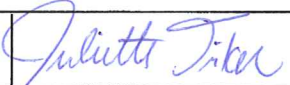
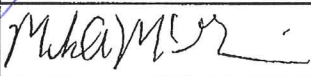
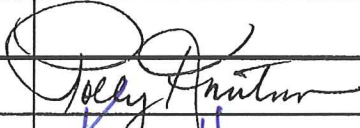
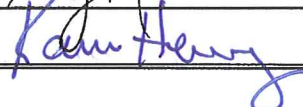


COVER SHEET FOR GRANT PROPOSALS

State Board of Education

SBOE PROPOSAL NUMBER: (to be assigned by SBOE)		AMOUNT REQUESTED: \$50,000	
TITLE OF PROPOSED PROJECT: Characterization of Antigens for a Staphylococcal Bovine Mastitis Vaccine			
SPECIFIC PROJECT FOCUS: Advancement of a novel nasal-spray vaccine to prevent mastitis in dairy cattle that is caused by the bacterial pathogen <i>Staphylococcus aureus</i>.			
PROJECT START DATE: 7-1-12		PROJECT END DATE: 6-30-13	
NAME OF INSTITUTION: Boise State University		DEPARTMENT: Biological Sciences	
ADDRESS: 1910 University Dr. Boise, ID 83725			
		E-MAIL ADDRESS: juliettetinker@boisestate.edu	PI PHONE NUMBER: 208-426-5472
NAME:		TITLE:	
SIGNATURE:			
PROJECT DIRECTOR	Juliette Tinker, Ph.D.	Assistant Professor	
CO-PRINCIPAL INVESTIGATOR	Mark McGuire, Ph.D.	Professor	
NAME:		SIGNATURE:	
Authorized Organizational Representative			
	Polly J Knutson, Director Office of Sponsored Programs University of Idaho		
	Karen Henry		

Characterization of Antigens for a Staphylococcal Bovine Mastitis Vaccine

- 1. Project institution:** Boise State University
- 2. Principal investigator:** Juliette Tinker, Ph.D. (Boise State)
Co-Principal investigator: Mark McGuire, Ph.D. (University of Idaho)
- 3. This technology has not been awarded a State Board of Education Incubation Fund in the past.**
- 4. Executive summary:**

The Gram positive bacterium, *Staphylococcus aureus*, is the most common cause of mastitis in dairy cattle worldwide. This infection is costly to the dairy industry; resulting in an estimated economic loss of \$184 per cow, and \$1.7 billion annually in the U.S. [2]. While a number of bacteria can cause clinical mastitis in dairy cattle, *S. aureus* can result in chronic sub-clinical infections that may cause reduced milk yield. Treatment for mastitis ranges from drying off procedures that often include antibiotic use, to the need for culling from the herd. Currently, there are two *S. aureus* vaccines available for use in dairy cattle. Both vaccines are based on injected whole cells, called bacterins. It is generally accepted that these vaccines have limited efficacy. Bacterins are not able to prevent new *S. aureus* infections, however some evidence suggests that they reduce the clinical severity of infected cows [3]. We have previously constructed and characterized an improved vaccine for bovine mastitis based upon a surface-exposed *S. aureus* antigen called iron regulated surface protein A (IsdA). This vaccine is unique in that it is fused to an adjuvant, or helper molecule, that will enable intranasal delivery [1]. The IsdA antigen has been found to be highly conserved in *S. aureus* isolates, upregulated during human disease and a promising vaccine target [4,5,6,7,8]. However, studies of the role of this antigen in bovine mastitis are limited. The sequence of the bovine *S. aureus* isolate RF122/ET3 suggests that IsdA is present, but contains significant differences

from sequenced human isolates [9]. After 3 years of benchtop preparation and preliminary characterization in mice and cows, a patent was filed on the IsdA vaccine in 2011. Now further laboratory work is necessary to promote the development of this vaccine using bovine milk and serum samples. We propose an extensive characterization of the expression and upregulation of IsdA during clinical and sub-clinical bovine mastitis to better understand the role of this vaccine antigen in disease. In addition, other surface proteins have been found in bovine isolates and represent important potential vaccine candidates. These include; clumping factor A (ClfA), fibronectin binding protein A (FnbpA) and iron regulated surface protein H (IsdH) [9,10,11]. We will characterize the expression and immune response to these additional *S. aureus* candidate antigens for incorporation into a multi-subunit bovine mastitis vaccine.

5. “Gap” project objective and total amount requested:

The objective of the proposed research is to promote the development of an *S. aureus* bovine mastitis vaccine. This vaccine will have improved efficacy over current licensed *S. aureus* vaccines and will be deliverable by nasal spray. Greater understanding of the role of bacterial antigens, and specifically the IsdA antigen, in clinical and subclinical bovine mastitis is essential for this development. We hypothesize that *isdA* expression is upregulated, and that antibodies are produced against IsdA, in cows during clinical infection with *S. aureus*. These results will strongly support the hypothesis that IsdA represents an important target antigen for a vaccine against bovine mastitis. In addition, we hypothesize that the expression of *clfA*, *fnbpA* and *isdH* are also upregulated during clinical infection and that these antigens represent important additional candidates for incorporation into an optimized *S. aureus* vaccine. Total amount requested: \$50,000.

6. Resource commitment and reflection of Boise State priorities:

The development of vaccine stimulants, or adjuvants, that can act from mucosal surfaces is a top priority for the advancement of veterinary vaccines. Nasal administration of vaccines may more effectively prevent disease from pathogens that enter via the mucosa, and these routes will also increase ease of administration and reduce costs. *S. aureus* is a highly significant target for vaccine development, as this organism is the agent of a heavy burden of chronic and clinical disease in dairy cattle worldwide. In addition, evidence exists that these isolates also represent a threat to human health [12,13]. Lastly, the development of new vaccines to promote veterinary health closely aligns with the translational goals of Boise State, and the expansion of a metropolitan research university of distinction.

7. Potential impact on the economy of Idaho:

According to a study in 2008 from Boise State's College of Business and Economics, Idaho is ranked third behind California and Wisconsin for the most milk and cheese produced in the nation [14]. In 2008, Idaho dairy farms produced over 12 million pounds of milk at an estimated \$2.1 billion. This industry also directly employed an estimated 13,180 people, and indirectly another 18,357 people. While it is believed that the number of dairies in Idaho has dropped in more recent years, the production of milk and cheese continues to be on the rise. Since 1997 the annual revenue from the sale of dairy products has exceeded that from the sale of potatoes. These reports indicate that the economics of the dairy industry will continue to be of great importance in this state, and thus the welfare of the animals is a top priority. An effective *S. aureus* vaccine to prevent mastitis will have a significant impact on the dairy industry. In addition, the

ease of administration and reduction of cost as a result of novel nasal delivery of this proposed vaccine will promote its use over other potential mastitis vaccine candidates.

8. Partnerships:

The primary partnership within the proposed research is that between two Idaho public universities; the University of Idaho and Boise State University. Dr. Mark McGuire and Dr. Tinker have collaborated since 2009 when work began on a USDA pilot award to characterize the immunogenicity of a subunit bovine mastitis vaccine. In addition, Dr. McGuire has significant involvement and collaboration with the dairy industry in Idaho. These connections will continue to promote future partnerships for this technology within the private sector.

9. The market opportunity:

Due to multiple agents of bovine mastitis as well as varying clinical and subclinical outcomes of disease, it is notoriously difficult to estimate industry losses as a result of *S. aureus* infection. However, a report from the National Mastitis Council in 1996 estimated the economic loss from bovine mastitis to be \$184 per cow, and \$1.7 billion annually in the U.S. [2]. These calculations include: reduced and lost milk production, replacement costs, extra labor, treatment and veterinary services for infected cows. The cost and limited efficacy of treatment has resulted in increased demand for preventative measures within the dairy industry, and this demand has coincided with an overall global demand for more veterinary vaccines to promote agriculture. According to a 2012 GBI Research report, the global market for veterinary vaccines has grown steadily at a rate of 1% annually since 2002. The largest market within this sector is the bovine vaccines market, which is estimated to be \$1.6 billion in the U.S. [15].

10. The technology:

We have previously reported the characterization of a potential *S. aureus* vaccine after intranasal immunization in mice [1]. This vaccine is based upon a non-toxic chimeric fusion of the *S. aureus* IsdA antigen to the A₂/B subunits of cholera toxin from *V. cholerae*.

Cholera toxin is a gold standard mucosal vaccine adjuvant that has been used in veterinary vaccines [16,17,18]. Fusion of IsdA to the A₂/B

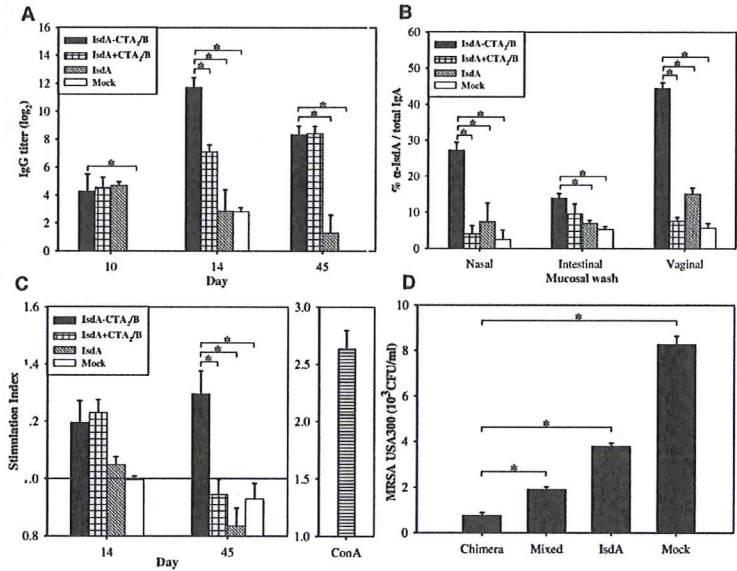


Figure 1. Characterization of the IsdA-CTA₂/B chimera after intranasal delivery. **A.** Endpoint titers of IsdA-specific serum IgG from days 10, 14 and 45. **B.** Percent IsdA-specific IgA out of total IgA in mucosal secretions from day 45. **C.** Stimulation index of splenocytes isolated from days 14 and 45 after a 4 day stimulation with IsdA. **D.** Effect of day 45 pooled serum on *S. aureus* adhesion to human epithelial cells *in vitro*. Significance ($p < 0.05$) between mice immunized with IsdA-CTA₂/B versus IsdA+CTA₂/B (*) is shown and all assays were performed in triplicate [1].

subunits of this toxin enables the replacement of toxic domains with the IsdA antigen to form a stable, holotoxin-like molecule that can be delivered via mucosal routes. Groups of 8 BALB/c mice were vaccinated intranasally with 2 doses of 50 µg of chimera, mixed IsdA and CTA₂/B, or purified antigen. IgG ELISAs on pooled sera revealed higher antigen specific IgG responses after the booster on day 10 that persisted until day 45 (Figure 1A). IgA responses from mucosal samples were also higher than those from control groups (Figure 1B). Ig subtyping and cytokine analysis were determined by ELISA, and cellular proliferation was analyzed by metabolic assay and flow cytometry (Figure 1C). Evidence suggested that a modest cellular response resulted in the production of largely Th-2 specific cytokines and Ig subtypes. Antibodies from day 45 were functional in the prevention of adhesion of distinct methicillin-sensitive and

methicillin-resistant strains of *S. aureus* to HeLa cell *in vitro* (Figure 1D). We have also initiated immunogenicity studies of the IsdA-CTA₂/B chimera in dairy cattle (Figure 2). 6 cows were vaccinated with 2 intranasal doses of 150 µg of IsdA-CTA₂/B or IsdA alone on days 0 and 14. Milk, serum and nasal secretions were isolated for assay of

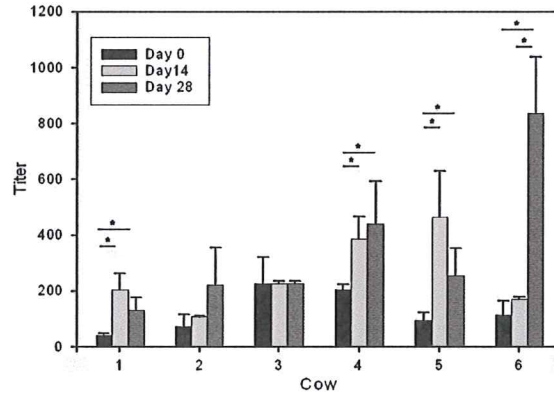


Figure 2. Immunogenicity of the IsdA-CTA₂/B chimera in cows. Endpoint titers of IsdA specific IgA in milk from days 0, 14 and 28. Cows 1, 2 and 3 were vaccinated with IsdA alone and cows 4, 5 and 6 were vaccinated with IsdA-CTA₂/B. Significance ($p < 0.05$) is shown (*) and all assays were performed in triplicate (*unpublished*).

antigen-specific humoral responses, and spleen cells removed for cellular responses. While bovine studies were complicated with previous and concurrent exposure to *S. aureus*, analysis of data is currently underway, and indicates a significant α -IsdA IgA response in milk from vaccinated cows.

11. Commercialization partners:

While a specific commercialization partner has not yet been identified for this technology, a patent has recently been submitted involving the use of enterotoxin A₂/B chimeras as mucosal mastitis vaccines (U.S. Patent Application No. 13/328,686). This patent, along the proposed studies and additional vaccination studies (planned proposal to the USDA, August 2012) will promote commercialization. In addition, Dr. Tinker is working closely with the Boise State Office of University and Industry Ventures to identify industry partners. This work has included the incorporation of this technology into Boise State MBA coursework (MBA585, spring 2012).

12. Specific project plan and detailed use of funds:

To test our hypothesis, we propose the following specific aims.

SPECIFIC AIM 1: Characterize the immune response to IsdA and the expression of *isdA* in healthy cows and cows with clinical and subclinical *S. aureus* mastitis.

Within the scope of this aim, we will isolate serum and milk from cows of known clinical status and analyze IsdA responses by ELISA. We will also use quantitative RT-PCR on milk samples from infected animals to detect *isdA* gene expression. The strain of *S. aureus* will be identified, and *isdA* will be sequenced. Lastly, we will perform opsonization studies using *E. coli* expressing IsdA and serum or milk from infected cows, to determine the functional capacity of the anti-IsdA responses.

SPECIFIC AIM 2: Assay the immune response to ClfA, IsdH and FnBPA, and gene expression, in healthy cows and cows with clinical and subclinical *S. aureus* mastitis.

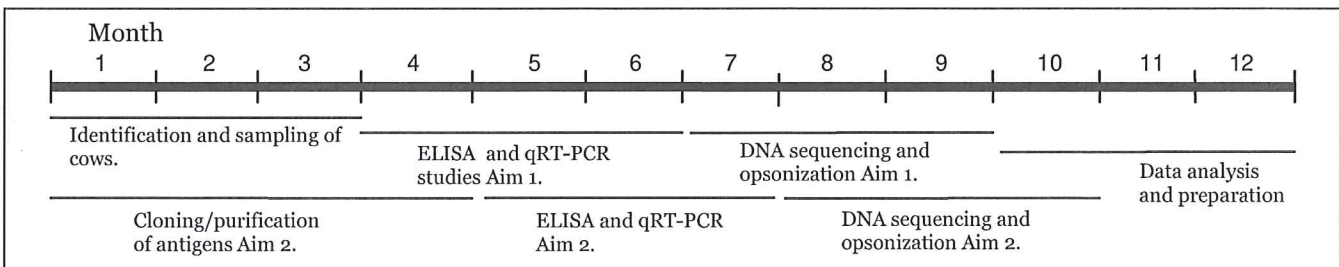
As described above, we will use serum and milk from healthy and infected cows, and identify antigen-specific responses by ELISA assay. Quantitative RT-PCR will also be performed on milk samples from infected animals to determine *clfA*, *fnbpA* and *isdH* expression. If results are significant, sequencing and opsonization assays will be performed.

Scope of work (University of Idaho): Cows will be sampled in two commercial dairy herds in Idaho. The Co-P.I. has conducted many field trials in the state and will find herds willing to participate. On the initial visit, milk from candidate cows (40 per herd) will be obtained according to procedures of the National Mastitis Council and cultured to determine growth of *Staphylococcus aureus*. Growth will be determined after 24 h of incubation. From the candidates, 3 groups (healthy, clinically infected, and subclinically infected) of 6 cows will be sampled. Cows will be deemed healthy if somatic cell count (SCC) is <200,000 cells/mL and no growth on culture plates occurs. Clinically infected cows will have SCC >500,000 cells/mL and growth on culture plates is

detected. Subclinically infected cows will have SCC >200,000 and <500,000 cells/mL with growth detected. The sampling for the selected cows includes milk from each quarter, and a blood sample. Thus, 18 cows per herd and 36 cows overall will be sampled. Milk will be analyzed for SCC (DSCC, Delaval, Sweden) and composition (Dairy Herd Improvement Association Lab). Cultures of milk will occur as for screening to confirm growth. A culture independent analysis for the presence of *S. aureus* will also be conducted using PCR amplification of the 16S rRNA gene will comparison to a standard curve of cultured *S. aureus* in order to quantify the number of bacteria present. Blood and milk samples will be shipped to Boise State for further analysis.

Scope of work (Boise State): Blood and milk will be analyzed by ELISA and quantitative RT-PCR. We have previously cloned and purified IsdA for use in ELISA assays. The antigens ClfA, IsdH and FnBPA will be cloned and purified using similar techniques. ELISA assays will follow standardized methods in the laboratory of the P.I. using purified proteins as capture antigens. Quantitive RT-PCR from milk samples will be performed using gene specific primers to amplify *isdA*, *clfA*, *isdH* and *fnbpa* from milk and a commercially available RT-PCR kit (Qiagen, Valencia, CA). A commercial vendor will be used for DNA sequencing (SeqWright, Houston, TX). Opsonization assays to determine functional antibody responses will use *E. coli* expressing antigens; IsdA, ClfA, FnbpA and IsdH, and will follow previously described methods [19].

Timeline and milestones:



Budget and justification (The University of Idaho):

Salaries and fringe benefits: We request \$3,652 of salary and \$1,205 in fringe for the Co-PI representing 4% of his time. Additionally, we request \$5,200 for an undergraduate student (520 h @ \$10/h) and \$208 in fringe. The undergraduate student will assist in sample collection, bacterial culture, DNA extraction, and PCR for quantification of *Staphylococcus aureus*.

Total salaries and fringe benefits \$10,265

Supplies: Costs are for reagents, media, culture plates and tubes for DNA extraction and PCR for quantification of *S. aureus* in milk (\$25 ea for 144 samples = \$3,600; screening 320 samples @ \$5 = 1,600), milk compositional analysis (\$10 ea for 144 samples = \$1,440), and sampling supplies (\$10 ea for 36 cows = \$360).

Total supplies \$7,360

Other Expenses:

We request support for the participating dairies (\$750 ea = \$1,500).

Total other expenses \$ 1,500

Travel:

We request support for travel to sample cows at the participating dairies. Costs include mileage (1,000 miles @ \$0.455/mile = \$455), per diem (4 days @ \$30/day = \$120) and 3 nights at a hotel (\$100/night = \$300). Sampling is proposed to take 0.5 day drive, 0.5 day screening, 1 day culture, 1.5 day sampling, and 0.5 day drive.

Total travel \$ 875

Total direct costs **\$20,000**

Budget and justification (Boise State):

Salaries and fringe benefits: We request \$6002 of salary and \$2100 in fringe for the P.I. representing 8% of her time. Additionally, we request \$4000 + \$400 fringe for a graduate student during the summer (400 h @ \$10/h), and \$4000.00 + \$160 fringe for an undergraduate during the academic year (400 h @ \$10/h). The graduate and undergraduate students will assist in protein purification, ELISA, qRT-PCR and opsonization assays.

Total salaries and fringe benefits \$16,663

Supplies: Costs are for reagents, media, culture plates, ELISA plates, secondary antibodies, qRT-PCR kits and tissue culture supplies. Small equipment will also include pipetmen.

Total supplies \$13,337

Total direct costs **\$30,000**

Total amount requested: \$50,000

13. Education and outreach:

These studies will move forward with significant involvement of top quality undergraduate and graduate researchers from both the University of Idaho and Boise State. A position for a Master's level graduate student will be advertised and the candidate recruited into the P.I.'s laboratory on a 3-year teaching assistantship.

Proposed funds will be used to support the student during the first year summer. This

student will be directly involved in the assay of bovine samples, including, the cloning and production of antigens, ELISA, opsonization assays and DNA sequencing. The graduate student will oversee and train a Biology undergraduate in the P.I.'s laboratory during the academic year. A top undergraduate in Animal and Veterinary Science will also be recruited into the Co-P.I.s laboratory to aid in the collection and assay of bovine samples at the University of Idaho. These students will have opportunities to present their work at local and regional conferences that include the INBRE, AAAS and ASM Intermountain conferences. In addition, Boise State students will participate in community outreach service-learning for vaccine education developed by the P.I. for coursework in Vaccinology (Biol 444/544) and Pathogenic Bacteriology (Biol 410/510).

14. Institutional and other sector support:

The P.I. and Co-P.I. have significant laboratory space and equipment (described below) and established research programs that are externally funded by the USDA, DoD, and the Gates Foundation. In addition, the Department of Biological Sciences at Boise State has fully supported the research program of the P.I. This has included a generous start-up package to supply needed equipment and materials for the lab, reduced teaching loads to allow faculty to pursue research, travel support for faculty and graduate students, and 3-year teaching assistantships to support Master's level graduate students. Lastly Boise State has recently initiated a new Ph.D. program in Biomolecular Sciences. This program will provide support for Ph.D. candidates on teaching assistantships with reasonable teaching loads, and will promote research in P.I.'s laboratory as well as the eligibility of the P.I. for larger federal research grants.


Tinker, HERC Incubation Fund

SUMMARY PROPOSAL BUDGET						
Name of Institution: Boise State University						
Name of Project Director: Juliette Tinker						
A. FACULTY AND STAFF						
Name/ Title	Rate of Pay	CAL	No. of Months		Dollar Amount Requested	
			ACA	SUM		
Juliette Tinker, Ph.D.	\$6002/mo			1	\$6000.00	
% OF TOTAL BUDGET:	12%	SUBTOTAL:			\$6000.00	
B. VISITING PROFESSORS						
Name/ Title	Rate of Pay	CAL	No. of Months		Dollar Amount Requested	
			ACA	SUM		
% OF TOTAL BUDGET:		SUBTOTAL:				
C. POST DOCTORAL ASSOCIATES / OTHER PROFESSIONALS						
Name/ Title	Rate of Pay	CAL	No. of Months		Dollar Amount Requested	
			ACA	SUM		
% OF TOTAL BUDGET:		SUBTOTAL:				
D. GRADUATE / UNDERGRADUATE STUDENTS						
Name/ Title	Rate of Pay	CAL	No. of Months		Dollar Amount Requested	
			ACA	SUM		
Undergraduate Assistant (TBA) Boise State (approx. 10 hrs/wk during academic year)	\$10/hr				\$4000.00	
Graduate Assistant (TBA) Boise State (full time, summer)	\$10/hr				\$4000.00	
% OF TOTAL BUDGET:	16%	SUBTOTAL:			\$8,000.00	

Tinker, HERC Incubation Fund

E. FRINGE BENEFITS						
Rate of Pay (%)		Salary Base			Dollar Amount Requested	
Juliette Tinker, PI (35% ave. fringe)		\$6002/mo			\$2100.00	
Graduate Summer BSU (10% ave. fringe)		\$4000/3 mo			\$400.00	
Undergraduate BSU (4% ave. fringe)		\$4000/12 mo			\$200.00	
SUBTOTAL:					\$2700.00	
F. EQUIPMENT: (List each item with a cost in excess of \$1000.00.)						
Item/Description						Dollar Amount Requested
SUBTOTAL:						
G. TRAVEL:						
Dates of Travel (from/to)	No. of Persons	Total Days	Transportation	Lodging	Per Diem	Dollar Amount Requested
SUBTOTAL:						
H. Participant Support Costs:						Dollar Amount Requested
1. Stipends						
2. Travel (other than listed in section G)						
3. Subsistence						
4. Other						
SUBTOTAL:						

Tinker, HERC Incubation Fund

I. Other Direct Costs:	Dollar Amount Requested
1. Materials and Supplies (animal housing, supplies for sampling/shipping, laboratory supplies, small equipment)	\$12,800.00
2. Publication Costs/Page Charges	\$500.00
3. Consultant Services (Include Travel Expenses)	
4. Computer Services	
5. Subcontracts (University of Idaho)	\$20,000.00
6. Other (specify nature & breakdown if over \$1000)	
SUBTOTAL:	\$33,300.00
J. Total Costs: (Add subtotals, sections A through I)	\$50,000.00
K. Amount Requested:	\$50,000.00
Project Director's Signature: 	Date: <i>5/10/12</i>

Appendices:

1. Facilities and Equipment

Tinker Lab: The P.I.'s laboratory consists of 700 sq. ft. of space on the second floor of the Science/Nursing Building Department of Biology, Boise State University. This laboratory contains the necessary equipment to perform the proposed assays, including two bacterial incubators, a tissue culture hood and tissue culture incubator, fume hood, laboratory benches with gas and air access, deionized water filter, two -20C freezers, a -80C freezer, a chromatography refrigerator, a refrigerated centrifuge and an inverted microscope. An additional 3000 sq. ft. of lab space has shared equipment including a walk-in warmroom, walk-in coldroom, autoclave facilities, and a core area which houses shared high-speed centrifuges, spectrophotometers, scintillation counters, ELISA plate readers, gel documentation systems, additional -80°C freezers and liquid nitrogen storage. In addition a microscopy core has been established in the department housing a new Zeiss Axiovert confocal microscope, and a flow-cytometry core has also recently been established within the Department.

Lactation (McGuire) Lab: The Co-PI's laboratory is part of the Agricultural Biotechnology building that opened in 2001. It is an open lab setting in which the PI has approximately 150 linear feet of bench space on a floor with 4 other research programs. Dr. McGuire's space includes 2 fume hoods, chemical media and glassware storage cabinets, compressed air, natural gas and vacuum lines and ultrapure water. Access to shared resources such as ultra- and high-speed centrifuges, autoclaves, ultrapure water, and cell culture facilities is present. The Agricultural Biotechnology building provides access to a substantial amount of shared equipment including high and ultracentrifuges, bacterial culture facilities, quantitative real-time PCR, various microscopes, imaging instruments and autoclaves. Dr. McGuire has an Agilent 6890 gas chromatograph and an Agilent 6890 GC with 5973 mass spectrometer for lipid analysis and assessment of stably labeled compounds. An Axon microarray scanner and an Applied Biosystems 7500 Fast real-time instrument are also present. An incubator with variable speed shaking is present for bacterial growth cultures.

2. Biographical sketches and individual support: (see attached)

References:

1. Arlian BM, Tinker JK (2011) Mucosal immunization with a *Staphylococcus aureus* IsdA-cholera toxin A2/B chimera induces antigen specific Th2-type responses in mice. *Clin Vaccine Immunol*.
2. (1996) *Current Concepts in Bovine Mastitis*. National Mastitis Council, Madison WI 4th ed.
3. Middleton JR, Ma J, Rinehart CL, Taylor VN, Luby CD, et al. (2006) Efficacy of different Lysigin formulations in the prevention of *Staphylococcus aureus* intramammary infection in dairy heifers. *J Dairy Res* 73: 10-19.
4. Clarke SR, Brummell KJ, Horsburgh MJ, McDowell PW, Mohamad SA, et al. (2006) Identification of in vivo-expressed antigens of *Staphylococcus aureus* and their use in vaccinations for protection against nasal carriage. *J Infect Dis* 193: 1098-1108.
5. Stapleton M, Wright L, Clarke S, Moseby H, Tarkowski A, et al. (2012) Identification of Conserved Antigens from Staphylococcal and Streptococcal Pathogens. *J Med Microbiol*.
6. Stranger-Jones YK, Bae T, Schneewind O (2006) Vaccine assembly from surface proteins of *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 103: 16942-16947.
7. Verkaik NJ, Boelens HA, de Vogel CP, Tavakol M, Bode LG, et al. (2010) Heterogeneity of the humoral immune response following *Staphylococcus aureus* bacteremia. *Eur J Clin Microbiol Infect Dis* 29: 509-518.
8. Kim HK, DeDent A, Cheng AG, McAdow M, Bagnoli F, et al. (2010) IsdA and IsdB antibodies protect mice against *Staphylococcus aureus* abscess formation and lethal challenge. *Vaccine* 28: 6382-6392.
9. Herron-Olson L, Fitzgerald JR, Musser JM, Kapur V (2007) Molecular Correlates of Host Specialization in *Staphylococcus aureus*. *PLoS ONE* 2: e1120.
10. Ster C, Beaudoin F, Diarra MS, Jacques M, Malouin F, et al. (2010) Evaluation of some *Staphylococcus aureus* iron-regulated proteins as vaccine targets. *Vet Immunol Immunopathol* 136: 311-318.
11. Ikawaty R, Brouwer, E.C., Van Duijkeren, E., Mevius, D., Verhoef, J., and A.C. Fluit. (2010) Virulence factors of genotyped bovine mastitis *Staphylococcus aureus* isolated in the Netherlands. *International Journal of Dairy Science* 5: 60-70.
12. Sakwinska O, Giddey M, Moreillon M, Morisset D, Waldvogel A, et al. (2011) *Staphylococcus aureus* host range and human-bovine host shift. *Appl Environ Microbiol* 77: 5908-5915.
13. Juhasz-Kaszanyitzky E, Janosi S, Somogyi P, Dan A, van der Graaf-van Bloois L, et al. (2007) MRSA transmission between cows and humans. *Emerg Infect Dis* 13: 630-632.
14. Church DHaJ (2009) *The Economic and Fiscal Impacts and the Idaho Dairy Farming and Dairy Product Manufacturing Industries 2008*. Boise State University College of Business and Economics.
15. Research GBI (2012) *Veterinary Vaccines Market to 2017- Europe to strengthen its position as the single largest market for animal vaccines due to highly organized livestock farms*. Industry reports Report code GBIHC166MR.

16. Cox E, Verdonck F, Vanrompay D, Goddeeris B (2006) Adjuvants modulating mucosal immune responses or directing systemic responses towards the mucosa. *Vet Res* 37: 511-539.
17. Holmgren J, Czerkinsky C, Eriksson K, Mharandi A (2003) Mucosal immunisation and adjuvants: a brief overview of recent advances and challenges. *Vaccine* 21 Suppl 2: S89-95.
18. Pizza M, M.M. Giuliani, M.R. Fontana, E. Monaci, G. Douce, G. Dougan, K.H.G. Mills, R. Rappuoli and G. Del Giudice. (2001) Mucosal vaccines: non toxic derivatives of LT and CT as mucosal adjuvants. *Vaccine* 19: 2534-2541.
19. Glowalla E, Tosetti B, Kronke M, Krut O (2009) Proteomics-based identification of anchorless cell wall proteins as vaccine candidates against *Staphylococcus aureus*. *Infect Immun* 77: 2719-2729.

CURRICULUM VITAE

Juliette Kay Tinker

Date of Preparation: 5/1/12

I. Personal Data

Home address:

2662 E. Brierfield Dr.
Eagle, ID 83616
(208) 319-1097

Professional address:

Department of Biological Sciences
Boise State University
1910 University Dr.
Boise, ID 83725
(208) 426-5472
juliettetinker@boisestate.edu

II. Education

B.A. in Biology and English, May 1994

Washington University, St. Louis, MO.

Ph.D. in Microbiology, December 2000

The University of Iowa, Iowa City, IA.

Thesis: Regulation of Type I Fimbrial Production In *Salmonella typhimurium*: The Characterization and Genetic Analysis of *fimY*, *fimU* and *fimW*.

Dr. Steven Clegg, Thesis Advisor

Post-doctoral fellowship, September 2000 –September 2004

Department of Microbiology, The University of Colorado Health Sciences Center, Denver, CO.

Dr. Randall Holmes, Principal Investigator

III. Current Position

Assistant Professor, Department of Biological Sciences, Boise State University, Boise, ID. January 2005- present.

IV. Areas of Research Interest

Vaccine development and pathogenic bacteriology

Current Research

- 1) The development of non-toxic *Vibrio cholerae* and *Escherichia coli* enterotoxins as adjuvants for mucosal vaccines.
- 2) Characterization of bacterial enterotoxin intracellular host trafficking.
- 3) Identification and characterization of novel bacterial enterotoxins from Gram negative pathogens.
- 4) Characterization of the antibacterial activity of zinc oxide nanoparticles.

V. Professional Affiliations

1997-present	American Society for Microbiology
2005-2007	Sigma Xi Scientific Research Society
2006-present	Mountain States Tumor and Medical Research Institute
2007-present	American Association for the Advancement of Science
2008-present	Idaho Academy of Sciences
2009-present	Phi Kappa Phi Honor Society
2011-present	International Society for Vaccines

VI. Teaching Activities

Washington University Teaching Assistant

1994 Developmental Biology, laboratory.

The University of Iowa Teaching Assistant/Tutor

1995 Molecular Biology for High School Students, lecture/laboratory.

1996 Health Sciences Microbiology for Dental and Pharmacy Students, laboratory.

1997 Pathogenic Bacteriology, laboratory.

1998 Principles of Infectious Diseases for Medical Students, laboratory.

The University of Colorado Post-doctoral Fellow

2001 Topics in Microbial Pathogenesis, 3 lectures.

2002 Medical Microbiology, 3 laboratory sessions.

Boise State University Assistant Professor

2005 Pathogenic Bacteriology lecture/laboratory (4 credits).
Introductory Microbiology lecture/laboratory (4 credits).

2006 Introductory Microbiology lecture/laboratory (4 credits).
Infection and Immunity seminar (2 credits).

2007 Pathogenic Bacteriology lecture/laboratory (4 credits).
Ecology of Infectious Disease seminar (2 credits).
Research in the Biological Sciences (1 credit)

2008 Vaccines and Vaccine Development lecture (3 credits).
Introductory Microbiology lecture/laboratory (4 credits).
Advanced Topics in Molecular Techniques seminar (2 credits).

2009 Pathogenic Bacteriology lecture/laboratory (4 credits)
Vaccines and Vaccine Development lecture (3 credits)

2010 Introductory Microbiology lecture/laboratory (4 credits)
Advanced Immunology Laboratory (2 credits)
Cancer Vaccines seminar (1 credit)

2011 Pathogenic Bacteriology lecture/laboratory (4 credits)
Microbial Toxins seminar (1 credit)
Vaccinology lecture (3 credits; service-learning course)

Other

2002-2005 Instructor for Westernaires, a non-profit horse riding organization for children, Golden, CO.

VII. Supervising Students in Research

The University of Iowa

1998 Co-teach summer Molecular Biology course to High School students

1998-2000 Train undergraduates enrolled in summer research projects.

The University of Colorado Health Sciences Center

2000-2004 Train undergraduates and graduate students in laboratory techniques.

2003 Train and mentor medical student with summer research fellowship from The University of Buffalo, NY.

Boise State University

2005 Graduate students, major advisor: Chadwick Davis.

Undergraduate students: Tabitha Sturgis, Liz Villaneuva, Felicia Martinez, Alonzo Rivas, Blake McDonald, Juliann Lucero, Kimberly Stevenson.

2006 Graduate students, major advisor: Chadwick Davis,
Graduate students, committee member: Alma Hodric, Holly Schultz, Brian Dufty.

Undergraduate students: Tabitha Sturgis, Liz Villaneuva, Alonzo Rivas, Jason Bell, Felica Martinez, Blake McDonald, Sara Murray, Jason Bell.

2007 Graduate students, major advisor: Chadwick Davis.

Graduate students, committee member: Alma Hodric, Brian Dufty, Ashley Masterson, Veera Vaas, Cory Hanley.

Undergraduate students: Liz Villaneuva, Brady Callahan, Rachel Nielsen, Britni Arlian, Sara Wilson, Rachael Shin, Justin Peer.

2008 Graduate students, major advisor: Chadwick Davis.

Graduate students, committee member: Ashley Masterson, Cory Hanley, Polina Kokoulina.

Undergraduate students: Britni Arlian, Sara Wilson, Brady Callahan, Christina Hayes, Caitlin Otto, Rachael Nielsen, Herbie Pollard.

2009 Post-doctoral fellow: Jenny Yan

Research technician: Britni Arlian

Graduate students, major advisor: Lavanya Vempati

Graduate students, committee member: Ashley Masterson, Polina Kokoulina, Cory Hanley, Emily Schmidt.

Undergraduate students: Britni Arlian, Caitlin Otto, Mary Zettick, Sheenah Bryant, Herbie Pollard, Jayashree Sanjeverman, Brady Callahan, Brad Morris, Ben Jaderholm.

2010 Post-doctoral fellow: Jenny Yan

Research technician: Britni Arlian

Graduate students, major advisor: Lavanya Vempati

Graduate students, committee member: Polina Kokoulina, Emily Schmidt, Ashley McCartney.

- Undergraduate students: Herbie Pollard, Brad Morris, Casey Denton, Benjamin Tverdy, Sheenah Bryant, Marita King, Kimberly Empey, Chris Barbey, Nathan Leavitt.
High school students: Kelly Rekeire
- 2011 Post-doctoral fellow: Jenny Yan
Research technician: Britni Arlian
Graduate students, major advisor: Lavanya Vempati
Graduate students, committee member: Panagiota Louka, Christopher Porterfield, Ashley McCartney.
Undergraduate students: Brad Morris, Sheenah Bryant, Casey Denton, George Hafez, Kelly Rekeire, Nathan Leavitt.
- 2012 Graduate student, major advisor: Lavanya Vempati
Graduate students, committee member: Panagiota Louka, Michelle Laskowski.
Undergraduate students: Brad Morris, Sheenah Bryant, Casey Denton, Melissa Reid, Shandra Levy.

VIII. Funding and Awards

Completed

- 1997-1999 NIH Parasitism Training Grant Trainee (#5T32AI07511).
2000 American Society for Microbiology student travel award. \$500.
- 2001-2003 Post-doctoral National Research Service Award (#T32AI07537).
2006 Boise State University Faculty Research grant. P.I., \$5000.
2006 Boise State University Graduate Student Research grant (Chadwick Davis) \$500.
2006 NSF Major Research Instrumentation grant. Confocal microscope. Co-P.I., \$348,786 (#CHE-0619793).
2006 Boise State University COAS Travel grant. P.I., \$500.
2007 Boise State University Faculty Research Associates Program grant. P.I., \$5310.
- 2007-2009 Mountain States Tumor Medical Research Institute Small Project Grant. Trafficking of fluorescent bacterial enterotoxins. P.I., \$5000, (#3447).
- 2007-2010 Merck AAAS Undergraduate Science Research Program grant. Co-P.I., \$60,000.
- 2008-2010 WWAMI- ITHS small pilot projects grant. The characterization of enterotoxin chimeras as Staphylococcal mucosal vaccines. P.I., \$10,000 (#3872).
2008 NSF Major Research Instrumentation grant. Fluorescence Activated Cell Sorter. Key personnel, \$503,775 (#0821233).
- 2009-2010 Mountain States Tumor Medical Research Institute Small Project Grant. Novel AB5 type toxins from gram negative pathogens. P.I., \$7500 (#4182).

2009-2010 Idaho State Board of Education Tech Incentive Grant. Development of a molecular immunology course. Co-P.I., \$122,000 (#T11-030).

Ongoing

2009-2012 Department of Defense. Idaho delegation appropriations grant. A West Nile Virus Vaccine. Co-P.I., \$940,000 (#W81XWH-09-1-0588).

2009-2012 USDA AFRI competitive seed grant. Enterotoxin-based mucosal vaccines to prevent bovine mastitis caused by *Staphylococcus aureus*. P.I., \$150,000 (#2009-01778).

2011 Boise State University Graduate Student Research grant (Lavanya Vempati) \$500.

2011 Boise State University Center for Teaching and Learning STEM mini-grant. Vaccinology course, fall 2011. P.I., \$1000.

2011-2012 Mountain States Tumor Medical Research Institute Small Project Grant. Characterization of ArtAB from *Salmonella enterica* Typhimurium. P.I., \$7500 (#5047).

2012-2014 U.S. Fish and Wildlife Services Pilot Project. Identification of *Yersinia pestis* in fleas and burrowing owls. Co-P.I., \$50,000.

2012-2012 Boise State Osher Institute Faculty Research grant. Preclinical trials of a mucosal *S. aureus* vaccine. P.I. \$4000.

Pending

2011 NIH RO3. Cholera toxin fusions as mucosal *Yersinia pestis* vaccines. P.I. \$137,721. Submitted 7/8/11. Score 32.

2012 NIH R15. Cholera toxin A2B chimeras as mucosal *Staphylococcus aureus* vaccines. P.I. \$344,000. Submitted 2/26/12.

IX. Publications

Tinker, J. K. and S. Clegg. 2000. Characterization of FimY as a coactivator of type I fimbrial expression in *Salmonella enterica* Serovar Typhimurium. *Infect. Immun.* 68(6):3305-3313.

Tinker, J. K., L.S. Hancox and S. Clegg. 2001. FimW is a negative regulator affecting type I fimbrial expression in *Salmonella enterica* Serovar Typhimurium. *J. Bacteriol.* 183(2):435-442.

Tinker, J.K. and S. Clegg. 2001. Control of FimY translation and type I fimbrial production by the arginine tRNA encoded by *fimU* in *Salmonella enterica* serovar Typhimurium. *Mol. Micro.* 40(3):757-768.

Yeh, K-S., **J.K. Tinker** and S. Clegg. 2002. FimZ binds to the *Salmonella typhimurium fimA* promoter and may regulate its own expression with FimY. *Microbiol. Immunol.* 46 (1): 1-10.

Tinker, J.K., J.L. Erbe, W.G.J. Hol and R.K. Holmes. 2003. Cholera holotoxin assembly requires a hydrophobic domain at the A-B₅ interface: mutational analysis and development of an *in vitro* assembly system. *Infect. Immun.* 71(7):4093-4101.

Tinker, J.K., J.L. Erbe and R.K. Holmes. 2005. Characterization of fluorescent chimeras of cholera toxin and *Escherichia coli* heat-labile enterotoxins

- produced by use of the twin arginine translocation system. *Infect. Immun.* 73(6):3627-3635.
- Feris, K., Otto, C., **Tinker, J.**, Wingett, D., Punnoose, A., Thurber, A., Kongara, M., Sabetian, M., Quinn, B., Hanna, C., and D. Pink. 2009. Electrostatic Interactions Affect Nanoparticle-Mediated Toxicity to Gram-Negative Bacterium *Pseudomonas aeruginosa* PAO1. *Langmuir* 16:26(6):4429-36.
- Tinker, J.K.***, Davis, C.T and B.A. Arlian. 2010. Purification and characterization of *Yersinia enterocolitica* and *Yersinia pestis*-cholera toxin A2/B chimeras. *Protein Expression and Purification.* 74(1):16-23.
- Arlian, B.A. and **J.K. Tinker***. 2011. Mucosal Immunization with a *Staphylococcus aureus* IsdA-cholera toxin A₂/B chimera induces antigen specific Th2 type responses in mice. *Clinical and Vaccine Immunology.* 18(9):1543-1551.

In preparation

- Tinker, J.K.***, Yan, J., Knipple, R., Wingett, D., and K. Cornell. May 2012. Construction and characterization of a domain-III – cholera toxin fusion as a novel West Nile vaccine. *PLOS Neglected Tropical Diseases.*
- Tinker, J.K.***, Bryant, S., Thurber, A., Reddy, K.M., Otto, C.C., Feris, K., Pink, D., Hanna C., and A. Punnoose. June 2012. Effects of reactive oxygen species scavengers on the antibacterial action of zinc oxide nanoparticles.
- Tinker, J.K.**, Knipple R. and K. Cornell. Aug 2012. Comparison of novel methods of purification of West Nile virus domain III for vaccine development. *Protein Expression and Purification.*

(*corresponding author).

X. Patents

- Cholera toxin chimera and its use as a staph vaccine. U.S. Patent pending.
Submitted 12/16/11. Application # 13328686

XI. Selected Abstracts (out of a total of 40)

- The role of FimY in the regulation of *Salmonella typhimurium* FimA expression. Tinker, J.K. Hancox, L., and S. Clegg. American Society for Microbiology General Meeting, Atlanta, GA., May 1998.
- Construction and characterization of *fimY* and *fimW* mutants affecting type I fimbrial expression in *Salmonella typhimurium*. Tinker, J.K. and S. Clegg. Gordon Research Conference on Molecular Mechanisms of Microbial Adhesion, Newport, RI., June, 1999.
- The characterization of FimW as a negative regulator of type I fimbrial expression in *Salmonella enterica* serovar Typhimurium. Tinker, J.K. and S. Clegg. American Society for Microbiology General Meeting, Los Angeles, CA., May, 2000.

- Analysis of mutations within the hydrophobic A-B interface of cholera toxin that effect assembly *in vivo* and *in vitro*. Tinker, J.K., Erbe, J.L., and R.K. Holmes. American Society for Microbiology General Meeting, Salt Lake City, UT., May, 2002.
- Construction of LcrV-enterotoxin fusions for use as potential mucosal *Yersinia* Vaccines. Davis, C.T., Tinker J.K. American Society for Microbiology General Meeting. Toronto, Canada. May, 2007.
- Construction of an LcrV-cholera toxin fusion for use as a potential *Yersinia* vaccine. Davis, C.T., Tinker J.K. AAAS Pacific Division Annual Conference, Boise, ID., June 2007.
- Construction of enterotoxin fusions for use as potential vaccines against methicillin-resistant *Staphylococcus aureus*. Arlian, B., Tinker, J.K. 6th Annual Idaho INBRE Research Conference, Moscow, ID, August 2007.
- Construction of a potential mucosal West Nile vaccine. Callahan, B. Cornell, K. and Tinker, J.K. 7 th Annual Idaho INBRE Research Conference, Boise, ID. August 2008.
- Methods for the Purification of Shiga Toxin Based Vaccines. Pollard, H., Arlian, B. and Tinker J.K. Boise State University Undergraduate Research Conference. Boise, ID. April 2009.
- Construction Of Enterotoxin Chimeras For Use As Potential Mucosal Staphylococcal Vaccines. Arlian, B.A., Callahan, B.C. and J.K. Tinker. American Society for Microbiology ICAAC Annual Meeting. San Francisco, CA. September 2009.
- Cholera toxin A2/B chimeras as potential West Nile vaccines. Yan, J.A. and J.K.Tinker. American Society for Microbiology Intermountain Branch annual meeting. Provo, UT. April 2010.
- Development of Shiga Toxin 1 Derivatives as Potential Mucosal Vaccine Adjuvants. Vempati, L. and J.K. Tinker. Boise State University Graduate Student Research Symposium. Boise, ID March 2011
- Purification of a Novel *Salmonella enterica* Typhimurium AB-type Enterotoxin. Morris, B.A and J.K. Tinker. Idaho Academy of Sciences Annual S yposium. Caldwell, ID March 2011 (3rd place award)
- Immunogenicity of a *Staphylococcus aureus* IsdA-Cholera Toxin A2/B Chimera after Intranasal Vaccination of Mice. Arlian, B.A. and J.K. Tinker. American Society for Microbiology Annual Intermountain Branch Meeting. Ogden, UT April 2011 (student award)
- Characterization of a novel *Salmonella enterica* Typhimurium AB-type Enterotoxin. Morris, B.A. and J.K. Tinker. ASM Intermountain Branch Meeting. April 7, 2012.

XII. Oral presentations and symposia

- The development of bacterial enterotoxin chimeras for use as potential vaccines. Tinker, J.K. Idaho INBRE Annual Research Conference. Nampa, ID. August 12, 2005.
- Development of cholera toxin and *E.coli* heat-labile toxin chimeras as potential mucosal vaccines. Davis, C., and Tinker, J.K. Western INBRE States Infectious Disease Symposium. Moscow, ID. April 23, 2006.
- The development of bacterial enterotoxin chimeras as potential vaccines. Tinker, J.K.. AAAS Pacific Division Annual Conference. Boise, ID, June 20, 2007 (Infectious disease symposium co-organizer).
- Bacterial enterotoxins as vaccines. Tinker, J.K. Albertson College, Department of Biology, invited by Dr. Ann Koga. May 9, 2007.
- Vaccines: current recommendations and future directions. Tinker, J.K. Boise State University Osher Institute symposium. January 21, 2008.
- Vaccines: current recommendations and the autism debate. Tinker, J.K. St. Luke's Hospital Infection Control Week. October 21, 2008.
- The use of bacterial enterotoxins as potential mucosal vaccines. Tinker, J.K. Ft. Dodge Animal Health, Ft. Dodge, IA. November 24, 2008.
- Vaccine safety: busting some myths. Tinker, J.K. St. Luke's Hospital Infection Control Week. October 21, 2009.
- Construction and characterization of an IsdA-cholera toxin chimera as Ba potential Staphylococcal mucosal vaccine. Arlian, B.A and Tinker, J.K. American Society for Microbiology Intermountain branch annual meeting. Provo, UT. April 11, 2010.
- Cholera toxin A₂/B chimeras as potential Staphylococcal vaccines. Tinker, J.K. AAAS Pacific Branch annual meeting. Ashland, OR. June 12, 2010.
- Cholera toxin A₂/B chimeras as mucosal Staphylococcal vaccines. Tinker, J.K. University of Washington ITHS Pilot Awards Symposium. Seattle, WA. Dec 17, 2010.
- Cholera toxin A₂/B chimeras as potential mucosal vaccines. Tinker, J.K. American Society for Microbiology Annual Intermountain Branch Meeting. Ogden, UT. April 9, 2011
- Construction and characterization of cholera toxin A₂/B chimeras as potential mucosal Staphylococcal vaccines. Tinker, J.K. Vaccine and ISV Annual Global Congress. Seattle, WA. October 2-4, 2011.
- Antibacterial activity of zinc oxide nanoparticles. Bryant, S., and J.K. Tinker. Idaho Academy of Sciences, Idaho Falls, ID. March 23, 2012.

XIII. University, professional and community service

University

- | | |
|--------------|--|
| 2005-present | Member, Biology Department Research Committee |
| 2007-present | Judge, College of Arts and Sciences Wallace G. Kay writing competition |
| 2008-2010 | Member, University Academic Grievance Committee |
| 2008-2011 | Member, University Foundation Scholars, Research and |

	Creative Committee
2010	Member, Faculty Connections Work-Life Group
2008-2010	Member, Institutional Biosafety Committee
2010-present	Chair, Institutional Biosafety Committee
2011-present	Member, Ph.D. Biomolecular Science Steering Committee
<u>Professional</u>	
2006	Journal reviewer, Idaho Academy of Sciences
2007	Chapter reviewer, Microbiology (Cowen, Talaro), McGraw Hill
2008	Symposium co-chair, AAAS Pacific Division, Boise, ID
2009-2010	Academic Advisory Board Member, Annual Editions Microbiology, McGraw Hill
2009-present	Journal reviewer, Journal of Biotech Research
2011-	ASM Intermountain Branch board member
2011	Grant Reviewer, M.J. Murdock Charitable Trust pilot grant
2011-present	Journal reviewer, Clinical and Vaccine Immunology
2012-present	Journal reviewer, Journal of Nanoparticle Research
<u>Community</u>	
2007	Boise State University Osher Institute lecture; "Vaccine Safety"
2008-present	Lecturer on vaccine safety for St. Luke's Hospital annual Infection Prevention Week
2009-present	Idaho Immunization Coalition member
2009	Vaccines and Vaccine Development service-learning course with the Idaho Immunization Program
2010-present	Central District Health Immunization Advisory Board member
2011-present	Vaccinology service-learning course with the Central District Immunization Board and the Idaho Immunization Program

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Juliette Kay Tinker		POSITION TITLE Assistant Professor	
eRA COMMONS USER NAME JTINKER			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Washington University, St. Louis, MO	B.A.	1994	Biology/English
University of Iowa, Iowa City, IA	Ph.D.	2000	Microbiology
University of Colorado, Denver, CO	Postdoctoral	2000-04	Bacterial enterotoxins

A. Personal Statement.

As a Ph.D. candidate at the University of Iowa, I characterized the regulatory mechanisms of *Salmonella* Type 1 fimbrial expression. As a post-doctoral fellow at the University of Colorado I studied the structure/function relationship of cholera toxin (CT) and began studies on the trafficking of fluorescent enterotoxin A₂/B chimeras. Since joining the Biological Sciences faculty at Boise State University, I have focused on the development of enterotoxins as mucosal adjuvants. I have received grants for, and published research on, the construction and characterization of cholera toxin fusions as mucosal vaccines.

B. Positions and Honors.**Positions**

1988-1990 Summer Research Assistant, University of Iowa, Iowa City, IA
 1993-1994 Undergraduate technician, Washington University, St. Louis, MO
 2000-2004 Post-Doctoral Fellow, University of Colorado Health Sciences Center, Denver, CO
 2005 – present Assistant Professor, Department of Biology, Boise State University, Boise, ID

Memberships

1997 - present Member, American Society of Microbiology
 2007-present Member, Mountain States Tumor Medical Research Institute
 2008-present Member, Idaho Academy of Sciences
 2009-present Member, Central District Health Immunization Advisory Board
 2010-present Chair, Institutional Biosafety Committee

Patents

U.S. pending No. 13/328,686 “Cholera toxin chimera and its use as a staph vaccine”

C. Peer Reviewed Publications.

1. **Tinker, J. K.** and S. Clegg. 2000. Characterization of FimY as a coactivator of type I fimbrial expression in *Salmonella enterica* Serovar Typhimurium. *Infect.Immun.* 68(6):3305-3313.
2. **Tinker, J. K.**, L.S. Hancox and S. Clegg. 2001. FimW is a negative regulator affecting type I fimbrial expression in *Salmonella enterica* Serovar Typhimurium. *J. Bacteriol.* 183(2):435-442.

3. **Tinker, J.K.** and S. Clegg. 2001. Control of FimY translation and type I fimbrial production by the arginine tRNA encoded by *fimU* in *Salmonella enterica* serovar Typhimurium. *Mol. Micro.* 40(3):757-768
4. Yeh, K-S., **J.K. Tinker** and S. Clegg. 2002. FimZ binds to the *Salmonella typhimurium fimA* promoter and may regulate its own expression with FimY. *Microbiol. Immunol.* 46 (1): 1-10.
5. **Tinker, J.K.**, J.L. Erbe, W.G.J. Hol and R.K. Holmes. 2003. Cholera holotoxin assembly requires a hydrophobic domain at the A-B₅ interface: mutational analysis and development of an *in vitro* assembly system. *Infect. Immun.* 71(7):4093-4101.
6. **Tinker, J.K.**, J.L. Erbe and R.K. Holmes. 2005. Characterization of fluorescent chimeras cholera toxin and *Escherichia coli* heat-labile enterotoxins produced by use of the twin arginine translocation system. *Infect. Immun.* 73(6):3627-3635.
7. Feris, K., Otto, C., **Tinker, J.**, Wingett, D., Punnoose, A., Thurber, A., Kongara, M., Sabetian, M., Quinn, B., Hanna, C., and D. Pink. 2009. Electrostatic Interactions Affect Nanoparticle-Mediated Toxicity to Gram-Negative Bacterium *Pseudomonas aeruginosa* PAO1. *Langmuir* 26(6) 4429-36.
8. **Tinker, J.K.**, Davis, C.T., and B.M. Arlian. 2010. Purification and characterization of *Yersinia enterocolitica* and *Yersinia pestis* LcrV-cholera toxin A₂/B chimeras. *Protein Expr Purif.* 74(1):16-23.
9. Arlian, B.M. and **J.K. Tinker**. 2011. Mucosal immunization with a *Staphylococcus aureus* IsdA-cholera toxin A₂/B chimera induces antigen specific Th2-type responses in mice. *Clin Vaccine Immunol* 18(9): 1543-1551.

In preparation:

Tinker, J.K., Yan, J., Knipple, R., Wingett, D., and K. Cornell. Construction and characterization of a domain-III – cholera toxin fusion as a novel West Nile vaccine. *PLoS Neglected Tropical Diseases* (May 2012).

Tinker, J.K., Bryant, S., Thurber, A., Reddy, K.M., Otto, C.C., Feris, K., Pink, D., Hanna C., and A. Punnoose. Effects of reactive oxygen species scavengers on the antibacterial action of zinc oxide nanoparticles (June 2012)

D. Research Support.

Ongoing

Department of Defense Idaho Delegations Grant	(#W81XWH091058)	9/09-8/12
Construction and characterization of mucosal West Nile vaccines based on the use of <i>Listeria</i> live vectors and cholera toxin non-toxic fusions.		
Role: Co-P.I.		
USDA AFRI Competitive Seed Grant.	(#2009-01778)	9/09-8/12
Construction and characterization of enterotoxin-based mucosal vaccines to prevent bovine mastitis caused by <i>Staphylococcus aureus</i> .		
Role: P.I.		
Mountain States Tumor Institute Small Program Grant	(#5047)	7/11-9/13
Characterization of ArtAB from <i>Salmonella enterica</i> Typhimurium.		
Role: P.I.		
U.S. Fish and Wildlife Service Pilot Project Grant		7/12-6/14
Identification of <i>Yersinia pestis</i> in fleas and burrowing owls in the Northwest.		
Role: Co-P.I.		
Boise State University Osher Faculty Research Grant		7/12-6/13
Preclinical mouse studies on a <i>Staphylococcus aureus</i> mucosal vaccine		
Role: P.I.		

Biographical Sketch

Mark A. McGuire

Professor
University of Idaho
Department of Animal & Veterinary Science
307 Ag Biotech
PO Box 442330
Moscow, ID 83844-2330
208-885-7683
mmcguire@uidaho.edu

Education

Degree	Institution and Location	Year Conferred	Major Field of Study
BS	University of Illinois, Urbana-Champaign	1984	Dairy Science
MS	University of Florida, Gainesville	1987	Dairy Science
PhD	Cornell University, Ithaca, NY	1994	Animal Science

Professional Experience

Year	Position	Institution and Location
1993-1995	Post-doctoral Research Associate	Cornell University, Ithaca, NY
1995-2001	Assistant Professor	University of Idaho, Moscow
2001-2008	Associate Professor	University of Idaho, Moscow
2008-present	Professor	University of Idaho, Moscow

Honors

National Milk Producers Federation Richard M. Hoyt Award, American Dairy Science Association, 1995
Finalist, International Life Sciences Institute North America Future Leader Award, 1996
Agway Inc. Young Scientist Award, American Dairy Science Association, 2001
University of Idaho Alumni of Excellence Mentor for Erin Mosley, 2004; Amelia Naher, 2008
Nominated, Outstanding Teacher in the College of Agricultural and Life Sciences, 2002, 2006
Nominated, Outstanding Researcher in the College of Agricultural and Life Sciences, 2002
Richard C. Heimsch Research Award, College of Agricultural and Life Sciences, 2007
University of Idaho Award for Excellence in Research or Creative Activity for 2006-2007

Membership in Professional Organizations/Societies

American Dairy Science Association, American Oil Chemists' Society, American Society of Animal Science, American Society for Microbiology, American Society for Nutrition, International Society for Research on Human Milk and Lactation

Relevant Publications (from over 75 refereed articles)

- Hunt, K.M., J.A. Foster, L.J. Forney, U.M.E. Schütte, D.L. Beck, Z. Abdo, L.K. Fox, J.E. Williams, M.K. McGuire, and M.A. McGuire. 2011. Characterization of the diversity and temporal stability of bacterial communities in human milk. *PLoS ONE* 6: e21313. Doi:10.1371/journal.pone.0021313.
- Park, J.Y., L.K. Fox, K.S. Seo, M.A. McGuire, Y.H. Park, F.R. Rurangirwa, W.M. Sischo, and G.A. Bohach. 2011. Comparison of phenotypic and genotypic methods for speciation of coagulase-negative staphylococcal isolates from bovine intramammary infection. *Vet. Microbiol.* 147:142-148.
- Park, J.Y., L.K. Fox, K.S. Seo, M.A. McGuire, Y.H. Park, F.R. Rurangirwa, W.M. Sischo, and G.A. Bohach. 2011. Detection of classical and newly described staphylococcal superantigens in coagulase-negative staphylococci isolates from bovine intramammary infections. *Vet. Microbiol.* 147:149-154.
- Hunt, K.M., J.E. Williams, B. Shafii, M.K. Hunt, R. Behre, R. Ting, M.K. McGuire, and M.A. McGuire. 2012. Mastitis is associated with increased free fatty acids, somatic cell count, and interleukin-8 concentrations in human milk. *Breastfeeding Medicine.* doi:10.1089/bfm.2011.0141.
- Hunt, K.M., J. Preuss, C. Nissan, C.A. Davlin, J.E. Williams, B. Shafii, A.D. Richardson, M.K. McGuire, L. Bode, and M.A. McGuire. 2012. Human milk oligosaccharides promote the growth of staphylococci. *Appl. Environ. Microbiol.* (In press).

Current Support

- United States Department of Agriculture, NRICGP, "Inflammatory and immunoregulatory roles of microbial superantigens in bovine mastitis," July 1, 2008 to June 30, 2012, G.A. Bohach, K.S. Seo, M.A. McGuire, \$375,000.
- IBEST Pilot Grant Program, "Relationships among time postpartum, related factors, and human milk microbiome", 6/01/10 to 5/31/12, M.A. McGuire, \$80,000.
- IDA/IDEAL and Elanco Animal Health, "Do alterations in retinoid metabolism affect mammary gland health during the periparturient period?" 7/10 to 6/13, P. Rezamand, M.A. McGuire, \$196,803.
- Gates Foundation Grand Explorations, "Induction of gut mucosal immunity via human milk microbiome," 11/1/10 to 5/31/12, M.K. McGuire (WSU), M.A. McGuire, \$100,000.