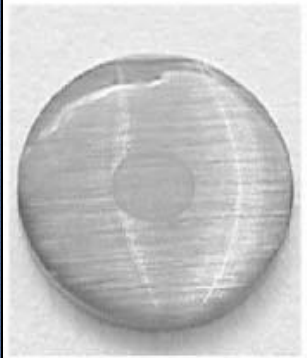


US EPA ARCHIVE DOCUMENT



# OECD Quantitative Method Testing Virus/Collaborative Study

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# Disclaimer

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- ⦿ *This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency Determination or Policy.*

# MLB's Priorities

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- ⦿ 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<sub>50</sub> versus plaque assay for determination of virus concentration.

# MLB's Priorities

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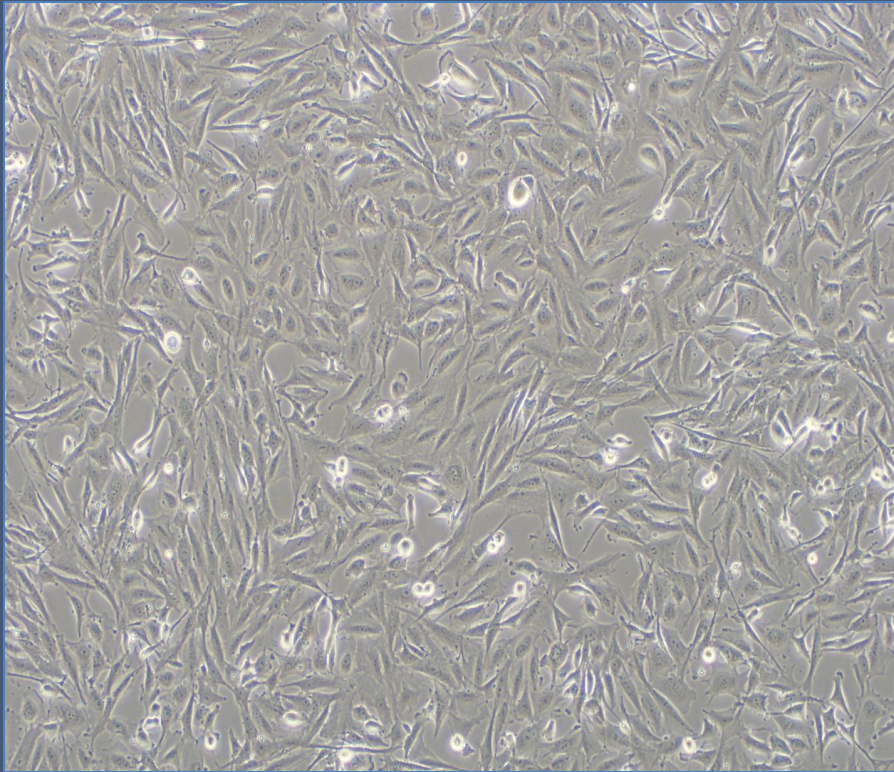
- ⦿ Revise the current OECD protocol for neutralization/interference assay.
- ⦿ Determination of most effective neutralizer volume
- ⦿ Reference standard development: high and low sodium hypochlorite treatments
- ⦿ Identification of test chemicals (and neutralizers) for collaborative study
- ⦿ Update/revise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

# Test System

- ⦿ TCID<sub>50</sub> 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.

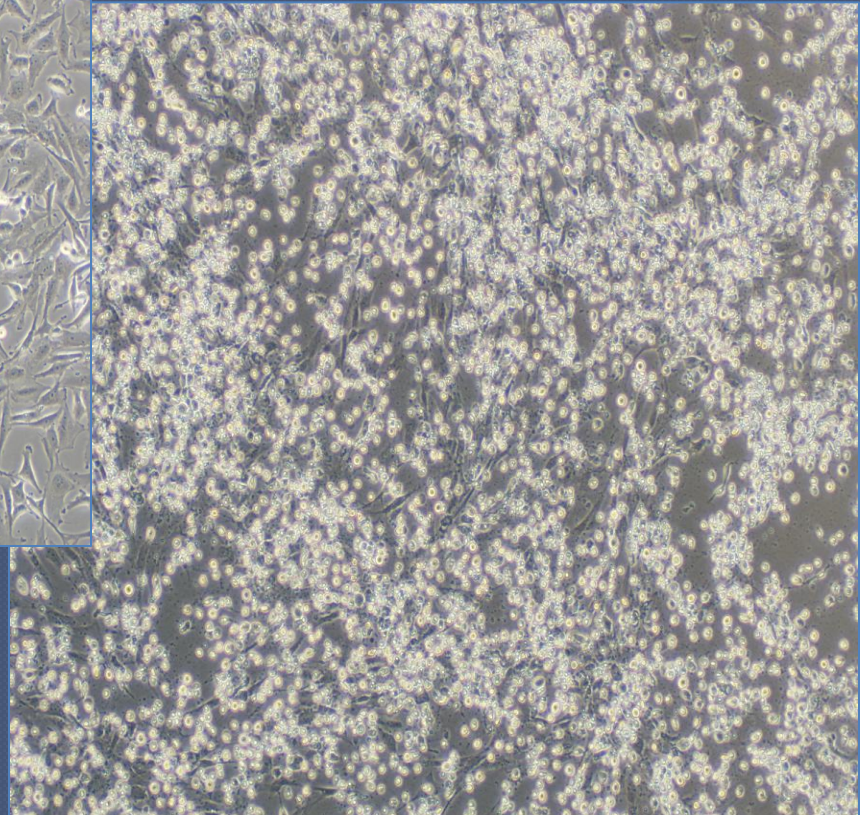


# Test System



Healthy CRFK monolayer

Feline calicivirus CPE on  
CRFK cell line



# Main revisions to the SOP

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- 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
- Attachment 5: Neutralization Confirmation, Testing for Cytotoxicity, Interference with Virus Infectivity, and Influence of Soil Load on Host Cells.



# Plans for a Collaborative Study

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- 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?

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