

400 gm with minimum recommended dose of vaccine. Repeat the inoculation with same number of dose after 28 days. Challenge the animal along with 5 control guinea pigs with 0.25 ml of virulent or 25 viable spore suspension of *Clostridium chauvoeii* in 5% calcium chloride not more than 10% of animal die from *Clostridium chauvoeii* infection within 5 days and all control animal die within 48 hrs of challenge.

3. Name of Vaccine: Enterotoxaemia

Sterility Test: FTM/SCDM

Safety Test: Inoculate recommended dose of vaccine subcutaneously in 6 healthy sheep about 18kg or in 10 healthy rabbit weighing 1.5 to 2 kg. Repeat the dose within the interval of 21 to 28 days.

Bleed the animal 10-14 days after the second dose of vaccine. Collect the serum and pooled separately. Mix 0.1 ml of serum and 0.1 ml of epsilon toxin 300 ml and incubate at 37°C for 30 mins. Inject 0.2 ml of mixture intravenously into 2 mice of 18 gm each. Mice should not die. The serum titre per ml capable of neutralizing 300 ml into 2 mice as positive control. The mice should die.

4. Name of Vaccine: Hemorrhagic septicemia

Sterility Test: FTM/SCDM

Safety Test: Inject intraperitoneally in 6 mice weighing not less than 18 gm with 0.5 ml of vaccine and animal for 5 days. Inject 2 seronegative cattle with twice the recommended dose of vaccine and observe for 10 min.

Potency Test: Inject intramuscularly into each of the 50 mice weighing not less than 18 gm of with 0.2 ml of vaccine. Repeat the dose 14 days later. After 7 days divide the mice into 10 groups of 5 each. Challenge the vaccinated and the control mice will 10^{-1} to 10^1 dilution of 8-12 hour. Old broth culture of virulent strain of *Pasturella multocida*. Observe the mice for 5 days and record the number of vaccinated control mice, dead ones. Calculate the median lethal dose (LD 50) of vaccinated and control by standard method. The protection provided by vaccine is calculated as number of protection unit.

Number of protection unit = LD50 in control animal.

(B) Testing Protocol for Viral Vaccines

1. Name of Vaccine: Anti-Rabies Vaccine (ARV)

Sterility Test: FTM/SCDM

Safety Test: 2 rabbits were inoculated with 5 ml of vaccine sub-cutaneously (S/C) and 0.5 ml of vaccine (I/P) in 5 mice

Potency Test: 20 mice were injected with 0.03ml of vaccine sub-cutaneously (S/C). After 21 days observation was taken and each 5 group of mice were challenged with different dilutions of virus namely 10^{-3} to 10^{-10} . LD₅₀ was calculated for vaccinated animals as well as for control separately.

2. Name of Vaccine: Swine Fever

Sterility Test: FTM/SCDM

Safety Test: Inoculate three 6-7 week old piglet with 10 field dose of vaccine and observe for 21 days.

Potency Test: 100 Pd 50

Recommended Virus Titer: 100 pd 50

Number of Doses: Pd 50/100

3. Name of Vaccine: Sheep Pox

Sterility Test: FTM/SCDM

Safety Test: Inoculate three sheep which are 8-12 months old with standard dose of vaccine S/C. Challenge along with two control sheep, 0.1 ml suspension of 10^1 to 10^6 dilutions of virulent and calculate the titer of challenge virus for vaccinated and control animals. The difference of log titer should be more than 2.5.

Recommended Virus Titer: Not less than 10-2.5 TCID 50 of virus per dose.

Number of Doses: TCID 50/10^{-2.5}

(C) Testing Protocol for Poultry Vaccines

1. Name of Vaccine: Ranikhet (R2B strain) Mesogenic

Sterility Test: FTM/SCDM

Safety Test: 20 chicks (08 weeks old) inoculated with standard dose S/C. Observe for 21 days. Challenge the chicks along with 8 controls with 10^5 LD 50 of virulent strain of NDV I/M and observe for 14 days.

Recommended Virus Titer: Not less than 10^5 EID 50 of virus per dose.

Number of Doses: EID 50/10⁵

2. Name of Vaccine: Marek's Vaccine

Sterility Test: FTM/SCDM

Safety Test: Administer 0.2 ml of 1:20 dilution of vaccine (I/M) in 21 days old chick. Keep 5 chicks as control observe for 21 days.

Potency Test: The vaccine sample shall be titrated in cell culture system and number of plaque forming unit are observed.

Recommended Virus Titer: Not less than 10^3 P.F.U. virus per dose.

Number of Doses: PFU/ 10³

3. Name of Vaccine: Fowl Pox

Sterility Test: FTM/SCDM

Safety Test: Administer 10 dose of SCDM vaccine to each 6-8 week of chick by skin clarification. Observe the bird for 21 days.

Potency Test: Immunize not less than 10 chick (6-8 week old) using the field dose by stick method and examine for tubs. After 21 days challenge each chick by skin scarification with fowl pox virulent and observe for 14 days. The vaccinated chick survive and showed no sign of disease except transient reaction of fowl pox within 6 days following the challenge.

Recommended Virus Titer: Not less than 10^3 PFU virus per dose.

Number of Doses: EID 50/10²

6. Media Used for Sterility Test

a) Robertson's Cooked Media:

- In Roux flask Nutrient broth with 2.5% Agar and EGG Albumin.
- Autoclave at 15 lbs for 45 minutes and filtered.
- Adjust pH to 7.4 by sodium hydroxide and hydrogen chloride.
- Add meat particle in distilled water.
- Distribute 140 ml to media flasks.
- Autoclave at 15lbs for 45 minutes.

b) Saboraud's Media:

- In Roux flask nutrient broth with 2.5 % agar and EGG

albumin.

- Autoclave at 15 lbs for 45 minutes and filter out.
- Adjust pH to 5.4 by sodium hydroxide and hydrogen chloride.
- Add 1% peptone and 0.4% glucose with distilled water.
- Distribute 140 ml to media flasks and autoclave at 15 lbs for 45 minutes.

7. Conclusion

The immune system recognizes vaccine agents as foreign, destroys them, and “remembers” them. When the virulent version of an agent comes along the body recognizes the protein coat on the virus, and thus is prepared to respond by neutralizing the target agent before it enters the cells and by recognizing and destroying infected cells before the agent can multiply to vast numbers.

Producing a usable, safe anti-viral vaccine involves a large number of steps which, unfortunately, cannot always be done for each and every virus. There is still much to be done and learned. The new methods of molecular manipulations have caused more than one scientist to believe that the vaccine technology is now entering a “golden age”. Refinements of existing vaccines are possible in the future. Rabies vaccine for example, produces side effects which make the vaccine unsatisfactory for mass immunization; therefore rabies vaccine is now used only in patients who have contracted the virus from an infected animal and are likely, without immunization, to develop the fatal disease.

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