain and/or distress.

Distress due to Busulfan/Fthyl palmitate, the cytotoxic regimen, or due to antibodies used to induce thrombocytopenia in rabbits, may weaken the animal and cause potential symptoms, such as: significant bleeding due to thrombocytopenia (hematomas); nonspecific drug-related adverse events—persistent anorexia, significant injection site reactions, significant decreased ambulation or listlessness, restlessness, repetitive locomotion, and abnormal vocalization.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see item 6 below)

Any rabbits showing any combination of these signs will be euthanized with euthanasia solution. All other procedures will occur under general anesthesia in these non-survival experiments, so no pain is anticipated. The potential painful procedures, i.e. the cannulation and laparotomy procedures, described in this protocol are essential for creating the conditions necessary to study the stability and efficacy of Multi-function Blood Substitution in vivo. However, every effort will be made to ensure maximum comfort of the animals under anesthesia. There will be a conscious effort by the P.I. and his staff to provide additional consideration for comfort and well being of the animals as is consistent with the scientific integrity of the study. The attending veterinarian will consulted regarding appropriate and humane use of anesthesia to alleviate the pain associated with the surgical procedures in this protocol.

Animals that appear distressed or moribund will be euthanized according to section V.D.7 of this protocol.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency  CFR
Column E Explanation

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1. Registration Number: 10-F-0002

2. Number_________272_________of animals used in this study.

3. Species (common name) Guinea Pig_________of animals used in the study.

4. Explain the procedure producing pain and/or distress.

Shigella vaccine candidates were evaluated by placing Shigella in the conjunctiva of guinea pigs' eyes, and then the severity of inflammation was scored.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The study of immune response to and protective efficacy of vaccine candidates directed against Shigella requires an accurate evaluation of the immune response raised by the administration of these vaccines. The use of analgesics, particularly opiates or narcotics, result in immunosuppression, which would invalidate the results of experiments testing immune responses as well as increasing the severity of the possible eye infection. Use of analgesics that are anti-inflammatory (e.g. aspirin) would also invalidate the model since we are studying a model for inflammation of epithelial cells by bacterial invasion.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., AHPHb, 9 CFR 113.162):

Agency____________________CFR________________
Column E Explanation

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1. Registration Number: 10-F 0002

2. Number _______ of animals used in this study.

3. Species (common name) _______ of animals used in this study.

4. Explain the procedure producing pain and/or distress:

Determine if virulent Brucella melitensis strain 16M can establish acute brucellosis in a non-human primate model that is indistinguishable from the aerosol route of infection when given by either conjunctival or nasal routes of infection at identical dosages. We expect the organisms to be internalized within the mucosa and subsequently become localized within resident phagocytic cells residing in the mucosa-associated lymphoid tissues. These cells or an unidentified cell population will transport the organisms via the reticuloendothelial (RE) system to target organs. It is expected that the organisms will replicate inside endothelial/adjacent isome of phagocytic cells and establish acute brucellosis identical to that seen in aerosol studies.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress would interfere with test results. (For federally mandated setting, see item 6 below)

If illness occurs following the administration of virulent Brucella in animals it will cause some distress. It is unclear whether such distress can be relieved with known treatments that may affect experimental results. Previous experience with rhesus monkeys given high respiratory doses of virulent Brucella indicates that clinical signs (incubation and weakness, progressive state of odorization, inappetence, responses to external stimuli, forced abdominal respirations or dysuria) have been manifested, illness is evident, however, the animals may recover spontaneously. Narcotic analgesics cause histamine release (Soma 1983). There is evidence for histamine involvement in the rhesus Brucella responses (Scheuer et al., 1985). Neuroleptanalgesics cause respiratory depression, bradycardia, and poor muscle relaxation (Swainbury et al., 1969). Butorphanol tartrate has respiratory depressant activity and causes significant increases in heart rate, blood pressure, and cardiac output (Lumb and Jones, 1964). Because of the potential for analgesics to contribute to or exacerbate the symptoms of shock, it is wise to use them in this protocol.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) see number and the specific section number (e.g., APHIS, 9 CFR 113.102).

Agency _______ CFR _______
Column E Explanation

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1. Registration Number: 10-F-0002

2. Number_________32_________of animals used in this study.

3. Species (common name): Guinea Pig _______of animals used in the study.

4. Explain the procedure producing pain and/or distress.

The guinea pigs will be exposed to subacute or to chronic low-dose Saman, Sarin, or VX. Neurological observations will be performed before, during, and following dosing.

Some nerve agent-exposed guinea pigs will be prepared using biotelemetry techniques for the collection and analysis of EEG, EKG, body temperature, and locomotor activity data. Guinea pigs will be anesthetized to achieve deep surgical anesthesia using a solution containing a mixture of Ketamine and Xylazine. Having achieved deep surgical anesthesia, either the surgical procedures or euthanasia will be conducted.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

Nerve agent exposed Guinea pigs may experience seizures. The pre- and post-seizure periods may be accomplished by distress. The relief of the pre-seizure period of distress is difficult to predict and pharmacological treatment is contraindicated if we are to determine the primary effects of nerve agent exposure.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.10(b).

Agency_________, CFR_________
Column E Explanation

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1. Registration number: 10-E-0002

2. Number of animals used in this study.

3. Species (common name): Same of animals used in the study.

4. Explain the procedure producing pain and/or distress.

Piglet model for both emetic and lethal response to Staph endotoxin (SE). Piglets are dosed orally with SE. A determination is made of the value of various potential drugs for prophylaxis against emesis (vomiting) and lethal shock. In addition, an evaluation of how these drugs can be administered after SE-challenge and still retain desired efficacy of response is determined.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The lethal shock that is induced by the lethal SE-challenge with the LD50 test in the positive control animals will necessarily cause pain to these animals. Positive controls are required to validate results. Analgesics would impact the physiological parameters, exacerbating the lethal shock or emesis induced by the SE and compromising analysis of collected data. If the experimental drugs proved their utility, the animals should experience relief, but should they not experience relief that indicates failure of the drug and is necessary for that reason. In all circumstances, the animals will be under constant veterinary care and will not be subject to any unnecessary pain.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulation (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency: CFR: