



United States Department of Agriculture

CVB – Policy, Evaluation, and Licensing

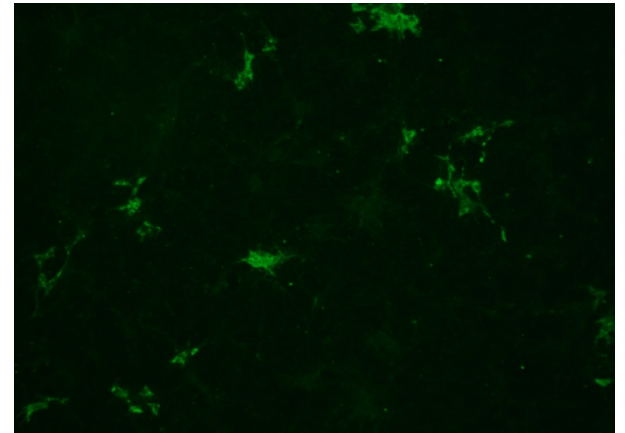
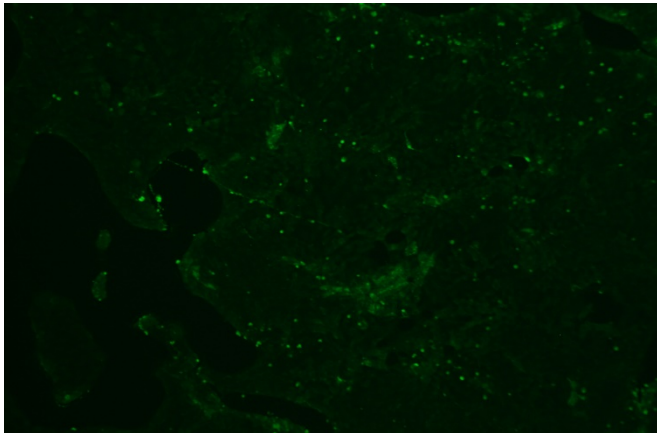
NIH Test for Rabies Potency

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Alethea M. Fry, MS

NIH Background

Developed in 1953, the NIH method meets the minimum standards for achieving compliance with US potency test regulations and establishes minimum recommended batch potency requirements ([Barth et al., 1988](#), [Wunderli et al., 2003a](#), [CFR, 2011](#)).



NIH Test Components/Considerations

- Reference vaccine
- Challenge virus
- Test serials (1-3)
- Animals



Reference Vaccine

- Lot of BPL inactivated rabies vaccine
- Collaborative studies with veterinary firms



Challenge Virus Standard

- Strain – CVS-11
- Tested in accordance with 9CFR 113.55, 113.27 and 113.28
- Identity confirmation



Test Serials

- Requests from Review team
 - Prelicense testing
- Testing based upon Inspection and Compliance requests
- Routine check and stability testing



NIH Test - Animals

Animals

- Animals weigh between 11-13 grams at arrival
- Typically weigh 13-16 grams at first vaccination
- A minimum of 200 mice are required to test 1 serial
- Test multiple lots (3) simultaneously to reduce animal usage

Test groups

- 4-5 groups to test 4-5 doses of the **reference** vaccine
- 5 groups to test 5 doses of the **test** vaccine.
- 4 groups to test CVS (back titration of **challenge** virus)

Vaccination of Animals

- Mice are vaccinated by the intraperitoneal route with 0.5 mL of test vaccine or reference preparation on Day 0 and Day 7.



Challenge Virus Standard (CVS)

- 7 days after the second vaccination mice are inoculated with a challenge dose of CVS Virus
(≥ 12 LD₅₀ per 30 μ L)
- Prepare three 10-fold dilutions (10^{-1} , 10^{-2} and 10^{-3}) to conduct a titration of the working CVS range.

Refinement - Use of Anesthesia

- CVB has incorporated the option of using general anesthesia in the NIH test
- Rodent general anesthesia machine
- Isoflurane with a calibrated vaporizer



NIH Test for Rabies Potency

- Mice are observed twice each day daily for 14 days for rabies infection or death
- Mice which die prior to or on day 5 post-challenge are considered nonspecific deaths.
- Animals that die or have clinical signs of rabies infection 6 -14 days post-challenge are considered as positive for rabies infection
- Rabies disease in mice can be recognized in 5 stages based upon work by Bruckner (Bruckner et.al 2003).

- Animals which exhibit stage 3 or greater are humanely euthanized.

- SAM 308 Outlines these stages
 - Stage 1: Ruffled fur and hunched back
 - Stage 2: Slow or circular movements
 - Stage 3: Shaky movements, trembling and convulsions
 - Stage 4: Signs of paralysis
 - Stage 5: Moribund animals

- Mice which survive the 14-day post challenge observation are humanely euthanized.

Test validity

- Tests are considered valid
 - if at least 70% of immunized mice receiving the lowest dilution (highest dose) of vaccine survive.
 - 70% of mice receiving the highest dilution (lowest dose) of vaccine die (lowest dose) of vaccine die

Calculations for serial potency

- ED_{50} of each test vaccine, reference and the LD_{50} of the CVS
- Relative Potency of the test vaccine is determined

Questions

