INSTITUTIONAL BIOSAFETY COMMITTEE MEETING
July 17, 2019
3 PM, Plant Biotechnology Bldg., Room 410

MEMBERS PRESENT: IBC Chair, David White; Vice Chair, Elizabeth Fozo; Paul Dalhaimer, George Dizikes, Doris D'Souza, Reza Hajimorad, Brittany Isabell, Jun Lin, Deidra Mountain, Jae Park, Ling Zhao

Ex-Officio – Linda Hamilton, Scott Moser, Brian Ranger, Jessica Woofter

MEMBERS ABSENT: Marc Caldwell, Lori Cole, Melissa Kennedy, Reggie Millwood

OTHERS PRESENT:

Opening:

The IBC Vice Chair called the meeting to order at 3:02 PM. The minutes of June 19, 2019 were reviewed and approved pending correction of Scott Moser's attendance.

Full Member Review IBC Registrations:

#IBC-05-238-2 (Feng Chen) Infectious Agents & Recombinant DNA, III-E-2-a, 3-year rewrite
Dr. Chen's research employs functional genomics and transgenic plant development to study various plant genes involved in resistance to insect pests or plant biomass production (bioenergy studies). Methyltransferase, acyltransferase, terpene synthase and other cell wall-related genes derived from plants (rice, poplar, tobacco, soybean and Arabidopsis), fungi (e.g. *Metarhizium spp.*), and amoebae (e.g. *Dictyostelium discoidium*) will be subcloned and assayed for specific enzymatic activities. Genes of interest will be cloned and mobilized onto binary vectors for Agrobacterium-mediated transformation of Arabidopsis, tobacco and soybean. GUS and GFP will be used as reporter genes. Transformants are to be further analyzed and regenerated. The registration also included the culturing of *Klebsiella pneumoniae* (environmental isolate; non-CRE strain) to be used as food for cultured amoebae. The committee voted approve the registration pending a statement clarifying terepenes in lay terms; an update to the biosafety cabinet certification; and correction of minor typographical errors.

#IBC-06-302-1 (Daniel Roberts) Infectious Agents & Recombinant DNA, III-E-2-a, 3-year rewrite
Dr. Roberts' registration covers molecular cloning of plant membrane transporters, regulatory protein kinases and calcium binding proteins from soybean, Arabidopsis and Medicago for structure-function analysis in *E.coli, Xenopus laevis* oocytes in culture, or *Pichia pastoris*. Briefly, oocytes from *X. laevis* will be used to allow the production of soybean, Medicago or Arabidopsis transporters to ascertain structural and functional relationships between the protein sequence and transport function in vitro. Additionally, the recombinant molecules will be used to investigate gene expression, characterize Arabidopsis mutants, and generate transgenic Arabidopsis plants

(fluorescent reporter systems, e.g. GFP). For the latter, standard Agrobacterium-mediated transformation techniques will be used. Finally, Nicotiana or Arabidopsis plants will be transiently transfected (Agrobacterium) with turnip mosaic virus UK-1 conjugated to a fluorescent protein marker so that infected tissues can be visualized by fluorescence microscopy. The committee voted approve the registration pending clarification of "cDNA"; the addition of the biosafety cabinet certification date; and the correction of minor typographical errors.

#IBC-07-313-1 (Elena Shpak) Recombinant DNA Registration, III-E-2-a, 3-year rewrite
Dr. Shpak's research involves the generation of transgenic *Arabidopsis thaliana* plants to investigate the mechanisms regulating plant size and shape. Recombinant constructs are designed so that growth-related genes are either overexpressed or turned off. Briefly, Agrobacterium strains carrying recombinant binary vectors are grown overnight and used to inoculate the aboveground parts of plants (dipping). Seeds are harvested and selected for transformants using antibiotic or herbicide selectable markers. Following analysis, seeds/plants are autoclaved and disposed. The committee approved the registration pending a rewrite of the nontechnical summary; removal of Walters Life Science Room D310 from listed laboratory spaces; updates to the biological spill response; the addition of lab coats and safety glasses; and the correction of minor typographical errors.

#IBC-13-404-1 (Steven Wilhelm) Recombinant DNA Registration, III-D-2-a, 3-year rewrite
Dr. Wilhelm's registration covers his research using insertion of a gene cassette encoding the cyanobacterial toxin, microcystin (from *Microcystis aeruginosa),* to *E. coli* Nissle1917 (B-strain) as well as the cyanobacterium *Synechococcus* PCC 7942 to examine the effects of heterologous expression in cells that do not make this toxin. Experiments involving potentially infectious and toxin producing agents will be conducted in a BSC using BSL-2 precautions and procedures. The committee approved the registration pending the addition of booties to PPE listed and checking BSL-2 for the laboratory biosafety containment level.

#IBC-16-441-2 (Jeremiah Johnson) Human Derived Materials, Infectious Agents & Recombinant DNA, III-D-1; D-2; D-4, 3-year rewrite
Dr. Johnson's research investigates the factors that affect *Campylobacter jejuni* colonization in natural chicken hosts. He will use a both wild type and genetically modified strains of *C. jejuni* to identify and characterize genes/proteins involved in colonization. Briefly, genes of interest (e.g. heme-utilization genes) will be knocked out via homologous recombination with insertion/deletion constructs (based on pGEM T-Easy). Complementation constructs will be used to restore the deleted gene/function. Finally, genes of interest will be subcloned and expressed in *E. coli* hosts for recombinant protein production (to be used in various downstream assays). Recombinant strains will be used to study heme utilization and mechanisms of resistance to bacteriostatic compounds. Additionally, wild type *C. jejuni* will be used in animal models to determine the effects of persistent infection on gut health. *Listeria monocytogenes*, *Salmonella enterica* Typhimurium, *Campylobacter coli*, and *Klebsiella pneumoniae* will be used as controls in several comparative assays. The committee approved the registration pending the addition of human-derived cell lines to the host/vector table; addition of the source of the fluorescent marker genes; clarification of Shiga toxin-producing *E. coli* are foodborne pathogens in the Technical Summary; including specific language about IACUC and IRB approval in the Technical Summary; update the biosafety certification date; and reference to how lab coats were laundered.

#IBC-16-442-2 (Heidi Goodrich-Blair) Infectious Agents & Recombinant DNA Registration, III-D-4-a, 3-year rewrite

Dr. Goodrich-Blair's research investigates the naturally occurring tripartite interaction between invertebrate nematodes in the genus *Steinernema*, their mutually beneficial bacterial symbionts in the genus *Xenorhabdu*s, and the larval stage invertebrate insects these pairs of organisms infect. Using standard bacterial genetic, molecular, and biochemical techniques, bacterial genes and gene products that play a role in either the beneficial or the pathogenic host interactions are identified and characterized. Briefly, mutants and recombinant strains of *Xenorhabdus* spp. are generated by transposon or site- directed mutagenesis, and the desired genetic alteration is typically obtained using selectable markers (e.g. antibiotic resistance genes). Mutants are assayed for their ability to associate with the nematode host (mutualism), or to suppress immunity and kill an insect host. *Salmonella typhimurium* and *Enterococcus faecalis* are used for controls in various assays. The committee voted to approve the registration as written.

#IBC-19-537-2 (Raul Almeida) Infectious Agents & Recombinant DNA, III-D-a, New registration

Dr. Almeida's registration covers the study of *Mycoplasma bovis* mastitis vaccine treatments for dairy herds. Studies will be performed both *in vitro* and *in vivo*. The *in vitro* research involves monitoring growth of mutant clones in milk and resistance to the lytic action of the blood defense system. The *in vivo* research involves the study of cows infected with the wild type parent strain (*M. bovis* PG45) and two less virulent mutants. Personnel involved in this project will be using disposable PPE which include disposable paper coveralls, disposable latex gloves, disposable goggles, disposable masks and disposable footwear covers in study areas. After used, disposable PPE will be collected in a dedicated bin lined with double autoclave bags, sterilized by autoclave and disposed. Personnel will require to have a full-body shower and change of clothes prior to tending to other non-study animals or dairying areas. Cows will be milked with separated individual milking machine equipment. After each milking, the milking machine will be cleaned and disinfected following site SOP. The floor corresponding to the milking area will be treated with chlorine after milking, to eliminate *M. bovis* mutant clones in milk that could be leaked during the milking process. Milk, as well as effluents from these cows, will be collected and packaged for disposal through the UTIA bio-waste contractor. At the end of the experiment, cows inoculated with the mutant clones will be euthanized at the CVM and carcasses will be destroyed through the CVM alkaline digester. The committee voted to approve the *in vitro* procedures but have tabled the *in vivo* procedures pending a rewrite of the Nontechnical Summary; clarification of separation of individual milking equipment; clarification of carcass treatment; addition of biosafety cabinet information; addition of storage information; correction of disinfectant shelf life to 24 hours; and the addition of statement indicating Advantra will not be handling the animal carcass for disposal.

Designated Member Review IBC Registrations: None

Old Business:

Administrative Report

i. Contingencies

Following up on the June 19, 2019 IBC Meeting, Dr. Jae Park's registration (#07-315-1) was updated to include the correction of minor typographical errors; clarification of which

plasmid is used for silencing; clarification of which method is used for knock-down/knockout and the target genes in technical summary; and the inclusion of contact times in the biological spill response. The registration was approved and closed on 7/1/2019. Dr. Tarek Hewezi's registration (#13-398-1) was updated to include the identification of stressors and antibiotic markers; the inclusion of contact times in the biological spill response; and updates to autoclave validation dates. The registration was approved and closed on 6/27/2019. Dr. Steven Wilhelm's registration (#13-404-1) was updated to include updated training date for personnel listed on the protocol; clarification of the mechanism of release for microcystin from the producing algae in the Technical Summary; and an update to the biosafety cabinet certification dates. The corrections were sent back to the committee for review at the July 17, 2019 IBC Meeting. Dr. Heidi Goodrich-Blair's registration (#16-442-2) was updated to include clarification of DNA sources; the inclusion of Shiga-like toxin and details in Section 6.2, entry 1; the removal of any non-microbe entries under Section 7.1 – Infectious Agents; the clarification of experimental details regarding the Shiga-like toxin and details regarding potential propagation/expression of this protein including quantities of culture; the deletion of facility references to Walters Life Sciences Bldg.; the modification of bleach contact time to 10 minutes in Section 9.7; the addition of the updated biological spill response; and the correction of the autoclave validation date. The corrections were sent back to the committee for review at the July 17, 2019 IBC Meeting.

ii. *Administrative Approvals*
Dr. Barry Rouse's registration (#06-280-2) was administratively approved for the addition of human brain microvascular epithelial cells to his registration on 8/12/2019 by the Biosafety Officer. Dr. Trinh Cong's registration (#17-450-2) was administratively approved for the addition of *Candida albicans* strain SC5314 and *Staphylococcus aureus* strains N315, RN4220, and Newman strain by the IBC Chair on 7/26/2019.

iii. *Administrative Terminations*
None.

iv. *Administrative Exemptions:*
None.

v. *Accidents, Injuries/Exposures:*
Select Agent diagnosis (*Clostridium botulinum*) from Necropsy was reported to CDC DSAT via Form 4.

vi. *Laboratory Report (Hamilton)*
None.

vii. *iMedRIS Update, Manual Reviews, & System Orientation (Woofter)*
None

Charter Revision Update
Brian reported to the committee that the charter revisions and SOPs have been completed and added to the website.

Biowaste Disposal Procedure Changes (Pending)
Brian notified the committee that the waste disposal program for the UTK/UTIA campuses is in process. EH&S met with Advantra to discuss new procedures and consolidating bills under one account. CVM would continue operate as before with their subaccounts.

New Business: None.

The meeting was adjourned at 4:38 PM. The next meeting scheduled for August 21, 2019 from 3-5 pm in Plant Biotechnology Room 410.