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ORDER FOR SUPPLIES OR SERVICES

PREVIOUS EDITION NOT USABLE

Uncovered by a White Coat Waste investigation

Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO

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ORDER FOR SUPPLIES OR SERVICES

Uncovered by a White Coat Waste investigation

Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO



Uncovered by a White Coat Waste investigation

PREVIOUS EDITION NOT USABLE

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TRANSPORTATION/TRAVEL/RELOCATION- TRANSPORTATION: CTHER Project Data: 136732.2024.100.HNAM5R33 22006 FHEIGHT OR EXPRESS TRANS.04/15/2024 Accounting Info: 08000420200RAD.2024.06.A100.HNAMSR3000 Ct.1.0057.601.9999.2006.6100001.9999 .9999.9999 Funded: 32,954.00 52.212-4 Contract Terms and Conditions Ecquired 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 Contract Terms and Conditions Required to Implement Statutes or Executive Orders Contract Terms and Conditions Required to Implement Statutes or Executive Orders Contract Terms and Conditions Required to Implement Statutes or Executive Orders Contract Terms and Conditions Required to Implement Statutes or Executive Orders Contract Terms and Conditions Required to Implement Statutes or Executive Orders Provisions of law or Executive Orders Contract Terms and Conditions Required to Implement Statutes or Executive Orders Provisions of law or Executive Orders Provisions in subdequent appropriations Provisions in Subdequent appro	(a)	(b)	ORDERE (c)	(d)	PRICE (e)	/	(1)	ACCEPTED (g)
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<pre>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 (RAR) clauses, which are incorporated in this contract by reference, to implement provisions of law or Executive orders applicable to acquisitions of commercial items: (a) 52.203-19, Prohibition on Requiring Certain Internal Confidentiality Agreements or Statements (Jan 2017) (section 743 of Division F, 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 IGA for Hub. L. 115-91). Continued</pre>	TH Ac 08 C. .9 F1	.22006 FREIGHT OR EXPRESS RANS.04/15/2024 ccounting Info: 8000420240RAD.2024.06.A100.HNAM5R3000 .I.00576.901.9999.22006.61000001.9999 9999.9999 unded: \$2,954.00	WHITE	/				
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PAGE NO

		SCHEDULE - CONT	INUATION	/				6	
IMPORTAN	T: Mar	rk all packages and papers with contract and/or order numbers.		/			1	/	
DATE OF OR	DER	CONTRACT NO.					ORDER NO	./HITE	
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Prescribed by GSA FAR (48 CFR) 53.213(f)

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<pre>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 H2 solicitations and contracts_ [(1) (1) 52.219-3, Notice of HDBZone 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) (1) 52.219-4, Notice of Frice Evaluation Preference for HDBZone Small Business Concerns (OCT 2014) (if the oftersor 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 (nos B-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 (i11) Alternate II (Nov 2011). For partial SB Set-asides_(15) (1) 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</pre>	/
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EM NO.	SUPPLIES/SERVICES	WĂ	QUANTITY	UNIT	UNIT		AMOUNT	QUANTITY
(a)	(b)		ORDERED (c)	(d)	PRICE (e)	/	(1)	ACCEPTED (g)
	(ii) Alternate I (Nov 2016) of 5	2.219-9.				/		/
	(iii) Alternate II (Nov 2016) of							1
	52.219-9.				NHITE			WHITE
	(iv) Alternate III (Nov 2016) of				TAOC			TAOD
	52.219-9.			1	VACTE			WACTE
	(v) Alternate IV (Aug 2018) of	52.219-9.			inore .			In the local day
	For Task/Delivery Orders, if appli	cable_						
	(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 WH	TE					
	Subcontracting (Nov 2011) (15 U.S.C	·	άŤ					
	637(a)(14)).		75					
	Includes 52.219-9 (20) 52.219-16	WP13	16				VVMSIC	
	Liquidated Damages; Subcontracting I	lan (Jan						
	1999) (15 U.S.C. 637(d)(4)(F)(i)).							
	For SDVOSB set aside (21) 52.219-	27.		\backslash				
	Notice of Service-Disabled Veteran-	Owned		<u></u>				1.411.11-72-72
	Small Business Set-Aside (Nov 2011)	(15			NHILE			VIHILE
	U S C (657 f)	(120			LAQ			GOAL
	All over micro purchase (22) 52	219-28		- 0	VASIE			NASIE
	Post Award Small Business Program	215 20,		1	\ \			
	Poroprosontation (Jul 2013) (15 U.S.	~		(
	(22(-)(2))							
	632(a)(2)).	20						
	_FOF EDWOSB Set_Aside_ (23) 52.219-	29, WHI	ΓE –					
	Notice of Set-Aside for Economicali	Y CO	AT.					
	Disadvantaged women-Owned Small Bus	iness	TE					
	(EDWOSB) Concerns (Dec 2015) (15 U.	s.c.						
	637(m)).	./						
	_ For WOSB set aside _ (24) 52.219-3	30,						
	Notice of Set-Aside for Women-Owned	Small		\mathbf{N}	/			
	Business (WOSB) Concerns Eligible U	nder the			VHITE			WHITE
	WOSB Program (Dec 2015) (15 U.S.C.	637(m)).		0	TAOC			COAT
	Over MPT (25) 52.222-3, Convict	Labor		- D	VASTE			MASTE
	(June 2003) (E.O. 11755).			1	1101-1			1011
	Over MPT (26) 52.222-19, Child			/				
	Labor; Cooperation with Authorities	and						
	Remedies (Jan 2018) (E.O. 13126).							
	Over \$10K (27) 52.222-21, Prohib	ition of	TE				WHITE	
	Segregated Facilities (Apr 2015).		άŤ					
	Over \$10K (28) 52.222-26, Equal		TE				MACTE	
	Opportunity (Apr 2015) (E.O. 11246)	. , , , , , , , , , , , , , , , , , , ,					19171310	
	(ii) Alternate I (Feb 1999) of							
	52.222-26.							
	Over \$150K except outside US (29)		\backslash	/			
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	(Oct 2015) (38 U.S.C. 4212).			1	OAT			COAT
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(a) (b) (c) (c) (c) (b) (c) (c) (c) (c) (c) (c) (c) (c) (c) <th>ITEM NO.</th> <th>SUPPLIES/SERVICES</th> <th>QUANTITY</th> <th>UNIT</th> <th>UNIT</th> <th></th> <th>AMOUNT</th> <th>QUANTITY</th>	ITEM NO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT		AMOUNT	QUANTITY
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Spectrumity for Workers with Disabilities (M1 2010 (29 US.C. 793). (M1) Alternate I (July 2014) of 52.222-37. Employment Reports on Veterans (FEB 2016) (38 U.S.C. 4212). 		Over \$15K (30) 52.222-36, Equal						/
<pre>(Jul. 2014) Alternate I (July 2014) of 52,222-35. </pre>		Opportunity for Workers with Disabilities			1			/
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(FEB 2016) (38 U.S.C. 4212). 		52.222-37, Employment Reports on Veterans		/				
<pre></pre>		(FEB 2016) (38 U.S.C. 4212).	/					
Employee Rights Under the National Labor Relations Act (Dec 2010) (20.0.13496). All_(33) (3) 52.222-50, Combating Trafficking in Forenon (37M 2019) (22 U.S.C. chapter 78 and E.O. 13627). (11) 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 Sligibility Verification (Orc 2015), (E. O. 12989). (Not applicable to the acquisition of commercially available off-the-shelf items as prescribed in 22.1803.) over Sl50K (55) (15) 52.223-9, Estimate of Percentage of Recovered Material Content for EPA;Designated ltems (May 2008) (42) U.S.C. 6962(10) (3) ((11)). (Not applicable to the acquisition of commercially available off-the-shelf items.) (11) Alternate T (May 2008) of 52.223-9 (42 U.S.C. 6962(10) (21) ((10). (Not applicable to the acquisition of commercially available off-the-shelf items.) (13) Alternate T (May 2008) of 52.223-9 (42 U.S.C. 6962(10) (21) ((10). (Not applicable to the acquisition of commercially available off-the-shelf items.) (14) Alternate T (May 2008) of 52.223-9 (20 (S.C. 6962(10) (21) (21). (Not applicable to the acquisition of commercially available off-the-shelf items.) (15) (5.0.13693). 		Over SAP (32) 52.222-40, Notification of						
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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO 10

OFORD	25R CONTRACT NO. 75N98024P01555				ORDER NO.		
INO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT	AMC	DUNT	QUANTITY
a)	(b)	(c)	(d)	(e)	/ (Ð	(g)
	government buys, contractor buys for				/		
	government, or contractor furnishes for			/			
	government use (38)(i) 52.223-13,			VHITE		V/I-	
	Acquisition of EPEAT®-Registered Imaging			TAO		dd	
	Equipment (JUN 2014) (E.O. 13423 and 13514)		1	VASTE		1772	
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	contractor buys for government, or	/	1				
	contractor furnishes for government use					/	
	(39)(i) 52.223-14, Acquisition of	ITE			WHIT		
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	(E.O.s 13423 and 13514).	STE			WAST	5	
	(ii) Alternate I (Jun 2014) of 52.223-14				1		
	Energy star or FEMP (40) 52.223-15,				/		
	Energy Efficiency in Energy-Consuming				/		
	Products (DEC 2007) (42 U.S.C. 82596).		$\langle \cdot \rangle$				
	For all personal computer products when			WHITE		WE	
	government buys, contractor buys for			COAL		QQ	
	government, or contractor furnishes for		1	VASTE		N/A	
	government use (41)(1) 52.223-16,		1			/	
	Acquisition of EPEATG-Registered Personal		1				
	computer Products (OCT 2015) (E.O. 13425						
	and 13514). (ii) Alternate I (Jun 2014) of 52 223-16					/	
	X All (42) 52 223-18 Encouraging	TE			WHITI		
	Contractor Policies to Ban Text Messaging	AL			COA	-	
	While Driving (AUG 2011) (E.O. 13513)	SIE			WAST	8	
	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)		Ν.	· · · · · · · · · · · · · · · · · · ·			
	Products that may contain high global	•		VHILE		WE	
	warming potential hydrofluorocarbons or			,QAL			
	refrigerant blends containing		11	VASIE		IVIT	
	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).	UTE .			WHITE	- É	
	(45)(i) 52.224-3, Privacy Training (JAN	14			COAT	-	
	2017) (5 U.S.C. 552a).	ATE .			MAGTI	5	
	X_For all service contracts_ (ii) Alternate	316			1171310	°.	
	I (JAN 2017) of 52.224-3.				/		
	MPT and \$25K (46) 52.225-1, Buy				/		
	American;Supplies (May 2014) (41 U.S.C.		\mathbf{i}	/			
	chapter 83).		N.	VHITE		WH	
	X \$25K to \$191K (47)(i) 52.225-3, Buy		Ċ	TAOC		CO	
	Continued		Ň	VASTE		11/11	
						1	
			1			/	
		/				/	
		/				/	
	WHITE WH	ITE			WHITE		
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Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO

	T: Mark all packages and papers with contract and/or orde	er numbers.	ITE			OPDERNO	/	-w
DATE OF ON	75N98024P01555					COAT		
ITEM NO.	SUPPLIES/SERVICES	WAS	QUANTITY	UNIT	UNIT	AMOUN	IT QUANTI	TY
(a)	(b)		(c)	(d)	(e)	(1)	(g)	
	American; Free Trade Agreemer	nts;Israeli				/		
	Trade Act (May 2014) (41 U.S	.C. chapter 83,						
	19 U.S.C. 3301 note, 19 U.S.	C. 2112 note,		1			WHITE	
	19 U.S.C. 3805 note, 19 U.S.	C. 4001 note,		1 1			COAT	
	Pub. L. 103-182, 108-77, 108	-78, 108-286,					MASTE	
	108-302, 109-53, 109-169, 10	9-283, 110-138,		17				
	112-41, 112-42, and 112-43.							
	(ii) Alternate I (May 201	4) of 52.225-3.	/	(
	(iii) Alternate II (May 2)	014) of						
	52.225-3. WHITE	WH	TE			WHITE		
	(iv) Alternate III (May 2)	014) of CO	AT			COAL		
	52.225-3.	WAS	TE			WASTE		
	Over \$191K (48) 52.225-5,	Trade						
	Agreements (FEB 2016) (19 U.	S.C. 2501, et				/		
	seq., 19 U.S.C. 3301 note).					/		
	X_AII (49) 52.225-13, Rest	rictions on						
	(F Q :s proglamations and	atatutos		1 1			WHITE	
	administered by the Office o	f Foreign					QOAT	
	Assets Control of the Depart	ment of the					NASTE	
	Treasury)	merre or che		1				
	Outside US performing secur	ity (50)						
	52.225-26. Contractors Perfo	prming Private					/	
	Security Functions Outside t	he United				AALLINE .		
	States (Oct 2016) (Section 8	62, as amended,	1 F			WHILE		
	of the National Defense Auth	orization Act	22			Grant.		
	for Fiscal Year 2008; 10 U.S	.C. 2302 Note).	16			WMSIG		
	Local area set asides (51) 52.226-4,				`		
	Notice of Disaster or Emerge	ncy Area				/		
	Set-Aside (Nov 2007) (42 U.S	.C. 5150).						
	Local area set asides (52) 52.226-5,		1			WHITE	
	Restrictions on Subcontracti	ng Outside		1			COAT	
	Disaster or Emergency Area (Nov 2007) (42		11			MACTE	
	U.S.C. 5150).			1			1111010	
	_If allowing for financing t	erms, insert if		$\langle -$				
	financial condition is accep	table security_		1				
	(53) 52.232-29, Terms for Fi	nancing of					/	
	Purchases of Commercial Item	ıs (Feb 2002)	TE			WHITE		
	(41 U.S.C. 4505, 10 U.S.C. 23	^{307(f))} . CO	AT			COAL		
	Qualified installment pay	ments (54)	TE			WASTE		
	52.232-30, Installment Payme	ents for	N			/		
	Commercial Items (Jan 2017)	(41 0.5.0.				/		
	4505, 10 U.S.C. 2307(f)).	nt by				/		
	Electronic Funds Transfer: St	istem for Award						
	Management (Oct 2018) (31 U	S C 3332)					WHITE	
	Continued	5.0. 55527.		9			COAL	
	continued			11			WASTE	
				/				
				(
							/	
	La		- 1			A A A A A A A A A A A A A A A A A A A		
	WHILE	WHI				0.00		
	TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17/H	-1))	2			50.00		

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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO 12

ATE OF OR	75N98024P01555				ORDER NO. HILE	
ITEM NO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT	AMOUNT	QUANTITY
(a)	(b)	(C)	(d)	(e)	(f)	(g)
	Exception applies (56) 52.232-34,				/	
	Payment by Electronic Funds Transfer;Other		$\left \right\rangle$		/	
	than System for Award Management (Jul 2013)					WHITE
	(31 U.S.C. 3332).					COAT
	Purchase card (57) 52.232-36, Payment by	7				Xorr
	Third Party (May 2014) (31 U.S.C. 3332).					NMOILE
	IT with Security (58) 52.239-1, Privacy					
	or Security Safeguards (Aug 1996) (5 U.S.C.		X			
	552a).					
	All that contain 52.219-9 Small Bus.	uure (MALLIPPE (`
	Subcontracting Plan (59) 52.242-5,				WHILE	
	Payments to Small Business Subcontractors	14			S. WATE	
	(JAN 2017) (15 U.S.C. 637(d) (12)).	4315			WASIE	
	Ocean transportation (60) (i) 52.247-64.					/
	Preference for Privately Owned U.SFlag					
	Commercial Vessels (Feb 2006) (46 U.S.C.		\mathbf{N}		/	
	Appx. 1241(b) and 10 U.S.C. 2631).					WILLITE
	(ii) Alternate I (Apr 2003) of 52.247-64	l.	1.1			A DAF
	(iii) Alternate II (Feb 2006) of					WYGTE
	52.247-64					MMSIE
	(c) The Contractor shall comply with the					
	FAR clauses in this paragraph (c),		1			
	applicable to commercial services, that the	e /				
	Contracting Officer has indicated as being	ure (WHITE	`
	incorporated in this contract by reference	315			COAT	
	to implement provisions of law or Executive	Carte -			WAATE	
	orders applicable to acquisitions of	1316			MMSIC	
	commercial/items:					
	[Contracting Officer check as appropriate.]					
	X Service contracts that are not exempted				/	
	(1) 52.222-17, Nondisplacement of Qualified	t l	<u></u>			WUITE
	Workers (May 2014) (E.O.13495).					COAT
	Subject to SCA (2) 52.222-41, Service		1.1			XATE
	Contract Labor Standards (Aug 2018) (41		1.1			MHOIL
	U.S.C. chapter 67).					
	Subject to SCA (3) 52.222-42, Statement		1			
	of Equivalent Rates for Federal Hires (May					
	2014) (29 U.S.C. 206 and 41 U.S.C. chapter	uire .			WHITE	`
	67).	OAT .			COAT	
	Subject to SCA (4) 52.222-43, Fair Labor	ATE .			XX OTE	
	Standards Act and Service Contract Labor	1010			11/1010	
	Standards-Price Adjustment (Multiple Year					
	and Option Contracts) (Aug 2018) (29 U.S.C.					
	206 and 41 U.S.C. chapter 67).		\mathbf{N}			
	Subject to SCA (5) 52.222-44, Fair Labor		N			WHITE
	Standards Act and Service Contract Labor		1			COAT
	Continued		Ŭ			WACTE
	In the second seco		1.7			
			1			
		/				
	WHITE WI	ITE			WHITE	
	TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H))				\$0.00	
HORIZED F	OR LOCAL REPODUCTION	STE			WASTE	OPTIONAL FORM 348 (Rev. 4/200
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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO

ATE OF OR	DER	CONTRACT NO.						ORDER NO.		
ITEM NO.	1	SUPPLIES/SERVICES	WAS	QUANTITY	UNIT	UNIT	l	AMOUNT	QUANTITY	
(a)		(b)		ORDERED (c)	(d)	PRICE	/	(0)	ACCEPTED	
	Sta	andards; Price Adjustment (May 2014)	(29				/			
	U.S	S.C. 206 and 41 U.S.C. chapter 67).					/			
	X	Maintenance that is commercially				VHITE			WHITE	
	ava	ailable (6) 52.222-51, Exemption from	m			TAOC			TAOD	
	App	lication of the Service Contract Lak	oor		1	VACTE			MACTE	
	Sta	andards to Contracts for Maintenance,	,		1	11010			/	
	Cal	ibration, or Repair of Certain			/					
	Equ	ipment;Requirements (May 2014) (41								
	U.S	S.C. chapter 67).								
	-	When using 52.222-52 (7) 52.222-53,	WH	TE						
	Exe	emption from Application of the Servi	ice	AT						
	Con	itract Labor Standards to Contracts f	for	TE						
	Cer	ctain Services;Requirements (May 201	4)				/			
	(41	U.S.C. chapter 67).								
		work is performed in US and 52.222-	6 or				/			
	52.	222-41 also are included (8) 52.222	2-35, 550			1				
	Min	num wages Under Executive Order 136	028			WHITE			WHITE	
	(De	ec 2015) (Executive Order 13658).				COAL			TAOD	
	-wn	ien clause 52.222-6 Construction Wage	e		- 6	VASTE			NASTE	
	ing	(1) (2)	NVO.		1	N 1			/	
	Und	Nor Executive Order 13706 (JAN 2017)	ive		/					
	/E	0 13706)								
	1.1.	Over \$25K for provision service or s	ale						`	
	of	food (10) 52.226-6. Promoting Excess	S	I.E.						
	Foo	d Donation to Nonprofit Organization	ns	Pr .						
	(Ma	v 2014) (42 U.S.C. 1792).		16						
	(d	 Comptroller General Examination 	of				/			
	Rec	cord. The Contractor shall comply wit	th				/			
	the	e provisions of this paragraph (d)	if							
	thi	s contract was awarded using other t	han		<u></u> ,	VUITE			WILITE	
	sea	aled bid, is in excess of the simplif	ied			VOIL C			COAT	
	acq	uisition threshold, and does not cor	ntain			Wate			MAGTE	
	the	e clause at 52.215-2, Audit and			1	MOIL			WHOIG	
	Rec	cords;Negotiation.			/					
	(1)	The Comptroller General of the Unit	ced							
	Sta	ates, or an authorized representative	e of							
	the	e Comptroller General, shall have acc	cess	TE						
	to	and right to examine any of the		ΔŤ.						
	Con	tractor;s directly pertinent record	s	TE						
	inv	volving transactions related to this								
	con	itract.					/			
	(2)	The Contractor shall make available	e at				/			
	its	s offices at all reasonable times the	•		\mathbf{i}	/				
	rec	cords, materials, and other evidence	for			VHITE			WHITE	
	exa	amination, audit, or reproduction, un	ntıl		0	TAO			COAT	
	Con	itinued			1	ASTE			WASTE	
					1	\				
					/					
		WHITE	WHI	TE				WHITE		
			CO	[******				0000		

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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

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EM NO.	Mark all packages and papers with contract and/or order numbers. CONTRACT NO. 75N98024P01555	ITE		ORD	ER NO.	
EM NO.	ER CONTRACT NO. 75N98024P01555			ORD	ER NO.	
EM NO. (a)		Carlo -			COAL	
1-3	SUPPLIES/SERVICES	QUANTITY ORDERED (c)	(d)	UNIT PRICE (e)	AMOUNT (f)	QUANTITY ACCEPTED
	3 years after final payment under this	(-)				(a)
X	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		100			WHITE COAT WASTE
i I	any resulting final termination settlement. Records relating to appeals under the				WHITE	
	disputes clause or to litigation or the	1 E			COAT	
	settlement of claims arising under or	75			WACTE	
	relating to this contract shall be made	10			Insis	
	available until such appeals, litigation,					
	or claims are finally resolved.					
	(3) As used in this clause, records include					\mathbf{X}
V	books, documents, accounting procedures and		1			WHITE
g	practices, and other data, regardless of					COAL
	require the Contractor to create or		11			MASTE
1	maintain any record that the Contractor		/			
	does not maintain in the ordinary course of					
7	business or pursuant to a provision of law.					
	(e) (1) Notwithstanding the requirements of	TE			WHITE	
	the clauses in paragraphs (a), (b), (c),	Ť			COAT	
i	and (d) of this clause, the Contractor is	TE			WACTE	
1	not required to flow down any FAR clause,	· ~ .			100000	
0	other than those in this paragraph (e)(1)					
	in a subcontract for commercial items.					
	Unless otherwise indicated below, the					\sim
e	extent of the flow down shall be as		- W			WHITE
g	required by the clause;		L .0			COAL
10	Ethics and Conduct (Oct 2015) (41 U.S.C.		111			WASTE
/	3509).					
/	(ii) 52.203-19, Prohibition on Requiring	- /				
0	Certain Internal Confidentiality Agreements					
0	or Statements (Jan 2017) (section 743 of	TE			WHITE	
1	Division E, Title VII, of the Consolidated	ÀT.			COAT	
i	and Further Continuing Appropriations Act,	TE			WASTE	
	2015 (Pub. L. 113-235) and its successor					
1	provisions in subsequent appropriations					
	acts (and as extended in continuing					
1	(iii) 52.204-23. Prohibition on Contracting					
- 2	for Hardware, Software, and Services		1 1			WHILE
	Continued		1			WHETE
170	MASIE WASIE					minare
/						
		/	1		/	
		/				
		TE -			WHITE	
<u>г</u>	TOTAL CARRIED FORWARD TO 1ST PAGE (ITEM 17(H))	\geq			\$0.00	

ORDER FOR SUPPLIES OR SERVICES

Uncovered by a White Coat Waste investigation

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ORDER FOR SUPPLIES OR SERVICES

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TE OF OR	RDER CONTRACT NO. 75N98024P01555	WH	AT	2		ORDER NO.		
EM NO.	SUPPLIES/SERVICES	WA.	QUANTITY	UNIT	UNIT	MÀ	AMOUNT	QUANTITY
(a)	(b)		(C)	(d)	(e)	/	(f)	(g)
	(xii) 52.222-40, Notification of Em	ployee				/		/
	Rights Under the National Labor Rel	ations		\mathbf{i}				/
	Act (Dec 2010) (E.O. 13496). Flow de	nwc						WHITE
	required in accordance with paragra	ph (f)						COAT
	of FAR clause 52.222-40.			1				MASTE
	(xiii) 52.222-41, Service Contract	Labor		1				
	Standards (Aug 2018) (41 U.S.C. cha	pter 67).		/				
	(xiv) (A) 52.222-50, Combating Traf	ficking	/					
	in Persons (Jan 2019) (22 U.S.C. ch	apter 78						
	and E.O 13627).	WH	TE			WH		
	(B) Alternate I (Mar 2015) of 52.22	2-50 (22	AT			CO		
	U.S.C. chapter 78 and E.O 13627).	WAS	TE			WA.		
	(xv) 52.222-51, Exemption from Appl	ication						
	of the Service Contract Labor Stand	ards to						
	Contracts for Maintenance, Calibrat	ion, or						
	Repair of Certain Equipment-Require	ements		\mathbf{i}				
	(May 2014) (41 U.S.C. chapter 67).							WHITE
	(XVI) 52.222-53, Exemption from App	lication						TAOD
	of the Service Contract Labor Stand	ards to		- 1				WASTE
	Contracts for Certain Services-Requ	lirements		1				
	(May 2014) (41 U.S.C. chapter 6/).			/				
	(XVII) 52.222-54, Employment Eligit	, ility						
	(uniii) 52 222 55 Minimum Magaa Un).						
	(XVIII) 52.222-55, Minimum Wages on	lder	TE			WH		
	Executive Order 13658 (Dec 2015).	, CO	AT.			CO		
	(X1X) 52.222-62, Paid Sick Leave 0	nder	TE			WA.		
	Executive Order 13/06 (Jan 2017) (E	.0.				/		/
	13706). () (D) 52 224 2 Duing an Euclidian	1.7.						
	(XX)(A) 52.224-3, Privacy framing	(Jan						
	(D) Alternate T (Jap 2017) of 52 22	1.2		\mathbf{N}				
	(B) Alternate I (Jan 2017) of 52.22	4-3. mina						WHITE
	(XXI) 52.225-20, Contractors Perior	the		- 9				COAL
	United States (Oct 2016) (Section 8	62 as		1				WASTE
	amended of the National Defense	02, as		1				
	Authorization Act for Fiscal Year 2	008.10	/	(
	H S C 2302 Noto)	000, 10						
	(xxii) 52 226-6. Promoting Excess B	boo'						
	Donation to Nonprofit Organizations	(May	TE			WH		
	2014) (42 U S C 1792) Flow down re	equired	AL			<u> </u>		
	in accordance with paragraph (e) of	FAR	316			WA.		
	clause 52.226-6.					/		1
	(xxiii) 52.247-64. Preference for F	rivatelv				/		
	Owned U.SFlag Commercial Vessels	(Feb						
	2006) (46 U.S.C. Appx, 1241(b) and	10						· · · · · · · · · · · · · · · · · · ·
	U.S.C. 2631). Flow down required in			y y				WHILE
	Continued			1.5				UDAL-
	WASTE WASTE			11				NHSIE
				1				
			/					
	WHILE		1.12	1		VV H		

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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

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	T: Mark all packages and papers with contract and/or order numbers.	/				MILITE	
DATE OF ORI	75N98024P01555				ORDEF	R NO.	
ITEM NO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT		AMOUNT	QUANTITY
(a)	(b)	ORDERED	(d)	PRICE		(0)	ACCEPTED
	accordance with paragraph (d) of FAR clause				1	· · · · · ·	
	52.247-64.						
	(2) While not required, the Contractor may		1	VHITE			WHITE
	include in its subcontracts for commercial			COAT			COAL
	items a minimal number of additional		6	VASTE			WASTE
	clauses necessary to satisfy its		1	\ \		/	
	Contractual obligations.		/				
	(End of clause)						
	Only Used if HCA waived Examination of					SALLIPPE	
	Records by Comptroller General Alternate I					WHILE	W
	(Feb 2000). As prescribed in					WARTE .	
	12.301(b)(4)(i), delete paragraph (d) from	316				WASIG	UVP
	the basic clause, redesignate paragraph (e)						
	as paragraph (d), and revise the reference						
	to ;paragraphs (a), (b), (c), or (d) of		\mathbf{i}	/			
	this clause; in the redesignated paragraph		1	VHITE			WHITE
	(d) to read ;paragraphs (a), (b), and (c)			TAOC			COAT
	of this clause.;		6	VASTE			WASTE
	Only used for ARRA Funds_Alternate II (Jan		1			/	
	substitute the following paragraphs (d) (1)						
	and $(e)(1)$ for paragraphs $(d)(1)$ and $(e)(1)$						
	of the basic clause as follows:					A / L L 1997	
	(d) (1) The Comptroller General of the	15				WHILE	W
	United States, an appropriate Inspector	85				WXOTE	i i i i i i i i i i i i i i i i i i i
	General appointed under section 3 or 8G of	16				WPISTE	
	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;		1	VHITE			WHITE
	(i) Examine any of the Contractor;s or any			COAL			COAL
	subcontractors; records that pertain to,		1	VASTE			WASTE
	and involve transactions relating to, this		1			/	
	(ii) Interview any officer or employee	1 1	(
	regarding such transactions.						
	(e) (1) Notwithstanding the requirements of	ire'				WHITE	14/1
	the clauses in paragraphs (a), (b), and	115				COAT	VVI CC
	(c), of this clause, the Contractor is not	TE				WACTE	й/2
	required to flow down any FAR clause in a					1011010	100
	subcontract for commercial items, other						
	than;						
	(i) Paragraph (d) of this clause. This			/			/
	paragraph flows down to all subcontracts,			VHITE			WHITE
	except the authority of the inspector		5	TAQ			CDAT
	concended		1	VASIE			WASIE
			/			/	\sim
		/	1			/	
	WHITE WH	TE				WHITE	W
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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO 18

ATE OF ORD	DER CONTRACT NO. 75N98024P01555				-	ORDER	NO. HITE	
ITEM NO.	SUPPLIES/SERVICES	- C	UANTITY	UNIT	UNIT		AMOUNT	QUANTITY
(a)	(b)	0	RDERED (c)	(d)	PRICE (e)	/	(f)	ACCEPTED (g)
	General under paragraph (d)(1)(ii) flow down; and (ii) Those clauses listed in this p (e)(1). Unless otherwise indicated the extent of the flow down shall k required by the clause; (A) 52.203-13, Contractor Code of Ethics and Conduct (Oct 2015)(41)	does not paragraph below, be as Business J.S.C.						IHITE OAT ASTE
	 3509). (B) 52.203-15, Whistleblower Protect Under the American Recovery and Reinvestment Act of 2009 (Jun 2010 (Section 1553 of Pub. L. 111-5). (C) 52.204-23, Prohibition on Cont for Hardware, Software, and Service Developed or Provided by Kaspersky Other Covered Entities (Jul 2018) 1634 of Pub. L. 115-91). 	ctions racting es Lab and (Section	HTH.					HITE
	 (D) 52.204-25, Prohibition on Confor Certain Telecommunications and Surveillance Services or Equipment 2019) (Section 889(a) (1) (A) of Pub 232). (E) 52.219-8, Utilization of Small Concerns (Oct 2018) (15 U.S.C. 637 and (3)), in all subcontracts that further subcontracting opportunities the subcontract (except subcontract) 	Video Video (AUG L. 115- Business (d) (2) offer es. If ts to	ELLE					ASTE
	<pre>small business concerns) exceeds \$ (\$1.5 million for construction of public facility), the subcontractor include 52.219-8 in lower tier subcontracting opportun (F) 52.222-21, Prohibition of Segr Facilities (Apr 2015). (G) 52.222-26, Equal Opportunity ((E.O. 11246).</pre>	700,000 any r must contracts ities. egated Sep 2016)						HITE OAT <i>ASTE</i>
	 (H) 52.222-35, Equal Opportunity f Veterans (Oct 2015) (38 U.S.C. 421) (I) 52.222-36, Equal Opportunity f Workers with Disabilities (Jul 201) U.S.C. 793). (J) 52.222-40, Notification of Emp Rights Under the National Labor Re Act (Dec 2010) (E.O. 13496). Flow of Continued 	or 2). or 4) (29 loyee lations down		20		/		
	WHITE	WHIT		/			WHITE	1315

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Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO 19

E OF ORDI	75N98024P01555				COAL	
M NO.	SUPPLIES/SERVICES	QUAN		UNIT	AMOUNT	QUANTITY
(a)	(b)	(c)	(d)	(e)	(f)	(g)
	required in accordance with paragraph (f)				/	
	of FAR clause 52.222-40.					
1	(K) 52.222-41, Service Contract Labor			WHITE		WHITE
- 2	Standards (Aug 2018) (41 U.S.C. chapter 6	7).		COAT		COAT
12	(L) _ALL_(1) 52.222-50, Combating			VACTE		MACTE
	Trafficking in Persons (Jan 2019) (22			MASIE		MPISTE
/	U.S.C. chapter 78 and E.O 13627).					
/	Outside US_(2) Alternate I (Mar 2015) of		X			
	52.222-50 (22 U.S.C. chapter 78 and E.O		/			
	13627).	NUTE			WHITE	
	(M) 52.222-51, Exemption from Application				COAT	
	of the Service Contract Labor Standards to	0			WXATE.	
	Contracts for Maintenance, Calibration, o	r			WASIE	
	Repair of Certain Equipment; Requirements					
	(May 2014) (41 U.S.C. chapter 67).					
\mathbf{X}	(N) 52.222-53, Exemption from Application	1			/	
<u>`</u> .	of the Service Contract Labor Standards to	0		· · · · · · · · · · · · · · · · · · ·		1411177
	Contracts for Certain Services; Requiremen	nts		WHILE		VIALLE
	(May 2014) (41 U.S.C. chapter 67).			LOAL		GOAL
- 11	(0) 52.222-54. Employment Eligibility			VASIE		MASIE
1	Verification (Oct 2015) (Executive Order		/			
/	12989).		X			
	(P) 52,222-55. Minimum Wages Under		/			
	Executive Order 13658 (Dec 2015)	/			· · · · · · · · · · · · · · · · · · ·	
	(0) 52,222-62. Paid sick Leave Under	VHILE			WHITE	
	Executive Order 13706 (Jan 2017) (E.O.	QAL.			COAL	
	13706)	ASIE			WASIE	
	(R) (1) 52 224-3. Privacy Training (Jan					
1.1	2017) (5 II S C 552a)					
	(2) Alternate I (Jan 2017) of 52 224-3				/	
	(S) 52 225-26 Contractors Performing			/		×
V	Private Security Functions Outside the			WHITE		WHITE
- 9	United States (Oct 2016) (Section 862 as			COAL		COAL
11	amended of the National Defense			VASIE		WASTE
1	Authorization Act for Fiscal Year 2008. 10	0	1			
/	H C C 2202 Note)	0	X			
	(T) 52 226-6 Promoting Excess Food		/			
	Donation to Nonprofit Organizations (May				/	
	2014) (42 U.S.C. 1792) Flow down required	YHITE			WHITE	
	in accordance with paragraph (a) of FAR	LAOC			COAL	
	alauso52 226-6	VASIE			NASIE	
	52 244-6 Subcontracts for Commercial Ite	me				
	(AUG 2019)					
	(II) 52 247-64 Preference for Privately				/	
	Owned II S - Flag Commercial Vessels (Feb			/		N
V	2006) (46 II S C Appy 1241(b) and 10			WHITE		WHITE
0	Continued			COAL		COAL
1	continuea			VASTE		WASTE
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RIZED FO						OPTIONAL FORM 348 (Rev.

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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO

DATE OF OR	DER CONTRACT NO. 75N98024P01555	AT	2		ORDER NO.	Ē	W
ITEM NO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT	A AN	IOUNT	QUANTITY
(a)	(b)	(c)	(d)	(e)	1	(f)	(g)
(2)	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	TE			WHIT	E TE	
	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	I.E.		NHITE COAT VASTE	WHIT	ц.	
	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		- and -	VHITE COAT ASTE	WAST	TE VOS	
	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		Soc	VHITE CAT IASTE	WHIT COA WAST	E LE	
	WHITE	TE			WHIT	E	
	COAT	[*****			0.00	0	

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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO

DATE OF ORI	DER CONTRACT NO.				1.5.64	ORDER	NO. HITE	
ITEMNO	/5N98024201555	00		Linux			COAL	QUALTTY
TIEM NO.	SUPPLIES/SERVICES		ORDERED		PRICE		AMOUNT	ACCEPTED
(a)	to, making Contractor employees ava	ailable	(C)	(a)	(e)	-/-	(0)	(g)
	for:	i fabic						
	(i) Formal and informal interviews	by EEO			ALL HTTE			MUNTE
	counselors or other Agency official	Ls			COAT			COAT
	processing EEO complaints;				VATE			Xort
	(ii) Formal or informal interviews	by EEO			MADIE			MPISTE
	investigators charged with investig	gating		/				
	complaints of unlawful discriminat.	ion filed	/	(
	by Federal employees;							
	(iii) Reviewing and signing approp:	riate WH	TE				WHITE	1
	affidavits or declarations summari	zing CO	AT				COAT	
	statements provided by such Contrac	ctor	TE				WASTE	l l
	employees during the course of EEO						1	
	investigations;							
	(iv) Producing documents requested	by EEO						
	counselors, EEO investigators, Age	ncy		1	/			\
	employees, or the ELOC in connection	on with a			VHITE			WHITE
	(v) Proparing for and providing to	atimony			COAL			TAOD
	in depositions or in hearings befor	estimony		- 0	VASIE			NASIE
	MSPB, EEOC and U.S. District Court	e che		1				
	(b) The Contractor shall include th	le					/	
	provisions of this clause in all							
	subcontract solicitations and subco	ontracts	~~ (WILLITE	`.
	awarded at any tier under this cont	ract.	1 두				COAT	
	(c) Failure on the part of the Co	ntractor	75				WACTE	
	or its subcontractors to comply wit	h the	16				WMSIG	
	terms of this clause may be grounds	for the						
	Contracting Officer to terminate th	nis						
	contract for default.				/			
	(End of clause)				VHITE			WHITE
	352.239-74 Electronic and Informat:	ion		- (COAT			COAT
	Technology Accessibility.			- V	VASTE			WASTE
	As prescribed in HHSAR 339.203-70(F	, (כ		1	\ \			
	Insert the following clause:						/	
	Accessibility (December 18 2015)	DGA						
	(a) Burguant to Section 508 of the						/	
	Rehabilitation Act of 1973 (29 U.S.	C WHI	TE				WHITE	
	794d), as amended by the Workforce	~· <u>CO</u>	BL I				COAL	S S
	Investment Act of 1998, all electro	nic and	16				WASIE	1
	information technology (EIT) suppl.	ies and						
	services developed, acquired, or ma	aintained						
	under this contract or order must c	omply			/			
	with the ¿Architectural and Transpo	ortation		i.	VHITE			WHITE
	Barriers Compliance Board Electron.	ic and		1	TAO			COAT
	Continued			Ŭ	ACTE			WAGTE
	The			1.1	1.010			1111010
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			/					
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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

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	wark all packages and papers with contract and/or order numbers.						
OF ORDER	CONTRACT NO.					ORDER NO.	
I NO.	SUPPLIES/SERVICES	WAS	QUANTITY	UNIT	UNIT	AMOUN	T QUANTITY
0	(b)		ORDERED	(d)	PRICE	(1)	ACCEPTED
, T	nformation Technology (EIT) Accessibi	lity	(0)	147	(*)		(3)
S	tandards: set forth by the Architectur	al				/	
	nd Transportation Barriers Compliance	. u I					
	and framsportation barriers compriance						WHITE
B	oard (also referred to as the ¿Access						COAL
Bo	oard;) in 36 CFR part 1194. Informatio	n					MASTE
al	bout Section 508 is available at			1			
h	ttp://www.hhs.gov/web/508. The comple	te					
te	ext of Section 508 Final Provisions ca	in be	/	r			
a	ccessed at						
h	ttp://www.access-board.gov/guidelines	-and-s	(MALLITTE	*
t.	andards/communications-and-it/about-t	he-sec	15			WHILE	
+	ion-508-standards					COAL	
	b) The Section 500 accordibility stars	larde	16			WASIE	
	by the section 500 accessibility stand	arus	N			/	
aj	ppicable to this contract or order ar	2				/	
10	dentified in the Statement of Work or					/	
S	pecification or Performance Work			\backslash			\setminus
S	tatement. The contractor must provide	any					W/HITE
ne	ecessary updates to the submitted HHS			1			COAT
P	roduct Assessment Template(s) at the e	end		1			MACTE
0	f each contract or order exceeding the			1			101010
s	implified acquisition threshold (see H	FAR					
2	.101) when the contract or order durat	ion	1	r 1			
i	s one year or less. If it is determined	d by					
t	he Government that EIT supplies and					NAVL UPTE	·
S	ervices provided by the Contractor do	not	1.5			WHILE	
C	onform to the described accessibility	CQ/	here a			COAL	
	tandards in the contract remodiation	of	16			WASIE	
5	he supplies or convises to the level of	£				/ `	
	ne supplies of services to the rever o	1 				/	
C	onformance specified in the contract w	VIII.				/	
b	e the responsibility of the Contractor	c at		\mathbf{i}		· · · · · · · · · · · · · · · · · · ·	
1	ts own expense.			1			WHITE
(c) The Section 508 accessibility stand	dards		- 0			COAT
aj	pplicable to this contract are:			1			WASTE
11				1			/
			1	1			
()	Contract staff must list applicable						
S	tandards)						
()	d) In the event of a modification(s) t	0 W H I	TE			WHITE	
tl	his contract or order, which adds new	EIT	Ť			COAT	
SI	upplies or services or revises the typ	e	TE			MAGTE	
0	f, or specifications for, supplies or		1.64			1111316	
S	ervices, the Contracting Officer may						
r	equire that the contractor submit a					/	
C	ompleted HHS Section 508 Product					/	
A	ssessment Template and any other			1			· · · · · · · · · · · · · · · · · · ·
21.	dditional information necessary to ass	siet		- V			WHITE
a	ontinued	JIGC		9			COAL
C	MASTE			11			WASTE
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OPTIONAL FORM 348 (Rev. 4/2006) Prescribed by GSA FAR (48 CFR) 53.213(f)

PORTAN	T: Mark all packages and papers with contract and/or order numbers.					
TE OF OR	DER CONTRACT NO.	ITE			ORDER NO.	
	75N98024P01555				COAT	
EM NO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT	AMOL	JNT QUANTITY
		ORDERED		PRICE		ACCEPTED
(a)	(b)	(C)	(d)	(e)	(1)	(g)
	the Government in determining that the EIT				/	
	supplies or services conform to Section 508			1		
	accessibility standards. Instructions for			VHITE		WHITE
	documenting accessibility via the HHS			TAO		COAT
	Section 508 Product Assessment Template may		1	VACTE		WACTE
	be found under Section 508 policy on the			inore.		101010
	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	TE			WILLITE	
	accessibility standards in the contract,	15			COAT	
	remediation of the supplies or services to				COAL	-
	the level of conformance specified in the	16			WASIC	
	contract will be the responsibility of the				/	
	Contractor at its own expense				/	
	(a) If this is an Indefinite Delivery				/	
	(e) II this is an indefinite belivery			1		
	Contract, a Blanket Purchase Agreement of a			WHITE		WHITE
	Basic Ordering Agreement, the task/delivery			COAL		COAL
	order requests that include EIT supplies or		6	VASTE		MASTE
	services will define the specifications and		1	\ \		
	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	TE			WHITE	
	additional information necessary to assist	Τ			COAT	
	the Government in determining that the EIT	TE			MACTI	
	supplies or services conform to Section 508				1010010	
	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		L.	VUITE		WHITE
	it is determined by the Government that EIT			COAT -		201F
	supplies and services provided by the		1	XAT		MAGTE.
	Contractor do not conform to the described		11	MASIE		WHSTE
	accessibility standards in the provided		/			
	documentation, remediation of the supplies					
	or services to the level of conformance					
	specified in the contract will be the				A A A A A A A A A A A A A A A A A A A	· · · · · · · · · · · · · · · · · · ·
	responsibility of the Contractor at its own	15			WHILE	
	evnense	34			UQAL	
	(End of clause)	16			NASIE	
	This of ofdioc,				/	
	FAR Deviation Clause				/	
	Executive Order 14042, Ensuring Adequate				/	
	COVID Safety Protocols for Federal		1	/		· · · · · · · · · · · · · · · · · · ·
	Contractors		V	VHITE		WHITE
	Continued		9	JAQ		COAL
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	WHILE WHI	1			VVIIII	

ORDER FOR SUPPLIES OR SERVICES

Uncovered by a White Coat Waste investigation

Prescribed by GSA FAR (48 CFR) 53.213(f)

PAGE NO



PAGE NO

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.





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 licens and compare the suspension or revocation of licens and compare the suspension or revocation of licens.

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



BILL OF LADING SHORT FORM -ORIGINAL NOT NEGOTIATED

SHIPPER NO.	157424	NUMBER OF SHIPPING UNITS	PKG TYPE	нм	DESCRIPTION OF ARTICLES, SPECIAL MARKS & EXCEPTIONS	cuiss - III COA	NMFC ITEM NO.	WEIGHT		CHARGES
Carrier Name	Marshall Farms Group, Lld, DBA TRRI	Thears		設置においた。	4 Beagle Dogs Male 14 Months	WAS	C.		WA	
ADDRESS	Marshall Blokesources	4	1-		4 Deagle Dogs, Male, 14 Months	/	1		/	
TY / STATE / ZIP	North Rose, NY 14516	-	1	1					/	
		-		/						
FULL NAME	National Institute of Health		W	DAT	COAT			COAT		
ADDRESS	9000 Rockville Pike		W/	ISTE	WASTE			WASTE		
ADDRESS	/				/ \		1	1		
ADDRESS			/	/						
TY / STATE / ZIP	Bethesda, MD 20892					1	1			
TELEPHONE	ITE	VHITE			A WE HERE SALAN A SAL		WITOTAL		TOTAL CHARGES	
BUS. REG. NO.	AL	COD FEE		ADDITIONAL INFO	COAL	SINGLE SHIPMENT	-	SHIPMENT CHARGE	S PREPAID UNLESS MARKI	DCOLL
D FREIGHT BILL TO	0	PREPAID		ROUTE NO.	WASIE	YES	6		COLLECT	_
NAME		COLLECT		DEPT. NO.	X	NO				
merked and label bansport accord	Ied/placarded and are in all respects in proper condition for ding to applicable international and national governmental regulations.	WHITE COAT VASTE	8	RECEIVED BY	WHITE COAT WASTE	WHIT COA WAST				

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

INTERSTATE / INTERNATIONAL HEALTH CERTIFICATE FOR DOGS



Species	50	Date	Ear Marshall Tag# ID #	(if applicable)	Name	Lot #	Vaccination Date	
				\setminus /			/	
BEAGLE	Cani M	4 2-20-23	1117923		RabVac3	E071891A	4-02-24	
BEAGLE	Cani M	2-21-23	1118423		RabVac3	E071891A	4-02-24	
BEAGLE	Cani M	4 2-23-23	1120002		RabVac3	E071891A	4-02-24	
BEAGLE	Cani M	4 2-25-23	1120974	MANTE	RabVac3	E071891A	4-02-24	
		14315		11/13/5		MMSIC		

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: Address: Street, City, Zip: Telephone: License: Signature, date 4/19/2024 WHITE COAT



Marshall BioResources

North Rose, NY 14516-9795

Ph 315-587-2295 Fx 315-587-2109

BILL TO:

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

Invoice

Invoice No: Date: Due Date: Cust ID: Currency:

IN318195 23-Apr-2024 23-May-2024 52985 USD

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

CUSTOMER PO NUMBER TERMS Net 30 Days 75N98024P01555 SHIP VIA SHIP DATE ORDER DATE SO NUMBER 4/16/2024 157424 Marshall Truck 4/23/2024 UOM UNIT PRICE EXTENDED ITEM QTY. Beagle Male 14 Months old EACH 4 Beagle Per Diem Fee EACH 4 NOTE4/16/2024 - 4/22/2024



NOTE: 6887993

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

COA WAST

Page: 1 of 1

53/24

RECORD OF SALE OF DOGS



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 licens provide the suspension or revocation of licens. The suspension of the subchapter of the suspension of the subchapter of the su

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

INTERSTATE / INTERNATIONAL HEALTH CERTIFICATE FOR DOGS

certificate / Order (Valider)	130134			
I. OWNER:				
Name:	Marshall BioResources - USA			
Address:	WASIB			
Street, City, Zip:	North Rose, NY 14516			
Federal ID #:	315-587-2295	115DA #	4 0000	
Means of transportation:	Marshall Truck	USDA #: 21-	A-0008	
II. CONSIGNEE				
Name	National Inst. of Health			
Address:	9000 Rockville Pike			
Street, City, Zip	Bethesda MD 20892			
Country	USA			
III. DESCRIPTION, IDENTIFIC	ATION & VACCINATION AGAINST RABI	ESWHITE		
Species Sex Birt	th Ear Marshall Microchip #	Vaccine	Lot # Vaccinati	ол
Date	Tag# ID # (if applicable)	Name	Date	
PENCIE Cool V 1 11				

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: Address: Street, City, Zip: Telephone: License:	North Rose, NY	14516 - USA		
Signature, date	WHITE	WH		
	WASTE	WAS		
Unco	overed by a White C	oat Waste ir	nvestigation	

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



BILL OF LADING SHORT FORM -ORIGINAL NOT NEGOTIATED

ar r cik										
SHIPPER NO.	158134	NUMBER OF	ATTO SAS	REAL	WHITE	WHI	re	1200	will	11217
TRAILER NO.		SHIPPING	PKG TYPE	НМ	DESCRIPTION OF ARTICLES, SPECIAL MARKS & EXCEPTIONS	CLASS	NMEC ITEM NO.	WEIGHT	RATE	CHARGES
Carrier Name	Marshall Farms Group, Ltd. DBA TRRT	UNITS			WASTE	WAS	TE		WA	
SHIPPER NAME	Marshall BioResources	1			1 Beagle Dog, Male, 15 Months	/			/	
ADDRESS									/	
CITY / STATE / ZIP	North Rose, NY 14516									
INSIGNEE										
FULL NAME	National Institute of Health									
ADDRESS	9000 Rockville Pike									
ADDRESS										
ADDRESS										
CITY / STATE / ZIP	Bethesda, MD 20892									
TELEPHONE	AT	COAT					WT TOTAL		TOTAL CHARGES	
BUS. REG. NO.		COD FEE		ADDITIONAL INFO	WASTE	SINGLE SHIPMENT	TE	SHIPMENT CHARGES		ED COLLECT:
D FREIGHT BILL TO		PREPAID		ROUTE NO.	/	YES			COLLECT	
NAME		COLLECT		DEPT. NO.		NO				
TELEPHONE								SPECIAL INSTRUCTIO	NS	
I hereby declare that described above by marked and labeled transport according	t the contents of this consignment are fully and accurately the proper shipping name and are classified, packaged, uplacarded and are in all respects in proper condition for g to applicable international and national governmental regulations.			RECEIVED BY	WHITE					



Marshall BioResources

North Rose, NY 14516-9795

Ph 315-587-2295 Fx 315-587-2109

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

Invoice

Invoice No: Date: Due Date: Cust ID: Currency:

IN318600 11-Jun-2024 11-Jul-2024 52985 USD

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

CUSTOMER PO NUMBERTERMS24-002634Net 30 DaysORDER DATESO NUMBER5/14/2024158134	SHIP VIA Marshall Truck	SHIP DATE 6/11/2024	
ITEM Beagle Male 15 Months old Beagle Per Diem Fee	QTY. UOM 1 EACH 1 EACH	UNIT PRICE EXT	ENDED
NOTE5/17/2024 - 6/10/2024 Freight - Marshall Truck	WHITE 1 EACH	WHITE COAT WASTE	
		WHITE	
i WASTE		LINASJUN 19 2	1024 J N
WHITE WHITE COAT COAT	WHIT	E	WHITE COAT
		Sales Total: Tax Total: Total (USD):	4,657.00 0.00 4,657.00
		WHITE COAT WASTE Pag	e: 1 of 1

	NATIONAL INSTITU ANIMAL STUDY PI (6/22/20	ITES OF HEALTH ROPOSAL [ASP] 018)	PROPOSAL # <u>CCM 23-02</u>
			EXPIRATION DATE 05/23/202
A. ADMINISTRAT	IVE DATA:		
Institute or Center (Clinical Center	WHITE	WHITE
Principal Investigate	or Steven Solomon, PhD	COAT WASTE	COAT
Building/Room_28/1	134_E-Mail ssolomon@cc.nih.g	gov_Telephone <u>301-435</u>	-2287 FAX <u>301-480-5493</u>
Emergency Treatm document.	ent and Animal Care instructio	ns shall be provided on	the attached form at the end of this
Division, Laboratory	y, or Branch: <u>Critical Care M</u> e	edicine Department	
\ <u></u>			
Project Title Non-R	esearch Holding and Mainte	enance of Canines	
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per	Renewal [X] or Modification [I individuals authorized to cond sonnel (i.e., Co-investigator(s)	enance of Canines] of Proposal Number <u>C</u> duct procedures involving)): A brief summary of the	CM20-02 g animals under this proposal e training and/or experience
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha	Renewal [X] or Modification [I individuals authorized to condisionnel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the supervalues achieved proficiency in those	enance of Canines] of Proposal Number <u>C</u> duct procedures involving): A brief summary of the perform in this ASP musi visor, mentor, or trainer vise procedures shall be in	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance included in that documentation.
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl	Renewal [X] or Modification [I individuals authorized to cond sonnel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the superv as achieved proficiency in thos hD (O) 301-435-2287 (C)	enance of Canines of Proposal Number <u>C</u> duct procedures involving b): A brief summary of the perform in this ASP musivisor, mentor, or trainer vise procedures shall be in 301-717-2494	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance included in that documentation.
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl B. ANIMAL REQU	Renewal [X] or Modification [I individuals authorized to cond sonnel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the superv as achieved proficiency in thos hD (O) 301-435-2287 (C)	enance of Canines] of Proposal Number <u>C</u> duct procedures involving): A brief summary of the perform in this ASP mus- visor, mentor, or trainer vise procedures shall be in 301-717-2494	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance included in that documentation.
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Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl B. ANIMAL REQU Species <u>canine</u> Age Source(s) <u>DVR ap</u>	Renewal [X] or Modification [I individuals authorized to condisionnel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the supervasion achieved proficiency in those hD (O) 301-435-2287 (C)	enance of Canines J of Proposal Number <u>C</u> duct procedures involving perform in this ASP mu- visor, mentor, or trainer v se procedures shall be in 301-717-2494 <u>6kg Sex M or F Stock of</u> Holding Location(s) <u>Bld</u>	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance ncluded in that documentation.
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl B. ANIMAL REQU Species <u>canine</u> Age Source(s) <u>DVR ap</u> Animal Procedure L	Renewal [X] or Modification [I individuals authorized to condisionnel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the supervase achieved proficiency in those hD (O) 301-435-2287 (C) IIREMENTS: e/Weight/Size 13-30 mo, 9-10 proved .ocation(s) n/a	enance of Canines] of Proposal Number <u>C</u> duct procedures involving): A brief summary of the perform in this ASP mu- visor, mentor, or trainer vise procedures shall be in 301-717-2494 <u>6kg Sex M or F Stock of</u> Holding Location(s) <u>Bld</u>	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance ncluded in that documentation.
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl B. ANIMAL REQU Species <u>canine</u> Age Source(s) <u>DVR ap</u> Animal Procedure L Estimated Number of	Renewal [X] or Modification [I individuals authorized to condisionel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the supervase achieved proficiency in those as achieved proficiency in those hD (O) 301-435-2287 (C) JIREMENTS: e/Weight/Size 13-30 mo, 9-10 proved .ocation(s) n/a of Animals:	enance of Canines] of Proposal Number <u>C</u> duct procedures involving): A brief summary of the perform in this ASP mu- visor, mentor, or trainer vise procedures shall be in 301-717-2494 <u>6kg Sex M or F Stock of</u> Holding Location(s) <u>Bld</u>	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance ncluded in that documentation.
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl B. ANIMAL REQU Species <u>canine</u> Age Source(s) <u>DVR ap</u> Animal Procedure L Estimated Number of 100	Renewal [X] or Modification [I individuals authorized to condisionel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the supervase achieved proficiency in those hb (O) 301-435-2287 (C) JIREMENTS: e/Weight/Size 13-30 mo, 9-10 proved .ocation(s) n/a of Animals: 100	enance of Canines] of Proposal Number <u>C</u> duct procedures involving): A brief summary of the perform in this ASP mu- visor, mentor, or trainer vise procedures shall be in 301-717-2494 <u>6kg Sex M or F Stock of</u> Holding Location(s) <u>Bld</u>	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance ncluded in that documentation.
Project Title <u>Non-R</u> Initial Submission [List the names of al and identify key per for procedures each available to the ACI each individual is/ha Steve Solomon, Pl B. ANIMAL REQU Species <u>canine</u> Age Source(s) <u>DVR ap</u> Animal Procedure L Estimated Number of <u>100</u> Year 1	Renewal [X] or Modification [I individuals authorized to condisionel (i.e., Co-investigator(s) in individual will be expected to UC. The name(s) of the supervasion achieved proficiency in those hD (O) 301-435-2287 (C) IREMENTS: e/Weight/Size 13-30 mo, 9-1(proved .ocation(s) n/a of Animals:	enance of Canines] of Proposal Number <u>C</u> duct procedures involving): A brief summary of the perform in this ASP mu- visor, mentor, or trainer visor, mentor, or trainer visor 301-717-2494 <u>6kg Sex M or F Stock of</u> Holding Location(s) <u>Bld</u> <u>100</u> Year 3	CM20-02 g animals under this proposal e training and/or experience st be documented and who will provide assurance included in that documentation.

Describe the methods and containment to be utilized if animals will be transported between facilities. Also

Uncovered by a White Coat Waste investigation

1

Non-Research Holding and Maintenance of Canines

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

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 <u>Guidelines for the Use of Non-Pharmaceutical Grade</u> <u>Compounds in Laboratory Animals</u>
 Blood Withdrawals (volume, frequency, withdrawal sites, and methodology)

Uncovered by a White Coat Waste investigation
	Non-Research Holding and Maintenance of Canines
99	Non-Survival Surgical Procedures (Provide details of survival surgical procedures in Section G.)
100	Radiation (dosage and schedule)
101	Methods of Restraint (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)
102	Animal Identification Methods (e.g., ear tags, tattoos, collar, cage card, etc.)
103	Other Procedures (e.g., survival studies, tail biopsies, etc.)
104	Potentially Painful or Distressful Effects, if any, the animals are expected to experience (e.g., pain
105	or distress, ascites production, etc.) For Column E studies provide: 1) a description of the
106	procedure(s) producing pain and/or distress; 2) scientific justification why pain and/or distress
107	cannot be relieved.
108	Experimental Endpoint Criteria (i.e., tumor size, percentage body weight gain or loss, inability to eat
109	or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity) must be specified
110	when the administration of tumor cells, biologics, infectious agents, radiation or toxic chemicals are
111	expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to
112	determine when euthanasia is to be performed. Death as an endpoint must always be scientifically
113	justified.
114	
115	Animal order requests for this ASP will be processed only when there is an additional approved
116	research study designated for future assignment of animals.
117	Animals purchased from approved vendors will be received by DVR personnel at bldg. 28, NIH, and
118	conditioned in accordance with NIH standard operating procedures. They will be evaluated for normal
110	health condition and neverological behavior indicating that the doas will adapt normally to handling
119	icalli condition and psychological ochavior indicating that the dogs with adapt normany to handning
120	and restraint by technicians. Vaccination requirements will be supplemented as required.
121	All routine veterinary and husbandry care will be provided by assigned DVR staff. All animals are
122	provided enrichment according to DVR SOP. Animals will be transferred to approved research
102	proposals prior to study
123	proposals prior to study.
124	
125	a supervise public process of several to fellowing
126	G. SURVIVAL SURGERY - If proposed, complete the following:

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

NUM	BER OF ANIMALS L	Year 1	Year 2	Year 3	
Х	X USDA Column C Minimal, Transient, or No Pain or Distress		100	100	100
-	USDA Column D	Pain or Distress Relieved By Appropriate Measures			1
	USDA Column E	Unrelieved Pain or Distress		WHILE	

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 <u>Guidelines for the Use of Non-Pharmaceutical Grade Compounds in</u> 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)

Κ.	HAZARDOUS AGENTS:			NONE	Х	(check if none)	
	Use of hazardous agents	requires the a	pproval of an IC sa	ifety specialist.			

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

Agent:

PRD #:

ABSL:

NONE X (check if none)

4

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

Recombinant DNA:

For guidance, see <u>NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid</u> Molecule<u>s FAQs</u>.

Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.



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)

Sterile?

6

N

For guidance, see NIH Policy Manual 3034 – <u>Working with Hazardous Chemicals</u> Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (<u>Title 29, Part 1910,1200(b)(3)(ii), CFR</u>)

List of Agents:

Material:

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.

Source:

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:

 I certify that I have attended an approved NIH investigator training course. Month/Year of Initial Course Completion: <u>9/1998</u>; Month/Year(s) of Refresher Training: <u>2/2020</u>.

 I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.

- I certify that all individuals working on this proposal who have animal contact are 3. participating in the NIH Animal Exposure Program (or equivalent, as applicable, for contract personnel).
- 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.
- 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.
- I will obtain approval from the ACUC before initiating any significant changes in this study. 6.

UTE		Digitally signed by Steven B.	
Principal Investigator:	Steven B. Solomon -S	Solomon -S	
Signature	GUAL	Date: 2023.05.22 13:25:05 -04'00' Date	COAL
S78	ANALS I K	WASTE	

0	CONCUPRENCES	PROPOSAL NUMBER	
Ο.	CUNCURRENCES:	PROPOSAL NUMBER	

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)

Signature	WHIT	Date	ITE M	
NIH Safety Repre material listed in th	sentative: (signature ne Hazardous Materia	e represents certification, complia I Section)	ance and concurrence for use of	
DOHS Safety Rep	oresentative			
Signature		Date		
DRS Safety Repre	esentative			
Signature	STE	Date	<u>W</u> ASTE	
Facility Manager:	(certification of resou	irce capability in the indicated fac	cility to support the	
proposed study)	Nama Sekou !	Savane -S Digitally signed by :	Sekou Savane -S -27:46 -04'00' Doto	
Facility	Name	Signature	Date	
Facility	Name	Signature	Date	
Facility	Name	Signature	Date	

Uncovered by a White Coat Waste investigation

7

-04'00'

Attending Veterinarian: Certification of Review

Digitally signed by Lisa G. Portnoy -S Name Lisa G. Portnoy -S Sign gtwre2023.05.25.16:01:09-04:00' Date

P. FINAL APPROVAL:

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



INSTRUCTIONS FO	R EMERGENCY ANIMAL TREATMENT AND CARE	
Principal Investigator:Steven Solomo	Date form completed: _5\22\23 Protocol Number: _CCM23-02 Office Phone: _301-435-2287 Home Phone: 301-717-2494	COAT WASTE
Protocol Title: Non-Research Quaran Use a separate form if care is different fo Species:canine	tine and Maintenance of Canines or each species Species:	4
Species:	Species:	``
Animal Housing Location: Use separate form if care differs by location	Bidg28 BidgBidg	
List of Procedures:		
(surgery, tumor implant, catheter)nor	10	<u> </u>
Work Tel: _301-435-2287 Hom	e or Emergency: Dr. Steven Solomon ne Tel: _301-717-2494_ Pager or Cell #: _301-717-2494	
Alternate Point of Contact in Case of Em	pergency:	
Work Tel:	Home Tel: Pager or Cell #:	- W ASTE
Potential or Expected Complications: _n	one	<u> </u>
Circumstances Requiring Contact:	significant injury/procedure, euthanasia	
Treatment (indicate appropriate respons Treatment determined by veterinarian: If NO, specify restrictions as fol	se): [X] Yes [] No llows:	
What drugs are contraindicated?		
Criteria for Euthanasia (indicate appropr At Vet discretion if poor condition, sever If NO, specify treatments or restrictions:	riate response) re pain or distress: [X] Yes [] No	
HITE WHIT	E WHITE	WHITE
Notify P.O.C. Requested euthanasia agent	*[X] Yes [] No	
and route of administration:		<u> </u>
		_ \
If Euthanasia is performed or animals are	e found dead:	
a. Contact P.U.C.		
n Dispose of carcass	COAL IXI Yes CC[1 No	
d. Submit to DVR for necronsy	I 1 Yes X1 No	
CAN number to use for submission:		
Additional Comments:		
Principal Investigator:	Divitally signed by Steven P	
Sleven D.	Solomon -S	
Solomon -S	Date: 2023.05.22 13:25:38 -04'00'	
Signature	Date	
		<u> </u>

* 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.

Uncovered by a White Coat Waste investigation

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 Bl	ldg/Rm: 28/119	Email: ssolomon@cc.nih.gov
PI Course completion dates: (Initial) 1998	(Refresher) 2022	WHITE
AU Course completion dates: (Initial)	(Refresher)	
1) Experience: Mimouse: Pirat: Didea		

xperience: M-mouse; R-rat; D-dog

Procedures	Species:	No experience	# Years	Other Comments on training:	
Handling and restraint	M,R,D		>20		1
Anesthesia:	/HITE		WHITE	Type: inhalational, intravenous	TE
Administration	M,R,D		>20	CO/	AT.
Monitoring	/ (M,R,D	د	>20 / () /	E WAQ	TE
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	VITIE	>20	WHILE	<u> </u>
Catheter placement:					
IVASIE	R,D	ASTE	>20	WASIE	
IA	R D		>20		
Intubation	R,D		>20		
Euthanasia	M,R,D		>20		
14/	LUTTE .		WHITE	WHI	TE
ä	OAT .		COAT		1

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.

J will be performing the following awake NHP procedures:

I am currently proficient in performing all of the awake NHP procedures that I've listed above, 4a)_ 0R

will provide my supervision and training until I am fully qualified to perform these awake NHP 4b) 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:

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.

Steven B. Solomon -S Digitally signed by Steven B. Solomon -S Date: 2023.04.18 15:18:42 04:00'

Date:

Date:

Principal Investigator signature:

ANIMAL STUDT PRO	POSAL IASPI	WASTE
(6/22/2018	3)	PROPOSAL # <u>CCM 23-04</u>
		APPROVAL DATE 8/25/23
WHITE WHITE COAT COAT	WHI COA	EXPIRATION DATE 8/25/26
A. ADMINISTRATIVE DATA:	/ /	
Institute or Center <u>Clinical Center</u>		
Principal Investigator Steven Solomon, Ph.D	HITE	WHITE
Building/Room_28/134_E-Mail_ssolomon@cc.nih.gov		5-2287_FAX_301-480-5493
Emergency Treatment and Animal Care instructions shall be provided on the	e attached form at the end of	this document.
Division, Laboratory, or Branch Clinical Center/Critic	cal Care Medicine	Department
Project Title: Functional and Structural Changes Ear	rly in Sepsis-induc	ed Cardiomyopathy
Initial Submission [] Renewal [X] or Modification [] of Proposal Nun	nber_CCM19-04
perform in this ASP must be documented and available to the	e ACUC. The name(s)	ocedures each individual will be expect) of the supervisor, mentor, or trainer wh ball be included in that degumentation
perform in this ASP must be documented and available to the provide assurance each individual is/has achieved proficiency	e ACUC. The name(s) in those procedures s	ocedures each individual will be expect) of the supervisor, mentor, or trainer wh hall be included in that documentation.
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Year l	Year 2			
	1 Cur	Year 3		
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4E:				
be provided by B wing through ith blankets to B	DVR or apy h the breeze Bldg 28/Rm	proved proto way in a tran 119.	col personnel technic asport cage to Bldg 1	cians. Anima 4E and
g 28 to 10/B1L0)2A:			
ted and ventilate 45-85 °F. Anima (301-496-2527) n Bldg. 28 to Bl ort through Bldg als will enter on	ed animal or als will be u) personnel. ldg 10. The lg 10. After n the SW sic	n the study c inder sedatio CCMD staf cart will be sanitization le of Bldg 10	art into a temperature n (see section 1) thro f will be present to n draped with a sheet o of the cart with a sui through the exit loc	-controlled aghout with ionitor and or blanket to table ated near the
to the Lab (B11 ve non-NIH staf. e hall.	L02A) and f ff access, C0	from the lab CMD staff w	o the MR1 scanner (ill ensure the route is	LDRR3T empty prior
acy, follow sign 3, 14, or West 1 le door, 100ft do	to B1 cafe 5), take to 5 own hall on	eteria (straig) 1st flr, go tov right.	nt to end of hall, righ vard short hall to end	t to end of , turn left and
ASIE		HASH	······································	WASIE
de no more than d how it might be rstand it. Acronyr iations, technical	a 300 word enefit human ms should be terms, and j	summary of the sand/or anime defined and argon.	e objectives of this wo als. This should be wri only used when neces	rk. Address ten so that a sary. Please
ints in medical t to 50%). Septic n becomes dysr sening cardiac fur sfunction leadin to solving this r ing cardiac MRI, epsis that simul well-known fact o contract (eject jury in survivor	therapy over c shock occ regulated re unction and ng to death medical qua , the effects lates the car t that sepsis tion fraction rs to non-su	r the decades urs when the sulting in org the associate remains a de indary. of sepsis on diac dysfunc causes reven and an inc rvivors, the c	, mortality from sept infection overtakes an injury and shock. ed shock state. (1-3) cades long still unsol the heart in our sedat tion seen during hum sible cardiac dysfund rease in the size of th ritical factor associat	ic shock he body's The heart is The actual ved mystery ed and han septic ction leading e ventricle. red with
in in fs tt in it	into solving this ing cardiac MRI, f sepsis that simu e well-known fac t to contract (ejec injury in survivor t time appeared t	into solving this medical qua ing cardiac MRI, the effects f sepsis that simulates the car e well-known fact that sepsis t to contract (ejection fraction injury in survivors to non-su t time appeared to be solely t	into solving this medical quandary. Sing cardiac MRI, the effects of sepsis on the sepsis that simulates the cardiac dysfunct e well-known fact that sepsis causes rever t to contract (ejection fraction) and an incu- injury in survivors to non-survivors, the c at time appeared to be solely the left ventra	into solving this medical quandary. Sing cardiac MRI, the effects of sepsis on the heart in our sedat of sepsis that simulates the cardiac dysfunction seen during hum e well-known fact that sepsis causes reversible cardiac dysfunc- t to contract (ejection fraction) and an increase in the size of the injury in survivors to non-survivors, the critical factor associat at time appeared to be solely the left ventricle's ability to fully

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recovery. These changes in ventricular size had previously been wrongly explained by either increases in 92 the filling of the heart (preload) or increased resistance to outflow (afterload). (4-6) We have shown that 93 changes in loading and afterload conditions are not responsible in this study and by exclusion, are related 94 rather to sepsis induced changes in the wall of the heart itself. Associated with recovery of the hearts 95 ability to eject blood or contract, we surprisingly showed for the first time, the left ventricular wall was 96 found to lose mass (15%) and develops an increased percentage of water (edema) over 92 h (2 to 3%). 97 This degree of edema is enough to fully explain the cardiac dysfunction seen during sepsis. There is no 98 biochemical (troponin levels) or histological (light and electron microscopy) evidence that this loss of 99/ mass is due to muscle cell loss (myocyte drop out) or damage from decrease tissue perfusion (ischemia). 100 The loss of mass occurs as the heart is recovering (the ejection fraction is returning to normal) suggesting 101 that it may represent a reparative remodeling of the heart. The most abundant cell type after myocytes is 102 endothelial cells. We hypothesize the microcirculation lined with endothelial cells are damaged but not 103 occluded by the edema early on leading to endothelial myocyte and interstitial edema seen on histology 104 and confirmed on MRI T2 images. Remodeling in response to damaged endothelial cells and potentially 105 some focal myofilament autolysis, shown to be a potentially protective mechanism, may be part of the 106 reparative process and results in restoration of vascular integrity and myocardial function. (7-9) We have 107 identified by MRI these changes described above occur at 48 and 96h of sepsis but we have not examined 108 by MRI the acute changes from time 0 to 48h when the development of the sepsis induced injury is 109 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) 110 after bacterial challenge in ventricular wall size, edema, and mass. We hypothesize we will see edema 111 associated with early chamber size decrease, worse in non-survivors. The worse edema results in a 112 restrictive like cardiomyopathy in non-survivors. After 24h, we hypothesize the loss of mass will begin, 113 with ventricular wall thinning and ventricular chamber size increase associated with survival and recovery. 114 Understanding in survivors verses non-survivors how damage occurs during sepsis resulting in cardiac 115 dysfunction and the reparative process will offer insight into the cause of all organ failure and help solve 116 this decades long mystery and allow us to design and test treatments to minimize damage or enhance 117 recovery. 118

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)

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125 1) There are currently no *in vitro* models that can simulate the biologic complexity and clinically 126 relevant endpoints encompassed by our *in vivo* pneumonia model of sepsis. *In vitro* models can address 127 narrow functional questions but they cannot mimic the complex systems and interactions necessary to test 128 our hypothesis. Computer simulations are beginning to show some promise in areas that have been well 129 defined (i.e., cardiac dynamics), but are still very incomplete in infectious diseases and the immunologic 130 response, given our imperfect understanding of these systems as a whole.

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

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Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy 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 155 a tracheostomy. This procedure has been developed and utilized repeatedly in the canine model at the NIH 156 over the past two decades. However, tracheostomy is a technically challenging procedure in the pig 157 because the animal's neck musculature makes it difficult to maintain patency. Consequently, this may 158 affect the ability of the stoma to be maintained, making it necessary to enroll additional training animals 159 for adapting the procedure to porcine anatomy (14). Further, to simulate human treatments in an intensive 160 care unit, we place percutaneous catheters and may need to replace them during the 92-hour study for 161 monitoring and treatments. Unlike porcine blood vessels, canine vessels are well suited for this. The 162 porcine vessels due to the thickness of the skin are difficult to access and tear with puncture much more 163 easily which can result in lethal hemorrhagic complications. 164

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.

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).

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

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	Functional and Stru	ictural Changes Farly in	Sensis-induced Cardiomyonathy	
197		NTAL DESIGN AND AN	IMAL PROCEDURES: Briefly explain	tha
100	experimental design and specifi	y all animal procedures	This description should allow the ACU	to understand
100	the experimental course of an a	animal from its entry into t	he experiment to the endpoint of the s	tudy
100	Specifically address the following	animal norr its entry into t	if persean()	luuy.
101	Specifically address the following	ig. (Ose additional sheeta	s if fieldssary.)	
191	and the stand in a substance on in still	latiana (aubatanana a a	infectious genetal adjuvante modient	
192	injections, inoculations or instil	lations (substances, e.g.	, mectious agents, adjuvants, medicat	lons,
193	drugs, etc.; dose, sites, volume	, route, diluent, and scher	Jules). ACOUS will address non-	
194	pharmaceutical grade compour	Ids IAW Guidelines for th	e Use or Non-Priarmaceutical Grade	
195	Compounds in Laboratory Anin	<u>tais</u> uoneu withdrowol sites	and methodology)	
190	Non-Suprival Surgical Procedur	es (Provide details of sur	vival surgical procedures in Section G	\checkmark
198	Radiation (dosage and schedule)		inta bargioa procedures in ecolion e.	/
199	Methods of Restraint (e.g., restra	aint chairs, collars, vests,	harnesses, slinos, etc.)	
200	Animal Identification Methods (e.g., ear tags, tattoos, col	ar, cage card, etc.)	
201	Other Procedures (e.g., survival	studies, tail biopsies, etc.		
202	Potentially Painful or Distressfu	I Effects, if any, the anim	als are expected to experience (e.g., r	bain
203	or distress, ascites production.	etc.) For Column E studie	s provide: 1) a description of the	
204	procedure(s) producing pain an	d/or distress; 2) scientific	justification why pain and/or distress	
205	cannot be relieved.		·	
206	Experimental Endpoint Criteria	i.e., tumor size, percenta	ge body weight gain or loss, inability to	o eat
207	or drink, behavioral abnormali	ties, clinical symptomatol	pay, or signs of toxicity) must be specif	fied
208	when the administration of tur	nor cells, biologics, infecti	ous agents, radiation or toxic chemical	is are
209	expected to cause significant s	symptomatology or are po	tentially lethal. List the criteria to be us	sed to
210	determine when euthanasia is	to be performed. Death a	is an endpoint must always be scientifi	icaliy
211	justified.			
212				
213				
214	Twenty-four sedated, trache	eostomized and mechar	ically ventilated purpose bred beag	gles (18-30
215	mo / 9-15 kg) will be studied up to	92 h. All animals will :	receive a bacterial dose of S. Aureu	s (0.5-1.5 x
216	10^9 CFU/kg) that results in a 50-70	% mortality. In this stu	dy we will compare the changes in	i cardiac
210	function and mass of survivors to r	on cuminate Two or t	bree animals will be enrolled each s	tudu wool
21/	function and mass of survivors to t	and the second s	rectantials will be embled each s	study week.
218	Animals will initially be sti	idled at baseline and re	peated at 0, 16, 50, 42, 54, 92 n 1011	owing
219	bacterial inoculation. At each time	point, cardiac MRI, he	modynamic measures and blood sat	mpling will be
220	done.			
221	Animals alive at 92h will b	e considered survivors	and after all studies are completed v	will be
272	euthanized (see Section J). Tissues	are collected from all a	inimals' post-mortem for further an	alvsis
222	including for pathology transcript	unics (messenger RNA) and cell sorting (flow cytometry)	WXATE
223	mentuning for pathology, transcript	Annes (messenger rear) and con sorting (now cytomous).	WMSIE
224				
225				
226	Procedure Timeline			
227	Prior to the beginning of the	e study a blood sample	(6 ml) will be taken from 1 of the s	tudy dogs.
228	(see below for details)			
229	COAL			
220	On the first day of each stu	dy, each animal will un	dervo one set of procedures which a	will he
250		1, cach ann an whiteh	aligo one set of procedures which	······································
231	performed in Bidg. 14E. Initially, a	ui study animais will be	e placed under general anestnesia (s	ee section 1).
232	Peripheral catheters (external jugul	ar, femoral arterial, cep	bhalic vein, and urinary) will be place	ced
233	percutaneously and a tracheostomy	will be surgically plac	ed to maintain a secure airway duri	ng prolonged
 12/	machanical ventilation with sedation	on (see section G) and t	hen returned to hldg 28 rm 119	WHITE
234	mechanical ventilation with sedatic	in (see section G) and t	non retained to blug. 20 mi 119.	
235	Propofol infusion is initiate	d after instrumentation	and maintained until analgesia (fen	(tanyl) and
110	addition (midazalam) infusions are	initiated and adjusted	to levels that can maintain adequate	sedation (see
230	Security influezorality influsions are		and a set of the factor is a set of the	Is a securitori (Sec
237	section I). A dexmedetomidine infi	ision will be added to s	upprement the rentanyl and midazo	iam infusions

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Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy 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 249 minutes and a bronchoscope (sterile at the beginning of each study, rinsed between animals) will be 250 advanced via the tracheal tube past the carina, into the right mainstem bronchus and then into a right lower 251 lobe segmental bronchus. A pulmonary artery occlusion catheter (Swan Ganz) will then be advanced via 252 253 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 10⁹ cfu/kg) will be administered via the 254 catheter into the subsegmental bronchus followed by 3 ml of saline. The balloon will then be deflated and 255 the catheter removed. The animal will then be mechanically ventilated with humidified air (Vela 256 ventilator, Carefusion and Conchatherm, Hudson Medical). 257

After intra-bronchial bacteria placement (T0), continuous monitoring of the animal's oxygen 258 saturation, arterial blood pressure and central venous pressure will begin. This time point will be 259 considered T0. A maintenance treatment regimen will be initiated until the development of sepsis (T4). 260 The maintenance treatment regimen will include a phenylephrine bolus or infusion (10 mg/250 ml; 0.5 ml 261 bolus or infusion titrated with a micro drip set) to maintain the animal's blood pressure at a MAP >80 262 mmHg, a maintenance intravenous fluid infusion (2 ml/kg/hr) of Normasol-M with 5% dextrose 263 supplemented with KCl (27mEq/l), and ventilatory support with tidal volume (TV) of 20 ml/kg, an oxygen 264 concentration (FiO2) of 25% and a positive end expiratory pressure (PEEP) of 5 cm H2O. The amount of 265 potassium (K) supplementation provides a small excess of K. For example, at 11 kg, hourly K requirement 266 = ~.07 meg/kg or ~.77 mEq, with an administration rate (0.022 L/hour * 40 mEq/L) = 0.88 mEq. (using 267 the standard dose would have resulted in undertreating with 20 mEq/L (= 0.44 mEq/hour). This 268 maintenance regimen will be maintained until T4. 269

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. 275 aureus, will be administered q24 to T92. Famotidine, an anti-acid (1 mg/kg, IV, q12) and heparin (3000 276 IU, sq, q8) will be administered for gastrointestinal ulcer and deep venous thrombosis prophylaxis. 277 respectively. General care for animals during the period of mechanical ventilation will be based on the 278 standard of care for critically ill animals requiring sustained mechanical ventilation in the clinical setting. 279 Every 4 h, the animal's mouth will be flushed with chlorhexidine solution and the eyes will be lubricated 280 with a sterile ophthalmic petroleum gel and the dog will be rotated. Sterile saline (3 ml) will be instilled in 281 the trachea followed by tracheal suctioning as needed. The inner cannula of the tracheostomy will be 282

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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.

296 MRJ Scan (LDRR 3T)

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Animals will be transferred from a transport cart and carried into the scanner and placed on the 298 bed. Ventilation and sedation will be transferred to MR compatible equipment (LTV1200) or extended 299 through the wave guide. The dog will be placed on a heated and padded surface. ECG and temperature 300 probes connected. A series of EKG-gated cardiac scan sequences will be performed to measure ventricular 301 chamber size, mass and edema. Depending on the sequences being performed, contrast (Gadavist, 1 302 mmol/ml) may be used. All fluids and sedation are continued during scanning. The animal's 303 hemodynamics and sedation is continuously monitored and recorded every 10 minutes (Appendix D). 304 After the scan sequence is complete (about 30 minutes), the animal is returned to the lab for continued 305 306 care.

308 CT Scan (1C560 or 1C562)

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

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Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy Animals will be transferred from the transport cart to the scanner bed. We will perform an EKG-312 gated cardiac CT angiograph with contrast (Isovue, Bracco). The scan will delineate the walls of the 313 ventricular chamber allowing us to measure changes in ventricular wall thickness. After the scan is 314 completed, the animal will be returned to the lab. 315

Pre-study blood sample 317

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318 One enrolled study animal will be brought from the 28 B-wing to the lab (28/119). Two highly 319 experienced technicians will handle and aseptically draw the blood sample. The sample site along the 320 brachial vein is prepared by removing the hair with an electric razor and cleaning with alternating three 321 scrubs of chlorhexidine or providone-iodine and 70% alcohol. A straight needle (23 ga) with a 6 ml 322 syringe catheter will be used to take a sample. The catheter will be removed and the vein compressed until 323 hemostasis is achieved (5-10 min). The animal will then be returned to B-wing. 324 325

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

Pain Monitoring and Management 327

In this model of intra-bronchial pneumonia, treatment with sedation and analgesia are utilized in 329 critical care veterinary practice to remove pain and distress, as during human illness. 330 The animals will be monitored continuously throughout the protocol by a CCMD staff member 331 with no other primary responsibilities. During the day (~0600-1700), additional CCMD staff members are 332 available in the building to assist as necessary. At night (~1700-0600), a CCMD technician will be in the 333 room with the other technician close enough to respond quickly (within 2 min). The animals will be 334 assessed for adequacy of sedation and analgesia every hour throughout the 92 h study. This frequency of 335 observation is consistent with the current practice at North Carolina State University Small and Large 336 Animal ICU (email exchange with ICU director Bernie Hanson, DVM). If there is any sign of inadequate 337 sedation or analgesia, the animal will be treated according to "Sedation regimen in response to purposeful 338 movement/Signs of distress" (see Section I). A supervisory or attending investigator will be available for 339 consultation at all times. If an event should occur that is not defined in the treatment algorithm or defined 340 in this protocol, Dr. Natanson and Dr. Portnoy or her designee will be notified. 341

Euthanasia criteria 343

Euthanasia criteria include: 1) an animal determined to be in pain and/or distress which cannot be 344 relieved with increased levels of propofol, fentanyl, midazolam, and dexmedetomidine by the facility or 345 Dr. Portnoy, Natanson or Solomon within 20 minutes; 2) seizure activity for greater than 2 minutes; 3) 346 uncontrolled hemorrhage from any orifice; or 4) oxygen saturation <40% for >30 minutes. Animals 347 selected for early euthanasia will be noted and the basis for euthanasia recorded in the medical record. At 348 any time during the study, animals noted to be in clear distress or pain by the facility or CC ACUC 349 veterinarian, CCMD supervisor or attending will be euthanized. 350

352 Monitoring of Bacterial Counts

Each week, the bacteria are grown and the amount is determined turbodimetrically. The bacteria 353 are diluted to 0.5-1.5 x 10⁹ CFU/kg for intrabronchial inoculation into the animal. The bacteria are then 354 plated out on two sets of growth plates. The next day, when the bacteria have grown on these plates, an 355 experienced microbiology laboratory technician counts the number of colonies to determine if the variance 356 is within 10-15% of the amount determined turbodimetrically. All procedures and counts are kept in a log 357/ book (28/121). 358

- Management and Coordination of Study Activities 360
 - Uncovered by a White Coat Waste investigation

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 364 the study and daily operations of the protocol. He will assign and schedule personnel who will conduct 365 procedures described in the ASP and coordinate with DVR facility management. Study scheduling and 366 animal assignments (confirmed by ear tattoo) will be given to DVR staff as soon as possible. The facility 367 veterinarian will manage the animals when they are in the veterinary facility. Clinical status of the animals 368 and schedule changes will be discussed between the investigative staff and the facility veterinarian as 369 needed during the study period. Dr. Lisa Portnoy is familiar with the model and will be directly monitoring 370 the study animals on a routine basis (daily whenever possible) and be informed of any previously unknown 371 characteristics or problems that occur with the model. Dr. Charles Natanson, an anesthesiologist, will be 372 consulted regarding anesthesia and sedation administration, monitoring procedures and the overnight call 373 374 for this protocol.

376 Animal ordering

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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.

381•Sex382•Breed383•Date of birth384•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.

391 Record maintenance

392 CCMD staff will maintain a folder of all laboratory data obtained for each animal which will be 393 available to all staff. Each animal has a separate medical record maintained with the forms used for 394 surgery and all other interactions. A note of the condition of the animal and the 92-hour monitoring form 395 will be included in the animal treatment record during each day of a study. In addition, notes will be made 396 in the medical record for activities by the staff and status of the animal.

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- G. SURVIVAL SURGERY If proposed, complete the following: None X Major____ Minor____
- Identify and describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized.
 (Use additional sheets if necessary):
 Who will perform surgery and what are their qualifications and/or experience?
- 365 3. Where will surgery be performed, Building and Room?
- While will suggery be performed, balance and recent to the use of post-operative analgesics, and identify the responsible individual:
 - Has survival surgery been performed on any animal prior to being placed on this study? Y/N If yes, please explain:
- 370 6. Will more than one survival surgery be performed on an animal while on this study? Y/N

Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy If yes, please justify:

lf y

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375

- 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.
- 381 All animals are anesthetized as described in section I.

382 External Jugular Catheter Placement

A catheter introducer (Maxxim Medical, Athens, TX, 8 French introducer) will be placed 383 percutaneously into the right external jugular vein. Through this introducer, a 7 French pulmonary artery 384 thermodilution catheter (Abbott Critical Care, Chicago, IL) will be advanced into the pulmonary artery via 385 the external jugular vein to measure PAP, PAOP, CVP and CO, sample blood and deliver fluids. A second 386 arterial catheter (20 ga, PTFE, Maxxim Medical, Athens, GA) will be placed percutaneously in the left 387 external jugular vein of each animal to deliver medications. The catheter has a smaller diameter than a 388 standard venous catheter and should be less intrusive to the dog and minimize any effect on the vessel. The 389 catheter will be sutured (1-0 to 4-0 monofilament) in place and flushed with heparinized saline. 390

391

393

392 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.

- 399 Cephalic Vein Catheter Placement
- 400

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401 An 18 ga. catheter (Maxxim Medical, Athens, TX) will be placed percutaneously for administration of 402 sedation.

403

404 *Tracheostomy* 405

A 5-6 cm skin incision is made on the cervical midline just posterior to the larynx. The incision is 406 deepened to the level of the sternohyoid muscles which are separated on the midline. The trachea is 407 dissected free from the adjacent tissues and a number 0 silk suture is passed around the trachea distal to the 408 proposed opening in the trachea where the tracheotomy tube will be placed. A U-shaped incision on the 409 ventral surface of the trachea is started a couple tracheal rings posterior to the larynx and extended 410 posteriorly for two or three cartilagenous rings. The flap is raised anteriorly as a door so that the 411 tracheostomy tube may be placed. The temporary tracheostomy tube is introduced into opening made in 412 the trachea and the cuff is inflated. The 0 silk suture that was preplaced earlier is tied just anterior to the 413 inflated cuff to keep the tube from accidentally being pulled out. The same silk suture is inserted through 414 the U-shaped tracheal flap and the tracheostomy tube is secured to the flap. The subcutaneous tissue and 415 skin are closed with 3-0 silk suture. 416

417							
418	Fole	y Urinary C	atheter Placement				
419							
420	A Fo	oley catheter	(Cook, Foley 8 Fr, 5	5 cm) will be introdu	ced into the distal	urethra and advanced u	ntil
421	the t	ip is position	ied in the bladder all	owing the urine to flo	w. After inflation of	of the balloon, the cathe	eter is
422	secu	red by suturi	ing to the skin (Coate	ed Vicryl 3-0 [Ethicon] or similar at the	discretion of the surgeo	m).
423		TE I	. WAG		WXCTF		(TE
424							
425	2.	Who will	perform surgery and	l what are their qualif	ications and/or exp	erience?	
426						1 1 500 01	
427	Dr. N	Marvin Thor	nas will perform the	tracheostomies. Dr. T	homas has perform	ied more than 500 of th	lese
428	proce	edures. Dr. 1	solomon will instill t	he bacteria. Ms. Feng	or Dr. Solomon w	ill place the arterial and	1
429	veno	us catheters	, and instrument the o	logs to measure press	ures, volume and i	lows. Dr. Solomon has	
430	perte	ormed bronc	hoscopies in over 50	0 animals and Mis. Fe	ng and Dr. Solomo	in nave placed vascular	
431	cathe	eters in more	; than 300 animals.				
432	_			1 0 11 10			
433	3.	Where w	ill surgery be perform	ned, Building and Roo	om?		
434	WHIT	E	WHI				
435	Bldg	: 14E/115A	and 113B				
436		5	WAST	6	WASIE	A	
437 438	4.	Describe analgesic	post-operative care r s, and identify the res	equired, including consible individual:	nsideration of the i	ise of post-operative	
439	n/a						
440							
441	5.	Has majo	or survival surgery be	en performed on any	animal prior to bei	ng placed on this study	?
442		Y/N	XCTE				
443		If	yes, please explain:				
444							
445	No						
446							
447	6.	- Will more	e than one major surv	vival surgery be perfo	rmed on an animal	while on this study?	
448		Y/N	WX8				
449		lf If	yes, please justify:				
450							
451	No						
452							
453		<u></u>	OAL	COAL		COAL	
454	H.	RECORDIN	G PAIN OR DISTRES	S CATEGORY - The AC	CUC is responsible f	or applying U.S. Governm	nent
455		Principle IV	.: Proper use of animal	s, including the avoidar	ce or minimization of	of discomfort, distress,	
456		and pain wh	en consistent with sou	Ind scientific practices, i	is imperative. Unles	s the contrary is	
457		established,	, investigators should o	consider that procedures	s that cause pain or k the appropriate ca	distress in numan	
458 450		Deings may and indicate	the approximate num	her of animals in each	Sum(s) should equa	It total from Section BMH	
455		and moleate	, the approximate hum		Barrita) Briedia Oque		
461	UF.	ANIMALS AF	RE INDICATED IN COL	UMN E, A SCIENTIFIC	JUSTIFICATION IS	S REQUIRED TO 🛛 \min	
462	EX	PLAIN WHY	THE USE OF ANEST	HETICS, ANALGESICS	, SEDATIVES OR 1	RANQUILIZERS	
463	DU	IRING AND/C	OR FOLLOWING PAIN		L PROCEDURES IS		
464				GULATED SPECIES, F		ETHE UMENT THIS FORM	
405 466				AL REPORT TO THE US	SDA. FOR ALL OTH	IER SPECIES. THE	
100	**1		HIE	WHITE	· · · · · · · · · · · · · · · · · · ·	WHITE	11
						COAL	

Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy 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

NUM	NUMBER OF ANIMALS USED EACH YEAR			Year 2	Year 3
ITE	USDA Column C	Minimal, Transient, or No Pain or Distress	E		WF
X	USDA Column D	Pain or Distress Relieved By Appropriate Measures	24		22
016	USDA Column E	Unrelieved Pain or Distress	6		1100

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 479 below and have found no valid alternatives to any procedures described herein which may cause more than 480 momentary pain or distress. All literature searches for alternatives to this animal model include conducting 481 a full Medline, Embase and Scopus search of the past 20 years of literature. The 2 search strategies 482 covered Alternative Sepsis Models and Alternative Sepsis Models and Pain, These searches have been 483 conducted within the last 2 months. No pertinent literature was found to answer the question that this study 484 addresses. Although other models exist, they are inappropriate for the present study as described in Section 485 E. 486

Details of database search strategies:

for Pubmed

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492 Alternative Sepsis Models

493 (animal use alternatives[majr] OR animal testing alternatives[majr] OR (replace[tiab] AND reduce[tiab] 494 AND refine[tiab]) OR (replacement[tiab] AND reduction[tiab] AND refinement[tiab]) OR (replacing[tiab] 495 AND reducing[tiab] AND refining[tiab]) OR animal replacement[tiab] OR animal reduction[tiab] OR 496 animal refinement[tiab] OR "three Rs"[tiab] OR 3Rs[tiab] OR NC3Rs[tiab]) AND (animal 497 experimentation[majr] OR "animal experimentation"[tiab] OR "experimental animal"[tiab] OR 498 "experimental animals" [tiab] OR animal welfare [majr] OR "animal welfare" [tiab] OR models, 499 animal[majr] OR "animal model"[tiab] OR "animal models"[tiab] OR disease models, animal[majr] OR 500 "animal disease model" [tiab] OR "animal disease models" [tiab] OR animals, laboratory [majr] OR 501 "laboratory animal"[tiab] OR "laboratory animals"[tiab] OR "animal testing"[tiab] OR dogs[mesh] OR 502 dog[tiab] or dogs[tiab] OR canine[tiab] OR canines[tiab] OR beagle[tiab] OR beagles[tiab] OR "sepsis 503 model"[tiab] OR "sepsis models"[tiab] OR "septic model"[tiab] OR "septic models"[tiab]) AND (bacterial 504 infections[majr] OR "bacterial infection"[tiab] OR "bacterial infections"[tiab] OR pneumonia, 505 bacterial[majr] OR "bacterial pneumonia"[tiab] OR "s. aureus pneumonia"[tiab] OR sepsis[majr] OR 506 sepsisitiab] OR shock, septic[mair] OR septic[tiab] OR septicemia[tiab] OR bacteremia[mair] OR 507 bacteremia[tiab] OR "blood stream infection"[tiab] OR "blood stream infections"[tiab] OR "bloodstream 508 infection"[tiab] OR "bloodstream infections"[tiab] OR endotoxins/blood[majr]) NOT mouse[ti] NOT 509 mice[ti] NOT murine[ti] NOT rat[ti] NOT rats[ti] NOT rodent[ti] NOT rodents[ti] 510 201 citation 511

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513	Alternative Sepsis Models and Pain			
514				
515	(animal use alternatives[mesh] OR ar	nimal testing alternatives[mesh	 OR (replace[tiab] AND reduce[tia 	ab]
516	AND refine[tiab]) OR (replacement[tiab] AND reduction[tiab] AN	D refinement[tiab]) OR (replacing[t	riab]
517	AND reducing[tiab] AND refining[ti	ab]) OR animal replacement[ti	ab] OR animal reduction[tiab] OR	
518	animal refinement[tiab] OR "three R	s"[tiab] OR 3Rs[tiab] OR NC3	Rs[tiab]) AND (animal	
519	experimentation[mesh] OR "animal e	experimentation"[tiab] OR "ex	perimental animal"[tiab] OR	
520	"experimental animals"[tiab] OR ani	mal welfare[mesh] OR "anima	I welfare"[tiab] OR models,	
521	animal[mesh] OR "animal model"[tia	ab] OR "animal models"[tiab]	OR disease models, animal[mesh] (OR
522	"animal disease model"[tiab] OR "an	imal disease models"[tiab] OF	animals, laboratory[mesh] OR	
523	"laboratory animal"[tiab] OR "labora	ttory animals"[tiab] OR "anim	al testing"[tiab] OR dogs[mesh] OR	ł
524	dog[tiab] or dogs[tiab] OR canine[tia	b] OR canines[tiab] OR beagl	e[tiab] OR beagles[tiab] OR "sensis	-
525	model"[fiab] OR "sensis models"[fiab]	ol OR "septic model"[tiab] OR	"septic models"[tiah]) AND (bacte	Tial
526	infections[mesh] OR "bacterial infect	tion"[tiab] OR "hacterial infec	tions"[tiab] OR pneumonia	
527	bacterial[mesh] OR "bacterial nneum	ionia"itiab] OR "s aureus pne	umonia"[tiab] OR sensis[mesh] OR	
528	sensis[tiah] OR shock sentic[mesh] (OR sentic[tiab] OR senticemia	[tiab] OR hacteremia[mesh] OR	
529	bacteremia[tiah] OR "blood stream in	fection"[tiah] OR "blood stre	am infections"[tiab] OR "bloodstres	, m
530	infection" [tish] OR "bloodstream inf	ections" [tiab] OR endotoxins/	blood[mesh]) AND (pain[mesh] OR	•
531	naintiah] OR nainstiah] OR nainfil	[tiah] OR distress[tiah] OR dis	tressing[tiab] OR discomfort[tiab] (OP
221	sufferitish) OR suffering[tish] OP M	aloxicam[tiab] OR opioids[tia	hi OR doop sodation[mosh] OR	Л
552	suffer[tiab] OR suffering[tiab] OR sed	ation[tish] OR ventilated[tish]	OR anesthesia and analogsia[mash	i
535	OP enerthesis[tigh] OP engloseis[tigh]	bl OP analossic[tiab] OP anal	on anestnesia and analgesia[mesii	·]
535	37 pitotions	b] OK analgeste[tiab] OK anal	gestes[tiab])	
535	57 citations			
530				
540 541 542 543	agent(s), the dosage, route, and sch grade compounds IAW <u>Guideline</u> Laboratory Animals.	edule of administration. ACUCs s s for the Use of Non-Pharma	will address non-pharmaceutical ceutical Grade Compounds in	
544 545	NONE(check if none)			
546	Range of drug dosing administration			
547	Propofol (4-12 mg/kg/h titrated to lev	vel, IV)		
548	Fentanyl (50-200 µg/h titrated to leve	el, IV)		
549	Midazolam (Versed) (5-40 mg/h titra	ted to level, IV)		
550	Dexmedetomidine (50-400 µg/h titrat	ted to level, IV)	WHITE	
551	Isoflurane (0.5-5%, titrated to effect)			
552				
553	*Note: the titrated dose is based on or	ur 20 year experience of an eff	ective dose range for sedating anim	als
554	between 9-15 kg which due to changi	ing severity of illness is dynan	nc throughout the study.	
555				
556	Pre-surgical induction cocktail: ketan	nine (5.5mg/kg IM), aceproma	zine (0.1mg/kg IM),	
557	Torbugesic (0.3mg/kg IM), atropine ((1.5ml/dog IM) followed by IV	/ propofol (6 mg/kg)	
558	WASTE WAST			
559	Animals will be kept NPO for 12 h pr	rior to anesthesia to minimize	the possibility of aspiration.	
560				
561	Animals will be maintained with proj	pofol during transport and MR	I. Propofol will be titrated to mainta	in
562	an effective plane of sedation. Mecha	nical ventilation will be provi-	ded by a portable MRI compatible	
	COAT		COAT	13

Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy 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.

566 Specifics of sedation administration

The animals will be induced with the pre-surgical cocktail (see above), intubated, and mechanically 567 ventilated. Anesthesia will be maintained with isoflurane (0.5 - 5% titrated to effect). After the above 568 procedures have been performed, isoflurane will be terminated and upon being able to breath 569 independently, sedation will be initiated with propofol (80 mg/h) and maintained for transfer to Bldg, 28. 570 Upon return of the animal to Bldg. 28, the animals will be ventilated (tidal volume: 20 ml/kg, FiO2-25%, 571 ventilation rate = 15 breaths/minute, $PEEP = 5 \text{ cmH}_2O$) with humidified air. Analgesia with fentanyl (50 572 µg/h infusion, 1 ml/h) and sedation with midazolam (9 mg/h infusion, 3 ml/h) will be initiated. Propofol 573 administration will be terminated after fentanyl and midazolam infusions are able to maintain adequate 574 sedation (see sedation infusion incrementation regimen below). A dexmedetomidine infusion (5-40 ug/h) 575 will be used to supplement the fentanyl and midazolam infusions if an animal requires additional sedation 576 throughout the study. The level of anesthesia will be evaluated continuously to assess the adequacy of the 577 578 fentanyl, midazolam and dexmedetomidine infusions and titrated upward until effective (see algorithm below) 579 580

- 581 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.
- 584 2) The eyeballs should remain central in the orbit.
- 585 3) The animal should be unresponsive to light tactile stimuli.
- 586 4) Palpebral reflexes not present (Criteria for reducing sedation).
- 588 Sedation Bolus regimen in response to purposeful movement/Signs of distress
- 589 (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
- 591

587

- 592 Sedation infusion incrementation regimen
- 593 (After initial bolus, increments should be made 20 minutes apart until an adequate plane of sedation is 594 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
- 597 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
- 600 601

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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

Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy pathology will be placed in double lined brown boxes. The boxes will be placed in the refrigerator.

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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
COAT	COAT	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.

618

Recombinant DNA:

NONE X (check if none)

For guidance, see <u>NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid</u> Molecules FAQs.

Include the NIH Institutional Biosafety Committee's risk-assessment language or attach a copy of the registration documents.

Recombinant DNA:

ABSL

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

RD #:

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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 – <u>Working with Hazardous Chemicals</u> Material safety data sheets for hazardous chemicals and drugs must be maintained readily accessible to laboratory and animal facility employees (<u>Title 29, Part 1910.1200(b)(3)(ii), CFR</u>)

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 <u>NIH Chemical Hygiene Plan</u> (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:		ાર	Sterner	
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S. Aureus	NIH 10D ICU	WASIL		Х	
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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 guestion? 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.

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	N. PRINCIPAL	INVESTIGATOR	CERTIFICATION	IS:		
1.	I certify that I	have attended a	an approved NIH ir	vestigator training	course.	
· •	Month/Year of	or initial Course u	Completion: <u>9/1998</u>	<u>s</u> ; Month/Year(s) proposed bergin i	of Refresher Fraining: <u>20</u>	2017
۷.	duplicative of	f previously repo	inted research	r proposed herein	is not unnecessarily	
3.	I certify that a	all individuals wo	rking on this propo	sal who have anin	nal contact are	
	participating	in the NIH Anima	al Exposure Progra	am (or equivalent,	as applicable, for	
	contract pers	onnel).				
4.	I certify that t	he individuals lis	ted in Section A ar	e authorized to co	nduct procedures	
	involving anir	mals under this p	proposal, have com	npleted the course	"Using Animals in	
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	reporting anim	mal welfare conc	erns. I further cert	ify that I am respo	nsible for the	
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814 Appendix A: Route from Loading dock to B1L02A (Lab) and to LDRR3T (B1D315) MRI

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PROPOSED CCMD APL SAT LAB RESTRICTED ROUTE

CC SPACE MANAGEMENT



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Appendix B: Laboratory Tests 821 822 823 Blood sampling (over 24 h) 824 Day 1 TEST <u>Day 2</u> <u>Day 3</u> Day 4 <u>Day 5</u> ABG 2 1 1 1 1 CBC 0.5 0.5 1 0.5 0.5 6 3 Chemistry 3 3 3 Troponin/CPK/BNP 6 3 3 3 3 Catecholamine/electrolytes 6 6 12 6 6 Blood culture/ 1 1 1 1 1 Total 28 14.5 14.5 14.5 14.5 **82**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.

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831 Appendix C: Treatment Algorithms (25)

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832 The results of these hemodynamic measurements listed in these Treatment Algorithms will dictate the subsequent treatments of the study animals. Oxygenation will be initiated at a concentration of 833 25% and a PEEP of 5 cm H₂O (see Oxygen Support Algorithm). The animal will be maintained at 834 these levels for a minimum of 15 minutes, and any subsequent interventions that are performed to 835 improve oxygenation will allow fifteen minutes to take effect. If the oxygen saturation falls below 836 92%, the oxygen concentration will be increased to 50%. If the oxygen saturation falls below 92%, 837 FiO_2 will be increased to 75% (PEEP=5). If after a minimum of 15 minutes the oxygen saturation 838 falls below 92%, the PEEP will be increased to 10 cm H₂O. If the oxygen saturation falls below 839 92%, the delivered oxygen concentration will be increased to 100%. If after a minimum of 15 840 minutes the oxygen saturation falls below 92%, the PEEP will be increased to 12 cm H₂0. The 841 oxygen saturation status will be monitored continuously and can be adjusted according to these 842 guidelines any time the oxygen saturation falls below 92%. Should oxygen saturation remain 843 above 93% for 6 h, the oxygen intervention will be decreased to the previous step (see Oxygen 844 Support Algorithm). If at any time the oxygen saturation falls below 85%, the next level of support 845 may be immediately initiated (i.e. unnecessary to wait 15 minutes to see the effects of a previous 846 treatment before giving additional support). 847

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Oxygen Support Algorithm



The blood gas measurements at T4 will be used to adjust the respiratory rate on the ventilator to control the pCO₂ of the blood and to assist in the maintenance of pH (see pCO_2 algorithm below). As shown in the pCO₂ algorithm, if the blood gas measures a pCO₂ 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 pCO₂ is less than 35 mmHg or a maximum of 30 breaths/minute is reached. To increase pCO₂ to 35 breaths/minute, the pCO₂ must be greater than 35 mmHg, MAP greater than 70 mmHg and peak ventilator pressure must be less than 35 cmH₂O. After the initial adjustments at T4, the pCO₂ will be monitored every 2 hours until T8 and then every 8 hours thereafter. If the pCO₂ 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).

Time point-based Fluid Support (T5 - T96)

Fluid Support



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

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CT / MRI Scan Record

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NMR Center Animal/Tissue proposal

Animal Study Proposal (ASP) # \underline{CC}	M23-04	Imag	ing Amendment #_	n/a 🔗	
ASP Title:	ral Changes E	arly in Se	psis-induced Ca	ardiomyopath	
Co-investigators involved in imaging: (list ONLY those people working in the	NMR Center, the	y are requi	red to attend MRI S	afety Training)	
Co-investigator Name	Email Addr	ess	Phone Number	Safety Training	
Verity Ford	verity.ford	@nih.gov	301-496-3091	9/1/2021	
Jasmine Holden	Jasmine.holde	en@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	
IASIE WASI	8	W	9518	WA	
Magnetic Resonance Imaging (MRI) A	14 H	Hours per Month			
Magnetic Resonance Imaging (MRI) T	<u></u> н	ours per Month			
MicroComputed Tomography (CT) in	2 H	Hours per Month			
MicroComputed Tomography (CT) in	vitro scanner		Hours per Month		
Ultrasound +/- Photoacoustic Imagin	g	- H	ours per Month		
Bioluminescence/Fluorescence	0	— Тн	ours per Month		
Room B1D-200A			ours per Month		
Clinical (human) MRI scanners	WHIT	Н	ours per Month		
Other:	MAST	н	ours per Month		
opproval of Scientific Director:					
laightan Chan	Digitally s	ianed by	Leighton		
Leignton Unar	Chan -S	W	HITE		
	Date: 202	3.08.30	18:26:51		
-0	-04'00'	/	10100101		
Signature of Scientific Direct	or			Date	
	\				

Animal Imaging Subjects: (check all tha	t apply)		
ive Animals: Mouse Rat	Rabbit NHP	Other, please	list: canine
Freshly Dead Tissue	ssue Tissue sourc	_{e_} choose tissue	source
nimal Housing: (list all areas where an	imals will be housed be	fore and after ima	nging)
Species (mouse, rat, etc.)	Building	Room #	Health Report
canine	28	119	WHITE
CO canine	CO 10	B1L02A	COAL
WASTE	WASTE		IVASIE
		/	
endoparasites and ectoparasites. Recent so veterinarian at least 48 hours prior to rode	ing roaent patnogens: coro entinel serum serology and nts entering the NMR Cente	na viruses (ivinv, RCV parasitology reports ir	v, SDAV), PVM, Sendai, must be provided to the Mi
erson responsible for animal(s) or tiss	ue samples while in the	e NMR Center:	
Steven Solomon		30	1-435-2287
ame: cloton colonion		Phone: VV	1-400-2207
ell/Pager: <u>301-717-2494</u>	Email: ssolomon(@cc.nih.gov	
ell/Pager: <u>301-717-2494</u> : Veterinarian providing emergency su	£mail: <u>ssolomon@</u> 	ĝcc.nih.gov	WHITE COAT WASTE
ell/Pager: <u>301-717-2494</u> : Veterinarian providing emergency su Lisa G. Portnoy -S -S Da Veterinarian Signature	Email: <u>ssolomon@</u> pport: <u>Lisa Portnoy</u> igitally signed by Lisa C iate: 2023.08.30 16:44:	DVM DVM Portnoy 40 -04'00' 301-	-434-5304
ell/Pager: 301-717-2494 Veterinarian providing emergency su Lisa G. Portnoy -S Veterinarian Signature 301-801-8024	Email: <u>ssolomon@</u> pport: <u>Lisa Portnoy</u> igitally signed by Lisa C ate: 2023.08.30 16:44;	@cc.nih.gov , DVM 3. Portnoy 40 -04'00' 301- portnoyl@	-434-5304 Phone mail.nih.gov
ell/Pager: 301-717-2494 Veterinarian providing emergency su Lisa G. Portnoy -S Veterinarian Signature 301-801-8024 Cell/pager	Email: <u>ssolomon@</u> pport: <u>Lisa Portnoy</u> igitally signed by Lisa (ate: 2023.08.30 16:44;	@cc.nih.gov , DVM 3. Portnoy 40 -04'00' 301- portnoyl@ email	-434-5304 Phone mail.nih.gov
ell/Pager: 301-717-2494 Veterinarian providing emergency su Lisa G. Portnoy -S S Veterinarian Signature 301-801-8024 Cell/pager azardous Agents and Special Safety Co	Email: <u>ssolomon(</u> <u>pport: Lisa Portnoy</u> igitally signed by Lisa C ate: 2023.08.30 16:44: oncerns: (Safety concern	@cc.nih.gov , DVM 3. Portnoy 40 -04'00' 301- portnoyl@ email ns for personnel or	-434-5304 Phone mail.nih.gov
ell/Pager: 301-717-2494 Veterinarian providing emergency su Lisa G. Portnoy -S S Veterinarian Signature 301-801-8024 Cell/pager azardous Agents and Special Safety Co No hazardous agents will be	Email: <u>ssolomon@</u> <u>pport: Lisa Portnoy</u> gitally signed by Lisa C ate: 2023.08.30 16:44: <u>prcerns: (Safety concern</u> <u>e in USe</u>	DVM DVM Portnoy 40 -04'00' 301- portnoyl@ email as for personnel or	-434-5304 Phone mail.nih.gov
ell/Pager: 301-717-2494 Veterinarian providing emergency su Lisa G. Portnoy -S S Veterinarian Signature 301-801-8024 Cell/pager azardous Agents and Special Safety Co No hazardous agents will be	Email: Ssolomon@ pport: Lisa Portnoy igitally signed by Lisa C ate: 2023.08.30 16:44: oncerns: (Safety concern e in USE	©cc.nih.gov , DVM G. Portnoy 40 -04'00' 301- portnoyl@ email ns for personnel or	-434-5304 Phone mail.nih.gov
ell/Pager: 301-717-2494 Veterinarian providing emergency su LiSA G. Portnoy -S S Veterinarian Signature 301-801-8024 Cell/pager azardous Agents and Special Safety Co No hazardous Agents will be ave read and understand the guidelines tp://intranet.nmrf.nih.gov). Tundersta ring live animal imaging, to analyze the sure that all personnel participating in a delines and policies of the NMR Center	Email: Ssolomon(pport: Lisa Portnoy igitally signed by Lisa C ate: 2023.08.30 16:44: oncerns: (Safety concern e in use s and policies of the Mo and that it is the respons ir own data, and backup animal studies in the Nf	©cc.nih.gov DVM DVM Portnoy 40 -04'00' 301- portnoyl@ email as for personnel of puse Imaging Facilit sibility of my invest o all data collected MR Center under r	-434-5304 Phone mail.nih.gov r animals) ity and NMR Center stigators to be present d in the center. 1 will my ASP will adhere to th
ell/Pager: 301-717-2494 Veterinarian providing emergency su Lisa G. Portnoy -S S Veterinarian Signature 301-801-8024 Cell/pager azardous Agents and Special Safety Co No hazardous agents will be ave read and understand the guidelines tp://intranet.nmrf.nih.gov). Tundersta ring live animal imaging, to analyze the sure that all personnel participating in a delines and policies of the NMR Center teven B. Solomon -S	Email: Ssolomon(pport: Lisa Portnoy igitally signed by Lisa C ate: 2023.08.30 16:44: oncerns: (Safety concerned in USE s and policies of the Mo and that it is the response ir own data, and backup animal studies in the NP Digitally signed Solomon -S Date: 2023.07.1	Cc.nih.gov DVM Dortnoy 40 -04'00' 301- portnoyl@ email as for personnel of buse Imaging Facilit sibility of my invest o all data collected MR Center under r by Steven B. 0 12:57:03 -04	-434-5304 Phone mail.nih.gov r animals) ity and NMR Center stigators to be present d in the center. I will my ASP will adhere to th My ASP will adhere to th
ell/Pager: 301-717-2494 Veterinarian providing emergency su LISA G. Portnoy -S S Veterinarian Signature 301-801-8024 Cell/pager azardous Agents and Special Safety Co No hazardous agents will be ave read and understand the guidelines tp://intranet.nmrf.nih.gov). Tundersta ring live animal imaging, to analyze the sure that all personnel participating in a delines and policies of the NMR Center teven B. Solomon -S Principal Investigator Signa	Email: Ssolomon(pport: Lisa Portnoy igitally signed by Lisa (ate: 2023.08.30 16:44: oncerns: (Safety concerned in use s and policies of the Mod animal studies of the Mod ir own data, and backup animal studies in the NM Digitally signed Solomon -S Date: 2023.07.1 iture	Cc.nih.gov DVM DVM Deportnoy 40 -04'00' 301- portnoyl@ email as for personnel of buse Imaging Facilit sibility of my invest o all data collected VIR Center under r by Steven B. 0 12:57:03 -04	-434-5304 Phone mail.nih.gov r animals) ity and NMR Center stigators to be present d in the center. I will my ASP will adhere to the 4'00' 7/10/2023 Date

Office Phone: 301-435-2287	completed: <u>10/2/2024</u> Protocol # Home Phone: <u>301-717-24</u>	: <u>CCM23-04</u> 194	
Protocol Title: Functional and Structural Changes E	arly in Sepsis-induced Cardia	emvopathy CC	
STE WASTE	WASTE	WA	
Use a separate form if care is different for each species Species:canine	Species:		
apecies	apecies		
Animal Housing Location: Bldg <u>28 B-wing</u> Use separate form if care differs by location Bldg	BITE		
List of Procedures: (surgery, tumor implant, catheter) <u>ir</u> fernoral, external jugular, urinary), tracheostomy	ntrabronchial inoculation of bacteria,	catheter placement	
Primary Point of Contact (P.O.C.) in Case of Emergency:	Steven Solomon		
Nork Tel: Home Tel:	Pager or Cell #: 30	1-717-2494	
Alternate Point of Contact in Case of Emergency: <u>Jasmine</u> Nork Tel: 301 496 4697	<u>e Holden</u> Barrar az Call # 20	1 975 9004	
Potential or Expected Complications: rebleeding	rager or Cell #: 30	<u>070-0004</u>	
At Gullett		S	
Dircumstances Requiring Contact: <u>unexpected event re</u>	equiring euthanasia		
Freatment (indicate appropriate response):			
reatment determined by veterinarian:	[x] Yes	[]No	
It NO, specify restrictions as follows:			
What drugs are contraindicated?	ITE	WHITE	
COAL	AL	COAL	
At Vet discretion if poor condition, severe nain or distress:		L I No.	
If NO, specify treatments or restrictions:		[] 100	
<u>//</u>		<u>·</u>	
Notify P.O.C.	*I I Yes	[x] No	
Requested euthanasia agent and route of admin	nistration:	Wł	
	COAT	CC	
Specific criteria for euthanasia:			
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead			
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C.	[]Yes	[x]No	
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C. Refrigerate carcass	[]Yes [x]Yes	[x] No [] No	
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C. Refrigerate carcass Dispose of carcass Submit to DVR for necroosy	[]Yes [x]Yes []Yes []Yes	[x] No [] No [x] No	
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C. Refrigerate carcass Dispose of carcass Submit to DVR for necropsy AN number to use for submission:8387332	[]Yes [x]Yes []Yes [x]Yes [x]Yes	[x] No [] No [x] No [] No	
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C. Refrigerate carcass Dispose of carcass Submit to DVR for necropsy AN number to use for submission:8387332 Additional Comments:	[] Yes [x] Yes [] Yes [x] Yes	[x] No [] No [x] No [] No	
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C. Refrigerate carcass Dispose of carcass Submit to DVR for necropsy CAN number to use for submission: 8387332 additional Comments: rincipal Investigator: STEVEN B. SOLOMO	[] Yes [x] Yes [] Yes [] Yes [x] Yes N -S Digitally signed by STEVEN B. S Date: 2024.10.02 11:00:46-04'0	[X] Na [] Na [X] Na [] Na DLOMON -S	
Specific criteria for euthanasia: Euthanasia is performed or animals are found dead: Contact P.O.C. Refrigerate carcass Dispose of carcass Submit to DVR for necropsy AN number to use for submission:8387332 Additional Comments: Frincipal Investigator: STEVEN B. SOLOMO Signature	N -S Digitally signed by STEVEN B. S Date: 2024.10.02 11:00:46 -04'0 Date:	[x] No [] No [x] No [] No DLOMON -5	

Functional and Structural Changes Early in Sepsis-induced Cardiomyopathy

Principal Investigator: <u>Sta</u>	even Solomon, Ph	D Date form co	mpleted: _6 <u>/22/20</u>	023 Protocol #	: <u>CCM23-xx</u>	
Office Phone: _ <u>301-43</u>	5-2287	5	Home Phone:	<u>_301-717-24</u>	194	
Protocol Title: Functiona	and Structura	l Changes Ear	ly in Sepsis-in	iduced Cardio	omyopathy	
SIC	WASI					
Use a separate form if cal	re is different for e	each species	Province			
Species:Ca	nine	<u> </u>	Species:			
opecies.	/		opeoies			
Animal Housing Location:	Bidg	28 B-wing				
ise separate form if cara differs by i	ocation Bldg	WH	I.E.			
ist of Procedurast (sur	iony tumor implant	cotheter) intr	abronchial inoculs	ation of hacteria	catheter placemen	*
femoral external jugular	jery, tumor anpian urinary), tracheost	t, cathetery <u>intra</u> tomv			cameter placemen	<u>it</u>
ionioidi oxionidi jugaturi		/				
Primary Point of Contact	(P.O.C.) in Case o	f Emergency: <u>Ste</u>	even Solomon	/		
Nork Tel: <u>301-435-228</u>	7 Home	Tel:	Pag	jer or Cell #: <u>30</u>	<u>1-717-2494</u>	_
Nork Tel: 301_496_469	Tin Case of Emerg	jency <u>: Jasmine F</u> -Tel [.]	Pan	er or Cell #: 30	1-875-8664	
Potential or Expected Co	mplications:	rebleeding	from catheter/tra	cheostomy site		WHILE
	SVA.		C.	VAL .		
Circumstances Requirin	g Contact: <u>unex</u>	pected event req	uiring euthanasia	SIE		
reatment (indicate appro	priate response): veterinarian:			[v] Yes	ELNo	
If NO. specify reading	strictions as follow	ws:			11100	
Specific treatment as foll	ows:			<u> </u>		
What drugs are contraine	dicated?	WHI	I E		COAL	
Pritorio for Euthanasia (in	dicate annronriate	(asoonse)			WYATE	
At Vet discretion if poor co	ndition, severe pa	in or distress:		[x] Yes	[] No	
If NO, specify tre	atments or restric	tions:				
	<u> </u>					
				*[] Yes		
 Noury F.O.C. TF • Requested eutha 	masia agent and :	route of adminis	stration:	[] 103	[x] NO	
 Specific criteria 1 	for euthanasia:			TAC		COAT
it.	WACT	C	177	ICTE		WACTE
f Euthanasia is perform	ed or animals are	found dead:			11.kl.	
I. Contact P.O.C.				[]Tes [x]Yee		
. Dispose of carcass				[]Yes	[x]No	
I. Submit to DVR for necre	opsy			[x]Yes	[] No	
CAN number to use for su	bmission:6	3387332	TE		WHITE	
COAT						
aggitional Comments:						
/		/	Dinitally sign	ed by Steven B. Sr	niomon -S	
Principal Investigator:	Steven B.	Solomon	-5 Date: 2023.0	8,25 11:07:09 -04'0	10'	
	Signature		··· /	Dat	<u>e</u>	
	Signature					
TE	WHITE		W	IITE.	·	WHITE
The veterinarian will ta	ke the appropriat	e action in an e	mergency if no r	response from t	the PI/POC is	COAT
received within 30 min	utes after an atte	mpt at notificat	ion is made.			
keterences						

Clinical Center	Training	and Experience Form
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Time: Eunctional and Structural Changes Early in Sepsis-induced Cardiomyopathy

SECTION A: General Information

Investigator Name: Verity Ford

4004	CC1 433	
n v r z ·		

Phone No: 301-496-3091

Phone No: 301-496-3091	Bldg/Rm: 28/119
PI Course completion dates: (Initial)	(Refresher)
AU Course completion dates: (Initial) 20	21 (Refresher)

Email: verity.ford@cc.nih.gov

Date: 07/28/2023

Date

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

Procedures	Species:	No experience	#Years	Other Comments or, training:	
Handling and restraint			<u></u>		
Änesthesía:	ute		MALLETE	Type: inhalational, infravenous	re l
Administration	D.R		2		
Monitoring	0 R	-	2		
Aseptic technique	S DR		2	WAS I	16
Injections:			AN COURT OF M	×	
SC	D.R.		2		
IM	D,R		2		
N /	D,R	×			
IP		AHITE		WHITE	
Catheter placement:	CA SECTION		· 通知管理: 图/中心		
IV/ Grs	DR	TO X	•	WACTE	
IA		1010		1111010	
Intubation	D.R	×	• • • • • • • • • • • • • • • • • • • •		/
Euthanasia	D;R		2		/
		-			
		• • • • • • • • • • • • • • • • • • • •	· · · · · · · · · · · · · · · · · · ·	7	/
Wh	11 E		WHITE	WHIT	IE III

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 Primales 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:

4.4.5	1	atternation and the second	- no domino	of of the owner all l	D piroop duroo that	100 Rotod above
4aj	ism curre	nuy protictent.	in periorning.	all of the awake MH	e procedures inal	The listed above

OR will provide my supervision and training until I am fully qualified to perform these awake NHP 4b)

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: Lunder	stand my	responsibilities f	or acquiri	ng training	on technique	es I am as	ked to perf	form on an	nimals as de	scribed	l in this
ASP,	but am not	currently	préficient in perf	orming. /	Additionally.	, if my suppo	rt role for	this ASP c	hanges, I	will submit a	new T	&E form
and a	icquire traini	ing prior to	performing any	r new pro	cedures.			HITE				NHITI

Animal User signature:

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

Principal Investigator signature:

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	WHITE
AU Course completion dates: (Initial)	(Refresher)	

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

Procedurés	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	M.R.D		>15	
Anesthesia:	urre		WILLITE	Type: inhalational, intravenous
Administration	M,R,D		>15	
Monitoring	M,R,D		>15	C MARTE
Aseptic technique	M.R.D		>15	INASI G
Injections:		C. MARCELLER	<u> 1988 - Secola d</u>	<u> </u>
SC	M,R,D		>15	
!M	M,R,D		>15	
iv v	M,R,D		」>15	
iP	M,R,D	WHITE	>15	WHITE
Catheter placement:				
IV/- (9775	R,D	1/1015	>15	117.1915
IA	R,D		>15	
Intubation	R,D		>15	
Euthanasia	M,R,D		>15	7
			<u> </u>	//
	HITE		WHITE	WHITE

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

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 Ni iP procedures:

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

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

SECTION C: Assurances

Yes /(Nd) I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those procedules described in the approved ASP.

Yes / Not Junderstand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, bet 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:

Digitally signed by Steven B. Solomon -S Steven B. Solomon -S

As the PI, Lassume 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. Digitally signed by Steven 8. Solomon Steven B. Solomon -S 4

Principal Investigator signature:

Date: 2023:07.10 11:08:04-04'06'

Date:

Date

SECTION A: General Information

Investigator Name: Charles Natanson

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

Phone No: 301-496-9770 Bldg/Rm: 28/119 PI Course completion dates: (Initial) 2000 AU Course completion dates: (Initial)

(Refresher) 2021 (Refresher)

Email: cnatanson@cc.nih.gov

Experience: M-mouse; R-rat; D-dog.

Procedures	Species;	No experience	# Years	Other Comments on training:
Handling and /estraint	D		>30	
Anesthesia:	1116		WHITE	Type: inhalational, intravenous
Administration	•) · · · · (D) · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	>30	n an general agente a transmissioner de la canada de la canada de la canada de la construir de la canada de la La canada de la construir de la canada de la c
Menitoring	D		>30	WASIE
Aseptic technique	D		>30	
Thjections:				
SC	D.	/	>30	
IM		/	>30	
IV	D		>30	
18	D		>30	WHILE
Catheter placement:	1. N. N. 198			
IV	D.	VASIE	>30	MASIE
IA	G	· · · · · · · · · · · · · · · · · · ·	>30	/
Intubation	D		>30	
Euthanásia	D		>30	//
			MALLITER	A A A A A T T T
			White	

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 Nonnumar. 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.

I will be performing the following awake NHP procedures.

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

OR. will provide my supervision and training until I am fully qualified to beform these -awake NHP 4b) procedures proficiently and independently.

SEGTION C: Assurances

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

Kes/ 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 protectent 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:

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 Digitallys give by Sleven B. Solomo

Date:

SECTION A: General Information

Investigator Name: Marcus Chen, MD

ASP#: CCM23-xx	Title:	Functional	and Structural	Changes Earl	y in Sepsis-induc	<u>ced Cardiomyo</u>	pathy
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Phone No: 301-496-007	7 Bidg/Rm:	10/B1D47	Email: chenmy@nh/bi.nih.gov	
PI Course completion dates:	(Initial) 9/2006 (Refre	sher) 8/7/2014	WHITE	
AU Course completion dates	: (Initial) 10/21/2019 (Refre	sher) 11/7/2022		

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

Procedures	Species:	No experience	# Years	Other Co	mments on training:	
Handling and restraint	M.R.D	X				/
Anesthesia:	JITE		WHITE		WH	ITE
Administration	M.R.D	X	· ····			·····
Monitoring	M,R,D	X	MASTE		17/4	STE
Aseptic technique	M,R,D	X	/		/	
Injections.		a de la compañía de l			/	
sc	MRO	X			/	
IM.	M.R.D	X			/	
IV	M,R,D	X		W/F	UTE	
IP	M,R,D	X	1	00	NAT .	
Cathoter placement:	0			100	16TE	
ÎV	RD	X		110	010	
IA	R,D	X				/
Intubation	RD	X		/		
Euthánasia	M,R.D	X				
· · · · · · · · · · · · · · · · · · ·	11112		WHITE			ITE
CC	JAL		COAL	··· · · · ·		AL

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 hor 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:

4.

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

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.

West No: Lunderstand my responsibilities for acquiring training on techniques Lam asked to perform on animals as described in this BSP, 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

Date

As the PI, Lassume 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:

SECTION A: General Information

Investigator Name: Lyn Colenda, DVM

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

Phone No: 301.443,8521 /301-640-6993	Bidg/Rm:	14E/119D	Email: colendal@mail.nih.gov
PI Course completion dates: (Initial) 6/1/98	(Refresher) 1/2	4/23	VHITE
AU Course completion dates: (Initial) 9/27/01	(Refresher) 1/2	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:			WHITE	WHITE
Administration	M,R,D	-	.39.	
Monitoring	M,R,D		39	WASIE
Aseptic technique	M.R.D		39	
injections:		n in steacht is	56.636 ⁻⁷⁷⁵ .636	
SC.	M.R,D		39	
1M	M.R.D		39	
N E	M.R.D	/ HITE	M/R-20 D-39	
P	M,R,D		39	COAL
Catheter placement:				MASTE
IV	R,D	R	D-40	
IA	RD	R	D-5	/
Intubation	R,D	R	D-39	
Euthanesia	M,R,D		M/R-48 D-39	
	····			
			COAL	<u> </u>
		<u>. i</u>	<u> </u>	

2) Or. Colenda ____isfully qualified in the techniques she will be performing on this ASP

3) Yes the 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 ('ve listed above,

SECTION C: Assurances

2002 PNo: I have read or will read the final, approved version of this ASP and will limit my activities to performance of only those accedures described in the approved ASP.

No: Lunderstand my responsibilities for acquiring training on techniques Lam 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:

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:

Uncovered by a White Coat Waste investigation

Date

SECTION A: General Information

Investigator Name: Melinda Fernandez

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

Phone No: 301-496-3091 Bidg/Rm: 28/119 (Refresher) PI Course completion dates: (Initial) (Refresher) 2020 AU Course completion dates: (Initial) 2005

Email: mfernandez@cc.nih.gov

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

Procedures	Species:	No experience	# Years	Other Comm	ents on training:	
Handling and restraint	M,R,D		>15		14/14	ITC
Anesthesia:	Calle .			Type: inhalat	ional, intravenous	A
Administration	M,R,D		>15			A-
Monitoring	M,R,D		>15		WAS	16
Aseptic technique	M.R,D		>15		/	
Injections:						
SC	M,R,D		>15			
IM	M.R.D		>15			
IV	M,R,D	AULITE	>15	VA/LU	TE	
IP	M,R,D	CAT.	>15		A T	
Catheter placement:					al-	
IV STE	R,D	MSIE	>15	WAS	516	
IA	R,D		>15	/		
Intubation	D		>15			
Euthanasia	M.R.D		>15	/		
		1		1		
W/H	ITE	1	WHITE		WHI	TE
00	AT		COAT		60	AT

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:

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

4b)

OR 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 finat, approved version of this ASP and will limit my activities to performance of only those procedures described in the approved ASP.

Xes/ 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:	Thei dasturast	COAT	Date: 7/1
WĂŚTE	I SIE O	WASTE	

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.

Steven B. Solomon -5 Digitally signed by Steven B. Solomon -5 Digitally signed by Steven B. Solomon -5 Digitally signed by Steven B. Solomon -5

Date:

Principal Investigator signature:

Incovered	by a	White	Coat	Nacto	invectigation
Uncovereu	by a	VV IIILE	Cual	vasie	Investigation

SECTION A: General Information

Investigator Name: Jasmine Holden

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

Phone No: 301-496-3091

PI Course completion dates: (Initial)

(Refresher)

Bldg/Rm: 28/119

Email: jasmine.holden@cc.nih.gov

AU Course completion dates: (Initial) 2013

(Refresher) 2022

Experience: M-mouse; R-rat; D-dog.

Procedures	Species:	No experience	# Years	Other Comments on training;
Handling and restraint	M.R.D	I	>5	
Anesthesia:			WHITE	Type: inhalational, intravenous
Administration	M.R.D	· · · · · · · · · · · · · · · · · · ·	>5	an a
Monitoring	M,R.D		>5	WASTE
Aseptic technique	M,R.D		>5	
Injections:	The Rock and St.			
SC	M.R.D	/	>5	
1M	M.R.D	/	>5	
IV	M,R,D		>5	
IP	M,R,D	INIC	>5	WHILE
Catheter placement:		ALLESS GERE	12.6 S	COAL
IVS	R,D	ASIE	>5	WASTE
	D		>5	
Intubation	M,R,D		>5	
Euthanasia	M,R,O		>5	
		ļ		
004	- F	<u> </u>	0047	0047

2) Dr. Steven Solomon, will provide supervision and training in the techniques I will be performing on this ASP until I am fully gualified 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) [will be performing the following awake NHP procedures:

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

OR will provide my supervision and training until I am fully qualified to perform these lawake NHP 4b) 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.

(E)/ No: (understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this X8P, 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. -1.1-

Animal User signature:	Aann		Date:	111 2023
MAGTE	MILGTE	ŭ X		i in a contraction of the contra

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 Dig sally signed by Steven B. Sciencer conduct these procedures independently.

Principal Investigator signature:

Steven B. Solomon -S Date: 2023.07.10 11:15:43 -04'00"

Date:

SECTION A: General Information

Investigator Name: Jing Feng

ASP#:	CCM23-xx
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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)	WHITE	
AU Course completion dates: (Initial) 2	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:			WHITE	Type: inhalational, intravenous
Administration	MAR,D		>15	
Monitoring	Mi, R, D,		>15	
Aseptic technique	M.R.D		>15	N/ASTU
Jajections:			1990 <u>- 20</u>	
SC	M,R,D	7	>15	
IM	M,R,D		>15	
JV VI	M,R,D	/	>15	
IP	M,R,D	VHITE	>15	WHITE
Catheter placement:				×
IV Contra	R,D	1. QTE	>15	WACTE
IA	R,D		>15	
Intubation	R,D		>15	
Euthanasia	M,R,D		>15	
	HTE		WHITE	WHITE

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)):

OR:

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:

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

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 projectures described in the approved ASP.

Xes/	/ No:	l under	stand my	responsibiliti	ies for acqu	iring training	on techniques	l am asked	to perform on	animals as des	cribed in this
VISP	h but a	im not	currently	proficient in	performing.	Additionally	if my support	role for this	ASP changes	, I will submit a	new T&F form
and	acquii	e traini	ng prior t	o performing	any new p	rocedures.		WHI	IE I		

Animal User signature:	Jing Feng -S	Dig (atty signed by ling rong -5 Date: 2023,07.10 (2:1 1-21 -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 Destally signed by Steven 8. Solomon -S Pate: 2023.07 1072.02 28-04 007

Date:

SECTION A: General Information

Investigator Name: Crystal Hite

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

Phone No: 301-496-3091	Bidg/Rm: 28/119	Email: crystal.hite@nih.gov	
PI Course completion dates: (Initial)	(Refresher)	COAL	
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	TE		WHITE	WHITE
Anesthesia:	AT.		COAT	Type: inhalational, intravenous
Administration	MRD		3	TIKOTE.
Monitoring	MRD		3	- mare
Aseptic technique	MRD		3	
injections:	Provide States	<u> (stekesks</u>	State & State	
SC	MRD		3	
IM	MRD	1	3	
IV ==	MRD	HITE	3	
(P	1	TAO		COAT
Catheter placement				
V IV C	MRD	19376	3	11/AS16
ÍA	MRD		3	
Intubation				
Euthanasia	MRD	1	3	
WHI:	TE		WHITE	WHITE
00	(F		COAT	COAT

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.

This ASP involves Nonhuman Primates procedures. If yes complete Section B. If no, go to Section C. es/Not

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:

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

OR will provide my supervision and training until I am fully qualified to perform these awake NHP 4b) 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.

Xes 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:

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 ligned by Steven B. Solomon -S Date: 2023.07.10 11:53.44-04:00

Date

Date:

SECTION A: General Information

Investigator Name: Dennis Du

ASP#: CCM23-xx	Title	Junctional and Structura	I Changes Early in Sepsis-induced Cardiomyopathy
Phone No: 301-496-3091	Į	Bldg/Rm: 28/119	Email: dennis.dugan@cc.nih.gov
PI Course completion da	ites: (ini	tial) (Refrest	ier) COAL
AU Course completion d	lates: (Ir	nitial) 2015 (Refres)	ler) 2021

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

Procedures	Species:	No experience	# Years	Other Comments on training:
Handling and restraint	R,D		>4	WHITE
Anesthesia				Type: inhalational, intravenous
Administration 777	R,D		>4	ŭXôTT.
Monitoring	R,D		>4	MM91 P
Aseptic technique	R.D.		>4	<u></u>
Injections:				
SC	R,D		>4	
IM	R,D		>4	
IV	D	HITE	>4	WHITE
CAPAT	6	×		COAT
Catheter placement:	NT WAR AND			NUM ATE
IV V		x		MASIC
		X		
Intubation		×		
Euthanasia	R,D		>4	
	/	1	\	
WHIT			WHITE	WHITE
COA	-	[COAT	COAT

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:

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

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

SECTION C: Assurances

Xes / 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.

Kes //No: I understand my responsibilities for acquiring training on techniques I am asked to perform on animals as described in this ASP, but an not currently proficient in performing. Additionally, if my support role for this ASP changes, I will submit a new 7&E form and acquire training prior to performing any new procedures.

Animal User signature:

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 stand by Staven B. Soloman -S: Date: 2023.07.10 : 1.55.27 -01.00

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-Pharmaccutical Grade Compounds: <u>ARAC guideline NPGC</u> 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 (<u>USDA 1997b</u>). 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 (<u>Wolff et al. 2003</u>); for example, the use of a non-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 (<u>NIH 2008</u>).

Pick one of the reasons listed below.

Substance Name	Pharmaceutical- grade (Yes, No or N/A) *	If "N" select reason from below
Propofol	Y WASIE	WA
Fentanyl	Y	
Midazolam	Y	WHITE
Dexmedetomidine	VASTE _Y	WASTE
Isoflurane	Y	
ketamine WHITE	Y WHITE	WI
acepromazine	Y WASTE	<u> </u>
Torbugesic	Y	
atropine	Y	
ceftriaxone	ОА <u>Ү</u>	COAT
		MPISTE \
WHITE	WHITE	WH

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 YesNoStatistical methods are appropriate for this study.X YesNoSafety 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, CCM16-04, 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: