

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

EH&S USE ONLY

PROTOCOL # 9565

EXPIRES: 6/4/04

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus macaque	78	CRPRC

Project Title Effect of Microbicides on Mucosal SIV Transmission

Overnight housing location: CRPRC Day use only :

Animals will be maintained by: Vivarium Investigator (If investigator maintained, attach husbandry SOP's.)

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

This project will involve developing a low-dose multiple SIV vaginal challenge system, and using that system to determine the protective effect of different microbicides applied to the vagina prior to viral challenge.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials (only if in the animal room):

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	SIV, SHIV
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	NIH, NIAID	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

It is widely believed that heterosexual transmission of HIV to women usually requires multiple exposures to a relatively weak (low titer) viral inoculum. The most widely used SIV vaginal transmission model used today involves inoculating mature female rhesus macaques once or twice with a very high titer inoculum. We hypothesize using a multiple low-dose inoculation model will provide a better model of HIV transmission, and thus allow investigation into the biology of transmission and the effect of microbicides on intravaginal transmission of SIV.

b) Procedures employed in this project:

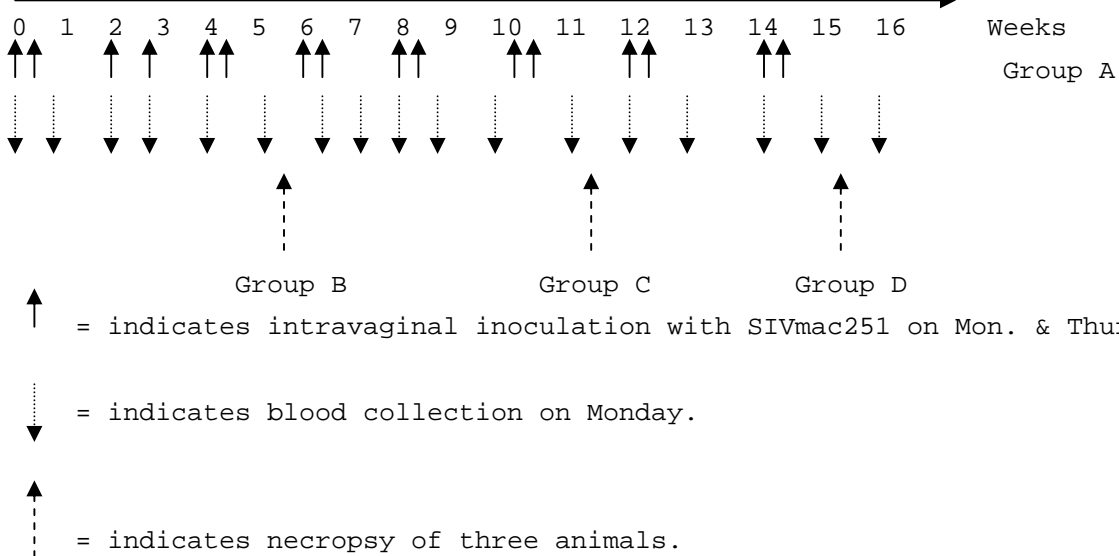
Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|-----------------------------------------------------------------------------|-----------------------------------------------------------|-------------------------------------------------------------------------------|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input checked="" type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

All animals used in this project will be multi-parous rhesus macaques with normal menstrual cycles. For all animals on this project, an accurate record of menstrual bleeding will be maintained. Animals in all groups will be fasted and anesthetized prior to all procedures.



The week number indicates the **END** of the week, so the arrow at #2 indicates the inoculation of animals at the beginning of week #3, and so on.

All animals will be inoculated on a Monday, Thursday schedule. Blood will be collected every Monday.

For groups A, E, F, M, and N- Inoculations will be wk 1, 3, 5, 7, 9, 11, 13, and 15. Blood will be drawn every Monday for the first 16 weeks and every four weeks thereafter. These animals will be necropsied 6 months after the first inoculation.

For groups B,G, J, O, and R- Inoculations will be wk 1, 3, and 5. Blood will be drawn every Monday for six weeks. These animals will be necropsied six weeks after the first inoculation.

For groups C, H, K, P, and S- Inoculations will be wk 1, 3, 5, 7, 9, and 11. Blood will be drawn every Monday for 12 weeks. These animals will be necropsied 12 weeks after the first inoculation.

For groups D, I, L, Q, and T- Inoculations will be wk 1, 3, 5, 7, 9, 11, 13, and 15. Blood will be drawn every Monday for 16 weeks. These animals will be necropsied 16 weeks after the first inoculation.

Group A- Multiple Low-titer SIV inoculation to determine infectious dose.

Animals will be inoculated intravaginally 16 times over a 16 week period with 10^3 TCID₅₀ of SIVmac251 each inoculation. Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic

infection status at designated timepoints(see figure above). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Before the development of clinical SAIDS, the animals will be euthanized and tissues (vagina, cervix, draining lymph node, axillary lymph node, mesenteric lymph node, and spleen) will be collected for virologic and immunologic analysis. We anticipate culling the animals at six months.

Group B- Multiple Low-titer SIV inoculation to determine infectious dose.

Animals will be inoculated intravaginally 6 times over a 6 week period with 10^3 TCID₅₀ of SIVmac251 each inoculation. Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status at designated timepoints(see figure above). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0 and 4 weeks post-inoculation. Following the 6th inoculation (see figure above), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group C- Characterization of SIV infection during and after multiple low-dose intravaginal SIV inoculations.

Animals will be inoculated 12 times intravaginally over a 12 week period with 10^3 TCID₅₀ of SIVmac251 each inoculation. Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status at designated timepoints(see figure above). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 12th inoculation (see figure above), week period the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group D- Characterization of SIV infection during and after multiple low-dose intravaginal SIV inoculations.

Animals will be inoculated 16 times intravaginally over a 16 week period with 10^3 TCID₅₀ of SIVmac251 each inoculation. Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status at designated timepoints(see figure above). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 16th inoculation (see figure, previous page), the animals will be euthanized and tissues (vagina, cervix, draining lymph node, axillary lymph node, mesenteric lymph node, and spleen) will be collected for virologic and immunologic analysis.

Group E- Protective effect of Caraguard on SIVmac251 high-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with Caraguard (a non-specific binding inhibitor) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the day of challenge, the animals will be anesthetized and treated with Caraguard as before, rested for 10-15 minutes and then challenged with a high dose of SIVmac251 (1 ml 10^5 TCID₅₀) intravaginally. This procedure will be repeated 3 days after the first inoculation. The post-SIV challenge sampling and procedures (including lymph node biopsies)will be the same as for Group A (see figure, previous page). We anticipate culling these animals six months after challenge.

Group F- Vehicle control for effect of Caraguard on SIVmac251 high-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina)with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a high dose of SIVmac251 (1 ml 10^5 TCID₅₀) intravaginally. This procedure will be repeated 3 days after the first inoculation. The post-SIV challenge sampling and procedures (including lymph node biopsies) will be the same as for Group A (see figure, previous page). We anticipate culling these animals six months after challenge.

Group G- Protective effect of Caraguard on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina)with Caraguard (a non-specific binding inhibitor)twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with Caraguard as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 6 times over six weeks as in Group B (see figure above) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group B (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0 and 4 weeks post-inoculation. Following the 6th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group H- Protective effect of Caraguard on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina)with Caraguard (a non-specific binding inhibitor)twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with Caraguard as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 12 times over 12 weeks as in Group C (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group C (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 12th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group I- Protective effect of Caraguard on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with Caraguard (a non-specific binding inhibitor)twice a week for 6 weeks prior to the SIVmac251 challenge (day 0).

Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with Caraguard as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 16 times over 16 weeks as in Group D (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group D (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 16th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group J- Vehicle control for effect of Caraguard on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 6 times over six weeks as in Group B (see figure, previous page). Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group B (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0 and 4 weeks post-inoculation. Following the 6th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group K- Vehicle control for effect of Caraguard on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 12 times over 12 weeks as in Group C (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group C (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 12th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group L- Vehicle control for effect of Caraguard on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be

infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 16 times over 16 weeks as in Group D (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group D (see figure, previous page). Lymph node biopsies (peripheral lymph node: axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 16th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group M- Protective effect of Buffer-gel on high dose SIVmac251 vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with Buffer-gel (a low pH virucide) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the day of challenge, the animals will be anesthetized and treated with Buffer-gel as before, rested for 10-15 minutes and then challenged with a high dose of SIVmac251 (1 ml 10^5 TCID₅₀) intravaginally. This procedure will be repeated 3 days after the first inoculation. The sampling and procedures (including lymph node biopsies) will be the same as for Group A (see figure, previous page). We anticipate culling these animals six months after challenge.

Group N- Vehicle control for effect of Buffer-gel on high dose SIVmac251 vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a high dose of SIVmac251 (1 ml 10^5 TCID₅₀) intravaginally. This procedure will be repeated 3 days after the first inoculation. The sampling and procedures (including lymph node biopsies) will be the same as for Group A (see figure, previous page). We anticipate culling these animals six months after challenge.

Group O- Protective effect of Buffer-gel on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with Buffer-gel (a low pH virucide) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with Buffer-gel as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 6 times over six weeks as in Group B (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in

Group B (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0 and 4 weeks post-inoculation. Following the 6th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group P- Protective effect of Buffer-gel on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina)with Buffer-gel (a low pH virucide)twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with Buffer-gel as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 12 times over 12 weeks as in Group C (see figure, previous page). Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group C (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation.Following the 12th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group Q- Protective effect of Buffer-gel on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with Buffer-gel (a low pH virucide)twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with Buffer-gel as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 16 times over 16 weeks as in Group D (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group D (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation.Following the 16th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group R- Vehicle control for effect of Buffer-gel on SIVmac251 multiple low-dose vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 16 times over 16 weeks as in Group B (see figure, previous page). Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group B (see figure, previous page). Lymph node biopsies (peripheral lymph

node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0 and 4 weeks post-inoculation. Following the 6th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group S- Vehicle control for effect of Buffer-gel on SIV vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 12 times over 12 weeks as in Group C (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group C (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 12th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

Group T- Vehicle control for effect of Buffer-gel on SIV vaginal challenge.

Animals in this group will be anesthetized and treated (1 ml will be infused into the vagina) with propylparaben (the vehicle used for most vaginal gel compounds currently used in humans) twice a week for 6 weeks prior to the SIVmac251 challenge (day 0). Cervicovaginal secretions, vaginal pH readings and microbiology swabs will be collected at the beginning of treatment and 4 weeks after the start of treatment. On the first day of challenge, the animals will be anesthetized and treated with propylparaben as before, rested for 10-15 minutes and then challenged with a low dose (10^3 TCID₅₀ in 1 ml each dose) intravaginal SIVmac251 inoculation. This procedure will be repeated 16 times over 16 weeks as in Group D (see figure, previous page) Blood (10 ml, not to exceed 12 ml/kg/month) from a femoral vein will be collected to assess systemic infection status as in Group D (see figure, previous page). Lymph node biopsies (peripheral lymph node:axillary or inguinal, approx. 1 gram/biopsy) will be collected at 0, 4 and 8 weeks post-inoculation. Following the 16th inoculation (see figure, previous page), the animals will be euthanized and tissues will be collected for virologic and immunologic analysis.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require.** The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
A	10^3 TCID ₅₀ SIVmac251 x 16	9	
B	10^3 TCID ₅₀ SIVmac251 x 6	3	
C	10^3 TCID ₅₀ SIVmac251 x 12	3	
D	10^3 TCID ₅₀ SIVmac251 x 16	3	
E	Caraguard/ 10^5 TCID ₅₀ SIVmac251 x 2	6	
F	Propylparaben/ 10^5 TCID ₅₀ SIVmac251 x 2	6	
G	Caraguard/ 10^3 TCID ₅₀ SIVmac251 x 6	3	

H	Caraguard/10 ³ TCID ₅₀ SIVmac251 x 12	3		
I	Caraguard/10 ³ TCID ₅₀ SIVmac251 x 16	3		
J	Propylparaben/10 ³ TCID ₅₀ SIVmac251 x 6	3		
K	Propylparaben /10 ³ TCID ₅₀ SIVmac251 x 12	3		
L	Propylparaben /10 ³ TCID ₅₀ SIVmac251 x 16	3		
M	Buffer-gel/10 ⁵ TCID ₅₀ SIVmac251 x 2	6		
N	Propylparaben/10 ⁵ TCID ₅₀ SIVmac251 x 2	6		
O	Buffer-gel/10 ³ TCID ₅₀ SIVmac251 x 6	3		
P	Buffer-gel/10 ³ TCID ₅₀ SIVmac251 x 12	3		
Q	Buffer-gel/10 ³ TCID ₅₀ SIVmac251 x 16	3		
R	Propylparaben/10 ³ TCID ₅₀ SIVmac251 x 6	3		
S	Propylparaben/10 ³ TCID ₅₀ SIVmac251 x 12	3		
T	Propylparaben/10 ³ TCID ₅₀ SIVmac251 x 16	3		

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p>Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.</p>
2	<p>Minor stress or pain of short duration</p> <p>Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress</p>
3	<p>Moderate to severe distress</p> <p>Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p>Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.</p>

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

- 1) The SIV/rhesus macaque model of HIV sexual transmission has become the recognized standard for studies on pathogenesis and prevention of HIV transmission. The reason for this is that the SIV is closely related to HIV biologically and genetically. Also the reproductive tract of the rhesus macaque is very similar to humans. Dr. [redacted] created this model and has published more than 50 peer-reviewed articles that involve studies using this model.
- 2) The groups using "high-dose" SIVmac251 inoculations (E,F,M,N) use 6 animals due to the established protocol using the "high dose" model

where we have established 6 to be the minimum number to yield statistically significant results.

Group A will consist of 9 animals in order to develop the low-dose inoculation procedure. This is the minimum number we feel will determine the range and variability we can expect using this low-dose model in out-bred rhesus macaques.

Groups B, C, and D use three monkeys per group. Although this is a small number, based on our experience with the groups above, we anticipate that we will be able to detect clear trends between animals killed early and animals killed later in the inoculation series.

Groups G, H, and I are testing the effect of Caraguard (a non-specific binding inhibitor) in preventing HIV infection as well as any effect repeated use may have in the female genital tract. Based on the results from unprotected groups and microbicide groups we are confident we will be able to detect clear trends between unprotected animals and those using a microbicide.

Groups J, K, and L are testing the effect of propylparaben alone (the vehicle) in preventing HIV infection as well as any effect repeated use may have in the female genital tract. Based on the results from the unprotected groups and microbicide groups, we are confident we will be able to detect clear trends between unprotected animals, those using a microbicide, and vehicle control.

Groups O, P, and Q are testing the effect of Buffer-gel (a low pH virucide) in preventing HIV infection as well as any effect repeated use may have in the female genital tract. Based on the results from unprotected groups and microbicide groups we are confident we will be able to detect clear trends between unprotected animals and those using a microbicide.

Groups R, S, and T are testing the effect of propylparaben alone (the vehicle) in preventing HIV infection as well as any effect repeated use may have in the female genital tract. Based on the results from the unprotected groups and microbicide groups, we are confident we will be able to detect clear trends between unprotected animals, those using a microbicide, and vehicle control.

Therefore, in order to compare groups the three monkeys using microbicide will be inoculated along with the vehicle control group enabling an effective comparison.

f) Surgery: If the project involves survival surgery, where will the surgery be conducted?

Building:

Room:

Who will be the surgeon?

g) Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
rhesus	Telazol	6-8 mg/kg	IM	Before all procedures
rhesus	Oxymorphone	1mg/kg	IM	As in the judgement of CRPRC vets

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

Any injection or venipuncture has the potential to cause minor pain or discomfort, but the animals are immobilized for the procedure and should not experience pain.

SIV infection of rhesus macaques results in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized before, or when, they experience 3 of the following: weight loss >15% in two weeks or >30% in 3 months; persistent hypothermia <96F even with heat supplementation; leukopenia (total WBC <3,000); lymphopenia (lymphocytes <800); anemia (hemoglobin <10); dehydration >10%; nonresponsive to therapy for opportunistic infections; persistent anorexia (>3 days); animal significantly obtunded. These criteria are based on CRPRC guidelines. In addition, the lymph node biopsies will result in some post-procedure pain.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

All possible efforts will be made to minimize animal pain and discomfort. Analgesics have no effect on the proposed studies and they will be administered at the discretion of the CRPRC veterinary staff. The SIV infected animals will be euthanized prior to or at the time they develop clinical signs of AIDS. The decision to euthanize will be based on the judgement of the CRPRC veterinarians.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?

5/15/01

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PubMed	unlimited	Vaginal pH, microbicide, HIV and SIV transmission,
Current contents	unlimited	Vaginal pH, microbicide, HIV and SIV transmission,

What were your findings with respect to alternative methodologies?

The only available animal model systems of HIV heterosexual transmission are the SIV/rhesus macaque, HIV/chimpanzee and FIV/cat. No other animal models are satisfactory for assessing the ability of microbicides to prevent vaginal HIV transmission. In-vitro systems are unsatisfactory for these studies. Various microbicides have been tested in the rhesus model using the high-dose inoculation system. We feel testing the efficacy of microbicides in the proposed low-dose model will better reflect the biology of HIV transmission.

Has this study been previously conducted?

Yes No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

Animals will be euthanized after systemic infection is documented or to collect tissues according to experimental design.

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
rhesus	IV	pentobarbital	60mg/kg	IV

m) **Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

All animals will be euthanized at the end of the project.

Antibody Production Project Description

If your project involves only antibody production, either polyclonal or monoclonal, you may complete this page in lieu of section c), project description.

c) Will these animals be used for antibody production?

Yes No

1. Polyclonal or Monoclonal antibodies?

If Monoclonal, will you be producing ascites tumors in the animals?

Yes No

2. What type(s) of antigen will be used?

Will the antigens be sterile?

3. What adjuvant will be used for the initial injection?

What adjuvant will be used for subsequent injections?

4. What route will be used for injections?

What anatomical location will be injected?

How many injections at one time?

How frequently will injections be given?

What volume will be injected at each site?

5. Polyclonal Blood collection Procedures:

Who will collect the blood?

From what anatomical location?

How frequently will blood be collected?

Volume?

Will the animals be sedated?

Yes No

6. Will monoclonal antibodies be produced in mice bearing ascites tumors?

Yes No

How often will the animals be assessed for abdominal distention?

How often will they be tapped?

How many times will they be tapped?

Will the animals be sedated for tapping?

Note: *If you are producing monoclonal antibodies using ascites tumors in mice, section j), alternatives, must explain why an in-vitro system is not suitable for your study.*

7. Sedation / Anesthesia for blood or ascites collection: If the animals will be sedated for either injections or collections, please indicate the species, drug, dose and route:

Species	Drug	Route	Dose (mg / kg)

h) What criteria will be used to determine that the animals should be euthanized rather than continue to be used?

Categories of Invasiveness in Animal Experiments
Use these categories when completing item d), Study Groups and Numbers

Each year, the US federal government requires a report from the campus in which animal projects are categorized as to degree of invasiveness. Please assist the IACUC in this determination by assigning the animal procedures in your project to one of the categories below. The *US Government Principles Regarding the Care and Use of Animals* state, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."

1. Experiments which cause little or no discomfort or stress.**

Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category 2); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose or decapitation; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

2. Experiments which cause minor stress or pain of short duration.

Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioral experiments on conscious animals that involve short-term, stressful restraint: short term exposure to noxious but non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.

3. Experiments which cause moderate to severe distress or discomfort

Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of adjuvants which cause clinically evident swelling or abscesses.

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress: the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems.

Note: procedures used in Category 3 studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence or grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

4. Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals

Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress: completely new biomedical experiments which have a high degree of invasiveness; behavioral studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the American Veterinary Medical Association; any procedures (e.g. the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g. when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

** The text of these categories has been freely adapted from a document originally published by the Canadian Council on Animal Care (CCAC).

