UTMB
The University of Texas Medical Branch

## ENVIRONMENTAL HEALTH AND SAFETY

## BIOLOGICAL AND CHEMICAL SAFETY PROGRAM

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**SENT TO:** Dr. Kathryn Harris

**Dept/Institute:** NIH - OBA

**FAX#:** (301) 496-9839

**NO OF PAGES:** 19 + cover page

**SENT BY:** **Dee Zimmerman**

☐ **URGENT** ☐ **Please respond** ☐ **Action** ☒ **For your information**

**MESSAGE:**

**The University of Texas System**
Nine Universities. Six Health Institutions. Unlimited Possibilities.

Institutional BioSafety Committee at Galveston



April 27, 2007

Dr. Kathryn Harris
Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892-7985

Dear Dr. Harris:

Attached please find the Institutional BioSafety Committee submission from Dr. David Walker. Dr. Walker submitted a UTMB Notification of Use for Biological Agents and rDNA (NOU) for a project that would involve the development of antibiotic resistant clones.

The NOU was reviewed by the IBC and tabled as it met the requirements as a major action under the Guidelines that requires review by the Office of Biotechnology. The project meets the criteria of Section III-A-1-a, the transfer of a drug resistance trait to microorganism that is not known to acquire the trait naturally.

Dr. Walker has provided information on the different antibiotics available and their clinical application including published references and the risk/benefit of the project.

Please let me know if you should require any additional information.

Sincerely,

Dee Zimmerman
IBC Coordinator
BioSafety Officer

# UNIVERSITY OF TEXAS MEDICAL BRANCH
## Notification of Use
## BSL3/4 and CDC/USDA Regulated Agents

The purpose of this document is to ensure adequate review of occupational safety and health precautions and the procedures for use, handling, storage, and disposal of biohazardous agents. As the Principal Investigator (P.I.) or Supervisor, you should be fully aware of the specific of potential hazards associated with the agents used in your work area.

| | | |
|---|---|---|
| Type of Submission: | ☒ New | ☐ Renewal |
| Type of Agents: | ☒ Biological Agent | ☒ r DNA<br>☐ r RNA |
| Select Agent: | ☐ Yes | ☒ No |

The information provided in this document is accurate to the best of my knowledge. I am familiar with, the agree to abide by the provisions set forth in this plan as approved by the UTMB Biological Safety and Institutional BioSafety Committees, the UTMB Safety Manual, the UTMB Institutional Handbook of Operating Procedures (IHOP), 42 CFR 73, 7 CFR 331, 9 CFR 121 and *BioSafety in Medical and Biomedical Laboratories,* NIH/CDC, 4th Edition.

When using recombinant DNA, I agree to comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules.*

I accept responsibility for training all laboratory workers involved in the research project described in this "Notification of Use" before commencing any work.

| | | | |
|---|---|---|---|
| David H Walker | Professor | 23989 | 12/4/06 |
| P.I. (Signature)<br>Responsible for Research | Title | Extension | Date Submitted |
| David H. Walker | | Pathology | 0609 |
| P.I. (Printed Name) | | Department | Route |

**BIOLOGICAL SAFETY COMMITTEE USE ONLY**

DATE APPROVED _____ DATE FOR RESUBMISSION _____

_____ _____

Chairman (Signature) Printed Name

NOU Number

_____

For EHS use only



Tabled 1/5/07

**SECTION I: General information**

List agent(s): *Rickettsia conorii and Rickettsia typhi*

**Attach a copy of the biological material safety data sheet if available, (http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/index. Html).**

1. Location: Building Keiller Room(s): 4.102A

3. Duration of use: 1/01/2007 -12/31/2011

4. Goal of the project: *Knockout of virulence genes to produce attenuated strain as vaccine candidate; proof of principle; pathogenesis research.*

5. Description of use: *The **purpose** of the project is to understand the pathogenesis of rickettsial diseases and the molecular biology of Rickettsia. The **objectives** are 1) to produce rickettsial DNA, RNA and proteins and 2) to transform R. conorii and R. typhi to chloramphenicol resistance. Rickettsial DNA and RNA will be used for PCR amplification, DNA sequencing, and/or DNA cloning. Rickettsial antigens will be used for protein analysis and antigen slides for diagnosis. Chloramphenicol resistant rickettsial clones will be analyzed for their virulence in mice to evaluate their pathogenicity and potential for vaccine. **Methods** include 1) cultivation of rickettsiae in mammalian cell lines such as Vero cell and L929 cell or embryonated chicken eggs; 2) purification of rickettsiae by centrifugation; 3) electroporation of R. conorii and R. typhi with plasmid vector containing chloramphenicol resistance gene, 4) purification of rickettsial DNA and RNA using commercial kits; 5) PCR amplification of rickettsial genes; cloning and DNA sequencing rickettsial genes; analysis of rickettsial proteins by SDS-PAGE and Western blot, 6) immunization of mice and/or guinea pigs with live rickettsiae, dead rickettsiae and/or rickettsial proteins.*

6. Can this agent infect humans? ☐ No ☒ Yes

   If yes, provide a description of potential mechanism of laboratory transmission: *In the past most laboratory infections were resulted from aerosol transmission by sonication of infected tissues in open area. Working inside the biological safety cabinet and wearing protective mask prevent aerosol transmission effectively. Rickettsia could be inoculated into skin by needle injection.*

   If yes, can it cause disease in healthy people? ☐ No ☒ Yes

   If Yes, is the infection associated with replication in humans or is it abortive (no progeny)?

   Replication ☒, or abortive ☐

7. Have you educated your staff regarding safe handling and decontamination procedures for the agents in this NOU? ☒ Yes ☐ No

8. Risk Assessment: Provide information on a separate sheet.
   a. Describe pathogenicity, including disease incidence and severity.
      *Rickettsia conorii causes Mediterranean spotted fever with severe headache, chills, fever, prostration, confusion, photophobia, vomiting and rash. Mediterranean spotted fever is endemic in Europe and Africa, but not in the Americas. Rickettsia typhi causes murine typhus, a similar febrile disease.*
   b. Describe route of transmission.
      *R. typhi is transmitted through flea and R. conorii is transmitted through tick bite.*
   c. Describe agent stability.
      *The stability of both R. typhi and R. conorii is poor. They die when outside of host cells except for a dormant form of R. typhi that has been found only in flea feces, which we will not be studying.*
   d. What is the infectious dose?
      *The infectious dose of Rickettsia is usually less than 10 organisms for animals.*
   e. What is the concentration (number of infectious organism per unit volume) and the volume of the concentrated material being handled? In cell culture the titer of *Rickettsia may reach to $10^6$ organism/ml or more. One to 20 culture flasks (25 to 150 $cm^2$) may be handled at one time.*
   f. What is the origin of the infectious material (may refer to geographic location, host or nature of source).
      *R. typhi is distributed worldwide, and it can be isolated from infected rats and their fleas, or infected patients worldwide. R. conorii is distributed in Europe and Africa and it may be isolated from infected ticks or patients from these areas.*
   g. What is the availability of data from animal studies (pathogenicity, infectivity and route of transmission in animal)?
      *These are described above in a and b.*
   h. Is there an effective prophylaxis or therapeutic intervention available? Specify prophylaxis or therapeutic intervention.
      *Prophylaxis is not recommended. Doxycycline is the choice of treatment.*

9. Response to "Fink Committee Report", experiment of concern: If answer yes, please explain in detail, use additional sheets as needed.

   a) Would this research demonstrate how to render a vaccine (if applicable) ineffective?
      ☒ No ☐ Yes:_____
      (Specify)

   b) Would this research confer resistance to therapeutically useful antibiotics and antiviral agents?
      ☐ No ☒ Yes: *Chloramphenicol is the second choice drug for treatment of rickettsial infection.*

   c) Would this research increase transmissibility of this pathogen?
      ☒ No ☐ Yes:_____
      (Specify)

   d) Would this research alter the host range of this pathogen?
      ☒ No ☐ Yes:_____
      (Specify)

e) Would this research enable the evasion of diagnostic/detection modalities of this agent?
☒ No ☐ Yes:_____
(Specify)

f) Would this research enable the weaponization of a biological agent or toxin?
☒ No ☐ Yes:_____
(Specify)

10. Is immunization required for work at the listed biosafety level? No ☒ Yes ☐
Is immunization recommended at the listed biosafety level? No ☒ Yes ☐

11. Is medical surveillance recommended prior to commencement of work? No ☒ Yes ☐

if yes, please explain (Post exposure management is considered part of occupational health exposure management) _____

_____

_____

Attach additional sheets if needed.

12. Experience and skill level of at-risk personnel. List names of P.I. and staff. (See instructions). Attach experience description.

*David H. Walker, M.D. (P.I. and faculty) has been involved in molecular and immunological research of infectious diseases sponsored by grants from CDC and NIH since 1979. He has extensive experience in clinical and research laboratories dating back to 1965 including BSL-3 and BSL-4 projects.*

*Xuejie Yu, M.D., Ph.D, has been working with rickettsiae for 23 years and has experience working in BSL3 for 20 years since 1987. He has worked with R. prowazekii, R. typhi, R. rickettsii, R. conorii, R. sibirica, R. japonica, R. australis, R. parkeri, Coxiella burnetii, Bartonella henselae and Bartonella quintana. His work included isolation of these bacteria from their vector and mammalian hosts; cultivation of the organisms in tissue culture; infection and immunization of laboratory animals; purification of bacterial DNA and RNA; DNA cloning and genomic sequencing. He has never been infected with any bacterial agent with which he has worked.*

*Jianzhi Zhang, M.D., MS, has been working with rickettsiae and other bacterial pathogens for 12 years since 1992. She has experience working with R. prowazekii, R. rickettsii, R. conorii, R. japonica, and R. australis in BSL3 laboratory for two years when she worked in the Unite des Rickettsia in the University of Mediterranean, Marseille, France. She has completed training for using the BSL3 at UTMB. Her work involved the isolation, cultivation of rickettsiae in tissue culture and animals; cloning and sequencing rickettsial DNA; analysis of host - pathogen interactions. Dr. Zhang has never been infected by any bacterial agent with which she has worked.*

13. What systems are you using to propagate or study the agent(s) listed?
*Cell culture, embryonated chicken eggs, and animal models (mouse for R. conorii and R. typhi).*

14. Check biosafety level: BSL 2 ☐ BSL 3 ☒ BSL 4 ☐

15. Check the protective clothing or equipment used when handling this agent:

- ☒ Lab coat/ gloves
- ☐ Safety Centrifuge/blender
- ☐ Cover gown/booties/gloves
- ☐ Face Shield
- ☐ Goggles
- ☐ Chemical Fume Hood – Room location _____
- ☐ Biological Safety Cabinet – Room location _4.102A Keiller
- ☒ N95 respirator during purification of *Rickettsia*
- ☐ Surgical mask
- ☐ PAPR (Racal) ☐ other _____

16. Method for disposal of biohazardous waste:

- ☐ Placed in red bag for disposal.
- ☐ Autoclaved then placed in regular trash.
- ☒ Autoclaved then placed in Yellow bag for incineration. (BSL 3 & 4 only)
- ☐ Chemical disinfection then placed in regular trash.
- ☐ Chemical disinfection of bulk liquid then poured down sanitary sewer.

17. List disinfectant(s) used for surface decontamination and spills:

☒ Cavicide ☒ Bleach ☐ Phenolic germicidal ☒ 70% alcohol
☐ MicroChem ☐ Other _____

**If you are using rDNA/RNA please fill out section II page 5.**
**If you are a Select agent please fill out section III page 8.**

**If you are planning any animal work please fill out section IV page 9.**

## SECTION II: Recombinant DNA / RNA

1. Goal of the project: Construction of knockout genes in *R. conorii* by homologous recombination.

1. rDNA / RNA Characteristics: (Attach additional sheets if needed)

| Source of DNA / RNA (Species) | Types of Vector(s) | Types of Host(s) | Seize of the insert/total genome | Nature of Inserted Sequences | Proteins(s) Produced |
|---|---|---|---|---|---|
| ***R. conorii*** | **Plasmid** | ***E. coli*** | **1kb/1.2Mb** | **Rickettsial genes chloramphenicol resistance gene** | **Rickettsial protein** |
| ***R. typhi*** | **Plasmid** | ***E. coli*** | **1kb/1.2Mb** | **Rickettsial genes, chloramphenicol resistance gene** | **Rickettsial protein** |
| | | | | | |
| | | | | | |
| | | | | | |

2. Please answer the following questions: (add additional sheet if necessary)

a) Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
☒ No ☐ Yes:_____
(Specify)

if Yes provide $LD_{50}$ ng/Kg of body weight: _____

b. Does the inserted gene encode a known oncogene?
☒ No ☐ Yes:_____
(Specify)

c. Does the viral DNA integrate into the host genome?
☒ No ☐ Yes:_____
(Specify)

d. Does the modification have the potential to increase the replication capacity of virus?
☒ No ☐ Yes:_____
(Specify)

e. Does the modification increase the pathogenicity of the agent?
☒ No ☐ Yes:_____
(Specify)

f. Does the inserted gene have the potential for altering the cell cycle?
☒ No ☐ Yes:_____
(Specify)

g. Does the modification change the host range of the agent?
☒ No ☐ Yes:_____
(Specify)

h. Use of infectious DNA / RNA?
☒ No ☐ Yes:_____
(Specify)

i. Use of defective DNA / RNA with Helper virus?
☒ No ☐ Yes:_____
(Specify)

j. Is there a probability of generating replication-competent viruses?
☒ No ☐ Yes *N/A*
(Specify)

k. Will the infectious DNA / RNA be used in tissue culture?
☐ No ☒ Yes: *Knockout rickettsiae will be selected and propagated in cell culture.*
(Specify)

l. Will the infectious DNA/RNA be use in whole animals?
☐ No ☒ Yes: *Knockout rickettsiae will be inoculated into animals to assess attenuation.*
(Specify)

m. Will the infectious DNA / RNA be used in whole plants?
☒ No ☐ Yes:_____
(Specify)

**If you are using a Select agent please fill out section III page 8.**

**If you are planning any animal work please fill out section IV page 9.**

**Section IV: Animal Use**

IACUC Protocol #: *0511074* Species *mouse and guinea pig*

**Attach a copy of the IACUC Flow diagram and the Hazardous Agent Form. (http://research.utmb.edu/iacuc/hazardous.doc)**

1. Does the infected animal present any human health risk after administration? No X Yes

   If yes, provide the following:

   Route of transmission/exposure: *Human infection resulting from laboratory-infected animals has never been reported in the literature. However, this does not exclude the potential of transmission of Rickettsia to human being through infected animals. Thus, general precautions should be taken when handling infected animals.*

   Precautions to be taken to prevent exposure: *Wear gloves and mask to handle infected animals and wash hands after handling infected animals. Let your physician know that you have worked with rickettsia-infected animals if you get a high fever, severe headache, and/or rash. We can assist with the diagnosis of rickettsial infection.*

2. What animal biosafety level is recommended?

   ☐ ABSL 2 ☒ ABSL 3 ☐ ABSL 4

3. Check the protective clothing level and equipment used when handling the infected animal

   ☐ ABSL 2 ☒ ABSL 3 ☐ ABSL 4
   ☒ Biological Safety Cabinet ☐ Chemical Fume Hood

   Respiratory protection
   ☐ Surgical Mask ☐ PAPR (Racal)
   ☒ N95 Respirator ☐ Other (specify) _____ _____

**Note: Investigators are reminded that when planning experiments with animals which will include the use of hazardous agents, please contact ARC staff at extension 28458 or 22334 two weeks prior to use of the agents in order to coordinate animal housing and husbandry concerns.**

**Information on the different antibiotics available and their clinical application including published references:**

*In vitro*, *Rickettsia conorii* and *R. typhi* are susceptible to doxycycline, fluoroquinolones (levofluoxacin in Europe, and ofloxacin), rifampin, and telithromycin (Table 1)(9).

Standard treatment for rickettsial infections has been tetracyclines or chloramphenicol. Other new antibiotics have been tested in patients recently. Patients suffering from Mediterranean spotted fever (MSF) have been successfully treated with fluoroquinolones (3, 6). Ciprofloxacin was found to exhibit the same efficacy as chloramphenicol in terms of duration of the fever (2). Thus, fluoroquinolones may be considered a safe alternative to tetracyclines for the treatment of rickettsial diseases of moderate severity. Clarithromycin and azithromycin are as effective as choramphenicol in a clinical trial of treatment of pediatric patients with mild MSF (Table 2)(1). Because of lack of adverse effects and a good compliance, clarithromycin and azithromycin can be considered valid alternative therapies to tetracyclines or chloramphenicol in the treatment of pediatric MSF (1).

Table 1. In vitro susceptibilities of *R. conorii* and *R. typhi* to antibiotics by plaque reduction (9)

| Antibiotics | Mic (µg/ml) | |
|---|---|---|
| | *R. conorii* | *R. typhi* |
| Amoxicillin | 128 | 128 |
| Ciprofloxacin | 0.5 | 1 |
| Doxycycline | 0.06 | 0.125 |
| Erythromycin | 8 | 1 |
| Gentamicin | >32 | >32 |
| Levofloxacin (levaqui) | 0.5 | 0.5 |
| Ofloxacin | 1 | 1 |
| Rifampin | 0.25 | 0.25 |
| Trimethoprim-sulfamethoxazole | >16/4 | >16/4 |
| Telithromycin | 0.5 | 0.5 |
| Thiamphenicol | 1 | 1 |

**Table 2: Efficacy and tolerability of the different drugs used in 415 patients with Mediterranean spotted fever.**

| | Chloramphenicol n = 107 | Clarithromycin n = 230 | Azithromycin n = 78 | p |
|---|---|---|---|---|
| Mean time to defervescence (h) | 40.62 | 41.94 | 47.13 | 0.093 |
| Hospitalization mean (days) | 5.4 | 3.4 | 4.1 | 0.018 |
| Prolonged fever / relapse (%) | 0 | 2 (0.9) | 9 (11.5) | < 0.01 |
| Side effects requiring drug discontinuation (%) | 0 | 16 (7) | 1 (1.3) | < 0.01 |

**Information regarding the risk/benefit of the project:**

**The risk of the project:**

In order to knock out rickettsial virulence determinants, in this project we will transform *R. conorii* and/or *R. typhi* to chloramphenicol resistance. The potential risks of using chloramphenicol as a selectable marker for transforming rickettsiae includes 1) exhausting the avilabity of antibiotics for treatment of rickettsial infections and 2) the transformed rickettsiae could be criminally used as a bioterrorism agent.

The first potential risk is exaggerated because of the notion that only tetracycline and chloramphenicol are effective for treatment of rickettsial diseases. The fact is that there are other new antibiotics that are effective for treatment of rickettsial diseases as described in the above information on the different antibiotics available and their clinical application. Ciprofloxacin, clarithromycin and azithromycin are all at least equally effective to chloramphenicol.

The second potential risk is overstated also because the rickettsial agents are strictly controlled. The persons working with rickettsiae are FBI cleared, and have access to the BSL3 laboratory where rickettsial cultures are handled is limited to authorized persons, and rickettsial stocks are secured in a locked-freezer in the BSL3 laboratory. Finally, this project will not generate new techniques for transforming rickettsiae. The technology for transforming rickettsiae is readily available in the literature (4, 5).

**The benefit of the project:**

Our purpose is to knock out rickettsial virulence determinants and to generate a vaccine for epidemic typhus. The benefit of this project is obtaining an effective vaccine for epidemic typhus. Epidemic typhus is caused by *R. prowazekii*, which can be used as a bioterrorism agent. The disease occurs in many places of the world including Africa, South America, Europe, and Asia. Recent outbreaks of epidemic typhus have been reported in Africa and European Russia (7, 8). An effective vaccine can eliminate epidemic typhus from endemic areas and prevent a worldwide outbreak. Existence of effective vaccine will also deter criminal use of *R. prowazekii* as bioterrorism agent. Finally, our work of gene knockout will identify the virulence factors of rickettsiae, which has been hampered because of lack of genetic manipulation tools.

Reference List


1. **Colomba, C., L. Saporito, V. F. Polara, R. Rubino, and L. Titone.** 2006. Mediterranean spotted fever: clinical and laboratory characteristics of 415 Sicilian children. BMC. Infect. Dis. 6:60.

2. **Gikas, A., S. Doukakis, J. Pediaditis, S. Kastanakis, A. Manios, and Y. Tselentis.** 2004. Comparison of the effectiveness of five different antibiotic regimens on infection with *Rickettsia typhi*: therapeutic data from 87 cases. Am. J. Trop. Med. Hyg. 70:576-579.

3. **Gudiol, F., R. Pallares, J. Carratala, F. Bolao, J. Ariza, G. Rufi, and P. F. Viladrich.** 1989. Randomized double-blind evaluation of ciprofloxacin and doxycycline for Mediterranean spotted fever. Antimicrob. Agents Chemother. 33:987-988.

4. **Qin, A., A. M. Tucker, A. Hines, and D. O. Wood.** 2004. Transposon mutagenesis of the obligate intracellular pathogen *Rickettsia prowazekii*. Appl Environ Microbiol 70:2816-2822.

5. **Rachek, L. I., A. Hines, A. M. Tucker, H. H. Winkler, and D. O. Wood.** 2000. Transformation of *Rickettsia prowazekii* to erythromycin resistance encoded by the *Escherichia coli ereB* gene. J. Bacteriol. 182:3289-3291.

6. **Raoult, D., H. Gallais, M. P. De, and P. Casanova.** 1986. Ciprofloxacin therapy for Mediterranean spotted fever. Antimicrob. Agents Chemother. 30:606-607.

7. **Raoult, D., V. Roux, J. B. Ndihokubwayo, G. Bise, D. Baudon, G. Marte, and R. Birtles.** 1997. Jail fever (epidemic typhus) outbreak in Burundi. Emerg. Infect. Dis. 3:357-360.

8. **Raoult, D., T. Woodward, and J. S. Dumler.** 2004. The history of epidemic typhus. Infect. Dis. Clin. North Am. 18:127-140.

9. **Rolain, J. M., L. Stuhl, M. Maurin, and D. Raoult.** 2002. Evaluation of antibiotic susceptibilities of three rickettsial species including *Rickettsia felis* by a quantitative PCR DNA assay. Antimicrob. Agents Chemother. 46:2747-2751.

**PI: David H. Walker, M.D.**_____

**Protocol #:** _____

**Date: 11/11/2005**_____

**HAZARDOUS AGENTS FORM**

**Instructions to Investigator:** If you will be using any hazardous substances (e.g., radioactive materials, toxic chemicals, biological agents) in your animals (*in vivo*) you must complete this form. ***Please fill out one form for each agent in the same category listed in question 1. For example, if you are using two chemical agents, please submit two forms. However, if you are using one chemical agent and one biological agent, please submit one form with both agents listed.*** Use of this form does not imply approval from the appropriate safety committee.

For more information concerning the approval of hazardous substances, contact Environmental Health and Safety (EH&S) at x21781, or visit their website at http://www.utmb.edu/ehs. **REMINDER:** If hazardous materials are being used *in vivo*, ARC must be notified via the Hazardous Agent Startup Form found on the ARC website at **http://research.utmb.edu/arc/hazard_start-up.htm two weeks prior to the use of agents. Failure to notify ARC could result in a delay in research commencement.**

1. Agent(s) category [*check one*]: *When applicable, date of UTMB Biological, Chemical or Radiation Safety Committee approval or date pending for submittal should be listed.*

   ☐ Chemical

   Answer questions 2 and 3, and complete Section A

   x☐ Biological

   Answer questions 2 and 3, and complete Section B

   ☐ Radioactive

   Answer questions 2 and 3, and complete Section C

   Attach a Material Safety Data Sheet (MSDS) or a product information sheet. If an MSDS for the chemical with which you are working does not appear in the MSDS database (http://www.utmb.edu/ehs), please call EH&S at x21781.

2. Give a project description:

Animals will be immunized with attenuated *R. prowazekii* or *R. rickettsii* and then challenged with homologous *Rickettsia* species (*R. prowazekii* or *R. rickettsii*) or heterologous *Rickettsia* (*R. conorii* or *R. typhi*). The aim of the study is to test if the attenuated *Rickettsia* vaccines are safe in animals and confer upon animals protective immunity.

3. List the species the agent(s) will be used in:

*Rickettsia prowazekii, R. rickettsii, R. conorii,* and *R. typhi*

## SECTION A – Chemical Agents

a. Name of chemical:

b. Provide chemical characteristics:

c. Dose per animal: mg/kg

d. Route of administration: [***Provide volume and concentration***]

**PI: David H. Walker, M.D.**_____

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**Date: 11/11/2005**_____

☐ Topical ☐ Oral ☐ Inhalation ☐ IC ☐ IP
☐ ID ☐ IM ☐ IV ☐ SQ

e. Is the agent metabolized?
☐ Yes ☐ No

f. Route of excretion:
☐ Respiratory ☐ Milk ☐ Urine ☐ Feces ☐ Saliva ☐ Other

g. Location of laboratory where dosing of animals will occur [*list bldg, room#*]:

h. Describe type and quantity of hazardous waste to be generated and method of disposal [*i.e., will animals, carcasses, or bedding contaminated with the following agents pose a hazardous risk when used* ***in vivo***?]:

i. Describe how contaminated materials are treated after usage (animals, bedding, glassware, bench tops, hoods, etc.):

## SECTION B – Biological Agents

a. Agent:

i: Will the study use recombinant DNA, viral vectors, gene transfer experiments, transgenic animals?
☐ Yes x☐ No

ii: Are the vectors replicative deficient?

iii: Discuss any safety concern associated with the vectors and also are there any toxins or virulence factors associated with the expression of transgene.

*Rickettsia* are infectious agents and need to be handled cautiously. *Rickettsia* are transmitted through arthropod bite, needle injection, and aerosol in certain conditions such as a bioterror attack. However, neither arthropod bite or aerosol transmission routine exists in the animal care facility. Animal care persons will also not involve in animal injection.

b. Dose per animal: mg/kg
$10^1 - 10^6$ organisms per animal
c. Route of administration: [***Provide volume and concentration***]
☐ Topical ☐ Oral ☐ Inhalation ☐ IC x☐ IP
☐ ID ☐ IM x☐ IV x☐ SQ

d. Is the agent metabolized:
☐ Yes x☐ No

e.Route of excretion:
☐ Respiratory ☐ Milk ☐ Urine ☐ Feces ☐ Saliva ☐ Other

**PI: David H. Walker, M.D.**_____

**Protocol #:** _____

**Date: 11/11/2005**_____

Organisms are not excreted from any of these route, but it can be transmitted by injection of infected animal blood or tissues.

f. Location of laboratory where dosing of animals will occur [*list bldg, room#*]:

Keiller Building ABSL3 laboratory

g. Describe type and quantity of hazardous waste to be generated and method of disposal [*i.e., will animals, carcasses, or bedding contaminated with the following agents pose a hazardous risk when used* ***in vivo***?]:

Animal carcasses contain rickettsial organisms, and it is unlikely that the bedding contains rickettsiae.

h. Describe how contaminated materials are treated after usage (animals, bedding, glassware, bench tops, hoods, etc.).

For caution, both animal carcasses and bedding should be autoclaved. Glassware, bench tops, hoods should be treated with 70% alcohol or Cavicide.

## SECTION C – Radioactive Material

a. Name of radionuclide:

b. Dose per animal: mg/kg

c. Half life:

d. Route of administration: [***Provide volume and concentration***]

☐ Topical ☐ Oral ☐ Inhalation ☐ IC ☐ IP

☐ ID ☐ IM ☐ IV ☐ SQ

e. Is the agent metabolized?

☐ Yes ☐ No

f. Route of excretion:

☐ Respiratory ☐ Milk ☐ Urine ☐ Feces ☐ Saliva ☐ Other

g. Location of laboratory where dosing of animals will occur [*list bldg, room#*]:

h. Describe type and quantity of hazardous waste to be generated and method of disposal [*i.e., will animals, carcasses, or bedding contaminated with the following agents pose a hazardous risk when used* ***in vivo***?]:

i. Describe how contaminated materials are treated after usage (animals, bedding, glassware, bench tops, hoods, etc.).

Signature----------Date----------

Principal Investigator

**PI: David H. Walker, M.D.**_____

**Protocol #:** _____

**Date: 11/11/2005**_____

(For office use only)

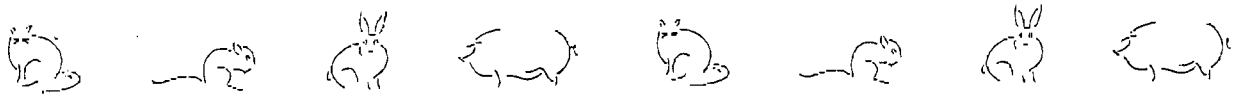
Animal Biosafety Level Classification:

☐ ABSL1 ☐ ABSL2 ☐ ABSL3 ☐ ABSL4 ☐ N/A

Chemical _____ Dose in chemical fume hood: _____

Radioactive _____ Dose in chemical fume hood: _____

**EH&S Officer:**



FORMS AND INSTRUCTIONS ARE ONLINE: **http://research.utmb.edu/iacuc/files.htm**

This form and any additional forms (see 3A on page 1 of this form) may be submitted online as an attachment to an email with one hard copy forwarded to the IACUC that includes the signature page. The IACUC cannot accept grant excerpts to satisfy questions. Answer all questions, and if not applicable to your study, please note "N/A" on this form. *New protocols are due by 5:00 pm on the first working day of each month for review in that month.*

# University of Texas Medical Branch Protocol for the Use of Live Vertebrate Animals for Research, Testing or Education

**Date:** 10-24-05 **Protocol #:**

_x_ **Initial Submission**
___ **Renewal**
___ **Amendment/Modification**

**(Please use the tab key to get to the next field)**

**1. A. Principal Investigator:** Walker David H. M.D.

Dept: Pathology Mail Route:0609 Daytime phone #: (409)772-3989 Emergency # for after hours: (409)744-2037

Fax #: (409)772-1850 Email: dwalker@utmb.edu

PI employee ID number: 44294

Account number for animal ordering: Pending Grant

**B. Co-Investigator: Xuejie Yu, M.D., Ph.D.**

**2. Project Title:** Vaccine for rickettsial diseases

**3. A. Please check below if your research protocol involves any of the following:**

| | | |
|---|---|---|
| ___ | Antibody production/adjuvant use | [*Complete Antibody Production Form*] |
| ___ | Breeding rodents in-house (on campus) | [*Complete Breeding Protocol Form*] |
| ___ | Ordering timed pregnant animals | Report quarterly to ARC – no Breeding Form required |
| ___ | Ordering animal(s) w/ litter | Report quarterly to ARC – no Breeding Form required |
| _x_ | "D" and/or "E" Level study | [*Refer to item* **4C**. *See* ***Appendices A&B***] |
| ___ | Death (without appropriate euthanasia) as an experimental endpoint | [*Complete item* **4C-#6**] |
| ___ | Decapitation/cervical dislocation without anesthesia | [*Provide rationale item* **7C**] |
| ___ | Hazardous materials use *in vivo* | [*Complete Hazardous Agents Form for any/all of the following]* |
| ___ | a) Chemicals (highly hazardous chemicals & select toxins) | **EHS/Chemical Safety Plan app. date:** |
| ___ | b) Radioactive materials | **EHS/Radiation Safety Plan app. date:** |
| _x_ | c) Biological agents & Recombinant DNA | **EHS/NOU approval date:** |
| ___ | Neuromuscular blocking agents | [*Complete Neuro. Blkg. Agents Form]* |
| ___ | Surgery | [*Complete Survival Surgery Form]* |
| ___ | Multiple survival surgery | [*Complete Survival Surgery Form]* |
| ___ | Tumor induction | [*Complete Tumor Induction Form]* |
| ___ | Transgenic/genetically modified animal use | [*Complete item* **5G**] |
| ___ | Wild animal capture or field studies | [*Complete Capture/Field Studies Form]* |

B. **Attach a COMPLETE OUTLINE OR FLOW CHART RELEVANT TO ANIMAL USE.**
*Provide a detailed outline description or flow chart of what is planned for the animals from start to finish of this experiment. This should include any other procedures not previously described, such as administration/injection of non-hazardous agents (e.g., estrogen, anesthetics for sedation or restraint purposes). You must include route of administration and amount. This description should indicate the number of test and control groups, the number of animals in each group, the sequence of the experimental manipulations in each group and the time between each manipulation. The numbers should also be reconciled with the numbers in sections 4A, 5E, and 9D.*

**1. Determining the median immunization dose of attenuated rickettsial strains or the 50% infectious dose to determine the challenge dose for the wild type rickettsiae.**

12 mice or 3 guinea pigs will be inoculated i.p. with attenuated or wild type *R. rickettsii* or *R. prowazekii* with 1, 10, $10^2$, $10^3$, $10^4$, $10^5$, or $10^6$ organisms for each animal. Four rickettsial strains with 7 concentrations each will be used to inoculate animals. Thus, 336 mice (12 mice/group x 4 rickettsia strains x 7 concentrations of each rickettsial strain) and 84 guinea pigs (3 guinea pigs/group x 4 rickettsia strains x 7 concentrations of each rickettsial strain) will be used in these experiments.

↓

The animals will be observed daily for clinical signs of illness for 2 weeks. Blood will be obtained from the animals by cardiac puncture from guinea pigs or retraorbital bleeding from mice weekly for 5 weeks. Serum and blood DNA will be used for antibody titration and PCR, respectively.

↓

All animals will be sacrificed at 5-6 weeks post-inoculation.

**2. Immunization with *R. rickettsii* or *R. prowazekii* vaccine**

40 mice or 5 guinea pigs will be immunized i.p. with 10 ID50 of attenuated *R. rickettsii*, attenuated *R. prowazekii*, virulent *R. rickettsii*, virulent *R. prowazekii*, or PBS control. A total of 200 mice and 25 guinea pigs will be used in this experiment

↓

Blood will be obtained weekly by cardiac puncture from guinea pigs or by retroorbital bleeding from mice

↓

6 – 8 weeks post-immunization, the animals will be challenged i.p. (guinea pigs) or i.v. (mice) with one of the following *Rickettsia* species: *R. rickettsii, R. conorii, R. prowazekii,* or *R. typhi.*

↓

Blood will be obtained weekly by cardiac puncture from guinea pigs or by retroorbital bleeding from mice

↓

On the third week post-challenge, the animals will be euthanized, and organs will be collected for histology and extraction of DNA.