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	FINAL	

4. TITLE AND SUBTITLE
Pilot Validation of a Hemodilution Technique to Estimate Blood Volume in Vivo

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5c. PROGRAM ELEMENT NUMBER
N/A

6. AUTHOR(S)

Wofford, Kenneth

5d. PROJECT NUMBER
HU0001-16-1-TS14

5e. TASK NUMBER
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N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road,
Bethesda, MD 20814-4712

8. PERFORMING ORGANIZATION REPORT NUMBER
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TriService Nursing Research
Program, 4301 Jones Bridge RD
Bethesda, MD 20814

10. SPONSOR/MONITOR'S ACRONYM(S)
TSNRP

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HU0001-16-1-TS14

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Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
N/A

14. ABSTRACT

Purpose: Perioperative fluid therapy is used to optimize tissue perfusion. Existing methods to quantify blood volume and estimate intravenous (IV) fluid requirements are either inaccurate or require specialized equipment and training. This study assessed the utility of mild hemodilution for quantifying blood volume.

Design: A prospective validation design was used. Blood volume was quantified utilizing two methods in a fixed order: radiodilution, then hemodilution as described in D'Angelo et al. (2014).

Methods: After IRB approval and informed consent, blood volume (BV) estimates were derived for each subject using radiodilution via the Daxor BVA-100, and from pre- and post-bolus hematocrit (HcT) using methods described in D'Angelo, et al. (2014). Hematocrit was assessed via 3 methods: Microimpedence via an i-STAT device and the Duke University Medical Center Lab, and total hemoglobin (SpHb, Masimo Corporation, Irvine, CA).

Sample: Thirty-three healthy, male subjects aged 18-35 years.

Analysis: Bland-Altman plots, the intraclass correlation, and Lin's Concordance Coefficient were utilized to compare the gold standard of radiodilution and the proposed method of mild hemodilution for each method of measuring HcT (i-STAT, Lab, and SpHb).

Findings: Mean BV estimates were 5568.6±902.2 ml via radiodilution, 8521.2±3526.5 ml via i-STAT, 16411.0±13774.5 via Duke lab, and 15882.0±22371.0 via the Masimo SpHb device. Agreement in estimated BV between methods was low (Lin's Concordance Coefficient 0.02-0.05). Crystalloid loss to extravasation or renal filtration necessary to account for the observed differences between methods ranged from 181.9 ml to 299.7 ml over 6 minutes.

Implications for Military Nursing: Hemodilution with normal saline was not useful in quantifying BV, most likely due to rapid movement of the saline out of the vascular compartment. Future attempts to estimate BV via hemodilution would need to use a fluid that is more resistant to extravascular movement (e.g., 6% Hetastarch or 5% albumin), or identify 3rd variables that enables precise estimation of the rate of extravascular movement.

15. SUBJECT TERMS
Blood volume, hemodilution technique, radiotracer dilution technique

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					Kesha Chandler
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UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED			301-319-0593

TriService Nursing Research Program Final Report Cover Page

Sponsoring Institution	TriService Nursing Research Program
Address of Sponsoring Institution	4301 Jones Bridge Road Bethesda MD 20814
USU Grant Number	HU0001-16-1-TS14
USU Project	N16-P10
Title of Research Study or Evidence-Based Practice (EBP) Project	Pilot Validation of a Hemodilution Technique to Estimate Blood Volume in Vivo
Period of Award	01JUL16-30JUN18
Applicant Organization	Henry Jackson Foundation
Address of Applicant Organization	6720-A Rockledge Dr., Suite 100 101 E. 27th St, Stop A9000 Bethesda, MD 20817-1834

Signatures

PI Signature  Date 05JUL18

Mentor Signature  Date 05JUL18

Table of Contents

Abstract

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TSNRP Research Priorities that Study or Project Addresses

Primary Priority

Force Health Protection:	☐ Fit and ready force ☒ Deploy with and care for the warrior ☐ Care for all entrusted to our care
Nursing Competencies and Practice:	☐ Patient outcomes ☐ Quality and safety ☐ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

Secondary Priority

Force Health Protection:	☐ Fit and ready force ☐ Deploy with and care for the warrior ☒ Care for all entrusted to our care
Nursing Competencies and Practice:	☒ Patient outcomes ☐ Quality and safety ☐ Translate research into practice/evidence-based practice ☐ Clinical excellence ☐ Knowledge management ☐ Education and training
Leadership, Ethics, and Mentoring:	☐ Health policy ☐ Recruitment and retention ☐ Preparing tomorrow's leaders ☐ Care of the caregiver
Other:	☐

Progress Towards Achievement of Specific Aims of the Study or Project

Findings related to each specific aim, research or study questions, and/or hypothesis:

The project had three specific aims:

Aim 1. Determine subject total blood volume using the gold standard DAXOR Blood Volume Analyzer100 Analysis System (Radiotracer Dilution Technique).

The team readily accomplished this aim. After IRB approval and three days of training the operation of the Daxor Blood Volume Anlyzer-100, we recruited 33 healthy male subjects. The subjects were young (mean age 23.8 ± 4.23 years) and racially diverse (white n = 19; African-American/Black n = 4; Asian/Pacific Islander n = 7; and Hispanic/Latino n = 3). Subjects were fit, with a mean body mass index of 23.5 ± 2.6 kg/m^2**.** The study procedures for quantifying blood volume via radiotracer worked as planned for 32 of 33 subjects, resulting in a mean radiodilution-estimated total blood volume (TBV) of 5529.7 ± 857.6 ml as compared to a predicted mean ideal BV of 5014.5 ± 393.3 ml. There was a significant correlation between radiodilution-estimated TBV and ideal BV (r = 0.58; p = 0.01)

Aim 2. Compute estimated subject total blood volume using venous blood hematocrit values drawn before and after an intravenous fluid bolus (Hemodilution Technique).

The team also readily accomplished this aim. Baseline hematocrits were drawn, a 500 cc bolus was administered over 12 minutes and repeat hematocrits drawn six minutes later without difficulty. The mean pre- and post-bolus hematocrits are depicted in Table 1.

Table 1. Mean Hematocrit Values Before and After Fluid Bolus

	Measure				
Measurement Method	Pre-Bolus HcT	Post-Bolus HcT	Measured Change	Expected Change	Difference
iSTAT	0.402 ± 0.032	0.386 ± 0.031	-0.020 ± 0.017	-0.037 ± 0.004	0.017 ± 0.016
Lab	0.411 ± 0.028	0.397 ± 0.028	-0.015 ± 0.010	-0.037 ± 0.004	0.022± 0.010
Masimo Total Hemoglobin (MMO)	0.414 ± 0.029	0.388 ± 0.032	-0.027 ± 0.016	-0.039 ± 0.004	0.012 ± 0.016

Thus, measured change in hematocrit was 0.012 to 0.022 points smaller than expected, with a highly variable difference (0.010-0.016). Accordingly, the hematocrit-derived estimated blood volumes calculated based on the formula in D'Angelo et al. (2015) were substantially higher than expected, with mean estimates of 6409.9 ± 8192.2 ml, 20,028.8 ± 21093.9, and 15,191 ± 21597.3 ml being derived using the iSTAT, Lab, and MMO, respectively.

Aim 3. Correlate inter-subject radiotracer dilution technique-derived total blood volume with hemodilution technique-derived total blood volume.

This aim has been met. To address this aim the team constructed Bland-Altman plots and calculated the intraclass correlation coefficient and Lin's concordance coefficient, comparing the radiodilution-derived estimated blood volume (the gold standard) to the hematocrit-derived estimated blood volume (the proposed method). The Bland Altman Plots (Figures 1-3) demonstrate substantial fixed and proportional bias, in which the mean PBVs predicted by the mathematical model were substantially higher then mean TBV, and the degree of deviation increased proportionally with both high and low blood bolumes. Intraclass correlation coefficients of TBV with PBV were 0.07, 0.02, and 0.04 for iSTAT, Lab, and MMO, respectively. Lin's Concordance Coefficient for TBV/PBV agreement was similarly small: 0.03, 0.02, and 0.02 for iSTAT, Lab, and MMO, respectively. Thus, none of the three methods were able to predict TBV with accuracy given the current conception of the model.

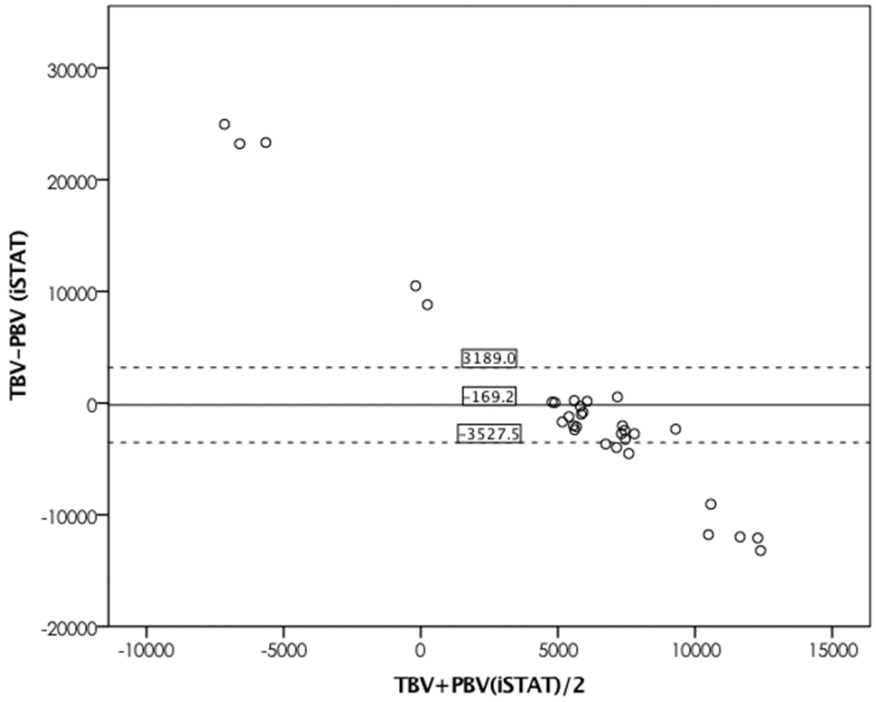

*Figure 1***.** Bland-Altman plot relating difference between TBV and PBV as predicted by iSTAT hematocrit.

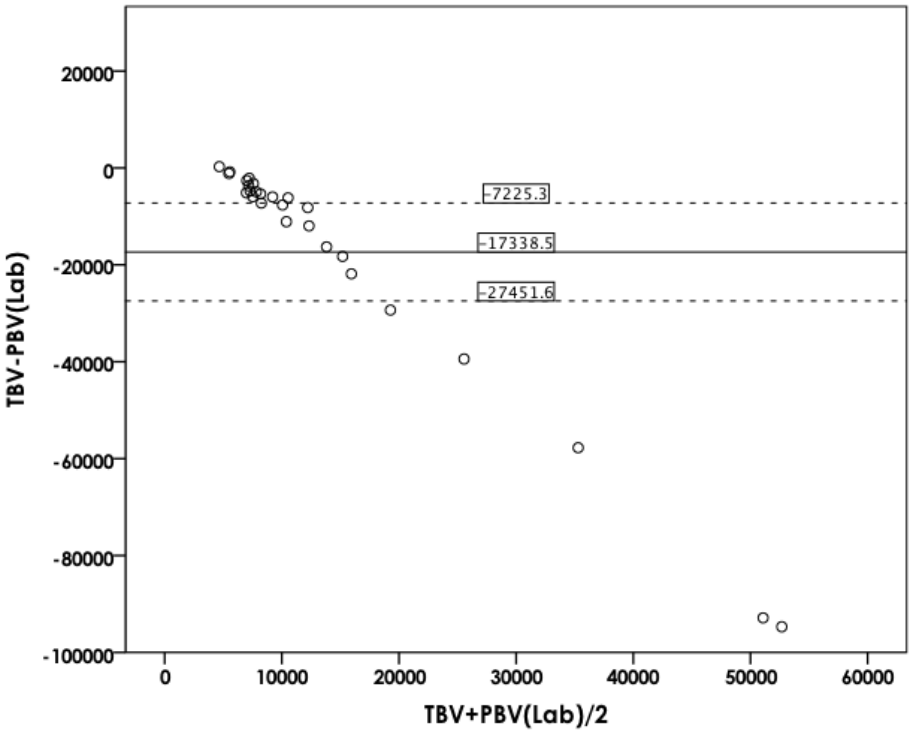

*Figure 2***.** Bland-Altman plot relating difference between TBV and PBV as predicted by Lab hematocrit.

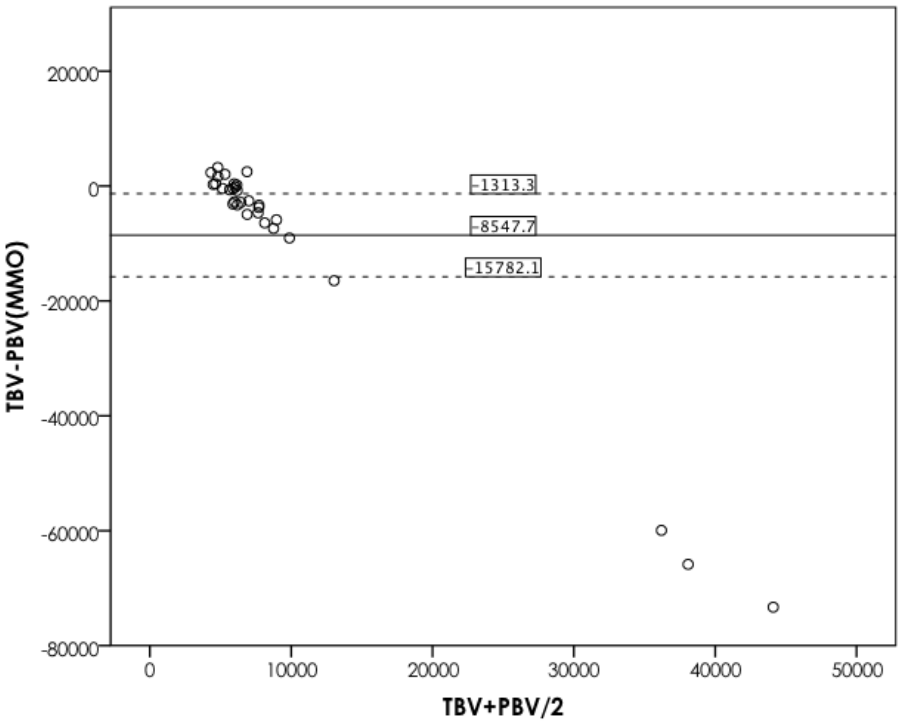

*Figure 3***.** Bland-Altman plot relating difference between TBV and PBV as predicted by MMO hematocrit.

The most likely explanation for the smaller than expected change in hematocrit is the rapid movement of crystalloid from the vascular space through filtration by the kidney or extravasation in other tissue compartments. Thus, the team performed a supplemental exploratory analysis to estimate the amount of crystalloid that would have to 'leak' from the vascular space to account for the observed difference between TBV and PBV for each method. The loss rates over the 12-minute period were 260.8 ± 203.0 ml based on the iSTAT estimates, 189.4 ± 192.1 ml based on the Lab estimates, and 317.0 ± 122.5 ml based on MMO estimates.

Relationship of current findings to previous findings:

Substantial differences were observed between blood volume as predicted by the D'Angelo et al (2015) model and blood volume as measured by the Daxor BVA-100. These differences were driven by smaller-than expected drops in hematocrit after fluid bolus administration, which in turn suggests a substantial loss of the normal saline bolus from the vascular space. The loss of crystalloid from the vascular space is a well-characterised phenomenon, but previous authors have reported much longer ½ times for saline retention in the vascular space. For example, after giving 25 ml/kg over 30 minutes (i.e., 1750 ml for a 70 kg subject, given at 58.33 ml/min), ½ times were estimated as 180 minutes by Norberg et al. (2007) and 110 minutes by Drobin et al. (2002). The present study used a much smaller volume of saline (7.14 ml/kg, for a 70 kg subject) given at a similar rate (41.67 ml/min) but observed much higher loss from the vascular space. The observed ½ times for normal saline from the vascular space for the present study would be approximately 11.5, 15.8, and 9.5 minutes when hematocrit was assessed via iSTAT, Lab, or MMO, respectively. Thus, the present study suggests that when normal saline is given in smaller, more ecologically valid boluses the ½ time may be much shorter than anticipated based on prior research examining the ½ time after large volume bolus. We continue to explore the literature to place this unexpected finding in the proper context.

Effect of problems or obstacles on the results:

In five subjects, miscommunications within the Duke lab led to the loss of one of the hematocrit samples and the loss of lab-hematocrit based data for that subject. In brief, during the conduct of the study the lab implemented a policy to decrease unnecessary clinical testing. This policy dictated that when any two orders and/or samples were arrived for the same patient within less than two hours, lab personnel were to run one, and discard and cancel the other. As our hematocrit samples arrived simultaneously in two tubes labeled 18 minutes apart, the lab personnel on duty that evening discarded one (in all four cases we were unable to ascertain which one) and ran the other. We discovered the new policy the next day, and were able to reach an agreement whereby our samples were handled separately. However, iSTAT and MMO data were still collected and valid for that subject. In a single subject, quantification of blood volume via radiodilution was impossible due to sample contamination. A filter in the micropipette had absorbed radiotracer from the previous subject's samples, and transferred it into the baseline sample for the omitted subject. Without a baseline sample to estimate baseline gamma activity in the subject's blood, we were forced to discard that subject's data.

We do not feel that this loss of data from these six subjects unduly impaired the execution of Specific Aim 3. We still had complete data for the iSTAT device and MMO for 32 subjects, and the patterns of fixed and proportional bias evidenced in the Bland-Altman plots (Figures 1-3)

are similar across all methods of assessing hematocrit. The loss of Daxor BVA-100 estimated blood volume from the single subject (leaving 32 with valid data) is unlikely to have affected the outcome, as the sample size calculation suggested that 30 subjects would be adequate to conduct the required analyses.

Limitations:

Key limitation of the project are the use of normovolemic subjects and the choice of normal saline as the crystalloid for hemodilution. The subjects were healthy and demonstrated a TBV approximately 500 cc greater than ideal. As TBV increases, the decrease in hematocrit from a given volume of crystalloid will grow smaller and thus, more difficult to measure. Additionally, isotonic crystalloids remain in circulation for a shorter period. For comparison, Waitzinger et al. (1998) reported the ½ time of 6% hydroxyethyl starch as 12 hours (compared to 180 and 110 minutes for normal saline reported by Norberg et al. (2007) and Drobin et al. (2002), respectively). Thus, the use of a colloid might have prevented the high loss rate observed in the present study, but would have decreased the ecological validity of the study as the goal was to use a nearly universally available, low cost fluid (normal saline) in a clinically relevant volume (500 ml) to provoke hemodilution.

Conclusion:

Specific Aims 1, 2, and 3 were unequivocally met by the study team, and the results of the study are clear: Small-volume hemodilution with normal saline was not useful in predicting total blood volume. However, the smaller than expected drops in hematocrit observed after hemodilution suggest that crystalloid loss from the vascular compartment is much larger and happens over a much shorter time scale than expected.

Significance of Study or Project Results to Military Nursing

As stated in the conclusion, small-volume hemodilution was not useful in estimating total blood volume, most likely due to larger than expected loss of normal saline from the vascular compartment. These findings further reinforce the current Tactical Combat Casualty Care TCCC Guidelines, which dictates blood products as the first-line treatment to correct hemorrhagic shock, and a colloid solution (currently 6% hydroxyethyl starch) when blood products are not immediately available (Butler et al., 2014).

After time to complete a more comprehensive review of the literature around this finding, we may propose to repeat the project, utilizing 6% hydroxyethyl starch rather than normal saline. Such an approach work hand-in-hand with the current TCCC Guidelines, as a 500 ml bolus of 6% hydroxyethyl starch is the recommended treatment for an unstable casualty when blood products are not immediately available (Butler et al., 2014). The use of 6% hydroxyethyl starch should overcome the high loss rate seen with the crystalloid, due to its much longer ½ time in the vascular compartment by (Drobin, et al., 2002, and Norberg, et al., 2007).

Changes in Clinical Practice, Leadership, Management, Education, Policy, and/or Military Doctrine that Resulted from Study or Project

None to date.

References Cited

Butler, F. K., Holcomb, J. B., Kotwal, R. S. et al . (2014) Fluid resuscitation for hemorrhagic shock in Tactical Combat Casualty Care: TCCC Guidelines Change 14-01. J Special Operations Medicine, 14 13-38.

D'Angelo, M., et al. (2015). A Theoretical Mathematical Model to Estimate Blood Volume in Clinical Practice. *Biological Research for Nursing*, 17(5).478-86. PMID: 25332464

Hahn R.G., Drobin D., & Zdolsek J. (2016). Distribution of crystalloid fluid changes with the rate of infusion: a population-based study. *Acta Anaesthesiolica Scandanavia* ; 60:569–578.

Norberg Å., Hahn R.G., Li H., et al. Population volume kinetics predicts retention of 0.9% saline infused in awake and isoflurane-anesthetized volunteers. *Anesthesiology* 2007; 107:24–32.

Waitzinger J., Bepperling F., Pabst G., et al. Pharmacokinetics and tolerability of a new hydroxyethyl starch (HES) specification (HES 130/0.4) after single-dose infusion of 6% or 10% solutions in healthy volunteers. *Clinical Drug Investigations* 1998; 16:151–160.

Summary of Dissemination

Type of Dissemination	Citation	Date and Source of Approval for Public Release
Publications	None	
Publications in Press	None	
Published Abstracts	None	
Podium Presentations	Wofford, K. A. (2018) 'Pilot Validation of a Hemodilution Technique to Estimate Blood Volume in Vivo.' Presented at the Daniel K. Inouye Graduate School of Nursing Research Colloquium at Uniformed Services University, Bethesda, MD.	
Poster Presentations	Wofford, K. A. (2018) 'Pilot Validation of a Hemodilution Technique to Estimate Blood Volume in Vivo.' Presented at the AANA Annual Congress, Boston, MA.	
Media Reports	None	

Other	None	

Reportable Outcomes

Reportable Outcome	Detailed Description
Applied for Patent	None
Issued a Patent	None
Developed a cell line	None
Developed a tissue or serum repository	None
Developed a data registry	None

Recruitment and Retention Table

Recruitment and Retention Aspect	Number
Subjects Projected in Grant Application	33
Subjects Available	34
Subjects Contacted or Reached by Approved Recruitment Method	34
Subjects Screened	123
Subjects Ineligible	89
Subjects Refused	1
Human Subjects Consented	33
Subjects Who Withdrew	0
Subjects Who Completed Study	33
Subjects With Complete Data	32
Subjects with Incomplete Data	1

Demographic Characteristics of the Sample

Characteristic	
Age (yrs)	23.8 ± 4.2
Women, n (%)	0 (0)
Race	
White, n (%)	19 (57.6)
Black, n (%)	4 (12.1)
Hispanic or Latino, n (%)	3 (9.1)
Native Hawaiian or other Pacific Islander, n (%)	0 (0)
Asian, n (%)	7 (21.2)
Other, n (%)	0 (0)
Military Service or Civilian	
Air Force, n (%)	0(0)
Army, n (%)	0(0)
Marine, n (%)	0(0)
Navy, n (%)	0(0)
Civilian, n (%)	33 (100)
Service Component	
Active Duty, n (%)	0 (0)
Reserve, n (%)	0 (0)
National Guard, n (%)	0 (0)
Retired Military, n (%)	0 (0)
Prior Military but not Retired, n (%)	0 (0)
Military Dependent, n (%)	0 (0)
Civilian, n (%)	33(100)

Final Budget Report

HENRY M. JACKSON FOUNDATION FOR THE ADVANCEMENT OF MILITARY MEDICINE
Advancing Military Medical Research

TASK BUDGET SUMMARY

Current as of: JUL2018

Organization: HJF Henry M. Jackson Foundation
Award #/Name: 65047 - PILOT VALIDATION
Award Manager: WOFFORD, KENNETH C
Award Period: 07/01/2016 to 06/30/2018
Project #/Name: 308674 - PILOT VALIDATION
Project Manager: WALLACE, PEARL SHANGE
Project Period: 07/01/2016 to 06/30/2018

Task # / Name: 1.00 - PILOT VALIDATION
Task Period: 07/01/2016 to 06/30/2018
Task Manager: NUNEZ, MELANIE B
Task Desc: N16-P10
Award Sponser: UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
Billing Analyst: LI, MING YIN
Primary Analyst: LIVAN, REYHAN
Ref Award#: HU0001-16-1-TS14

Current As of	Award/Project/Task Number	Task Budgetary Control	Category Group	Expenditure Category	Budgetary Control	Current Month Expenses	Budget	Open Commitment	Task-To-Date Expenses	Total Funds Used	Balance Available	Percentage Available
07/09/2018	65047 - 308674 - 1.00	Absolute	DIRECT	EQUIPMENT	Absolute	0.00	75,010.00	0.00	75,010.00	75,010.00	0.00	0.00
				SUPPLIES	Absolute	0.00	19,026.00	1,906.90	14,086.96	15,993.86	3,032.14	15.94
				SUBAWARDS	Absolute	0.00	194,984.00	48,950.86	146,033.14	194,984.00	0.00	0.00
				TOTAL DIRECT :		**0.00**	**289,020.00**	**50,857.76**	**235,130.10**	**285,987.86**	**3,032.14**	**1.05**
			INDIRECT	USU OFF-SITE OVERHEAD	Advisory	0.00	3,761.34	70.56	3,571.61	3,642.17	119.17	3.17
				COMPANY-WIDE G & A	Advisory	0.00	13,424.00	270.91	12,695.50	12,966.41	457.59	3.41
				SUBAWARD RATE	Advisory	0.00	2,066.18	543.35	1,522.83	2,066.18	0.00	0.00
				TOTAL INDIRECT :		**0.00**	**19,251.52**	**884.82**	**17,789.94**	**18,674.76**	**576.76**	**3.00**
				TOTAL TASK :		**0.00**	**308,271.52**	**51,742.58**	**252,920.04**	**304,662.62**	**3,608.90**	**1.17**

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