NIH Test for Rabies Potency

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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
  \( (\geq 12 \text{ LD}_{50} \text{ per } 30\muL) \)

- Prepare three 10-fold dilutions \((10^{-1}, 10^{-2} \text{ and } 10^{-3})\) 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
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- 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.
Calculations for serial potency

- $\text{ED}_{50}$ of each test vaccine, reference and the $\text{LD}_{50}$ of the CVS

- Relative Potency of the test vaccine is determined
Questions