OECO Quantitative Method Testing Virus/Collaborative Study

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MLB’s Priorities

- Preparation and storage of a stable virus stock.
- Care and storage of the test cell line.
- Dilution of virus stock needed to obtain appropriate control carrier counts (e.g., 5.0-6.0 logs/carrier).
- Virus recovery: TCID$_{50}$ versus plaque assay for determination of virus concentration.
MLB’s Priorities

- Revise the current OECD protocol for neutralization/interference assay.
- Determination of most effective neutralizer volume
- Reference standard development: high and low sodium hypochlorite treatments
- Identification of test chemicals (and neutralizers) for collaborative study
- Update/revise MLB’s SOP MB-25 (OECD Quantitative Method) to accommodate testing virus
Test System

- **TCID\(_{50}\)** approach = median tissue culture infective dose; that amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated
  - **Cell Line:** Crandell Rees Feline Kidney (ATCC # CCL-94).
  - **Virus:** Feline calicivirus (ATCC # VR-782)

- **Virus-induced cytopathic effects (CPE):**
  - The CPE associated with FCV is visually evident by the presence of small, rounded cells, with a slight granular look that may have detached from the monolayer.
Test System

Healthy CRFK monolayer

Feline calicivirus CPE on CRFK cell line
Main revisions to the SOP

- Attachment 1: Keeping CRFK Cell Line Stocks by Freezing in Liquid Nitrogen
- Attachment 2: Reviving CRFK Cell Line From Liquid Nitrogen Storage
- Attachment 3: Sub-culturing CRFK Cell Line for Work with Viruses
- Attachment 4: FCV Propagation, Harvest and Titration
MLB to complete standardization & demonstration studies, SOP and study plan within 8 months

- Request stakeholder support/input to resolve technical issues and enhance assay

Launch collaborative study in October/November timeframe

EPA will be the lead lab (will provide protocol, carriers, test chemicals)

Seek 3-4 volunteer labs

To be conducted in phases
Step-Wise Process

- Phase 1 – readiness
  - Kickoff meeting(s)
  - Establish virus stock/titer
  - Establish cell line stock
  - Control carrier counts – report back

- Phase 2 – neutralization assay on one test chemical; report back

- Phase 3 – reference standard (proficiency and responsiveness); report back

- Phase 4 – method performance with four actives with a range of presumed efficacy
Typical Test design

- Four treated carriers and three control carriers per test chemical
  - Inoculum to include three part soil
- Two treatments for the sodium hypochlorite reference standard
  - Standardize contact time @ 5 min
  - OECD hard water as the diluent
  - Three replications
  - Meet anticipated LR before proceeding
- Neutralization assay on one test chemical – conduct one time
- Four test chemical treatments – side-by-side desirable, randomized order
  - Standardize contact time @ 5 min
  - OECD hard water as the diluent
  - Three replications per test chemical
  - One set of controls may be used for multiple treatments
Questions/Comments?