ORDER FOR SUPPLIES OR SERVICES

PAGE 1 OF PAGES 24

IMPORTANT: Mark all packages and papers with contract and/or order numbers.

1. DATE OF ORDER	
2. CONTRACT NO. *(If any)*	75N98024P01555
3. ORDER NO.	
4. REQUISITION/REFERENCE NO.	6942967

5. ISSUING OFFICE *(Address correspondence to)*

National Institutes of Health
Offices of Research Services
Bldg 13 Room 3W46
Bethesda, MD 20892

6. SHIP TO:

a. NAME OF CONSIGNEE

[redacted]

b. STREET ADDRESS

[redacted]

c. CITY	d. STATE	e. ZIP CODE
Bethesda	MD	20814

7. TO:

a. NAME OF CONTRACTOR
MARSHALL FARMS USA:1106448

b. COMPANY NAME

c. STREET ADDRESS
[redacted]

d. CITY	e. STATE	f. ZIP CODE
NORTH ROSE	NY	145169795

f. SHIP VIA

8. TYPE OF ORDER

[X] a. PURCHASE
REFERENCE YOUR:

Please furnish the following on the terms and conditions specified on both sides of this order and on the attached sheet, if any, including delivery as indicated.

[ ] b. DELIVERY

Except for billing instructions on the reverse, this delivery order is subject to instructions contained on this side only of this form and is issued subject to the terms and conditions of the above-numbered contract.

9. ACCOUNTING AND APPROPRIATION DATA	10. REQUISITIONING OFFICE
See Schedule	ORACLE

11. BUSINESS CLASSIFICATION *(Check appropriate box(es))*

[ ] a. SMALL [X] b. OTHER THAN SMALL [ ] c. DISADVANTAGED [ ] d. WOMEN-OWNED [ ] e. HUBZone
[ ] f. SERVICE-DISABLED VETERAN-OWNED [ ] g. WOMEN-OWNED SMALL BUSINESS (WOSB) ELIGIBLE UNDER THE WOSB PROGRAM [ ] h. EDWOSB

12. F.O.B. POINT: Destination

13. PLACE OF		14. GOVERNMENT B/L NO.	15. DELIVER TO F.O.B. POINT ON OR BEFORE *(Date)*	16. DISCOUNT TERMS
a. INSPECTION	b. ACCEPTANCE			
Destination	Destination		04/15/2024	

17. SCHEDULE ***(See reverse for Rejections)***

ITEM NO. (a)	SUPPLIES OR SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	UEI: DVGAR51JKM99 Continued ...					

18. SHIPPING POINT	19. GROSS SHIPPING WEIGHT	20. INVOICE NO.	17(h) TOTAL *(Cont. pages)*

21. MAIL INVOICE TO:

a. NAME	NIH Commercial Accts	$12,986.00
b. STREET ADDRESS (or P.O. Box)	Paid By: NIH Commercial Accounts Br 6701 Rockledge Drive 3rd Floor	17(i) GRAND TOTAL
c. CITY: Bethesda	d. STATE: MD	e. ZIP CODE: 20892-7784

SEE BILLING INSTRUCTIONS ON REVERSE

$12,986.00

22. UNITED STATES OF AMERICA BY *(Signature)*	23. NAME *(Typed)*
	[redacted] TITLE: CONTRACTING/ORDERING OFFICER

AUTHORIZED FOR LOCAL REPRODUCTION
PREVIOUS EDITION NOT USABLE

OPTIONAL FORM 347 (Rev. 2/2012)
Prescribed by GSA/FAR 48 CFR 53.213(f)

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52.212-4 Contract Terms and Conditions ¿ Commercial Items (May 2015)

"(a) Inspection/Acceptance. The Contractor shall only tender for acceptance those items that conform to the requirements of this contract. The Government reserves the right to inspect or test any supplies or services that have been tendered for acceptance. The Government may require repair or replacement of nonconforming supplies or reperformance of nonconforming services at no increase in contract price. If repair/replacement or reperformance will not correct the defects or is not possible, the Government may seek an equitable price reduction or adequate consideration for acceptance of nonconforming supplies or services. The Government must exercise its post-acceptance rights-

(1) Within a reasonable time after the defect was discovered or should have been discovered; and

(2) Before any substantial change occurs in the condition of the item, unless the change is due to the defect in the item.

(b) Assignment. The Contractor or its assignee may assign its rights to receive payment due as a result of performance of this contract to a bank, trust company, or other financing institution, including any Federal lending agency in accordance with the Assignment of Claims Act (31 U.S.C. 3727). However, when a third party makes payment (e.g., use of the Governmentwide commercial purchase card), the Contractor may not assign its rights to receive payment under this contract.

(c) Changes. Changes in the terms and conditions of this contract may be made only by written agreement of the parties.

(d) Disputes. This contract is subject to 41 U.S.C. chapter 71, Contract Disputes. Failure of the parties to this

Continued ...

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contract to reach agreement on any request for equitable adjustment, claim, appeal or action arising under or relating to this contract shall be a dispute to be resolved in accordance with the clause at Federal Acquisition Regulation (FAR) 52.233-1, Disputes, which is incorporated herein by reference. The Contractor shall proceed diligently with performance of this contract, pending final resolution of any dispute arising under the contract.

(e) Definitions. The clause at FAR 52.202-1, Definitions, is incorporated herein by reference.

(f) Excusable delays. The Contractor shall be liable for default unless nonperformance is caused by an occurrence beyond the reasonable control of the Contractor and without its fault or negligence such as, acts of God or the public enemy, acts of the Government in either its sovereign or contractual capacity, fires, floods, epidemics, quarantine restrictions, strikes, unusually severe weather, and delays of common carriers. The Contractor shall notify the Contracting Officer in writing as soon as it is reasonably possible after the commencement of any excusable delay, setting forth the full particulars in connection therewith, shall remedy such occurrence with all reasonable dispatch, and shall promptly give written notice to the Contracting Officer of the cessation of such occurrence.

(g) Invoice.

A. The Contractor shall submit invoices to the Department of Treasury's Invoice Processing Platform (IPP)

Admin Office:

National Institutes of Health
OD - Office of Logistics and
Acquisition Operations
Bethesda, MD 20892-7511

Period of Performance: 04/21/2024 to 04/21/2025

Continued ...

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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
1	01Beagle, male, 1429 months Catalog # : beagle1 Delivery To: 134/Steven SOLOMON Product/Service Code: 8820 Product/Service Description: LIVE ANIMALS, NOT RAISED FOR FOOD Project Data: 136732.2024.100.HNAM5R33 [redacted] [redacted].26083 RESEARCH ANIMAL-DIR USE.04/15/2024 Accounting Info: 08000420240RAD.2024.06.A100.HNAM5R3000 C.I.00576.901.9999.26083.61000001.9999 .9999.9999					
2	02Per Diem Fee Catalog # : perdiem Delivery To: 134/Steven SOLOMON Product/Service Code: 8820 Product/Service Description: LIVE ANIMALS, NOT RAISED FOR FOOD Project Data: 136732.2024.100.HNAM5R33 [redacted] [redacted].26083 RESEARCH ANIMAL-DIR USE.04/15/2024 Accounting Info: 08000420240RAD.2024.06.A100.HNAM5R3000 C.I.00576.901.9999.26083.61000001.9999 .9999.9999					
3	03Shipping and handling Catalog # : shipping Delivery To: 134/Steven SOLOMON Product/Service Code: V119 Product/Service Description: Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $12,986.00

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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	TRANSPORTATION/TRAVEL/RELOCATION- TRANSPORTATION: OTHER Project Data: 136732.2024.100.HNAM5R33 [redacted] [redacted].22006 FREIGHT OR EXPRESS TRANS.04/15/2024 Accounting Info: 08000420240RAD.2024.06.A100.HNAM5R3000 C.I.00576.901.9999.22006.61000001.9999 .9999.9999 Funded: $2,954.00 52.212-4 Contract Terms and Conditions ¿ Commercial Items (May 2015) 52.212-5 Contract Terms and Conditions Required to Implement Statutes or Executive Orders¿Commercial Items. As prescribed in 12.301(b)(4), insert the following clause: Contract Terms and Conditions Required to Implement Statutes or Executive Orders -- Commercial Items (AUG 2019) (a) The Contractor shall comply with the following Federal Acquisition Regulation (FAR) clauses, which are incorporated in this contract by reference, to implement provisions of law or Executive orders applicable to acquisitions of commercial items: (1) 52.203-19, Prohibition on Requiring Certain Internal Confidentiality Agreements or Statements (Jan 2017) (section 743 of Division E, Title VII, of the Consolidated and Further Continuing Appropriations Act 2015 (Pub. L. 113-235) and its successor provisions in subsequent appropriations acts (and as extended in continuing resolutions)). (2) 52.204-23, Prohibition on Contracting for Hardware, Software, and Services Developed or Provided by Kaspersky Lab and Other Covered Entities (Jul 2018) (Section 1634 of Pub. L. 115-91). Continued ...					

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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	(3) 52.204-25, Prohibition on Contracting for Certain Telecommunications and Video Surveillance Services or Equipment. (AUG 2019) (Section 889(a)(1)(A) of Pub. L. 115-232). (4) 52.209-10, Prohibition on Contracting with Inverted Domestic Corporations (Nov 2015) (5) 52.233-3, Protest After Award (AUG 1996) (31 U.S.C. 3553). (6) 52.233-4, Applicable Law for Breach of Contract Claim (OCT 2004) (Public Laws 108-77, 108-78 (19 U.S.C. 3805 note)). (b) The Contractor shall comply with the FAR clauses in this paragraph (b) that the contracting officer has indicated as being incorporated in this contract by reference to implement provisions of law or Executive orders applicable to acquisitions of commercial items: ___Over SAP (1) 52.203-6, Restrictions on Subcontractor Sales to the Government (Sept 2006), with Alternate I (Oct 1995) (41 U.S.C. 4704 and 10 U.S.C. 2402). ___Over $5.5m and 120 days performance (2) 52.203-13, Contractor Code of Business Ethics and Conduct (Oct 2015) (41 U.S.C. 3509)). ___ARRA (3) 52.203-15, Whistleblower Protections under the American Recovery and Reinvestment Act of 2009 (June 2010) (Section 1553 of Pub. L. 111-5). (Applies to contracts funded by the American Recovery and Reinvestment Act of 2009.) ___Over $35K (4) 52.204-10, Reporting Executive Compensation and First-Tier Subcontract Awards (Oct 2015) (Pub. L. 109-282) (31 U.S.C. 6101 note). ___(5) [Reserved]. ___Over $2.5M (6) 52.204-14, Service Contract Reporting Requirements (Jan 2014) (Pub. L. 111-117, section 743 of Div. C). ___IDIQ $2.5M (7) 52.204-15, Service Contract Reporting Requirements for Indefinite-Delivery Contracts (Jan 2014) (Pub. L. 111-117, section 743 of Div. C). Continued ...					

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	___Over $30K (8) 52.209-6, Protecting the Government¿s Interest When Subcontracting with Contractors Debarred, Suspended, or Proposed for Debarment. (Oct 2015) (31 U.S.C. 6101 note). ___Over $500K and 52.209-7 is checked (9) 52.209-9, Updates of Publicly Available Information Regarding Responsibility Matters (Oct 2018) (41 U.S.C. 2313). ___(10) [Reserved]. _For HZ solicitations and contracts_ (11)(i) 52.219-3, Notice of HUBZone Set-Aside or Sole-Source Award (Nov 2011) (15 U.S.C. 657a). ___(ii) Alternate I (Nov 2011) of 52.219-3. ___Full and open (12)(i) 52.219-4, Notice of Price Evaluation Preference for HUBZone Small Business Concerns (OCT 2014) (if the offeror elects to waive the preference, it shall so indicate in its offer) (15 U.S.C. 657a). ___(ii) Alternate I (JAN 2011) of 52.219-4. ___(13) [Reserved] _For total SB Set-Asides (no SB-type below selected)_ (14)(i) 52.219-6, Notice of Total Small Business Set-Aside (Nov 2011) (15 U.S.C. 644). ___Non-manufacturer rule waiver (ii) Alternate I (Nov 2011). ___FPI included (iii) Alternate II (Nov 2011). _For partial SB Set-asides_ (15)(i) 52.219-7, Notice of Partial Small Business Set-Aside (June 2003) (15 U.S.C. 644). ___(ii) Alternate I (Oct 1995) of 52.219-7. ___(iii) Alternate II (Mar 2004) of 52.219-7. _When value over the SAT, is in US, and not personal services_ (16) 52.219-8, Utilization of Small Business Concerns (Oct 2018) (15 U.S.C. 637(d)(2) and (3)). _Unrestricted and subcontracting possible over $700K (17)(i) 52.219-9, Small Business Subcontracting Plan (Aug 2018) (15 U.S.C. 637(d)(4)). Continued ...					

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	___(ii) Alternate I (Nov 2016) of 52.219-9. ___(iii) Alternate II (Nov 2016) of 52.219-9. ___(iv) Alternate III (Nov 2016) of 52.219-9. ____(v) Alternate IV (Aug 2018) of 52.219-9. _For Task/Delivery Orders, if applicable_ (18) 52.219-13, Notice of Set-Aside of Orders (Nov 2011)(15 U.S.C. 644(r)). ___8(a) (19) 52.219-14, Limitations on Subcontracting (Nov 2011) (15 U.S.C. 637(a)(14)). ___Includes 52.219-9 (20) 52.219-16, Liquidated Damages¿Subcontracting Plan (Jan 1999) (15 U.S.C. 637(d)(4)(F)(i)). _For SDVOSB set aside_ (21) 52.219-27, Notice of Service-Disabled Veteran-Owned Small Business Set-Aside (Nov 2011) (15 U.S.C. 657 f). ___All over micro purchase (22) 52.219-28, Post Award Small Business Program Rerepresentation (Jul 2013) (15 U.S.C. 632(a)(2)). _For EDWOSB Set-Aside_ (23) 52.219-29, Notice of Set-Aside for Economically Disadvantaged Women-Owned Small Business (EDWOSB) Concerns (Dec 2015) (15 U.S.C. 637(m)). _ For WOSB set aside _ (24) 52.219-30, Notice of Set-Aside for Women-Owned Small Business (WOSB) Concerns Eligible Under the WOSB Program (Dec 2015) (15 U.S.C. 637(m)). ___Over MPT (25) 52.222-3, Convict Labor (June 2003) (E.O. 11755). ___Over MPT (26) 52.222-19, Child Labor¿Cooperation with Authorities and Remedies (Jan 2018) (E.O. 13126). ___Over $10K (27) 52.222-21, Prohibition of Segregated Facilities (Apr 2015). ___Over $10K (28) 52.222-26, Equal Opportunity (Apr 2015) (E.O. 11246). ____(ii) Alternate I (Feb 1999) of 52.222-26. ___Over $150K except outside US (29) 52.222-35, Equal Opportunity for Veterans (Oct 2015)(38 U.S.C. 4212). Continued ...					

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	___Over $15K (30) 52.222-36, Equal Opportunity for Workers with Disabilities (Jul 2014) (29 U.S.C. 793). ____(ii) Alternate I (July 2014) of 52.222-35. ___When including 52.222-35(above)(31) 52.222-37, Employment Reports on Veterans (FEB 2016) (38 U.S.C. 4212). ___Over SAP (32) 52.222-40, Notification of Employee Rights Under the National Labor Relations Act (Dec 2010) (E.O. 13496). _All_ (33) (i) 52.222-50, Combating Trafficking in Persons (JAN 2019) (22 U.S.C. chapter 78 and E.O. 13627). ____(ii) Alternate I (Mar 2015) of 52.222-50, (22 U.S.C. chapter 78 and E.O. 13627). _Over SAP_except for commercial off the shelf_ (34) 52.222-54, Employment Eligibility Verification (Oct 2015). (E. O. 12989). (Not applicable to the acquisition of commercially available off-the-shelf items or certain other types of commercial items as prescribed in 22.1803.) ___Over $150K (35)(i) 52.223-9, Estimate of Percentage of Recovered Material Content for EPA¿Designated Items (May 2008) (42 U.S.C. 6962(c)(3)(A)(ii)). (Not applicable to the acquisition of commercially available off-the-shelf items.) ___(ii) Alternate I (May 2008) of 52.223-9 (42 U.S.C. 6962(i)(2)(C)). (Not applicable to the acquisition of commercially available off-the-shelf items.) _When buying items listed in 23.804(a) (too many to list)_ (36) 52.223-11, Ozone-Depleting Substances and High Global Warming Potential Hydrofluorocarbons (JUN 2016) (E.O. 13693). _For maintenance, service, repair or disposal of Refrigeration/AC units_ (37) 52.223-12, Maintenance, Service, Repair, or Disposal of Refrigeration Equipment and Air Conditioners (JUN 2016) (E.O. 13693). ___For all imaging equipment (copiers/printers/fax machines) when Continued ...					

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	government buys, contractor buys for government, or contractor furnishes for government use (38)(i) 52.223-13, Acquisition of EPEAT®-Registered Imaging Equipment (JUN 2014) (E.O. 13423 and 13514). ___(ii) Alternate I (Oct 2015) of 52.223-13. ___For all Television when government buys, contractor buys for government, or contractor furnishes for government use (39)(i) 52.223-14, Acquisition of EPEAT®-Registered Television (Jun 2014) (E.O.s 13423 and 13514). ___(ii) Alternate I (Jun 2014) of 52.223-14. ___Energy star or FEMP (40) 52.223-15, Energy Efficiency in Energy-Consuming Products (DEC 2007) (42 U.S.C. 8259b). ___For all personal computer products when government buys, contractor buys for government, or contractor furnishes for government use (41)(i) 52.223-16, Acquisition of EPEAT®-Registered Personal Computer Products (OCT 2015) (E.O. 13423 and 13514). ___(ii) Alternate I (Jun 2014) of 52.223-16. X_ All (42) 52.223-18, Encouraging Contractor Policies to Ban Text Messaging While Driving (AUG 2011) (E.O. 13513). _For products that may contain high global warming potential hydrofluorocarbons as a propellant, or as a solvent_ (43) 52.223-20, Aerosols (JUN 2016) (E.O. 13693). _Products that may contain high global warming potential hydrofluorocarbons or refrigerant blends containing hydrofluorocarbons as a foam blowing agent, such as building foam insulation or appliance foam insulation_ (44) 52.223-21, Foams (JUN 2016) (E.O. 13693). ___(45)(i) 52.224-3, Privacy Training (JAN 2017) (5 U.S.C. 552a). X_For all service contracts_ (ii) Alternate I (JAN 2017) of 52.224-3. ___MPT and $25K (46) 52.225-1, Buy American¿Supplies (May 2014) (41 U.S.C. chapter 83). _X_ $25K to $191K (47)(i) 52.225-3, Buy Continued ...					

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	American¿Free Trade Agreements¿Israeli Trade Act (May 2014) (41 U.S.C. chapter 83, 19 U.S.C. 3301 note, 19 U.S.C. 2112 note, 19 U.S.C. 3805 note, 19 U.S.C. 4001 note, Pub. L. 103-182, 108-77, 108-78, 108-286, 108-302, 109-53, 109-169, 109-283, 110-138, 112-41, 112-42, and 112-43. ___(ii) Alternate I (May 2014) of 52.225-3. ___(iii) Alternate II (May 2014) of 52.225-3. ___(iv) Alternate III (May 2014) of 52.225-3. ___Over $191K (48) 52.225-5, Trade Agreements (FEB 2016) (19 U.S.C. 2501, et seq., 19 U.S.C. 3301 note). _X_ All (49) 52.225-13, Restrictions on Certain Foreign Purchases (June 2008) (E.O.¿s, proclamations, and statutes administered by the Office of Foreign Assets Control of the Department of the Treasury). _Outside US performing security_ (50) 52.225-26, Contractors Performing Private Security Functions Outside the United States (Oct 2016) (Section 862, as amended, of the National Defense Authorization Act for Fiscal Year 2008; 10 U.S.C. 2302 Note). ___Local area set asides (51) 52.226-4, Notice of Disaster or Emergency Area Set-Aside (Nov 2007) (42 U.S.C. 5150). ___Local area set asides (52) 52.226-5, Restrictions on Subcontracting Outside Disaster or Emergency Area (Nov 2007) (42 U.S.C. 5150). _If allowing for financing terms, insert if financial condition is acceptable security_ (53) 52.232-29, Terms for Financing of Purchases of Commercial Items (Feb 2002) (41 U.S.C. 4505, 10 U.S.C. 2307(f)). ___Qualified installment payments (54) 52.232-30, Installment Payments for Commercial Items (Jan 2017) (41 U.S.C. 4505, 10 U.S.C. 2307(f)). ___All (55) 52.232-33, Payment by Electronic Funds Transfer¿System for Award Management (Oct 2018) (31 U.S.C. 3332). Continued ...					

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	___Exception applies (56) 52.232-34, Payment by Electronic Funds Transfer¿Other than System for Award Management (Jul 2013) (31 U.S.C. 3332). ___Purchase card (57) 52.232-36, Payment by Third Party (May 2014) (31 U.S.C. 3332). ___IT with Security (58) 52.239-1, Privacy or Security Safeguards (Aug 1996) (5 U.S.C. 552a). _All that contain 52.219-9 Small Bus. Subcontracting Plan _ (59) 52.242-5, Payments to Small Business Subcontractors (JAN 2017)(15 U.S.C. 637(d)(12)). ___Ocean transportation (60)(i) 52.247-64, Preference for Privately Owned U.S.-Flag Commercial Vessels (Feb 2006) (46 U.S.C. Appx. 1241(b) and 10 U.S.C. 2631). ___(ii) Alternate I (Apr 2003) of 52.247-64. ____(iii) Alternate II (Feb 2006) of 52.247-64 (c) The Contractor shall comply with the FAR clauses in this paragraph (c), applicable to commercial services, that the Contracting Officer has indicated as being incorporated in this contract by reference to implement provisions of law or Executive orders applicable to acquisitions of commercial items: [Contracting Officer check as appropriate.] X Service contracts that are not exempted (1) 52.222-17, Nondisplacement of Qualified Workers (May 2014) (E.O.13495). ___Subject to SCA (2) 52.222-41, Service Contract Labor Standards (Aug 2018) (41 U.S.C. chapter 67). __Subject to SCA (3) 52.222-42, Statement of Equivalent Rates for Federal Hires (May 2014) (29 U.S.C. 206 and 41 U.S.C. chapter 67). ___Subject to SCA (4) 52.222-43, Fair Labor Standards Act and Service Contract Labor Standards-Price Adjustment (Multiple Year and Option Contracts) (Aug 2018) (29 U.S.C. 206 and 41 U.S.C. chapter 67). ___Subject to SCA (5) 52.222-44, Fair Labor Standards Act and Service Contract Labor Continued ...					

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	Standards¿Price Adjustment (May 2014) (29 U.S.C. 206 and 41 U.S.C. chapter 67). _X_ Maintenance that is commercially available (6) 52.222-51, Exemption from Application of the Service Contract Labor Standards to Contracts for Maintenance, Calibration, or Repair of Certain Equipment¿Requirements (May 2014) (41 U.S.C. chapter 67). ___When using 52.222-52 (7) 52.222-53, Exemption from Application of the Service Contract Labor Standards to Contracts for Certain Services¿Requirements (May 2014) (41 U.S.C. chapter 67). _If work is performed in US and 52.222-6 or 52.222-41 also are included_ (8) 52.222-55, Minimum Wages Under Executive Order 13658 (Dec 2015) (Executive Order 13658). _When Clause 52.222-6 Construction Wage Rate or 52.222-41 (number 2 above) are included _ (9) 52.222-62, Paid Sick Leave Under Executive Order 13706 (JAN 2017) (E.O. 13706). ___Over $25K for provision service or sale of food (10) 52.226-6, Promoting Excess Food Donation to Nonprofit Organizations (May 2014) (42 U.S.C. 1792). (d) Comptroller General Examination of Record. The Contractor shall comply with the provisions of this paragraph (d) if this contract was awarded using other than sealed bid, is in excess of the simplified acquisition threshold, and does not contain the clause at 52.215-2, Audit and Records¿Negotiation. (1) The Comptroller General of the United States, or an authorized representative of the Comptroller General, shall have access to and right to examine any of the Contractor¿s directly pertinent records involving transactions related to this contract. (2) The Contractor shall make available at its offices at all reasonable times the records, materials, and other evidence for examination, audit, or reproduction, until Continued ...					

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DATE OF ORDER	CONTRACT NO.	ORDER NO.
	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	3 years after final payment under this contract or for any shorter period specified in FAR subpart 4.7, Contractor Records Retention, of the other clauses of this contract. If this contract is completely or partially terminated, the records relating to the work terminated shall be made available for 3 years after any resulting final termination settlement. Records relating to appeals under the disputes clause or to litigation or the settlement of claims arising under or relating to this contract shall be made available until such appeals, litigation, or claims are finally resolved. (3) As used in this clause, records include books, documents, accounting procedures and practices, and other data, regardless of type and regardless of form. This does not require the Contractor to create or maintain any record that the Contractor does not maintain in the ordinary course of business or pursuant to a provision of law. (e)(1) Notwithstanding the requirements of the clauses in paragraphs (a), (b), (c), and (d) of this clause, the Contractor is not required to flow down any FAR clause, other than those in this paragraph (e)(1) in a subcontract for commercial items. Unless otherwise indicated below, the extent of the flow down shall be as required by the clause¿ (i) 52.203-13, Contractor Code of Business Ethics and Conduct (Oct 2015) (41 U.S.C. 3509). (ii) 52.203-19, Prohibition on Requiring Certain Internal Confidentiality Agreements or Statements (Jan 2017) (section 743 of Division E, Title VII, of the Consolidated and Further Continuing Appropriations Act, 2015 (Pub. L. 113-235) and its successor provisions in subsequent appropriations acts (and as extended in continuing resolutions)). (iii) 52.204-23, Prohibition on Contracting for Hardware, Software, and Services Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

IMPORTANT: Mark all packages and papers with contract and/or order numbers.

DATE OF ORDER	CONTRACT NO.	ORDER NO.
	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	Developed or Provided by Kaspersky Lab and Other Covered Entities (Jul 2018) (Section 1634 of Pub. L. 115-91). (iv) 52.204-25, Prohibition on Contractin for Certain Telecommunications and Video Surveillance Services or Equipment. (AUG 2019) (Section 889(a)(1)(A) of Pub. L. 115-232). (v) 52.219-8, Utilization of Small Business Concerns (Oct 2018) (15 U.S.C. 637(d)(2) and (3)), in all subcontracts that offer further subcontracting opportunities. If the subcontract (except subcontracts to small business concerns) exceeds $700,000 ($1.5 million for construction of any public facility), the subcontractor must include 52.219-8 in lower tier subcontracts that offer subcontracting opportunities. Other Covered Entities (Jul 2018) (Section 1634 of Pub. L. 115-91). (v) 52.219-8, Utilization of Small Business Concerns (Oct 2018) (15 U.S.C. 637(d)(2) and (3)), in all subcontracts that offer further subcontracting opportunities. If the subcontract (except subcontracts to small business concerns) exceeds $700,000 ($1.5 million for construction of any public facility), the subcontractor must include 52.219-8 in lower tier subcontracts that offer subcontracting opportunities. (vi) 52.222-17, Nondisplacement of Qualified Workers (May 2014) (E.O. 13495). Flow down required in accordance with paragraph (l) of FAR clause 52.222-17. (vii) 52.222-21, Prohibition of Segregated Facilities (Apr 2015) (viii) 52.222-26, Equal Opportunity (Sept 2016) (E.O. 11246). (ix) 52.222-35, Equal Opportunity for Veterans (Oct 2019) (38 U.S.C. 4212). (x) 52.222-36, Equal Opportunity for Workers with Disabilities (Jul 2014) (29 U.S.C. 793). (xi) 52.222-37, Employment Reports on Veterans (Feb 2016) (38 U.S.C. 4212) Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

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	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	(xii) 52.222-40, Notification of Employee Rights Under the National Labor Relations Act (Dec 2010) (E.O. 13496). Flow down required in accordance with paragraph (f) of FAR clause 52.222-40. (xiii) 52.222-41, Service Contract Labor Standards (Aug 2018) (41 U.S.C. chapter 67). (xiv) (A) 52.222-50, Combating Trafficking in Persons (Jan 2019) (22 U.S.C. chapter 78 and E.O 13627). (B) Alternate I (Mar 2015) of 52.222-50 (22 U.S.C. chapter 78 and E.O 13627). (xv) 52.222-51, Exemption from Application of the Service Contract Labor Standards to Contracts for Maintenance, Calibration, or Repair of Certain Equipment-Requirements (May 2014) (41 U.S.C. chapter 67). (xvi) 52.222-53, Exemption from Application of the Service Contract Labor Standards to Contracts for Certain Services-Requirements (May 2014) (41 U.S.C. chapter 67). (xvii) 52.222-54, Employment Eligibility Verification (Oct 2015) (E.O. 12989). (xviii) 52.222-55, Minimum Wages Under Executive Order 13658 (Dec 2015). (xix) 52.222-62, Paid Sick Leave Under Executive Order 13706 (Jan 2017) (E.O. 13706). (xx)(A) 52.224-3, Privacy Training (Jan 2017) (5 U.S.C. 552a). (B) Alternate I (Jan 2017) of 52.224-3. (xxi) 52.225-26, Contractors Performing Private Security Functions Outside the United States (Oct 2016) (Section 862, as amended, of the National Defense Authorization Act for Fiscal Year 2008; 10 U.S.C. 2302 Note). (xxii) 52.226-6, Promoting Excess Food Donation to Nonprofit Organizations (May 2014) (42 U.S.C. 1792). Flow down required in accordance with paragraph (e) of FAR clause 52.226-6. (xxiii) 52.247-64, Preference for Privately Owned U.S.-Flag Commercial Vessels (Feb 2006) (46 U.S.C. Appx. 1241(b) and 10 U.S.C. 2631). Flow down required in Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

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DATE OF ORDER	CONTRACT NO.	ORDER NO.
	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	accordance with paragraph (d) of FAR clause 52.247-64. (2) While not required, the Contractor may include in its subcontracts for commercial items a minimal number of additional clauses necessary to satisfy its contractual obligations. (End of clause) ========== _Only Used if HCA waived Examination of Records by Comptroller General_ Alternate I (Feb 2000). As prescribed in 12.301(b)(4)(i), delete paragraph (d) from the basic clause, redesignate paragraph (e) as paragraph (d), and revise the reference to ¿paragraphs (a), (b), (c), or (d) of this clause¿ in the redesignated paragraph (d) to read ¿paragraphs (a), (b), and (c) of this clause.¿ _Only used for ARRA Funds_Alternate II (Jan 2017). As prescribed in 12.301(b)(4)(ii), substitute the following paragraphs (d)(1) and (e)(1) for paragraphs (d)(1) and (e)(1) of the basic clause as follows: (d)(1) The Comptroller General of the United States, an appropriate Inspector General appointed under section 3 or 8G of the Inspector General Act of 1978 (5 U.S.C. App.), or an authorized representative of either of the foregoing officials shall have access to and right to¿ (i) Examine any of the Contractor¿s or any subcontractors¿ records that pertain to, and involve transactions relating to, this contract; and (ii) Interview any officer or employee regarding such transactions. (e)(1) Notwithstanding the requirements of the clauses in paragraphs (a), (b), and (c), of this clause, the Contractor is not required to flow down any FAR clause in a subcontract for commercial items, other than¿ (i) Paragraph (d) of this clause. This paragraph flows down to all subcontracts, except the authority of the Inspector Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

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DATE OF ORDER	CONTRACT NO.	ORDER NO.
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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	General under paragraph (d)(1)(ii) does not flow down; and (ii) Those clauses listed in this paragraph (e)(1). Unless otherwise indicated below, the extent of the flow down shall be as required by the clause¿ (A) 52.203-13, Contractor Code of Business Ethics and Conduct (Oct 2015) (41 U.S.C. 3509). (B) 52.203-15, Whistleblower Protections Under the American Recovery and Reinvestment Act of 2009 (Jun 2010) (Section 1553 of Pub. L. 111-5). (C) 52.204-23, Prohibition on Contracting for Hardware, Software, and Services Developed or Provided by Kaspersky Lab and Other Covered Entities (Jul 2018) (Section 1634 of Pub. L. 115-91). (D) 52.204-25, Prohibition on Contracting for Certain Telecommunications and Video Surveillance Services or Equipment. (AUG 2019) (Section 889(a)(1)(A) of Pub. L. 115-232). (E) 52.219-8, Utilization of Small Business Concerns (Oct 2018) (15 U.S.C. 637(d)(2) and (3)), in all subcontracts that offer further subcontracting opportunities. If the subcontract (except subcontracts to small business concerns) exceeds $700,000 ($1.5 million for construction of any public facility), the subcontractor must include 52.219-8 in lower tier subcontracts that offer subcontracting opportunities. (F) 52.222-21, Prohibition of Segregated Facilities (Apr 2015). (G) 52.222-26, Equal Opportunity (Sep 2016) (E.O. 11246). (H) 52.222-35, Equal Opportunity for Veterans (Oct 2015) (38 U.S.C. 4212). (I) 52.222-36, Equal Opportunity for Workers with Disabilities (Jul 2014) (29 U.S.C. 793). (J) 52.222-40, Notification of Employee Rights Under the National Labor Relations Act (Dec 2010) (E.O. 13496). Flow down Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	required in accordance with paragraph (f) of FAR clause 52.222-40. (K) 52.222-41, Service Contract Labor Standards (Aug 2018) (41 U.S.C. chapter 67). (L) _ALL_(1) 52.222-50, Combating Trafficking in Persons (Jan 2019) (22 U.S.C. chapter 78 and E.O 13627). _Outside US_(2) Alternate I (Mar 2015) of 52.222-50 (22 U.S.C. chapter 78 and E.O 13627). (M) 52.222-51, Exemption from Application of the Service Contract Labor Standards to Contracts for Maintenance, Calibration, or Repair of Certain Equipment¿Requirements (May 2014) (41 U.S.C. chapter 67). (N) 52.222-53, Exemption from Application of the Service Contract Labor Standards to Contracts for Certain Services¿Requirements (May 2014) (41 U.S.C. chapter 67). (O) 52.222-54, Employment Eligibility Verification (Oct 2015) (Executive Order 12989). (P) 52.222-55, Minimum Wages Under Executive Order 13658 (Dec 2015). (Q) 52.222-62, Paid sick Leave Under Executive Order 13706 (Jan 2017) (E.O. 13706). (R) (1) 52.224-3, Privacy Training (Jan 2017) (5 U.S.C. 552a). (2) Alternate I (Jan 2017) of 52.224-3 (S) 52.225-26, Contractors Performing Private Security Functions Outside the United States (Oct 2016) (Section 862, as amended, of the National Defense Authorization Act for Fiscal Year 2008; 10 U.S.C. 2302 Note). (T) 52.226-6, Promoting Excess Food Donation to Nonprofit Organizations. (May 2014) (42 U.S.C. 1792). Flow down required in accordance with paragraph (e) of FAR clause52.226-6. 52.244-6, Subcontracts for Commercial Items (AUG 2019) (U) 52.247-64, Preference for Privately Owned U.S.-Flag Commercial Vessels (Feb 2006) (46 U.S.C. Appx. 1241(b) and 10 Continued ...					

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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	U.S.C. 2631). Flow down required in accordance with paragraph (d) of FAR clause 52.247-64. HHSAR CLAUSES 352.222-70 Contractor Cooperation in Equal Employment Opportunity Investigations. As prescribed in HHSAR 322.810(h), the Contracting Officer shall insert the following clause: Contractor Cooperation in Equal Employment Opportunity Investigations (December 18, 2015) (a) In addition to complying with the clause at FAR 52.222-26, Equal Opportunity, the Contractor shall, in good faith, cooperate with the Department of Health and Human Services (Agency) in investigations of Equal Employment Opportunity (EEO) complaints processed pursuant to 29 CFR part 1614. For purposes of this clause, the following definitions apply: (1) Complaint means a formal or informal complaint that has been lodged with Agency management, Agency EEO officials, the Equal Employment Opportunity Commission (EEOC), or a court of competent jurisdiction. (2) Contractor employee means all current Contractor employees who work or worked under this contract. The term also includes current employees of subcontractors who work or worked under this contract. In the case of Contractor and subcontractor employees, who worked under this contract, but who are no longer employed by the Contractor or subcontractor, or who have been assigned to another entity within the Contractor¿s or subcontractor¿s organization, the Contractor shall provide the Agency with that employee¿s last known mailing address, e-mail address, and telephone number, if that employee has been identified as a witness in an EEO complaint or investigation. (3) Good faith cooperation cited in paragraph (a) includes, but is not limited Continued ...					

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	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	to, making Contractor employees available for: (i) Formal and informal interviews by EEO counselors or other Agency officials processing EEO complaints; (ii) Formal or informal interviews by EEO investigators charged with investigating complaints of unlawful discrimination filed by Federal employees; (iii) Reviewing and signing appropriate affidavits or declarations summarizing statements provided by such Contractor employees during the course of EEO investigations; (iv) Producing documents requested by EEO counselors, EEO investigators, Agency employees, or the EEOC in connection with a pending EEO complaint; and (v) Preparing for and providing testimony in depositions or in hearings before the MSPB, EEOC and U.S. District Court. (b) The Contractor shall include the provisions of this clause in all subcontract solicitations and subcontracts awarded at any tier under this contract. (c) Failure on the part of the Contractor or its subcontractors to comply with the terms of this clause may be grounds for the Contracting Officer to terminate this contract for default. (End of clause) 352.239-74 Electronic and Information Technology Accessibility. As prescribed in HHSAR 339.203-70(b), insert the following clause: Electronic and Information Technology Accessibility (December 18, 2015) (a) Pursuant to Section 508 of the Rehabilitation Act of 1973 (29 U.S.C. 794d), as amended by the Workforce Investment Act of 1998, all electronic and information technology (EIT) supplies and services developed, acquired, or maintained under this contract or order must comply with the ¿Architectural and Transportation Barriers Compliance Board Electronic and Continued ...					

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	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	Information Technology (EIT) Accessibility Standards¿ set forth by the Architectural and Transportation Barriers Compliance Board (also referred to as the ¿Access Board¿) in 36 CFR part 1194. Information about Section 508 is available at http://www.hhs.gov/web/508. The complete text of Section 508 Final Provisions can be accessed at http://www.access-board.gov/guidelines-and-standards/communications-and-it/about-the-section-508-standards. (b) The Section 508 accessibility standards applicable to this contract or order are identified in the Statement of Work or Specification or Performance Work Statement. The contractor must provide any necessary updates to the submitted HHS Product Assessment Template(s) at the end of each contract or order exceeding the simplified acquisition threshold (see FAR 2.101) when the contract or order duration is one year or less. If it is determined by the Government that EIT supplies and services provided by the Contractor do not conform to the described accessibility standards in the contract, remediation of the supplies or services to the level of conformance specified in the contract will be the responsibility of the Contractor at its own expense. (c) The Section 508 accessibility standards applicable to this contract are: _____ (Contract staff must list applicable standards) (d) In the event of a modification(s) to this contract or order, which adds new EIT supplies or services or revises the type of, or specifications for, supplies or services, the Contracting Officer may require that the contractor submit a completed HHS Section 508 Product Assessment Template and any other additional information necessary to assist Continued ...					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

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ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	the Government in determining that the EIT supplies or services conform to Section 508 accessibility standards. Instructions for documenting accessibility via the HHS Section 508 Product Assessment Template may be found under Section 508 policy on the HHS website: (http://www.hhs.gov/web/508). If it is determined by the Government that EIT supplies and services provided by the Contractor do not conform to the described accessibility standards in the contract, remediation of the supplies or services to the level of conformance specified in the contract will be the responsibility of the Contractor at its own expense. (e) If this is an Indefinite Delivery contract, a Blanket Purchase Agreement or a Basic Ordering Agreement, the task/delivery order requests that include EIT supplies or services will define the specifications and accessibility standards for the order. In those cases, the Contractor may be required to provide a completed HHS Section 508 Product Assessment Template and any other additional information necessary to assist the Government in determining that the EIT supplies or services conform to Section 508 accessibility standards. Instructions for documenting accessibility via the HHS Section 508 Product Assessment Template may be found at http://www.hhs.gov/web/508. If it is determined by the Government that EIT supplies and services provided by the Contractor do not conform to the described accessibility standards in the provided documentation, remediation of the supplies or services to the level of conformance specified in the contract will be the responsibility of the Contractor at its own expense. (End of clause) FAR Deviation Clause Executive Order 14042, Ensuring Adequate COVID Safety Protocols for Federal Contractors Continued ...					

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	75N98024P01555	

ITEM NO. (a)	SUPPLIES/SERVICES (b)	QUANTITY ORDERED (c)	UNIT (d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED (g)
	September 30, 2021 PART 52¿SOLICITATION PROVISIONS AND CONTRACT CLAUSES ***** Subpart 52.2¿Text of Provisions and Clauses ***** [52.223-99 Ensuring Adequate COVID-19 Safety Protocols for Federal Contractors.					

TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H)) $0.00

Statement of Work

Specific Requirements

Specifically, the contractor shall provide and deliver the following:

4, Beagles, 14-29 months old, 10-15 kg, males which meet the specifications further described herein.

All animals under this contract will be required to have an updated weight at the time of shipment and undergo a veterinary exam prior to shipment to ensure the health of the animals.

Technical requirements

1. **Beagle**

A. Disease Prevention and Health Status:

All animals provided under this contract shall be in overt good health upon arrival at the NIH. In addition the animals must be research naive, physically sound and healthy, free of wounds, external parasites, and clinical signs of disease prior to shipment. No animal shall be shipped to the NIH that has a history of illness without the prior approval of the COR. A record of each individual animal shall accompany shipment to include date of birth, sex, and all medical records of clinical tests and results, treatments, and physical exams including body conditioning score. All animals shall be identified by individual tattoo numbers or ID tags.

Once an animal has been designated for the NIH and vaccinations are required by the vendor, all vaccines must be approved by the COR to ensure there will be no impact to the intended research.

B. Animal Acceptance:

All animals shall be delivered in a physically sound and healthy condition and shall be free of wounds, external parasites and clinical signs of disease. Animals with clinical signs of disease will be rejected. Only animals judged healthy based on an examination by a veterinarian upon arrival in the vendor vehicle, will be acceptable. This judgment will be based on a physical examination and observation of the animal's general appearance and state of nutrition. Animals judged as unacceptable will be rejected and returned to the contractor at no expense to the Government.

All animals shall be with all applicable regulatory documents in accordance with USDA, and a State issued Health Certificate.

Animals will not be accepted from the vendor whose colony(s) are experiencing reportable diseases as defined by the CDC or mortality or morbidity of greater than 5% for a cohort group of animals without the prior approval of the COR.

C. Transportation

Transportation shall be in compliance with CDC and Animal Welfare Act requirements Title 9, Subchapter A, Part 3, Transportation Standards. The contractor shall be responsible for meeting all requirements associated with shipment and delivery of animals. The contractor shall maintain close liaison with carrier personnel in order to assure the animals receive proper care, in accordance with USDA standards and AAALAC, International standards during transit. Animals shall be delivered in properly ventilated, escape proof, crates (cages). Each crate (cage) shall have separate water and feed containers for each compartment. Crates shall be in accordance with USDA, CDC and IATA guidelines. Standard commercial marking shall be used and shall be in compliance with USDA, and IATA guidelines. Shipping crates shall be marked, labeled or tagged as applicable and shall include (a) contractors name and address; (b) delivery order number, name and address; and (c) total number of cartons in shipment.

RECORD OF SALE OF DOGS

SELLER	BUYER	DATE OF SALE	PAGE
Marshall BioResources [redacted] North Rose, NY 14516 U. S. A.	National Inst. of Health 9000 Rockville Pike Bethesda MD 20892	4-23-24	1 OF 1
		6887993	

DEALER'S LICENSE #	BUYER'S USDA LICENSE OR RES. FACIL REG #	MARSHALL ORD #	CUSTOMER PO#	QUANTITY SHIPPED
21-A-0008		157424		8 Dogs: 8 Male, 0 Female

DOG #	SEX	BIRTH	WGT-KG	TYPE	COLOR
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	Black & White & Tan
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	"
1117923	M	2-20-23	13.6	Beagle	"
1118423	M	2-21-23	12.0	Beagle	"
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	"
1120002	M	2-23-23	13.2	Beagle	"
1120974	M	2-25-23	12.6	Beagle	"
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	"

DOG #	SEX	BIRTH	WGT-KG	TYPE	COLOR

Delivery by: Truck Freight

RECEIVED BY	SIGNATURE	TITLE	DATE

This record is required by law (7 USC 2131-2156). (9 CFR, Subchapter A, Parts 1, 2 and 3). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year or a fine of not more than $1000, or both.

DATE	04/23/2024
PRO. NO.	
B/L NO.	
P.O. NO.	Ref# 6887993

MARSHALL

BILL OF LADING SHORT FORM – ORIGINAL NOT NEGOTIATED

SHIPPER

SHIPPER NO.	157424
TRAILER NO.	
Carrier Name	Marshall Farms Group, Llc. DBA TRRT
SHIPPER NAME	Marshall BioResources
ADDRESS	[redacted]
CITY / STATE / ZIP	North Rose, NY 14516

CONSIGNEE

FULL NAME	National Institute of Health
ADDRESS	9000 Rockville Pike
ADDRESS	[redacted]
ADDRESS	
CITY / STATE / ZIP	Bethesda, MD 20892
TELEPHONE	
BUS. REG. NO.	

SEND FREIGHT BILL TO

NAME	
TELEPHONE	

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name and are classified, packaged, marked and labeled/placarded and are in all respects in proper condition for transport according to applicable international and national governmental regulations.

NUMBER OF SHIPPING UNITS	PKG TYPE	HM	DESCRIPTION OF ARTICLES, SPECIAL MARKS & EXCEPTIONS	CLASS	NMFC ITEM NO.	WEIGHT	RATE	CHARGES
4			4 Beagle Dogs, Male, 14 Months					
					WT TOTAL		TOTAL CHARGES	

COD FEE		ADDITIONAL INFO		SINGLE SHIPMENT	
PREPAID		ROUTE NO.		YES	
COLLECT		DEPT. NO.		NO	

SHIPMENT CHARGES PREPAID UNLESS MARKED COLLECT:

COLLECT	

SPECIAL INSTRUCTIONS

DATE RECEIVED	4/23/24
RECEIVED BY	[redacted]

NEW YORK STATE - DEPARTMENT OF AGRICULTURE AND MARKETS - DIVISION OF ANIMAL INDUSTRY

INTERSTATE / INTERNATIONAL HEALTH CERTIFICATE FOR DOGS

Certificate / Order Number : *157424*

I. OWNER:

Name: ***Marshall BioResources - USA***
Address: [redacted]
Street, City, Zip: ***North Rose, NY 14516***
Telephone: [redacted]
Federal ID #: *USDA #:* ***21-A-0008***
Means of transportation: ***Marshall Truck***

II. CONSIGNEE

Name ***National Inst. of Health***
Address: ***9000 Rockville Pike***
Street, City, Zip ***Bethesda MD 20892***
Country ***USA***

III. DESCRIPTION, IDENTIFICATION & VACCINATION AGAINST RABIES

Species	Sex	Birth Date	Ear Tag#	Marshall ID #	Microchip # (if applicable)	Vaccine Name	Lot #	Vaccination Date
[redacted]								
BEAGLE	Cani M	2-20-23		1117923		RabVac3	E071891A	4-02-24
BEAGLE	Cani M	2-21-23		1118423		RabVac3	E071891A	4-02-24
BEAGLE	Cani M	2-23-23		1120002		RabVac3	E071891A	4-02-24
BEAGLE	Cani M	2-25-23		1120974		RabVac3	E071891A	4-02-24
[redacted]								

IV. CLINICAL EXAMINATION

The above listed animals did not originate within an area under quarantine for Rabies, or from a site where Rabies has been detected and by reasonable investigation have not been exposed to Rabies, all within at least 6 months before shipment. Animals old enough to receive rabies vaccine were immunized as shown above with an inactivated or killed vaccine, and for canine distemper. They were found clinically free from symptoms of any contagious, infectious, or communicable disease. I also certify that the animals in this shipment are, to the best of my knowledge, acclimated to air temperatures as low as 10 degrees F.

V. NAME AND QUALIFICATION OF UNDERSIGNED (approved veterinarian/ approved official)

Approval of this certificate indicates our belief in the honesty and competency of the veterinarian signing same and is not a guarantee of health.

Name: [redacted]
Address:
Street, City, Zip: ***North Rose, NY 14516 - USA***
Telephone: [redacted]
License:

Signature, date

[redacted]

4/19/2024

Invoice

Invoice No: IN318195
Date: 23-Apr-2024
Due Date: 23-May-2024
Cust ID: 52985
Currency: USD

Marshall BioResources
[redacted]
North Rose, NY 14516-9795

Ph 315-587-2295
Fx 315-587-2109 [redacted]

BILL TO:
National Institute of Health
2115 E Jefferson St
Suite 4B 432
Bethesda MD 20892-0001

SHIP TO:
National Institute of Health
9000 Rockville Pike
Bethesda MD 20892-0001

CUSTOMER PO NUMBER	TERMS	SHIP VIA	SHIP DATE
75N98024P01555	Net 30 Days		
ORDER DATE	**SO NUMBER**		
4/16/2024	157424	Marshall Truck	4/23/2024

ITEM	QTY.	UOM	UNIT PRICE	EXTENDED
Beagle Male 14 Months old	4	EACH	[redacted]	
Beagle Per Diem Fee	4	EACH		
NOTE 4/16/2024 - 4/22/2024				
Freight				

May 23, 2024 [redacted] $11,439.60

PAID
MAY 23 2024

NOTE: 6887993

Sales Total:	**11,439.60**
Tax Total:	**0.00**
Total (USD):	**11,439.60**

5/3/24

RECORD OF SALE OF DOGS

SELLER	BUYER	DATE OF SALE	PAGE
Marshall BioResources [redacted] North Rose, NY 14516 U. S. A.	National Inst. of Health 9000 Rockville Pike Bethesda MD 20892	6-11-24	1 OF 1

DEALER'S LICENSE #	BUYER'S USDA LICENSE OR RES. FACIL REG #	MARSHALL ORD #	CUSTOMER PO#	QUANTITY SHIPPED
21-A-0008		158134	24-002634	1 Dogs: 1 Male, 0 Female

DOG #	SEX	BIRTH	WGT-KG	TYPE	COLOR	DOG #	SEX	BIRTH	WGT-KG	TYPE	COLOR
1144598	M	3-20-23	14.6	Beagle	Black & White & Tan						

Delivery by: Truck Freight

RECEIVED BY	SIGNATURE	TITLE	DATE

This record is required by law (7 USC 2131-2156). (9 CFR. Subchapter A. Parts 1. 2 and 3). Failure to maintain this record can result in a suspension or revocation of license and/or imprisonment for not more than 1 year, or a fine of not more than $1000, or both.

NEW YORK STATE - DEPARTMENT OF AGRICULTURE AND MARKETS - DIVISION OF ANIMAL INDUSTRY

INTERSTATE / INTERNATIONAL HEALTH CERTIFICATE FOR DOGS

Certificate / Order Number : *158134*

I. OWNER:

Name: *Marshall BioResources - USA*
Address: [redacted]
Street, City, Zip: *North Rose, NY 14516*
Telephone: *315-587-2295*
Federal ID #: [redacted] *USDA #:* *21-A-0008*
Means of transportation: *Marshall Truck*

II. CONSIGNEE

Name *National Inst. of Health*
Address: *9000 Rockville Pike*
Street, City, Zip *Bethesda MD 20892*
Country *USA*

III. DESCRIPTION, IDENTIFICATION & VACCINATION AGAINST RABIES

Species	Sex	Birth Date	Ear Tag#	Marshall ID #	Microchip # (if applicable)	Vaccine Name	Lot #	Vaccination Date
[redacted]								
BEAGLE	Cani M	3-20-23		1144598		RabVac3	E071891A	4-30-24
[redacted]								

IV. CLINICAL EXAMINATION

The above listed animals did not originate within an area under quarantine for Rabies, or from a site where Rabies has been detected and by reasonable investigation have not been exposed to Rabies, all within at least 6 months before shipment. Animals old enough to receive rabies vaccine were immunized as shown above with an inactivated or killed vaccine, and for canine distemper. They were found clinically free from symptoms of any contagious, infectious, or communicable disease. I also certify that the animals in this shipment are, to the best of my knowledge, acclimated to air temperatures as low as 10 degrees F.

V. NAME AND QUALIFICATION OF UNDERSIGNED (approved veterinarian/ approved official)

Approval of this certificate indicates our belief in the honesty and competency of the veterinarian signing same and is not a guarantee of health.

Name: [redacted]
Address:
Street, City, Zip: *North Rose, NY 14516 - USA*
Telephone: [redacted]
License:

Signature, date [redacted]

[redacted]

[redacted]

DATE	06/11/2024
PRO. NO.	
B/L NO.	
P.O. NO.	PO # 24-002634

MARSHALL

BILL OF LADING SHORT FORM – ORIGINAL NOT NEGOTIATED

SHIPPER

SHIPPER NO.	158134
TRAILER NO.	
Carrier Name	Marshall Farms Group, Ltd. DBA TRRT
SHIPPER NAME	Marshall BioResources
ADDRESS	[redacted]
CITY / STATE / ZIP	North Rose, NY 14516

CONSIGNEE

FULL NAME	National Institute of Health
ADDRESS	9000 Rockville Pike
ADDRESS	[redacted]
ADDRESS	
CITY / STATE / ZIP	Bethesda, MD 20892
TELEPHONE	
BUS. REG. NO.	

SEND FREIGHT BILL TO

NAME	
TELEPHONE	

NUMBER OF SHIPPING UNITS	PKG TYPE	HM	DESCRIPTION OF ARTICLES, SPECIAL MARKS & EXCEPTIONS	CLASS	NMFC ITEM NO.	WEIGHT	RATE	CHARGES
1			1 Beagle Dog, Male, 15 Months					
					WT TOTAL		TOTAL CHARGES	

COD FEE		ADDITIONAL INFO		SINGLE SHIPMENT	SHIPMENT CHARGES PREPAID UNLESS MARKED COLLECT:
PREPAID		ROUTE NO.		YES	COLLECT
COLLECT		DEPT. NO.		NO	

SPECIAL INSTRUCTIONS

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name and are classified, packaged, marked and labeled/placarded and are in all respects in proper condition for transport according to applicable international and national governmental regulations.

DATE RECEIVED 6-11-24

RECEIVED BY [redacted]

Invoice

Marshall BioResources
[redacted]
North Rose, NY 14516-9795

Ph 315-587-2295
Fx 315-587-2109
[redacted]

Invoice No:	**IN318600**
Date:	11-Jun-2024
Due Date:	11-Jul-2024
Cust ID:	52985
Currency:	USD

BILL TO:
National Institute of Health
2115 E Jefferson St
Suite 4B 432
Bethesda MD 20892-0001

SHIP TO:
National Institute of Health
9000 Rockville Pike
Bethesda MD 20892-0001

CUSTOMER PO NUMBER	TERMS	SHIP VIA	SHIP DATE
24-002634	Net 30 Days		
ORDER DATE	**SO NUMBER**		
5/14/2024	158134	Marshall Truck	6/11/2024

ITEM	QTY.	UOM	UNIT PRICE	EXTENDED
Beagle Male 15 Months old	1	EACH	[redacted]	
Beagle Per Diem Fee	1	EACH		
NOTE 5/17/2024 - 6/10/2024				
Freight - Marshall Truck	1	EACH		

Sales Total:	**4,657.00**
Tax Total:	**0.00**
Total (USD):	**4,657.00**

NATIONAL INSTITUTES OF HEALTH
ANIMAL STUDY PROPOSAL [ASP]
(6/22/2018)

PROPOSAL # CCM 23-02

APPROVAL DATE 05/23/2023

EXPIRATION DATE 05/23/2026

A. ADMINISTRATIVE DATA:

Institute or Center Clinical Center

Principal Investigator Steven Solomon, PhD

Building/Room 28/134 E-Mail ssolomon@cc.nih.gov Telephone 301-435-2287 FAX 301-480-5493

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

Division, Laboratory, or Branch: Critical Care Medicine Department

Project Title Non-Research Holding and Maintenance of Canines

Initial Submission [ ] Renewal [X] or Modification [ ] of Proposal Number CCM20-02
List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., Co-investigator(s)). A brief summary of the training and/or experience for procedures each individual 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 individual is/has achieved proficiency in those procedures shall be included in that documentation.

Steve Solomon, PhD (O) 301-435-2287 (C) 301-717-2494

B. ANIMAL REQUIREMENTS:

Species canine Age/Weight/Size 13-30 mo, 9-16kg Sex M or F Stock or Strain Beagle or Mongrel

Source(s) DVR approved Holding Location(s) Bldg 28 B-wing

Animal Procedure Location(s) n/a

Estimated Number of Animals:

100	100	100	=	300
Year 1	Year 2	Year 3		TOTAL

C. TRANSPORTATION:

Transportation of animals must conform to all NIH and Facility guidelines/policies. Describe the methods and containment to be utilized if animals will be transported between facilities. Also

include the route and elevator(s) to be utilized if animals will be transported within the Clinical Center.

Any transportation will be provided by DVR transportation services

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D. STUDY OBJECTIVES: Provide no more than a 300 word summary of the objectives of this work. Address why this work is important and how it might benefit humans and/or animals. This should be written so that a non-scientist can easily understand it. Acronyms should be defined and only used when necessary. Please eliminate or minimize abbreviations, technical terms, and jargon.

The holding protocol allows for a ready pool of dogs for approved studies which require significant lead time (4-6 weeks). In addition, animals that are not adaptable or do not meet the criteria for one protocol can be held on this protocol until they match the criteria for a future protocol.

E. RATIONALE FOR ANIMAL USE: 1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used. 4) If applicable, justify why this study uses only animals of the same sex in all experimental groups. (Use additional sheets if necessary)

1) The holding protocol allows for a ready pool of dogs for studies. In addition, animals that do not meet the criteria for one protocol can be held on this protocol until they match the criteria for a future protocol.

2) A large animal model allows hemodynamic monitoring (thermodilution balloon flow directed pulmonary catheters, direct measurements of pressures, volumes and flows, changes in myocardial function by echocardiography) as is used in humans to measure and subsequently follow cardiac function. It is not possible, in small animals, to perform serial hemodynamic interventions over days or to measure the left ventricular ejection fraction that defines the cardiovascular abnormalities of human septic shock. In addition to the reasons above, this species was selected because it is easy to work with given size and temperament. A clinically relevant model of sepsis has been developed using this species.

3) The number of dogs, 100, reflects the number of dogs to be used in projected protocols that will be submitted in the next year. Each future protocol will use approximately 20-40 dogs. These numbers allow us to keep a ready pool of dogs available for approved protocols. These numbers also include dogs that may need to be held until they meet the criteria for future protocols.

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: Briefly explain the experimental design and specify all animal procedures. 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: (Use additional sheets if necessary.)

Injections, Inoculations or Instillations (substances, e.g., infectious agents, adjuvants, medications, drugs, etc.; dose, sites, volume, route, diluent, and schedules). ACUCs will address non-pharmaceutical grade compounds IAW Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals

Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)

Non-Survival Surgical Procedures (Provide details of survival surgical procedures in Section G.)
Radiation (dosage and schedule)
Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)
Animal Identification Methods (e.g., ear tags, tattoos, collar, cage card, etc.)
Other Procedures (e.g., survival studies, tail biopsies, etc.)
Potentially Painful or Distressful Effects, if any, the animals are expected to experience (e.g., pain or distress, ascites production, etc.) For Column E studies provide: 1) a description of the procedure(s) producing pain and/or distress; 2) scientific justification why pain and/or distress cannot be relieved.
Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, 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 symptomatology 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.

Animal order requests for this ASP will be processed only when there is an additional approved research study designated for future assignment of animals.

Animals purchased from approved vendors will be received by DVR personnel at bldg. 28, NIH, and conditioned in accordance with NIH standard operating procedures. They will be evaluated for normal health condition and psychological behavior indicating that the dogs will adapt normally to handling and restraint by technicians. Vaccination requirements will be supplemented as required.

All routine veterinary and husbandry care will be provided by assigned DVR staff. All animals are provided enrichment according to DVR SOP. Animals will be transferred to approved research proposals prior to study.

G. SURVIVAL SURGERY - If proposed, complete the following:
None X Major_____ Minor_____

1. Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized. (Use additional sheets if necessary):
2. Who will perform surgery and what are their qualifications and/or experience?
3. Where will surgery be performed, Building and Room?
4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:
5. Has survival surgery been performed on any animal prior to being placed on this study? Y/N
   If yes, please explain:
6. Will more than one survival surgery be performed on an animal while on this study? Y/N
   If yes, please justify:

H. RECORDING PAIN OR DISTRESS CATEGORY - *The ACUC is responsible for applying U.S. Government Principle IV.: 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 or categories 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

Non-Research Holding and Maintenance of Canines

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 USED EACH YEAR			Year 1	Year 2	Year 3
X	**USDA Column C**	Minimal, Transient, or No Pain or Distress	100	100	100
	USDA Column D	Pain or Distress Relieved By Appropriate Measures			
	USDA Column E	Unrelieved Pain or Distress			

Describe your consideration of alternatives to procedures listed for Column D and E, and your determination that alternatives were not available. [Note: Principal investigators must certify in paragraph N.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.] Delineate the methods and sources used in the search below. **Database references must include the databases (2 or more) searched, the date of the search, period covered, and keywords used.**

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION: For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration. ACUCs will address non-pharmaceutical grade compounds IAW Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

NONE X (check if none)

J. METHOD OF EUTHANASIA 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 Guidelines on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

NONE X (check if none)

K. HAZARDOUS AGENTS: **NONE** X (check if none)
Use of hazardous agents requires the approval of an IC safety specialist.

Biological Agents with Pathogenic Potential: **NONE** X (check if none)
For guidance, see ORS/DOHS Biological Safety and Compliance. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Agent:	PRD #:	ABSL:

Additional occupational health and/or animal facility handling safety considerations:

Recombinant DNA: **NONE** X (check if none)
For guidance, see NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules FAQs.
Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Non-Research Holding and Maintenance of Canines

Recombinant DNA:	RD #:	ABSL:
Additional occupational health and/or animal facility handling safety considerations:		

Ionizing Radiation: (Radionuclides & radiation producing equipment) NONE [X] (check if none)

For guidance, see ORS/DRS/Policies/Radiation Safety Protocols Animal Studies Proposal Requirements

Yes, I will use radionuclides or radiation producing equipment as part of the experimental procedures on the ASP and all operators will be registered with Division of Radiation Safety. If an irradiator is to be used, then all individual users must comply with Division of Radiation Safety requirements for irradiator training, and all individual assessors will comply with applicable security requirements for escorts and proxy card access approval.

List of Radionuclides:

Radiological safety considerations:

Hazardous Chemicals or Drugs: **NONE_X____**(check if none)

For guidance, see NIH Policy Manual 3034 – Working with Hazardous Chemicals
Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (Title 29, Part 1910.1200(b)(3)(ii), CFR)

List of Agents:

Additional occupational health and/or animal facility handling safety considerations:

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS: **NONE X** (check if none)
List cells/tissues, sera/antibodies, viruses/parasites/bacteria, and non-synthetic biochemicals that will be introduced into research animals.

Material:	Source:	Sterile? Y	N

If derived from rodents, has the material been tested, e.g. MAP/RAP/HAP/PCR? (If Yes, attach copy of results)		
Have the tested materials been passed through rodents outside of the animal facility in question?		
Is the material derived from the original MAP/RAP/HAP/PCR tested sample?		
I certify that to the best of my knowledge that the above is complete and correct, and that the material remains uncontaminated with rodent pathogens.		

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY: **NONE X** (check if none)
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.

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. I certify that I have attended an approved NIH investigator training course.
   Month/Year of Initial Course Completion: 9/1998 ; Month/Year(s) of Refresher Training: 2/2020
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.

3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (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 completed the course "Using Animals in Intramural Research: Guidelines for Animal Users" will complete refresher training as required, and 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); and 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 momentary pain or distress, whether it is relieved or not.
6. I will obtain approval from the ACUC before initiating any significant changes in this study.

Principal Investigator: Signature Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.05.22 13:25:05 -04'00' Date_____

O. CONCURRENCES: PROPOSAL NUMBER_____(LEAVE BLANK)

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit and sex as a biological variable. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____ Signature _____Date_____

Clinical Center Biostatistics and Clinical Epidemiology Service certification of statistical review, required for Clinical Studies and selected Pilot Studies.

Signature_____ Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative

Signature_____ Date_____

DRS Safety Representative

Signature_____ Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)

Facility_____Name Sekou Savane -S Digitally signed by Sekou Savane -S Date: 2023.06.06 11:27:46 -04'00' Signature_____Date_____
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____
Facility_____Name_____Signature_____Date_____

Facility Veterinarian: Certification of Review

Name Michael C. Eichner -S Digitally signed by Michael C. Eichner -S Date: 2023.05.26 11:35:47 -04'00' Signature _____Date_____

Attending Veterinarian: Certification of Review

Name Lisa G. Portnoy -S Signature Digitally signed by Lisa G. Portnoy -S Date: 2023.05.25 16:01:09 -04'00' Date _____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson _____ Signature Dima Hammoud -S Digitally signed by Dima Hammoud -S Date: 2023.05.23 11:44:37 -04'00' Date _____

INSTRUCTIONS FOR EMERGENCY ANIMAL TREATMENT AND CARE

Principal Investigator: __Steven Solomon_____ Date form completed: _5\22\23_____
Protocol Number: _CCM23-02_____
Office Phone: _301-435-2287_____
Home Phone: _301-717-2494_____

Protocol Title: __ Non-Research Quarantine and Maintenance of Canines _____
Use a separate form if care is different for each species
Species:____canine_____ Species: _____
Species: _____ Species: _____

Animal Housing Location: Bldg ___28_____
Use separate form if care differs by location Bldg _____
Bldg _____

List of Procedures:
(surgery, tumor implant, catheter) __none_____
Primary Point of Contact (P.O.C.) in Case of Emergency: Dr. Steven Solomon
Work Tel: _301-435-2287__ Home Tel: _301-717-2494_ Pager or Cell #: _301-717-2494
Alternate Point of Contact in Case of Emergency:
Work Tel: _____ Home Tel: _____ Pager or Cell #: _____

Potential or Expected Complications: _none_____

Circumstances Requiring Contact: _____significant injury/procedure, euthanasia_____

Treatment (indicate appropriate response):
Treatment determined by veterinarian: [X] Yes [ ] No
If NO, specify restrictions as follows: _____
Specific treatment as follows: _____
What drugs are contraindicated? _____

Criteria for Euthanasia (indicate appropriate response)
At Vet discretion if poor condition, severe pain or distress: [X] Yes [ ] No
If NO, specify treatments or restrictions: _____

Notify P.O.C. *[X] Yes [ ] No
Requested euthanasia agent
and route of administration: _____
Specific criteria for euthanasia: _____

If Euthanasia is performed or animals are found dead:

a. Contact P.O.C.	[X] Yes	[ ] No
b. Refrigerate carcass	[ ] Yes	[X] No
c. Dispose of carcass	[X] Yes	[ ] No
d. Submit to DVR for necropsy	[ ] Yes	[X] No

CAN number to use for submission: _____

Additional Comments: _____

Principal Investigator: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.05.22 13:25:38 -04'00'

Signature Date

* The veterinarian will take the appropriate action in an emergency if no response from the PI/POC is received within 30 minutes after an attempt at notification is made.

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Steven Solomon

ASP#: CCM23-xx **Title: Non-Research Holding and Maintenance of Canines**

Phone No: 301-496-3091 **Bldg/Rm: 28/119** **Email: ssolomon@cc.nih.gov**
PI Course completion dates: (Initial) 1998 **(Refresher) 2022**
AU Course completion dates: (Initial) **(Refresher)**

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D		>20	
Anesthesia:				Type: inhalational, intravenous
Administration	M,R,D		>20	
Monitoring	M,R,D		>20	
Aseptic technique	M,R,D		>20	
Injections:				
SC	M,R,D		>20	
IM	M,R,D		>20	
IV	M,R,D		>20	
IP	M,R,D		>20	
Catheter placement:				
IV	R,D		>20	
IA	R,D		>20	
Intubation	R,D		>20	
Euthanasia	M,R,D		>20	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) Yes/No: This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

****

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component. date(s): ; ;

2) Yes/No There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**

3) I will be performing the following awake NHP procedures:

4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above,

OR

4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

****

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.

Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature:_____ **Date:**_____

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.04.18 15:18:42 04'00' **Date:**_____

NATIONAL INSTITUTES OF HEALTH
ANIMAL STUDY PROPOSAL [ASP]
(6/22/2018)

PROPOSAL # CCM 23-04

APPROVAL DATE 8/25/23

EXPIRATION DATE 8/25/26

A. ADMINISTRATIVE DATA:

Institute or Center Clinical Center

Principal Investigator Steven Solomon, Ph.D

Building/Room 28/134 E-Mail ssolomon@cc.nih.gov Telephone 301-435-2287 FAX 301-480-5493

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

Division, Laboratory, or Branch Clinical Center/Critical Care Medicine Department

Project Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Initial Submission [ ] Renewal [X] or Modification [ ] of Proposal Number CCM19-04

List the names of all individuals authorized to conduct procedures involving animals under this proposal and identify key personnel (i.e., Co-investigator(s)). A brief summary of the training and/or experience for procedures each individual 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 individual is/has achieved proficiency in those procedures shall be included in that documentation.

Steven Solomon, PhD (principal investigator)	435-2287	301-717-2494
Charles Natanson, MD (Co-principal investigator)	496-9770	301-675-8157
Marcus Chen, MD	496-0077	
Marvin Thomas, MD	496-5993	
Lyn Colenda, DVM	443-8521	
Verity Ford, MD	496-3091	
Melinda Fernandez, MD	496-3091	
Jasmine Holden	496-3091	
Jing Feng	496-3091	
Crystal Hite	496-3091	
Dennis Dugan	496-3091	

B. ANIMAL REQUIREMENTS:

Species: Canine Age/Weight/Size: Arrival criteria: 18-30 mo/9-15 kg

Sex: male Stock or Strain: Beagle

Source(s): Any approved source

Holding Location(s): Bldg 28, B-wing

Animal Procedure Location(s) Building/Room 14E/115A and 113(instrumentation); 28 /119 and 10/B1L02A (study); LDRR3T B1D315 (MRI), 1C560 or 1C562

Estimated Number of Animals: 24 = 24

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

For procedures in bldg. 14E:

Transportation to bldg. 14E will be provided by DVR or approved protocol personnel technicians. Animals will be transported from Bldg 28 B wing through the breezeway in a transport cage to Bldg 14E and returned on a cart and covered with blankets to Bldg 28/Rm119.

Transportation from Bldg 28 to 10/B1L02A:

CCMD staff will wheel the sedated and ventilated animal on the study cart into a temperature-controlled area of a DVR vehicle between 45-85 °F. Animals will be under sedation (see section I) throughout with transportation provided by DVR (301-496-2527) personnel. CCMD staff will be present to monitor and manage the animal in transit from Bldg. 28 to Bldg 10. The cart will be draped with a sheet or blanket to conceal the animal during transport through Bldg 10. After sanitization of the cart with a suitable disinfectant (e.g. opticide), animals will enter on the SW side of Bldg 10 through the exit located near the B1NMR loading dock entrance.

See map (Appendix A) for route to the Lab (B1L02A) and from the lab to the MR1 scanner (LDRR3T – B1D315). Any area that may have non-NIH staff access, CCMD staff will ensure the route is empty prior to moving the animal through the hall.

Lab (B1L02A) to CT (1C560 or 1C562)

Leave [defunct] inpatient pharmacy, follow signs to B1 cafeteria (straight to end of hall, right to end of hall, right, left to elevator (cab 13, 14, or West 15), take to 1st flr, go toward short hall to end, turn left and 1st right past elevator, thru double door, 100ft down hall on right.

D. STUDY OBJECTIVES: Provide no more than a 300 word summary of the objectives of this work. Address why this work is important and how it might benefit humans and/or animals. This should be written so that a non-scientist can easily understand it. Acronyms should be defined and only used when necessary. Please eliminate or minimize abbreviations, technical terms, and jargon.

Despite continued improvements in medical therapy over the decades, mortality from septic shock remains unacceptably high (30% to 50%). Septic shock occurs when the infection overtakes the body's immune system and inflammation becomes dysregulated resulting in organ injury and shock. The heart is one of many organs injured worsening cardiac function and the associated shock state. (1-3) The actual mechanism of this multiorgan dysfunction leading to death remains a decades long still unsolved mystery. The heart may offer an insight into solving this medical quandary.

We recently investigated, using cardiac MRI, the effects of sepsis on the heart in our sedated and ventilated pneumonia model of sepsis that simulates the cardiac dysfunction seen during human septic shock. Our study confirmed the well-known fact that sepsis causes reversible cardiac dysfunction leading to a reduced ability of the heart to contract (ejection fraction) and an increase in the size of the ventricle. (1) When comparing the heart injury in survivors to non-survivors, the critical factor associated with survival we showed for the first time appeared to be solely the left ventricle's ability to fully dilate during

recovery. These changes in ventricular size had previously been wrongly explained by either increases in the filling of the heart (preload) or increased resistance to outflow (afterload). (4-6) We have shown that changes in loading and afterload conditions are not responsible in this study and by exclusion, are related rather to sepsis induced changes in the wall of the heart itself. Associated with recovery of the hearts ability to eject blood or contract, we surprisingly showed for the first time, the left ventricular wall was found to lose mass (15%) and develops an increased percentage of water (edema) over 92 h (2 to 3%). This degree of edema is enough to fully explain the cardiac dysfunction seen during sepsis. There is no biochemical (troponin levels) or histological (light and electron microscopy) evidence that this loss of mass is due to muscle cell loss (myocyte drop out) or damage from decrease tissue perfusion (ischemia). The loss of mass occurs as the heart is recovering (the ejection fraction is returning to normal) suggesting that it may represent a reparative remodeling of the heart. The most abundant cell type after myocytes is endothelial cells. We hypothesize the microcirculation lined with endothelial cells are damaged but not occluded by the edema early on leading to endothelial myocyte and interstitial edema seen on histology and confirmed on MRI T2 images. Remodeling in response to damaged endothelial cells and potentially some focal myofilament autolysis, shown to be a potentially protective mechanism, may be part of the reparative process and results in restoration of vascular integrity and myocardial function. (7-9) We have identified by MRI these changes described above occur at 48 and 96h of sepsis but we have not examined by MRI the acute changes from time 0 to 48h when the development of the sepsis induced injury is occurring. In this study, we will use MRI to look at the early changes at 6 h and then every 12 h (to 54 h) after bacterial challenge in ventricular wall size, edema, and mass. We hypothesize we will see edema associated with early chamber size decrease, worse in non-survivors. The worse edema results in a restrictive like cardiomyopathy in non-survivors. After 24h, we hypothesize the loss of mass will begin, with ventricular wall thinning and ventricular chamber size increase associated with survival and recovery. Understanding in survivors verses non-survivors how damage occurs during sepsis resulting in cardiac dysfunction and the reparative process will offer insight into the cause of all organ failure and help solve this decades long mystery and allow us to design and test treatments to minimize damage or enhance recovery.

E. RATIONALE FOR ANIMAL USE: 1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used. 4) If applicable, justify why this study uses only animals of the same sex in all experimental groups. (Use additional sheets if necessary)

1) There are currently no *in vitro* models that can simulate the biologic complexity and clinically relevant endpoints encompassed by our *in vivo* pneumonia model of sepsis. *In vitro* models can address narrow functional questions but they cannot mimic the complex systems and interactions necessary to test our hypothesis. Computer simulations are beginning to show some promise in areas that have been well defined (i.e., cardiac dynamics), but are still very incomplete in infectious diseases and the immunologic response, given our imperfect understanding of these systems as a whole.

2) Many relevant ethical issues limit our ability to undertake controlled or invasive investigations in humans. Therefore, it is necessary to have animal models of septic shock, a highly lethal disease, for mechanistic and therapeutic investigations to validate interventions potentially suitable for human trials. One illustration of this point is our study in a canine sepsis model investigating the drug HA-1A, which had been approved for use in Europe and Australia and was in the approval process at the U.S. FDA (10). This study showed that HA-1A increased late mortality and our results led to a reexamination of the data

in humans which confirmed harmful effects. The results of our study in this canine model of sepsis prompted additional clinical trials that led to the removal of HA-1A from worldwide use.

We compared the appropriateness of two large animal models for this study, canine and porcine. To determine the most appropriate choice, we identified six main areas of concern by searching the literature. These include: 1) documented relevance to humans, 2) physiologic similarity to the human, 3) anatomic similarity to the human, 4) species specific study design considerations, 5) application of study results to clinically relevant findings, and 6) relevance to humans of other therapies planned as major research endeavors in the sepsis model.

The canine is the only large or small animal model shown to reproduce the distinct pattern of cardiovascular dysfunction seen over 7-10 days in human septic shock (12). It is also the only species where results of new sepsis therapies have been confirmed to have outcomes similar to human septic shock (11). Neither of these critical findings has been shown to be true for porcine models.

Further, anatomically, unlike the porcine heart, the canine heart has intrinsic collateral coronary vasculature similar to humans (13, 14). This difference in coronary circulation may cause the myocardial effects of sepsis to be expressed differently in a porcine and a canine model (13, 14). The similarity between human and canine coronary circulation also makes this large animal model more applicable to the human clinical state.

Species-specific study design issues center on the protocol requiring placement and maintenance of a tracheostomy. This procedure has been developed and utilized repeatedly in the canine model at the NIH over the past two decades. However, tracheostomy is a technically challenging procedure in the pig because the animal's neck musculature makes it difficult to maintain patency. Consequently, this may affect the ability of the stoma to be maintained, making it necessary to enroll additional training animals for adapting the procedure to porcine anatomy (14). Further, to simulate human treatments in an intensive care unit, we place percutaneous catheters and may need to replace them during the 92-hour study for monitoring and treatments. Unlike porcine blood vessels, canine vessels are well suited for this. The porcine vessels due to the thickness of the skin are difficult to access and tear with puncture much more easily which can result in lethal hemorrhagic complications.

Pharmacological agents for the treatment of human conditions are initially tested in murine models (mice or rats) by pharmaceutical companies. If found to be effective in a small animal model, it is then tested in a large animal model. The canine model is one of the most commonly used models to investigate new pharmaceutical agents prior to beginning human investigational trials and is more predictive than rodent models (15). Therefore, a canine model of septic shock is a more relevant and accepted model than a porcine model to examine the effects of newly developed treatments for sepsis.

We have performed studies in canines using this model of sepsis for over 15 years and have established the clinical similarities of this model.

For all the reasons stated above, we have selected a canine model as the most appropriate animal for this study.

3) This study will use a dose of Staphylococcal Aureus (*S. Aureus*) bacteria resulting in a 50-70% mortality. The primary endpoint of this study will be survival with secondary endpoints to include quantifying cardiovascular, pulmonary, renal and hepatic injury. Historically, 6-8 animals per group (survivors vs. non-survivors) were used as a basis to perform a power analysis to determine an appropriate sample size which has ranged up to 12 animals per group (protocols: CCM15-03, CCM16-03, CCM16-04, CCM17-01, CCM19-04).

4) We study males to limit differences associated with hormonal changes that may influence immune response and due to the extent of our historical dataset.

F. **DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES**: Briefly explain the experimental design and specify all animal procedures. 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: (Use additional sheets if necessary.)

Injections, Inoculations or Instillations (substances, e.g., infectious agents, adjuvants, medications, drugs, etc.; dose, sites, volume, route, diluent, and schedules). ACUCs will address non-pharmaceutical grade compounds IAW Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals
Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)
Non-Survival Surgical Procedures (Provide details of survival surgical procedures in Section G.)
Radiation (dosage and schedule)
Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)
Animal Identification Methods (e.g., ear tags, tattoos, collar, cage card, etc.)
Other Procedures (e.g., survival studies, tail biopsies, etc.)
Potentially Painful or Distressful Effects, if any, the animals are expected to experience (e.g., pain or distress, ascites production, etc.) For Column E studies provide: 1) a description of the procedure(s) producing pain and/or distress; 2) scientific justification why pain and/or distress cannot be relieved.
Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, 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 symptomatology 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.

Twenty-four sedated, tracheostomized and mechanically ventilated purpose bred beagles (18-30 mo / 9-15 kg) will be studied up to 92 h. All animals will receive a bacterial dose of *S. Aureus* (0.5-1.5 x 10^9 CFU/kg) that results in a 50-70% mortality. In this study, we will compare the changes in cardiac function and mass of survivors to non-survivors. Two or three animals will be enrolled each study week.

Animals will initially be studied at baseline and repeated at 6, 18, 30, 42, 54, 92 h following bacterial inoculation. At each time point, cardiac MRI, hemodynamic measures and blood sampling will be done.

Animals alive at 92h will be considered survivors and after all studies are completed will be euthanized (see Section J). Tissues are collected from all animals' post-mortem for further analysis including for pathology, transcriptomics (messenger RNA) and cell sorting (flow cytometry).

Procedure Timeline

Prior to the beginning of the study a blood sample (6 ml) will be taken from 1 of the study dogs. (see below for details)

On the first day of each study, each animal will undergo one set of procedures which will be performed in Bldg. 14E. Initially, all study animals will be placed under general anesthesia (see section I). Peripheral catheters (external jugular, femoral arterial, cephalic vein, and urinary) will be placed percutaneously and a tracheostomy will be surgically placed to maintain a secure airway during prolonged mechanical ventilation with sedation (see section G) and then returned to bldg. 28 rm 119.

Propofol infusion is initiated after instrumentation and maintained until analgesia (fentanyl) and sedation (midazolam) infusions are initiated and adjusted to levels that can maintain adequate sedation (see section I). A dexmedetomidine infusion will be added to supplement the fentanyl and midazolam infusions

if additional sedation is required. A bolus of propofol or midazolam will be used for acute changes in the plane of sedation until sedation levels can be adjusted.

After achieving adequate sedation and analgesia, the animals will be transported to 10/B1L02A (see Section C). In all studies, the animals are maintained on padded tables (24" x 36" and 5" deep). A primary concern and potential complication is airway compromise. These open tables will allow maximal access to monitoring the animals. Although the animals will not be completely in an enclosure, a 5" lip will act as a safety while they are sedated and ventilated.

Initial baseline measures are performed including hemodynamic [mean arterial pressure (MAP), pulmonary artery pressure (PAP), pulmonary artery occlusion pressure (PAOP), cardiac output (CO), and heart rate (HR)] and laboratory parameters (ABG, CBC, chemistry, troponin CPK, BNP, catecholamines and electrolytes) and cardiac MRI (Figure 1).

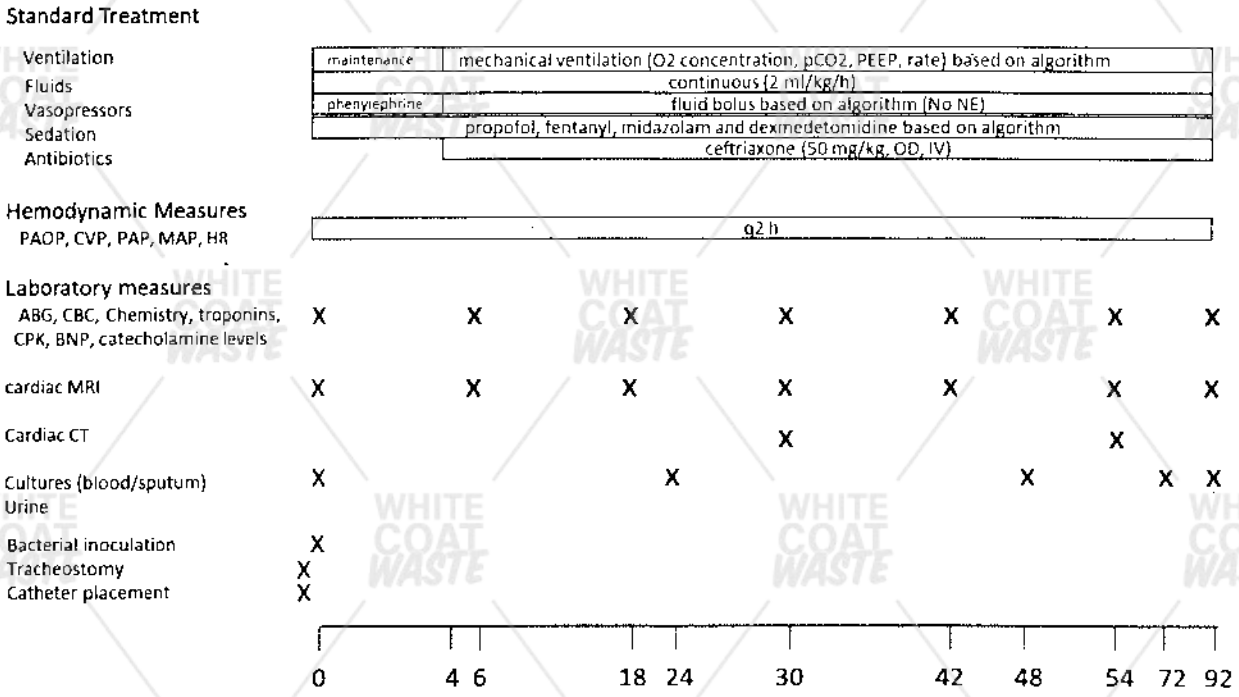
Following baseline measures, the animal will be hyper-oxygenated with 100% oxygen for two minutes and a bronchoscope (sterile at the beginning of each study, rinsed between animals) will be advanced via the tracheal tube past the carina, into the right mainstem bronchus and then into a right lower lobe segmental bronchus. A pulmonary artery occlusion catheter (Swan Ganz) will then be advanced via the suction port of the bronchoscope and wedged with the balloon inflated into a sub-segmental bronchus. Then, 0.5-3 ml solution of a known amount of *S. aureus* (0.5-1.5 x 10^9 cfu/kg) will be administered via the catheter into the subsegmental bronchus followed by 3 ml of saline. The balloon will then be deflated and the catheter removed. The animal will then be mechanically ventilated with humidified air (Vela ventilator, Carefusion and Conchatherm, Hudson Medical).

After intra-bronchial bacteria placement (T0), continuous monitoring of the animal's oxygen saturation, arterial blood pressure and central venous pressure will begin. This time point will be considered T0. A maintenance treatment regimen will be initiated until the development of sepsis (T4). The maintenance treatment regimen will include a phenylephrine bolus or infusion (10 mg/250 ml; 0.5 ml bolus or infusion titrated with a micro drip set) to maintain the animal's blood pressure at a MAP >80 mmHg, a maintenance intravenous fluid infusion (2 ml/kg/hr) of Normasol-M with 5% dextrose supplemented with KCl (27mEq/l), and ventilatory support with tidal volume (TV) of 20 ml/kg, an oxygen concentration (FiO2) of 25% and a positive end expiratory pressure (PEEP) of 5 cm H2O. The amount of potassium (K) supplementation provides a small excess of K. For example, at 11 kg, hourly K requirement = ~.07 meq/kg or ~.77 mEq, with an administration rate (0.022 L/hour * 40 mEq/L) = 0.88 mEq. (using the standard dose would have resulted in undertreating with 20 mEq/L (= 0.44 mEq/hour). This maintenance regimen will be maintained until T4.

Four hours after bacterial inoculation (T4), the phenylephrine will be turned off to determine what therapy levels will be needed. This ends the "maintenance" phase and begins the "treatment" phase. Treatment Algorithms (see Appendix C) have been designed to address changes in oxygen saturation, blood gases, hemodynamic measurements resulting in changes in fluid support, and body temperature algorithms will dictate the subsequent treatments of the animal.

At T4, ceftriaxone (50 mg/kg IV once daily (OD)), a broad spectrum antibiotic effective against *S. aureus*, will be administered q24 to T92. Famotidine, an anti-acid (1 mg/kg, IV, q12) and heparin (3000 IU, sq, q8) will be administered for gastrointestinal ulcer and deep venous thrombosis prophylaxis, respectively. General care for animals during the period of mechanical ventilation will be based on the standard of care for critically ill animals requiring sustained mechanical ventilation in the clinical setting. Every 4 h, the animal's mouth will be flushed with chlorhexidine solution and the eyes will be lubricated with a sterile ophthalmic petroleum gel and the dog will be rotated. Sterile saline (3 ml) will be instilled in the trachea followed by tracheal suctioning as needed. The inner cannula of the tracheostomy will be

cleaned with chlorhexidine and then rinsed with sterile saline two times each day or more frequently if secretions accumulate.



MRI, CT, Hemodynamic and Sampling timeline

Standard hemodynamic parameters will be measured every 2 h to 92 h. MRI, Cardiac output (CO) measured using thermodilution, ABG, CBC, chemistry, troponin, CPK, BNP, catecholamines and electrolytes will be collected at 0, 6, 18, 30, 42, 54, 92 h following bacterial inoculation (Appendix B). Blood and sputum cultures, and urinary output will be collected every 24 h. CT of the heart will be performed at 30 h and 54 h to get a better delineation of changes in wall thickness.

MRI Scan (LDRR 3T)

Animals will be transferred from a transport cart and carried into the scanner and placed on the bed. Ventilation and sedation will be transferred to MR compatible equipment (LTV1200) or extended through the wave guide. The dog will be placed on a heated and padded surface. ECG and temperature probes connected. A series of EKG-gated cardiac scan sequences will be performed to measure ventricular chamber size, mass and edema. Depending on the sequences being performed, contrast (Gadavist, 1 mmol/ml) may be used. All fluids and sedation are continued during scanning. The animal's hemodynamics and sedation is continuously monitored and recorded every 10 minutes (Appendix D). After the scan sequence is complete (about 30 minutes), the animal is returned to the lab for continued care.

CT Scan (1C560 or 1C562)

CT scans will not be performed as part of this protocol until the Department of Radiation Safety has signed off on the procedure and personnel.

Animals will be transferred from the transport cart to the scanner bed. We will perform an EKG-gated cardiac CT angiograph with contrast (Isovue, Bracco). The scan will delineate the walls of the ventricular chamber allowing us to measure changes in ventricular wall thickness. After the scan is completed, the animal will be returned to the lab.

Pre-study blood sample

One enrolled study animal will be brought from the 28 B-wing to the lab (28/119). Two highly experienced technicians will handle and aseptically draw the blood sample. The sample site along the brachial vein is prepared by removing the hair with an electric razor and cleaning with alternating three scrubs of chlorhexidine or providone-iodine and 70% alcohol. A straight needle (23 ga) with a 6 ml syringe catheter will be used to take a sample. The catheter will be removed and the vein compressed until hemostasis is achieved (5-10 min). The animal will then be returned to B-wing.

DVR will be notified prior to removing the animal from B-wing and after it is returned.

Pain Monitoring and Management

In this model of intra-bronchial pneumonia, treatment with sedation and analgesia are utilized in critical care veterinary practice to remove pain and distress, as during human illness.

The animals will be monitored continuously throughout the protocol by a CCMD staff member with no other primary responsibilities. During the day (~0600-1700), additional CCMD staff members are available in the building to assist as necessary. At night (~1700-0600), a CCMD technician will be in the room with the other technician close enough to respond quickly (within 2 min). The animals will be assessed for adequacy of sedation and analgesia every hour throughout the 92 h study. This frequency of observation is consistent with the current practice at North Carolina State University Small and Large Animal ICU (email exchange with ICU director Bernie Hanson, DVM). If there is any sign of inadequate sedation or analgesia, the animal will be treated according to "Sedation regimen in response to purposeful movement/Signs of distress" (see Section I). A supervisory or attending investigator will be available for consultation at all times. If an event should occur that is not defined in the treatment algorithm or defined in this protocol, Dr. Natanson and Dr. Portnoy or her designee will be notified.

Euthanasia criteria

Euthanasia criteria include: 1) an animal determined to be in pain and/or distress which cannot be relieved with increased levels of propofol, fentanyl, midazolam, and dexmedetomidine by the facility or Dr. Portnoy, Natanson or Solomon within 20 minutes; 2) seizure activity for greater than 2 minutes; 3) uncontrolled hemorrhage from any orifice; or 4) oxygen saturation <40% for >30 minutes. Animals selected for early euthanasia will be noted and the basis for euthanasia recorded in the medical record. At any time during the study, animals noted to be in clear distress or pain by the facility or CC ACUC veterinarian, CCMD supervisor or attending will be euthanized.

Monitoring of Bacterial Counts

Each week, the bacteria are grown and the amount is determined turbodimetrically. The bacteria are diluted to 0.5-1.5 x 10^9 CFU/kg for intrabronchial inoculation into the animal. The bacteria are then plated out on two sets of growth plates. The next day, when the bacteria have grown on these plates, an experienced microbiology laboratory technician counts the number of colonies to determine if the variance is within 10-15% of the amount determined turbodimetrically. All procedures and counts are kept in a log book (28/121).

Management and Coordination of Study Activities

The lab meets prior to each study to review any issues from the previous studies, changes in the upcoming study and scheduling staff coverage. Our staff will coordinate with the facility veterinarian, DVR staff and CC APD and coordinator as necessitated by the study.

Dr. Steven Solomon will be responsible for managing and supervising the laboratory schedule for the study and daily operations of the protocol. He will assign and schedule personnel who will conduct procedures described in the ASP and coordinate with DVR facility management. Study scheduling and animal assignments (confirmed by ear tattoo) will be given to DVR staff as soon as possible. The facility veterinarian will manage the animals when they are in the veterinary facility. Clinical status of the animals and schedule changes will be discussed between the investigative staff and the facility veterinarian as needed during the study period. Dr. Lisa Portnoy is familiar with the model and will be directly monitoring the study animals on a routine basis (daily whenever possible) and be informed of any previously unknown characteristics or problems that occur with the model. Dr. Charles Natanson, an anesthesiologist, will be consulted regarding anesthesia and sedation administration, monitoring procedures and the overnight call for this protocol.

Animal ordering

On all animal orders the following will be specified on the order request with the DVR purchasing agent to be checked prior to order placement. An affirmative response from a CCMD PI to the DVR purchasing agent that the below specifications were met will be needed to approve a purchase and delivery.

- Sex
- Breed
- Date of birth
- Weight

The PI or designee, whenever possible, will receive the animals with DVR staff to assure that the animal delivered meets the criteria specified on the purchase order and in the protocol. If this is not possible, a separate confirmation will occur by CCMD staff within 24 h following delivery and any deviations from the specified criteria will be reported to the CC-ACUC and DVR.

Record maintenance

CCMD staff will maintain a folder of all laboratory data obtained for each animal which will be available to all staff. Each animal has a separate medical record maintained with the forms used for surgery and all other interactions. A note of the condition of the animal and the 92-hour monitoring form will be included in the animal treatment record during each day of a study. In addition, notes will be made in the medical record for activities by the staff and status of the animal.

G. SURVIVAL SURGERY - If proposed, complete the following:
None X Major_____ Minor_____

1. Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized. (Use additional sheets if necessary):
2. Who will perform surgery and what are their qualifications and/or experience?
3. Where will surgery be performed, Building and Room?
4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:
5. Has survival surgery been performed on any animal prior to being placed on this study? Y/N
   If yes, please explain:
6. Will more than one survival surgery be performed on an animal while on this study? Y/N

If yes, please justify:

1. Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized.

All procedures are performed using aseptic technique including gown, gloves, booties, mask and head cover. **The eyes of the animals will be lubricated with a sterile ophthalmic petroleum gel.** Catheter (femoral and jugular) and tracheostomy sites are prepared by removing the hair and preparing the site with alternating three scrubs of chlorhexidine or providone-iodine and 70% alcohol. The urinary catheter site is cleaned with providone-iodine prior to placement.
All animals are anesthetized as described in section I.

External Jugular Catheter Placement

A catheter introducer (Maxxim Medical, Athens, TX, 8 French introducer) will be placed percutaneously into the right external jugular vein. Through this introducer, a 7 French pulmonary artery thermodilution catheter (Abbott Critical Care, Chicago, IL) will be advanced into the pulmonary artery via the external jugular vein to measure PAP, PAOP, CVP and CO, sample blood and deliver fluids. A second arterial catheter (20 ga. PTFE, Maxxim Medical, Athens, GA) will be placed percutaneously in the left external jugular vein of each animal to deliver medications. The catheter has a smaller diameter than a standard venous catheter and should be less intrusive to the dog and minimize any effect on the vessel. The catheter will be sutured (1-0 to 4-0 monofilament) in place and flushed with heparinized saline.

Femoral Arterial Catheter Placement

A single femoral artery catheter will be placed in each animal to measure femoral artery pressure. Under anesthesia, catheter sites in the groin are prepped as described above. A 20 gauge PTFE arterial catheter (Maxxim Medical, Athens, TX) will be placed percutaneously into the femoral artery. The catheter will be sutured (1-0 to 4-0 monofilament) in place and flushed with heparinized saline.

Cephalic Vein Catheter Placement

An 18 ga. catheter (Maxxim Medical, Athens, TX) will be placed percutaneously for administration of sedation.

Tracheostomy

A 5-6 cm skin incision is made on the cervical midline just posterior to the larynx. The incision is deepened to the level of the sternohyoid muscles which are separated on the midline. The trachea is dissected free from the adjacent tissues and a number 0 silk suture is passed around the trachea distal to the proposed opening in the trachea where the tracheotomy tube will be placed. A U-shaped incision on the ventral surface of the trachea is started a couple tracheal rings posterior to the larynx and extended posteriorly for two or three cartilagenous rings. The flap is raised anteriorly as a door so that the tracheostomy tube may be placed. The temporary tracheostomy tube is introduced into opening made in the trachea and the cuff is inflated. The 0 silk suture that was preplaced earlier is tied just anterior to the inflated cuff to keep the tube from accidentally being pulled out. The same silk suture is inserted through the U-shaped tracheal flap and the tracheostomy tube is secured to the flap. The subcutaneous tissue and skin are closed with 3-0 silk suture.

Foley Urinary Catheter Placement

A Foley catheter (Cook, Foley 8 Fr, 55 cm) will be introduced into the distal urethra and advanced until the tip is positioned in the bladder allowing the urine to flow. After inflation of the balloon, the catheter is secured by suturing to the skin (Coated Vicryl 3-0 [Ethicon] or similar at the discretion of the surgeon) .

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

Dr. Marvin Thomas will perform the tracheostomies. Dr. Thomas has performed more than 500 of these procedures. Dr. Solomon will instill the bacteria. Ms. Feng or Dr. Solomon will place the arterial and venous catheters, and instrument the dogs to measure pressures, volume and flows. Dr. Solomon has performed bronchoscopies in over 500 animals and Ms. Feng and Dr. Solomon have placed vascular catheters in more than 300 animals.

3. Where will surgery be performed, Building and Room?

Bldg. 14E/115A and 113B

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

n/a

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

No

6. Will more than one major survival surgery be performed on an animal while on this study?
Y/N._____
If yes, please justify:

No

H. RECORDING PAIN OR DISTRESS CATEGORY - *The ACUC is responsible for applying U.S. Government Principle IV.: 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 or categories 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 USED EACH YEAR			Year 1	Year 2	Year 3
	USDA Column C	Minimal, Transient, or No Pain or Distress			
X	**USDA Column D**	Pain or Distress Relieved By Appropriate Measures	24		
	USDA Column E	Unrelieved Pain or Distress			

Describe your consideration of alternatives to procedures listed for Column D and E, and your determination that alternatives were not available. [Note: Principal investigators must certify in paragraph N.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.] Delineate the methods and sources used in the search below. **Database references must include the databases (2 or more) searched, the date of the search, period covered, and keywords used.**

I certify that I have reviewed the pertinent scientific literature and the sources and/or databases as noted below and have found no valid alternatives to any procedures described herein which may cause more than momentary pain or distress. All literature searches for alternatives to this animal model include conducting a full Medline, Embase and Scopus search of the past 20 years of literature. The 2 search strategies covered *Alternative Sepsis Models* and *Alternative Sepsis Models and Pain,* These searches have been conducted within the last 2 months. No pertinent literature was found to answer the question that this study addresses. Although other models exist, they are inappropriate for the present study as described in Section E.

Details of database search strategies:

for Pubmed

Alternative Sepsis Models

(animal use alternatives[majr] OR animal testing alternatives[majr] OR (replace[tiab] AND reduce[tiab] AND refine[tiab]) OR (replacement[tiab] AND reduction[tiab] AND refinement[tiab]) OR (replacing[tiab] AND reducing[tiab] AND refining[tiab]) OR animal replacement[tiab] OR animal reduction[tiab] OR animal refinement[tiab] OR "three Rs"[tiab] OR 3Rs[tiab] OR NC3Rs[tiab]) AND (animal experimentation[majr] OR "animal experimentation"[tiab] OR "experimental animal"[tiab] OR "experimental animals"[tiab] OR animal welfare[majr] OR "animal welfare"[tiab] OR models, animal[majr] OR "animal model"[tiab] OR "animal models"[tiab] OR disease models, animal[majr] OR "animal disease model"[tiab] OR "animal disease models"[tiab] OR animals, laboratory[majr] OR "laboratory animal"[tiab] OR "laboratory animals"[tiab] OR "animal testing"[tiab] OR dogs[mesh] OR dog[tiab] or dogs[tiab] OR canine[tiab] OR canines[tiab] OR beagle[tiab] OR beagles[tiab] OR "sepsis model"[tiab] OR "sepsis models"[tiab] OR "septic model"[tiab] OR "septic models"[tiab]) AND (bacterial infections[majr] OR "bacterial infection"[tiab] OR "bacterial infections"[tiab] OR pneumonia, bacterial[majr] OR "bacterial pneumonia"[tiab] OR "s. aureus pneumonia"[tiab] OR sepsis[majr] OR sepsis[tiab] OR shock, septic[majr] OR septic[tiab] OR septicemia[tiab] OR bacteremia[majr] OR bacteremia[tiab] OR "blood stream infection"[tiab] OR "blood stream infections"[tiab] OR "bloodstream infection"[tiab] OR "bloodstream infections"[tiab] OR endotoxins/blood[majr]) NOT mouse[ti] NOT mice[ti] NOT murine[ti] NOT rat[ti] NOT rats[ti] NOT rodent[ti] NOT rodents[ti]
201 citation

Alternative Sepsis Models and Pain

(animal use alternatives[mesh] OR animal testing alternatives[mesh] OR (replace[tiab] AND reduce[tiab] AND refine[tiab]) OR (replacement[tiab] AND reduction[tiab] AND refinement[tiab]) OR (replacing[tiab] AND reducing[tiab] AND refining[tiab]) OR animal replacement[tiab] OR animal reduction[tiab] OR animal refinement[tiab] OR "three Rs"[tiab] OR 3Rs[tiab] OR NC3Rs[tiab]) AND (animal experimentation[mesh] OR "animal experimentation"[tiab] OR "experimental animal"[tiab] OR "experimental animals"[tiab] OR animal welfare[mesh] OR "animal welfare"[tiab] OR models, animal[mesh] OR "animal model"[tiab] OR "animal models"[tiab] OR disease models, animal[mesh] OR "animal disease model"[tiab] OR "animal disease models"[tiab] OR animals, laboratory[mesh] OR "laboratory animal"[tiab] OR "laboratory animals"[tiab] OR "animal testing"[tiab] OR dogs[mesh] OR dog[tiab] or dogs[tiab] OR canine[tiab] OR canines[tiab] OR beagle[tiab] OR beagles[tiab] OR "sepsis model"[tiab] OR "sepsis models"[tiab] OR "septic model"[tiab] OR "septic models"[tiab]) AND (bacterial infections[mesh] OR "bacterial infection"[tiab] OR "bacterial infections"[tiab] OR pneumonia, bacterial[mesh] OR "bacterial pneumonia"[tiab] OR "s. aureus pneumonia"[tiab] OR sepsis[mesh] OR sepsis[tiab] OR shock, septic[mesh] OR septic[tiab] OR septicemia[tiab] OR bacteremia[mesh] OR bacteremia[tiab] OR "blood stream infection"[tiab] OR "blood stream infections"[tiab] OR "bloodstream infection"[tiab] OR "bloodstream infections"[tiab] OR endotoxins/blood[mesh]) AND (pain[mesh] OR pain[tiab] OR pains[tiab] OR painful[tiab] OR distress[tiab] OR distressing[tiab] OR discomfort[tiab] OR suffer[tiab] OR suffering[tiab] OR Meloxicam[tiab] OR opioids[tiab] OR deep sedation[mesh] OR sedate[tiab] OR sedated[tiab] OR sedation[tiab] OR ventilated[tiab] OR anesthesia and analgesia[mesh] OR anesthesia[tiab] OR analgesia[tiab] OR analgesic[tiab] OR analgesics[tiab])
37 citations

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION: For animals indicated in Section H, Column D, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration. ACUCs will address non-pharmaceutical grade compounds IAW Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals.

NONE____(check if none)

Range of drug dosing administration
Propofol (4-12 mg/kg/h titrated to level, IV)
Fentanyl (50-200 µg/h titrated to level, IV)
Midazolam (Versed) (5-40 mg/h titrated to level, IV)
Dexmedetomidine (50-400 µg/h titrated to level, IV)
Isoflurane (0.5-5%, titrated to effect)

*Note: the titrated dose is based on our 20 year experience of an effective dose range for sedating animals between 9-15 kg which due to changing severity of illness is dynamic throughout the study.

Pre-surgical induction cocktail: ketamine (5.5mg/kg IM), acepromazine (0.1mg/kg IM), Torbugesic (0.3mg/kg IM), atropine (1.5ml/dog IM) followed by IV propofol (6 mg/kg)

Animals will be kept NPO for 12 h prior to anesthesia to minimize the possibility of aspiration.

Animals will be maintained with propofol during transport and MRI. Propofol will be titrated to maintain an effective plane of sedation. Mechanical ventilation will be provided by a portable MRI compatible

ventilator (Carefusion LTV1200). Any changes in the plane of sedation will be addressed with a bolus infusion (30 mg) of propofol followed by an adjustment in the infusion rate.

Specifics of sedation administration

The animals will be induced with the pre-surgical cocktail (see above), intubated, and mechanically ventilated. Anesthesia will be maintained with isoflurane (0.5 – 5 % titrated to effect). After the above procedures have been performed, isoflurane will be terminated and upon being able to breath independently, sedation will be initiated with propofol (80 mg/h) and maintained for transfer to Bldg. 28. Upon return of the animal to Bldg. 28, the animals will be ventilated (tidal volume: 20 ml/kg, FiO2- 25%, ventilation rate = 15 breaths/minute, PEEP = 5 cmH_2O) with humidified air. Analgesia with fentanyl (50 µg/h infusion, 1 ml/h) and sedation with midazolam (9 mg/h infusion, 3 ml/h) will be initiated. Propofol administration will be terminated after fentanyl and midazolam infusions are able to maintain adequate sedation (see *sedation infusion incrementation regimen* below). A dexmedetomidine infusion (5-40 µg/h) will be used to supplement the fentanyl and midazolam infusions if an animal requires additional sedation throughout the study. The level of anesthesia will be evaluated continuously to assess the adequacy of the fentanyl, midazolam and dexmedetomidine infusions and titrated upward until effective (see algorithm below)

Algorithm for Criteria for adequacy of sedation

1) The animal should be breathing comfortably in synchrony with the ventilator with jaw tone present but without voluntary limb movement.
2) The eyeballs should remain central in the orbit.
3) The animal should be unresponsive to light tactile stimuli.
4) Palpebral reflexes not present **(Criteria for reducing sedation)**.

Sedation Bolus regimen in response to purposeful movement/Signs of distress
(If insufficient sedation after 3 minutes move to next step until purposeful movement ceases)

1) Propofol – 3 ml bolus (30 mg), repeat up to 3 times for each event

Sedation infusion incrementation regimen
(After initial bolus, increments should be made 20 minutes apart until an adequate plane of sedation is reached)

1) Increase midazolam rate by 1 ml/h (3 mg/ml) AND fentanyl by 0.5 ml/h (50 ug/ml),
   *Repeat until rate of 7 ml/h midazolam and 3 ml/h fentanyl
2) Increase midazolam rate by 1 ml/h (3 mg/ml) to 8 ml/h
3) Increase dexmedetomidine rate by 1 ml/h (5 ug/ml) to effect

---

J. METHOD OF EUTHANASIA 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 Guidelines on Euthanasia, provide justification why such methods must be used. Indicate the method of carcass disposal if not as MPW.

NONE____(check if none)

The animals will already be sedated before euthanasia. A pentobarbital and phenytoin mixture [Euthanasia – D 75 mg/kg of Sodium Pentobarbital (390 mg/ml) IV] will be given to euthanize the animal. Death will be verified immediately following injection by confirming the absence of heart sounds, respiratory effort, papillary response, and an arterial blood pressure. Carcasses being sent to veterinary

pathology will be placed in double lined brown boxes. The boxes will be placed in the refrigerator.

K. HAZARDOUS AGENTS: **NONE____**(check if none)
Use of hazardous agents requires the approval of an IC safety specialist.

Biological Agents with Pathogenic Potential: **NONE___**(check if none)
For guidance, see ORS/DOHS Biological Safety and Compliance. Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Agent: *S. Aureus*	PRD #: 7644	ABSL: 2 for preparation and inoculation

Additional occupational health and/or animal facility handling safety considerations:

BSO Comments: 7644 amend 230728. Work with Staphylococcus aureus is previously approved at BSL-2. A 1-2-3 poster must be displayed in the laboratory and all personnel on this registration must maintain annual lab safety training/refresher through DOHS (https://www.safetytraining.nih.gov/). Care should be taken to minimize worker exposure to aerosols, such as working in a certified Class II BSC and using gasketed bucket covers when centrifuging infectious material. Work with the bacteria in animals as described in the ASP renewal entitled " *Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy*" (CCM23-04) is approved at the established ABSL-2 at the time of administration, followed by ABSL-1 housing and practices post delivery. AEP for personnel on animal studies. No recombinant work is submitted, reviewed, or approved in this amendment.

Recombinant DNA: **NONE X** (check if none)
For guidance, see NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules FAQs.
Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Recombinant DNA:	RD #:	ABSL:

Additional occupational health and/or animal facility handling safety considerations:

Ionizing Radiation: (Radionuclides & radiation producing equipment)

NONE__ ___ (check if none)

For guidance, see ORS/DRS/Policies/Radiation Safety Protocols Animal Studies Proposal Requirements

CT angiograph with contrast (Isovue, Bracco)

Hazardous Chemicals or Drugs:

NONE_____(check if none)

For guidance, see NIH Policy Manual 3034 – Working with Hazardous Chemicals
Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (Title 29, Part 1910.1200(b)(3)(ii), CFR)

List of Agents: Isoflurane, torbugesic

Additional occupational health and/or animal facility handling safety considerations:

All chemicals should be handled with prudent practices by trained personnel who know how to properly prepare, label, and dispose of chemical compounds

according to NIH Waste Disposal Guide. Before handling any chemical compound, personnel should read and understand the Safety Data Sheets (SDS) and the NIH Chemical Hygiene Plan (CHP). Keep a copy of the SDS and CHP readily available for review. Isoflurane will be scavenged in a chemical fume hood, downdraft table, anesthesia machine Activated Charcoal Adsorption Filter Capture system, local active exhaust ventilation system, or possible a combination of these methods as approved by the Division of Occupational Health and Safety (DOHS).

Sodium Pentobarbital and Torbugesic are suspected reproductive hazards. NIH Department of Occupational Health & Safety (DOHS) and Occupational Medical Service (OMS) is available for counseling women of childbearing age and any personnel who seek additional information about the use of these hazardous compounds. Refer personnel who require counseling or request additional information about the use of the hazardous compounds to OMS and DOHS.

CDC/NIOSH offers the following recommendations to help prevent exposures to illicit drugs, including fentanyl:

- Always wear nitrile gloves when illicit drugs may be present and change them properly when they become contaminated.
- Wear respiratory protection if powdered illicit drugs are visible or suspected.
- Avoid performing tasks or operations that may cause illicit drugs to become airborne.

- Do not touch the eyes, nose, or mouth after touching any surface that may be contaminated, even if wearing gloves.
- Wash hands with soap and water after working in an area that may be contaminated, even if gloves were worn. Do not use hand sanitizer or bleach.

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS: **NONE**___(check if none)
List cells/tissues, sera/antibodies, viruses/parasites/bacteria, and non-synthetic biochemicals that will be introduced into research animals.

Material:	Source:	Sterile? Y	Sterile? N
S. Aureus	NIH 10D ICU		X

If derived from rodents, has the material been tested, e.g. MAP/RAP/HAP/PCR? (If Yes, attach copy of results)		
Have the tested materials been passed through rodents outside of the animal facility in question?		
Is the material derived from the original MAP/RAP/HAP/PCR tested sample?		
I certify that to the best of my knowledge that the above is complete and correct, and that the material remains uncontaminated with rodent pathogens.		

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY: **NONE X** (check if none)

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.

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS:

1. I certify that I have attended an approved NIH investigator training course. Month/Year of Initial Course Completion: 9/1998 ; Month/Year(s) of Refresher Training: 2/2017
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (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 completed the course "Using Animals in Intramural Research: Guidelines for Animal Users" will complete refresher training as required, and 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); and 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 momentary pain or distress, whether it is relieved or not.
6. I will obtain approval from the ACUC before initiating any significant changes in this study.

Principal Investigator:

Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.08.25 11:06:14 -04'00'

Signature_____ Date_____

O. CONCURRENCES: PROPOSAL NUMBER_____(LEAVE BLANK)

Laboratory/Branch Chief: (certification of review and approval on the basis of scientific merit and sex as a biological variable. Scientific Director's signature required for proposals submitted by a Laboratory or Branch Chief)

Name_____Signature_____Date_____

Clinical Center Biostatistics and Clinical Epidemiology Service certification of statistical review, required for Clinical Studies and selected Pilot Studies.

Name_____Signature_____ Date_____

NIH Safety Representative: (signature represents certification, compliance and concurrence for use of material listed in the Hazardous Material Section)

DOHS Safety Representative

Signature Brittany E. Tillman -S Digitally signed by Brittany E. Tillman -S Date: 2023.08.24 16:53:31 -04'00' Date_____

DRS Safety Representative

Signature Lawrence J. Koenig -S Digitally signed by Lawrence J. Koenig -S Date: 2023.08.24 12:06:58 -04'00' Date_____

Facility Manager: (certification of resource capability in the indicated facility to support the proposed study)

Facility_____Name_____Signature Sekou Savane -S Digitally signed by Sekou Savane -S Date: 2023.08.25 12:53:25 -04'00' Date

Facility_____Name_____Signature_____Date

Facility_____Name_____Signature_____Date

Facility_____Name_____Signature_____Date

Facility Veterinarian: Certification of Review

Name Marvin L. Thomas III -S Signature Digitally signed by Marvin L. Thomas III -S Date: 2023.08.25 15:39:00 -04'00' Date_____

Attending Veterinarian: Certification of Review

Name Lisa G. Portnoy -S Signature Digitally signed by Lisa G. Portnoy -S Date: 2023.08.23 13:59:26 -04'00' Date_____

P. FINAL APPROVAL:

Certification of review and approval by the Animal Care and Use Committee Chairperson

Chairperson Dima Hammoud -S Signature Digitally signed by Dima Hammoud -S Date: 2023.08.25 09:02:52 -04'00' Date_____

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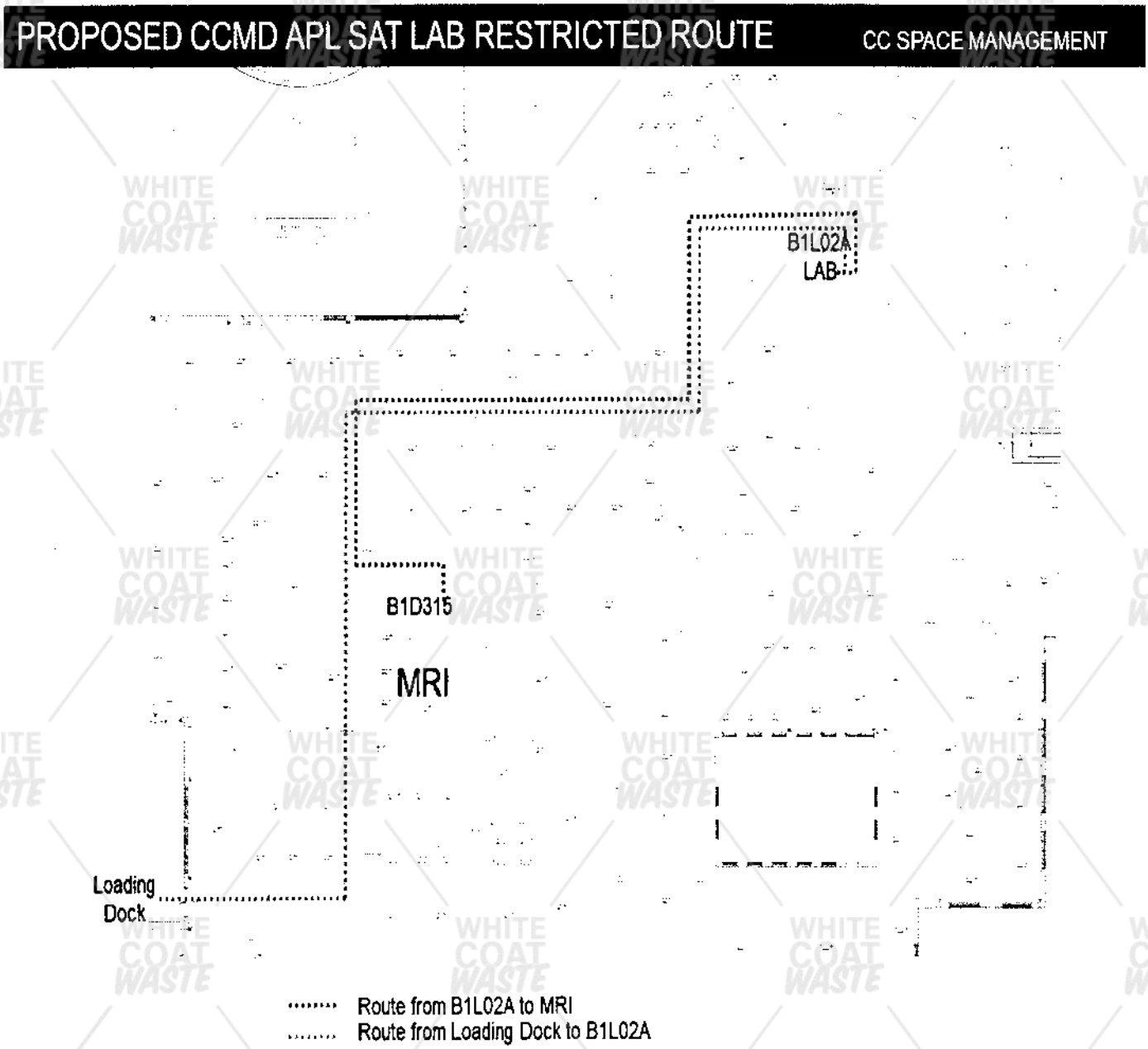
17. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.zoetis.co.nz/_locale-assets/doc/sds/torbugesic-_1-july-2019_.pdf

Appendix A: Route from Loading dock to B1L02A (Lab) and to LDRR3T (B1D315) MRI



Appendix B: Laboratory Tests

Blood sampling (over 24 h)

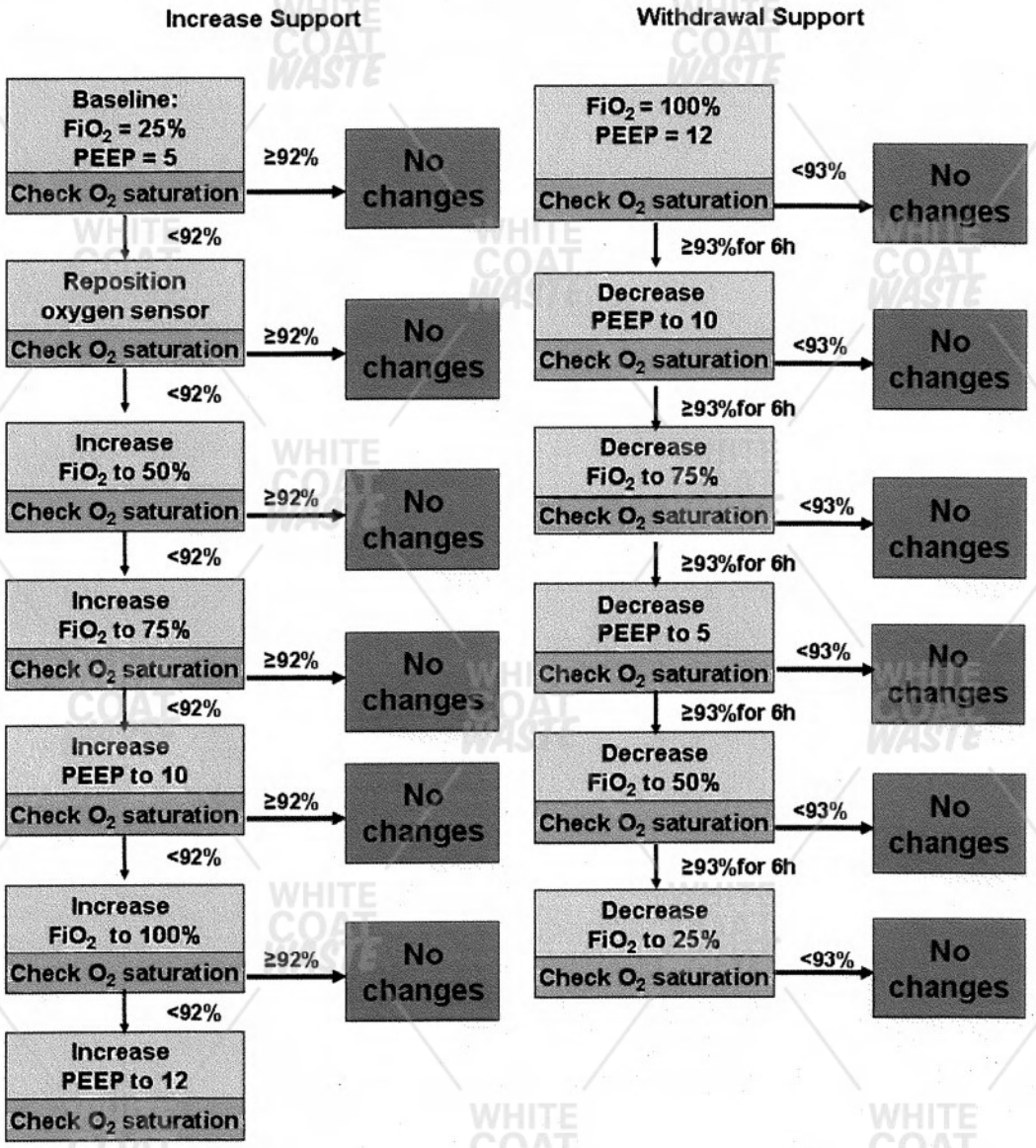
TEST	Day 1	Day 2	Day 3	Day 4	Day 5
ABG	2	1	1	1	1
CBC	1	0.5	0.5	0.5	0.5
Chemistry	6	3	3	3	3
Troponin/CPK/BNP	6	3	3	3	3
Catecholamine/electrolytes	12	6	6	6	6
Blood culture	1	1	1	1	1
Total	28	14.5	14.5	14.5	14.5

For each sample 2-3 ml is withdrawn from the catheter, the blood is drawn and then the initial 2-3cc is returned and the line is flushed with heparinized saline.

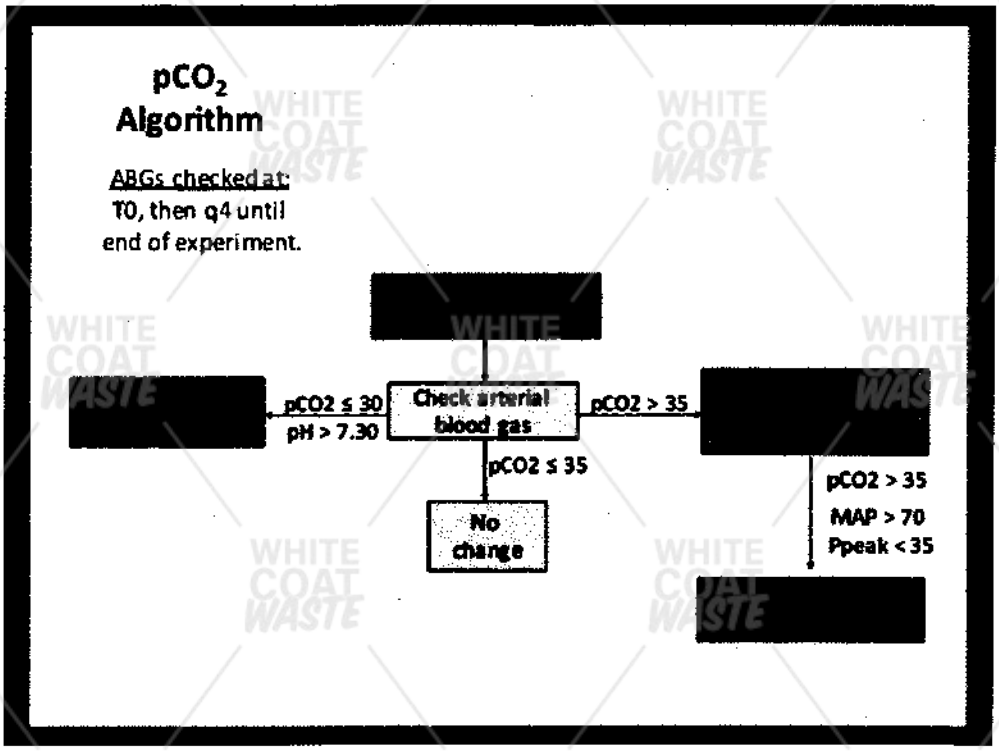
831 **Appendix C: Treatment Algorithms (25)**

832 The results of these hemodynamic measurements listed in these *Treatment Algorithms* will dictate
833 the subsequent treatments of the study animals. Oxygenation will be initiated at a concentration of
834 25% and a PEEP of 5 cm H_2O (see *Oxygen Support Algorithm*). The animal will be maintained at
835 these levels for a minimum of 15 minutes, and any subsequent interventions that are performed to
836 improve oxygenation will allow fifteen minutes to take effect. If the oxygen saturation falls below
837 92%, the oxygen concentration will be increased to 50%. If the oxygen saturation falls below 92%,
838 FiO_2 will be increased to 75% (PEEP=5). If after a minimum of 15 minutes the oxygen saturation
839 falls below 92%, the PEEP will be increased to 10 cm H_2O. If the oxygen saturation falls below
840 92%, the delivered oxygen concentration will be increased to 100%. If after a minimum of 15
841 minutes the oxygen saturation falls below 92%, the PEEP will be increased to 12 cm H_20. The
842 oxygen saturation status will be monitored continuously and can be adjusted according to these
843 guidelines any time the oxygen saturation falls below 92%. Should oxygen saturation remain
844 above 93% for 6 h, the oxygen intervention will be decreased to the previous step (see *Oxygen*
845 *Support Algorithm*). If at any time the oxygen saturation falls below 85%, the next level of support
846 may be immediately initiated (i.e. unnecessary to wait 15 minutes to see the effects of a previous
847 treatment before giving additional support).
848

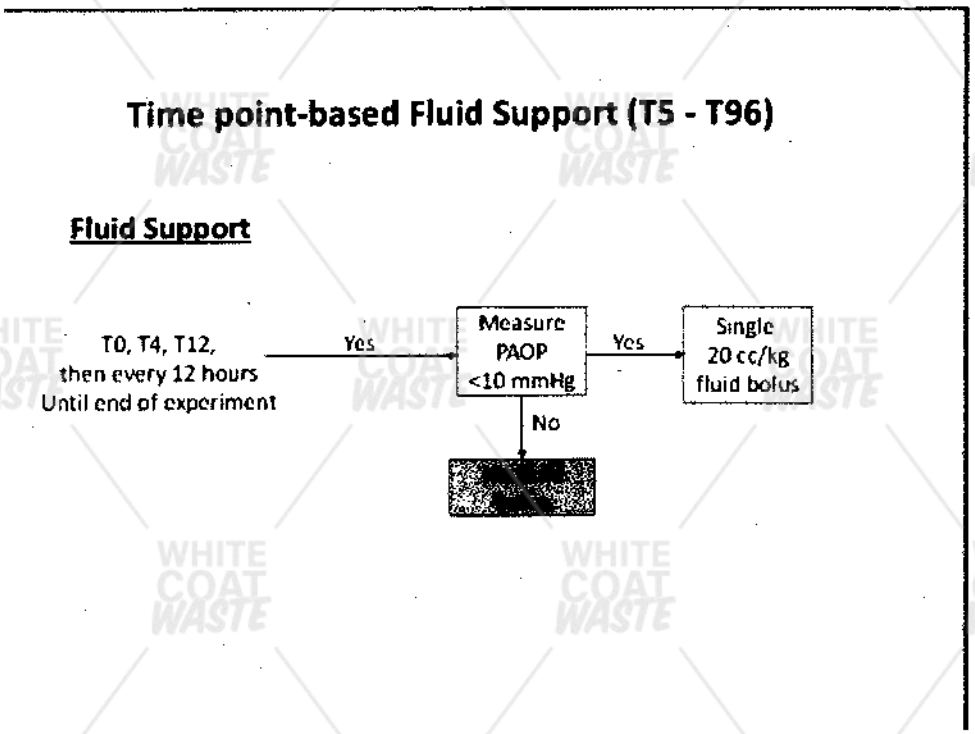
Oxygen Support Algorithm



The blood gas measurements at T4 will be used to adjust the respiratory rate on the ventilator to control the pCO_2 of the blood and to assist in the maintenance of pH (see *pCO_2 algorithm* below). As shown in the pCO_2 algorithm, if the blood gas measures a pCO_2 that is above 35 mmHg, the respiratory rate will be increased 5 breaths/minute. A repeat blood gas will be obtained in fifteen minutes. This cycle will be repeated until the pCO_2 is less than 35 mmHg or a maximum of 30 breaths/minute is reached. To increase pCO_2 to 35 breaths/minute, the pCO_2 must be greater than 35 mmHg, MAP greater than 70 mmHg and peak ventilator pressure must be less than 35 cmH_2O. After the initial adjustments at T4, the pCO_2 will be monitored every 2 hours until T8 and then every 8 hours thereafter. If the pCO_2 is less than 35 mmHg and the pH is below 7.3, no changes are required. If at a given time point the pCO_2 is less than 35 and the pH is above 7.3, the respiratory rate will be decrease 5 breaths/minute.



At T4, the pulmonary artery occlusion pressure (PAOP) will be measured. If the PAOP is less than 10 mmHg, a fluid bolus (0.9 % NaCl) of 20 ml/kg will be given (see *Initial Hemodynamic Support (T4)* algorithm). If after the infusion, PAOP remains below 10 mmHg, an additional fluid bolus can be given, up to a total of 3 fluid boluses. At subsequent time points (T6, T8, T10, T12, and then every 4 hours) if PAOP <10 mmHg, a single intravenous fluid bolus (20 ml/kg) will be administered (see *Time point-based fluid support* algorithm).



During the period of ventilation, the animals will be kept warm by wrapping them in a heated water blanket, heated air blanket (Bair Hugger, 3M) and other heavy blankets (see *Temperature Algorithm*). Humidity will be maintained using a servo controlled heater to heat and humidify the air (ConchaTherm III, Hudson RCI-AB). If the core temperature (measured by Swan Ganz catheter) is <36.5 °C a heated water blanket, heated air and cloth blanket are applied. If the temperature is between 36.5 and 37.5 °C, only the heated water blanket is applied. If the temperature >37.5 °C, the heated water blanket is turned off.

Temperature Algorithm

Temperature (°C)	
<36.5:	Apply heated water, air and cloth blankets
36.5 to 37.5:	Only water or cloth blanket
>37.5:	Turn off or remove heat source

CT / MRI Scan Record

Date: ID: Species: Canine DOB: Wt (kg): Sex: M Investigator: Solomon

Protocol#: 23-xx

Expiration:

Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Pre-Transportation Settings

Sedation	Dose	Route
Versed		
Fentanyl		
Normal		
Propofol		
Medetomidine		

Ventilator Settings	
PEEP	
FiO_2	
RR	
Volume	

Other Drugs	Dose	Route
Epinephrine		
NaCl		

Time	HR	MAP	B/P	Resp Rate	Temp (C)	SpO2 (%)	P[1]	V[1]	F[1]	NM[1]	M[1]	Comments

[1]P = Propofol, F=Fentanyl, V=Versed, M=Medetomidine, NM=Normsal

NMR Center Animal/Tissue proposal

Institute: CC **Principal Investigator:** Steven Solomon, PhD

Animal Study Proposal (ASP) # CCM23-04 **Imaging Amendment #** n/a

ASP Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Co-investigators involved in imaging:

*(list **ONLY** those people working in the NMR Center, they are required to attend MRI Safety Training)*

Co-investigator Name	Email Address	Phone Number	Safety Training
Verity Ford	verity.ford@nih.gov	301-496-3091	9/1/2021
Jasmine Holden	Jasmine.holden@cc.nih.gov	301-496-3091	11/12/2019
Melinda Fernandez	MFernandez@cc.nih.gov	301-496-3091	8/13/2019
Crystal Hite	crystal.hite@nih.gov	301-496-3091	5/4/2022

Time Requested on Imaging Equipment:

(Time on animal MR imagers is typically assigned in 6 hour blocks)

Magnetic Resonance Imaging (MRI) Animal Scanners	14	Hours per Month
Magnetic Resonance Imaging (MRI) Tissue Scanner		Hours per Month
MicroComputed Tomography (CT) *in vivo* scanner	2	Hours per Month
MicroComputed Tomography (CT) *in vitro* scanner		Hours per Month
Ultrasound +/- Photoacoustic Imaging		Hours per Month
Bioluminescence/Fluorescence		Hours per Month
Room B1D-200A		Hours per Month
Clinical (human) MRI scanners		Hours per Month
Other:		Hours per Month

Approval of Scientific Director:

Leighton Chan -S

Digitally signed by Leighton Chan -S
Date: 2023.08.30 18:26:51 -04'00'

Signature of Scientific Director Date

Updated 2021

NMR Center Animal/Tissue proposal

Animal Imaging Subjects: *(check all that apply)*

Live Animals: ☐ Mouse ☐ Rat ☐ Rabbit ☐ NHP ☑ Other, please list: canine

☐ Freshly Dead Tissue ☐ Fixed Tissue Tissue source choose tissue source

Animal Housing: *(list all areas where animals will be housed before and after imaging)*

Species (mouse, rat, etc.)	Building	Room #	Health Report
canine	28	119	
canine	10	B1L02A	

NOTE: The NMR Center excludes the following rodent pathogens: corona viruses (MHV, RCV, SDAV), PVM, Sendai, endoparasites and ectoparasites. Recent sentinel serum serology and parasitology reports must be provided to the MIF veterinarian at least 48 hours prior to rodents entering the NMR Center.

Person responsible for animal(s) or tissue samples while in the NMR Center:

Name: Steven Solomon **Phone:** 301-435-2287

Cell/Pager: 301-717-2494 **Email:** ssolomon@cc.nih.gov

IC Veterinarian providing emergency support: Lisa Portnoy, DVM

Lisa G. Portnoy -S Digitally signed by Lisa G. Portnoy -S Date: 2023.08.30 16:44:40 -04'00' 301-434-5304

Veterinarian Signature Phone

301-801-8024 portnoyl@mail.nih.gov

Cell/pager email

Hazardous Agents and Special Safety Concerns: (*Safety concerns for personnel or animals)*

No hazardous agents will be in use

I have read and understand the guidelines and policies of the Mouse Imaging Facility and NMR Center (http://intranet.nmrf.nih.gov). I understand that it is the responsibility of my investigators to be present during live animal imaging, to analyze their own data, and backup all data collected in the center. I will ensure that all personnel participating in animal studies in the NMR Center under my ASP will adhere to the guidelines and policies of the NMR Center.

Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 12:57:03 -04'00' 7/10/2023

Principal Investigator Signature Date

Updated 2021

INSTRUCTIONS FOR EMERGENCY ANIMAL TREATMENT AND CARE

Principal Investigator: Steven Solomon, PhD Date form completed: 10/2/2024 Protocol #: CCM23-04
Office Phone: 301-435-2287 Home Phone: 301-717-2494

Protocol Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Use a separate form if ***care is different*** for each species
Species: canine **Species:** _____
Species: _____ **Species:** _____

Animal Housing Location: Bldg 28 B-wing
Use separate form if care differs by location Bldg _____

List of Procedures: (surgery, tumor implant, catheter) intrabronchial inoculation of bacteria, catheter placement (femoral, external jugular, urinary), tracheostomy

Primary Point of Contact (P.O.C.) in Case of Emergency: Steven Solomon
Work Tel: 301-435-2287 **Home Tel**: _____ **Pager or Cell #**: 301-717-2494
Alternate Point of Contact in Case of Emergency: Jasmine Holden
Work Tel: 301-496-4697 **Home Tel**: _____ **Pager or Cell #**: 301-875-8664
Potential or Expected Complications: _____ rebleeding from catheter/tracheostomy site

Circumstances Requiring Contact: unexpected event requiring euthanasia

Treatment (indicate appropriate response):
Treatment determined by **veterinarian**: [x] Yes [ ] No
If NO, specify **restrictions** as follows: _____
Specific treatment as follows: _____
What **drugs** are **contraindicated?** _____

Criteria for **Euthanasia** (indicate appropriate response)
At Vet discretion if poor condition, severe pain or distress: [x] Yes [ ] No
If NO, specify treatments **or** restrictions:
_____

- Notify P.O.C. *[ ] Yes [x] No
- Requested **euthanasia agent** and **route of administration**: _____
- Specific **criteria** for **euthanasia**: _____

If Euthanasia is performed or animals are found dead:

a. Contact P.O.C.	[ ] Yes	[x] No
b. Refrigerate carcass	[x] Yes	[ ] No
c. Dispose of carcass	[ ] Yes	[x] No
d. Submit to DVR for necropsy	[x] Yes	[ ] No

CAN number to use for submission: 8387332

Additional Comments:
_____

Principal Investigator: STEVEN B. SOLOMON -S Digitally signed by STEVEN B. SOLOMON -S Date: 2024.10.02 11:00:46 -04'00'

Signature Date

*** The veterinarian will take the appropriate action in an emergency if no response from the PI/POC is received within 30 minutes after an attempt at notification is made.**

INSTRUCTIONS FOR EMERGENCY ANIMAL TREATMENT AND CARE

Principal Investigator: Steven Solomon, PhD Date form completed: 6/22/2023 Protocol #: CCM23-xx
Office Phone: 301-435-2287 Home Phone: 301-717-2494

Protocol Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Use a separate form if ***care is different*** for each species

Species: canine **Species:** _____
Species: _____ **Species:** _____

Animal Housing Location: Bldg 28 B-wing
Use separate form if care differs by location Bldg _____

List of Procedures: (surgery, tumor implant, catheter) intrabronchial inoculation of bacteria, catheter placement (femoral, external jugular, urinary), tracheostomy

Primary Point of Contact (P.O.C.) in Case of Emergency: Steven Solomon
Work Tel: 301-435-2287 **Home Tel**: _____ **Pager or Cell #**: 301-717-2494
Alternate Point of Contact in Case of Emergency: Jasmine Holden
Work Tel: 301-496-4697 **Home Tel**: _____ **Pager or Cell #**: 301-875-8664
Potential or Expected Complications: rebleeding from catheter/tracheostomy site

Circumstances Requiring Contact: unexpected event requiring euthanasia

Treatment (indicate appropriate response):

Treatment determined by **veterinarian**:	[x] Yes	[ ] No
If **NO**, specify **restrictions** as follows: _____		
Specific treatment as follows: _____		
What **drugs** are **contraindicated**? _____		

Criteria for **Euthanasia** (indicate appropriate response)

At Vet discretion if poor condition, severe pain or distress:	[x] Yes	[ ] No
If NO, specify treatments or restrictions: _____		
• Notify P.O.C.	*[ ] Yes	[x] No
• Requested **euthanasia agent** and **route of administration**: _____		
• Specific **criteria** for **euthanasia**: _____		

If Euthanasia is performed or animals are found dead:

a. Contact P.O.C.	[ ] Yes	[x] No
b. Refrigerate carcass	[x] Yes	[ ] No
c. Dispose of carcass	[ ] Yes	[x] No
d. Submit to DVR for necropsy	[x] Yes	[ ] No

CAN number to use for submission: 8387332

Additional Comments:
_____

Principal Investigator: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.08.25 11:07:09 -04'00'
Signature Date

*** The veterinarian will take the appropriate action in an emergency if no response from the PI/POC is received within 30 minutes after an attempt at notification is made.**

References

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Verity Ford

ASP#: CCM23-xx Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 Bldg/Rm: 28/119 Email: verity.ford@cc.nih.gov
PI Course completion dates: (Initial) (Refresher)
AU Course completion dates: (Initial) 2021 (Refresher)

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint				
Anesthesia:				Type: inhalational, intravenous
Administration	D,R		2	
Monitoring	D,R		2	
Aseptic technique	D,R		2	
Injections:				
SC	D,R		2	
IM	D,R		2	
IV	D,R	x		
IP				
Catheter placement:				
IV	D,R	x		
IA				
Intubation	D,R	x		
Euthanasia	D,R		2	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) **Yes/No:** This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component. date(s): ;
2) **Yes/No** There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above,
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: Verity Ford Date: 07/28/2023

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Date: 7/25/23

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Steven Solomon

ASP#: CCM23-xx **Title:** Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 **Bldg/Rm: 28/119** **Email: ssolomon@cc.nih.gov**
PI Course completion dates: (Initial) 2000 (Refresher) 2022
AU Course completion dates: (Initial) (Refresher)

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D		>15	
Anesthesia:				Type: inhalational, intravenous
Administration	M,R,D		>15	
Monitoring	M,R,D		>15	
Aseptic technique	M,R,D		>15	
Injections:				
SC	M,R,D		>15	
IM	M,R,D		>15	
IV	M,R,D		>15	
IP	M,R,D		>15	
Catheter placement:				
IV	R,D		>15	
IA	R,D		>15	
Intubation	R,D		>15	
Euthanasia	M,R,D		>15	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) Yes/No: This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component date(s):
2) Yes/No There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above.
OR
4b) _____ _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes / No I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 11:03:11 -04'00' **Date:**

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 11:08:04 -04'00' **Date:**

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Charles Natanson

ASP#: CCM23-xx Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-9770 Bldg/Rm: 28/119 Email: cnatanson@cc.nih.gov
PI Course completion dates: (Initial) 2000 (Refresher) 2021
AU Course completion dates: (Initial) (Refresher)

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	D		>30	
Anesthesia:				Type: inhalational, intravenous
Administration	D		>30	
Monitoring	D		>30	
Aseptic technique	D		>30	
Injections:				
SC	D		>30	
IM	D		>30	
IV	D		>30	
IP	D		>30	
Catheter placement:				
IV	D		>30	
IA	D		>30	
Intubation	D		>30	
Euthanasia	D		>30	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) Yes/No This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component, date(s) ;
2) Yes/No There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures.
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above.
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes/ No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes/ No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature:_____ Date: July 10, 2023

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07 0 11:21:25 -04'00' Date:_____

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Marcus Chen, MD

ASP#: CCM23-xx Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-0077 Bldg/Rm: 10/B1D47 Email: chenmy@nhlbi.nih.gov
PI Course completion dates: (Initial) 9/2006 (Refresher) 8/7/2014
AU Course completion dates: (Initial) 10/21/2019 (Refresher) 11/7/2022

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D	X		
Anesthesia:				
Administration	M,R,D	X		
Monitoring	M,R,D	X		
Aseptic technique	M,R,D	X		
Injections:				
SC	M,R,D	X		
IM	M,R,D	X		
IV	M,R,D	X		
IP	M,R,D	X		
Catheter placement:				
IV	R,D	X		
IA	R,D	X		
Intubation	R,D	X		
Euthanasia	M,R,D	X		

2) Dr. Chen is fully qualified in the techniques he will be performing on this ASP (operating the CT scanner). He will have no contact with the study animal.

3) Yes/No: This ASP involves Nonhuman Primates procedures. If yes complete Section B. If no, go to Section C.

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component date(s): ; ;
2) Yes/No There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. If Yes – complete 3 and 4. If no, go to Section C.
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above.
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: _____ Date: 7/26/2023

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: _____ Date: 7/26/23

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Lyn Colenda, DVM

ASP#: CCM23-xx Title: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301.443.8521 /301-640-6993 Bldg/Rm: 14E/119D Email: colendal@mail.nih.gov
PI Course completion dates: (Initial) 6/1/98 (Refresher) 1/24/23
AU Course completion dates: (Initial) 9/27/01 (Refresher) 1/24/23

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D		50	
Anesthesia:				
Administration	M,R,D		39	
Monitoring	M,R,D		39	
Aseptic technique	M,R,D		39	
Injections:				
SC	M,R,D		39	
IM	M,R,D		39	
IV	M,R,D		M/R-20 D-39	
IP	M,R,D		39	
Catheter placement:				
IV	R,D	R	D-40	
IA	R,D	R	D-5	
Intubation	R,D	R	D-39	
Euthanasia	M,R,D		M/R-48 D-39	

2) Dr. Colenda is fully qualified in the techniques she will be performing on this ASP

3) Yes/No: This ASP involves Nonhuman Primates procedures. If yes complete Section B. If no, go to Section C.

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component date(s): ; ;
2) Yes/No There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. If Yes – complete 3 and 4. If no, go to Section C.
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above,
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.

Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: Lyn Colenda Date: 2/27/23

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: _____ Date: 2/28/23

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Melinda Fernandez

ASP#: CCM23-xx **Title:** Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 **Bldg/Rm: 28/119** **Email: mfernandez@cc.nih.gov**
PI Course completion dates: (Initial) **(Refresher)**
AU Course completion dates: (Initial) 2005 **(Refresher) 2020**

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D		>15	
Anesthesia:				Type: inhalational, intravenous
Administration	M,R,D		>15	
Monitoring	M,R,D		>15	
Aseptic technique	M,R,D		>15	
Injections:				
SC	M,R,D		>15	
IM	M,R,D		>15	
IV	M,R,D		>15	
IP	M,R,D		>15	
Catheter placement:				
IV	R,D		>15	
IA	R,D		>15	
Intubation	D		>15	
Euthanasia	M,R,D		>15	

2) **Dr. Steven Solomon** will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) **Yes/(No)** This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component. date(s): ; ;
2) **Yes/No** There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above,
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

(Yes)/ No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
(Yes)/ No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: Melinda Fernandez **Date:** 7/10/2023

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 12:06:07 -04'00' **Date:**

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Jasmine Holden

ASP#: CCM23-xx **Title:** Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 **Bldg/Rm: 28/119** **Email: jasmine.holden@cc.nih.gov**
PI Course completion dates: (Initial) **(Refresher)**
AU Course completion dates: (Initial) 2013 **(Refresher) 2022**

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D		>5	
Anesthesia:				Type: inhalational, intravenous
Administration	M,R,D		>5	
Monitoring	M,R,D		>5	
Aseptic technique	M,R,D		>5	
Injections:				
SC	M,R,D		>5	
IM	M,R,D		>5	
IV	M,R,D		>5	
IP	M,R,D		>5	
Catheter placement:				
IV	R,D		>5	
IA	D		>5	
Intubation	M,R,D		>5	
Euthanasia	M,R,D		>5	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) Yes/No: This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component, date(s): ;
2) Yes/No There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above,
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: _____ **Date:** 7/1/2023

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 11:15:43 -04'00' **Date:** _____

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Jing Feng

ASP#: CCM23-xx **Title:** Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 **Bldg/Rm: 28/119** **Email: fengjing2@cc.nih.gov**
PI Course completion dates: (Initial) **(Refresher)**
AU Course completion dates: (Initial) 2002 **(Refresher) 2022**

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M,R,D		>15	
Anesthesia:				Type: inhalational, intravenous
Administration	M,R,D		>15	
Monitoring	M,R,D		>15	
Aseptic technique	M,R,D		>15	
Injections:				
SC	M,R,D		>15	
IM	M,R,D		>15	
IV	M,R,D		>15	
IP	M,R,D		>15	
Catheter placement:				
IV	R,D		>15	
IA	R,D		>15	
Intubation	R,D		>15	
Euthanasia	M,R,D		>15	

2) **Dr. Steven Solomon** will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) **Yes/No:** This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component date(s): ;
2) **Yes/No** There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above,
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: Jing Feng -S Digitally signed by Jing Feng -S Date: 2023.07.10 12:11:21 -04'00' **Date:** _____

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 12:32:23 -04'00' **Date:** _____

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Crystal Hite

ASP#: CCM23-xx **Title:** Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 **Bldg/Rm: 28/119** **Email: crystal.hite@nih.gov**
PI Course completion dates: (Initial) **(Refresher)**
AU Course completion dates: (Initial) 2020 **(Refresher)**

1) Experience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint				
Anesthesia:				Type: inhalational, intravenous
Administration	M R D		3	
Monitoring	M R D		3	
Aseptic technique	M R D		3	
Injections:				
SC	M R D		3	
IM	M R D		3	
IV	M R D		3	
IP				
Catheter placement:				
IV	M R D		3	
IA	M R D		3	
Intubation				
Euthanasia	M R D		3	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) Yes/No: This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component date(s): ; ;
2) **Yes/No** There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**
3) I will be performing the following awake NHP procedures:
4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above.
OR
4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.
Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: _____ **Date:** 7/10/23

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 11:53:44 -04'00' **Date:** _____

Clinical Center Training and Experience Form

SECTION A: General Information

Investigator Name: Dennis Du

ASP#: CCM23-xx **Title:** unctional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Phone No: 301-496-3091 **Bldg/Rm:** 28/119 **Email:** dennis.dugan@cc.nih.gov
PI Course completion dates: (Initial) **(Refresher)**
AU Course completion dates: (Initial) 2015 **(Refresher)** 2021

1) Experience: M-mouse, R-rat D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	R,D		>4	
Anesthesia:				Type: Inhalational, intravenous
Administration	R,D		>4	
Monitoring	R,D		>4	
Aseptic technique	R,D		>4	
Injections:				
SC	R,D		>4	
IM	R,D		>4	
IV	D		>4	
IP		x		
Catheter placement:				
IV		x		
IA		x		
Intubation		x		
Euthanasia	R,D		>4	

2) Dr. Steven Solomon will provide supervision and training in the techniques I will be performing on this ASP until I am fully qualified to perform these animal activities independently.

3) Yes/No: This ASP involves Nonhuman Primates procedures. **If yes complete Section B. If no, go to Section C.**

SECTION B: Nonhuman Primate (NHP) Procedures

1) Nonhuman Primate Safety Course: (IC component date)
(Facility component. date(s):

2) **Yes/No** There will be "awake" NHP procedures performed as a part of this protocol, e.g. squeezing up for injections, pole/collar, restraint chairs, operant procedures, etc. **If Yes – complete 3 and 4. If no, go to Section C.**

3) I will be performing the following awake NHP procedures:

4a)_____I am currently proficient in performing all of the awake NHP procedures that I've listed above.

OR

4b) _____will provide my supervision and training until I am fully qualified to perform these awake NHP procedures proficiently and independently.

SECTION C: Assurances

Yes / No: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.

Yes / No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but am not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new T&E form and acquire training prior to performing any new procedures.

Animal User signature: _____ **Date:** 7/11/2023

As the PI, I assume the responsibility to ensure that this Animal User's training and experience for procedures he/she will be performing under this ASP has been or will be assessed, and if this person is not proficient in performing these procedures, training will be provided, and proficiency verified, before the person is allowed to conduct these procedures independently.

Principal Investigator signature: Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.07.10 11:55:27 -04'00' **Date:** _____

CHEMICAL COMPOUND ATTACHMENT

CHEMICAL COMPOUND ATTACHMENT

List all chemical compounds (from sections "F", "I", and "J") administered to live animals. If they are not pharmaceutical grade as required by USDA Policy #3. USDA policy #3

Please refer to the ARAC Guideline on the use of NON-Pharmaceutical Grade Compounds: ARAC guideline NPGC

For any compounds that are not considered pharmaceutical grade and fall under criteria 4 described below, please use the justification form provided.

From The Guide:

> *Use of Non-Pharmaceutical-Grade Chemicals and Other Substances* The use of pharmaceutical-grade chemicals and other substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals. They should therefore be used, when available, for all animal-related procedures (USDA 1997b). The use of non-pharmaceutical-grade chemicals or substances should be described and justified in the animal use protocol and be approved by the IACUC (Wolff et al. 2003); for example, the use of a non-pharmaceutical-grade chemical or substance may be necessary to meet the scientific goals of a project or when a veterinary or human pharmaceutical-grade product is unavailable. In such instances, consideration should be given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use (NIH 2008).

Pick one of the reasons listed below.

Substance Name	**Pharmaceutical-grade (Yes, No or N/A) ***	**If "N" select reason from below**
Propofol	Y	
Fentanyl	Y	
Midazolam	Y	
Dexmedetomidine	Y	
Isoflurane	Y	
ketamine	Y	
acepromazine	Y	
Torbugesic	Y	
atropine	Y	
ceftriaxone	Y	

CHEMICAL COMPOUND ATTACHMENT

Reasons for using non-pharmaceutical grade drugs:

1. Compounds are not commercially available as pharmaceutical grade.
2. Compounds are available pharmaceutical grade but not in a formulation suitable for this study(e.g., tablet form when injectable is required).
3. Non----pharmaceutical grade compounds have been used previously and are needed to directly compare new results with the same compounds.
4. Other–provide brief justification below.

Non-Pharmaceutical Grade compound Justification:

CCMD SCIENTIFIC CONTINUING REVIEW OF ANIMAL PROTOCOLS

(Prior to ACUC Review)

PROTOCOL TITLE: Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

PRINCIPAL INVESTIGATOR: _ Steven Solomon, Ph.D

CO-INVESTIGATOR(S): Charles Natanson, MD (Co-principal investigator) Verity Ford, MD, Melinda Fernandez, MD, Jasmine Holden, Jing Feng, Crystal Hite, Dennis Dugan

ANIMALS: SPECIES Canine **STOCK/STRAIN** Beagle

DATE PROTOCOL REVIEWED AT CCMD SENIOR STAFF MEETING:
July 13, 2023, 11:30am

SCIENTIFIC REVIEW COMMITTEE PARTICIPANTS: CCMD members - Anthony Suffredini MD, Henry Masur MD, Robert Danner MD, Daniel Chertow MD, Nitin Seam MD, Sameer Kadri MD, Shreya Kanth MD, Jeffrey Strich MD, Parizad Torabi-Parizi, MD, Andrew Platt, MD (NIAID)

Requires outside review	___ Yes _X No
Statistical consultation provided:	X Yes ___ No
Returned to PI for changes:	___ Yes _X No
Requires resubmission to CCMD Scientific Review Committee:	___ Yes _X No

Protocol Design

X Yes ___No The protocol is designed to answer an important question.
X Yes ___No The protocol is designed to address the research question(s).
X Yes ___No The hypothesis is clearly stated.
X Yes ___No The hypothesis is reasonable and well justified.
X Yes ___No The expertise of the investigators is suitable for this protocol.

Study Analysis

X Yes ___No Statistical methods are appropriate for this study.
X Yes ___No Safety monitoring is planned and adequate.

PRESENTATION BY THE PI/AI:

Dr Charles Natanson presented the protocol to the committee members:

Background information:
Using cardiac MRI, in sedated and ventilated pneumonia model of sepsis in canines that simulates the cardiac dysfunction seen during human septic shock we confirmed the that sepsis causes reversible cardiac dysfunction that leads to a reduced ejection fraction and an increase in

the size of the ventricle. When comparing the heart injury in survivors to non-survivors, the critical factor associated with survival appeared to be the left ventricle's ability to fully dilate during recovery. These changes in ventricular size had previously been explained by either increases in the filling of the heart (preload) or increased resistance to outflow (afterload). In contrast to earlier work by ourselves and others, we have shown that changes in loading and afterload conditions are not responsible for these changes and are related to sepsis-induced changes in the wall of the heart.

Associated with recovery of the hearts ability to eject blood or contract, we showed for the first time, that the left ventricular wall was found to lose mass (15%) and develops an increased percentage of water (edema) over 92 h (2 to 3%). This degree of edema may fully explain the cardiac dysfunction seen during sepsis. There is no biochemical (troponin levels) or histological (light and electron microscopy) evidence that this loss of mass is due to muscle cell loss (myocyte drop out) or damage from decrease tissue perfusion (ischemia).

The loss of mass occurs as the heart is recovering (the ejection fraction is returning to normal) consistent with the notion it represents sloughing of damaged tissue and is part of the recovery process. The most abundant cell type after myocytes is endothelial cells. We hypothesize the microcirculation lined with endothelial cells are damaged but not occluded by the inflammation early on leading to endothelial myocyte and interstitial edema seen on histology and confirmed on MRI T2 images. Sloughing of damaged endothelial cells and potentially some focal myofilament autolysis, a potentially protective mechanism, may be part of the reparative process and results in restoration of vascular integrity and myocardial function.

We have identified by MRI the changes described above occur at 48 and 96h of sepsis but we have not examined by MRI the acute changes from time 0 to 48h when the development of the sepsis-induced injury is occurring. Understanding how damage occurs during sepsis resulting in cardiac dysfunction and the reparative process will offer insight into the cause of all organ failure and help solve this decades long mystery and allow us to design and test treatments to minimize damage or enhance recovery.

Objectives:

In the current study, we will use MRI to look at the early changes at 6 h and then every 12 h (to 54 h) after bacterial challenge in ventricular wall size, edema, and mass. We hypothesize we will see edema associated with early chamber size decrease, worse in non-survivors. The worse edema results in a restrictive-like cardiomyopathy in non-survivors. After 24h, we hypothesize the loss of mass will begin, with ventricular wall thinning and ventricular chamber size increase associated with survival and recovery.

Study design:

This study will use a dose of *Staphylococcal aureus* (*S. Aureus*) bacteria resulting in a 50-70% mortality. The primary endpoint of this study will be survival with secondary endpoints to include quantifying cardiovascular, pulmonary, renal and hepatic injury. Historically, 6-8 animals per group (survivors vs. non-survivors) were used as a basis to perform a power analysis to determine an appropriate sample size which has ranged up to 12 animals per group (protocols: CCM15-03, CCM16-03, CCM16-04, CCM17-01, CCM19-04).

Outcome measures:
Animals will initially be studied at baseline and repeated at 6, 18, 30, 42, 54, 92 h following bacterial inoculation. At each time point, cardiac MRI, hemodynamic measures and blood sampling will be done. Animals alive at 92h will be considered survivors and after all studies are completed will be euthanized (see Section J). Tissues are collected from all animals post-mortem for further analysis including for pathology, transcriptomics (messenger RNA) and cell sorting (flow cytometry).

SUMMARY SCIENTIFIC REVIEW DISCUSSION:

Q. Why is a CT scan also necessary with the MRI?
A. It provides complementary information on wall structure and dimensions to the MRI.

Q.When will you be able to obtain cardiac tissue?
A. The cardiac tissue for analysis will be taken at the time of death.

Q. What tests will be done to address your hypothesis that there is loss of endothelial cells?
A. We plan to test for changes in cardiac endothelial cells with the Agbor lab in the NHLBI using circulating cell free DNA with epigenetic markings that identify the cell sources from cardiac endothelium. We will also perform flow cytometry (Torabi-Parizi lab) on the blood to measure changes in circulating endothelial cells. Cardiac tissue will be obtained to look for markers of apoptosis, gene expression and phosphorylated proteins

SCIENTIFIC REVIEW COMMITTEE VOTE:

Total = 9; For – 9, Opposed – 0 ,Abstained – 1 (The chair did not vote)

Chairman Scientific Review Certification:

This is a scientifically meritorious study and ready for ACUC review: X_ Yes ___ No

Anthony Suffredini, M.D.
Chairman Scientific Review: Suffredini, Anthony (NIH/CC/CCMD) [E] Digitally signed by Suffredini, Anthony (NIH/CC/CCMD) [E] Date: 2023.07.15 10:08:30 -04'00' **Date:**