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14. ABSTRACT In future conflicts demanding prolonged care in place prior to casualty evacuation, the need for advanced organ support systems to sustain life in the forward setting will be essential. Current organ support systems require administration of systemic anticoagulation to prevent bleeding/thrombotic complications related to extracorporeal blood circulation within the medical devices. However, in the setting of combat trauma systemic anticoagulation is contraindicated. Trauma-specific coagulation management guidelines and strategies for extracorporeal organ support in the combat care setting are necessary for delivery of artificial organ support in the forward setting. **Objective:** Investigate key biomarkers identified as drivers of coagulation derangement following trauma alone, as well as during artificial organ support alone – that have not been investigated in the setting of trauma and organ support together. **Hypothesis:** A targeted coagulation panel will identify specific impairments to hemostasis during organ support in the early post-trauma (72h) phase.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION:

This report serves as an annual report of activities and progress made by Dr. Teryn Roberts, PhD, Principal Investigator at AREVA/The Geneva Foundation and the AREVA team towards completion of work awarded to The Geneva Foundation as part of federal grant W81XWH-22-1-0028, titled "Proactive Targeted Coagulation Management During Trauma and Acute Respiratory Distress Syndrome." The purpose of this project is to investigate a novel panel of coagulation biomarkers that may be integral mediators following trauma and during extracorporeal life support (ECLS). We utilize a library of blood samples from already planned/funded studies of combat-relevant trauma and ARDS in AREVA's established large animal swine model with 72-hours follow-up, including simulated ground and aeromedical evacuation. This library of samples contains: 1) Injured control group with polytrauma and conventional mechanical ventilation (no ECLS) (n=12); 2) ECLS only group with healthy subjects placed on ECLS without polytrauma injury (n=20 total, 10 receiving systemic anticoagulation, 10 without systemic anticoagulation); 3) Polytrauma + ECLS group receiving ECLS immediately post-injury as an adjunct to mechanical ventilation (n=24 total, 12 with systemic anticoagulation, 12 without systemic anticoagulation). We will investigate 1) von Willebrand factor multimer distribution and functionality, 2) platelet receptor shedding, and 3) thrombin generation potential in these distinct cohorts. The objective is to identify the differential effects of trauma and ECLS on these coagulation mediators/tests, as well as determine whether the combined stimuli of trauma and ECLS have an additive or antagonistic effect on these potential drivers of bleeding/thrombosis. This discovery proposal represents a dramatic shift from conventional generalized, reactive, non-specific coagulation management protocols for ECLS to proactive targeted, injury/disease-specific coagulation management.

Dr. Roberts as the project PI fully appreciates the opportunity to execute this award, and how this project is contributing to her career growth as a DOD investigator. This project has brought the opportunity to mentor 10+ medical students who have shared that the research they are performing will impact their clinical careers and has increased their interest in participating in clinical research going forward. This work is also inspiring follow-on submissions through PRMRP and the DOD to apply the research methods utilized here to clinical trials. The project tasks and objectives are being completed according to the SOW timeline. Results of this project are already being presented at international meetings, and Dr. Roberts contributed to a chapter of the premier clinical handbook for ECLS, the *ELSO Red Book*, based on her expertise through this project and other related DOD-funded efforts on which she serves as a Co-Investigator. Thus, this work is already impacting clinical care.

2. KEYWORDS:

Coagulation, bleeding, thrombosis, hemostasis, trauma, hemorrhage, coagulopathy, ECLS, ARDS, organ support, combat casualty care

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Evaluate hemostasis using a novel, targeted coagulation panel (assessment of ultra-long von Willebrand Factor [UVWF] multimers, platelet receptor shedding, thrombin generation potential) during ECLS (extracorporeal life support) in healthy controls versus traumatically wounded.	**Timeline**(Months)	**Percent complete (%)**	**Status:**
Major Task 1: Prepare equipment and test assays for novel coagulation panel execution for Aim 1 study.			
Subtask 1.1: Purchase equipment, install and receive training	1-6	100%	Complete Y1
Subtask 1.2: Purchase assay kits and validate using subset test samples	1-6	100%	Complete Y1

Subtask 1.3: Personnel training for coagulation panel execution	1-6	100%	Complete Y1
Subtask 1.4: Inventory plasma samples from parent study for Aim 1 assessment of novel panel in healthy controls during ECLS versus traumatically wounded during ECLS. Approvals for sample collection and storage already achieved as a part of parent study.	1-18	75%	In progress
MILESTONE: Equipment, materials and samples prepared to begin coagulation panel execution for Aim 1 study.	6	100%	Complete Y1
Major Task 2: Execute targeted coagulation panel (assessment of ultra-long von Willebrand Factor [UVWF] multimers, platelet receptor shedding, thrombin generation potential) using library of samples including healthy subjects receiving ECLS and traumatically wounded receiving ECLS (N=48 subjects, ~300 samples)			
Subtask 2.1: Perform ULVWF panel, platelet receptor shedding panel, and thrombin generation potential sample analysis	6-18	60%	In progress
Subtask 2.2: Perform statistical analysis and data interpretation	18-24	30%	In progress
Subtask 2.3: Report findings, prepare conference abstracts and manuscript preparation.	18-24	30%	In progress
MILESTONE: Analyze and report on novel coagulation panel outcomes during ECLS in healthy and trauma subjects	24	15%	In progress
Specific Aim 2: Evaluate hemostasis in trauma injured supported by standard of care versus trauma injured with standard of care + adjunct ECLS support			
Major Task 3: Prepare equipment and test assays for novel coagulation panel execution for Aim 2 study.			
Subtask 3.1: Purchase assay kits and validate using subset test samples	1-6	100%	Complete Y1
Subtask 3.2: Inventory plasma samples from parent study for Aim 2 assessment of novel panel trauma injured treated with and without ECLS. Approvals for sample collection and storage already achieved as a part of parent study.	1-18	50%	Complete for Trauma, Trauma+ECLS in progress
MILESTONE: Equipment, materials and samples prepared to begin coagulation panel execution for Aim 2 study.	6	85%	In progress
Major Task 4: Execute targeted coagulation panel (assessment of ultra-long von Willebrand Factor [UVWF] multimers, platelet receptor shedding, thrombin generation potential) using library of samples involving trauma injury treated with standard of care alone versus with adjunct ECLS (N=36 swine subjects in parent study, ~225 plasma samples)			
Subtask 4.1: Perform ULVWF panel, platelet receptor shedding panel, and thrombin generation potential sample analysis	6-18	50%	Complete for Trauma, Trauma+ECLS in progress
Subtask 4.2: Perform statistical analysis and data interpretation	18-24	5%	Not started
Subtask 4.3: Report findings, prepare conference abstracts and manuscript preparation.	18-24	5%	Not started
MILESTONE: Analyze and report on novel coagulation panel outcomes after trauma in subjects treated with standard of care versus with adjunct ECLS	24		In progress
Specific Aim 3: Apply investigative coagulation panel to compare subjects receiving ECLS with systemic anticoagulation versus no anticoagulation			
Major Task 5: Prepare equipment and test assays for novel coagulation panel execution for Aim 3 study.			
Subtask 5.1: Purchase assay kits and validate using subset test samples	1-6	100%	Complete Y1
Subtask 5.2: Inventory plasma samples from parent study for Aim 2 assessment of novel panel trauma injured treated with and without ECLS. Approvals for sample collection and storage already achieved as a part of parent study.	1-18	60%	In progress
MILESTONE: Equipment, materials and samples prepared to begin coagulation panel execution for Aim 3 study.	6	85%	In progress
Major Task 6: Execute targeted coagulation panel (assessment of ultra-long von Willebrand Factor [UVWF] multimers, platelet receptor shedding, thrombin generation potential) using library of samples involving ECLS subjects receiving systemic anticoagulation versus no anticoagulation (N=44 swine subjects in parent study, ~275 plasma samples)			
Subtask 6.1: Perform ULVWF panel, platelet receptor shedding panel, and thrombin generation potential sample analysis	6-18	50%	In progress
Subtask 6.2: Perform statistical analysis and data interpretation	18-24	10%	In progress

Subtask 6.3: Report findings, prepare conference abstracts and manuscript preparation.	18-24	10%	In progress
MILESTONE: Analyze and report on novel coagulation panel outcomes in subjects receiving ECLS with and without systemic anticoagulation.	24	5%	In progress
Specific Aim 4: Compare results of investigative coagulation panel to bleeding/thrombotic outcomes and classic coagulation metrics acquired in the parent study from which the library of samples for analysis was acquired.			
Major Task 7: Gather conventional coagulation outcome results from parent study subjects			
Subtask 7.1: Quantify and characterize instance of bleeding and thrombotic complications in the subjects from which the library of plasma samples from the parent study was acquired (N=56 subjects from parent study)	6-20	33%	Complete for Trauma Control, others in progress
Subtask 7.2: Tabulate results of conventional coagulation testing obtained in the parent study from which the library of discovery samples was acquired.	12-20	5%	In progress
MILESTONE: Parent study results acquired, and pertinent data tabulated			
Major Task 8: Perform statistical analysis and interpretation to assess correlation of novel coagulation panel results with conventional coagulation tests; and associate novel coagulation panel results with bleeding and thrombotic outcomes.			
Subtask 8.1: Statistical analysis and interpretation of results correlating conventional coagulation tests with novel coagulation panel outcomes.	18-24	10%	In progress
Subtask 8.2: Statistical analysis and interpretation of results evaluating novel coagulation panel outcomes relative to clinical bleeding and thrombotic complications.	18-24	0%	Not started
Subtask 8.3: Report findings, prepare conference abstracts and manuscript preparation.	22-24	0%	Not started
Subtask 8.4: Prepare final reports	24	0%	Not started
MILESTONE: Analyze and report on relevance of novel proactive, targeted coagulation panel relative to conventional coagulation tests and bleeding/thrombotic outcomes.			

What was accomplished under these goals?

<u>Specific Aim 1: Evaluate hemostasis using a novel, targeted coagulation panel (assessment of ultra-long von Willebrand Factor [UVWF] multimers, platelet receptor shedding, thrombin generation potential) during ECLS (extracorporeal life support) in healthy controls versus traumatically wounded.</u>

1) **Major Activity 1: Prepare equipment and assays for Aim 1 coagulation panel.**
   a) **Objective 1.1-1.3:** Purchase equipment and assay kits, validate using subset test samples, train personnel to execute.
      - **Major activities:** Key equipment including the Sebia Hydrasys H2 for measuring vWF multimer distribution and Calibrated Automated Thrombogram (CAT) for measuring thrombin generation potential were received and installed at AREVA. Specialists performed on-site training and personnel mastered the techniques. A set of test samples were assembled from historical studies involving smoke inhalation and burn injury treated with mechanical ventilation and adjunct ECLS to validate the instruments for use with swine plasma samples. We determined that both the Sebia and CAT instruments operate without modification using the swine samples. We discovered that in swine that were systemically anticoagulated with heparin, we would need to apply a heparin reversal agent when performing the CAT analysis – otherwise, no thrombin generation could be detected due to the presence of the heparin. We performed a validation study and selected polybrene as a heparin reversal agent to be applied to the samples to elucidate the underlying thrombin generation potential without inhibition of heparin.

        In addition to instrument setup and validation, we selected sample kits for all kit-based assays for the coagulation panel. We performed dilution testing and validation to determine optimal conditions to perform the assays. Two of the initially selected assays did not yield positive results with our swine samples, so we identified an alternative vendor source. We identified optimal assay kits that were compatible with our swine samples for all markers.

The results from the initial instrument and assay testing and setup that was performed with plasma from swine with smoke inhalation and burn injury were assembled to an exciting and informative abstract that was presented at the 2022 European Extracorporeal Life Support Organization International Meeting (see Products page 16).

- **Results/developments/achievements:** Instrument and assay setup for the project is completed. Additionally, we generated results from our swine sample test set (smoke inhalation and burn injury) to generate an exciting abstract that was presented at the 10th EuroELSO Congress. **Figure 1** summarizes the results obtained during optimization of the vWF activity assay and ADAMTS-13 activity assay that were reported at the conference. We found that collagen binding activity of vWF was significantly elevated following smoke and burn injury at 72-hours treated with conventional mechanical ventilation, compared to smoke and burn injured swine treated with ECLS and uninjured swine placed on ECLS. There was no significant change between groups in the activity of ADAMTS-13, an enzyme that degrades vWF from large- to smaller, less-functional molecular weight multimers. This objective is completed.

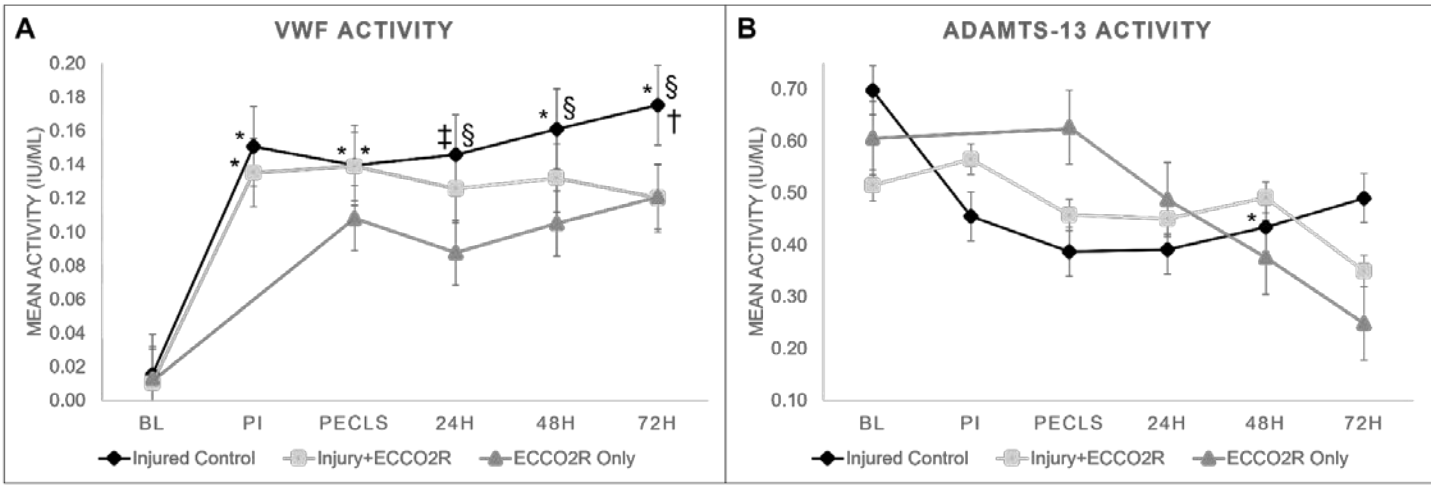

Figure 1: Mean ± standard error of activity of vWF (Panel A) and ADAMTS-13 (Panel B) reported over 72-hours. Treatment groups include Injured Control (diamonds), Injury+ECCO$_2$R (square), and healthy ECCO$_2$R Only (triangle). Significant difference indicators are as follows: * for change from baseline, † for change between Injured Control and Injury+ECCO$_2$R, § for change between Injured Control and ECLS Only, ‡ for change between Injury+ECCO$_2$R and ECLS Only. No post-injury value is shown for the healthy ECCO$_2$R Only group because no injury was given.

b) **Objective 1.4:** Inventory plasma samples from parent studies for Aim 1 comparison of healthy controls receiving ECLS versus trauma wounded receiving ECLS.

- **Major activities:** 15 of 20 subject samples have been inventoried for the ECLS healthy control group. Of note, a large library of subjects receiving ECLS for 72-hours without underlying disease/injury is a unique and invaluable resource of the AREVA Program provided to this project, as clinically healthy subjects are not placed on ECLS and it is only utilized in the presence of injury/disease which can confound the coagulation results. Likewise, AREVA is one of the only facilities that can conduct round-the-clock multi-day animal ICU studies carried out a prolonged field care timeline of 72 hours utilizing animal models that accurately represent combat trauma care scenarios. The remaining samples are actively being collected and analyzed as the parent studies are executed at AREVA. The parent study is scheduled to be completed by 01 Sept 2023, so we anticipate that all samples will be analyzed expediently as the parent study progresses and will rapidly be completed once the study is finished.
- **Results/developments/achievements:** This objective is ongoing and is being completed as the parent studies are executed at AREVA.

2) **Major Activity 2: Execute targeted coagulation panel (assessment of high molecular weight von Willebrand Factor [HMWM] multimers, platelet receptor shedding, thrombin generation potential) using library of samples including healthy subjects receiving ECLS and traumatically wounded receiving ECLS (N=48 subjects, ~300 samples)**

a) Objective 2.1-2.3: Perform vWF multimer panel, platelet receptor shedding panel, and thrombin generation potential sample analysis.

- **Major activities:** The panel has been completed for 15 of 20 planned subjects in the healthy ECLS group. The parent study from which the trauma+ECLS (n=24) subject samples are to be acquired from was initially delayed at AREVA but has now begun and scheduled to be completed by Sept 2023. We will continue to collect samples and execute the panel as the samples become available. A subset of the preliminary results from the n=15 healthy ECLS subjects was included in an abstract selected for presentation at the 2023 CNHS Keystone ECMO and the Advanced Therapies for Cardiac and Respiratory Failure 39th Annual Symposium (will take place 27 Feb 23).
- **Results/developments/achievements:** In the preliminary analysis (n=15 of 20 subjects) in the healthy ECLS group, we see a significant trend indicating increased percentage of low molecular weight multimers (LMWM) of vWF with time on ECLS, accompanied by a significant decrease in high molecular weight multimers (HMWM). LMWM are less functional/do not exhibit the same platelet binding activity and potential as the HMWM; and excessive LMWM relative to HMWM results in Acquired von Willebrand Syndrome and bleeding. However, we saw elevation in total vWF antigen levels, and did not see a reduction in the functional collagen binding activity of vWF in these subjects (**Figure 2**). This could mean that despite increase in the amount of less functional low molecular

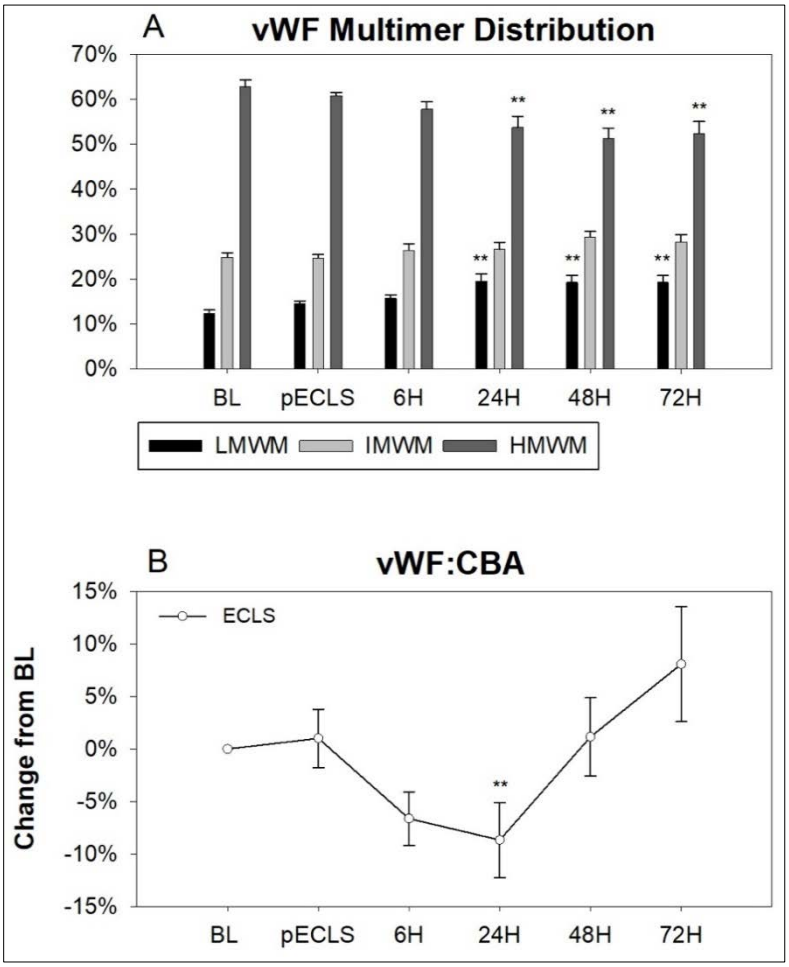

Figure 2: (A) Percentage of von Willebrand Factor (vWF) as low-, medium- and high-molecular-weight multimers (LMWM, IMWM, and HMWM). (B) Percent change in vWF collagen binding activity (vWF:CBA). **Indicates significant difference compared to baseline

weight multimers, there is no functional/clinical effect as the vWF levels may increase to accommodate that change in the protein size distribution.

Regarding platelet receptor shedding, we compared circulating levels of soluble platelet collagen binding receptor glycoprotein VI (sGPVI) to levels of metalloproteinases ADAM-10 and ADAM-17,

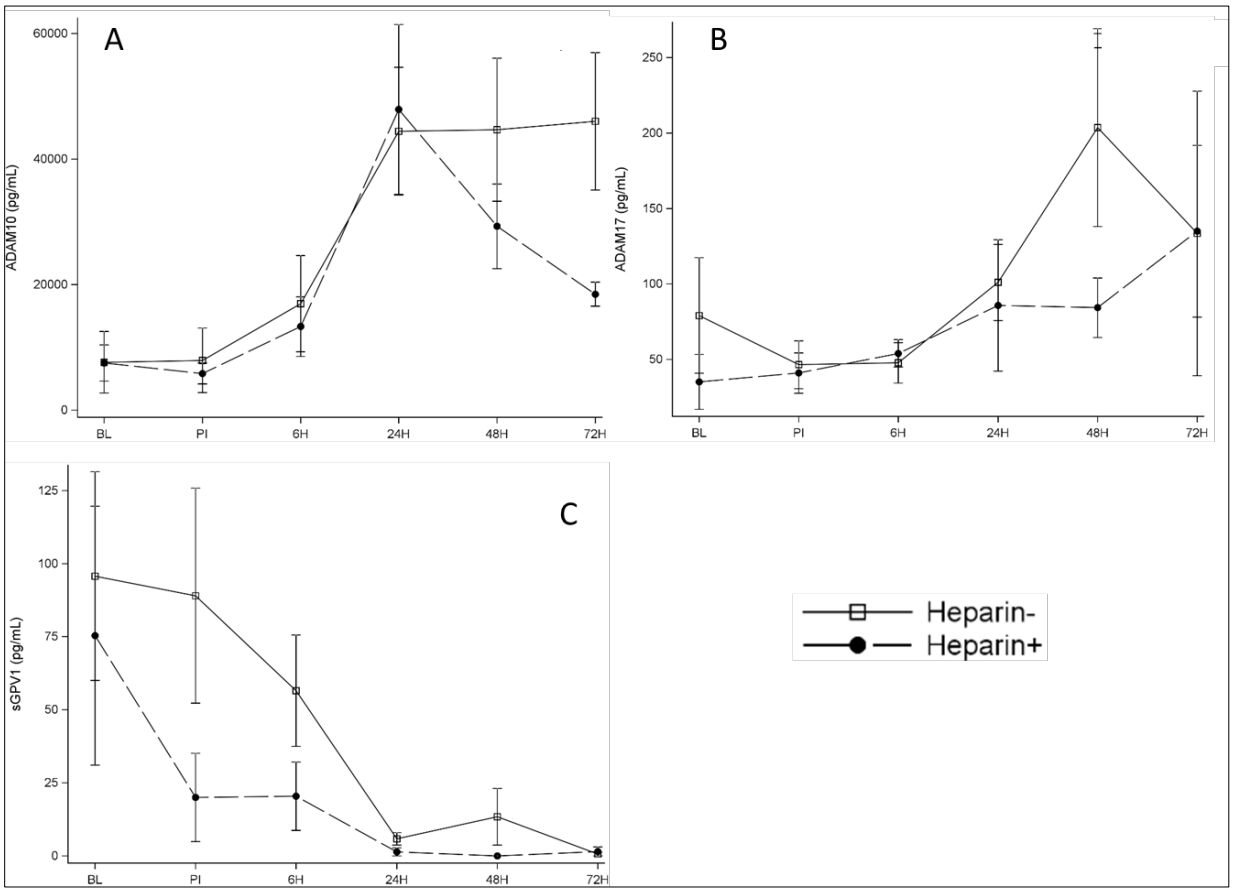

Figure 3. A) ADAM-10, **B)** ADAM-17, and **C)** soluble glycoprotein VI (sGPVI) levels in plasma collected from uninjured swine placed on extracorporeal life support for 72 hours. Subjects were categorized as having received systemic heparin anticoagulation (Heparin+, n=7) or not receiving heparin (Heparin-, n=8). No statistically significant within or between groups changes observed.

which are sheddases that proteolytically remove these binding sites from the platelet surface, inhibiting the platelet's function. In healthy ECLS subjects that were systemically anticoagulated with heparin (n=7) vs subjects that did not receive systemic anticoagulation (n=8), we observed a trend towards elevated sheddase levels in both groups over time. However, we also saw a trend towards decline in systemic sGPV1 levels over time in both groups (**Figure 3**). This finding will require further investigation and interpretation once we compare to the results of the trauma injured subjects. This could be one of the innovative descriptors of coagulation status that we are seeking to identify through this project – more to follow in next reporting period.

Conventional coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) measure time to start of thrombin formation; however they do not provide any information on the total thrombin generation potential of the sample – thus we are applying the CAT assay to evaluate estimated thrombin generation potential and lag time to thrombin generation in the setting of ECLS, first in uninjured subjects and later in injured subjects placed on ECLS. Thrombin generation potential and lagtime to thrombin generation were not statistically different between the uninjured ECLS subjects that were anticoagulated with heparin versus non-anticoagulated subjects; however,

there is a numerical trend towards elevated thrombin generation potential in subjects that were administered heparin (**Figure 4**).

We will compare the uninjured ECLS animals to trauma ECLS animals once the trauma ECLS studies are completed. Portions of this work were accepted for presentation at the 2023 CNHS Keystone ECMO and the Advanced Therapies for Cardiac and Respiratory Failure 39th Annual Symposium. Of note, under the training of Dr. Roberts, all of these assays were performed by medical school students and research technicians performing internships while applying for medical school admittance with no prior extensive research or laboratory experience with molecular biology techniques.

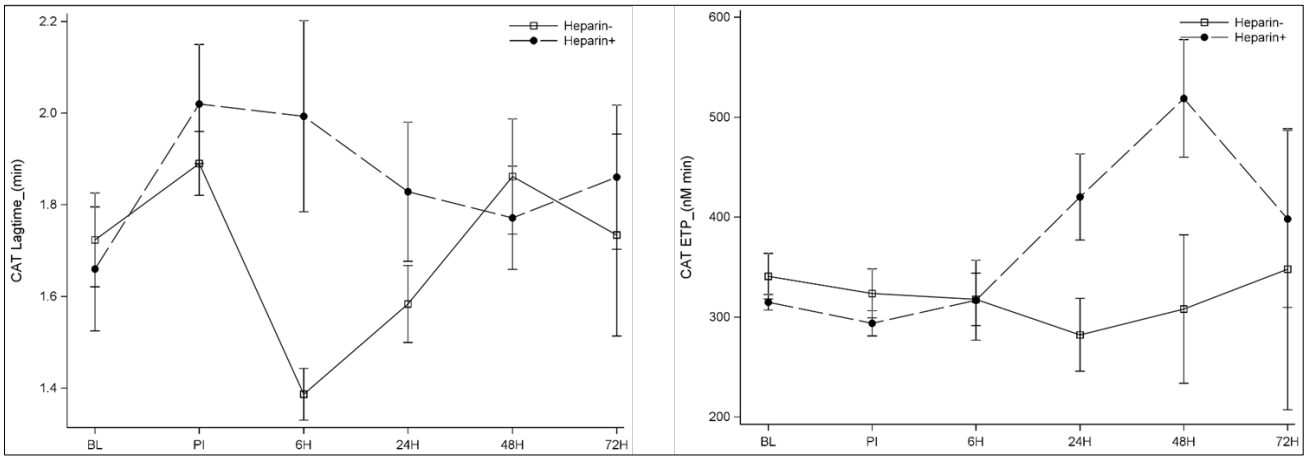

Figure 4. Lagtime (Left Panel) and estimated thrombin potential (ETP) (Right Panel) over 72-hours in uninjured subjects placed on extracorporeal life support. Subjects were categorized as having received systemic heparin anticoagulation (Heparin+, n=7) or not receiving heparin (Heparin-, n=8). No statistically significant within or between groups changes observed.

<u>Specific Aim 2: Evaluate hemostasis in trauma injured supported by standard of care versus trauma injured with standard of care + adjunct ECLS support.</u>

1) Major Activity 3: Prepare equipment and assays for Aim 1 coagulation panel.

a) Objective 3.1-3.2: Purchase equipment and assay kits, validate using subset test samples, train personnel to execute.

- **Major activities:** Equipment and materials/assays prepared, and personnel trained as described above under Specific Aim 1.
- **Results/Developments/Achievements:** Completed.

2) Major Activity 4: Execute targeted coagulation panel using library of samples involving trauma injury standard of care versus trauma injury + adjunct ECLS

a) Objective 4.1-4.3: Analyze samples and report findings.

- **Major activities:** All samples have been collected and analyzed for the trauma injury standard of care group (N=12 of 12 complete). Collection of trauma + ECLS samples is ongoing as described under Specific Aim 1.
- **Results/Developments/Achievements:** Analysis of trauma/standard of care subjects is completed. Medical students were trained to perform the analysis. They reported a subset of the results comparing von Willebrand factor assays in the trauma subjects to the uninjured ECLS animals at the 2023 Latino Medical Student Association Southwest Conference where they won first place award for top research presentation in the Translational Medicine category. The sample analysis for the trauma + ECLS group will be ongoing as the studies are completed. We do not anticipate delays.

<u>Specific Aim 3: Apply investigative coagulation panel to compare subjects receiving ECLS with systemic anticoagulation versus no anticoagulation.</u>

1) Major Activity 5: Prepare equipment and assays for Aim 1 coagulation panel.

a) Objective 5.1-5.2: Purchase equipment and assay kits, validate using subset test samples, train personnel to execute.

- **Major activities:** Equipment and materials/assays prepared, and personnel trained as described above under Specific Aim 1.
- **Results/Developments/Achievements:** Completed.

2) Major Activity 6: Execute targeted coagulation panel using library of samples involving ECLS subjects receiving anticoagulation versus no anticoagulation.

a) Objective 6.1-6.3: Analyze samples and report findings.

- **Major activities:** The panel is complete for 15 of 20 planned subjects in the healthy ECLS group. The parent study from which the trauma+ECLS (n=24) subject samples are to be acquired from was initially delayed at AREVA but has now begun and scheduled to be completed by Sept 2023. We will continue to collect samples and execute the panel as the samples become available. A subset of the preliminary results from the n=15 healthy ECLS subjects is reported above under Specific Aim 1 (pages 9-11)
- **Results/Developments/Achievements:** Preliminary results reported above under Specific Aim 1, Major Activity 2 (pages 9-11). This Aim is ongoing and anticipated to be completed on time with the SOW.

Specific Aim 4: Compare results of investigative coagulation panel to bleeding/thrombotic outcomes and classic coagulation metrics acquired in the parent study from which the library of samples for analysis was acquired.

1) Major Activity 7: Gather coagulation outcomes from parent studies.

a) Objective 7.1-7.2: Quantify/characterize incidence of bleeding/thrombotic complications in subjects from parent studies and tabulate results of conventional coagulation testing obtained in the parent studies.

- **Major activities:** For all subjects acquired from the parent studies to date (27 of 56 planned subjects), the conventional coagulation results have been tabulated and collected. We are awaiting final analysis of histological results from a veterinary pathologist to finish categorizing the bleeding and thrombotic complications. This work is ongoing. The results for the remaining planned subjects are being acquired continuously at AREVA as the parent studies are executed.
- **Results/Developments/Achievements:** We have assembled all available data from the parent studies for the subjects that have been performed (N=12 of 12 in Trauma only group, N=15 of 20 in ECLS only group). We are continuing to collect the remaining samples/subject data and anticipate that this should be completed in a timely manner once the parent studies are completed in Sept 2023.

2) Major Activity 8: Perform statistical analysis and interpretation to assess correlation of novel coagulation panel results with conventional coagulation tests; and associate novel coagulation panel results with bleeding and thrombotic outcomes.

a) Objective 8.1-6.4: Perform statistical analyses and report findings.

- **Major activities:** With data acquired from the samples that have been analyzed, we performed a preliminary correlation comparing results of the novel panel utilized in this study (vWF multimer distribution analysis, platelet receptor shedding analysis, and thrombin generation potential) to conventional coagulation tests (hematocrit, platelet count, thromboelastography (TEG), prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, von Willebrand factor antigen test, and activated clotting time (ACT) that were acquired as a part of the parent studies. Of note, the instrumentation and effort to acquire the conventional coagulation results would amount to ~$1M research funding that is currently carried on other existing/ongoing DoD funded research efforts, and directly benefits this Discovery Study. This preliminary look includes 126 observations. Significance correlations were identified using alpha=0.05. Correlations were defined as weak for Spearman R <0.50; moderate for Spearman R >50 and <0.75; strong for Spearman R >75.
- **Results/Developments/Achievements:** Results from the preliminary correlation analysis are summarized in Table 1 (Correlation results table is also included as Appendix A). To summarize, we see some unique and logical relationships in each panel test subset, such as elevated circulating shed

platelet receptors associated with more rapid clotting times. We will investigate additional relationships once we have analyzed the remaining study sample sets.

Panel Subset	Initial Correlation Findings (n=126 observations)
vWF Multimer Analysis	-Percentage of high molecular weight multimers of vWF had a weak but significant negative correlation with vWF antigen percentage, while intermediate- and low- vWF multimer percentage had a weak positive correlation with vWF antigen percentage. -vWF collagen binding activity had a strong positive correlation with vWF antigen percentage (meaning stronger binding activity with higher vWF antigen levels). -ADAMTS-13 activity had a weak positive correlation with platelet count, TEG Angle, TEG MA, and aPTT. ADAMTS-13 activity had a weak negative correlation with vWF antigen (lower enzymatic cleavage of vWF when less is present).
Platelet Receptor Shedding Analysis	-sGPVI (soluble platelet receptor) had moderate positive correlation with hematocrit, platelet count, and TEG angle. sGPVI had moderate negative correlation with TEG R/Reaction Time, and weak negative correlations with PT and ACT. Cumulatively, this suggests that clotting times are shorter/more rapid with higher levels of this shed platelet receptor in circulation. -ADAM10 concentration had moderate negative correlations with hematocrit and platelet count. -ADAM17 did not correlate with any conventional coagulation metrics.
Thrombin Generation Potential (CAT)	-Estimated total thrombin potential (ETP) and peak thrombin potential had significant weak positive correlation with TEG clot strength (TEG MA) and fibrinogen concentration. No correlation with hematocrit, platelet count, PT/aPTT, ACT were noted. -Factor XII levels had a weak positive correlation with vWF antigen levels.

Table 1. Preliminary correlation analysis comparing novel coagulation panel outcomes collected in this project to conventional coagulation tests that were collected in the parent studies from which samples were acquired. Significance was accepted using alpha=0.05. Correlations were defined as weak for Spearman R <0.50; moderate for Spearman R 50>and<0.75; strong for Spearman R >75.

What opportunities for training and professional development has the project provided?

Dr. Roberts trained and mentored six post-baccalaureate pre-medical students (Isabella Garcia, Cassandra Niemeyer, Ryley Zapien, Hunter Allen, Ji Lee, Shubhneet Warar) employed as research technicians at the AREVA lab throughout year 1 of this project. The technicians are in process of applying to medical school while learning essential research and translational medical skills at AREVA. They had no prior experience with advanced molecular biology techniques or any of the instruments utilized in this study but have now mastered these techniques. Dr. Roberts worked hands-on with all students to teach them these techniques. She also trained and mentored the students in collaboration with AREVA Program Director Dr. Andriy Batchinsky as they wrote their first research abstract that was accepted for presentation at a major international meeting, the 10th Annual EuroELSO Congress, where Dr. Roberts presented results from this project (see Products p 16).

Additionally, several medical students from the UIW School of Osteopathic Medicine had the opportunity to participate in the AREVA Student Research Internship Program. Dr. Roberts worked with 8 UIW medical students, in particular Ms. Pamela Villalobos and Mr. Eric Bigon, who were trained to conduct molecular biology-based assays and to operate the Sebia and CAT instruments. The students were also trained to write research abstracts and prepare scientific posters and slideshows for meetings. Eric and Pam presented the work they performed as a part of this project at the Latino Medical Student Association Southwest Meeting where they were awarded first place in the Translational Research division. They also submitted an abstract to the 2023 CNHS Keystone ECMO and the Advanced Therapies for Cardiac and Respiratory Failure 39th Annual Symposium which was selected for podium presentation (will take place 27 Feb 23). Under Dr. Batchinsky's

direction, AREVA now offers a year-round volunteer program for these medical students where they participate in large scale translational research studies and are engaged weekly in laboratory meetings and lecture sessions.

How were the results disseminated to communities of interest?
All medical students that participated in the project are currently enrolled in clinical rotations. The students shared that their research experience at AREVA is shaping their performance in the clinical realm and providing practical application to their classroom studies. Participation in this research project is already impacting their clinical careers and they will be more likely to participate in translational research activities as clinicians in the future.

The results were reported at the following international and regional scientific and medical research meetings:

-10th Annual EuroELSO Congress 2022 (London, England; 06 May 2022)

-2023 Latino Medical Student Association Southwest Regional Conference (Fort Worth, TX; 03-04 Feb 2023).

-2023 CNHS ECMO and the Advanced Therapies for Cardiac and Respiratory Failure 39th Annual Symposium (Keystone, CO; 27 Feb 2023)

The results have been submitted for consideration to be presented at the 2023 Military Health System Research Symposium.

Dr. Roberts and the AREVA team hosted a visit with the Sebia, Inc. application specialists to inform them of this research project. Sebia is primarily involved in highly specific clinical applications for von Willebrand disorders, but were unaware of the potential impact of their technology in the translational research space.

This work is inspiring subsequent PRMRP application submissions to extend this panel to human/clinical cohorts.

Dr. Roberts and AREVA Director Dr. Batchinsky were approached by the Extracorporeal Life Support Organization (ELSO) to contribute a chapter on bleeding, thrombosis, and blood-biomaterial interactions during ECLS to the 6th edition of the *ELSO RedBook*. The *RedBook* serves as an SOP/handbook for clinicians across disciplines of extracorporeal organ support; thus, the project is already contributing to clinical care through incorporation of Dr. Robert's experience in this book. (Book details listed p 16 under "Products")

What do you plan to do during the next reporting period to accomplish the goals?

In the next reporting period, sample analysis will continue until all subjects in the Uninjured ECLS group and the Trauma+ECLS group are completed. The sample analysis is ongoing continuously while the parent studies are being executed at AREVA.

We will perform all statistical analyses and begin preparation of manuscripts summarizing these results. We will also continue to prepare abstracts for scientific meetings as we finish evaluating the final study groups.

We will prepare and submit the final report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
This project represents a dramatic shift from conventional generalized, reactive, non-specific coagulation management protocols for ECLS to proactive targeted, injury/disease-specific coagulation management strategies in multiple disease states. The need for this paradigm shift has been evident during the ongoing COVID-19 pandemic where COVID-19 positive patients supported by ECMO had significant instance of

bleeding events (49%) that are suggested to be of unique/distinctive origin relative to non-COVID ECMO bleeding complications. We anticipate that novel products resulting from this work will include: 1) CPG for ECLS in combat-relevant trauma specifically, 2) Novel coagulation panel for evaluation of ECLS hemocompatibility; 3) Database of mechanistic, novel coagulation metrics from translational research studies conducted at PFC timeline during early ECLS for trauma with and without systemic anticoagulation; 4) Identification of novel targets for development of field care/out of hospital post-trauma coagulation assessment instrument.

What was the impact on other disciplines?
ECLS and other forms of organ support are routinely used in the civilian sector/outside of combat trauma, and all forms of ECLS could benefit from more targeted coagulation management protocols that are specific to underlying disease/coagulopathy. Further, this project will inform general trauma management (without ECLS/organ support). This could lead to identification of new coagulation tools to be developed for the point-of-care or forward surgical setting.

As mentioned above, Dr. Roberts and AREVA Director Dr. Batchinsky were approached by the Extracorporeal Life Support Organization (ELSO) to contribute a chapter on bleeding, thrombosis, and blood-biomaterial interactions during ECLS to the 6th edition of the ELSO RedBook. The RedBook serves as an SOP/handbook for clinicians across disciplines of extracorporeal organ support; thus, the project is already contributing to clinical care through incorporation of Dr. Robert's experience in this book. (Book details listed p 16 under "Products")

What was the impact on technology transfer?
The AREVA team is introducing Sebia, Inc. to the translational research space and educating them on the potential impact of their technology (Sebia instrument) in this area.

What was the impact on society beyond science and technology?
The training opportunities provided to medical students through this project are teaching these future clinicians how to interpret and communicate scientific literature to an array of audiences. This will benefit them in their future careers as they communicate with patients and families in the clinical setting.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change
Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
The AREVA team experience and unfortunate facility flooding incident on 06 Jan 2023 where water from a laboratory located above our laboratory floor leaked into our instrument room and onto the Sebia Hydrasis instrument. Upon discovering the flooding, the instrument was removed from the equipment room and Sebia was notified. Sebia sent a field service engineer to our site to evaluate the instrument and perform maintenance to ensure that the instrument is functioning properly. This issue caused ~3 week delay in instrument use but will not affect timeline of any deliverables for this project. The issue is resolved.

The parent projects at AREVA from which the samples used in this study are acquired have entered a no cost extension due to experimental backlog following COVID-19 related delays/facility closures and animal vendor delays, as well as hiring and training-retraining of new personnel. We are continuing to analyze all samples as they become available, and we do not anticipate a delay in timely completion of the project according to the SOW timeline.

Changes that had a significant impact on expenditures
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.
Roberts TR, Harea GT, Zang Y, Devine RP, Maffe P, Handa H, Batchinsky AI. A dual-action nitric oxide-releasing slippery surface for extracorporeal organ support: Dynamic in vitro hemocompatibility evaluation. *J Biomed Mater Res. B: Appl Biomaters.* 2023; 111(4): 923-932.

Roberts TR, Garren MS, Wilson SN, Handa H, Batchinsky AI. Development and in vitro whole blood hemcompatibility screening of endothelium-mimetic multifunctional coatings. *ACS Appl Bio Mater.* 2022; 5(5): 2212-2223.

Roberts TR, Seekell RP, Zang Y, Harea GT, Zhang Z, Batchinsky AI. In vitro hemocompatibility screening of a slippery liquid impregnated surface coating for extracorporeal organ support applications. *Perfusion.* 2022; epub ahead of print. DOI: 10.1177/02676591221095469

Melvin AC, Wick TV, Zang Y, Harea GT, Cancio LC, Reynolds MM, Batchinsky AI, Roberts TR. Development and blood compatibility of a stable and bioactive metal-organic framework composite coating for blood-circulation tubing. *ACS Biomater Sci Eng.* 2022; 8(8): 3438-3449.

Vedula EM, Isenberg BC, Santos J, Lai W, Lewis DJ, Sutherland D, Roberts TR, Harea GT, Wells C, Teece B, Urban J, Risoleo T, Solt D, Leazer S, Chung K, Sukavaneshvar S, Batchinsky AI, Borenstein JT. Multilayer scaling of a biomimetic microfluidic oxygenator. *ASAIO.* 2022; 68(10): 1312-1319.

Books or other non-periodical, one-time publications.

Ryerson LM, Annich G, Batchinsky AI, Martucci G, Roberts TR, Thiagarajan RR, Vandenbriele C, MacLaren G. Adverse Effects of Extracorporeal Life Support. In: MacLaren G, Brodie D, Lorusso R, Peek G, Thiagarajan R, Vercaemst L, eds. *Extracorporeal Life Support: The ELSO Red Book 6th Edition.* Ann Arbor, MI, USA: Extracorporeal Life Support Organization; 2022:97-111.

Other publications, conference papers and presentations.

Warar S, Zapien R, Niemeyer C, Batchinsky AI. Roberts TR. Comparison of vWF and ADAMTS-13 activity after smoke inhalation and burns managed with and without ECCO2R. *10th Annual EuroELSO Congress 2022.* May 04, 2022; London, UK.

Villalobos PA, Bigon EB, Batchinsky AI, Roberts TR. Evaluation of von Willebrand factor distribution over 72-hours of ICU care in swine with polytrauma versus veno-venous extracorporeal life support. *Latino Medical Student Association Southwest Regional Conference.* Feb 03-04, 2023; Fort Worth, TX, USA. **Selected for first-place presentation award in Translational Research Category

Villalobos PA, Bigon EB, Batchinsky AI. Roberts TR. Evaluation of von Willebrand Factor multimer distribution over 72-hours of ICU care in swine with veno-venous extracorporeal life support. *CNHS*

ECMO and the Advanced Therapies for Cardiac and Respiratory Failure 39th Annual Symposium. 27 Feb 2023; Keystone, CO, USA. (podium presentation).

Batchinsky AI, Roberts TR – co-chaired session "Advanced Medical Devices for Lung Support" at the CNHS ECMO and the Advanced Therapies for Cardiac and Respiratory Failure 39th Annual Symposium. 27 Feb 2023; Keystone, CO, USA

- **Website(s) or other Internet site(s)**
  Nothing to report.

- **Technologies or techniques**
  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Nothing to report.

- **Other Products**
  - Data/database of extensive coagulation markers in translation research studies collected over 72-hours.
  - Development of novel criteria for description and identification of bleeding and bleeding severity during ICU care for translational research application.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Teryn Roberts, PhD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-2460-6432
Nearest person month worked:	1.4
Contribution to Project:	Oversaw study conduct, led study execution, data and sample analysis, experiment planning, and report/abstract/manuscript preparations. Oversaw personnel training.

Name:	Ryley Zapien
Project Role:	Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.3
Contribution to Project:	Biosample inventory and analysis, data collection and entry.

Name:	Pamela Villalobos
Project Role:	Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.6
Contribution to Project:	Biosample inventory and analysis, data collection and entry. Assist in data analysis and interpretation. Assist with scientific abstract preparations and presentations.

Name:	Cassandra Niemeyer

Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.1
Contribution to Project: Biosample inventory and analysis, data collection and entry.

Name: Pamela Villalobos
Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.6
Contribution to Project: Biosample inventory and analysis, data collection and entry. Assist in data analysis and interpretation. Assist with scientific abstract preparations and presentations.

Name: Yan Gernhofer
Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.2
Contribution to Project: Biosample inventory and analysis, data collection and entry.

Name: Isabella Garcia
Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.2
Contribution to Project: Biosample inventory and analysis, data collection and entry.

Name: Chelsea Flanagan
Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Biosample inventory and analysis, data collection and entry. Assist in data analysis and interpretation. Assist with scientific abstract preparations and presentations.

Name: Eric Bigon
Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Biosample inventory and analysis, data collection and entry. Assist in data analysis and interpretation. Assist with scientific abstract preparations and presentations.

Name: Aboozar Ali
Project Role: Research Laboratory Technician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 0.1
Contribution to Project: Biosample inventory and analysis, data collection and entry.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: Appendix A – Correlation Results Table