Polio technique identical with this one.

Live Rubella Vaccine
Potency Determination

Division of Virology
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Materials:

1. Microtiter plates: Falcon plastic tissue culture, flat-bottomed, sterile.
2. Reference vaccine: BoB Lot #1 Titer -
3. Test vaccine: At least two vials
4. Diluent: Eagle's MEM + 2% fetal bovine serum
5. Cells: RK-13, suspended in diluent to approximately 200,000 cells/ml.

~~Different~~ For Polio Rhes. Monk. Kidney — MEM+2% fetal
HEp 2 —— ~~Med. described by Sabin except that fetal bovine~~ BME +2% fetal bovine ser ~~is used~~ — No chicken serum

Method:

1. Rinse a fully sheeted culture of RK-13 cells twice with 0.25% trypsin in Saline A. Incubate cell-side down until cell monolayer is completely trypsinized. Resuspend trypsinized Vero cells in 3-5 times the original volume of medium (Eagles + 2% FBS) to give the appropriate cell concentration. Add one drop of cell suspension, approximately 0.05 ml to each well to give approximately 10,000 cells/well. Place plates in CO_2 incubator, 34-36° C, for at least 30 minutes.

2. Each vaccine, as well as the reference, is tested in duplicate and titrated separately. The usual dilution sequence is 10^{-2} to 10^{-5} in whole log steps.

3. In a wire rack dilution blanks are set up for the required number of vaccines (1.8 ml diluent in sterile 13 x 100 tubes).

4. For single dose containers, using a 1 ml plastic syringe and a 25 gauge 5/8" needle, reconstitute one vial of vaccine in 0.7 ml distilled water, mix and place contents in a sterile tube. Using a 1 ml disposable glass pipette take up 0.3 ml of the undiluted vaccine and add 0.2 ml to the first dilution blank (10^{-1}). Mix thoroughly on mixer and repeat to the 10^{-2} dilution.

5. Mix and using a fresh 1 ml pipette take up 1.0 ml, transfer 0.2 ml to the 10^{-3} dilution and 0.1 ml to each of eight weeks on the microtiter plate containing the RK-13 cells. Repeat procedure through the 10^{-5} dilution for each sample to be tested. Wipe off excess fluid on plate with sterile gauze pads.

6. Place plates in covered plastic box containing a dampened gauze pad to prevent excessive drying. Incubate in CO_2 at 34-36° C.

7. The presence of absence of CPE is recorded beginning at 4 days. Final reading may be made at 7-11 days.

8. A 50% endpoint is calculated by the Karber method.

9. For release, the titer of the vials tested must average a minimum of 3.0 $\log_{10}$ (1000 $TCID_{50}$) per human dose based upon the potency of the reference standard as established in RK-13 microtest plates at the Bureau of Biologics. A test is deemed invalid if a) the reference titer deviates more than 0.5 $\log_{10}$ from the established titer or b) the variation between vaccine vials in >0.5 $\log_{10}$.

10. Appropriate precautions must be taken to protect all virus samples from light.