Laboratory Orientation and Safety
Laboratory Safety

- Prevention of physical injuries and laboratory acquired infections with zoonotic disease agents
- **Laboratory safety is every employee’s responsibility!**
- No live agents in this course…..but work as though you are working with live agents
What is a Zoonotic Disease?

- “Diseases and infections naturally transmitted between vertebrate animals and man, with or without an arthropod intermediate” — WHO
- Of the >1,700 known pathogens affecting humans, 49% are zoonotic
- Of the 156 pathogens associated with emerging diseases, 73% are zoonotic
- Asian HPAI H5N1 has been confirmed in >270 people with >160 deaths
  - Human infections a result of direct contact with birds
  - Limited evidence of human-to-human spread
  - No known laboratory associated infections
Biosafety in Microbiological and Biomedical Laboratories (BMBL)

Biosafety Levels (BSL) Defined

- **BSL-1**: Level to handle a microorganism **not known to cause disease in humans**, with minimal community risk
- **BSL-2**: Level to handle a microorganism that **causes human disease**, with minimal community risk
- **BSL-3**: Level to handle a microorganism that **causes serious (or potentially lethal) human disease** (e.g. Asian HPAI H5N1)
- **BSL-4**: Level to handle a microorganism that **causes life threatening disease** in humans
Containment/Barriers

- “Box within a box” concept
- Equipment – “primary barriers”
  - Biological safety cabinets (BSCs) – Class II
  - Aerosol-resistant centrifuge cup holders
  - Horsefall-type bird cages
- Facilities – “secondary barriers”
  - Building design to control traffic
  - Air flow/HEPA filtration
  - Sewage/waste treatment
Class II BSC

- Provides personnel, environment, and specimen protection
Class II BSC Use:

Before you begin

- Check to be sure the cabinet has recently been certified (annually)
- Turn on cabinet and run for 2 or 3 minutes before using to filter the air and establish airflow patterns
- Check to be sure that the sash is in the correct position (if unit has sliding sash)
- Make sure the UV light is off whenever anyone is in the room
Class II BSC Use:

Before you begin:

- Organize cabinet to segregate clean items from contaminated ones.
- Choose disinfectant that will inactivate the target organism but not cause corrosion of metals in cabinet.
Class II BSC Use:  

Working in the Cabinet

- Only one person should work in the cabinet at a time
- Operator should be seated; armpits level with the bottom of the window
- Use good aseptic technique...always
- Use a limited number of slow movements
- Never block the grilles!
Class II BSC Use:

Working in the Cabinet

- Minimize activity around the cabinet
  - People walking behind cabinet and doors opening cause air turbulence and disturb airflow in cabinet
- Minimize activity inside the cabinet
  - Enter or exit the cabinet from straight on, then allow air in the cabinet to stabilize
Class II BSC Use: 

*After the procedure*

- Place contaminated supplies in an enclosed container (with disinfectant) and remove from cabinet.
- Disinfect all inner surfaces of cabinet.
- Purge cabinet for 2 to 3 minutes, then close the cabinet window completely - if possible, leave the cabinet running.
- Do not store equipment or supplies in or on the cabinet.
Standard Laboratory Safety Practices

- Use mechanical pipetting devices (no mouth pipetting)
- Wear disposable gloves/wash hands frequently
- Avoid touching eyes, face
- Decontaminate work surfaces after each activity
- Clean and disinfect spills and splashes promptly
- Restrict or limit access to laboratory
- Prohibit eating, drinking and smoking
- Proper disposal of waste materials
Standard Laboratory Safety Practices (cont’d)

- Safe use/disposal of sharps
  - Place in biohazard sharps containers only
  - **DO NOT** place in paper waste containers
- Use appropriate Personal Protective Equipment (PPE) as needed
Personal Protective Equipment (PPE) suitable for HPAI H5N1

- Eye - safety glasses, goggles
- Hands - gloves
- Bodies/arms - lab coat with long sleeves
- Nose/mouth - N-95 (or equivalent) respirator
  - Several types available
  - Requires fit testing
- BSL-3 enhanced for *in vivo* work and virus isolation
Fit-Testing (N-95 Respirator)

- Respirator will not protect you if they don’t fit properly!
- Must be conducted prior to use
- Must be conducted at least annually
- Must be retested if respirator fit or wore changes (e.g. type/style of respirator, or change in weight)
Required Laboratory Dress for this Course

- Safety glasses
- Lab coat
- Disposable gloves
- Leave lab coat and safety glasses in lab – do not wear in halls
Quality Assurance Program: Equipment Monitoring/Calibration

- Routine monitoring of all equipment
  - Internal external monitoring
  - ISO 17025 standard
- Pipetting devices
- BSCs (annually or if moved)
- Incubators/water baths
- Centrifuges
- Thermocyclers (PCR machines)
Conclusions

- Biosafety levels (BSL) help to determine the extent of protection needed when working with zoonotic microorganisms.
- Containment is a combination of basic and special practices, safety equipment and facilities – “box within a box” concept.
- Proper containment allows workers to handle zoonotic microorganisms safely while protecting themselves and the environment.
- QA programs provide increased confidence in test results.
Diagnostic Tests Demonstrated

- Virus/antigen/RNA detection
  - Virus isolation
  - Virus identification – HA, HI
  - Antigen capture, AGI D
  - Real time RT-PCR
- Antibody detection
  - AGI D
  - ELISA
- Unknown specimens