Version 2 **ORIGINAL**

NATIONAL INSTITUTES OF HEALTH ANIMAL STUDY PROPOSAL 2nd draft received 08/09/2010 Sent out for pre-review 08/16/2010 Rec'd. back 08/23/2010 Sent to PI 08/24/2010 Rec'd. back 09/02/2010	**Leave Blank** Proposal #: **LID 15** Approval Date: **11/1/10** Expiration Date: **10/31/13** **Annual Review Due** 1st Year: **10/31/11** 2nd Year: **10/31/12**

For information about NIAID ACUC policies and procedures, see NIAID Animal Care and Use Committee
For general information, see NIH Policy Manual 3040-2.
See the NIAID ACUC ASP Form with instructions for detailed information regarding completion.

Emergency Treatment and Animal Care instructions (IEATC) shall be provided on the attached form at the end of this document.

A. ADMINISTRATIVE DATA:

Institute or Center	**NIAID**
Principal Investigator	**Kim Y. Green, Ph.D. (Lisbeth K. Green)**
Building/Room	**50/6318**
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Fax/Email	**301 480-5031 / kgreen@niaid.nih.gov**
Division, Laboratory, or Branch	**Laboratory of Infectious Diseases**
Project Title	**Norovirus Infection in Chimpanzees**

Renewal [x] of Proposal Number **LID 15**
For Modifications use the NIAID ACUC Amendment Form

List the names of all individuals authorized to conduct procedures involving animals under this proposal, and complete a T & E Form for each person; identify key personnel (i.e., Co-investigator(s)).
A brief summary of the training and/or experience for procedures each co-investigator will be expected to perform in this ASP must be documented and available to the ACUC. The name(s) of the supervisor, mentor, or trainer who will provide assurance each co-investigator is/has achieved proficiency in those procedures shall be included in that documentation (T & E Form).
For guidance see, NIAID ACUC Training & Safety Requirements OACU Training History Search

PI (if doing hands-on animal work): **Dr. Kim Y. Green (no direct contact with animals)**

Co-investigator(s): **Dr. Robert Purcell, Dr. Karin Bok, Dr. Stanislav Sosnovtsev (no direct contact with animals)**

Other investigators:
BIOQUAL Staff: Dr. Anthony Cook (head veterinarian), Dr. F. Salih Muhammad, Dr. Wendeline Wagner, Charlene Shaver, Zach Pippins, Raymond Tchoua

B. ANIMAL REQUIREMENTS:

Species:	**Chimpanzee (*Pan troglodytes*)**
Stock/Strain:	**Not applicable for chimpanzees**
Age/Weight/Size:	**Juvenile, 9 to 36 months, 6 - 22 Kilograms**
Sex:	**Male or Female**
Source(s):	**NIAID DIR contract with the New Iberia Research Center or other members of the Chimpanzee Breeding and Research Program Management Committee (CBRPMC)**
Animal Holding Location(s):	**BIOQUAL, Inc., Research Blvd facility**
Animal Procedure Location(s):	**BIOQUAL, Inc., Research Blvd facility**

Number of Animals:

Species	Year 1	Year 2	Year 3	Total
Chimpanzee	**4 carryover 4 new**	**4 carryover 4 new**	**4 carryover 4 new**	**24 "chimp-years" 16 individuals**

C. TRANSPORTATION: Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within the Clinical Center, also include the route and elevator(s) to be utilized.

Not applicable.

D. STUDY OBJECTIVES:

1. Provide no more than a 300 word summary of the objectives of this work. Why is this work important/interesting? How might this work benefit humans and/or animals? This should be written so that a non-scientist can easily understand it. Please eliminate or minimize abbreviations, technical terms, and jargon. Where necessary, they should be defined.
2. For renewal proposals, provide a brief summary of recent progress made using the last three-years' animals.
3. For new and renewal proposals, a brief synopsis of the scientific rationale or foundation for the study may optionally be provided.

1. Summary of Objectives: Studies conducted under LID 15 over the past several years have established chimpanzees as a valid model in which to study Norwalk virus infectivity, evolution, tissue and cellular tropisms, and vaccine development. In our present three-year renewal request, we want to extend these findings and accomplish the following objectives:

a. Establish the infectivity titer of human stool samples containing norovirus strains other than Norwalk virus.

b. Continue to examine the genetic diversity and "evolution" of the population of Norwalk virus shed in the stool after infection to search for adaptive mutations.

c. Continue immunohistochemical studies to identify permissive cells and tissues in the chimpanzee. Cells from tissue biopsies and blood will be used as a source of viral RNA for microarray gene expression analyses.

d. Analyze the extent of cross-reactivity of the antibody response to the Norwalk virus prototype with other strains of human noroviruses as new assays are developed.

e. Evaluate methods of immunoprophylaxis (i.e., protection by vaccination or other immunological treatment) or antiviral treatment. Most work in the next three years will focus on testing vaccine formulations and delivery as well as the mechanisms of protection. We will test for neutralizing antibodies, and whether they can be used to prevent or ameliorate infection in the chimpanzee model. We will evaluate promising anti-viral compounds for efficacy as they become available.

f. Determine the infectivity of a full-length cDNA clone of Norwalk virus or other human noroviruses. Differently-designed cDNA constructs of Norwalk virus (such as those with adaptive mutations or those transmitted from chimpanzee to chimpanzee), or cDNA clones derived from other norovirus strains may finally prove successful.

g. Determine the infectivity of inocula of known but uncharacterized intestinal viruses (principally caliciviruses other than noroviruses) and study enteric disease states for which an infectious etiology has been suspected but not proven (e.g., ulcerative colitis).

2. Summary of Recent Progress:

We have successfully and reproducibly infected chimpanzees with Norwalk virus for the past three years. We have verified that the virus can replicate in the small intestine and liver, and it is possible that additional sites of replication may exist in the host. These observations have informed our studies to identify a permissive cell culture system in the laboratory. We have shown that this animal model can be applied to the development of norovirus vaccine candidates by proving that VLPs can induce protective immunity. We have obtained intestinal and liver biopsies to begin the elucidation of the host response to Norwalk virus infection at the molecular level with gene microarray studies. These data, in part, have been presented at two international meetings (Third International Calicivirus Meeting in France and the Enteric Vaccines Meeting in Spain), and a presentation will be made at the Fourth International Calicivirus Meeting in Santa Cruz, Chile in October, 2010. A manuscript has been written and is under review.

3. Rationale:

In the absence of a cell culture system, the chimpanzee model has proven to be an essential component of our research program. It has led to the first proof-of-principle data for the potential efficacy of a norovirus VLP vaccine. For the first time, permissive cells in the gut tissue have been visualized, and the liver has been shown to be a site of norovirus replication. These studies should continue to give insight into the development of strategies to lessen the burden from norovirus gastroenteritis.

E. RATIONALE FOR USE OF ANIMALS: 1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used.

1. **Rationale for animal use: A cell culture system for Norwalk virus and other human noroviruses remains unavailable, in spite of numerous attempts to develop one. Two publications (Duizer, E., Schwab, K.J., Neill, F.H., Atmar, R.L., Koopmans, M., and Estes, M.K. J. Gen Virology 85: 79-87, 2004, and Malik, Y.S., Maherchandani, S., Allwood, P.B., and Goyal, S.M. J Applied Research 5: 312-317, 2005) document some of the efforts in this area over the last several years. A recent paper published in the journal Virology (2010 Jul 26) entitled, "Norwalk virus does not replicate in human macrophages or dendritic cells derived from the peripheral blood of susceptible humans" by Lay *et al.* illustrates the continuing unsuccessful efforts to propagate these viruses in *in vitro* systems.**

   Recently, a norovirus infectivity (but not propagation) assay based on three-dimensional cell culture in a bioreactor has been reported (Straub, T.M. *et al.*, In vitro cell culture infectivity assay for human noroviruses. Emerg Infect Dis 13: 396-403, 2007), but the technology has not been confirmed in an independent laboratory in spite of numerous attempts. Amplification of human noroviruses in an animal model or in human volunteers is presently the only way to obtain additional virus and study viral replication. In addition, studies of the immune response to a virus require an intact animal.

2. **Appropriateness of chimpanzees: Chimpanzees remain an important animal model in the study of human noroviruses because of their close genetic relatedness with humans. Chimpanzees are susceptible to subclinical Norwalk virus infection: virus shedding is prolonged, and strong immune responses are detected.**

   A gnotobiotic piglet or calf model for the study of human noroviruses developed in the laboratory of Dr. Linda Saif and colleagues has been reported (Cheetham *et al.* Pathogenesis of a genogroup II human norovirus in gnotobiotic piglets. J. Virology 80: 10372-10381, 2006; Souza *et al.* Pathogenesis and immune responses in gnotobiotic calves after infection with the genogroup II.4-HS66 strain of human norovirus. J Virology 82: 1777-1786, 2008), but virus shedding is at best transient and antibody responses are low. Replication of human noroviruses in non-human, non-chimpanzee primates (rhesus macaques or pigtail macaques) has been reported (Rockx et al. Experimental norovirus infection in non-human primates. J Med Virology 75: 313-320, 2005 and Subekti et al. Experimental infection of *Macaca* nemestrina with a Toronto Norwalk-like virus of epidemic viral gastroenteritis, J Med Virology 66: 400-406, 2002). However, there have been no follow-up reports from these groups and we and others have not been able to confirm human norovirus infection in monkeys. Human noroviruses do not replicate in rodents.

   The original report showing that chimpanzees are susceptible to Norwalk virus infection was published in 1978 (Wyatt *et al.* Experimental infection of chimpanzees with the Norwalk agent of epidemic viral gastroenteritis. J. Medical Virology 2:89-96, 1978), and we have confirmed these studies over the last three years. Technologies for the study of the human host response to norovirus infection are compatible between the human and chimpanzee genomes (e.g., microarrays) and these are being developed and pursued in our laboratory. Progress has been made in establishing the infectious dose of Norwalk virus pools and in demonstrating the evolution of the Norwalk virus genome while replicating in the animal. We have also generated preliminary evidence that an animal undergoing infection with Norwalk virus develops resistance to subsequent Norwalk virus challenge. In summary, we have shown the chimpanzee to be reproducibly susceptible to Norwalk virus infection, and new technologies are being applied to elucidate the host response at the molecular level and to begin to evaluate vaccine candidates. The high degree of identity between human and chimpanzee genomes makes this animal the closest model available in lieu of human volunteer studies.

   In Objective g, we propose searching for unknown or suspected human enteric agents in the chimpanzee animal model. The discovery of new disease agents that cannot be propagated in cell culture may lead to insight into the etiology of serious enteric illnesses caused by suspected (but unproven) infectious agents such as ulcerative colitis. These studies are not possible in adult volunteers and the genetic relatedness of chimpanzees to humans makes this animal model potentially invaluable for agent discovery and characterization.

3. **Justification for the number of animals used: The number of animals listed represents the maximum number of animals that will be considered for this protocol. They principally represent chimpanzees that are carried over from year-to-year. We generally carry over 4 animals, and bring in 4 new animals in each year. Animals will be studied in groups of from one (challenge studies) to three (vaccine trials), with an average of 2 - 3 animals per experiment, depending upon the goals of the study. For a typical vaccine "proof of principle" study, two animals will receive a test vaccine, and one animal will receive a placebo (3 total per protocol). In a challenge experiment or infectivity titration experiment, 1 or 2 animals will be tested.**

   Over the next three years, we plan to test at least 2 additional vaccine candidates (2 x 3= 6 animals). Over the next three years, at least three new (previously untested) human norovirus strains (stool filtrate or DNA clone) will be tested per year

in one animal each (3 x 1=3). At least two experiments will involve the testing of monoclonal antibodies for neutralization in 1 animal at a time (2 x 1 = 2). These protocols are the smallest numbers of animals possible to assess the infectivity of an inoculum or efficacy of a treatment or vaccine, and animals that we fail to infect may be recycled to the next protocol. A tentative list of protocols, along with the numbers of animals and re-purposing strategy is attached as an appendix.

Since noroviruses are ubiquitous pathogens and we cannot predict what proportion of available chimpanzees will have preexisting serologic evidence of immunity, individual experimental protocols will be tailored to the needs of the experiment and the availability of suitable animals.

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: Briefly explain the experimental design and specify all animal procedures performed. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following:

1. **Briefly explain the experimental design.** An outline, table or flowchart presentation might be helpful

Juvenile animals (pre-screened by an ELISA to be negative for Norwalk virus-specific serum antibodies) will be inoculated by the alimentary or intravenous (IV) route with a human stool filtrate containing either Norwalk virus or other noroviruses to assess infectious dose, or following administration of vaccine, therapeutic test compound or placebo to the animal. Some animals will be inoculated with a virus-containing filtrate that has been pretreated with an antibody preparation (e.g., monoclonal antibody) in order to assess whether the antibody neutralized virus infectivity. The infectious dose has been established for Norwalk virus - we will continue to examine additional human norovirus strains. We have found that the IV route results in reproducible infection of chimpanzees with Norwalk virus. (Please include somewhere in F.1 that you may test MAb for neutralization of virus inocula.)

The animals will be monitored for clinical signs of gastroenteritis (vomiting, diarrhea, fever, and anorexia). Stool samples will be collected and assayed for the presence of the virus by PCR. Serum samples will be collected before and after challenge for analysis of a serum antibody response to the virus and biochemical changes that may be associated with gastrointestinal disease. Intestinal biopsies (jejunal and colonic) will be obtained for analysis of cells that are permissive for Norwalk virus infection. In addition, intestinal biopsies will be used for brush border enzymatic analyses and microarray analyses. Liver biopsies (that we have shown now to be positive for the presence of norovirus genomic RNA after challenge) will be obtained in order to test for the presence of cells permissive for norovirus infection and to examine the host response to infection. In some protocols, bone marrow biopsies will be obtained for use in the isolation of norovirus-specific antibodies in our research laboratory with phage display technology.

Note: Caliciviruses are ubiquitous. Juvenile animals must be used to ensure that animals receiving Norwalk or other noroviruses have the lowest possible pre-existing anti-norovirus antibody titers prior to challenge.

Additional attempts will be made to determine the infectivity of a full-length cDNA clone of Norwalk virus or other human noroviruses by inoculating it into the jejunal mucosa via an endoscope. We will use differently-designed cDNA constructs of Norwalk virus (such as those with adaptive mutations or those transmitted from chimpanzee to chimpanzee), or cDNA clones derived from other norovirus strains.

The infectivity of clinical samples of known but uncharacterized intestinal viruses (principally caliciviruses other than noroviruses), putative infections with gastrointestinal clinical signs but from which a defined etiologic agent has not yet been recovered (e.g., outbreaks of gastroenteritis and/or diarrhea from which recognized agents have not been recovered), and specimens obtained from disease states for which an infectious etiology has been suspected but not proven (Crohn's disease, ulcerative colitis, and regional enteritis) will be tested by the intravenous inoculation route, which has been successful for norovirus samples (whereas the alimentary route was not successful). In these experiments, a detailed description of the clinical signs associated with the original illness will be provided to the facility veterinarian prior to the challenge experiment. In the event of an acute adverse event, the facility veterinarian will be entrusted with care management (see IEATC).

Brief outline of typical procedure for each animal:

a. **Identify norovirus-naïve animal.**

b. **Administer norovirus via alimentary or IV route (in some cases, following administration of vaccine candidate or treatment to the animal, or after incubation of the virus inoculum with antibodies to test for neutralization).**

c. **Monitor at least daily for clinical signs. Collect daily stool specimens, weekly serum or blood samples, and**

intermittent intestinal or colonic biopsies or bone marrow samples as needed. In some experiments, liver biopsies will be collected to evaluate virus spread in the animal.

d. Following collection of relevant samples, retire animal from protocol.

2. Injections or inoculations (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules)

Noroviruses will be administered to chimpanzees by either the alimentary or intravenous routes, depending on the specific protocol. Alimentary administration (via stomach tube) to sedated animals of 15 ml of distilled water (with or without 400 mg $NaHCO_3$) will be done two to three minutes prior to alimentary administration of virus inoculum (1 to 5 ml of human stool filtrate). The alimentary administration of stool filtrate will be performed no more than two times in an individual animal at intervals no shorter than one month.

Intrajejunal mucosal inoculation (via endoscope) of norovirus (infection) or norovirus cDNA and RNA transcripts (transfection) may be done. The volume to be inoculated by endoscope will be up to one 1 ml and will contain 100 to 1000 micrograms of nucleic acid suspended in sterile water or pharmaceutical-grade normal saline solution. No adjuvant or carrier for the DNA or RNA will be used. No needle is required. Animals will be fully anesthetized, and a flexible video endoscope will be used to perform the intramucosal inoculations in the jejunum. (Details in Section G).

Animals will be inoculated intravenously with various inocula containing Norwalk virus or other noroviruses. In initial experiments for a new inoculum, a reverse titration will be performed, beginning with approximately 1 viral genome equivalent and repeating the inoculation with tenfold-higher concentrations at monthly intervals until infection is achieved. Animals will be followed for clinical signs of illness, virus shedding, and serologic evidence of infection. If animals are infected, they may be re-challenged with the same or a larger dose of virus one or more months after the initial infection in order to characterize susceptibility to re-exposure, which has been reported for human volunteers. Inocula will consist of 0.5 - 1 ml of virus suspension, administered via the femoral or saphenous vein. The diluent for the norovirus inocula will be pharmaceutical-grade normal saline solution containing 1% plasma from a norovirus antibody-negative chimpanzee.

For Study Objective "e": In studies evaluating vaccine efficacy, the vaccine or placebo will be injected intramuscularly (or administered by the oral route) in two separate doses (50 µg of vaccine in each dose), one month apart. At one month after the second dose of vaccine, a virus challenge dose will be administered. Virus shedding in stool and serum antibody responses will be analyzed to assess whether infection with the challenge virus occurred. Protection will be defined as the absence of both virus shedding and a serologic response to the challenge virus. For assessment of neutralization activity of antibodies, the antibody (approximately 1 mg diluted in pharmaceutical-grade normal saline) will be incubated with the virus inoculum overnight at 4 degrees C. The mixture (1 ml volume) will then be administered to the animal by the I.V. or alimentary route.

For Study Objective "g": Clinical sample inocula may consist of serum, plasma, tissue homogenate, fecal filtrate or bile (diluted to reduce toxicity). Volume of the inoculum would be from 0.1 mL to 100 mL for serum or plasma (the high-end volume will require exchange plasmapheresis), and 0.1 mL to 1 mL for the other inocula. Inocula will be diluted in pharmaceutical-grade normal saline solution. Inoculation by the intravenous route would occur once per experiment except for reverse titrations, which would be repeated at intervals of two weeks to six months, depending upon the experiment.

Endoscopic jejunal intramucosal transfection:
Animals will be fully anesthetized, and a flexible video endoscope will be used to perform the intramucosal inoculations in the jejunum. The endoscope will be introduced via the orogastric route into the jejunum. The mucosa will be visualized and a small diameter piece of tubing will be inserted through the endoscope's biopsy channel. The tip of the tubing will be rounded off in order to prevent trauma to the jejunum. The tubing will be passed through the proximal end of the biopsy channel until it encounters jejunal mucosa. It will then be gently advanced into the upper layer of mucosal cells, and the norovirus sample will be injected through the tubing into the mucosa. No needle is required. The entire process can easily be visualized with the endoscopic video to ensure the tubing does not advance beyond the mucosa. The intrajejunal mucosal administration of nucleic acid via an endoscope will be performed no more than one time on an individual animal.

3. Blood withdrawals (volume, frequency, withdrawal sites, and methodology)

Plasmapheresis or phlebotomy may be performed at intervals from weekly to every other week. For Study Objective "g", we need antibody-free plasma for use in the inoculum diluent. We use this only rarely in our protocols.

During Plasmapheresis:
1. Chimpanzees will be anesthetized with the appropriate anesthetic agent (BIOQUAL SOP 1522). An IV catheter will be

placed in a peripheral vein, and the catheter will be attached to a plasma transfer pack unit (150 ml to 300 ml), collecting 0.15 ml Acid Citrate Dextrose solution per 1 ml whole blood (i.e., 1.5 ml/10 ml).
2. The collected blood sample will be centrifuged in a floor-mounted refrigerated centrifuge at 4° C for 10 minutes at 2,000 rpm. A plasma extractor will be then used to remove the plasma from the sample. The point at which the separation is stopped will be determined visually by the technician.
3. The same amount of normal saline as plasma removed (approximately 35-50 ml) will be added to the red blood cells (RBCs) and is mixed gently. This material will be then given through the IV recipient set to the animal.
4. All used material will be placed in clear plastic bags and the material will be disposed of as biohazardous waste (BIOQUAL SOP 430 and 510) and removed by a licensed contractor. Any potentially contaminated cage surface and the cart surface will be sprayed with a 10% bleach solution (made fresh daily) or a 70 % isopropyl alcohol solution.
5. During procedure chimpanzees will be observed closely for any signs of distress or respiratory arrest until the animal recovers.

In some experiments, we will obtain daily blood samples for at least one week after challenge in order to investigate the presence of viral RNA in serum.

The volume of blood withdrawn will not exceed established guidelines with respect to percentage of body weight. Chronic blood sampling will not exceed > 20% blood volume/month = 1.2% of body weight or 12 ml/kg/month; BIOQUAL, Inc., SOPs 1533 (methodology) and 1534 (blood withdrawal limits). Blood will be taken via Vacutainer 21-gauge x 1"-long blood collection needle or Abbott Butterfly 23-gauge x ¾"-long Vacutainer evacuated blood collection tube holder using Vacutainer evacuated blood collection tubes.

CBC and serum protein will be evaluated at appropriate intervals (monthly).
For example, for a 15 kg animal:
a. Phlebotomy, weekly, 15 to 40 ml, femoral vein. Serum and peripheral blood leukocytes (PBLs) will be separated and analyzed.
b. Plasmapheresis, weekly to every other week, 150 to 300 ml, femoral and saphenous veins.

For plasmapheresis, the RBCs (resuspended in pharmaceutical-grade normal saline) will be filtered through a blood filter to remove any clots, and returned to the animal.

4. **Minor surgical procedures** (that do not penetrate and expose a body cavity)

None.

5. **<u>Non-survival</u> surgical procedures** (Provide details of survival surgical procedures in Section G.)

None.

6. **Radiation** (dosage and schedule)

None.

7. **Methods of restraint** (e.g., restraint chairs, manual restraint, chemical restraint, collars, vests, harnesses, slings, etc.)

Squeeze mechanism in home cage, followed by anesthesia for alimentary tract administrations, phlebotomies, IV injections, and endoscopic procedures, will be used. Animals will be anesthetized with Ketamine or Telazol (Section I).

8. **Animal identification methods** (e.g., ear tags, tattoos, collar, cage card, etc.)

Chest or thigh tattoo

9. **Other procedures** (e.g., breeding, genotyping, etc.)

<u>Stool sample collection</u>:
This will occur from cage, or via rectal swab when the animal is anesthetized.

10. **Potentially Painful or Distressful Effects**, if any, the animals are expected to experience (e.g., pain or discomfort, ascites production, etc.), by experimental procedure if applicable. For Column E studies provide: a. A description of the procedure(s) producing pain and/or distress; b. Scientific justification why pain and/or distress can not be relieved.

Noroviruses cause acute gastroenteritis in humans. Clinical signs of gastroenteritis include vomiting, diarrhea, fever, malaise, cramping, and anorexia. We have not yet observed these clinical signs in chimpanzees following human norovirus challenge, but the possibility exists that certain viruses might induce these clinical signs.

Any clinical signs of pain or distress or gastroenteritis will be relieved by appropriate means.

11. Experimental endpoint criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical signalment, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation, or toxic chemicals are expected to cause significant clinical signs or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified. List by experimental procedure if applicable.

No clinical signs of illness are anticipated, judging from previous experience. However, animals will be assessed for fever, (by rectal thermometer when anesthetized for blood withdrawal and for other procedures, or at least monthly) and occurrence of gastrointestinal manifestations such as vomiting, diarrhea, abdominal cramping, and anorexia, and appropriate therapy will be administered. The types of therapy appropriate for treating gastrointestinal manifestations of human norovirus infection that we will consider using include use of dietary restriction, fluid therapy, and targeted treatment with agents such as histamine blockers (i.e. cimetidine, 5 to 10 mg/kg, IM or PO, bid or tid) and motility modifiers (i.e. metoclopramide, 0.3 mg/kg, IM, SQ, or PO, tid).

12. Disposition of Animals at the End of the Study (for those animals not meeting Experimental Endpoint Criteria above, e.g. retired breeders, etc.) not expected to experience clinical signs.

Noroviruses characteristically cause acute infection without clinical disease in chimpanzees and the virus is cleared after a period of shedding that can last a few days to several weeks. Long-term sequelae are not expected in the healthy chimpanzees studied in these protocols. The cessation of norovirus shedding will be part of the required endpoint for removal of the animal from a protocol. After ending the protocol, animals may be recycled to other studies or retired. In the event that a chronic norovirus infection is detected (which has been documented only in immunocompromised hosts), the animal would need to be maintained in a biocontainment environment because there is presently no treatment. We have protocols planned to evaluate neutralizing antibodies and antiviral drugs directed against norovirus, and if these prove efficacious, we would design a protocol and request permission to treat the animal in an effort to clear the virus.

13.

G. MAJOR SURVIVAL SURGERY - If proposed, complete the following.
NONE __ (check if none)

1. Describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized.

Bone Marrow Biopsy:

Up to 0.5 ml of bone marrow may be taken from the trochanteric fossae or the iliac crest of an anesthetized animal using an Illinois sternal/iliac bone marrow aspiration needle. Bone marrow aspiration procedures will be performed as sterile procedures. The hair will be removed from the site by clipping. The skin will be scrubbed 3 times with Betadine scrub, alternating with alcohol. The skin will be then painted with final Betadine solution prior to the procedure. Post-procedure analgesics (Meloxicam) will be administered over the next 5 days. After 5 days, analgesics will be administered as needed for discomfort, as instructed by the attending veterinarian. Post procedure, analgesics will be administered over the next 12 to 24 hours. After 24 hours, analgesics will be administered as needed for discomfort, as instructed by the attending veterinarian. The biopsy procedure may be repeated weekly for one month, at different locations (sites on the animal), or monthly for up to six months. The bone marrow will serve as a source of norovirus-specific immune cells for further study (e.g., creation of phage-display recombinant antibody libraries). Bioqual SOP 1528.

Endoscopic jejunal biopsy:

Animals will be fasted overnight, but allowed water ad lib. Animals will be anesthetized with Ketamine or Telazol, placed in lateral recumbency, and the endoscope advanced through the mouth and into the alimentary tract. Animals will be maintained on a heating pad and given IV fluids during the procedure. The endoscope will be advanced to the level of the jejunum and small pinch biopsies obtained. The animal is then given Yohimbine, IV to reverse the effects of Xylazine and monitored until full recovery. This procedure is done in humans routinely when needed. Ten to fifteen (10 to 15) pinch biopsies will be obtained from each animal. While we expect to perform only one endoscopic jejunal biopsy procedure per animal, unexpected histopathology in the intestinal biopsies taken to identify cells permissive for virus growth or unanticipated chronic effects of norovirus replication in the chimp host may require multiple biopsy procedures per animal - up to 6 to 10 over an 18-month period, as is done with mucosal immunity studies in macaques. No analgesics are

usually given after the procedure, but if animals exhibit discomfort, Ibuprofen (Motrin) is given as needed. Bioqual SOP 1529.

Colonic Biopsy:

Animals will be anesthetized as above. An enema may be administered to clean out the rectum, if required, prior to introduction of the endoscope. The endoscope will be advanced into the rectum and then into the colon. Small pinch biopsies will be obtained as described for jejunal biopsy. The animal will be returned to its home pen and observed closely by a trained technician until it is up and behaving normally. Animals will be given metronidazole 35 mg/kg SQ, once, immediately following the biopsy procedure, and then 25 mg/kg BID orally for 5 days following this procedure. A similar antimicrobial may be given instead, since animals often do not find oral metronidazole palatable. No analgesics are usually given after the procedure, but if animals exhibit discomfort, Ibuprofen (Motrin) is given as needed. Bioqual SOP 1577.

Liver Biopsy:

We have found that Norwalk virus RNA is present in chimpanzee liver tissue during infection, and efforts will be made to determine whether replication in this tissue might play a role in pathogenesis. Percutaneous Liver Biopsy (direct or ultrasound-guided) may be repeated weekly for six months, or more often (twice weekly) for short-term experiments (up to two weeks' duration). The liver biopsy will be percutaneous, trans-diaphragmatic, using a liver biopsy 16-gauge needle set. The skin will be clipped and surgically prepped using betadine and 70% isopropyl alcohol, alternating, for a total of three scrubs. Then a betadine solution will be applied and left on. We use sterile gloves, needles, etc. We do not initially use ultrasound because we use the central part of the right lobe, which is large and there are no anatomical structures to avoid. We do use the ultrasound if we have initial trouble getting sample, because sometimes an animal develops fibrous connective tissue at the site, in which case we use the ultrasound to see how to best position the biopsy needle. A maximum of 3 tries may be attempted per animal to get an appropriate liver biopsy sample. No analgesics are usually given after the procedure, but if animals exhibit discomfort, Ibuprofen (Motrin) is given as needed. Liver function tests will be performed as an adjunct to the liver biopsy analysis (Bioqual SOP 1510).

2. Who will perform surgery and what are their qualifications and/or experience?

Dr. Anthony L. Cook graduated from Tuskegee University School of Veterinary Medicine in 1998. He then attended the University of Florida laboratory animal residency program and received his Master's in Pathobiology in 2002. Since 2002, he has worked at BIOQUAL, Inc., mostly with nonhuman primates, rabbits, and rodents. He became a diplomate with the American College of Laboratory Animal Medicine (ACLAM) in 2005. He has 10 years of experience in surgery and with nonhuman primates, working with infectious diseases, vaccine discovery and biodefense agents. He has over 10 years experience performing bone marrow aspiration, colonic biopsy, liver biopsy and jejunal biopsy.

Dr. Wendeline Wagner graduated from veterinary school and became licensed in 1990 and completed an NIH sponsored laboratory animal residency in 1996. She gained experience in small animal and exotic practice, radiology, emergency and critical care, and general and orthopedic surgery. She has worked with all laboratory animal species, but has specialized in non-human primates, specifically in infectious disease models such as HIV/SIV vaccines and treatments and biodefense agents, since 1998.
Some of the research procedures that these models require and that Dr. Wagner has experience in are: surgical lymph node biopsies both peripheral and via laparotomy; intestinal biopsy via pinch or surgical wedge resection or endoscopy; vaginal and cervical biopsy, cerebral spinal fluid collection, bone marrow collection, bronchial alveolar lavage, liver biopsy via percutaneous needle collection or wedge resection, and various necropsy techniques including whole animal perfusion.

Dr. F. Salih Muhammad received a Bachelor of Science in Animal and Poultry Science from Tuskegee University in 2002 and in 2006, he received a Doctor of Veterinary Medicine from Tuskegee University. Dr. Muhammad is fully ACLAM (American College of Laboratory Animal Medicine) Board Eligible and passed his Specialty Boards in 2010. He carried out his post-doctoral studies during a residency at the Wake Forest University School of Medicine. Dr. Muhammad joined BIOQUAL in July, 2008 as an Assistant Director of BIOQUAL's Research Boulevard facility. Under the direction of Dr. Cook, Dr. Muhammad has over 3 years experience performing bone marrow aspiration, colonic biopsy, liver biopsy and jejuna biopsy.

3. Where will surgery be performed (Building and Room)?

BIOQUAL Research Boulevard animal procedure locations

4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:

Analgesics are usually not necessary for colonic, jejunal and liver biopsy procedures. If there is evidence of discomfort, the animals are given Ibuprofen (Motrin), 20 mg/kg, PO SID. Prior to the bone marrow procedure, the animals are given Ketoprofen, 5 mg/kg IM. After the procedure, the animals are given Meloxicam at an initial loading dose of 0.2 mg/kg, IM, SID, and then 0.1 mg/kg IM, SID for maintenance. Post procedure analgesics are given for 5 days. Post-operative care is administered and supervised by Dr. Anthony L Cook, Dr. F. Salih Muhammad and Charlene Shaver, Project Manager.

5. Has major survival surgery been performed on any animal prior to being placed on this study? Y / N [**N**]
If yes, please explain:

6. Will more than one major survival surgery be performed on an animal while on this study? Y / N / Not Applicable [**NA**]
If yes, please justify: **Note: These are not major surgical procedures.**

H. RECORDING PAIN OR DISTRESS CATEGORY - The ACUC is responsible for applying U.S. Government Principle IV, contained in Appendix 3 of the NIH Manual 3040-2: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. 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.
Check the appropriate category(ies) and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. FOR USDA REGULATED SPECIES, PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA FOR ALL OTHER SPECIES, THE JUSTIFICATION FOR SUCH STUDIES MUST BE PROVIDED IN SECTION F. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT.

	Number of animals to be used each year		
	YEAR 1	YEAR 2	YEAR 3
USDA Column C – Slight (Minimal), Momentary (Transient), or No Pain or Distress	**0**	**0**	**0**
USDA Column D - Pain or Distress Relieved By Appropriate Measures	**8**	**8**	**8**
USDA Column E - Unrelieved Pain or Distress	**0**	**0**	**0**

Describe in a brief narrative your consideration of alternatives to all procedures listed for Column D and E that may cause more than momentary or slight pain or distress to the animals, and your determination that alternatives that allow the attainment of the goals of the research were not available. Consideration must be given to alternative procedures, refinement of current procedures, and reduction in the number of animals used. Delineate below the methods and sources used in the search for and consideration of alternative procedures and method refinements. **Database references must include databases (2 or more) searched, the date of the search, period covered, and keywords used. Expert consultations must include the consultant's name and qualifications and the date and content of consult.** It is the responsibility of the Principal Investigator to justify why alternative procedures and refinements are contraindicated. Guidance and resources will be found in NIAID ACUC web document Consideration of Alternatives to Painful/Distressful Procedures.

A review of databases (MEDLINE, PIC, and Icosascan), information services (internet), knowledge of the field (from professional meetings, memberships, and frequent contact with non-NIH scientists who are prominent in the field, such as Dr. Mary Estes, Baylor College of Medicine, and Dr. Ian Clarke, Southampton University), and input from colleagues, (including Dr. Albert Kapikian, LID, NIAID) who reviewed this ASP all confirm the widely-known absence of a permissive cell culture system for the human noroviruses.

Key words used in searches included Norwalk virus, norovirus, calicivirus, Caliciviridae, unknown agents of gastroenteritis, and gastroenteritis. The dates covered by the searches range from 1972 (the date of the discovery of Norwalk virus) to the present: September 2, 2010.

There is no established animal model for human norovirus gastrointestinal disease. Recent reports of an infectivity assay in three-dimensional cell cultures and the application of potential new animal models for the study of human noroviruses such as gnotobiotic piglets and calves (summarized above in Section E.2) have yet to be confirmed and published in a laboratory other than that of the reports' origins. However, even if these new models are independently confirmed, a major advantage of the

chimpanzee model is the close genetic relatedness of chimpanzees to humans. All available human gene-based assay systems are applicable to the study of chimpanzees, and these assays will likely yield data more closely reflective of the human response to norovirus infection. Taken together, the database searches and consultations confirm that no current methodology exists that is proven and readily available for the assay of human norovirus infectivity other than the administration of virus to human volunteers or chimpanzees.

Note: Principal investigators must certify in Section N, Certification 5, that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION – For animals indicated in Section H, Column D, and for animals anesthetized to prevent distress, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and frequency of administration.
NONE __ (check if none)

Sound medical and surgical management of patients requires that choices be available for anesthesia and analgesia in order to obtain the desired response. A variety of modern anesthetic/analgesic regimens are available for nonhuman primate use alone, or in combination. Dosages provided below are approximate and may be altered, if deemed necessary by the attending veterinarian.

Ketamine HCL 100 mg/ml, 0.1 ml/kg (10 to 15 mg/kg), IM, q. 30 min.
Ketamine HCL + Acepromazine 91 mg/ml: 0.91 mg/ml, 0.1 ml/kg, IM, q. 45 min.
Ketamine HCL + Xylazine 72.7 mg/ml: 5.45 mg/ml, 0.08 ml/kg to 0.12 ml/kg, IM, q. 35-45 min.
Telazol, 3 to 6 mg/kg, IM
Butorphanol tartrate, 0.025 mg/kg, IM, q. 3-6 h.
Nalbuphine 10mg/ml, 0.05 to 0.10 ml/kg, IM, q. 3-6h.
Buprenorphine 0.3 mg/ml, 0.03 to 0.10 ml/kg, IM, q. 6-12h.
Pentazocine 30 mg/ml, 0.06 to 0.16 ml/kg, IM, q. 6-12h.
Isoflurane, endotracheal tube, to effect.
Midazolam, 5 mg, IM, q. 3 to 6h.
Meloxicam, 0.2 mg/kg loading dose; 0.1 mg/kg maintenance, IM, SID for 5 days.
Ibuprofen (Motrin), 20 mg/kg, PO, SID.
Yohimbine, 0.13 mg/kg IV, slowly.
Ketoprofen, 5 mg/kg, IM, q. 6-8 h.

J. METHOD OF EUTHANASIA AND/OR DISPOSITION OF ANIMALS AT END OF STUDY Indicate the proposed method, and, if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Guideline on Euthanasia, provide scientific justification why such methods must be used, e.g., cervical dislocation of non-anesthetized mice. Indicate the method of carcass disposal if not as MPW
NONE ___ (check if none)

This ASP describes non-lethal experiments. Animals may be recycled from this NIAID DIR ASP to other NIAID DIR ASPs. Animals will be returned to the New Iberia Research Center or other CBRPMC member institutions following completion of use under NIAID DIR ACUC-approved ASPs. Animals with evidence of chronic infection will remain in biocontainment at Bioqual until a permanent biocontainment housing solution is identified.

If medically necessary, animals will be anesthetized with Ketamine, followed by a rapid IV overdose of sodium pentobarbital (100 mg/kg) until cardiac arrest occurs.

Successful euthanasia will be confirmed by cardiac auscultation.

K. HAZARDOUS AGENTS: Use of hazardous agents requires the approval of an IC safety specialist. Registration Documents for the use of recombinant DNA or potential human pathogens may be required to be attached at the discretion of the ACUC. However, in most instances, the NIAID ACUC only requires HPRD and Recombinant DNA RD numbers to have been issued prior to ASP approval.
NONE ___ (check if none)

	Yes	No	List agents and registration document number (if applicable)
Radionuclides:		**x**	
Biological Agents with Human Pathogenic Potential:	**x**		**Norwalk virus and other human calicivirus strains (HPRD 1408)** **Human Blood, Body Fluids, and Tissues (HPRD 3915)**

Hazardous Chemicals or Drugs:		**x**	
Recombinant DNA:	**x**		**RD-01-VIII-06 (cDNA clones of the RNA genomes of 'Norwalk-like' viruses in the family *Caliciviridae*)**
Study conducted at Animal Biosafety Level (ABSL):	**ABSL-2 with ABSL-3 practices**		

Describe the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study. For studies involving the administration of radioisotopic materials to animals, provide the DRS number for the Authorized User. Also describe methods for removal of radioactive waste and, if applicable, the monitoring of radioactivity. Use of volatile anesthetics requires a description of scavenging methods used.

This study will be conducted using ABSL-3 practices. Standard BIOQUAL chimpanzee husbandry procedures incorporate ABSL-3 practices.

Additional safety considerations:

Animals on these studies will be isolated from other animals in the facility. Technicians, animal caretakers, and veterinarians will take precautions (mask, gloves, and gown) in order to prevent the introduction of infectious agents to and between chimpanzees and to protect themselves against infection with the agent being studied. At a minimum, animal excreta will be autoclaved before release from the facility.

Occupational Health and Safety considerations (vaccinations, precautions, etc):

Noroviruses are infectious to humans, so care will be taken in the facility in order to avoid transmission of the virus to personnel and other animals, including the use of protective clothing and strict infection control procedures. Noroviruses characteristically cause a self-limiting acute gastroenteritis in humans of 24 to 28 hours' duration. There are no vaccines available.

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS (e.g., cell lines, antiserum, etc.): All biologicals to be used in animals must be listed here, regardless of species. Biologicals for use in rodents must be reviewed by the CMB QA Office before they can be used. If the biological is intended for use in rodents, has a CMB Biological Assessment Form for the material been submitted to the CMB QA Office? If yes, please list the CMB QA assessment code. If no, please use the link above to obtain the submission form.
NONE ___ (check if none)

Material Name:	Source:	Sterile/Attenuated? Y/N	For use in rodents? Y/N If yes, list CMB QA assessment code.
Stool material containing infectious noroviruses	**Human stools from adult volunteers and patients involved in outbreaks**	**N**	**N; CMB QA assessment not required for biological use in NHP**
Samples containing unknown infectious agents	**Human stool or tissue samples from individuals with gastroenteritis of unknown etiology**	**N**	**N; CMB QA assessment not required for biological use in NHP**

I certify that the MAP/RAP/HAP/PCR tested materials to be used in rodents have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP/RAP/HAP/PCR tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.

KG Initials of Principal Investigator

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY: List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs.
NONE ___ (check if none)

Animals may be pair-housed as long as animals are clearly identified and both are participating on study with the same inoculum.

BIOQUAL environmental enrichment program for nonhuman primates will be maintained throughout the study (BIOQUAL SOP 601, 602, 610, 611).

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS (See instructions for further guidance.):

1. I certify that I have attended an approved NIH investigator training course. OACU Training History Search

   Year of Course Attendance: **8/17/98**

   Year (s) of Refresher Training: Refresher **01/26/2009**

2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research. Provide a brief narrative describing the means used in your determination. In addition, for renewal proposals provide citations of your published work under this ASP or describe advances made during the previous 3 year ASP cycle.

   This research project is generating new data that is unique to the norovirus field. Dr. Karin Bok has presented findings at the Third International Calicivirus Meeting in Cancun, Mexico, November, 2007 and the Enteric Vaccines Meeting in Spain, 2009. We have written and submitted a manuscript for publication. A summary of some noteworthy findings include:

   1. Norwalk virus is reproducibly infectious when given to chimpanzees by the IV route.
   2. Shedding of Norwalk virus in the feces is prolonged (up to one month) in some animals; we have been able to quantify the level of shedding over time using real-time PCR.
   3. Norwalk virus undergoes evolution following replication in the chimpanzee gut for prolonged periods, as shown by the sequence analysis of viral genomes present in stool samples collected at different timepoints post-infection.
   4. Microarray analysis of RNA from intestinal biopsies is being used to define the characteristics of the immune response in the chimpanzee (e.g., innate versus adaptive). This knowledge will be useful in the development of vaccine strategies.
   5. Bone marrow biopsies have provided material from which we have generated the first Norwalk virus-specific antibody library using phage-display technology. These antibodies may have potential in treating severe or prolonged norovirus illness, or in the prevention of disease in individuals at risk following the onset of an outbreak (such as gastroenteritis outbreaks that occur in nursing homes).
   6. Chimpanzees are protected from re-infection with Norwalk virus infection following an initial Norwalk virus infection.
   7. Norovirus VLPs can induce protective immunity when utilized as vaccines.
   8. Norovirus VLPs induce homotypic immunity, but not heterotypic immunity, in the studies conducted thus far.

3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (AEP), (or equivalent, as applicable, for contract personnel)

4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal; have attended the course "Using Animals in Intramural Research: Guidelines for Animal Users," will complete refresher training as required, and have received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); procedures for reporting animal welfare concerns. I further certify that I am responsible for the professional conduct of all personnel listed in Section A.

5. FOR ALL COLUMN D AND COLUMN E PROPOSALS (see Section H): I certify that I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) as noted in Section H, and have found no valid alternative to any procedures described herein which may cause more than slight or momentary pain or distress, whether it is relieved or not.

6. For all proposals involving the administration of chemicals and/or bioactive compounds to animals: I certify that whenever possible, in accord with USDA Animal Care Policy 3 and ARAC Guidelines for the Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals, pharmaceutical-grade chemicals/compounds will be used. The use of non-pharmaceutical-grade chemicals/compounds will be based on scientific necessity and non-availability of acceptable veterinary or human pharmaceutical-grade material, not on cost. Sufficient detail will be provided in the proposal for ACUC review, including the chemical or experimental-use grade of the compound, its formulation, and vehicle.

7. I will obtain approval from the ACUC before initiating any significant changes in this study. I will obtain approval from the NIAID ACUC-authorized official before initiating any minor changes in this study. (See NIH PM 3040-2, F.4.f.d)

Principal Investigator: **Kim Green** Signature: _____ Date: _____

O. CONCURRENCES: Proposal Number **LID 15**

Laboratory/Branch Chief certification of review and approval on the basis of scientific merit. The Scientific Director's signature is required for proposals submitted by a Laboratory or Branch Chief.

Name: **Jeffrey Cohen** Signature: Date :

Division of Occupational Health and Safety Representative certification of review and concurrence. (Required of all studies utilizing hazardous agents.)

Name: **Jeffrey Potts** Signature: Date :

Facility Manager certification of resource availability in the facility indicated to support the proposed study.

Facility: **Bioqual at Research Blvd** Name: **Brad Finneyfrock** Signature: Date:

COMMENTS:

Facility Veterinarian certification of review:

Facility: **Bioqual at Research Blvd** Name: **Anthony Cook** Signature: Date:

Attending Veterinarian certification of review:

Name: **Daniel Paré** Signature: Date :

O. FINAL APPROVAL:

CHAIRPERSON: **Ted A. Torrey** Signature: Date :