International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

To Study the Production and Standardization of Veterinary Vaccines

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Abstract: ***Protecting animals from infection is a major obligation of every veterinarian's work in order to preserve animal welfare while assuring human health. Highly infectious animal diseases can reduce the performances of food producing animals and may have a great economical impact on many industries. A reliable supply of pure, safe, potent, and effective vaccines is essential for maintenance of animal health and the successful operation of animal health programmes. Immunisation of animals with high quality vaccines is the primary means of control for many animal diseases. The requirements and procedures described here are intended to be general in nature and to be consistent with published standards that are generally available for guidance in the production of veterinary vaccines.***

Keywords: Veterinary Vaccines, Immunity, Prevention, Production, Standardization, Haemorrhagic septicemia, Black Quarter, Anthrax Spore, Enterotoxeamia, Sheep pox, Anti-Rabies Vaccine, Swine Fever, New Castle Disease, Marek's Disease Vaccine, Fowl Pox Vaccine.

1. Introduction

A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe or its toxins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and remember it, so that the immune system can more easily recognize and destroy any of these micro-organisms that it later encounters. The term *vaccine* is derived from Edward Jenner's 1796 use of the term cow pox which, when administered to humans, provided them protection against smallpox.

The principle of vaccination is to induce a primary immune response in the vaccinated subject so that following the exposure of a pathogen; a rapid secondary immune response is generated leading to the accelerated elimination of the organism and protection from clinical disease. Success depends on the generation of memory T and B cells and the presence of neutralizing antibody in the serum.

Types of Vaccines

A. Bacterial Vaccines
- Haemorrhagic septicemia
- Black Quarter
- Anthrax Spore
- Enterotoxaemia

B. Viral Vaccines
- Sheep Pox
- Rabies Vaccine
- Swine Fever

C. Poultry Vaccines
- New Castle Disease
- Marek's Disease Vaccine
- Fowl Pox Vaccine

1. Haemorrhagic Septicaemia
Name: Haemorrhagic septicaemia alum precipitated vaccine
Causative Agent: *Pasteurella multocida*
Description: P-52 strain of *Pasteurella multocida*, formaldehyde inactivated alum precipated
Immunity: One year biannual vaccine
Dose Route: Cow, Buffalo(5 ml), Sheep, Pig, goat (2.5 ml) s/c
Storage: One year at 4ºc to 8ºc
Presentation: 250 ml in vials

2. Black Quarter
Name: Black Quarter Vaccine
Causative Agent: *Clostridium chauvoei*
Description: Clostridium chauvoei strain- 49 formaldehyde inactivated monovalent vaccine
Immunity: One year
Dose Route: Cow, Buffalo (5ml), Sheep goat (3ml) s/c
Storage: One year at 4ºc to 8ºc
Presentation: 250 ml in vials

3. Anthrax Spore
Name: Anthrax spore vaccine
Causative Agent: *Bacillus anthracis*
Description: Suspension of live spores of avirulent strain of *Bacillus anthracis* in 50% glycerine saline
Viable Count: Not less than 10 million spores per ml of vaccine
Immunity: One year
Dose Route: Cow, Buffalo, Sheep (1ml), Goat (0.25 ml), Camel (2ml), Elephnat (3ml)
Storage: One year at 4ºc to 8ºc
Presentation: 100 ml/250 ml in vials

4. Enterotoxaemia
Name: Enterotoxaemia vaccine
Causative Agent: *Clostridium welchii*, Type D formaldehyde inactivated, Alum precipitated bacterial vaccine
Immunity: One year, Biannual vaccination in endemic areas
Dose Route: Sheep 2.5 ml subcutaneous (s/c)
Storage: One year at 4ºc to 8ºc
Presentation: 250 ml in vials

5. Sheep Pox
Name: Sheep Pox vaccine
Causative Agent: *Capripox virus*

Description: SPV/RF Roumann (Franner) strain of sheep pox virus propagated in lamb testes cells and maintained in freeze dried form
Virus Content: Minimum of 10,000 TCID50 of viral antigen per dose of vaccine
Immunity: 6 months
Dose Route: Sheep (1ml) subcutaneous
Storage: One year at 4ºc to 8ºc

6. Rabies Vaccine
Name: ARV (Anti Rabic Vaccine Single Dose)
Causative Agent: *Rhabdo viriadae*
Description: Whitish turbid liquid slight odour of phenol 20% BPL (β propeo lactone) inactivated sheep brain emulsion 5 ml
Immunity: One year
Dose Route: For prophylactic immunization of dogs 5 ml s/c. First dose is 3 months age booster after that 6 months yearly
Storage: 4ºc to 8ºc
Presentation: 5 ml vials

7. Swine Fever
Name: Swine fever vaccine lapinised
Causative Agent: Swine fever virus
Description: 10% spleen suspension of rabbits infected with a modified swine fever virus in yolk buffer base and freeze dried
Immunity: One year
Dose Route: 1 ml s/c in swines, pigs
Storage: One month in refrigeration at 4ºc and one year at -20ºc in deep freezer
Presentation: Number of doses contained in vials is indicated with each supply.

8. New Castle Disease
Name: Ranikhet Disease vaccine
Causative Agent: R2B virus
Description: Contains live New castle disease virus of Mukteshwar strain of chick embryo origin
Immunity: Life long
Dose Route: 0.5 ml per bird of 6 weeks via S/C or I/M
Storage: One year at -20ºc
Presentation: Each vial contains 200 doses

9. Marek's Disease Vaccine
Name: Marek's Disease vaccine
Causative Agent: Herpes virus
Description: Suspension of cell free lyophilized HVT-FC 126 strain of herpes virus of turkey grown in chicken embryo fibroblast cultures
Immunity: A single vaccination one day old chick provide life long immunity
Dose Route: 0.2 ml in one day old chick via S/C or I/M
Storage: One year from date of manufacture at 4ºc
Presentation: Available in 125/250/500 doses vials

10. Fowl Pox Vaccine
Name: Freeze dried fowl pox vaccine
Causative Agent: Fowl pox
Description: A suspension of a modified living virus prepared from the chorio-allanotic membrane of the infected embryo and is freeze dried
Immunity: Six months
Dose Route: Two punctures are made in wing web with modified swing needle or vaccination lancets dipped in the reconstituted vaccine. The vaccinated birds should be examined for "Takes" for 7 days
Storage: For one year at -20ºc and for one month at 4ºc
Presentation: Each vial contains 100 doses

2. Common Media Used for the Preparation of Different Vaccines

A) Growth Media:
- EHSS: 100 ml
- LAH: 5 gm
- Yeast Extract: 0.15 gms
- Sodium Bicarbonate: 0.75 gms
- Serum: 100 ml
- Distilled Water: 1000 ml

B) Maintenance Media:
- EHSS: 100 ml
- LAH: 5 gms
- Yeast Extract: 0.15 gms
- Sodium Bicarbonate: 1.5 gms
- Serum: 20 ml
- Distilled Water: 1000 ml

C) Eagle's High Salt Solution (EHSS):
- Sodium Chloride: 14 gms
- Potassium Chloride: 0.80 gms
- Calcium Chloride: 0.40 gms
- Magnesium Sulphate: 0.40 gms
- Glucose: 5 gms
- Phenol Red Solution: 10 ml
- Distilled Water: 200 ml
- Trypsin Solution: 1 gm
- Phosphate Buffer Solution: 400 ml

D) Phosphate Buffer Solution:
- Sodium Chloride: 16 gms
- Potassium Chloride: 0.40 gms
- Sodium Dihydrogen Phosphate: 2.80 gms
- Mono potassium phosphate: 0.40 gms
- Calcium Chloride: 0.20 gms
- Magnesium Chloride: 0.42 gms

E) Trypsin Solution:
- Trypsin (1:250): 0.1 gm
- Phosphate Buffered Saline (PBS): 400 ml

F) Phenol Red Solution:
- Phenol Red Powder: 0.40 gms
- Distilled Water: 400 ml
- O.5 N Sodium Hydroxide: Q.S.

G) Minimum Essential Medium (MEM):
- MEM Dry Medium: 1 vial
- Sodium Bicarbonate: 2.20 gms
- Antibiotics: Q.S.
- Serum: 20 ml

- Distilled Water: 1 litre

H) Trypsin Versene Solution:
- Sodium Chloride: 4 gms
- Potassium Chloride: 0.20 gms
- Sodium Bicarbonate: 0.29 gms
- Dextrose: 0.50 gms
- Trypsin: 0.25 gms
- Versene: 0.10 gms
- Phenol Red Solution: 12.50 ml
- Distilled Water: 500 ml

I) Sucrose Phosphate Glutamic Acid (SGPA):
- Sucrose: 37.31 gms
- Dipotassium Phosphate: 0.625 gms
- Sodium Dihydrogen Phosphate: 6.296 gms
- Glutamic Acid: 0.415 gms
- Bovine Albumin: 5 gms
- Antibiotics: Q.S.
- Distilled Water: 500 ml

3. Media Used for the Production of Bacterial Vaccines

1. Media Preparation For Haemorrhagic Septicemia
- Meat was boiled in equal quantity of water in order to remove fatty material.
- After that filtration was done with muslin cloth.
- Chemicals such as 1% peptone, 0.5% sodium chloride and water was added in equal proportions.
- The above mixture was boiled and pH was adjusted at 7.4.
- Finally autoclave was done at 15 lbs pressure for 45 minutes at 121ºc.

2. Media Preparation For Black Quarter Vaccine

a) Production Flask:
- 4 parts of meat and 1 part of liver was mixed together and equal quantity of water was added to it.
- The above mixture was filtered out and 0,06% of thioglycolic acid and 1% peptone was added.
- To the above solution 0.5% sodium chloride and 0.4% dipotassium hydroxyphosphate was added.
- Then it was boiled and pH was adjusted at 8.4.
- Finally autoclave was done at 15 lbs for 45 minutes at 121ºc.

b) Seed Flasks:
- Meat particles were boiled with distilled water.
- Then 1% glucose was added and pH was adjusted at 8.4.
- Finally autoclave was done.

3.1 Protocol for the Production of Haemorrhagic Septicemia

Production of Seed Culture
- Pasturella multocida P52 phase I was inoculated in buffalo calf by intra- muscular (I/M) or sub-cutaneous (S/C).
- The blood was collected from calf's heart at phase I.
- Blood was tested for purity.
- Plating is performed in blood agar to grow colonies of P. multocida organism.
- Blood agar slants were prepared from which vaccine was produced.

Production Protocol for Vaccine (Alum Precipitated)
- Inoculation of seed flasks of broth with P. mutocida from slant.
- Incubation for 18 hrs at 37ºc.
- Testing for purity of seed flasks.
- Inoculation of production flasks with material from pure seed flasks.
- Incubation for 18 hrs at 37ºc temperature
- Testing for purity of production flasks.
- Addition of 0.5 % formaline to each flasks.
- Incubation for 48 hrs at 37ºc temperature.
- Pooling of the material in drum from mature flasks.
- Addition of 1% alum.
- pH adjustment at 7.2- 7.4 by adding sodium hydroxide solution.
- Sterility test of drum pulled material.
- If sterile, filling and sealing in bottles in 250 ml quantity.
- Storage at 4ºc till supply.

3.2 Protocol for the Production of Anthrax Vaccine

- The stock seed ie avirulent anthrax spore vaccine strain *Bacillus anthracis* is streaked on plain agar and incubated for 18 hrs at 37ºc.
- The pure colony character is observed under low magnification.
- Single colony showing medusa head appearance is picked up and incubated in nutrient broth and incubated for 24 hrs.
- Purity by hanging drop and smear examination.
- Non-motile, Gram's positive showing long chains were inoculated from plate to plain agar slant for stock seed.
- Inoculate roux flask spread evenly by to and fro movement and incubate for 96 hours at 37ºc.
- Roux flasks showing pure growth are selected and harvested by adding 10-15 ml normal saline solution with sterilized glass beads. And then the harvested material is collected.
- 50% of glycerine saline was added by (w/w) to harvest material and pH was adjusted to 7.2.
- The above material was kept at room temperature for 21 days.
- Safety by inoculating dilute solution (1:5) into two healthy guinea pigs 0.5 ml and 0.2 ml (s/c) and observed for 7 days.
- Spore count of glycerinated harvest in different dilutions.
- After satisfactory report for safety and potency of harvest and bottling of anthrax spore vaccine in 200 ml vials 50% glycerine was added and harvested to spore count pooled material which should contain atleast 15 million spores per ml.
- Each bottle is filled with 200 ml vaccine and stored in cold temperature after labeling.

3.3 Protocol for the Production of Enterotoxaemia Vaccine

- Innoculation of seed flask with Clostridium welchii type 'D'
- Incubation for 6hrs at 37ºc.
- Purity test on blood agar slant and examination for morphological characters of organisms by smear examination.
- Inoculation of production flasks with actively growing culture of Clostridium welchii type 'D' and add 0.2% sterilized dextrose.
- Incubation for 24 hrs at 37ºc.
- Purity test and smear examination.
- pH adjustment of individual flask to 7.
- Proto-toxins is ready which is tested in mice.
- 0.25% of trypsin is added for conversion of proto-toxin to epsilon.
- Incubation for 1.30 hrs at 37ºc.
- Epsilon toxin tested in mice in mice in different dilution viz. 1000, 2000, 4000, 8000.
- Formalization with 0.5% formalin for conversion of epsilon toxin to toxoid.
- Incubation for 11- 18 days at 37ºc.
- Test for toxicity in mice.
- Pulling of formalized material.
- Sterility test for pooled vaccine for aerobic, anaerobic and fungal contaminant.
- Sterility is observed for 7 days.
- Bottling of sterile vaccine in 250 ml/ bottle.
- Vaccine is stored at 2-4ºc.

3.4 Protocol for the Production of Black Quarter Vaccine

- Inoculation of seed flasks with pure seed strain of Clostridium chauvoei.
- Incubation for 24-48 hrs at 37ºc.
- Purity test on blood agar slant and examination of morphological characters of organiss by smear.
- Inoculation of production flasks with Clostridium chauvoei culture and addition of 30 ml of 50% glucose.
- Incubation for 3-5 days at 37ºc.
- Formalisation by 0.5% of formalin.
- Purity test and smear examination.
- Incubation for 24-96 hrs at 37ºc.
- Pooling of formalized material.
- pH adjustment at 7.4.
- Sterility test of pooled vaccine for aerobic, anaerobic and fungal contaminants.
- Sterility is observed for 7 days.
- Bottling in 250 ml bottle and stored at 4-10ºc.

3.5 Protocol for the Production of Sheep Pox Vaccine

- Testicular tissue collected aseptically from healthy lamb at pre puberty age after moving fascia and capsule and washed thrice in chilled protein basal solution.
- Mixing of tissue and washing with phosphate buffer solution twice and with 25% trypsin once.
- Supernatant was taken after each trypsinization (37ºc, 20 minutes, for 3-4 times) in 5-10 ml growth media and centrifuged at 1000 rpm for 10 minutes at 4ºc after filtration through muslin cloth.
- Cell suspension is prepared to have 6.58*106/ ml after washing of the cells with phosphate buffer solution twice and with growth media once and disturbed in roux flask in 900 ml bottle kept at 37ºc.
- Monolayer completion in 72-96 hrs then washing with prewormed phosphate buffer solution and addition of 5 ml trypsin versin glutamic acid solution at 37ºc.
- Trypsin decanted as cells start decanting and 10% growth medium is added and flask shaken to remove the cells. The cell clumps are broken with pipette and dispersed in double the original volume 200 ml of growth medium cell concentration 104.5 to 105/ml.
- Growth media discarded after secondary cell monolayer completion at 48-72 hrs and monolayer is washed with prewarmed phosphate buffer solution.
- Adsorption for 45 minutes at room temperature after infecting monolayer with diluted seed virus.
- Inoculation decanted and maintenance media is added to each flask and kept at 37ºc.
- More than 80% CPE (roundening and detachment by 120-144 hrs flask transferred to -20ºc.
- After 3 cycles of thawing at 37ºc and freezing at -20ºc an equal quantity of chilled sterile stabilizer is added and distributed in sterilized vials in 0.5 ml quantity.
- After freeze drying, sealing labeling of vials.
- Finally storage at a temperature between 4ºc-8ºc.

3.6 Protocol for the Production of Anti-Rabies Vaccine (ARV)

- Selection of long eared, young healthy sheep without permanent teeth and above 12 kg body weight.
- After final selection the body temperature and other illness was recorded, shearing and clipping of hairs from head region.
- Intra-cisternal inoculation of sheep with (1:100) dilution of fixed rabies virus (0.5 ml).
- Slaughter of sheep and collection of brain aseptically in separate containers after performing sterility test of brain on blood agar slant and weigh brain individually.
- Storage in low temperature at -20ºc till emulsion.
- Preservatives are added between 48-72 hrs after emulsion; such as Thiomersal is added at 1: 1000 dilution and Phenol is added at 0.02% dilution and required quantity of normal saline solution was also added to make 5% sheep brain emulsion.
- Pooling of sterile flasks in big containers.
- Finally, pooled sample was tested for virulence test in mice and toxicity test in guinea pigs.

3.7 Protocol for the Production of Swine Fever Vaccine

- Healthy rabbits of 1kg -1.5 kg were taken and 10-2 dilution of seed virus was injected intra-muscularly (I/M) in the ear vein.
- Temperature was noted for continuous 6 days.
- After 5 to 6 days rabbits which have 2ºF higher temperature than normal were selected and harvested.
- Spleen and mesenteric lymph nodes was collected aseptically under a laminar air flow.

- 10% spleen suspension and 10% egg yolk was made in phosphate buffer saline (PBS).
- The above solution was filtered out to remove coarse particles.
- The above suspension was filtered in 0.5 ml per vial.
- Finally the vial was freeze dried, sealed and stored at -20ºc in a deep freezer.

3.8 Protocol for the Production of Ranikhet Regular Vaccine (R2b)

- Nine to ten days old embryonated eggs were selected
- A mark was done at the air sac and at a place where there is no blood vessel and was punched with a pin.
- The above eggs were then inoculated with R2B seed virus in normal saline solution with 0.1 ml/egg by chlorioannantoic sac route.
- The eggs were then incubated at 37ºc for 48 hrs.
- The dead eggs were selected on second day for harvest
- The eggs were then chilled for 2 hrs.
- The dead embryos and chlorioallantoic fluid were collected separately and marked as spot.
- Embryos and chlorioallantoic fluid were then blended and filtered.
- The above suspension was filled at 0.5 ml per vial.
- Finally freeze drying, sealing was done and the suspension was stored at -20ºc.

3.9 Protocol for the Production of Marek's Disease Vaccine

- Chick embryo tissue collected from 9-10 days old embryo after removing head, legs and viscera and washed with PBS (Phosphate Buffer Saline) solution.
- After mincing the tissue, wash 3 times with PBS (Phosphate Buffer Saline) solution and once with 0.25% trypsin.
- After each trypsinisation supernatant is taken at 37ºc for 10 minutes for 3-4 times and centrifuge at 1000 rpm for 10 minutes at 4ºc.
- Cells were then mixed with MEM (Minimum Essential Medium) and again centrifuge two times.
- Cells were collected and distributed to Roux flasks with 90 ml growth media (GM) and kept at 37ºc.
- After monolayer completion occurred media was discarded and the layer was washed with PBS.
- Infection is given at 1 P.F.U/100 cells.
- After adsorption at 37ºc for 45 minutes, maintenance media is added to each flask and kept for incubation at 37ºc.
- CPE (Cytopathic Effect) ie roudening and plaque formation completed after 72 hrs of infection.
- Media was discarded and a sheet was detached with the help of (T.P.V.G) Trypsin Versus Glutamic Acid and then 10-20 ml SPGA (Sucrose Phosphate Glutamic Acid) was added to each flasks and collected and then centrifuged at 1000 rpm for 10 minutes at 4ºc.
- Cells were then centrifuged twice with SGPA (Sucrose Phosphate Glutamic Acid) then collected at 6-10 ml each SPGA flask and kept at -20ºc.
- Material was pooled after thawing ultra-sonication (3 times) was done then finally it was filled in a sterilized vials in 0.5 ml quantity.
- After freeze-drying, sealing was done and then it was labeled and stored at -20ºc.
- Sterility test and titration of the final product.

3.10 Protocol for the Production of Fowl Pox Vaccine

- 12 days old embryonated eggs were selected and at a point was marked over the air sac and another point avoiding any blood vessels.
- Suction was applied through the hole at the air sac.
- Depending upon Chlorio-Allantoic Membrane (CAM) 0.1 ml of 10-2 dilution of seed virus was inoculated in the false air sac (CAM route).
- The eggs were then incubated for 4 days at 37ºc discharging the dead eggs daily.
- The live eggs were then selected on the 4th day.
- The eggs were then chilled for 2hrs.
- The CAM was collected and blended on nutrient broth tube.
- The above suspension was then filled at 0.5 ml per vial.
- Finally the vial was freeze dried, sealed and stored at -20ºc.

4. Quality Control

To protect both the purity of the vaccine and the safety of the workers who make and package the vaccine, conditions of laboratory cleanliness are observed throughout the procedure. All transfers of virus and media are conducted under sterile conditions, and all instruments used are sterilized in an autoclave before and after use. Workers performing the procedures wear protective clothing which includes disposable gowns, gloves, booties, hair nets, and face masks. The manufacturing room themselves are specially air conditioned so that there is a minimal number of a particle in the air.

5. Standardization of Vaccines

(A) Testing Protocol for Bacterial Vaccines

1. Name of Vaccine: Anthrax
Sterility Test: FTM/SCDM
Safety Test: Carry out test in goat administer twice the dose of vaccine (2 million spore) in each of two sero negative goat and observe animal for 10 days.
Potency Test: Carry out the test in 5 healthy sheep weighing not less than 20 kg. Inject 1/10 of the dose of vaccine in each animal and observe it for 2 days.
Challenge each sheep with at least 100 ml of two control sheep with 10 ml of pathogenic strain of *Bacillus anthracis*. Observe animal for 10 days. All vaccinated animal survive and all control animal die from anthrax. During observation period determine the number of viable spore by plate count.

2. Name of Vaccine: Black Quarter
Sterility Test: FTM/SCDM
Safety Test: Inject two healthy guinea pigs weighing 300-400 gm with recommended dose 9 s/c. Observe animal for 7 days.
Potency Test: Inject ten healthy guineas pig weighing 300-

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 37ºc 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: Hamorragic septicemia
Sterility Test: FTM/SCDM
Safety Test: Inject intraperitonically 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}. LD_{50} 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 $50/10^{5}$
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/ 10^{3}

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 $50/10^{2}$

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) Saboruaud'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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