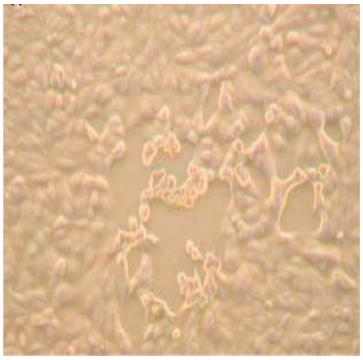
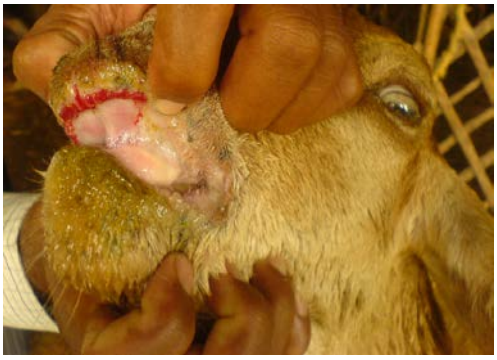
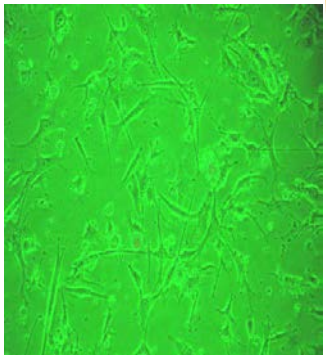
Laboratory Manual VMC 321

Systematic Veterinary Virology

LABORATORY MANUAL
FOR
VETERINARY MICROBIOLOGY

SYSTEMATIC VETERINARY VIROLOGY

VMC-321 (New Syllabus)

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LABORATORY MANUAL
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CERTIFICATE

Certified that this is a bonafide record of practical work done in the laboratory for the course of **Systematic Veterinary Virology** course No. VMC-321 during the year_____.

Name of the student: _____

Registration No.: _____

Exam seat No.: _____

Course Teacher

SEMESTER END EXAMINATION

Evaluated The Practical Record Submitted For The SEMESTER END Practical Examination Held On _____.

Course Teacher **Sectional Head**

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Practical No.1

PREPARATION OF EQUIPMENTS & GLASSWARE FOR VIROLOGICAL WORK

Preparation of new glassware:

New glasswares require special attention. Because resistant spores which may be present in the straw and other packaging material and also because it tends to give off free alkali which may be sufficient to interfere with the growth of certain organisms.

- New glasswares should be treated with/placed in 1% HCl/Picric acid overnight. Washed under tap water followed by distilled water. Air-dry and subject to sterilization.

- Screw capped bottles are subjected to special cleansing process by the makers whereby the surface alkali is removed and above treatment is unnecessary. The bottles may be used without treatment of acid.

Washing of glasswares for tissue culture:

Tissue culture / cells are very sensitive to minute traces of toxic substances.

- Autoclave the glasswares (test tubes, Rous flask) with rubber bang after use. Remove bangs and rinse in hot running tap water.
- Boil for 10 minutes in demineralised water in boiler with flakes of soap. Brush the tubes / Rous flask after removing from the boiler.
- Rinse in hot running tap water.
- Wash with hot demineralized water containing neutral detergent (inorganic detergent) followed by through washing by emptying and filling.
- Allow to stand overnight.
- Rinse in hot running tap water at least 4 times.
- Rinse 3 times in distilled water. Drain and dry in drying cabinet.
- Cover with aluminium foil or paper.
- Sterilized in Hot air oven i.e., 160 ^{O}C for 1 hour.

Discarded cultures can be placed in 3% Lysol after use/transferred directly to boiling.

Cleaning of pipettes:

- If contaminated with infective material use 3%; Lysol solution for overnight.
- Rinse under tap water.
- Steep overnight in dichromate/ sulphuric acid cleaning fluid. (Dissolve 63 gm of sodium/potassium dichromate by heating with 35 ml water. Cool and concentrated H_2SO_4 to 1 liter.
- Wash with tap water in an automatic pipette washer.
- Connect the pipette to a water pump and draw thorough distilled water followed by acetone.
- Air until dry.
- Top end is plugged with cotton wool.
- Place the pipette in copper/steel pipette holder/cylinders.
- Place in hot-air-oven at $160^{O}C$ for 1 hour.

Accurately calibrated volumetric glassware should never be heated in hot-air-oven, since expansion and contraction of the glass makes the graduations inaccurate.

Exercise:

Q1.Enlist the different instruments used in virological procedures.

Write the use and method of sterilization.

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Practical No. 2

PREPARATION OF MEDIA, SOLUTIONS, BUFFERS AND THEIR STERILIZATION

Preparation of media,solutions & buffers:

A.Transport media/preservatives, Anticoagulants for collection of blood:

50% Glycerine Phosphate Buffer Saline(GPB).

First prepare M/25 Phosphate Buffer

Phosphate Buffer Saline(PBS)(pH 7.4).

Saline (pH 7.4-7.6) as follows:

Sodium chloride	8.0gm
Potassium chloride	0.2 gm
Di sodium hydrogen phosphate	1.15 gm
Potassium Di-hydrogen phosphate	0.20 gm
Distilled water	1000ml

Add equal volume of sterile neutral glycerin to sterile M/25 PBS (pH 7.4-7.6) to prepare 50/5 GPBS of pH 7.4. Add 0.1ml of 1% phenol red solution to 100 ml of that solution so as to give a final concentration of phenol red to 0.001%. The solution when sterile should have reddish tinge. Yellow color indicates contamination. Each vial must contain 10mlk of PBS for collection of tissues.

Anticoagulants for collection of blood:

Heparin: 5-6 IU units/ml of blood

EDTA: 1-2 mg /ml of blood

Oxalate phenol glycerin OCG solution: 1 part to 2 parts of blood.

Blood is transported in chilled condition.,but should never frozen for both clinical examination and for virus isolation. Antibiotics can be added.

Sterilization of of tissue culture media / thermolabile liquids :

The various types of filters used for clarifying or to remove the bacteria, fungi from the thermolabile liquids,media,solutions & buffers are as follows:

1. Earthenware Candles e.g., Berkfeld, Chamberland filters.
2. Asbestos Paper Disks e.g., Seitz Filter.
3. Sintered Glass Filters.
4. Membrane Filters.

1.Earthenware Candles

Berkfeld Filters:

Made from kieselguhr, a fossil diatomaceous earth found in deposits in Germany.

Filters are of coarse type owing to the size of the granules forming the substance the substance of filter.

Made in three grades of porosity:

V: Veil (the coarsest) do not allow the *Serratia marscens,* the test bacteria to pass through).

W: Wenig (the finest).

N: Normal (the intermediate)

Filters can be sterilized by steaming/autoclaving. Filters should be brushed with a stiff nailbrush and than boiled in distilled water. When clogged with organic matter heated to redness in a muffle furnace and allowed to cool slowly.

Chamberland Filters:

Made up of unglazed porcelain and are produced in various grades of porosity, the finest grade allows only small viruses such as FMD virus, Circovirus.

Most porous grades **L_{1a}, L_2,** and **L_3** are comparable with **V, N,** and **W** candles respectively.

2. Asbestos Paper Disk Filters.

Seitz Filters:

Disk of Asbestos is inserted into a metal holder (14 cm in diameter-Large size).

Various sizes are available.

Made in three grades of porosity:

K : Clarifying.

N : Normal.

EK: Special grade.

Do not allow *Serratia marscens,* the test bacteria to pass.

For sterilization the filter is loosely assembled wit

and the delivery tube passed through a rubber bang when filtering flask if used. The whole assembly is wrapped in Kraft paper and sterilized in autoclave. Plug the filtration flask and the side arm is fitted with an air filter.

Before using flush the disk with sterile saline and then screw down tightly the metal holder.

3.Sintered Glass Filters:

Made up of finely ground glass fused sufficiently to make small particles adhere, giving uniform average pore diameter (APD).

Manufactured in three grades of porosity:

Grade 5 : Finest. **Grade**

3 : Coarsest. **Grade5/3:**

Special grade

After use sintered glass filters are washed with running water in the reverse direction. They should be cleaned with warm sulphuric acid + potassium nitrate.

4. Membrane Filters:

Two types of cellulose acetate membrane filters are available:

-Older type (Gradocol membrane) is composed of cellulose nitrate whereas the

-Modern membrane filters in use nowadays are made up of Cellulose acetate.

Gradocol membranes: Made in different grades with average pore diameter ranging from 3um to 10 nm. Used to determine the size of many viruses.

Modern membrane filters (Cellulose acetate):

Developed by Millipore Filter Corporation in America.

Made in variety of sizes from 1.7cm to 14 cm and can be fitted into metal or glass holder/syringe filter holder.

Cellulose acetate filters are composed of two layers, a basal layer with pores of 3-5um and an upper layer with pores of 0.5-1.0 um in diameter.

Sterilized by autoclaving at $121^{O}C$ for 35-40 mins.

Cellulose acetate membranes are less absorptive when compared with Seitz filter and the rate of filtration is high. Also do not alter the pH of media passed through.

Exercise:

Q1. Write the composition of MEM & HBSS in detail.

Q2. Draw well labeled diagram showing the process of filtration using negative pressure.

Q3. How to prepare the growth medium for animal cell culture.

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Practical No. 3

DEMONSTRATION OF PRIMARY CELL CULTURE, PREPARATION AND SUBCULTURING OF THE CELL LINE

Material Collected: Scabs in sterile containers on ice, scabs in 50%buffered glycerin.

Preparation Of Lamb Kidney (LK) Cells:

1. Collect lamb kidney from a (young) wool sheep breed aseptically in a beaker containing Hank's Balanced Salt Solution.
2. Transfer the kidneys to sterile petri dish, peel off the capsule and cut the kidneys half longitudinally. Cut the cortex portion with sterilized curved scissors and mince finely.
3. Transfer the minced tissue in a 500ml-trypsinizing flask and wash thrice with HBSS.
4. Add trypsin (1:250 dilution) solution to the minced tissue and allow to stir about 30-40 minutes at room temperature.
5. After trypsinization, filter cell suspension through 3 layers of cheesecloth and pour cell suspension in a conical graduated centrifuge tube of 50ml capacity.
6. Centrifuge the suspension in a refrigerated centrifuge for 10 minutes at 1500 RPM. Decant the supernatant fluid, add equal amount of fresh BSS to the tube, mix the contents and repeat washing twice.
7. Collect 2 ml cell suspension and add to the 25 cm^2 tissue culture flask with suitable growth media.
8. Incubate at $37^{O}C$(CO_2 incubator) for 3-5 days.
9. Examine the monolayer cell sheet under inverted microscope and preserve for further processing by replacing the growth medium with maintenance medium.

Exercise:

Q1. Enlist the continuous cell lines used for the cultivation of animal viruses.

Q2. How to prepare the Chicken Embryo Fibroblast Cell Culture. Write the procedure in detail.

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Practical No. 4

CHICK EMBRYO INOCULATION AND HARVESTING OF VIRUS:

ALLANTOIC & CHORIO- ALLANTOIC ROUTE

CULTIVATION OF RD VIRUS BY ALLANTOIC ROUTE OF INOCULATION

Material required: Spleen, lung, kidney tissue from RD suspected bird, 10-11 day old embryonated chicken eggs 5 nos. , drill machine, egg candler, egg incubator, syringes, needles, forceps, scissors, petridish, tincture of iodine, melted paraffin, phosphate buffer saline, penicillin & streptomycin.

Procedure:

1. Candle the egg and mark an area of CAM away from the from the embryo and amniotic cavity .The area should be free from large blood vessels and about 3 mm below the base of the air-cell. Make a pencil mark in the area at the point of inoculation.
2. Make another mark on the upper end of the air sac of the eggs.
3. Drill a small hole through the shell at each mark but do not pierce the shell membrane.
4. Apply tincture of iodine to the holes and allow drying.
5. .With sterile precautions, make a small puncture in the shell membrane in the hole over the air sac. The hole over the air-cell is necessary for air vent to allow the equalization of pressure produced by the inoculum within the egg and to prevent the inoculum and embryo fluid from escaping through the hole on the side of the egg.
6. Inoculate 0.2 ml inoculum through the hole in the side of the egg to a depth of about ¼ inch. Use 1ml tuberculin syringe with 27-gauge ½ inch needle.
7. Seal the two holes with melted paraffin or suitable liquid adhesive.
8. Incubate the eggs for 4-5 days.

9. For collection of allantoic fluid(harvesting), apply disinfectant to the shell over air sac. Break the shell over air sac with forceps and remove the shell to a distance of about 8-10 mm from the top of the air sac.

10. With the help of a 10 ml syringe and 22-gauge 1-inch needle, collect about 5ml of allantoic fluid from the cavity through the air sac opening and expel the fluid in the container.

CULTIVATION OF FOWL POX VIRUS BY CHORIO ALLANTOIC MEMBRANE ROUTE OF INOCULATION

Fowl poxvirus can be isolated by the inoculation of suspected material into embryonated chicken eggs. Approximately 0.1 ml of tissue suspension of skin or diphtheritic lesions, with the appropriate concentration of antibiotics, is inoculated on to the chorioallantoic membranes (CAMs) of 9–12 days old developing chicken embryos. These are incubated at 37°C for 5–7 days, and then examined for focal white pock lesions or generalized thickening of the CAMs.

Material required: drill machine, egg candler, egg incubator, syringes, needles, forceps, scissors, petridish, tincture of iodine, melted paraffin, phosphate buffer saline, penicillin & streptomycin.

Procedure:

1. Candle the eggs and mark the position of the embryo.
2. Keep the long axis of the egg in horizontal position with embryo uppermost; mark an equilateral triangle with each side about 1 cm, equidistant between the ends of the egg.
3. With the help of small carborundum disc, cut the eggshell at the marks but do not pierce the shell membrane.
4. With needle, pierce the shell membrane over the air sac.
5. Apply tincture iodine to the grooves cut by the carborundum disc and allow drying.

6. Gently remove the triangle of shell to expose shell membrane without rupturing the chorioallantoic membrane (CAM).
7. Using a teasing needle, pierce the exposed shell membrane on the side of the egg but do not pierce the CAM.
8. Place 0.1 ml of inoculum (0.1 ml of tissue suspension of skin or diphtheritic lesions) over the pierced point on the side of the egg and create a slight vacuum with a small rubber at the hole over the air sac by sucking the air through the air bulb. Inoculum over the shell membrane will pass through the opening in the shell membrane on the side of the side of the egg allowing the CAM to drop from the shell membrane underneath the triangular area and the inoculum will be taken in due to negative pressure created at the opening i.e., triangular area on the side of the egg.
9. Close the triangular opening in the shell on the side as well as over the air sac with the help of cut shell flap removed and melted paraffin wax or suitable adhesive tape.
10. Incubate the egg for 5-7 days at 37^{O} C maintaing the humidity.
11. Candle the eggs daily & discard the mortality of embryo within 24 hours of inoculation.
12. After 5-7 days remove all the eggs from the incubator, kill the embryos by chilling and collect CAM. Wash the CAM in a petridish with normal saline and examine.

Exercise:

Q1.Explain the selection of eggs for isolation of virus.

Q2.Explain-SPF eggs. Write the source for SPF eggs in India.

Q3.Write the findings on Chorioallantoic membrane (CAM) with poxvirus isolation.

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Practical No. 5

CHICK EMBRYO INOCULATION AND HARVESTING OF VIRUS: YOLK SAC ROUTES

CULTIVATION OF AVIAN ENCEPHALITIS VIRUS IN EMBRYONATED CHICKEN EGGS BY YOLK SAC ROUTE

Material required:

Brain tissue from the birds infected with AE virus- processed inoculum (10% emulsion in PBS-Practical No.3), incubated 7-8 day old embryonated chicken eggs, egg candler, egg incubator, syringes, needles, forceps, scissors, petridish, tincture of iodine, melted paraffin, phosphate buffer saline, penicillin, streptomycin & centrifuge machine.

Procedure:

1. Candle the egg with long axis in the horizontal plane and locate the yolk sac. Make a mark on the shell over the yolk sac about half way from the small end of the egg to the apex of the curvature of the shell.
2. Drill a small hole through the shell at the mark avoiding piercing of the shell membrane.
3. Using a 1 ml tuberculin syringe fitted with a 27 gauge needle, 1.25 needle insert the full length needle ion the long axis through the hole and inject the inoculum (0.2 ml of 10% emulsion of brain in PBS from suspected bird).
4. Seal the hole with melted paraffin/ suitable adhesive tape.
5. Incubate the egg for 5-7 days at 37^{O} C maintaing the humidity.
6. Candle the eggs daily & discard the mortality of embryo within 24 hours of inoculation.
7. Remove the dead embryos, if any and all the remaining embryos after 3 days of incubation, chill them and collect yolk, yolk sac and brain of the embryos.
8. Preserve the material in sterilized screw capped tubes in the deep freeze till further use.

Exercise:

Q1. Enlist the inoculation routes for isolation of various virus.

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Practical No. 6

ANIMAL INOCULATION FOR CULTIVATION OF VIRUSES

GUINEA PIG FOOT PAD INOCULATION METHOD FOR ISOLATION OF FMD VIRUS

Material Required /Clinical specimen:

1 ml of lymph from an unruptured or recently ruptured cattle tongue vesicle / epithelium samples.Oesophageal-pharyngeal fluid collected by means of a probang cup.

Two month old Guinea pig 2 Nos., 25 gauge needle, syringe etc.

Preparation of inoculum:

Take out the frozen specimen from deep freeze and thaw at room temperature.Transfer the specimen (ruptured mucosal /tongue epithelium/tissue collected) to the mortar.Cut into small pieces .Add small quantity of sterile sand & PBS (to make it 10-20% specimen) and grind into a fine paste.Centrifuge and collect supernatent.Add the antibiotics to avoid contamination i.e.,Penicillin @ 10,000 units and streptomycin @ 10 mg per ml and incubate for 30 minutes.

Inoculation Procedure :

1. Prepare one hind foot pad of each guinea pig.Clean the foot pad with 70% alcohol and dry.
2. Mark one longitudinal line on the sterilized foot pad of each guinea pig.
3. Intradermally inoculate 0.2 ml of above prepared inoculum with 25 gauge needle at five equyidistant spots on the marked line on foot pads.
4. The Guinea pigs are maintained on good bedding and observed daily for the the lesions on the foot pad and clinical signs.
5. Lesions in positive cases will be evident on 4^{th} or 5^{th} day of inoculation.

   The skin from fully developed lesions is removed and stored in deep freezer till further processing.

Exercise:

Q1. Describe the clinical signs and lesions of FMD in cattle.

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Practical No. 7

INFECTION OF CELL CULTURES WITH VIRUS AND STUDY OF CYTOPATHIC EFFECTS DEMONSTRATIONS

Preparation of inoculum:

Take out the frozen specimen from deep freeze and thaw at room temperature.Transfer the specimen (tissue collected) to the mortar.Cut into small pieces .Add small quantity of sterile sand & PBS (to make it 10-20% specimen) and grind into a fine paste.Centrifuge and collect supernatent.Add the antibiotics to avoid contamination i.e.,Penicillin @ 10,000 units and streptomycin @ 10 mg per ml and incubate for 30 minutes.Use for inoculation.

Preparation of blood for isolation of virus:

Blood collected using suitable anticoagulant is stored at $4^{O}C$ till further processing.(some viruses loose the infectivity when stored at 4 ^{O}C). At the time of inoculation take out the blood from refrigerator bring it to the room temperature, either centrifuge it to collect the buffy coat used in many virus isolation procedures or subject it to **Ultra–sonicator**, centrifuge , collect the supernatant . Add the antibiotics to avoid contamination i.e.,Penicillin @ 10,000 units and streptomycin @ 10 mg per ml and incubate for 30 minutes.Use for inoculation.

Pass on the inoculum through Sintered glass filter,cellulose acetate membrane filters with average pore diameter of 1.2u,0.8u and 0.2 u to remove the debris,bacterial cell etc, to prevent the contamination i.e., bacterial and fungal.

Inoculation:

1. One ml of clarified biopsy preparation supernatant is inoculated on to a 25 cm^2 tissue culture flask of 90% confluent LT or LK cells.
2. Allow to absorb for 1 hour at 37°C.
3. The culture is then washed with warm PBS and covered with 10 ml of a suitable medium, such as GMEM, containing antibiotics and 2% fetal calf serum.

4. The flasks should be examined daily for **14 days for evidence of cytopathic effect (CPE)**, and the medium is replaced if it appears to be cloudy. Infected cells develop a characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and eventually r**ounding of cells and margination of the nuclear chromatin, characterized** by grape-like clusters of rounded cells gathered around a hole in the monolayer; sometimes giant cells with several nuclei may be observed. Experience is needed to recognize this characteristic appearance.
5. If no CPE is apparent by day 14,blind passage must be made. The culture should be freeze/thawed three times, and clarified supernatant inoculated on to fresh LT or LK culture.
6. The cell cultures are observed daily for CPE, which usually appears within 3 days after inoculation.
7. After third passage, if there is no CPE, declare the sample negative for presumptive isolation of virus using cell culture.

Exercise:

Q1. Enlist the Cytopathic Effect (CPE) for various animal viruses.

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Practical No. 8

STUDY OF VIRAL INCULSIONS: DETECTION OF NEGRI BODIES IN BRAIN IMPRESSION SMEARS STAINED BY SELLER'S STAIN

For demonstration of Negri bodies accurately in paraffin sections as well as in impression smears Seller's stain is the simplest and preferred method. Best results are obtained when brain tissue is fresh as decomposition makes demonstration of Negri bodies difficult.

Seller's stain:

Stock solutions:

a. Methylene blue	1.0 G
Methanol	100 ml
b. Basic Fuchsin	1.0 G
Methanol	100 ml

Working solutions:

Methylene blue	6.0 ml
Basic Fuchsin	20.0 ml
Methanol	50 ml

Staining Method:

1. For Impression smears:

i. Prepare the impression smear.

ii. Immerse the moist smear in the working solution for about 5 seconds.

iii. Rinse in tap water. & Dry at room temperature without using blotting paper.

iv. Mount the slide and read.

2. For Paraffin sections:

i. Free the paraffin by serially dipping in 100% to 50% alcohol.

ii. Rinse in distilled water.

iii. Immerse the slide in the working solution for 2 to 10 minutes.

iii. Wash in running tap water & Mount the slide and read.

Exercise:

Q1. Perform the test and write the findings.

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Practical No. 9

COLLECTION , PRESERVATION AND TRANSPORT OF CLINICALSPECIMEN FOR VIROLOGICAL INVESTIGATIONS

1.PPR: Eye (ocular swab), mouth and rectal swabs in PBS on ice, about 10ml or more blood at the height of body temperature in anticoagulant, Prescapular lymph nodes, spleen, tonsil etc. in HBSS and on ice. Tissue materials from 5 to 6 or more animals be collected and dispatched for better picture of disease / outbreak. Cotton buds can be used as swabs.

2.Foot And Mouth Disease: Vesicular fluid from unruptured oral vesicles and curetted epithelium from fresh lesions oeso-pharyngeal fluid in 50% PBS preferably on ice. About 10 ml blood at the height of body temperature in EDTA/heparin.

3.Bovine Spongiform Encepahalopathy: Tissues from brain stem involving medulla oblongata at the level of obex, posterior cerebellar peduncle, rostral quadrigemina body and spinal cord in 10% formal saline for histopathological examination and for prion detection very thin pieces of above sites of brain in frozen state.

4.Bovine Virus Diarrhea / Mucosal Disease: Blood in EDTA, paired serum samples, semen, intestinal swabs, lymph nodes and spleen on ice.

5.Bovine Malignant catarrhal Fever (BMCF) : Blood in EDTA, Paired serum samples, all internal organs including cornea, skin, muzzle on ice.

6.Rabies: Half portion of brain, salivary gland in 50% phosphate buffered glycerin in leak proof hard box and the rest half portion in 10% neutral formol saline solution. Alternative and preferable small pieces from hippocampus and brain (cerebellum, medulla, cerebrum spinal cord) in 50% buffered glycerin and on ice separately duly sealed and packed in thick polybags and hard box labeled **"SUSPECTED FOR RABIES"**. If available, fresh smears from brain may be stained with Seller's stain.

7.Pox Disease: Scabs in sterile containers on ice, scabs in 50%buffered glycerin.

8.Bovine Herpes Virus1,2, 3,/IBR/IPV/Bovine Mammilitis / Parainfluenza 3/Adenovirus :

Paired serum sample on ice, swabs from vagina and nasal lesions and pieces of trachea, liver, turbinate bone, lung on ice. From bulls semen and preputial washing in transport medium and paired serum on ice.

9.Enzootic bovine leucosis: Blood in EDTA, tumor tissues, lymph nodes, abomasum, right auricle of heart, spleen, intestine, liver, kidney, lung and uterus.

10.African Swine Fever: Blood in heparin or EDTA, spleen, tonsil, kidney lymph nodes, and bones on ice for virus isolation, paired serum samples.

11.Classical swine fever: Heparinized 20 ml blood at the height of temperature in sterile vials or test tube on ice from live animal. Heart blood, pieces of spleen, lymph nodes, pancreas (10 to 15 g each) in 250% GPB.. Tissue materials from 5 to 6 or more animals may be collected in order to give confirmatory diagnosis/true picture of disease. Materials for isolation and serological tests may be collected in sterile vials on ice without adding glycerin.

12.Transmissible gastroenteritis (TGE): Faeces, small intestine, lung, udder on ice for virus isolation and serological tests

13.Porcine reproductive and respiratory (PRRS):

Lung, liver, lymph nodes, tonsils, spleen, heart, brain, ascitic fluid and paired sera on ice

14.Aujeszky's disease (Pseudorabies): One half of brain, skin and subcutaneous tissue in sterile container on wet ice or in buffer glycerin for virus isolation. Paired serum sample.

15.Bluetongue disease: Blood at the height of body temperature in heparin (6-10 units/ml), paired sera in sterile containers on ice. Spleen, Lymph nodes on ice for virus isolation.

16.Rift valley fever: Blood, liver, spleen, brain, aborted fetus on ice for virus isolation.

17.Caprine arthritis encephalitis/ Maedi/Visna disease: Paired serum, joint capsule, lung, brain etc. on ice.

18.Canine Distemper: Pieces of lung, brain etc. on ice. Impression smears from liver, pieces of liver and spleen on ice.

19.Infectious canine hepatitis: Impression smears from liver fixed in methanol. Spleen and liver in sterile containers on ice.

20.Canine parvovirus infection: Rectal swabs and faeces in PBS, pieces of intestine, heart on ice.

21.Equine influenza: Nasal swabs in PBS or Hank's solution on ice paired serum.

22.Equine Infectious anemia: Paired serum sample,spleen on ice/blood on ice.

23.African horse sickness: 20 ml unclotted whole blood in EDTA, paired serum samples, spleen, brain, lung in 50% buffered glycerin.

24.Equine rhinopneumonitis: Nasal swabs, liver, lung, spleen, thymus from aborted

fetus, paired serum samples, blood from acute clinical cases.

25.Ranikhet disease: Freshly dead / moribund bird on ice, portion of liver, spleen, trachea, bronchi, lung in 50% buffered glycerin saline on ice.

26.Marek's disease: Live birds in acute stage of disease, feather follicles from chest and neck region in transport medium, paired serum samples .

27.Avian influenza: Intestinal contents, faeces, cloacal swabs, oro-nasal swabs, samples from trachea, lungs, liver, air sacs, intestine, spleen, kidney, brain, heart, separately as a pool , paired serum samples.

28.Infectious bursal disease (Gumboro disease): Live affected chick/bird, bursa of Fabricious in transport medium, paired serum sample .

29.Infectious bronchitis: Swab from exudates, lung, paired serum sample.

30.Infectious stunted chick syndrome: Liver, duodenal portion of intestine with pancrease, spleen on ice.

31.Leechi heart disease (Hydro pericardium syndrome):

Liver, spleen, thymus, bursa, kidney, heart on ice.

Exercise:

Q1. Explain the importance of 'cold chain'.

Q2.Write the preservatives used for collection of material for the isolation of virus.

******

Practical No. 10

DIAGNOSIS OF *Peste des petits ruminants* (PPR): ELISA

Collection of samples

Samples for virus isolation must be kept chilled in transit to the laboratory. In live animals, swabs are made of the conjunctival discharges and from the nasal and buccal mucosae. During the very early stage of the disease, whole blood is also collected in anticoagulant for virus isolation, polymerase chain reaction (PCR) and haematology (either ethylene diamine tetra-acetic acid or heparin can be used as anticoagulant, though the former is preferred for samples that will be tested using PCR). At necropsy, samples from two to three animals should be collected aseptically from lymph nodes, especially the mesenteric and bronchial nodes, lungs, spleen and intestinal mucosae, chilled on ice and transported under efrigeration. Samples of organs collected for histopathology are placed in 10% neutral buffered formalin. It is good practice to collect blood for serological diagnosis at all stages, but particularly later in the outbreak.

Immunocapture enzyme-linked immunosorbent assay

Advice on the use and applicability of the immunocapture enzyme-linked immunosorbent assay (icELISA) method is available from the OIE Reference Laboratories for PPR. The method described is available as a commercial kit.

The icELISA (Libeau et al., 1994) using two monoclonal antibodies (MAb) raised to the N protein allows a rapid identification of PPRV. The instructions provided by kit supplier should be followed, but the following shows a typical procedure for the test.

i) Microtitre ELISA plates are coated with 100 µl of a capture MAb solution (diluted according to the instructions of the kit supplier). Coating may be overnight at 4°C or for 1 hour at 37°C.
ii) After washing, 50 µl of the sample suspension is added to each of two wells, and two block (control) wells are filled with buffer.
iii) Immediately add 25 µl of a detection biotinylated MAb for PPR and 25 µl of streptavidin/peroxidase to two wells.
iv) The plates are incubated at 37°C for 1 hour with constant agitation.
v) After three vigorous washes, 100 µl of ortho-phenylenediamine (OPD) in 0.03% (v/v) hydrogen peroxide is added, and the plates are incubated for 10 minutes at room temperature.
vi) The reaction is stopped by the addition of 100 µl of 1 N sulphuric acid, and the absorbance is measured at 492 nm on a spectrophotometer/ELISA reader.

The cut-off above which samples are considered to be positive is calculated from the blank control as three times the mean absorbance values of the control wells.

The test is very specific and sensitive (it can detect 100.6 TCID50/well of PPRV). The results are obtained in 2 hours.

A sandwich form of the immunocapture ELISA is widely used in India (Singh et al., 2004): the sample is first allowed to react with the detection MAb and the immunocomplex is then captured by the MAb or polyclonal antibody adsorbed on to the ELISA plate.

The assay shows high correlation to the cell infectivity assay (TCID50) with a minimum detection limit of 103 TCID50/ml.

(www.oie.int.org Prescribed protocol)

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Practical No. 11

DIAGNOSIS OF FOOT AND MOUTH DISEASE : COMPLEMENT FIXATION TEST

Foot and mouth disease is endemic in India and a large number of outbreaks occur every year. The presence of FMD virus in the field samples is routinely confirmed by typing of suspected material/isolated virus. Any control strategy to curtain the disease requires knowledge of prevalence of various serotypes in a particular geographic area. For this various serological tests have been used. Viz. CFT, SNT, ELISA, PCR, etc.
for typing of virus.

The complement fixation test is considered to be cumbersome and time consuming even then it is used for the detection of foot and mouth disease virus antigen in vesicular fluid for diagnosis and typing of the virus. (Nowadays replaced by Double Antibody Sandwich ELISA)

Principle: Complement system gets activated following antigen antibody reaction .The FMD virus when treated with type specific antisera in the presence of complement, bring about the lysis of immune complex. The lysis will not be visible to the naked eyes. Therefore in order to make this reaction visible an indicator system consisting of sheep RBC and hemolysin is employed in the complement fixation test.

The complement fixation test employs two systems of antigen – antibody reaction.:

1. Test system: Type specific Antisera and Virus antigen (vesicular fluid).
2. Indicator system: Hemolysin (antibody against sheep RBC) and Sheep RBC.

The complement is the system of factors that can fix in any one of the above antigen-antibody systems and subsequently results into either lysis of RBC (hemolysis) or no lysis of RBC (no hemolysis).

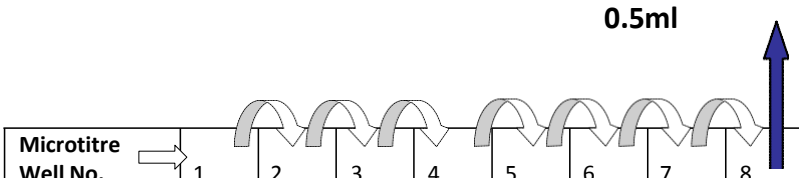
If the clinical material contains the virus homologus to type specific antisera, there will be antigen-antibody reaction, leads to activation and complement fixation in the test system.

If the clinical material does not contain the virus homologus to type specific antisera, there will be no antigen-antibody reaction in the test system & there will be no activation and complement fixation in the test system. The complement would remain free and subsequently react in the indicator system resulting into lysis of RBC.The negative complement fixation test thus will be observed as hemolysis.

Complement Fixation Test:

Material Required: Virus antigen (vesicular fluid), Antisera, Guinea pig complement, hemolysin, 2% sheep RBC suspension. Serological test tubes, metal rack, veronal NaCl diluent, pipettes, rubber bulbs, water bath micro diluter, 96 well U shaped bottom microtitre plates., pipettes and microtips etc.

The protocol for complement fixation test is as follows:



Microtitre Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Veronal NaCl Diluent	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**
AntiseraType specific	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**					
Virus Antigen	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**		**0.5 ml**				
Complement (2/3 unit)	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**		
Hemolysin(2 unit)	**0.5 ml**	**0.5 ml**	**0.5ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**		**0.5 ml**	**0.5 ml**	
Veronal NaCl Diluent											0.5 ml	1.0 ml	1.0 ml	1.5 ml
2% sheep RBC	**0.5 ml**	**0.5 ml**	**0.5ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0. 5ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**	**0.5 ml**
Dilutions	1:2	1:4	1:8	1:16	1:32	1:64	1;128	1:256	1:512	1:1024				
Control									Ab *	Agb *	Hs**	Co *	Hm *	RBC *

*Control.

Deliver 0.5ml of antisera to the separate row of wells for each FMD type virus.

If the clinical material contains the virus homologus to type specific antisera, there will be antigen-antibody reaction, leads to activation and complement fixation in the test system.

And for the titres reciprocal of dilution showing the 50% hemolysis is taken as complement fixing titre of the serum

Interpretation:

Antibody control Antigen control Hemolytic system control	Tube No. 9 Tube No. 10 Tube No. 11	Haemolysis
Complement control Hemolysin control Sheep RBC control	Tube No. 12 Tube No. 13 Tube No. 14	No Hemolysis

Exercise:

Q1. Name the prevalent FMD Serotypes in your region.

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Practical No. 12

DIAGNOSIS OF RANIKHET DISEASE: HAEMAGGLUTINATION TEST & HAEMAGGLUTINATION INHIBITION TEST

Some of the viruses belonging to Paramyxovirus, Orthomyxovirus, Reovirus, Enterovirus & Pox viruses agglutinate suspensions of washed RBC's of various species of animals. This haemagglutination property (ability to agglutinate erythrocytes) has been successfully used for identifying the viruses.

Principle of Haemagglutination Test:

Some of the viruses contain in their outer coat virus coded glycoproteins (Haemagglutinin) capable of binding to the mucoprotein receptors (Sialic acid) over the surface of the erythrocytes. Such viruses bridge the gap between the erythrocytes leads to the formation of lattice. This reaction is seen in micro plates/Perspex plates in the form of mat formation.

Material Required: Perspex plate1ml pipettes, rubber bulb, beaker, conical flask, Pasteur pipette, centrifuge, measuring cylinder.

Reagents: 1% Chicken RBCs, Phosphate buffer saline (pH7.2), Allantoic fluid (collected from ECE on inoculation of suspected material for RD virus isolation)

Procedure:

1. Add 0.5 ml each of PBS in all the wells in a row of Perspex plate with the help of 1ml pipette and rubber bulb.
2. Add 0.5 ml of undiluted allantoic fluid in the first well (only).
3. With the help of pipette mix PBS and allantoic fluid (containing RD virus) from the first well properly and transfer 0.5ml from 1^{st} well to 2^{nd} well (Two fold dilution). Again in 2^{nd} well mix the contents with the help of pipette and transfer 0.5ml from 2^{nd} well to 3^{rd} well. Repeat till 9^{th} well and discard 0.5ml (diluted allantoic fluid containing RD virus) from 9th well.
4. Add 0.5 ml of 1% chicken RBC suspension to all the wells including 10^{th} well (RBC control).

5. Shake the plate well to mix the contents and incubate the plate at room temperature.
6. Read the test at intervals of 15,30,45 and 60 minutes.

Interpretation:

1. The presence of haemagglutinating virus in the allantoic fluid will result in mat formation (layer of uniformly agglutinated cells covering the bottom). The mat formation depends on the concentration of virus present in the suspension.

2. A negative test consists of a compact, sharply demarcated disc of sedimented red blood cells at the bottom of the wells i.e., button formation.

3. The end point of HA activity of the RD virus is the highest dilution of the virus showing mat formation i.e., haemagglutination. The virus titre i8s expressed as HA unit contained in undiluted allantoic fluid. HA titre is the reciprocal of the highest dilution showing mat formation i.e., haemagglutination.

Protocol for Haemagglutination Test: **0.5ml**

(Diluted allantoic fluid)

Well No. → **Reagent** ↓	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9	**10**
	0.5ml	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	0.5ml	
PBS	**0.5ml**	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Allantoic Fluid	0.5ml								**Discard 0.5ml**	
Dilution of virus	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
1%Chicken RBC	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Control Result										**RBC Control**
Mat/ Button										

HAEMAGGLUTINATION INHIBITION TEST

The haemagglutination inhibition test is used for assess the titres of haemagglutinating-inhibiting antibodies against RD virus in the serum of suspected/healthy bird used for diagnosis and monitoring the vaccine titres.

Principle of Haemagglutination Inhibition Test:

HI-Test is based on the haemagglutination property of RD virus. Haemagglutinin binds to the mucoprotein receptors (Sialic acid) over the surface of the erythrocytes& bridges the gap between the erythrocytes leads to the formation of lattice. This reaction is seen in microtitre / Perspex plates in the form of mat formation. But In the presence of antibodies (in the serum), abs interacts with the virus and thus makes the virus unavailable for agglutination of red blood cells. Red blood cells settles at the bottom of the wells thereby giving i.e., button formation in the presence of haemagglutinating-inhibiting antibodies against RD virus.

Material Required: Perspex plate, 1ml pipettes, rubber bulb, beaker, conical flask, Pasteur pipette, centrifuge, measuring cylinder.

Reagents: 1% Chicken RBCs, Phosphate buffer saline (pH7.2), 4HA Unit RD virus, and Test serum)

Procedure:

1. Add 0.5 ml each of PBS in all the wells in a row of Perspex plate with the help of 1ml pipette and rubber bulb.
2. Add 0.5 ml of undiluted Test serum in the first well (only) .
3. With the help of pipette mix PBS and Test serum in the first well properly and transfer 0.5ml from 1^{st} well to 2^{nd} well (Two fold dilution). Again in 2^{nd} well mix the contents with the help of pipette and transfer 0.5ml from 2^{nd} well to 3^{rd} well. Repeat till 8^{th} well and discard 0.5ml (diluted test serum) from 8^{th} well.
4. Add 0.5ml each of 4 HA unit virus upto 9^{th} well (Virus control).
5. Allow it to stand for 10 minutes at room temperature.
6. Add 0.5 ml each of 1% chicken RBC suspension to all the wells including 10^{th} well (RBC control).

7. Shake the plate well to mix the contents and incubate the plate at room temperature.
8. Read the test at intervals of 15,30,45 and 60 minutes.

Interpretation:

1. The presence of haemagglutinating-inhibiting antibodies against RD virus in the test serum binds to the RD virus and makes RD virus unavailable for haemagglutination of Red blood cells. Resulting into sedimented red blood cells at the bottom of the wells i.e., button formation.
2. A negative test consists of absence of haemagglutinating-inhibiting antibodies against RD virus in the test serum results in the availability of RD virus for haemagglutination of Red blood cells. Resulting into mat formation (layer of uniformly agglutinated cells covering the bottom).
3. The end point of haemagglutination inhibition test. The titre is expressed as HI titre of haemagglutinating-inhibiting antibodies against RD virus in the test serum .HI titre is the reciprocal of the highest dilution showing button formation i.e., haemagglutination inhibition.

Protocol for Haemagglutination Inhibition test: **0.5ml**

(Diluted test serum)

Well No. ► Reagent ▼	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9	**10**
	0.5ml	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	**0.5ml**	0.5ml	
PBS	**0.5ml**	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Test serum	**0.5ml**							**Discard 0.5ml**		
4HA unit RD virus	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Dilution of Test serum	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
1%Chicken RBC	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Control									**Virus control**	**RBC control**
Result Button/ Mat										

Exercise:

Q1. Write the use and composition of Alserver's solution.

Q2. Calculate 4HA unit of RD Virus.

Q3. Calculate the HI titres of given sera samples and interprete the Results based on the history of vaccination in broilers.

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Practical No. 13

DIAGNOSIS OF BLUE TONGUE DISEASE: AGID

Anti-BTV antibody generated in infected animals can be detected by using Agar gel immune-diffusion (a prescribed test for international trade). The AGID test to detect anti-BTV antibodies is simple to perform However, one of the disadvantages of the AGID used for BT is its lack of specificity in that it can detect antibodies to other orbiviruses particularly those in the EHD serogroup. The preferred protocol, is as follows:

Test procedure:

1. A 2.8 mm thick layer of 0.9% agarose in 0.85% NaCl is prepared and circular wells 5.0 mm in diameter and 3.0 mm apart are cut out with six wells arranged around a central well.
2. Place BTV readymade soluble antigens (procured from the reference laboratories) in the central well.
3. Three positive and three test sera are placed in alternate peripheral wells surrounding the antigen in the central well and the plates are incubated at 20-25°C in a humid environment for 24 hours.
4. A series of precipitin lines form between the antigen and known positive sera and lines generated by strong positive test sera will join up with those of the positive controls. With weak positive samples, the control lines bend toward the antigen and away from the test sample well, but may not form a continuous line between the control test wells. With negative samples, the precipitin lines will continue into the sample wells without bending toward the antigen.
5. All weak positive samples and other samples that produce questionable results should be repeated using wells that are 5.3 mm in diameter placed 2.4 mm apart or retested using the competitive ELISA.

Exercise:

Q1. Prepare the protocol for the above test.

Q2. Write the interpretation of the test.

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Practical No. 14

DIAGNOSIS OF INFECTIOUS BURSAL DISEASE : AGID

Agar gel immunodiffusion test

The AGID test is the most useful of the serological tests for the detection of specific antibodies in serum, or for detecting viral antigen or antibodies in bursal tissue.

For detection of antigen in the bursa of Fabricius, the bursae should be removed aseptically from about ten chickens at the acute stage of infection.

The bursae are minced using two scalpels in scissor movement, then small pieces are placed in the wells of the AGID plate against known positive serum.

Preparation of agar

Dissolve sodium chloride (80 g) and phenol (5 g) in distilled water (1 litre). Add agar (12.5 g) and steam until the agar has dissolved. While the mixture is still very hot, filter it through a pad of cellulose wadding covered with a few layers of muslin. Dispense the medium in 20 ml volumes into glass bottles and store at 4°C until required for use.

The linear pattern of wells is preferred although a hexagonal pattern may be used. Each test serum or bursa should be placed adjacent to a positive control antibody (AB) or antigen (AG), respectively.

Wells, 3 mm deep, 6 mm in diameter, and 3 mm apart, are used.

Test procedure

1. Prepare plates from 24 hours to 7 days before use. Dissolve the agar by placing in a steamer or boiling water bath. Take care to prevent water entering the bottles.
2. Pour the contents of one bottle into each of the required number of 9 cm plastic Petri dishes laid on a level surface.
3. Cover the plates and allow the agar to set.
4. Cut three vertical rows of wells 5 mm in diameter and 3 mm apart, using a protocol and tubular cutter

5. Remove the agar from the wells using a pen and nib, taking care not to damage the walls of the wells.
6. Using a pipette, dispense 50 µl of the test sera into the wells .
7. Dispense small pieces of finely minced test bursa suspension by means of curved fine pointed forceps/pipette into the wells, to just fill the wells.
8. Dispense 50 µl of the positive and negative control reagents into the relevant wells.
9. Incubate the plates at between 22°C and 37°C for up to 48 hours in a humid chamber to avoid drying the agar.
10. Examine the plates against a dark background with an oblique light source after 24 and 48 hours.

Exercise:

Q1. Explain: Preparation of antigen from infected bursa of Fabricius.

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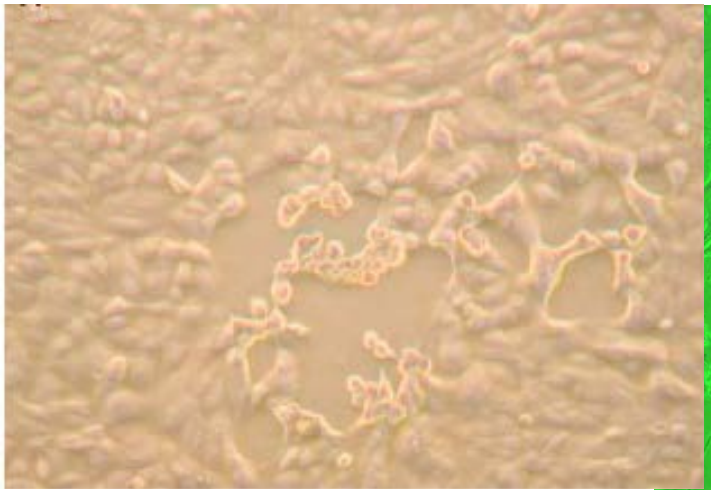
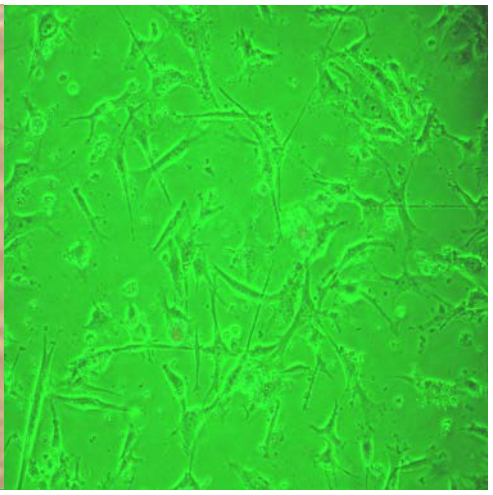
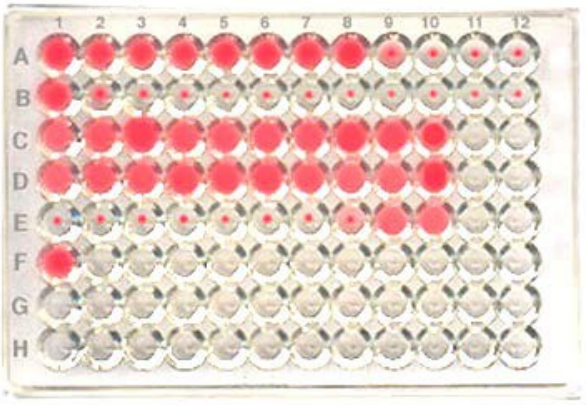


Infected Vero (monkey kidney cell-line)-Note the CPE

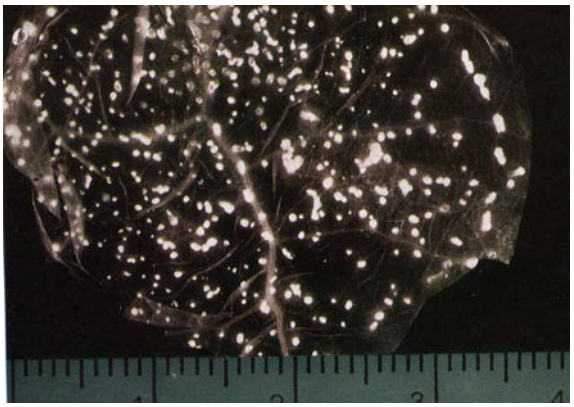


BHK-21 Cell line

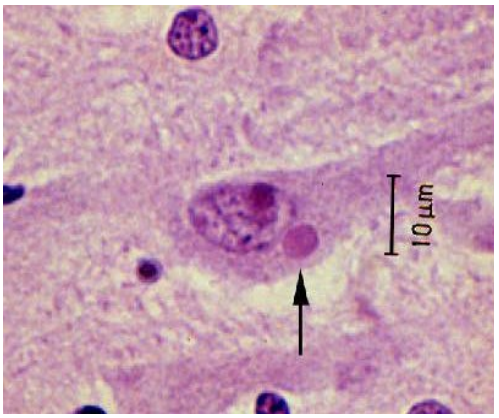


Haemagglutination Inhibition Test: Note The Button & Mat Formation

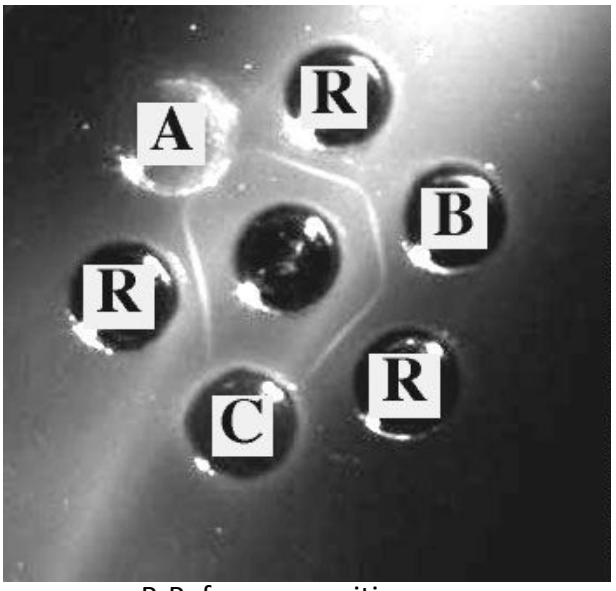
Goatpox- Note the scab lesions



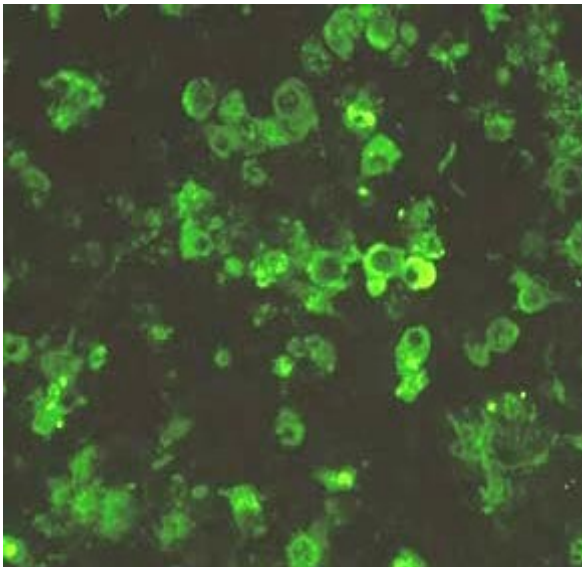
Characteristic Pock Lesion on CAM - Pox virus



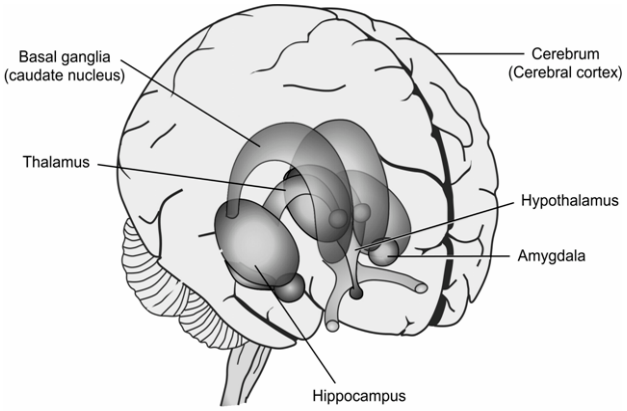
Negri body :neuron cell in trigeminal ganglion



R-Reference positive sera
Central well- antigen; A,B & C -Test samples



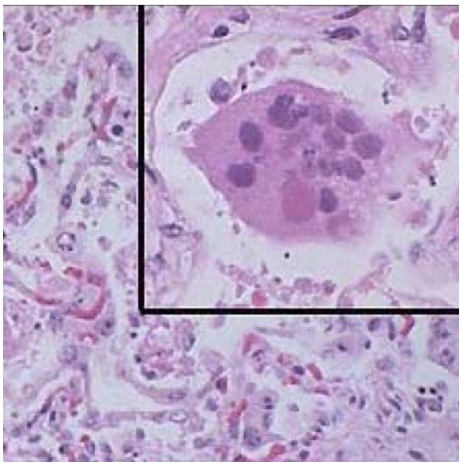
Fluorescent Antibody Technique
: Diagnosis of Rabies



Note the Hippocampus region of brain

Guinea pig: Footpad inoculation-FMD virus

Syringe Filter



Fusion & Syncitia Formation
(http://www.tau.ac.il/lifesci/departments/biotech/members/rozenblatt/fig10)

Practical No. 15

CANINE PARVOVIRUS: HAEMAGGLUTINATION TEST

Clinical samples.

Fecal samples and intestinal tissue samples from CPV suspect dogs should be prepared as 10% (wt/vol) suspensions in phosphate-buffered saline (PBS) (pH 7.2)

Haemagglutination Test: (As described by Carmichael et al.)

1. The samples were serially diluted twofold in PBS (0.2 M) in V-bottom plates. First, 50 microliters of PBS was added to each well of the plate.
2. In the first column, 50 µl of sample (fecal suspension or cell culture supernatant) was added. The sample was mixed five times, and 50 µl was transferred to the next well.
3. Each sample was diluted from 1:2 through 1:4,096. Then, 50 µl of PBS was added to each well.
4. The HA test was performed using porcine erythrocytes (0.5%).
5. The corners of the plate were tapped four or five times to mix the erythrocytes.
6. The plates were covered with lids and incubated at 4 to 7°C for 2 to 4 h.
7. Positive agglutination was indicated by mat formation, and the button indicated lack of agglutination.
8. The titer was calculated as the reciprocal of the last well with agglutination. After overnight incubation, some positive samples can elute.

Exercise:

Q1. Perform the test and write results..

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Practical No. 16

INFECTIOUS BOVINE RHINOTRACHEITIS
(IBR dot-ELISA)
(Prescribed Test Protocol by OIE.int.org)

Collection and processing of samples

Nasal swabs are collected from several (from five to ten) affected cattle in the early phase of the infection. These cattle still have serous rather than mucopurulent nasal discharge. In cases of vulvovaginitis or balanoposthitis, swabs are taken from the genitals. The swabs should be vigorously rubbed against the mucosal surfaces. The prepuce can also be washed with saline; the washing fluid is then collected. The specimens are suspended in transport medium (cell culture medium containing antibiotics and 2–10% BoHV-1-free fetal bovine serum to protect the virus from inactivation), cooled at 4°C, and rapidly submitted to the laboratory.

During necropsy, mucous membranes of the respiratory tract, and samples of the tonsil, lung and bronchial lymph nodes are collected for virus detection. In cases of abortion, the fetal liver, lung, spleen, kidney and placental cotyledons are examined. Samples should be kept on ice and sent to the laboratory as quickly as possible.

After arrival at the laboratory, swabs are agitated at room temperature for 30 minutes in the transport medium to elute virus. Following removal of the swabs, the transport medium is clarified by centrifugation at 1500 g for 10 minutes. Tissues are homogenised to a 10–20% (w/v) suspension in cell culture medium before centrifugation at 1500 g for 10 minutes

Diagnostic Method: The ImmunoComb® test is based on solid phase “dot”-ELISA technology. Antigen is applied to test ‘spots’ on the solid phase, which is a comb-shaped plastic card (the Comb).

The serum specimen to be tested is diluted in a buffer in the first well of a multi-chamber developing plate. The test spots on the Comb are then incubated with the sera in the developing plate. Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spot.

The Comb is then transferred to a well, where unbound antibodies are washed from the antigen spots. In the next step, the Comb is allowed to react with an anti-cow IgG Alkaline Phosphates conjugate, which will bind to antigen-antibody complexes at the test spots. After 2 more washes, the Comb is moved to the last well, where a color result develops via an enzymatic
reaction. The intensity of the color result of test spots corresponds directly to
the antibody level in the test specimen.

IBR dot-ELISA:

Specificity	86.2%
Sensitivity	94.6%

Preferred Method of Diagnosis: Serology is used to evaluate antibody responses to infection by the viruses associated with BRD. Serologic techniques are also helpful in evaluating the effectiveness of vaccination programs.

Interpretation: The level of antibodies (i.e., antibody titer) is determined according to the intensity of the test color result.

Positive and negative control serum samples are included in the ImmunoComb® Bovine IBR-PI3-BRSV Antibody Test Kit.

The negative control consists of non-immune sera and should be read as zero. Specimens with colorless (white) or faint color result (i.e., less than S1) are considered negative.

The positive control spot on the Comb should develop a distinct grey color that is scored S3. The positive control has been calibrated to correspond approximately to 200 ELISA units (0.2 Optical Density). Specimens with identical or darker grey color results (S3 – S5) are considered positive.

Proper evaluation of the humoral immune response to infection is performed by comparing test results in paired serum samples, which are obtained at the acute and convalescent stages of illness. Negative serology would be expected to be found in unvaccinated and unexposed herds. Any seroconversion (S1 or greater) would indicate infection, while S3 or greater is considered significant in previously vaccinated animals.

Applications:

1. To determine IBR infection in cattle by measuring IgG antibody titer.
2. To evaluate passive (maternal) antibody levels in calves to IBR Virus.
3. To evaluate humoral immunity status of herds for assistance in designing vaccination programs forIBR.

References:

Peters, A., R. (1987). Vaccines for respiratory disease in cattle. Vaccine,5(3), 164.

Tittle, D. (2000). A quantitative measure of the concentration of immunoglobulin G1, and antibodies to Infectious Bovine Rhinotracheitid, Parainfluenza 3 and Bovine Respiratory Syncytial Viruses in bovine colostrum and post-suck calf sera. Farm Animal Elective Project: Royal Veterinary College, University of London, England.

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VACCINATION SCHEDULE FOR PREVENTION OF VIRAL DISEASES IN LIVESTOCK AND POULTRY

The vaccination is the most effective way of preventing the viral diseases. The vaccination schedule is not fixed one .One schedule may not work at different places (region/country). The optimal design of veterinary vaccination programme depends on the incidence of disease in the area (epidemiology of virus) and the characteristics of the vaccine.

As a guideline the following vaccination schedule can be used for the prevention of viral diseases . Taking into the consideration the incidence of disease and other properties, the optimal vaccination schedule can be designed coupled with the field experience.

Some of the principles underlying decisions on vaccination schedules are outlined and tables of schedules for the vaccination of various domestic animals are provided.

Schedule for vaccination against viral diseases of cattle

Vaccine	Age of vaccination	Booster Dose
Attenuated virus vaccines		
Bovine viral diarrhea virus	4-9 months of age (single dose)	Annual
Infectious Bovine Rhinotracheitis virus	3-4 months of age (single dose)	Annual
Rinderpest virus	6-8 months off age (single dose)	None
Parainfluenza virus 3	4-6 months of age (two doses with a 4 week interval)	Annual
Inactivated Virus Vaccines		
Foot & Mouth disease viruses	4 weeks of age (single dose)	4-12 months
Infectious Bovine Rhinotracheitis virus	3-4 months of age (two doses at a 4 week interval) single dose)	6-12 months
Rota virus	6-8 weeks and 2 weeks before end of gestation	Each pregnancy
Corona virus	6-8 weeks and 2 weeks before end of gestation	Each pregnancy
Parainfluenza virus 3	6 weeks, 3 months, 7 months of age	Annual

Schedule for vaccination against viral diseases of sheep & goats

Vaccine	Age of vaccination	Booster Dose
Attenuated virus vaccines		
Blue tonguevirus polyvalent vaccine	3-6 months of age (two doses with a 3 week interval)	Annual
Sheep pox virus	3 months of age (single vaccine)	2 years
Peste des petits ruminants virus	3 months of age (single vaccine)	None
Orf virus	2-3 months of age (single vaccine)	None
Inactivated Virus Vaccines		
Foot & mouth disease viruses	3-4 months of age (single vaccine)	None

Schedule for vaccination against viral diseases of swine

Vaccine	Age of vaccination	Booster Dose
Attenuated virus vaccines		
Hog cholera virus	8 weeks of age (single dose)	None
Pseudorabies virus	All ages (two doses with a 4 week interval)	4-6 months
Inactivated Virus Vaccines		
Pseudorabies virus	4 weeks of age (two doses with a 4 week interval)	5-6 months
Porcxine polioencephal;it is virus	8-12 weeks of age 8-13 (two doses with a 4 week interval)	None
Porcine parvovirus	Before mating (two doses with a 4 week interval)	Annual
Influenza viruses	Any age (two doses with a 4 week interval)	Annual

Schedule for vaccination against viral diseases of horses

Vaccine	Age of vaccination	Booster Dose
Attenuated virus vaccines		
Equine herpes virus 1 (Equine abortion virus	3 months of age (three doses with 2 and 6 month intervals)	9-12 months
African Horse sickness viruses (polyvalent)	6-8 months of age (single dose)	2-3 years
Inactivated Virus Vaccines		
Influenza viruses (bivalent)	3-5 months of age (three doses with 2 and 6 month intervals)	Every 3-6 months
Equine herpes virus 1 (Equine abortion virus & Equine herpes virus 4 (Equine rhinopneumonitis virus)	10 weeks of age (three doses with 1 and 6 month intervals)	6-12 months

Schedule for vaccination against viral diseases of dogs

Vaccine	Age of vaccination	Booster Dose
Attenuated virus vaccines		
Canine Distemper virus	6-8 weeks of age (three doses with 3 week intervals)	Every two years
Canine hepatitis virus	6-8 weeks of age (three doses with 3 week intervals)	Every two years
Canine parvovirus 2	6 weeks of age (three doses with 3 week intervals)	Annual
Inactivated Virus Vaccines		
Rabies virus	12 weeks of age (single dose)	Annual or every 3 Years
Canine parvovirus 2	6-8 weeks of age (three doses with 3 week intervals)	Annual
Canine coronavirus	9-16 weeks of age (three doses with 3 week intervals)	Annual
Parainfluenza viruses	6-8 weeks of age (three doses with 3 week intervals)	Annual

Schedule for vaccination against viral diseases of cats

Vaccine	Age of vaccination	Booster Dose
Attenuated virus vaccines		
Feline panleukopenia virus	6-12 weeks of age (three doses with 3 week intervals)	Annual or every 3 Years
Feline calcivirus	6-12 weeks of age (three doses with 3 week intervals)	Annual
Feline herpes virus	6-12 weeks of age (three doses with 3 week intervals)	Annual
Inactivated Virus Vaccines		
Feline panleukopenia virus	6-12 weeks of age (three doses with 3 week intervals)	Annual
Rabies virus Feline	12 weeks of age (single dose)	Annual or every 3 Years
calcivirus Feline	6-12 weeks of age (three doses with 3 week intervals)	Annual
herpes virus	6-12 weeks of age (three doses with 3 week intervals)	Annual
Feline leukemia virus (subunit)	9 weeks of age (2 doses with 3 –15 wk int.)	Annual
Feline leukemia virus	10 weeks of age (two doses with a 1 month intervals)	Annual

Exercise: Q1. Design the vaccination schedule for cattle and dogs based on the incidence of disease in your region.

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APPENDIX

1.Anticoagulants for collection of blood:

Heparin: 5-6 IU units/ml of blood

EDTA: 1-2 mg /ml of blood

Oxalate phenol glycerin OCG solution: 1 part to 2 parts of blood.

Blood is transported in chilled condition.,but should never frozen for both clinical examination and for virus isolation. Antibiotics can be added.

2.Transport media/preservatives:

50% Glycerine Phosphate Buffer Saline(GPB).

First prepare M/25 Phosphate Buffer

Phosphate Buffer Saline(PBS)(pH 7.4).

Saline (pH 7.4-7.6) as follows:

Sodium chloride	8.0gm
Potassium chloride	0.2 gm
Di sodium hydrogen phosphate	1.15 gm
Potassium Di-hydrogen phosphate	0.20 gm
Distilled water	1000ml

Add equal volume of sterile neutral glycerin to sterile M/25 PBS (pH 7.4-7.6) to prepare 50/5 GPBS of pH 7.4. Add 0.1ml of 1% phenol red solution to 100 ml of that solution so as to give a final concentration of phenol red to 0.001%. The solution when sterile should have reddish tinge. Yellow color indicates contamination. Each vial must contain 10mlk of PBS for collection of tissues.

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Systematic Veterinary Virology

Practical

Manual

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