Wadsworth Center Institutional Biosafety Committee
November 24, 2025
Meeting Minutes

ATTENDANCE

In attendance: Robert Glaser (Chair), David Hill (BSO), Joseph Wade, Steven Zink, Seth Blumerman, Mike Perry, Trent Gemmill, and Carlos deNoronha. Absent: Christina Egan and Kirsten St. George. A quorum of the committee was present. Michael Boucher was a guest attendee. He provided an overview of his work on *Cryptococcus* and answered questions from the committee. The meeting was held remotely via Teams.

MEETING MINUTES

The minutes were reviewed and approved by all IBC members except one member who did not attend the meeting and abstained from voting on the minutes.

NEW APPLICATIONS

1. PI: Michael Boucher
   Project Title: High-throughput dissection of fungal pathogenesis.
   Application number: 25-005R
   Applicable NIH Guideline: Section III-D-1a
   Experiments using RG-2 agents as host-vector systems.
   Objective: Develop and utilize functional genomic tools to understand the biology of the human fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*.
   Approach: Genome-wide loss-of-function mutagenesis screens (existing libraries and CRISPR-based approaches) to identify genes needed for infection in mice. Targeted loss-of-function, complementation, and tagging mutagenesis of candidate virulence genes to validate role in virulence. No gain-of-function mutations other than functionally neutral tagged-gene fusions.
   Source organisms; genes being cloned/targeted; gene function(s)
   RG-2
   Cryptococcus neoformans
   Cryptococcus gattii
   All genes in genome; phenotypic selection for genes with a role in virulence; gene functions TBD.
   Note: BMBL designates RG-2 for both species. PHAC designates *C. gattii* as RG-3 but provides directive that BSL-2 containment is permitted if there is low aerosol risk.
   RG-1 and not associated with infection of humans
   Various bacteria, fungi, plants.
   Genes that are routinely used for 1) selectable drug resistance in fungi (none used clinically or agriculturally), 2) nutrient prototrophy, 3) gene expression control. None of sequences have pathogenic properties on their own.
   RG-2, RG-1, and not associated with infection of humans
   Various sequences from bacteria and bacteriophages.
   Components of CRISPR/Cas and bacteriophage integrase systems. None of sequences have pathogenic properties on their own.
   Not associated with infection of humans
   Various nonpathogenic organisms (jellyfish, sea pansies, lancelets)
   Various reporter genes. None of sequences have pathogenic properties on their own.
   Host organisms
   RG-2
   Cryptococcus neoformans
   Cryptococcus gattii

Not associated with infection of humans

Mus musculus (IACUC review scheduled for December, 2025)

Cultured murine and human macrophages

Risk Assessment

- *C. neoformans* is an opportunistic pathogen, while *C. gattii* can be a primary pathogen of humans and animals.
- There is no known person-to-person or animal-to-animal transmission of either *C. neoformans* or *C. gattii*. They are both thought to be exclusively environmentally acquired.
- All mutations are loss-of-function with respect to gene function or are functionally neutral tagged gene fusions. As such, there are no anticipated phenotypic changes of concern, such as increase virulence or transmissibility or dangerous gain-of-function phenotypes.
- Should a gene's function be in a pathway that negatively regulates virulence or transmissibility, a loss-of-function genetic mutation could, in principle, create a gain-of-function phenotypic change. Based on past experimental evidence, however, increases in virulence caused by loss of a negative regulator would be negligible.
- Neither *C. neoformans* or *C. gattii* are Category 1 pathogens, so the proposed research does not constitute potential dual-use research.
- Neither *C. neoformans* or *C. gattii* are pathogens of pandemic potential, nor would the genetic changes being proposed create pandemic potential.

Containment

- The IBC voted unanimously to require that 1) the proposed experiments be performed at a BSL-2 level of containment, and 2) that any work with *C. gattii* be performed using primary containment of a BSC.

Training

- The PI is a new investigator and confirmed that the lab works at a BSL-2 level of containment and that lab members receive training appropriate to both the level of containment and the organisms being used.
- The lab is currently working with the Biosafety Officer and Safety Office to develop a lab-specific BSL-2 safety plan that will be reviewed and approved by both the Biosafety Officer and the IBC Chair when completed. All biosafety plans include annual safety training.

2. PI: Haixin Sui

Project Title: Mechanistic studies of pneumococcal cyclic-di-AMP binding protein CabP.

Application number: 25-002R

Applicable NIH Guideline: Section III-D-2a.

Experiments in which DNA from Risk Group 2 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector system.

Objective: Define the functional roles of the cyclic-di-AMP binding protein CabP and its associated functional partners in cyclic-di-AMP signaling related to *S. pneumoniae* physiology and pathogenesis using structural methods.

Approach: Express specific *S. pneumoniae* genes in *E. coli*; purify proteins and study structure *in vitro*. No work with live *S. pneumoniae*.

Source organisms; genes being cloned; gene function

RG-2

Streptococcus pneumoniae

Gene sequences coding for cyclic-di-AMP binding proteins CabP and TrkH.

RG-1

E. coli (commercial strains)

Routine gene sequences coding for protein expression cassettes and plasmid amplifying systems.

Host organisms

RG-1

E. coli (numerous commercial strains)

Risk Assessment

- The *S. pneumoniae* gene products being expressed do not present occupational or environmental biosafety risks.
- There are no anticipated phenotypic changes of concern or dangerous gain-of-function phenotypes in *E. coli* that express the *S. pneumoniae* gene products being studied.
- *E. coli* is not a Category 1 pathogen, so the proposed research does not constitute potential dual-use research.
- *E. coli* is not a pathogen of pandemic potential, nor is *E. coli* being modified in such a way that it would acquire increased pandemic potential.

Containment

- The IBC voted unanimously that the proposed experiments can be performed safely using BSL-1 practices.

Training

- The PI confirms that lab members receive training appropriate to both the level of containment and the organisms being used.

3. PI: Joseph Wade
   Project Title: Plasmid conjugation study.
   Application number: 25-003R
   Applicable NIH Guideline: Section III-D-2a.
   Experiments in which DNA from Risk Group 2 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector system.
   Objective: Determine the roles on conjugation efficiency of plasmid-encoded H-NS and Hha from clinical isolates of *S. enterica* and *E. coli*.
   Approach: Capture plasmids from clinical isolates by conjugation between clinical isolates of *Salmonella enterica* and *Escherichia coli* with *E. coli* MG1655, then mutate the *hns* and *hha* genes to determine their impact on conjugation efficiency. Epitope tag the genes to allow ChIP experiments to identify their potential DNA binding sites.
   Source organisms; genes being cloned; gene function
   RG-2
   Salmonella enterica (clinical isolates)
   Escherichia coli (clinical isolates)
   Whole plasmid capture by conjugation; no cloning involved.
   RG-1
   E. coli strain MG1655
   Mutated *hns* and *hha* genes and epitope-tagged H-NS and Hha.
   Host organisms
   RG-1
   E. coli strain MG1655
   Risk Assessment
   - Mutation of the *hns* and/or *hha* genes could increase conjugation frequency of the captured plasmids, but such a phenotype is not anticipated to be of biosafety concern or to create dangerous gain-of-function phenotypes.
   - Neither *S. enterica* nor *E. coli* are Category 1 pathogens, so the proposed research does not constitute potential dual-use research.
   - *E. coli* MG1655, a non-pathogenic strain, is not a pathogen of pandemic potential, nor is the strain being modified in such a way that it would acquire increased pandemic potential.
   - The plasmids being captured have been fully sequenced and have no virulence genes. The antibiotic genes on the captured plasmids are naturally occurring.

Containment

- While the experiments after initial plasmid capture could be performed safely using BSL-1 practices, the IBC voted unanimously to require all proposed experiments be performed at a BSL-2 level of containment because of the biosafety risk of setting up conjugation reactions between viable, infectious clinical isolates of *S. enterica* and *E. coli* with *E. coli* MG1655.

Training

- The PI confirms that lab members receive training appropriate to both the level of containment and the organisms being used.
- The lab is confirmed to have an approved lab-specific BSL-2 safety plan that includes annual safety training.

ADDENDUM REQUESTS - none

RENEWAL APPLICATIONS

1. PI: Todd Gray
   Project Title: Mycobacterial genetics.
   Application number: 25-004R
   Applicable NIH Guideline: Section III-D-1a
   Experiments using RG-2 agents as host-vector systems.
   Objectives and Approaches:
   a) Identify and characterize short open reading frames and their encoded small proteins using transcriptomic profiling approaches and plasmid reporters.
   b) Characterize the regulation, function, and physiologic role for ESX-4 secretion systems using transcriptomic profiling approaches and targeted deletion mutations.
   c) Identify mechanism and effects of kin vs non-kin interactions using protein-protein interaction assays with purified proteins expressed in *E. coli.*
   Source organisms; genes being cloned; gene function
   RG-3
   Mycobacterium tuberculosis (source of cloned sequences; no work with RG-3 Mtb)
   RG-2
   Mycobacterium smegmatis
   Mycobacterium marinum
   Mycobacterium abscessus
   Sequences:
   a) Open reading frames and their encoded small proteins; functions unknown.
   b) Genes constituting the ESX-4 secretion system.
   c) Genes identified phenotypically as potentially being involved in kin-non-kin mating interactions; functions unknown or TBD.
   RG-1
   E. coli
   Mycobacterial shuttle vectors; antibiotic resistance genes and regulatory regions routinely used for cloning and not
   Not associated with infection of humans
   Various mycobacteriophage used for genetic manipulation of mycobacteria.
   Host organisms
   RG-2
   Mycobacterium smegmatis
   Mycobacterium marinum
   Mycobacterium abscessus
   Mycobacterium tuberculosis (RG-2 attenuated strains)

RG-1

E. coli (commercial strains)

Risk Assessment

- None of the gene products being studied present occupational or environmental biosafety risks.
- There are no anticipated phenotypic changes that would raise biosafety concerns for any of the various mycobacterial host species being used in the proposed experiments, and no dangerous gain-of-function phenotypes will be created.
- No mycobacteria species are Category 1 pathogens, so the proposed research does not constitute potential dual-use research.
- The mycobacteria species being used as host species are not pathogens or have pandemic potential, nor will they be modified in such a way as to create or increase pandemic potential.
- None of the antibiotics used for selection purposes are in medical use and have no clinical relevance in the BSL2 species listed.

Containment

- The IBC voted unanimously to require that the proposed experiments be performed at a BSL-2 level of containment.

Training

- The PI confirmed that the lab works at a BSL-2 level of containment and that lab members receive training appropriate to both the level of containment and the organisms being used.
- The lab is confirmed to have an approved lab-specific BSL-2 safety plan that includes annual safety training.

2. PI: Rajendra K. Agrawal

Project Title: Structural aspects of protein synthesis.

Application number: 25-006R

Applicable NIH Guideline: Section III-F-8, Appendix C-II.

Experiments which use *E. coli* K-12 host-vector systems.

Objective: Elucidate various aspects of the mechanism of protein synthesis.

Approach: Express wildtype and mutant ribosome-binding protein factors from humans in *E. coli* and determine their structure and function using cryo-electron microscopy.

Source organisms; genes being cloned; gene function

Not associated with infection in humans

Homo sapiens

Ribosome binding proteins.

Host organisms

RG-1

E. coli (commercial strains)

Risk Assessment

- The human gene products being expressed do not present occupational or environmental biosafety risks.
- There are no anticipated phenotypic changes of concern or dangerous gain-of-function phenotypes in *E. coli* that express the human gene products being studied.
- *E. coli* is not a Category 1 pathogen, so the proposed research does not constitute potential dual-use research.
- *E. coli* is not a pathogen of pandemic potential, nor is *E. coli* being modified in such a way that it would acquire increased pandemic potential.

Containment

- The IBC voted unanimously that the proposed experiments can be performed safely using BSL-1 practices.

Training
- The PI confirms that lab members receive training appropriate to both the level of containment and the organisms being used.

3. PI: Janice Pata
Project Title: HIV-1 reverse transcriptase.
Application number: 25-007R
Applicable NIH Guideline: Section III-D-2a.
Experiments in which DNA from Risk Group 3 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector system.
Objective: Elucidate the function of the reverse transcriptase, protease and nucleocapsid proteins in HIV-1.
Approach: Express HIV-1 genes in *E. coli*; purify proteins and study structure *in vitro*. No work with live virus.
Source organisms; genes being cloned; gene function
RG-3
HIV-1
Gene sequences coding for the reverse transcriptase, protease and nucleocapsid proteins.
Host organisms
RG-1
E. coli (commercial strains)
Risk Assessment
- The HIV-1 gene products being expressed do not present occupational or environmental biosafety risks.
- There are no anticipated phenotypic changes of concern or dangerous gain-of-function phenotypes in *E. coli* that express the HIV-1 gene products being studied.
- *E. coli* is not a Category 1 pathogen, so the proposed research does not constitute potential dual-use research.
- *E. coli* is not a pathogen of pandemic potential, nor is *E. coli* being modified in such a way that it would acquire increased pandemic potential.
Containment
- The IBC voted unanimously that the proposed experiments can be performed safely using BSL-1 practices.
Training
- The PI confirms that lab members receive training appropriate to both the level of containment and the organisms being used.

4. PI: Janice Pata
Project Title: Structure and function of DNA replication proteins.
Application number: 25-008R
Applicable NIH Guideline: Section III-D-2a.
Experiments in which DNA from Risk Group 3 and Risk Group 2 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector system.
Objective: Elucidate the structure and function of DNA polymerases.
Approach: Express polymerase and associated replication protein genes in *E. coli*; purify proteins and study their structure *in vitro*. No work with live organisms other than *E. coli*.
Source organisms; genes being cloned; gene function
RG-3
Mycobacterium tuberculosis
Gene sequences coding polymerase and associated replication proteins. No work with live bacteria.
RG-2
Staphylococcus aureus

Gene sequences coding polymerase and associated replication proteins. No work with live bacteria.

Not associated with infection of humans

Sulfolobus solfataricus

Sulfolobus acidocaldarius

Gene sequences coding polymerase and associated replication proteins.

Host organisms

RG-1

E. coli (commercial strains)

Risk Assessment

- The polymerase and associated replication proteins being expressed do not present occupational or environmental biosafety risks.
- There are no anticipated phenotypic changes of concern or dangerous gain-of-function phenotypes in *E. coli* that express the polymerase and associated replication proteins gene products being studied.
- *E. coli* is not a Category 1 pathogen, so the proposed research does not constitute potential dual-use research.
- *E. coli* is not a pathogen of pandemic potential, nor is *E. coli* being modified in such a way that it would acquire increased pandemic potential.

Containment

- The IBC voted unanimously that the proposed experiments can be performed safely using BSL-1 practices.

Training

- The PI confirms that lab members receive training appropriate to both the level of containment and the organisms being used.

5. PI: Alex Ciota

Project Title: Genetic correlates of arbovirus fitness and transmission.

Application number: 25-009R

Applicable NIH Guideline: Section III-D-1a

Experiments using RG-3 and RG-2 agents as host-vector systems.

Objective: Elucidate the influence of intrinsic genetic and extrinsic environmental factors on arbovirus evolution.

Approach: Use basic recombinant DNA technologies and reverse genetics to clone arboviral RNA genomes into DNA systems that can be manipulated genetically and create mutations in genes to investigate parameters of viral evolution in tissue culture and mosquitos.

Source organisms; genes being cloned; gene function

Flaviviruses

RG-3

Deer Tick virus

Full or partial genome DNA copies in bacterial plasmids.

RG-2

West Nile virus

Zika virus

Dengue virus

Full or partial genome DNA copies in bacterial plasmids.

Orthobunyavirus

RG-2

Cache Valley virus

Full or partial genome DNA copies in bacterial plasmids.

RG-1

E. coli (commercial strains)

Host organisms

RG-1

E. coli (commercial strains)

Not associated with infection in humans

Culex, Aedes and Anopheles species mosquitoes

Various mosquito and vertebrate tissue culture cells

Risk Assessment

- Viral RNA/rDNA genomes pose no health risk until transfected into cells to rescue infectious virus.
- The experiments proposed look at infection in mosquito vectors, not mammalian hosts. Discussion of how genetic variation in the virus might impact 'virulence' or 'transmissibility' is with respect to phenotypic effects in mosquito vectors not disease phenotypes in humans.
- The cloned viruses that will be studied are recovered from field-collected mosquitoes, so the viruses represent a snapshot of viral genetic variation that is already circulating in nature. As such, the potential phenotypes produced by the recovered viruses reflect the range of phenotypic variation that is already occurring in mosquito vectors in nature, from attenuation to increased transmissibility or virulence.
- Cloned viruses most often have genetic differences at multiple loci. To better understand genotype-phenotype relationships, some viruses will be created that have an individual, or a subset of, targeted genetic changes, or 'sub-variants' chosen from amongst the multiple genetic differences observed between viruses. Based on past experiments, viruses containing such targeted 'sub-variant' genetic changes produce phenotypes that are less severe than phenotypes of 'parent' viral genotypes that contain multiple genetic changes. So, there is no expectation that any of the phenotypes produced by viruses containing targeted sub-variant genetic changes will be any more severe than the phenotypic variation that is already occurring in nature. No viruses will be created that would reasonably be anticipated to produce a dangerous gain-of-function phenotype in a mosquito host.
- WNV is a Category 1 pathogen, and while some of the viruses that will be studied will have genetic differences that may increase virulence or transmissibility in an infected mosquito vector, the range of phenotypic variation will be no greater than what occurs in nature. For this reason, the IBC concluded that the proposed experiments do not constitute dual-use research of concern.
- Humans are dead-end hosts for arboviral infections, so none of the arboviruses being studied have pandemic potential.

Containment

- The IBC voted unanimously to 1) require that the proposed cloning and tissue culture-based experiments be performed at either a BSL-2 or BSL-3 level of containment as appropriate for the RG-2 and RG-3 viruses being studied, respectively; and 2) require that work with infected mosquitoes for both RG-2 and RG-3 viruses be performed using ACL-3 containment.

Training

- The PI confirms that the lab works at a BSL-2, BSL-3, or ACL-3 level of containment, as appropriate, and that lab members receive training appropriate to both the level of containment and the organisms being used.
- The lab is confirmed to have lab-specific BSL-2, BSL-3 and ACL-3 safety plans that include annual safety training.

5. PI: Kathleen McDonough

Project Title: Bacterial pathogenesis, gene expression and drug resistance.

Application number: 25-010R

Applicable NIH Guideline: Section III-D-2a

Experiments using RG-3 and RG-2 agents as host-vector systems.

Objective: Identify and characterize gene regulatory pathways in bacterial pathogens that play a central role in how pathogens respond to host-associated environments, particularly as relates to bacterial evolution and the development of drug resistance.

Approach: Application of a variety of standard recombinant DNA technologies, including the use of native gene promoter sequences to express reporter genes, ectopic expression of native genes for genetic complementation, and expression of native gene flanking sequences to generate gene knockouts with insertion of selectable marker genes. Also, recombinant DNA technologies are used in non-pathogenic bacteria to express gene promoters and open reading frames from pathogenic bacteria for the purpose of investigating the function of gene regulatory factors.

Source organisms; genes being cloned; gene function

RG-2

Mycobacterium tuberculosis (attenuated strains MC26020, MC27000, MC26202, MC26030, MC26230)
Mycobacterium bovis (attenuated BCG vaccine strain)
Mycobacterium smegmatis
Mycobacterium leprae
Yersinia pestis (attenuated RG-2 strains lacking LCR virulence plasmid or *Pgm* locus; exempt from SA regulations)
Yersinia pseudotuberculosis
Genes of specific interest include: *mc11*, *abmR*, *Rv0805*, *lysG*, *dosR*, *Rv2623* and recA, DosR regulon members, transcription factors and cAMP-associated genes. Various gene promoter and gene flanking sequences.

RG-1

E. coli (commercial strains)

Host organisms

RG-1

E. coli (commercial strains)

RG-2

Mycobacterium tuberculosis (attenuated strains MC26020, MC27000, MC26202, MC26030, MC26230)
BCG (vaccine strain made from attenuated *Mycobacterium bovis*)

RG-3

Mycobacterium tuberculosis

Risk Assessment

- None of the gene products being studied present occupational or environmental biosafety risks.
- There are no anticipated phenotypic changes that would raise biosafety concerns for any of the *Mycobacterium* or *Yersinia* host species being used in the proposed research. Genetic changes are loss-of-function or marker genes constructs, and no dangerous gain-of-function phenotypes will be created.
- The *Yersinia pestis* being used is an attenuated RG-2 strain that is neither a Select Agent nor a Category 1 pathogen, so the proposed research using *Yersinia pestis* does not constitute potential dual-use research.
- The committee acknowledged the elevated risk from exposure to *Pgm(-)* attenuated *Yersinia pestis* strains for individuals that have hereditary hemochromatosis.
- No *Mycobacterium* species are Category 1 pathogens, so the proposed research with *Mycobacterium* species does not constitute potential dual-use research.
- None of the proposed experiments will enhance the virulence or transmissibility of the mycobacterial hosts being studied. Combined with the existing global distribution of *M. tuberculosis* (infecting 1/4-1/3 of global population) and slow disease progression, the proposed work will not create bacterial strains with enhanced pandemic potential.

Containment

- The IBC voted unanimously that the appropriate level of containment for the proposed research is BSL-2 or BSL-3 as appropriate for the Risk Group of the organism being studied.
- The IBC concluded that there is no reasonable expectation that the proposed experiments will enhance the virulence or transmissibility of the mycobacterial hosts being studied. However, to be maximally

conservative and to protect against possible reductions in BCG attenuation, BCG hosts containing cloned Mtb genes must be handled at a BSL-3 level of containment.

Training

- The PI confirms that the lab works at a BSL-2 or BSL-3 level of containment, as appropriate, and that lab members receive training appropriate to both the level of containment and the organisms being used.
- The lab is confirmed to have both approved lab-specific BSL-2 and BSL-3 safety plans that includes annual safety training.
- The IBC requested that the PI inform the committee if/when work begins with *Pgm*(-) *Yersinia pestis* so that the elevated risk to individuals with hereditary hemochromatosis can be discussed with lab staff before the work begins.