Diagnositic techniques of HPAI

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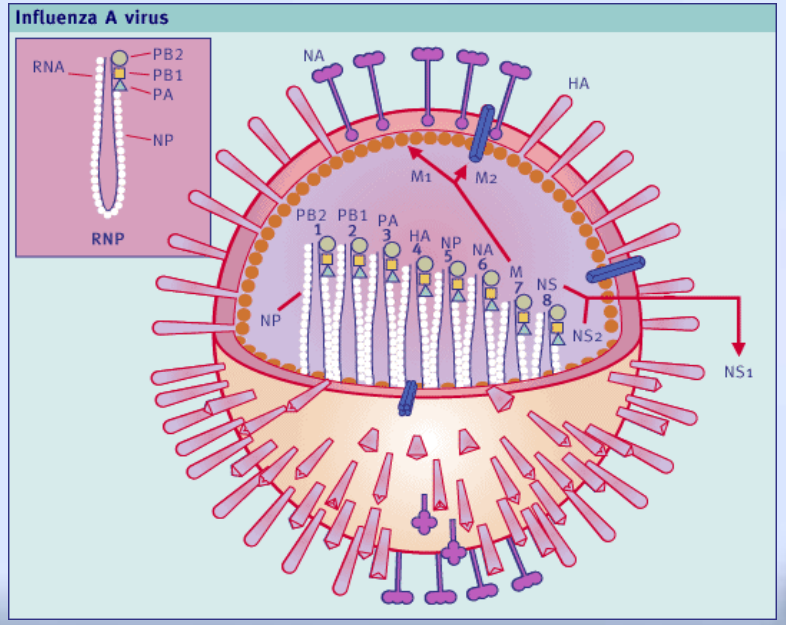
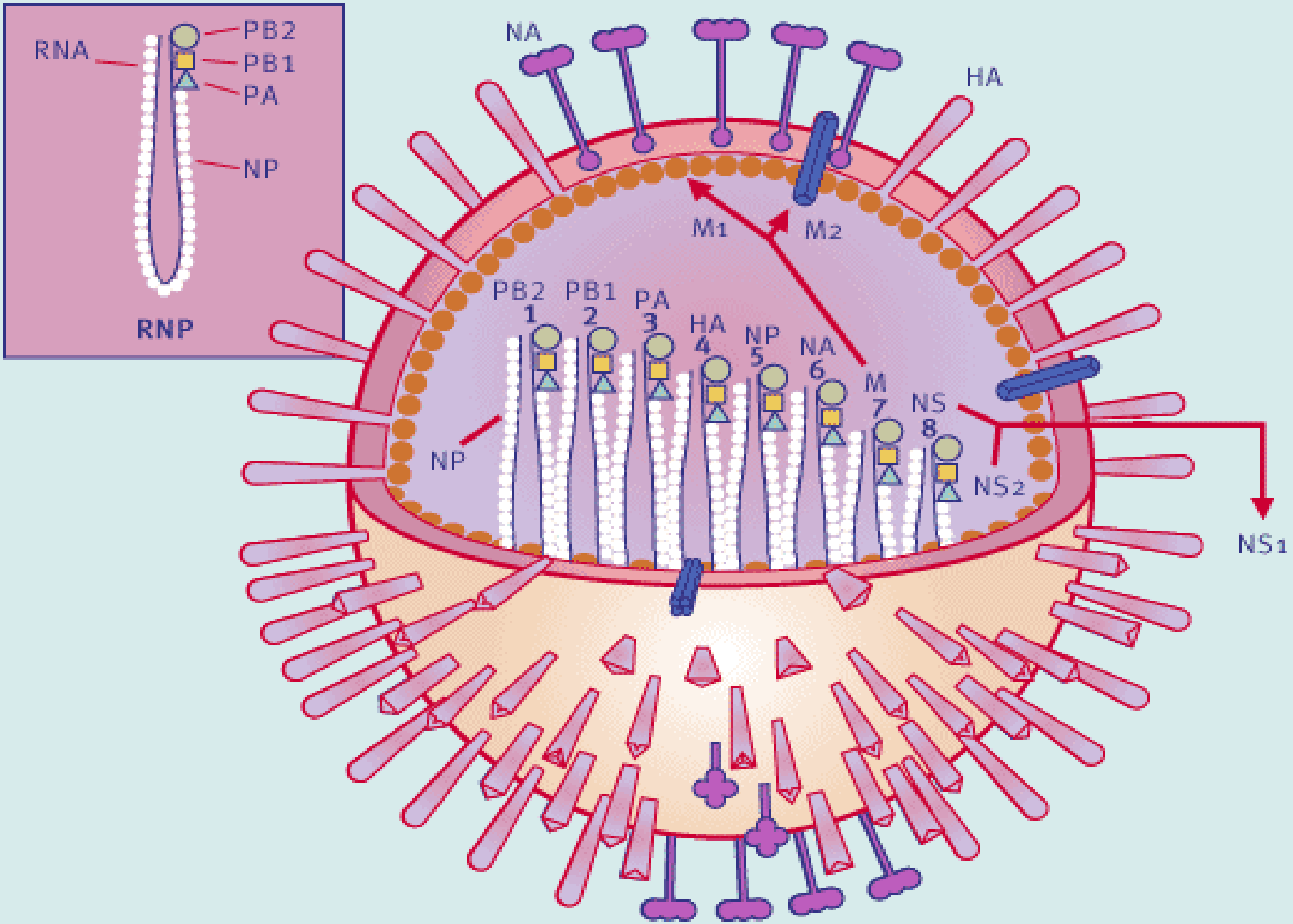
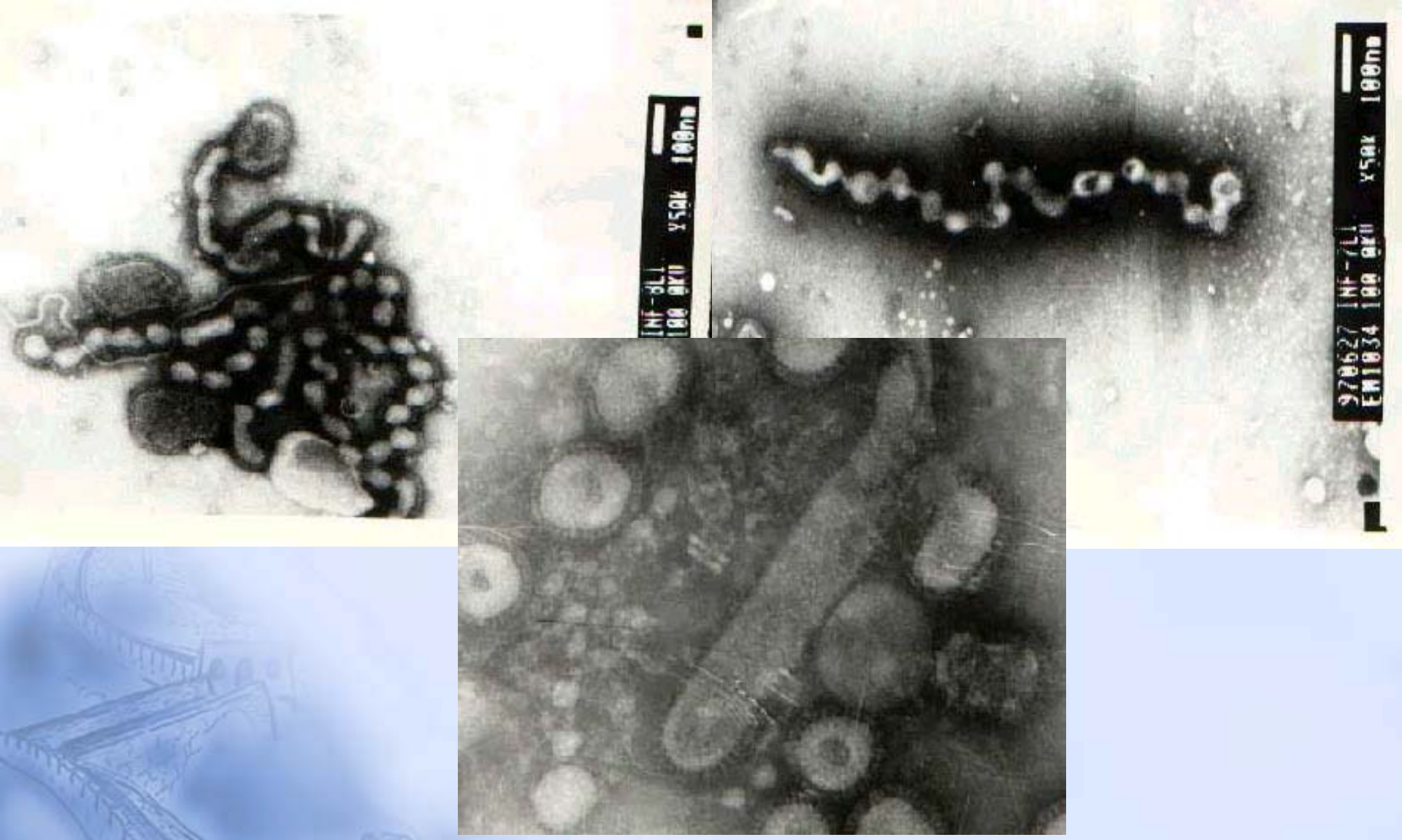
Etiological agent

Influenza viruses are RNA viruses in the family Orthomyxoviridae. The genome of influenza A contains 8 single stranded, negative strand segments of RNA. Only type A Influenza viruses are known to infect birds naturally. Viruses of 16 HA and 9 NA influenza A subtypes have been isolated from avian species . Influenza A viruses having infected poultry are divided into two groups, highly pathogenic avian influenza viruse(HPAIV) and low pathogenic avian influenza viruse(LPAIV), according to the pathogencity to chicken.



Influenza A virus





Morphous of AIV below electronmicroscope

Diagnosis method of HPAI

- Epidemiologic characteristics
- Clinical signals
- Pathologic anatomy
- Histology changes
- Etiological diagnosis
- Serological tests

Epidemiologic characteristics

Highly pathogenic avian influenza is a fulminating, contagious and fatal disease in domestic poultry that is of international significance. The disease is most severe in winter and spring although it can occur the whole year. It can infect a wide range of host, including chicken, turkey, duck, goose, quail, pheasant and dove. The morbidity and mortality of chicken and turkey is higher than that of aquatic poultry. The mortality in aquatic poultry ranged from 0-80%.

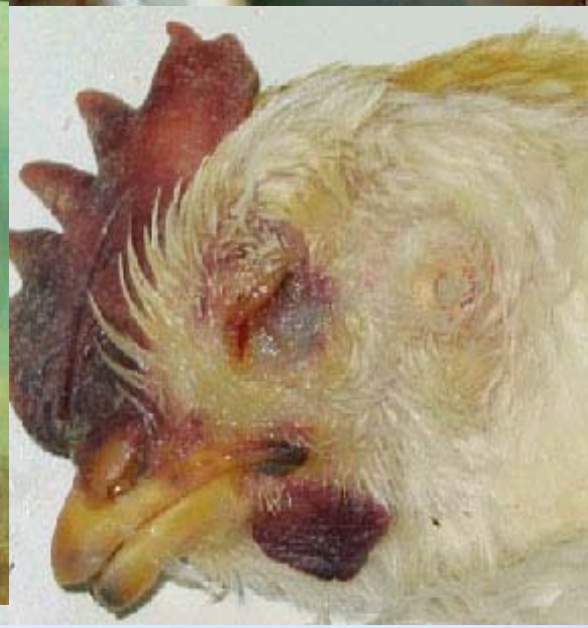
The distribution of avian influenza is influenced by many factors, including feeding, distribution of wild birds, the place of origin of poultry production, migration route and season. The infected aquatic birds can contaminated water, which place important role in the epidemiology of avian influenza.

The mortality of HPAIV may be as high as 100%

鸭、鹅等水禽感染后则死亡率差别较大

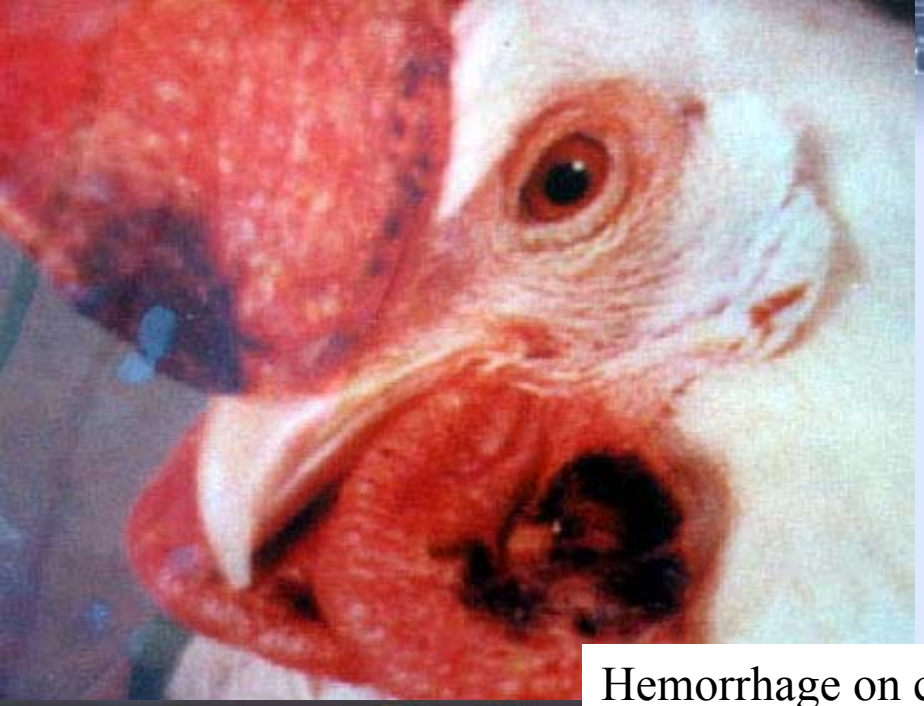
HPAI Clinical symptom

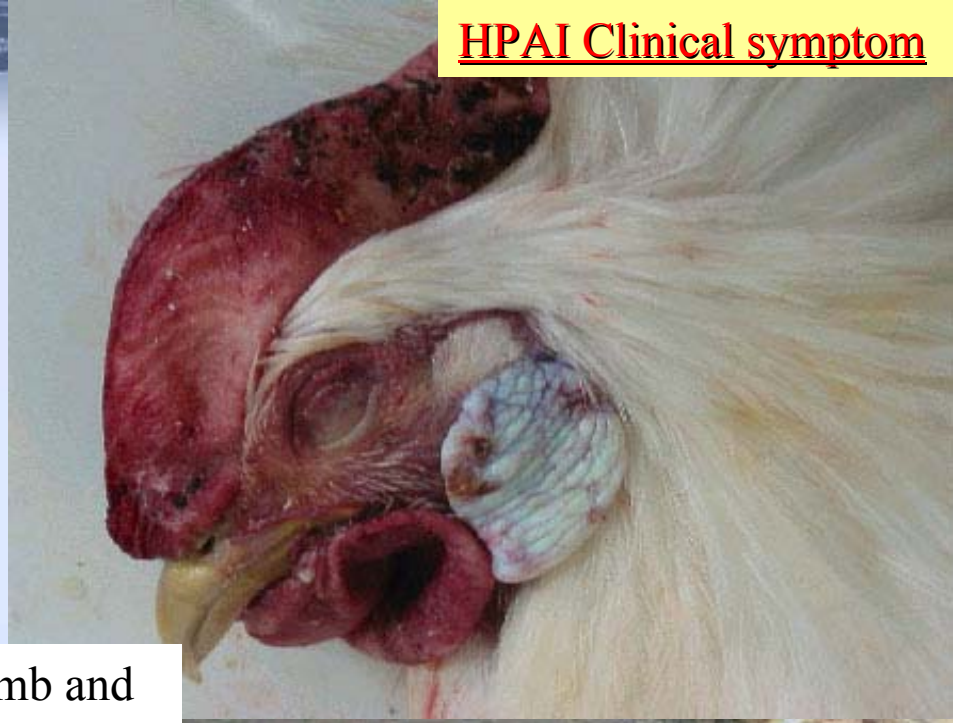
Dead abruptly, severe depression, yellow and green excreta, sharp drop in egg production and consumption of feed and water



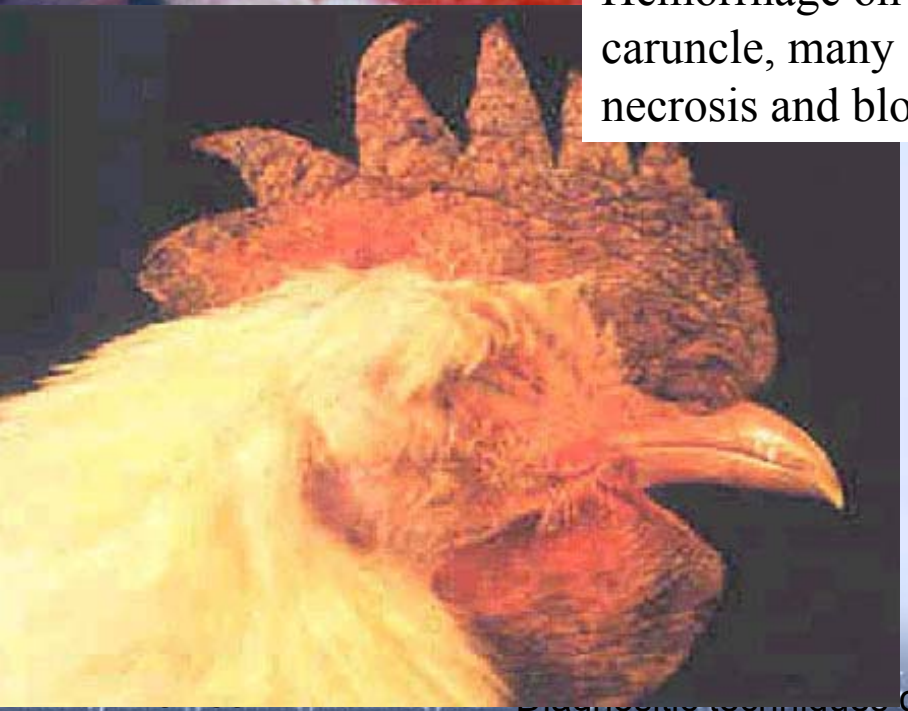
Edema on head and face, cyanosis on comb and caruncle

HPAI Clinical symptom

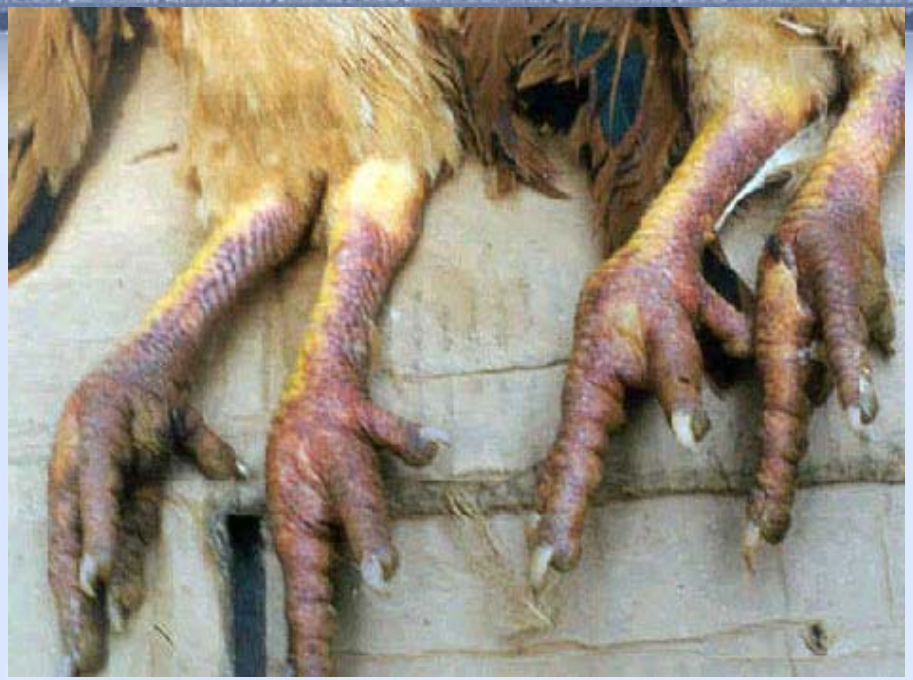




Hemorrhage on comb and caruncle, many spot necrosis and block necrosis

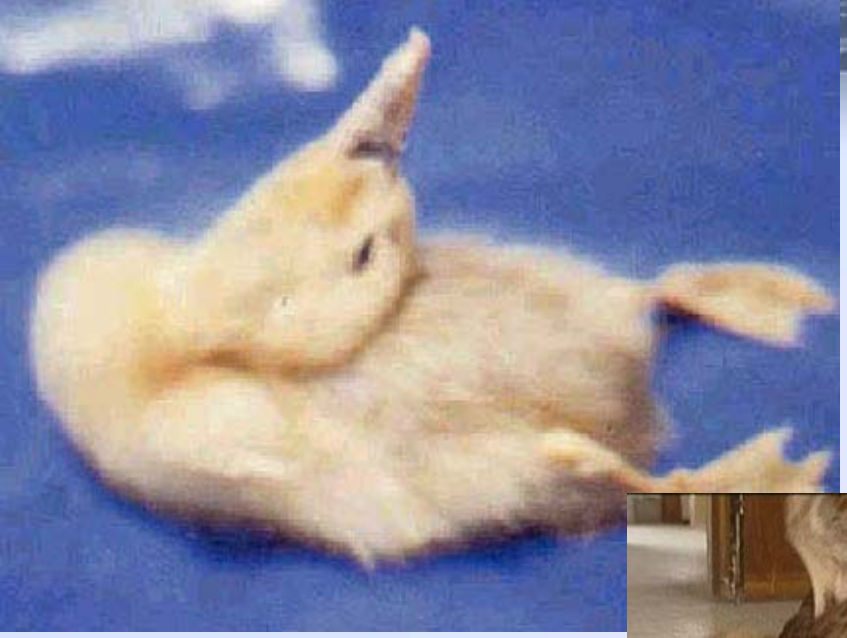


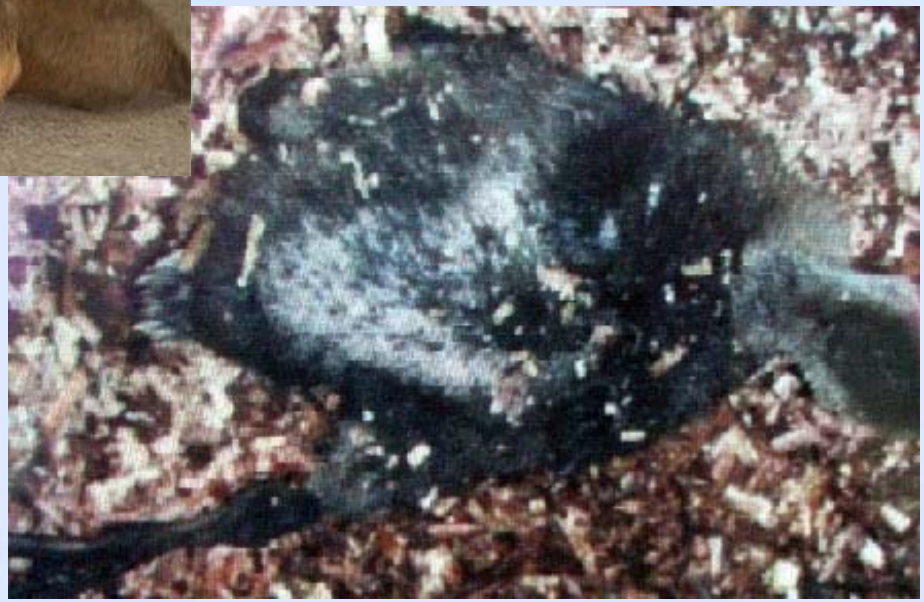
HPAI Clinical symptom



Hemorrhage on foot squama

HPAI Clinical symptom





Opisthotonus in ducks and geese, swell on head, tear and diarrhea

Keratitis, swell on eyelid, blindness

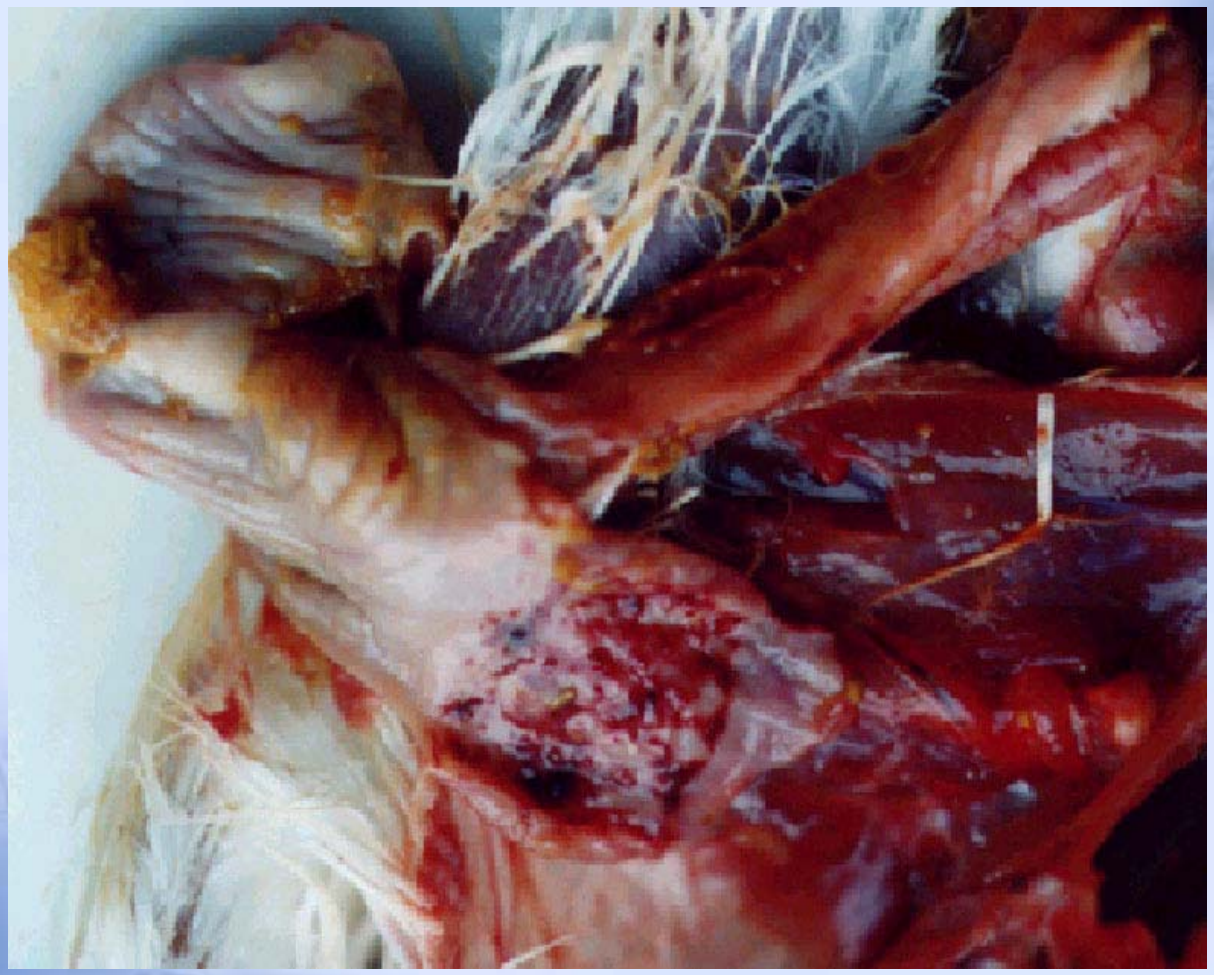
HPAI Clinical symptom

neurosis symptom of Barheaded goose and pigeon

neurosis symptom of wild bird

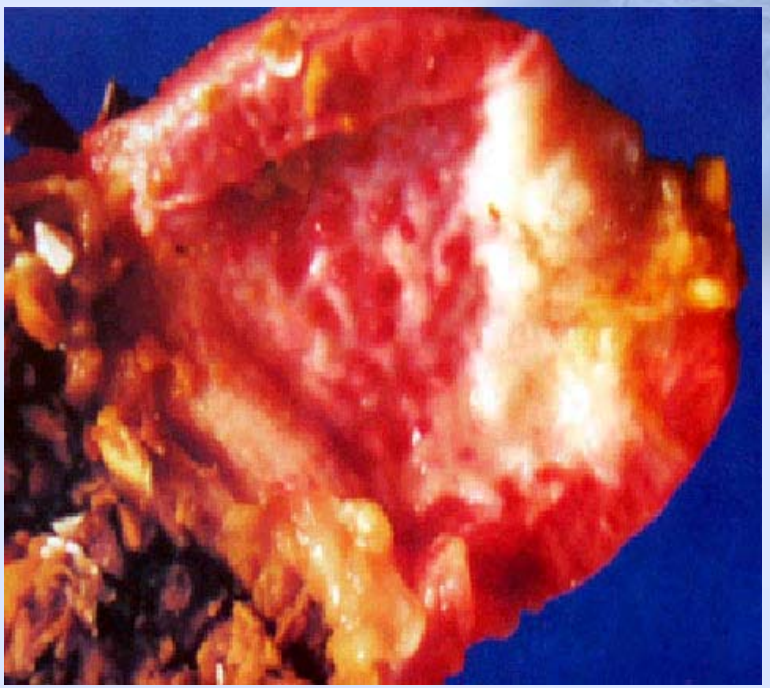
Pathoanatomical changes

Anatomy: Extensive hemorrhage in organs and mucous membrane

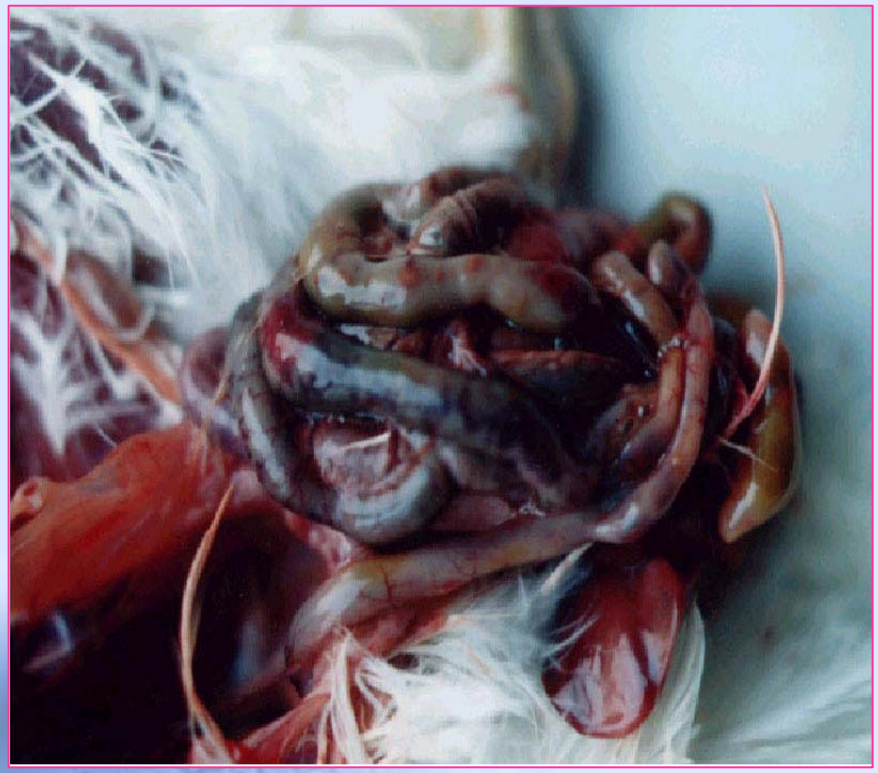


Hemorrhage on the border of proventriculus papillae and muscular ventriculus

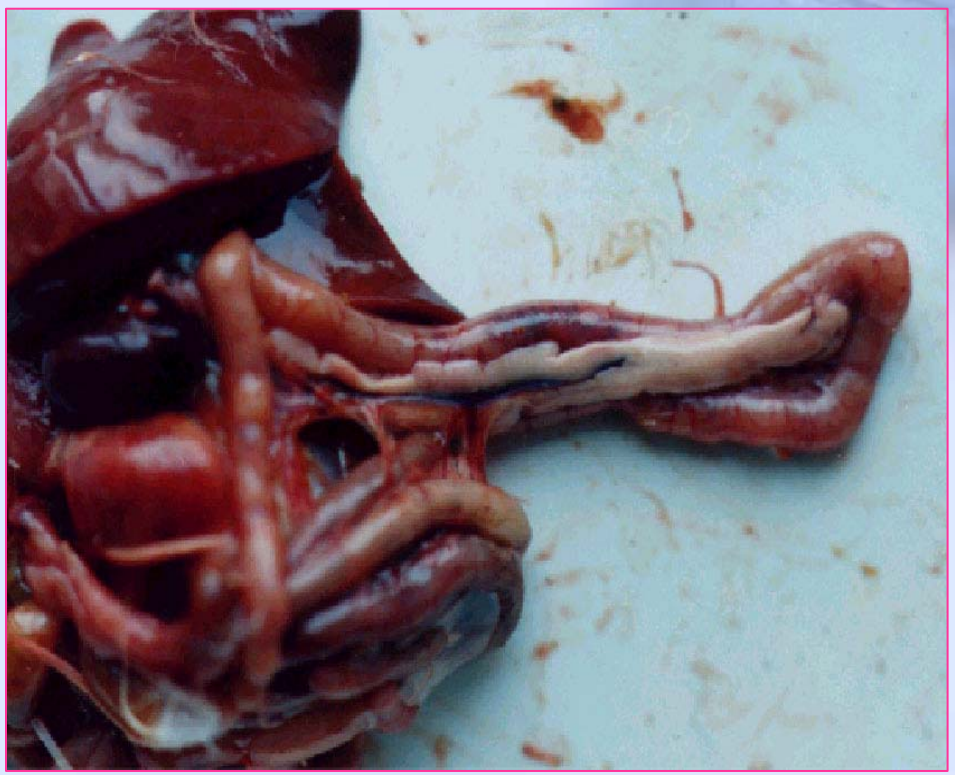
Hemorrhage and canker on proventriculus papillae, hemorrhage on muscular ventriculus, suppurative secretion on mucous membrane



HPAI pathologic changes of the dissection

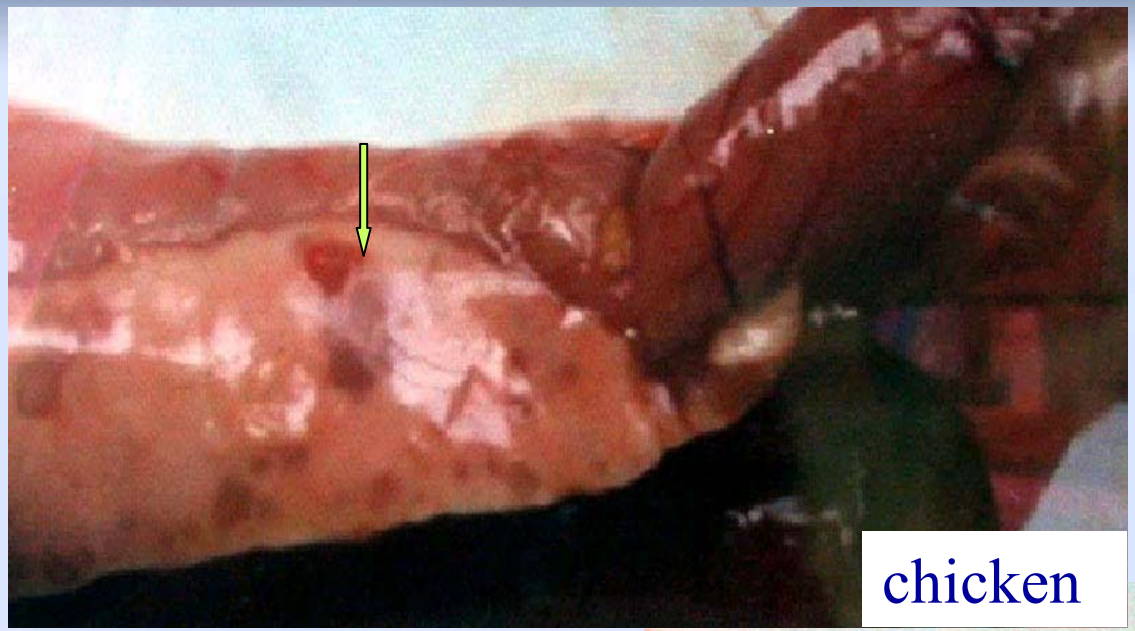


Extensive hemorrhage on mucous membrane of digestive tract

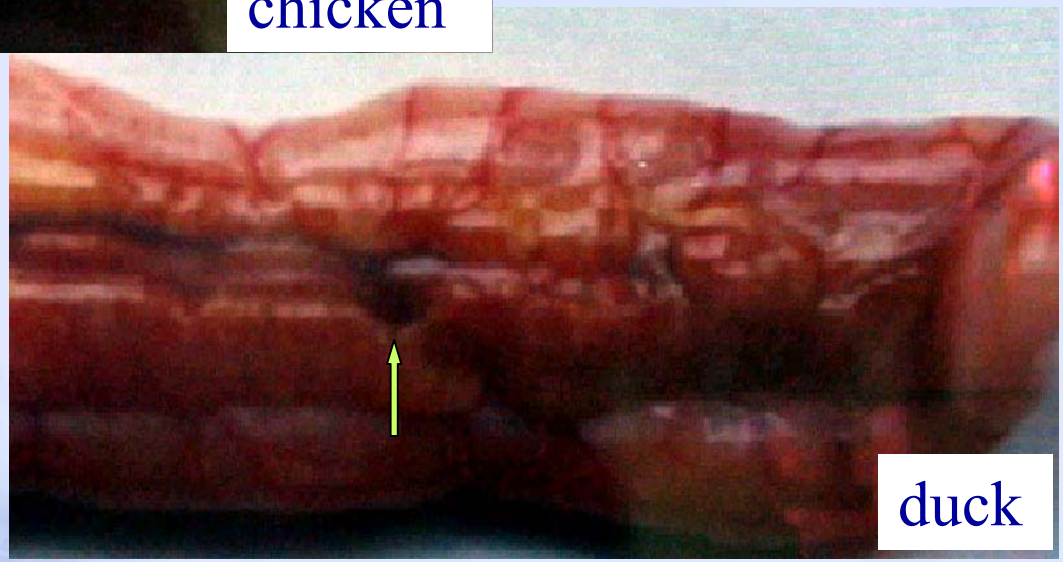


Severe hemorrhage on duodenum

HPAI pathologic changes of the dissection



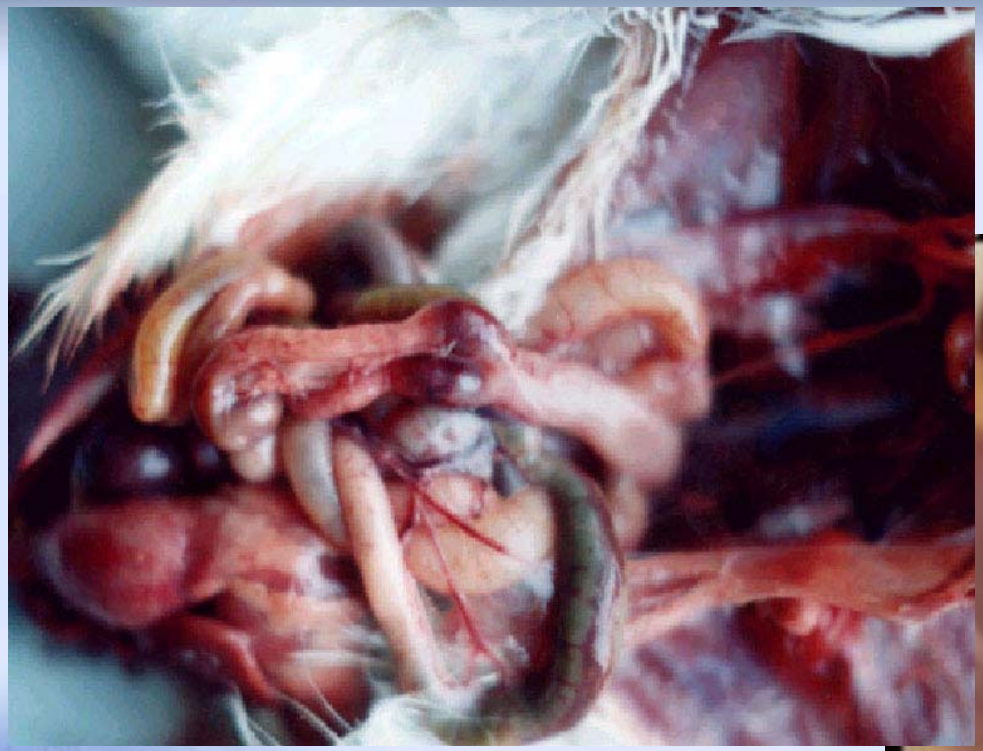
chicken



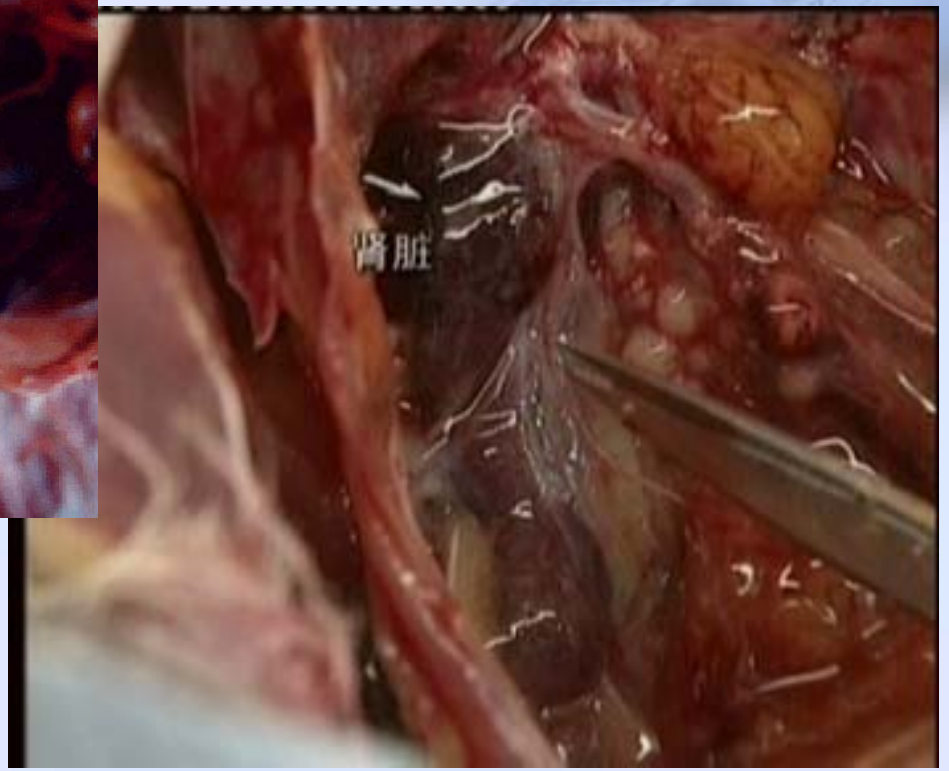
duck

Spot hemorrhage or strip hemorrhage on pancreas and necrosis

HPAI pathologic changes of the dissection

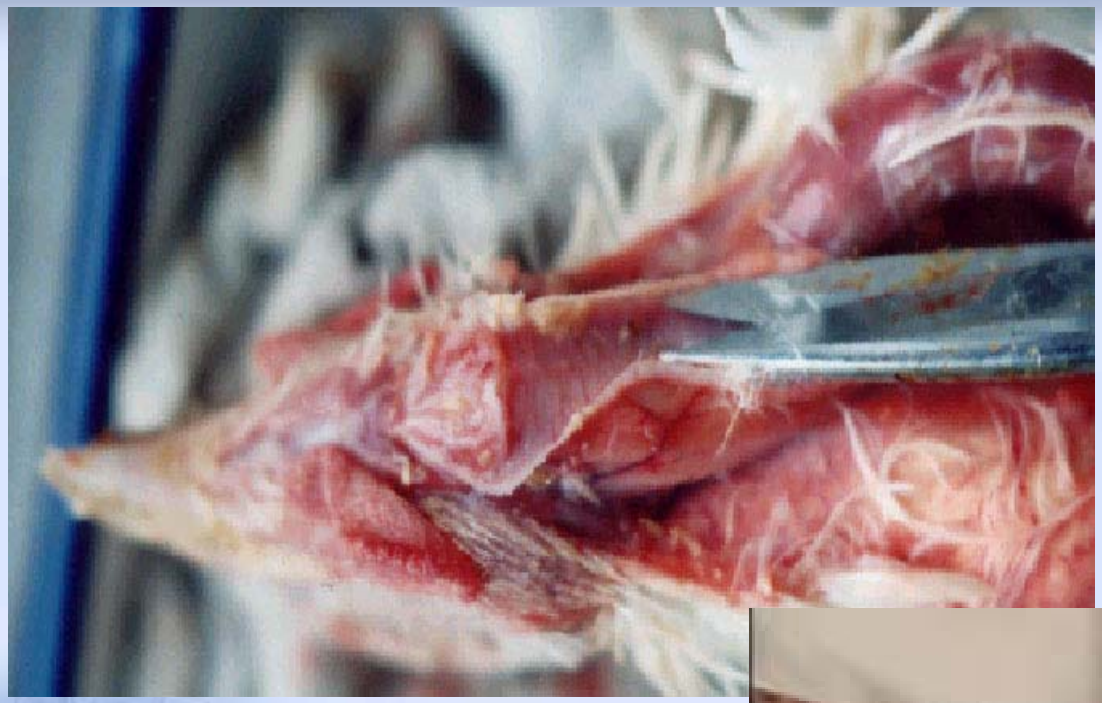


Severe hemorrhage on caecum tonsil



Swell on kidney, deposit of urates

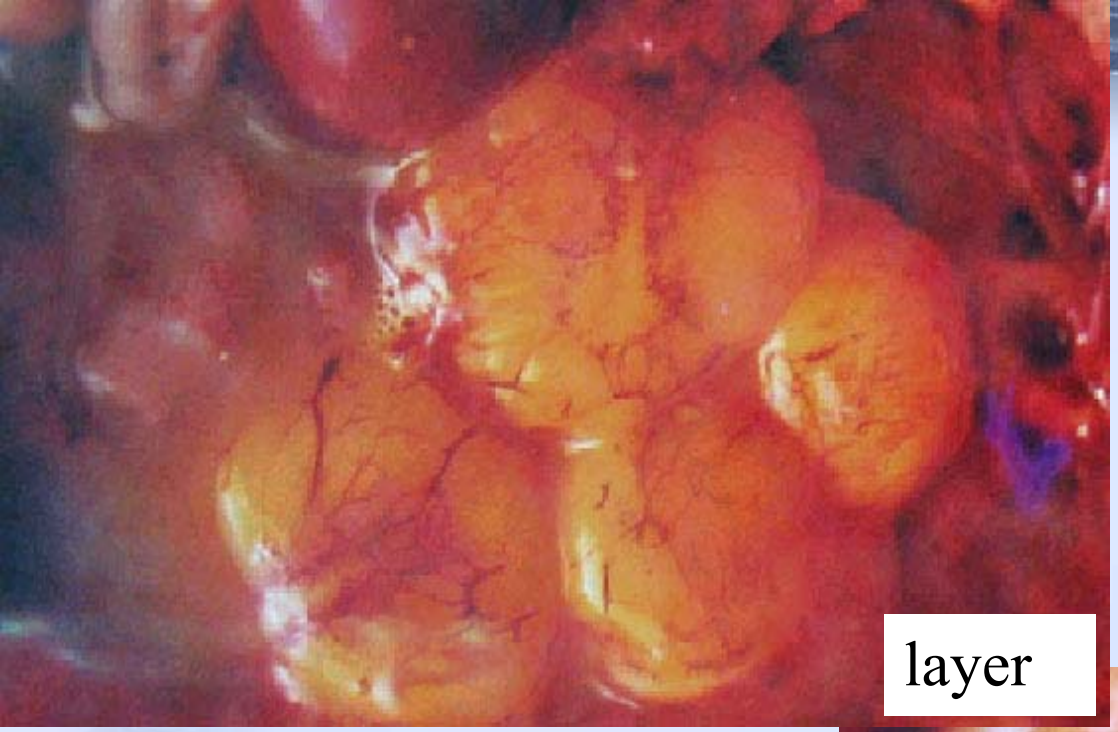
HPAI pathologic changes of the dissection



Hyperemia, hemorrhage and mucoid on larynx and trachea

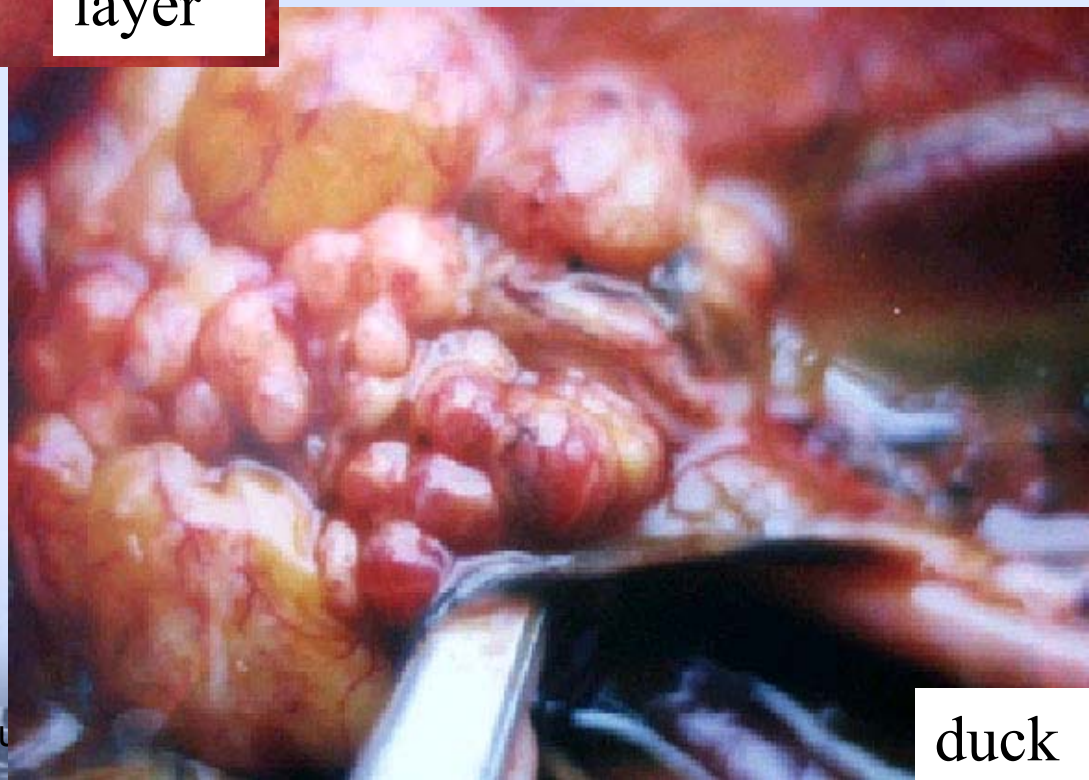
Hemorrhage and swell on thymus

HPAI pathologic changes of the dissection



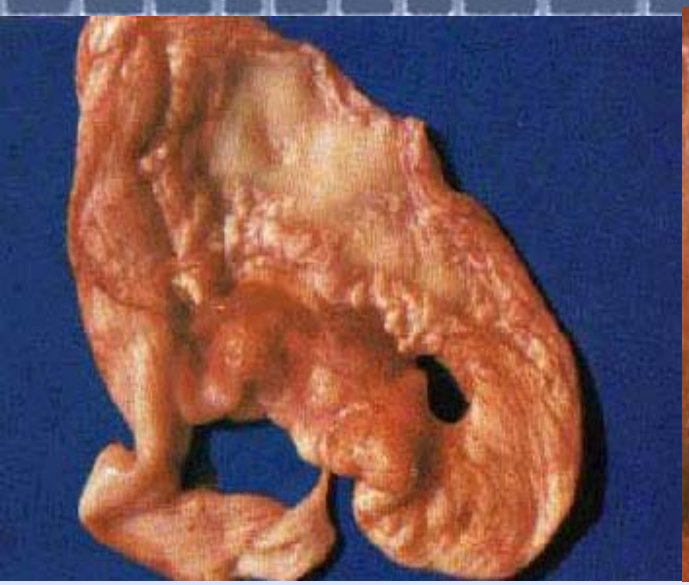
layer

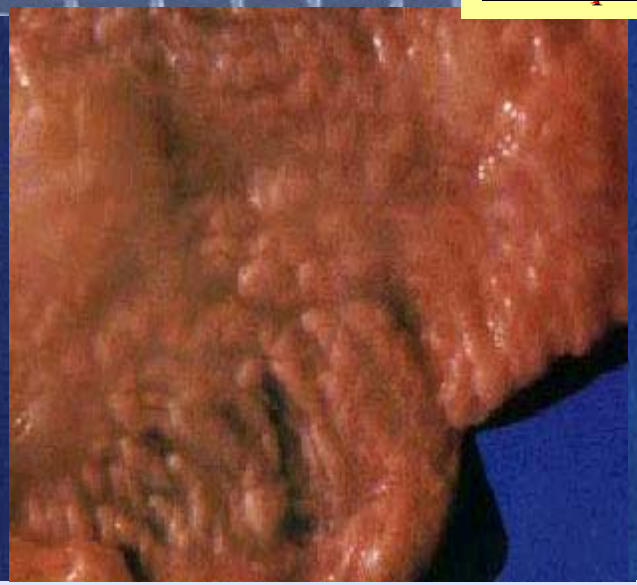
Hyperemia, hemorrhage and atrophy of follicle, yolk sac peritonitis

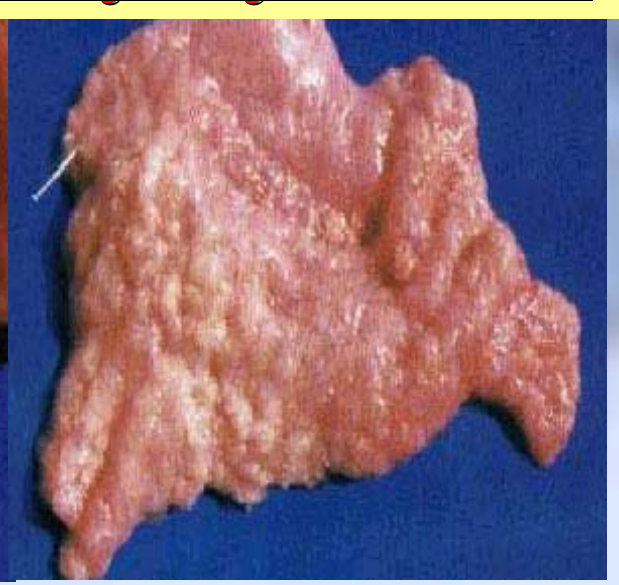


duck

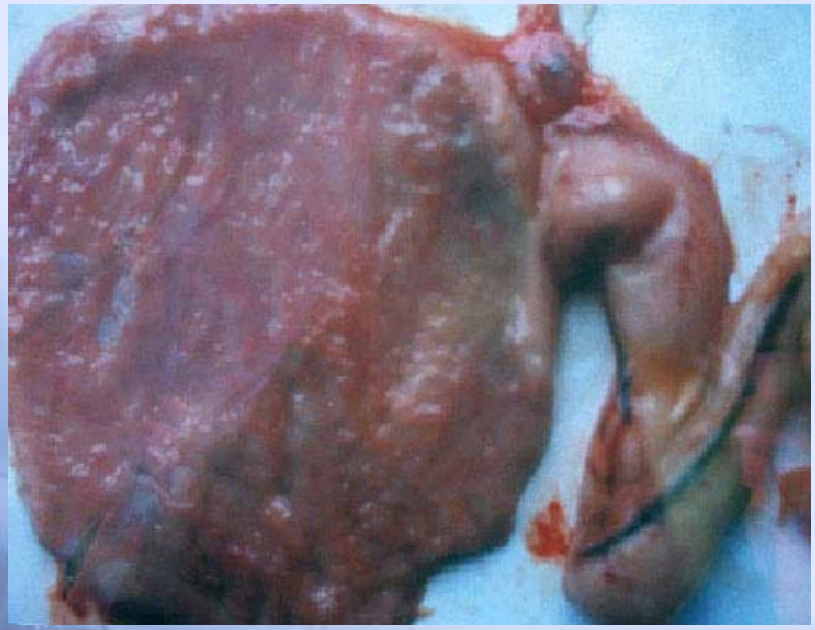
HPAI pathologic changes of the dissection



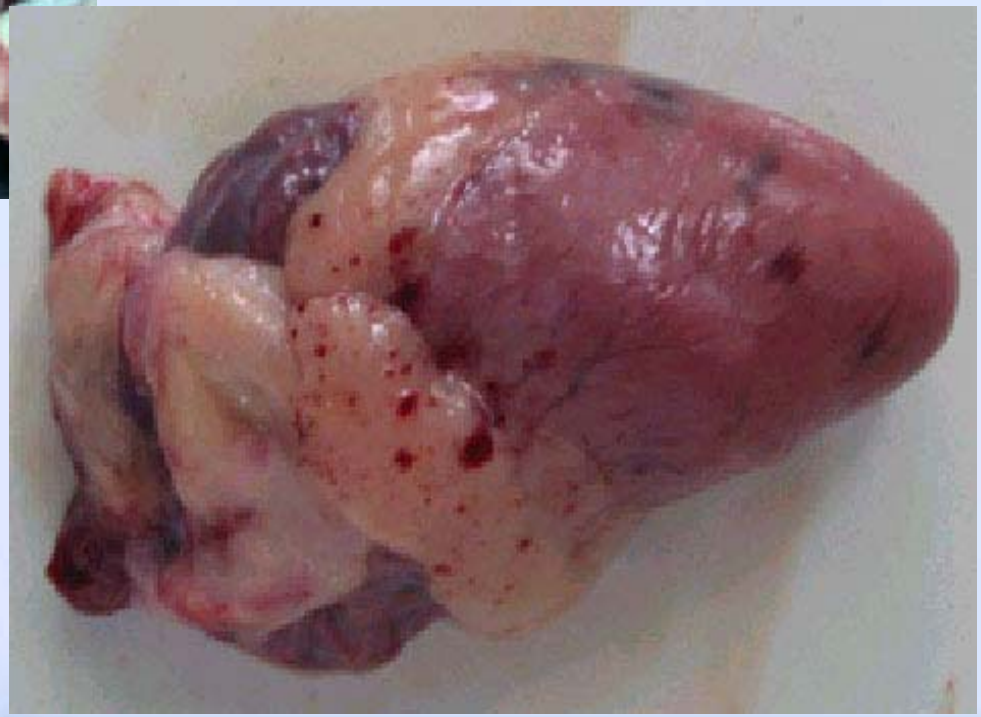




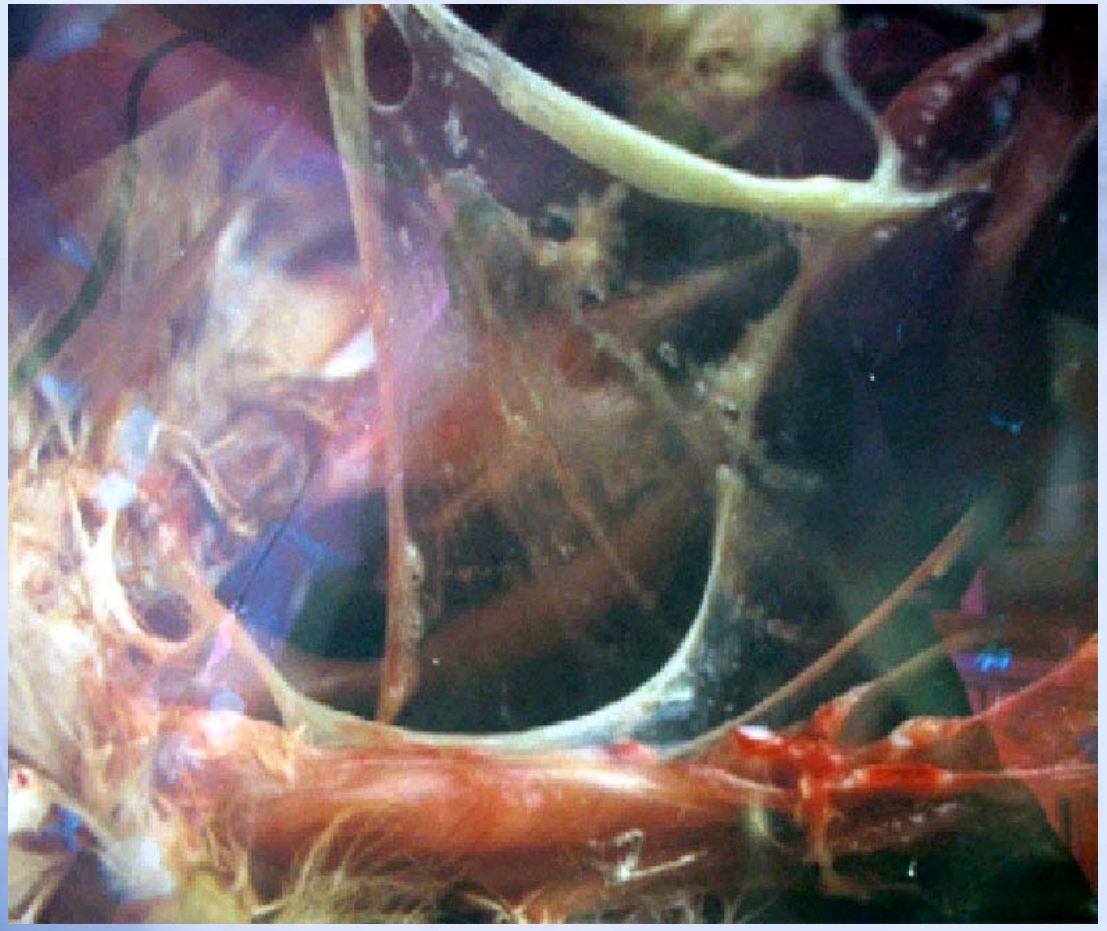
Hyperemia on ovary and oviduct, ivory secretion in oviduct



HPAI pathologic changes of the dissection



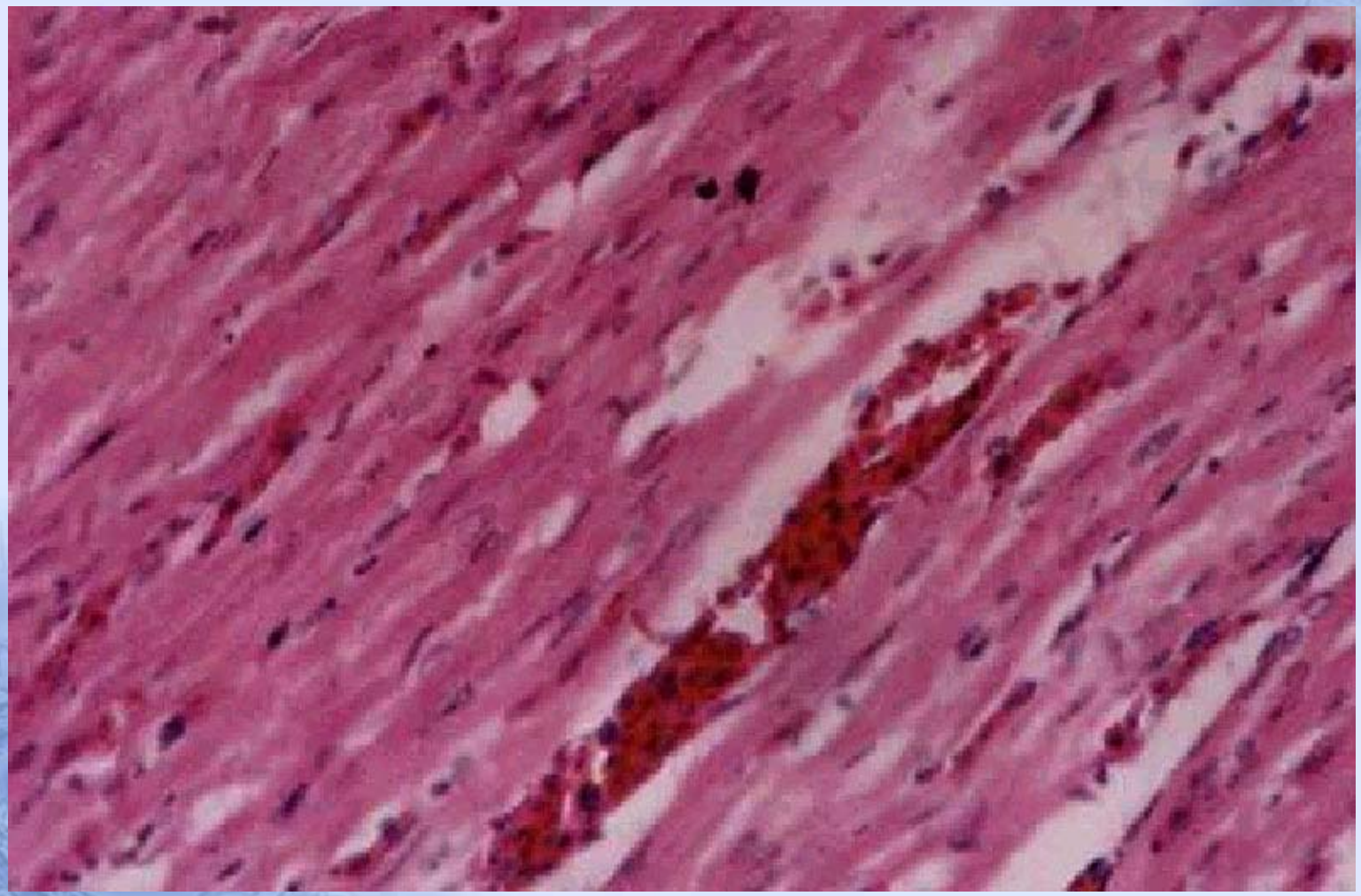
Hemorrhage on cordis fat, ivory strip necrosis on cardiac muscle



Air sac inflammation, serous and caseous secretion on air sac

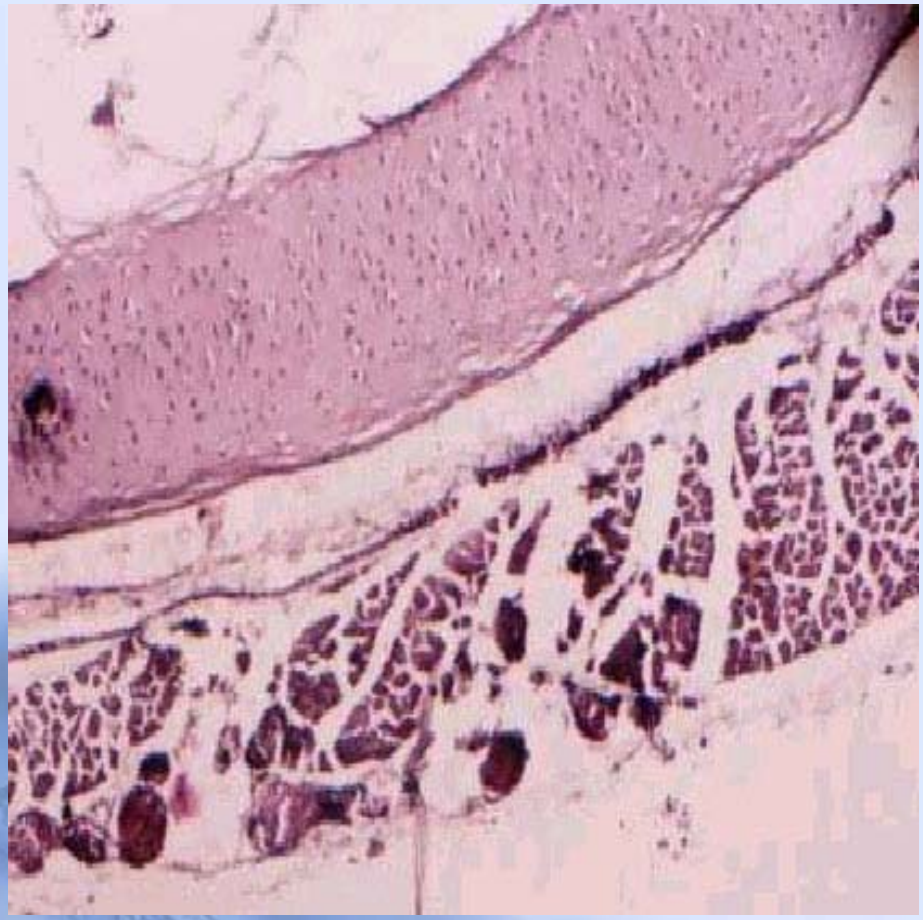
Pathoanatomical changes

Histology: Hemorrhage, hyperemia and necrosis in skin and internal organs (liver, spleen, pancreas, lung and kidney)

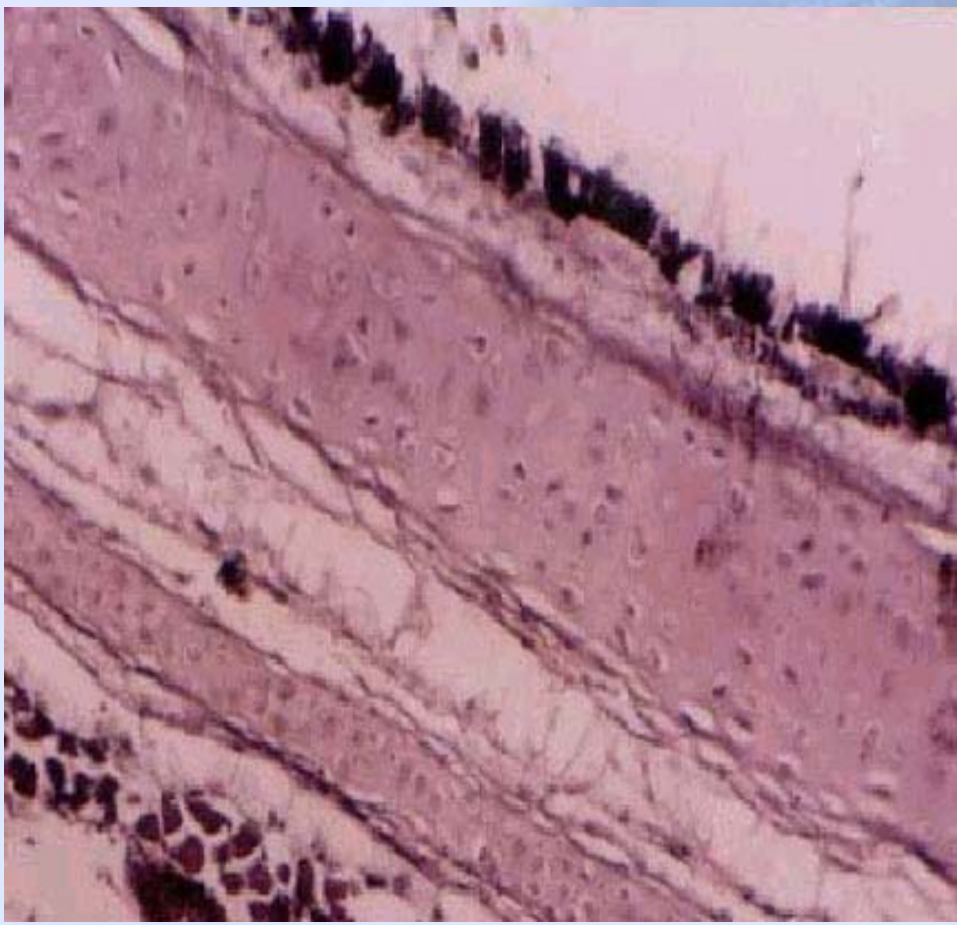


心肌　心肌纤维消失、横纹消失，点状出血。小血管充血

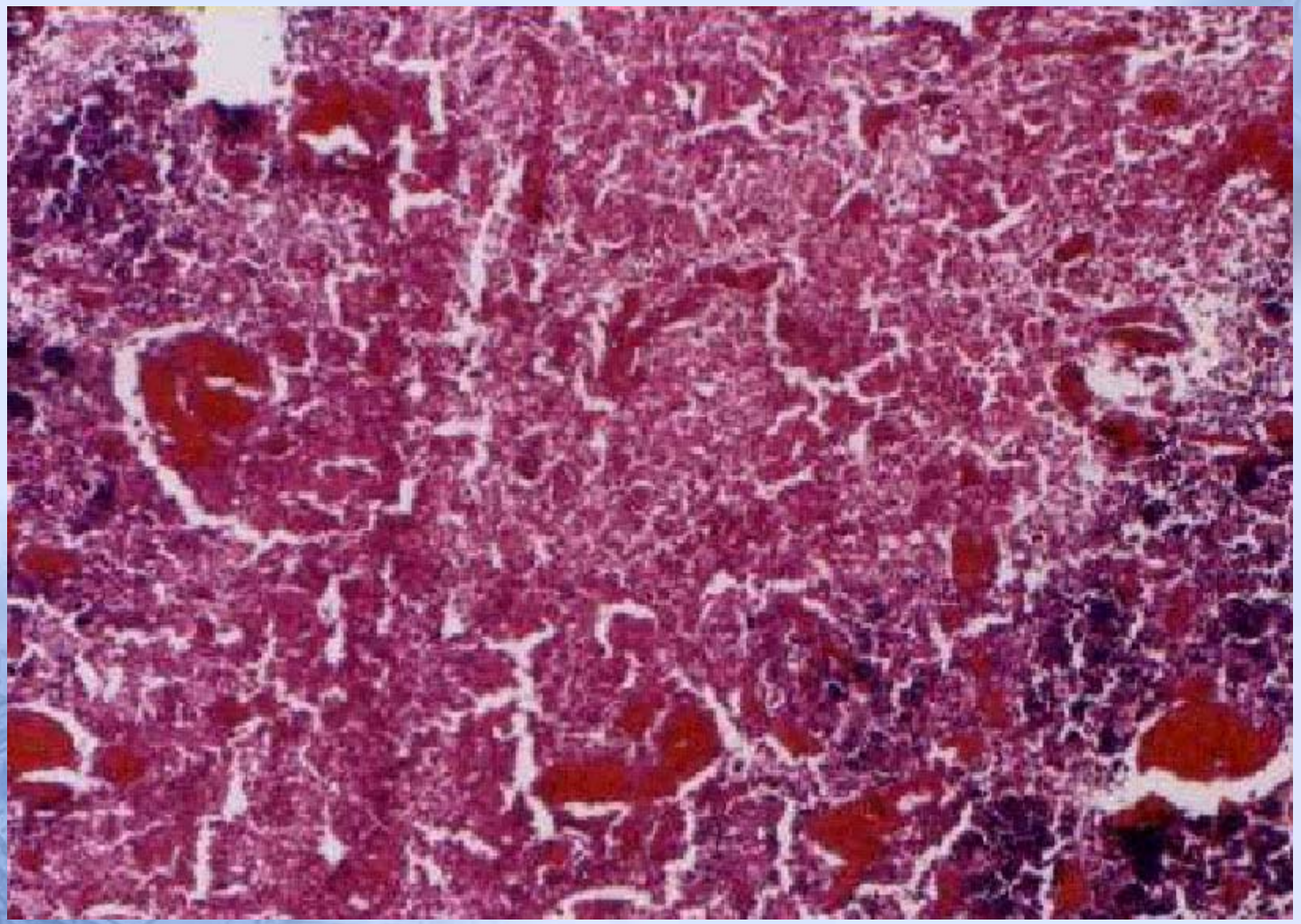
Cardiac muscle　Necrosis of muscle fibers、obliteration of rhabdium，focal hemorrhage and smaller vena filled with blood. HE40×3.3



气管 肌层血管出血
Trachea **the muscle layer blood vessel bleeds. HE 10×3.3**

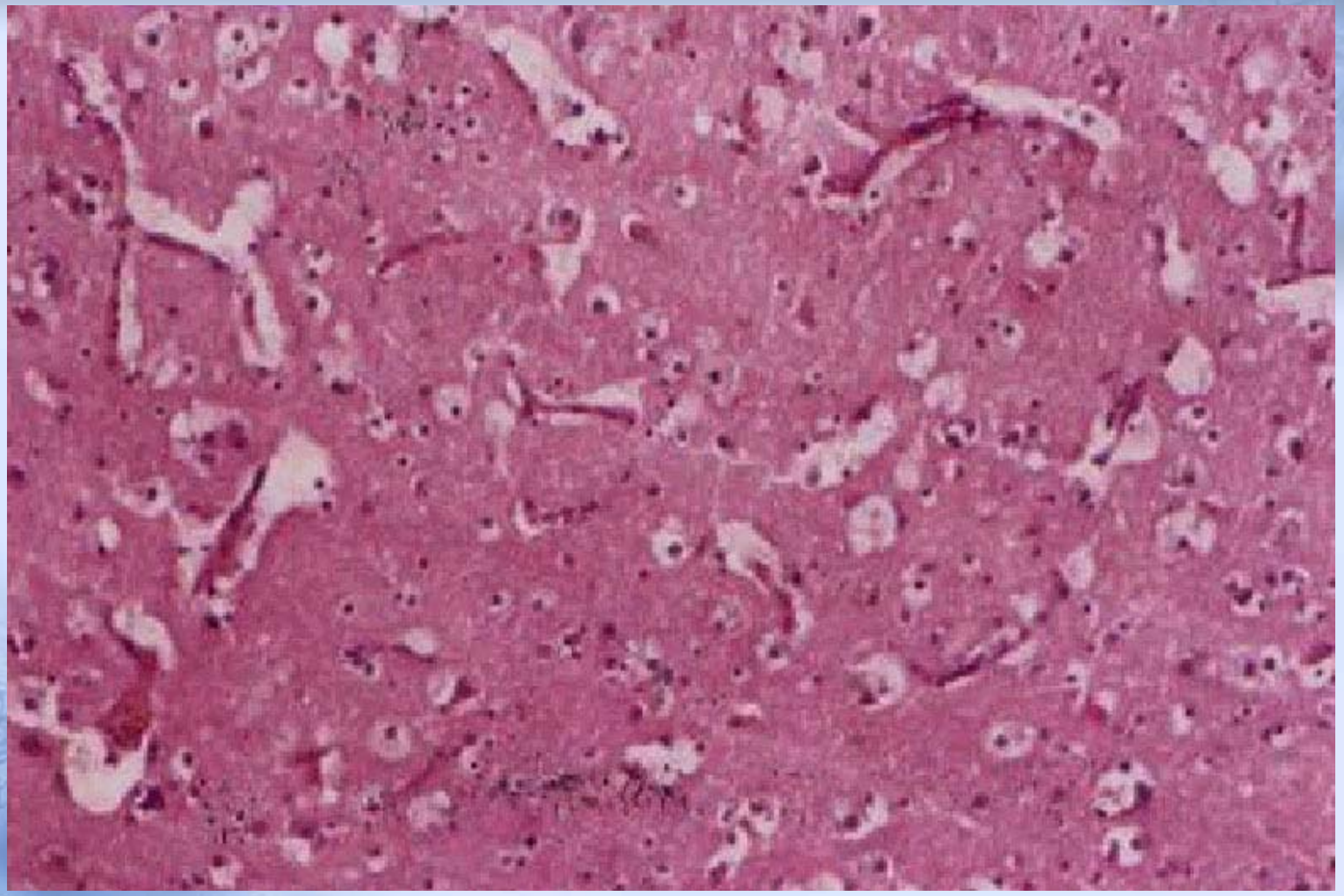


肌层血管出血
The muscle layer blood vessel bleeds. HE 20×3.3



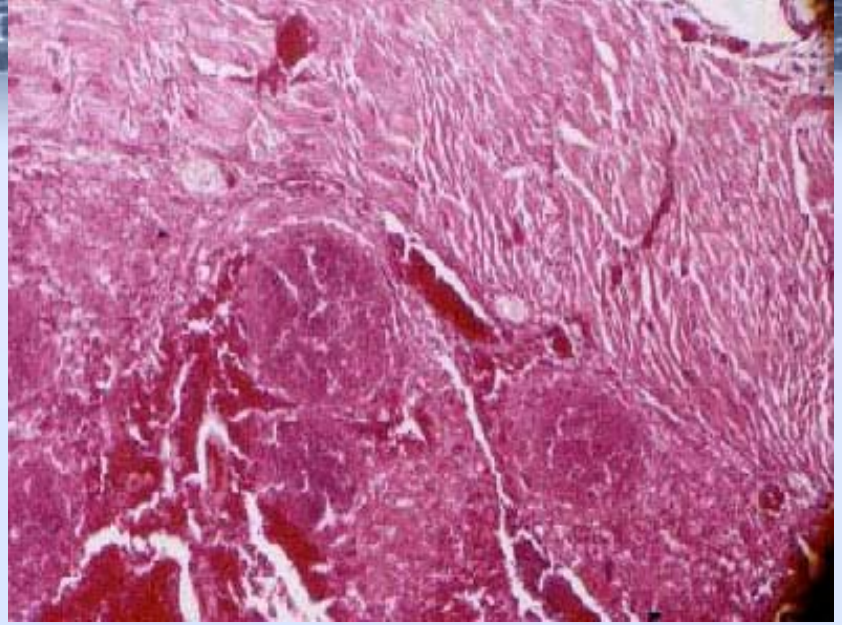
胸腺　淋巴细胞坏死，广泛性血管内凝血　　HE10×3.3

Thymus　Leukomonocyte necrosis , catholicity intravascular coagulation



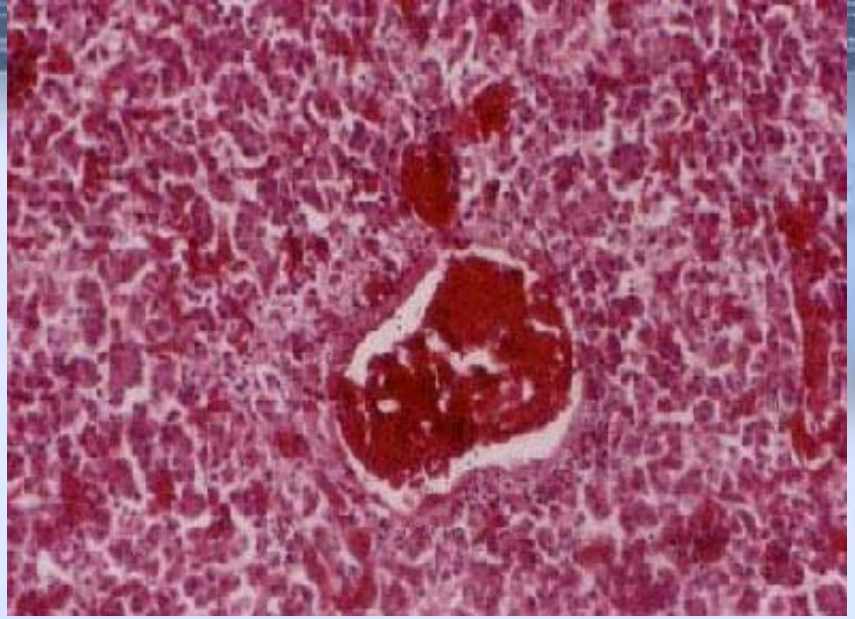
脑　神经细胞水肿、坏死呈空泡状，小血管充血
HE20×3.3

Brain　Neurocyte edema、necrosis to submit vacuolus，smaller vascular engorgement。

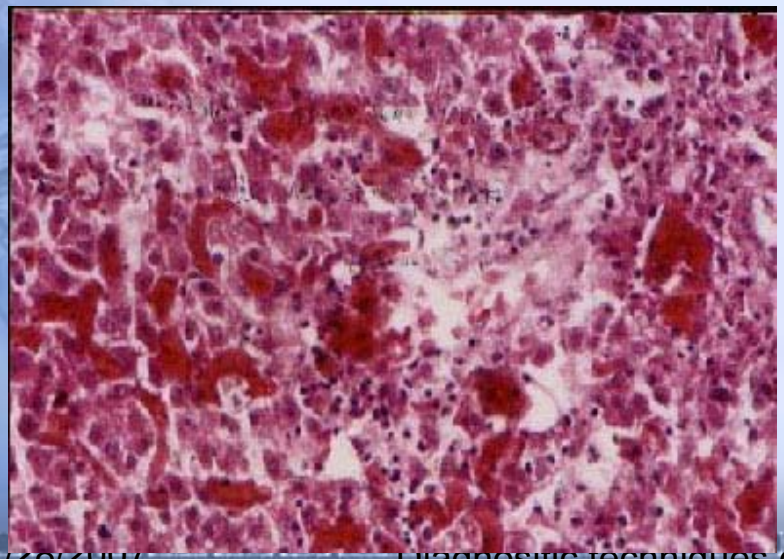


肝细胞坏死，窦壮隙小血管充血，淋巴样细胞浸润
Hepatocyte necrosis，sinusoids of hepatic lobule are filled with blood, lymphoid cell infiltration。HE 20×3.3

肝

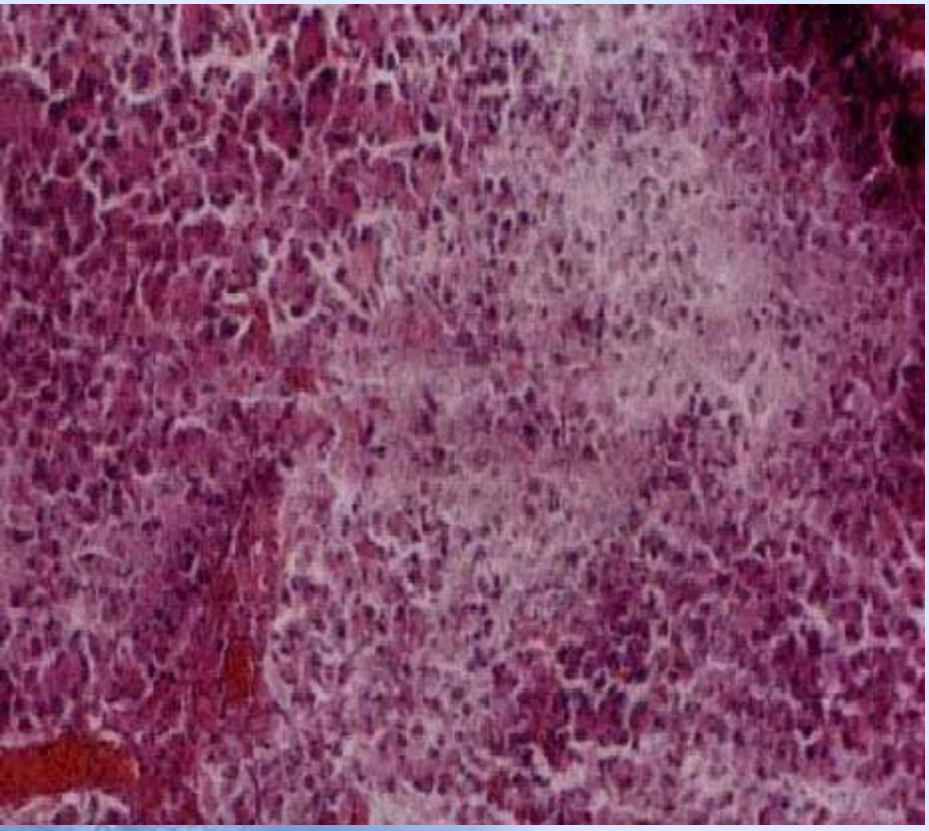


肝细胞坏死，窦壮隙小血管充血，淋巴样细胞浸润 Hepatocyte necrosis，sinusoids of hepatic lobule are filled with blood, lymphoid cell infiltration。 HE 20×3.3



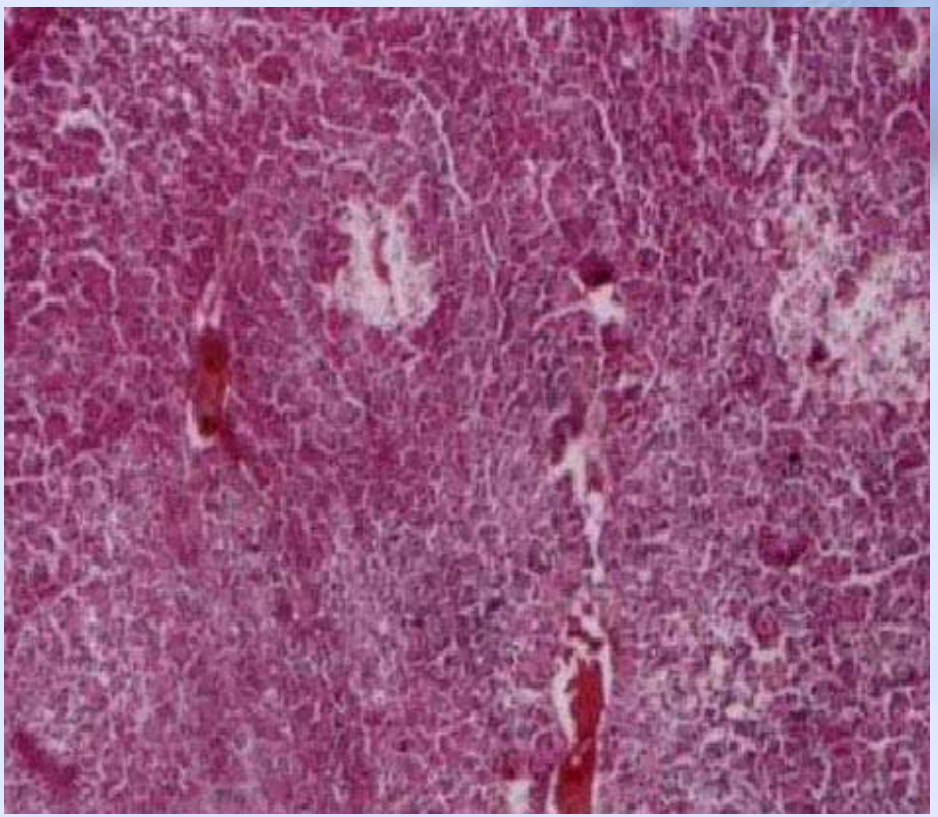
肝细胞呈灶状坏死，肝小叶内有淋巴样细胞润
Focal necrosis of hepatocyte , HE 40×3.3

lymphoid cell infiltrate within hepatic lobules。



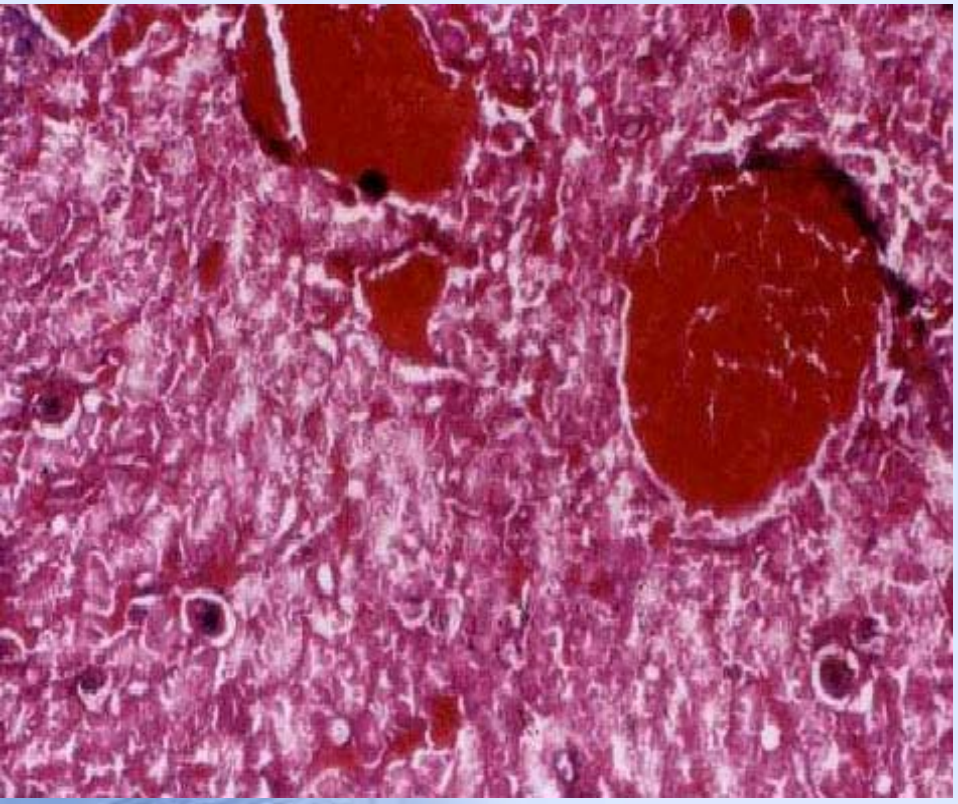
胰腺 腺泡细胞呈灶状坏死，小血管充血

Pancreas Acinar cell submit focal necrosis, smaller vascular engorgement HE 10×3.3



胰腺细胞坏死、呈空泡状，胰岛充血，小血管充血

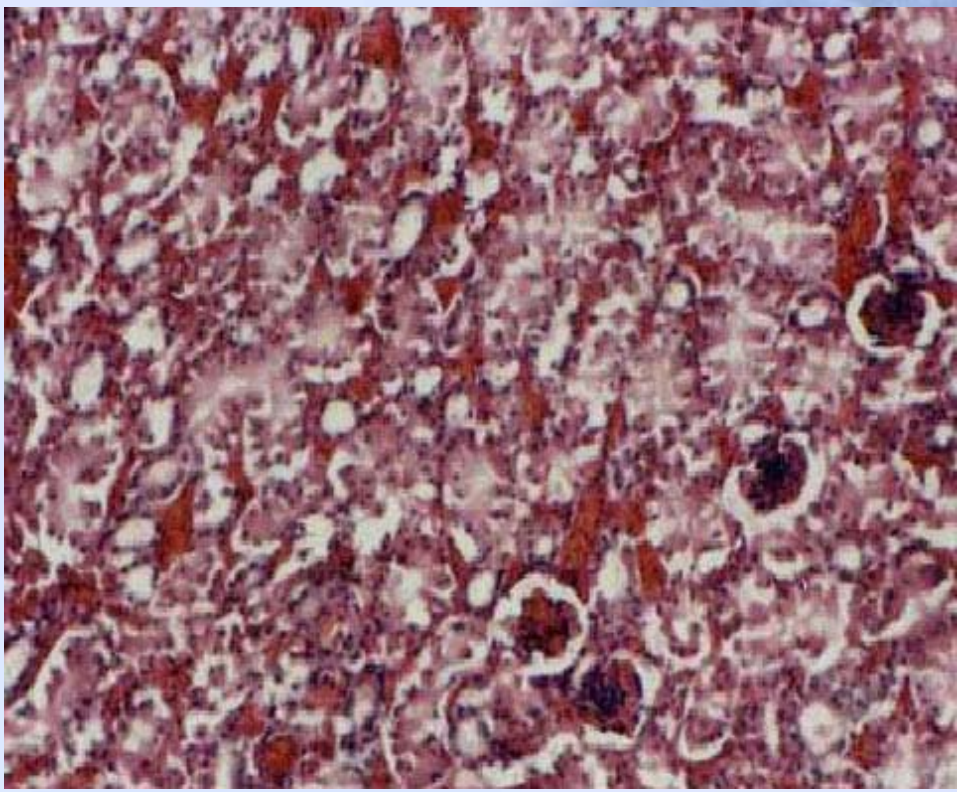
Acinar cell necrosis to submit vacuolus, glomeruli pancreatici congestion,smaller vascular engorgement HE 10×3.3



肾脏　肾小球出血、水肿，曲细尿管细胞坏死，小血管充血，有大的出血灶，淋巴样细胞浸润

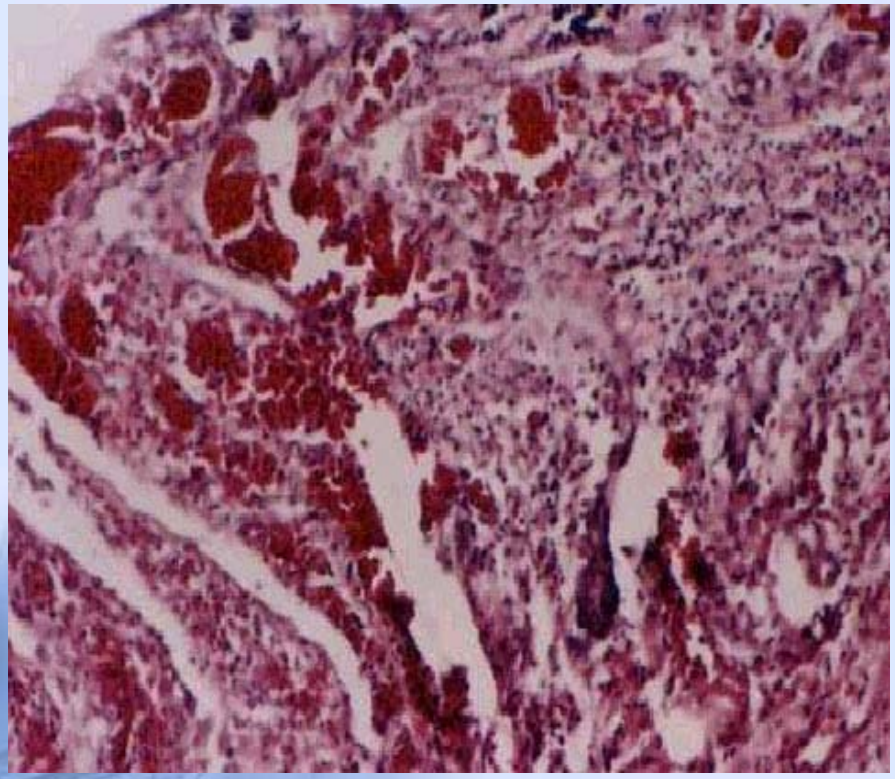
Rena Glomerulus appear hemorrhage、edema, renal tubules cells necrosis, small vessels congestion with lymphoid cell infiltration

HE 10×33



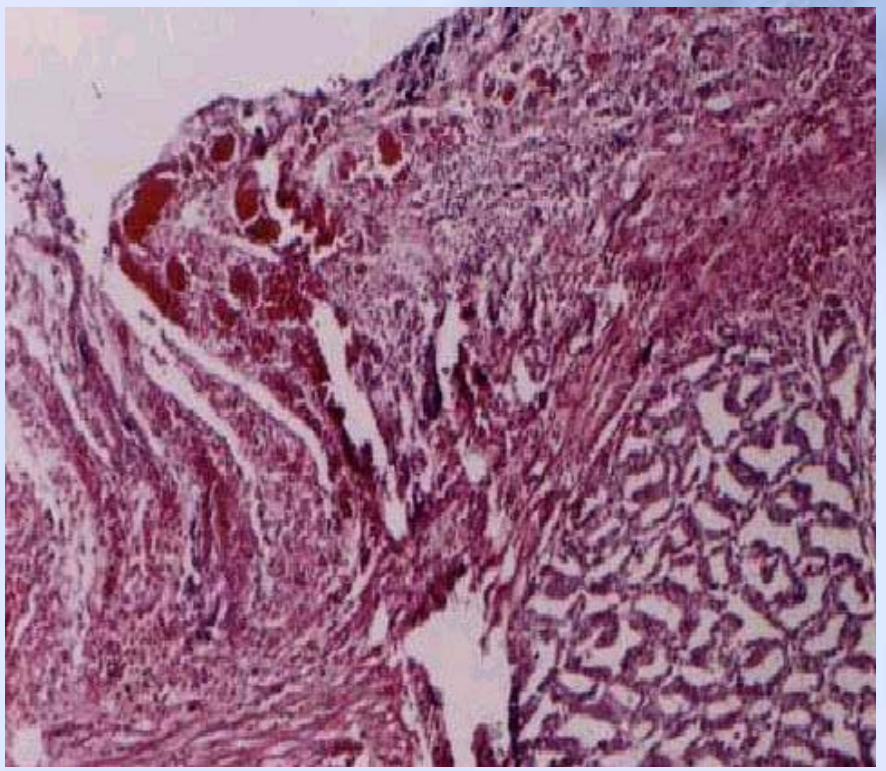
肾脏　肾小球水肿，曲细尿管细胞坏死。小血管充血

Rena Glomerulus appear edema, renal tubules cells necrosis, small vessels congestion HE 20×3.3



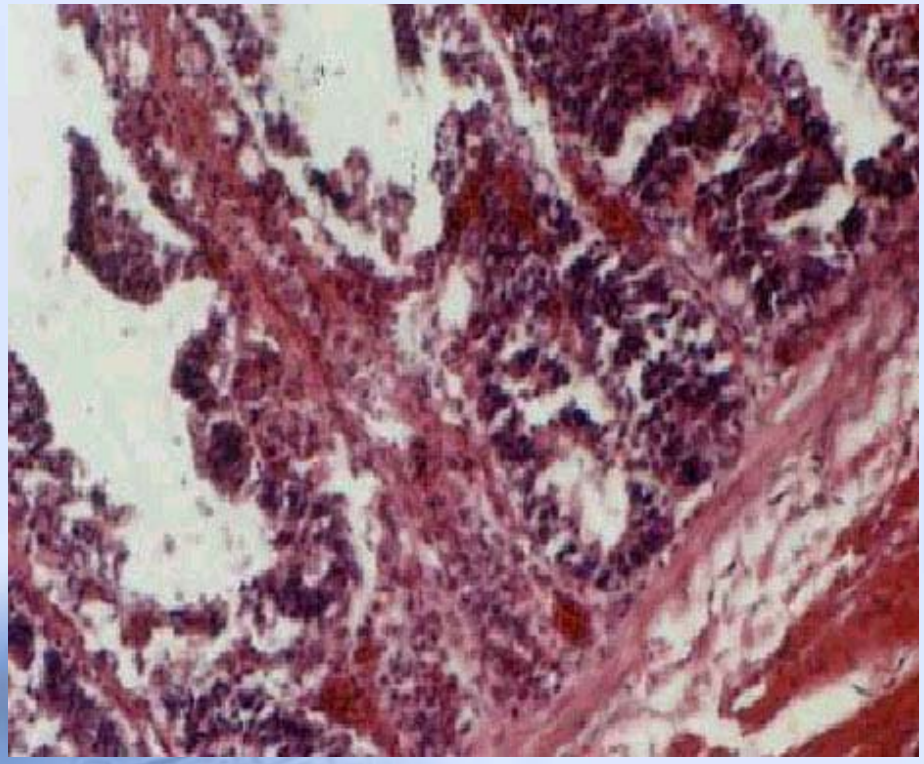
腺胃 粘膜上皮坏死、灶状出血。小血管充血，有大量淋巴样细胞浸润

Glandular stomach **epithelium mucosae necrosis and focal hemorrhage. Small vessels congestion and lymphoid cell infiltratio.** HE40×3.3



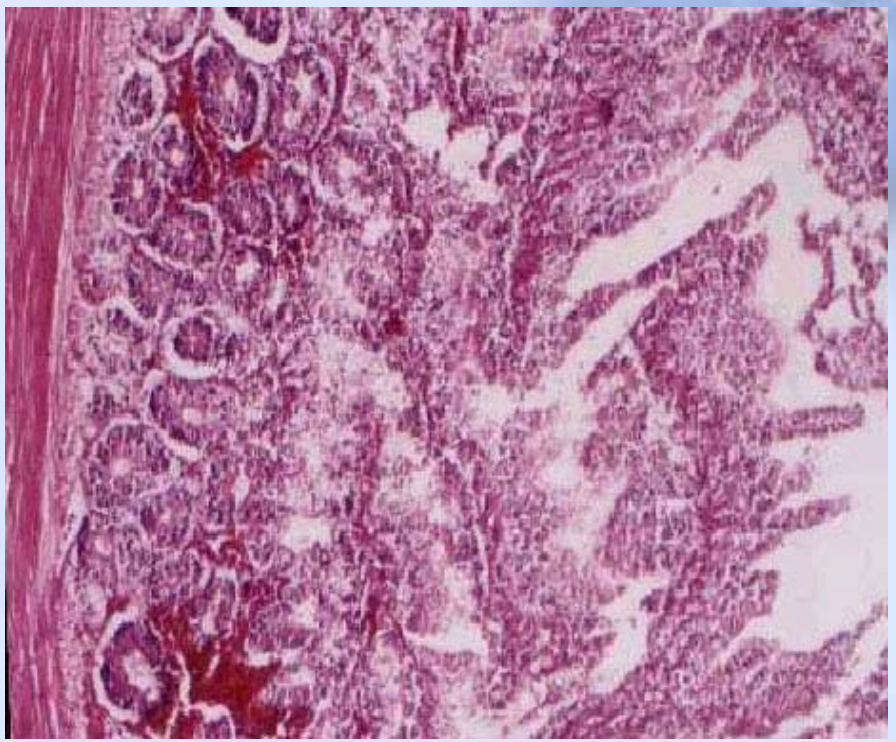
腺胃 粘膜上皮坏死、出血，小血管充血，有淋巴样细胞浸润

Glandular stomach **epithelium mucosae necrosis and focal hemorrhage. Small vessels congestion and lymphoid cell infiltratio.** HE 10×3.3



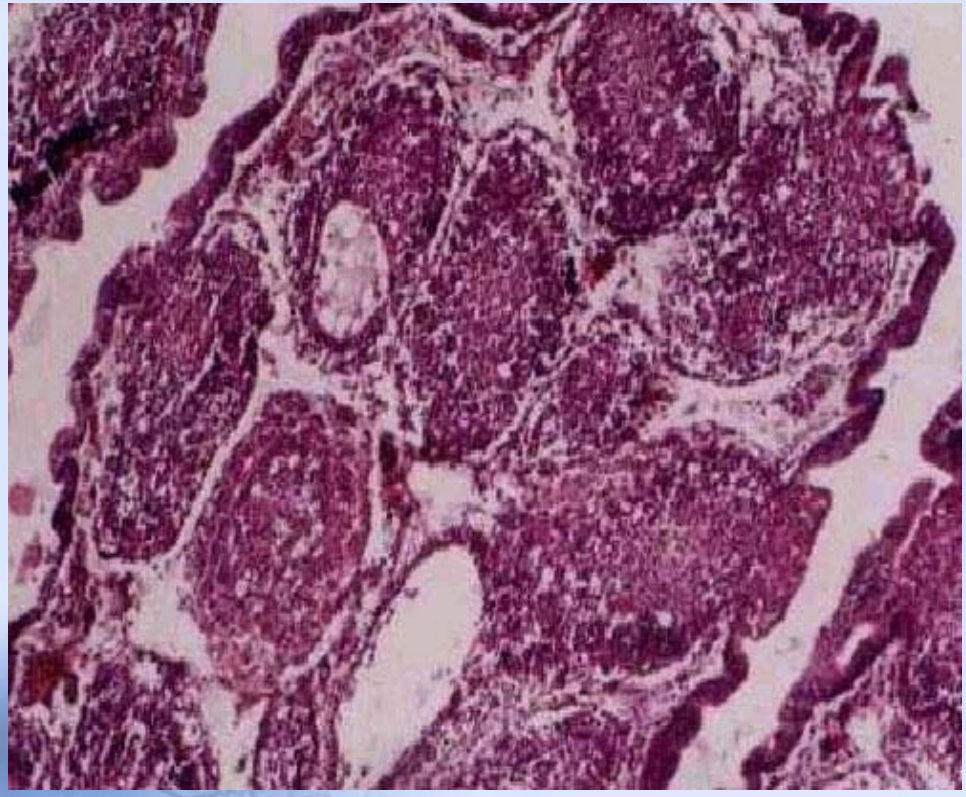
十二指肠 肠绒毛坏死、脱落，肠腺坏死，有淋巴样细胞浸润。固有层出血，肌层水肿

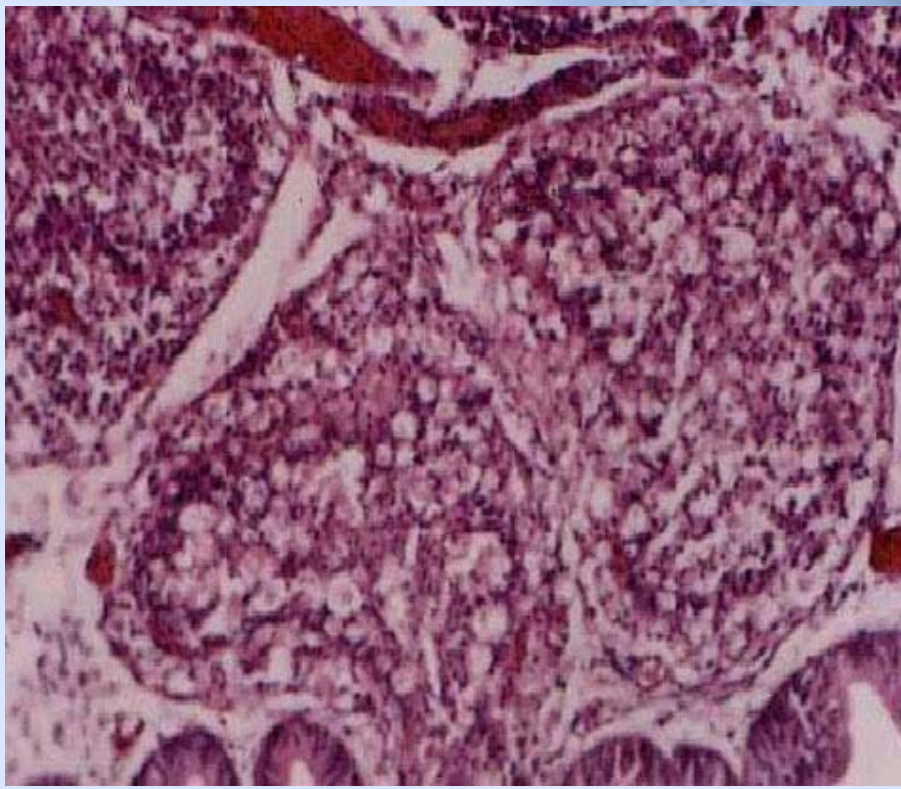
Duodenum intestinal villus necrosis to fall off, intestinal gland necrosis , Lymphoid cells infiltrated. lamina propria bleeding, muscular layer edema. HE20×3.3



十二指肠 肠绒毛坏死、崩解、消失 HE20×3.3

Duodenum intestinal villus necrosis to fall off, disintegrate and disappear.





法氏囊 淋巴滤泡坏死，呈空泡状，淋巴细胞外逸

Bursa of Fabricius folliculus lymphaticus necrosis to submit vacuolus，leukomonocyte submit to escape.

法氏囊 淋巴细胞坏死、崩解，数量减少，间质小血管淤血

Bursa of Fabricius folliculus lymphaticus necrosis and disaggregation , quantity of leukomonocyte decrease, small vessels congestion within interstitium.HE 20×3.3S

Pathological changes of duck

The duck of impatient death, which whole blood vessels were congestion and hemorrhage. Beak and head were filled with bleed. Web was filled with bleed, subcutaneous tissue，especially subcutaneous tissue of abdomen were congestion and fat tissue were with small bleeding point.

The liver was swollen with focal hemorrhage, which texture was crisp with soil yellow color.

The spleen was swollen to bleed greatly, the surface contain grey color necrosis point. Bursa of Fabricius was bleeding.

The most thymus gland were atrophies and bleeding.

The coronal fat were blooding, the myocardium has grey color necrosis points.

The pancreas has bleed points.

Parts of cases within gland stomach and muscle stomach has bleeding.

Duodenum filled with blood and hemorrhage.

Mucous membrane with interrupted 2~5 cm or so within jejunum and ileum were hemorrhage. Pleura filled with blood severity and adhere to thin yellow cellulose.

The gall bladder is greatly swollen, being filled with gall. Gallbladder enlarged and filled with bile.

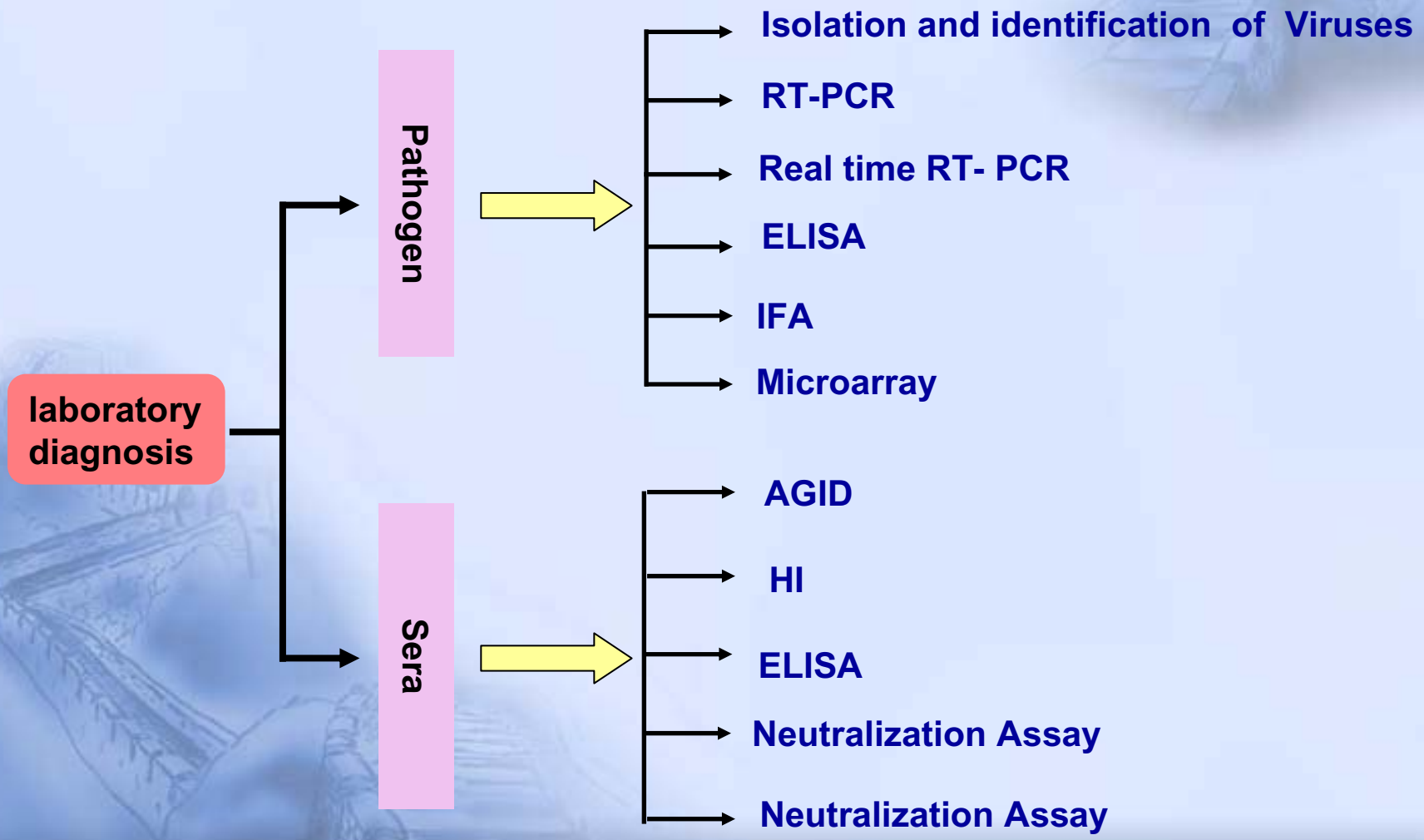
Annulus tracheae were hemorrhage.

Fall ill of egged duck were main pathological changes in the ovary, bigger ovarian follicle filled with blood and congestion. Some ovarian follicle atrophy and present purple grape kind.

Pathological changes of goose

The most sick goose filled with blood and hemorrhage, Subcutaneous tissue were hemorrhage. Myocardials of most case contain grey color necrosis spot. Endocardium has hemorrhage spot. In most case lung were congestion and hemorrhage. The pancreas has focal hemorrhage、 necrosis spot and liquefaction spot. Glandular stomach secretion increased. Parts of case mucosas were bleed. The juncture of glandular stomach muscular stomach were bleed. Egg bubble of egg laying female goose breaks in belly cavity and the egg bubble film were bleed and transform and sometimes the bleed spot were seen. Serous membrane of Ovary were hemorrhage and filled with blood and coagulated protein. With long course of disease goose ovarian follicle to dry up and shrink, egg bubble film were bleed、 hemorrhage and transform. Bursa of Fabricius in the sick poult goose were hemorrhage. The spleen, liver is greatly swollen to hemorrhage and hyperemia. The bowel were hemorrhage or hemorrhagic ulcer. Eye Conjunctival hemorrhage and nictitating membrane hyperemia and were bleeding which was so called for “red eye ”disease"

laboratory diagnosis of AIV



Isolation of influenza Viruses

Virus isolation is a highly sensitive and very useful technique for the diagnosis of viral infection when used with clinical specimens of good quality. In fact, isolation of a virus in eggs or cell culture along with subsequent identification by immunologic or genetic techniques (or by electron microscopy) are standard methods for virus diagnosis. One important advantage of virus isolation is that the virus is available for further antigenic and genetic characterization, and also for vaccine preparation or drug-susceptibility testing if required.

Agar Gel Immunodiffusion Test (AGID)

Principle

The AGID test is based on the principle of concurrent migration of antigen and antibody toward each other through an agar medium. The medium contains a high salt concentration, which enhances the formation of the antigen-antibody immune complex precipitate. As all influenza A viruses have antigenically similar nucleocapsid and antigenically similar matrix antigens, AGID tests are used to detect antibodies to these antigens. Concentrated virus preparations containing either or both type antigens are used in such tests.

Agar Gel ImmunodiffusionTest (AGID)

Advantage:

Easy method and convenient manipulation

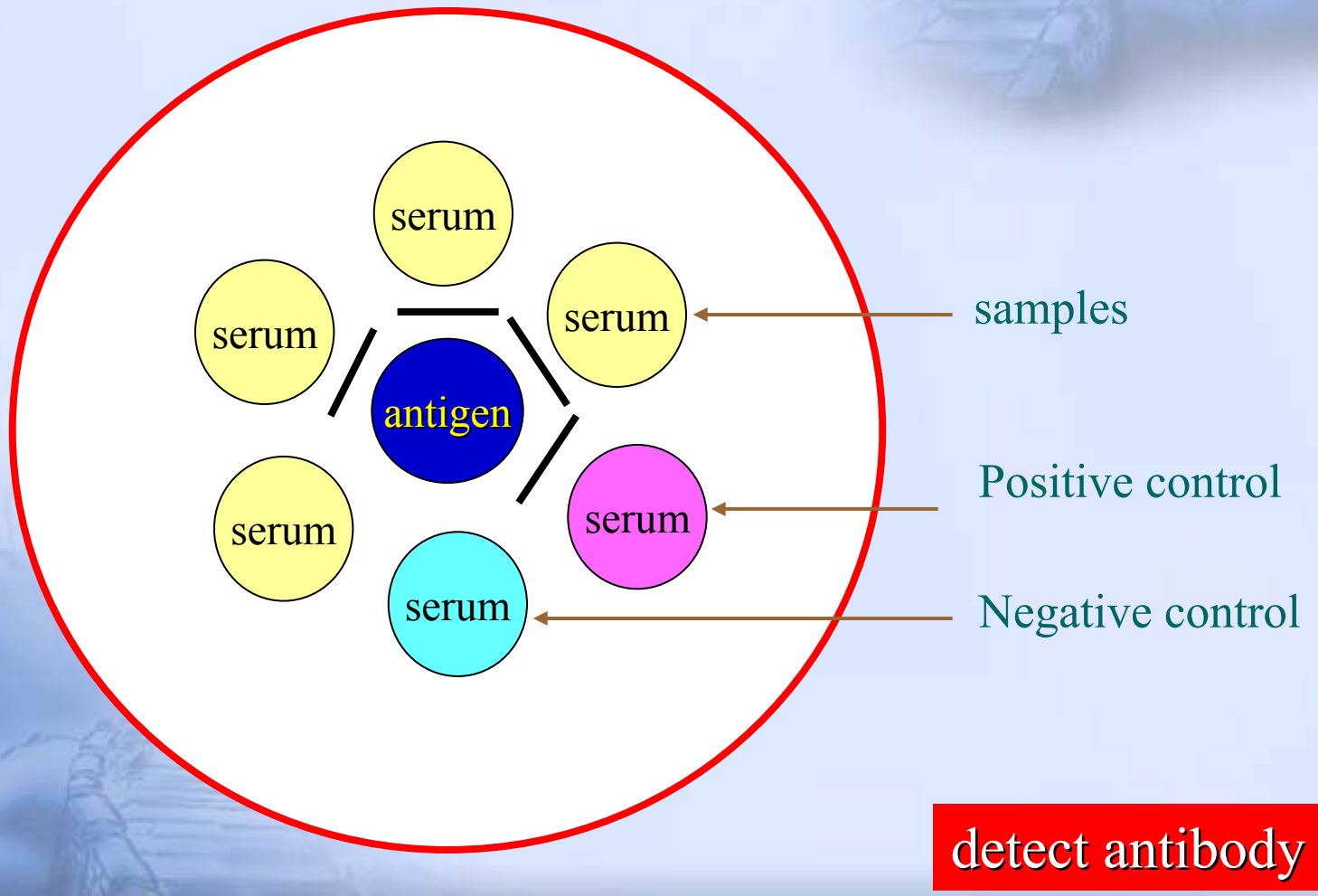
Applied to detection of antigen or antibody of all type A influenza viruses

Disadvantage:

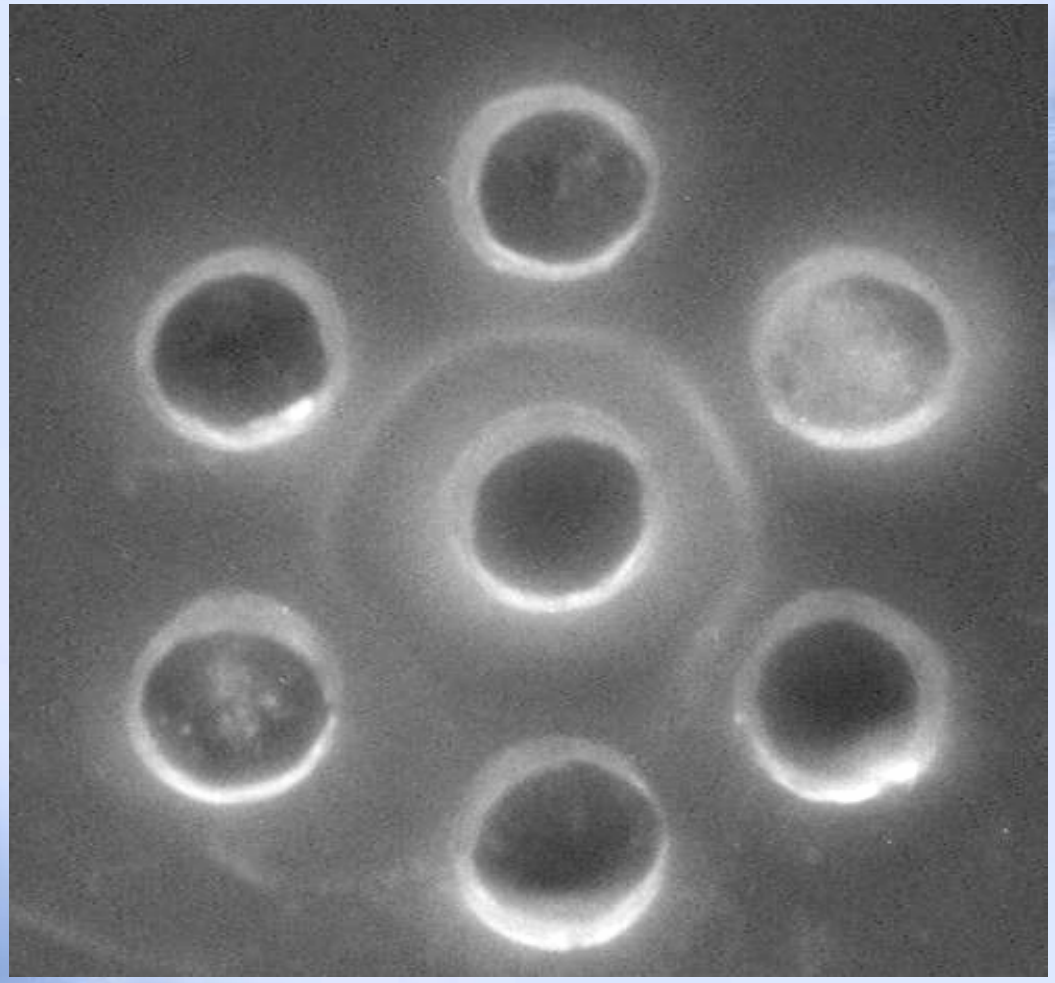
Low sensibility compared with HI test.

Not practicable to detection of AI antibody of aquatic poultry

The AGID schematic diagram of AIV



detect antibody



The results of AGID tests

Hemagglutination and Hemagglutination Inhibition Test

Principle：

The hemagglutinin (HA) protein agglutinates erythrocytes; hence, the derivation of its name. The traditional method for identifying influenza field isolates takes advantage of this property. Specific attachment of antibody to the antigenic sites on the HA molecule interferes with the binding between the viral HA and receptors on the erythrocytes. This effect inhibits hemagglutination and is the basis for the hemagglutination inhibition (HAI) test.。

Hemagglutination and Hemagglutination Inhibition Test

Advantage:

The HAI assay is widely used in WHO global influenza surveillance.

Practicable to identification of all influenza virus isolates

Practicable to detection of serum antibodies of infected or immunized poultry

Disadvantage:

Disadvantages of the HAI test include the need to remove nonspecific inhibitors which naturally occur in sera, to standardize antigen each time a test is performed, and the need for specialized expertise in reading the results of the test.

Neuraminidase Inhibition Assay

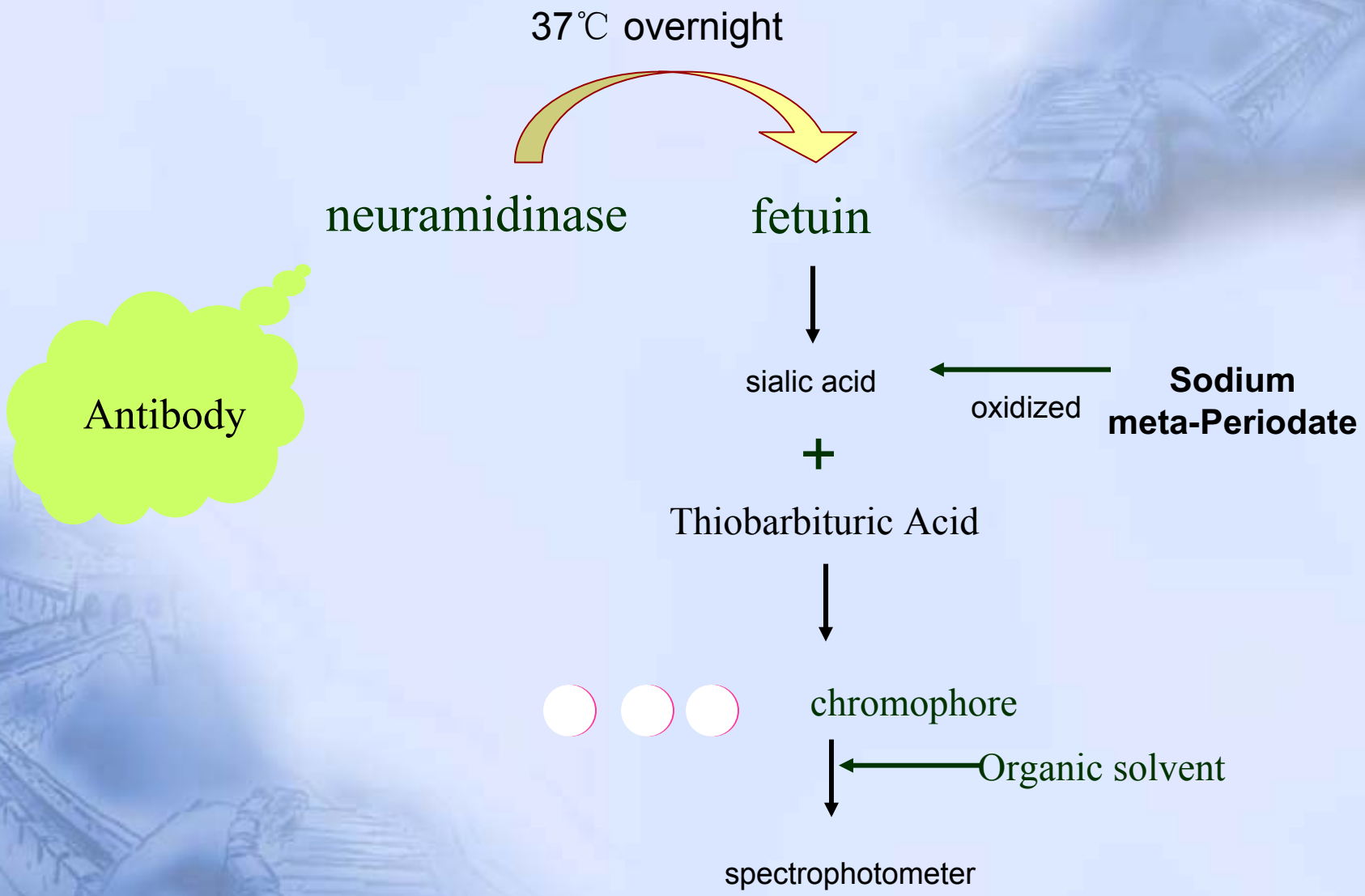
Principle

NA is the enzyme that cleaves terminal sialic acid residues from cell surface receptors of the influenza virus. This activity enables release of virions from infected cells and removes sialic acid from newly synthesized HA and NA molecules. The first step in the procedure is to estimate the amount of NA activity in your influenza virus sample. In this assay the viral neuraminidase acts on the substrate and releases salic acid. Arsenite reagent is added to stop enzyme activity. The amount of sialic acid liberated is determined chemically with the thiobarbituric acid that produces a pink color in proportion to free sialic acid. The amount of color is measured in a spectrophotometer at wavelength 549nm.

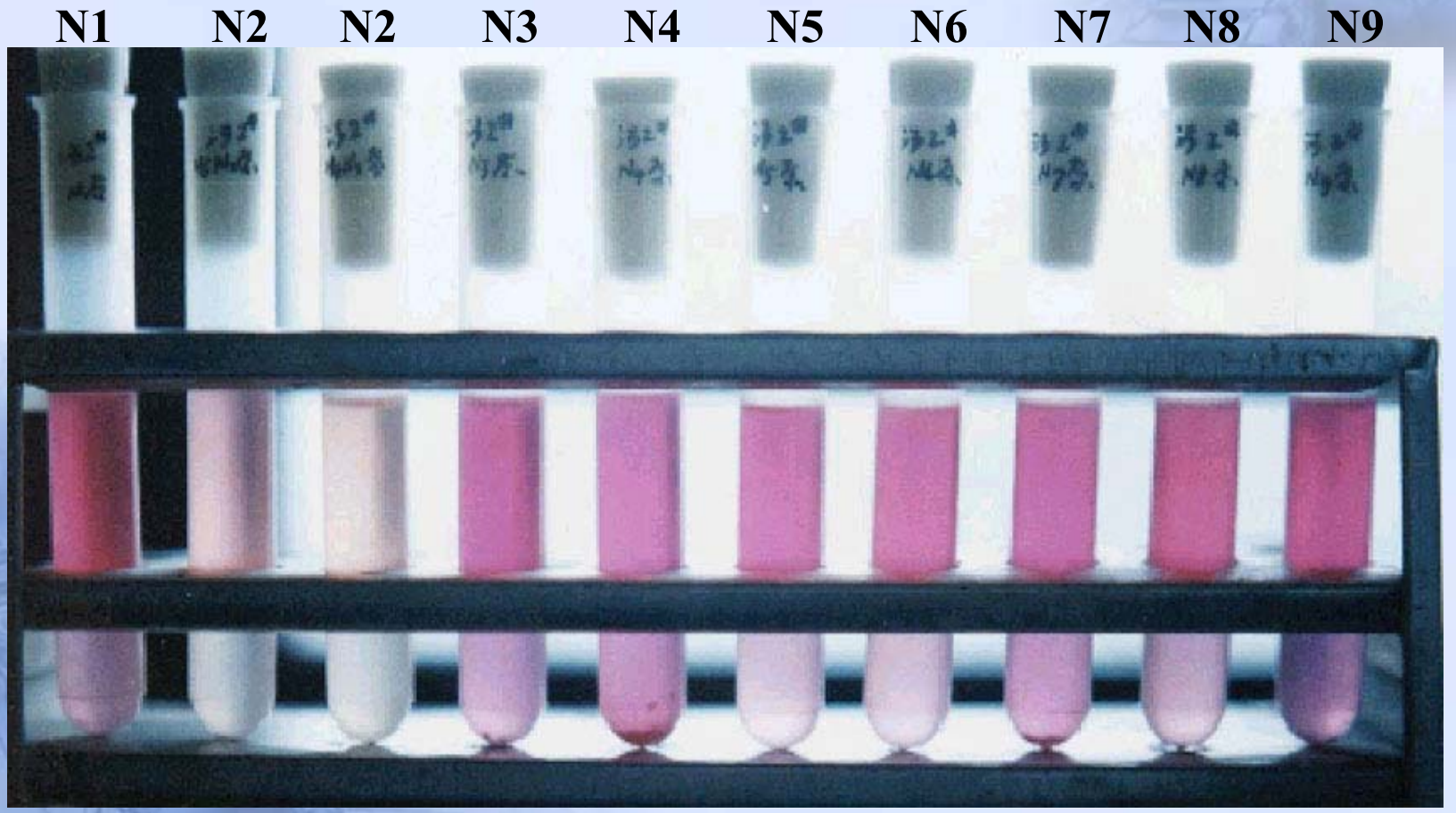
Neuraminidase Inhibition Assay

Principle

The next step in the procedure is to standardize the reference antisera to each of the nine NA subtypes. This serum can be prepared in animals to the isolated NA when it is monospecific for that NA. If monospecific sera is not available then antiserum to the whole virus is used. The third step in the procedure is the identification of the NA subtype of an unknown influenza virus usingthe specific standardized antisera. In the NAI test the antibodies in the serum discriminate between the different NA subtypes and inhibit the action of the viral enzyme on the fetuin substrate and therefore no pink color is developed in the test.


37℃ overnight
neuramidinase
fetuin
Antibody
sialic acid
oxidized
Sodium meta-Periodate
+
Thiobarbituric Acid
chromophore
Organic solvent
spectrophotometer

The results of Neuraminidase Inhibition Assay



The Intravenous Pathogenicity Test (IVPI) for Influenza Viruses

As the terms highly pathogenic avian influenza (HPAI) and ‘fowl plague’ refer to infection with virulent strains of influenza A virus, it is necessary to assess the virulence of an isolate for domestic poultry. Whereas all virulent strains isolated to date have been either of the H5 or H7 subtype, most H5 or H7 isolates have been of low virulence. It is very important to assess whether the virulent strains isolated is highly pathogenic virulent strains or not.

Method of the IVPI test

The IVPI test is carried out as follows:

1. Working in a High Security Laboratory, harvest infective allantoic fluid from Specific Pathogen Free (SPF) eggs previously inoculated with virus. The hemagglutinating titre must be >1/16 (>24 or >log2 4 when expressed as the reciprocal).

2. Dilute the harvested material 1:10 in sterile isotonic saline. (Antibiotics must not be used so as to avoid the i/v inoculation of birds with relatively high concentrations of antibiotics.) Any suspicion of bacterial contamination in allantoic fluid is checked by bacterial culture on nutrient agar plates prior to IVPI test.

   All the following in-vivo procedures should ideally be done in a category 3 high security animal facility.

3. Inoculate 0.1ml of the diluted/undiluted virus intravenously into each of 10 six-week-old chickens. These birds should be hatched from eggs obtained from an SPF flock. Inoculate two chicks with 0.1ml of the diluent to act as controls.

4.Examine the birds for clinical signs at intervals of 24 hours over a ten day period.

5.At each observation, each bird is scored 0 if normal, 1 if sick, 2 if severely sick, 3 if dead. (The judgement of sick and severely sick birds is a subjective clinical assessment. Normally, 'sick' birds would show one of the following signs and 'severely sick' more than one of the following signs: respiratory involvement, depression, diarrhoea, cyanosis of the exposed skin or wattles, oedema of the face and/or head, nervous signs. Dead individuals must be scored as 3 at each of the remaining daily observations after death).

6. The intravenous pathogenicity index (IVPI) is the mean score per observation over the 10-day period. An index of 3.00 means that all birds died within 24 hours, an index of 0.00 means that no bird showed any clinical sign during the 10-day observation period. Calculate the pathogenicity index as shown in the example below: (The numbers recorded are the numbers of birds showing clinical signs on the specified day.)

Clinical signs.	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Total	Score
Normal	10	10	0	0	0	0	0	0	0	0	20×0	=0
Sick	0	0	3	0	0	0	0	0	0	0	3×1	=3
Paralysed	0	0	4	5	1	0	0	0	0	0	10×2	=20
Dead	0	0	3	5	9	10	10	10	10	10	67×3	=201
											Total	=224

The index is calculated as the mean score per bird per observation. In the above example this would be: 224/100=2.24

The OIE criteria for classifying an avian influenza virus as highly pathogenic：

1.Any influenza virus that is lethal for six ,seven or eight of eight 4-8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free,infective allantoic fluid.

2.The following additional test is reqired if the isolate kills from one to five chickens but is not of the H5 or H7 subtype: growth of the virus in cell culture with cytopathic effect or plaque formation in the absence of trypsin.If no growth is observed,the isolate is not considered to be a HPAI isolate.

3.For all H5 and H7 viruses of low pathogenicity and for other influenza viruses,if growth is observed in cell culure without trypsin ,the amino acid sequence of the connecting peptide of the haemagglutinin must be determined.If the sequence is similar to that observed for other HPAI isolates, the isolate being tested will be considered to be highly pathogenic.

ELISA diagnostic technique of AIV

For virus

Coating antibody

For antibody

Coating antigen

samples

sera

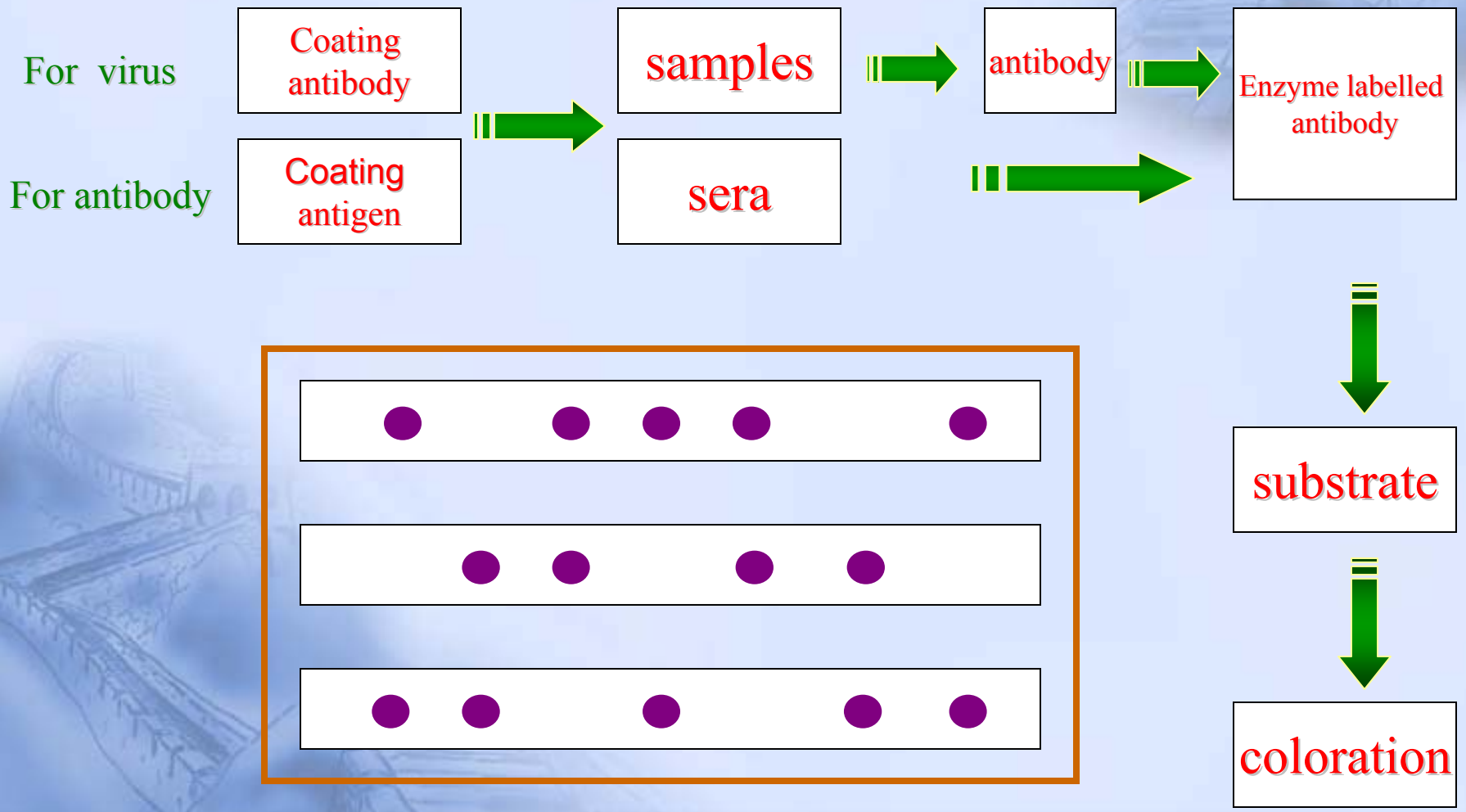
antibody

Enzyme labelled antibody

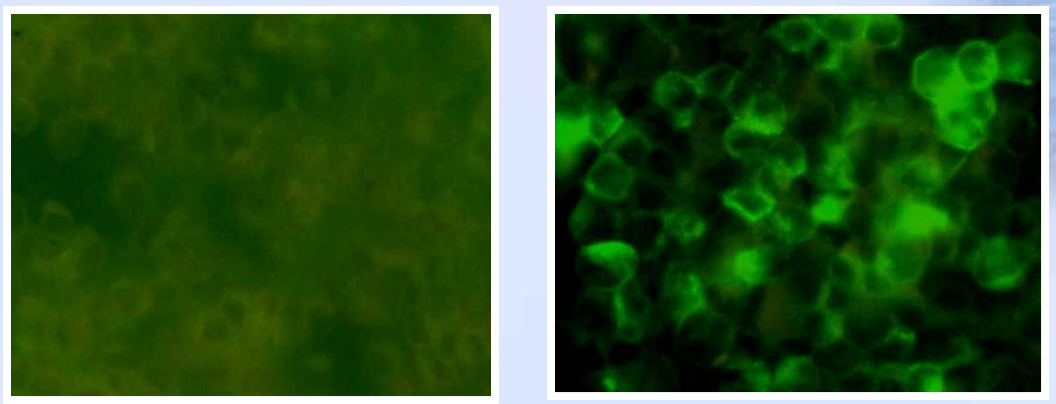
substrate

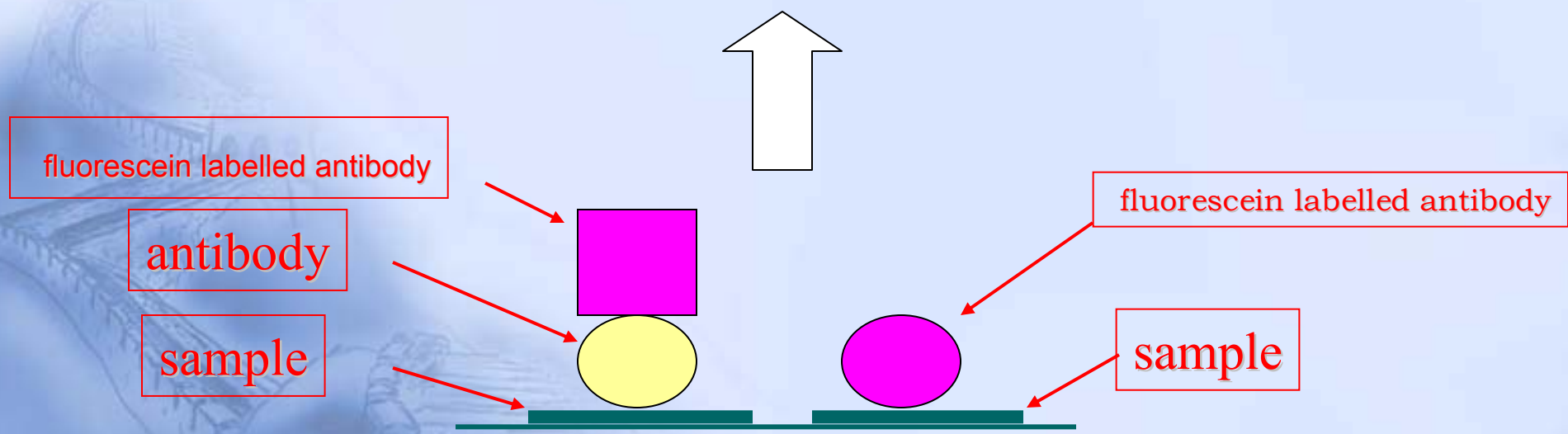
coloration

Dot-ELISA diagnostic technique of AIV

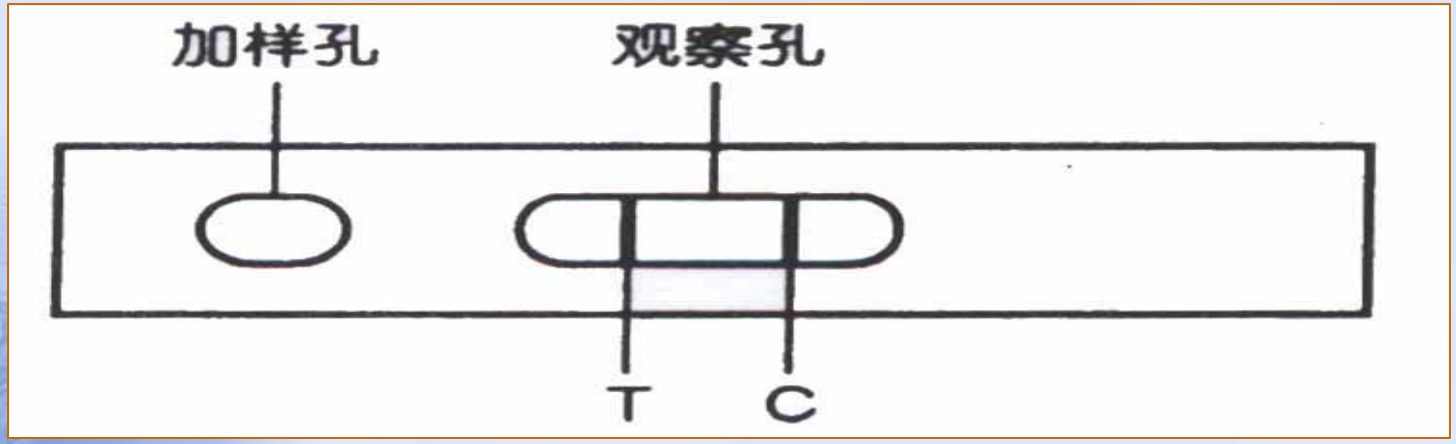


Immunofluorescence diagnostic technique of AIV





Colloidal gold technique for AIV



Neutralization Assay （NT）

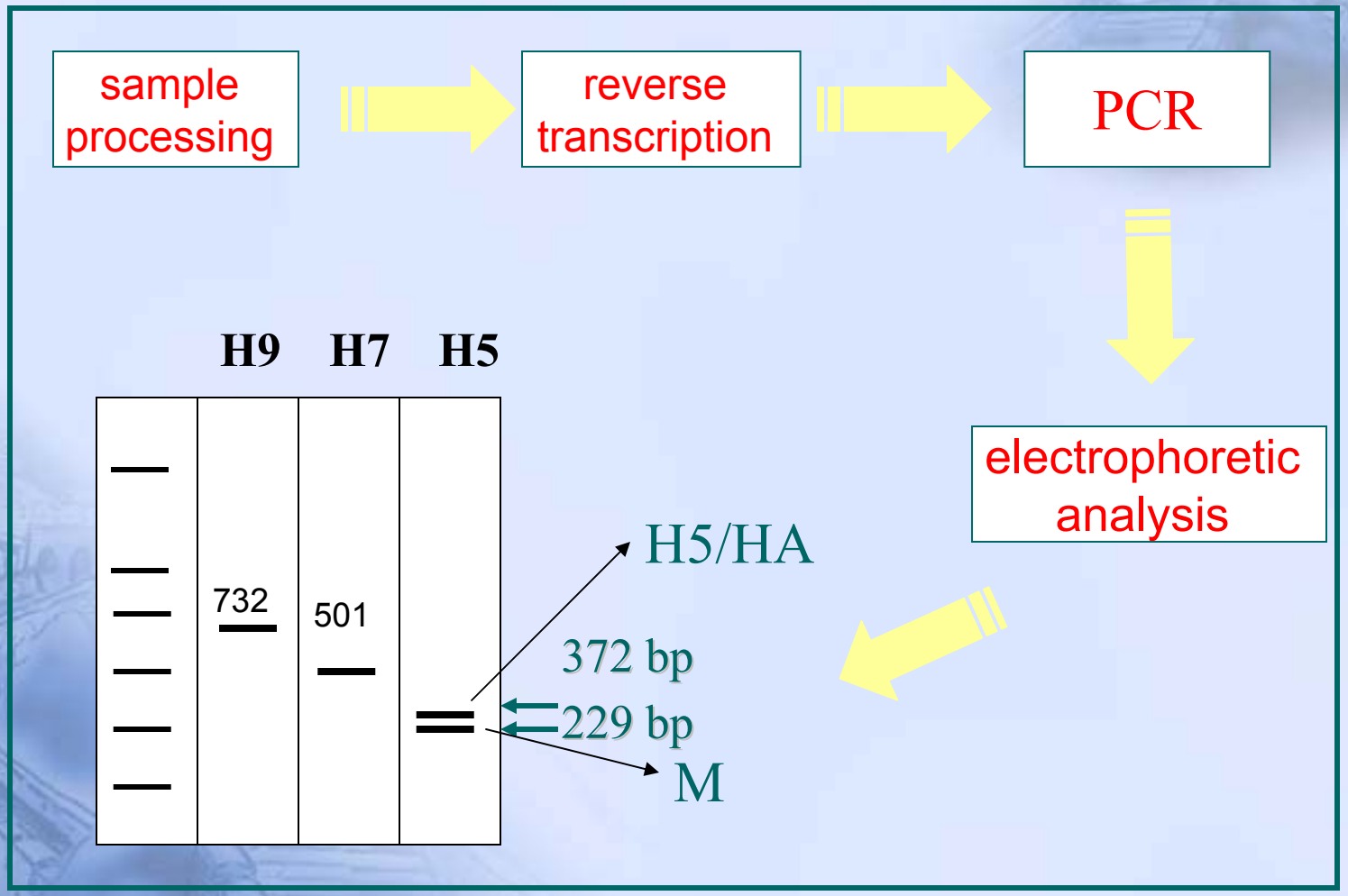
The virus neutralization test is a sensitive and specific assay applicable to the identification of virus-specific antibody in animals and humans. The neutralization test is performed in two stages consisting of (1) a virus-antibody reaction step, in which the virus is mixed and inoculated with the appropriate antibody reagents, and (2) an inoculation step, in which the mixture is inoculated into the appropriate host system (e.g. cell cultures, embryonated eggs, or animals) to detect residual virus infectivity. The absence of infectivity constitutes a positive neutralization reaction and indicates the presence of virus-specific antibodies in human or animal sera.

neutralization test （NT）

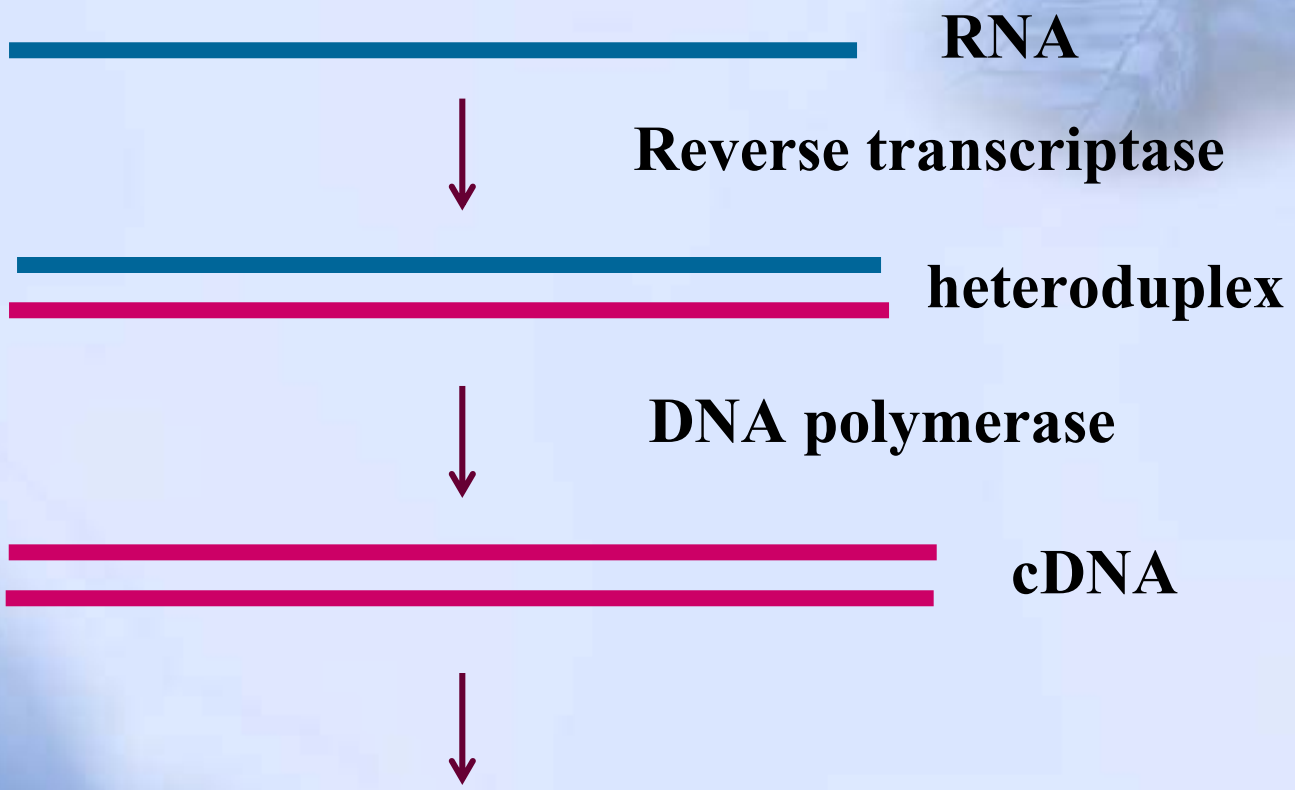
In the neutralization test, it is expected that serum neutralizing antibodies to influenza virus HA will inhibit the infection of MDCK cells with virus. Serially diluted sera are pre-incubated with a standardized amount of virus prior to the addition of MDCK cells.

There are different options for the detection of virus neutralisation. In conventional neutralisation tests, as described here, the neutralisation of virus activity is based on directly visualising the suppression of CPE under an inverted microscope. These are more easily set up with a new virus, but take 3-4 days for a result to be obtained, and are more labor intensive and are less sensitive. Neutralisation tests with influenza differ from that done with other viruses in that the culture medium needs to be serum free and contain trypsin to allow influenza viruses to undergo productive replication.

RT-PCR diagnostic technique of AIV



RT－PCR


RNA
Reverse transcriptase
heteroduplex
DNA polymerase
cDNA
PCR amplification

RNA Template

TRIZOL LS Reagent(Invitrogen, product) is recommended to extract total RNA from influenza virus according to the manufacture's instructions.

1. Homogenize tissue samples in 0.75ml of TRIZOL LS Reagent 0.25ml allantoic fluid or per 0.25ml of tissre srspension using a glass-Teflon or power homogenizer. If tissue sample volume is 0.25ml, adjust the volume to 0.25ml with water.
2. The mixture was shaken and incubated for 5 minutes at 15 to 30℃ to permit the complete dissociation of nucleoprotein complexes. Add 0.2ml of chloroform per 0.75ml of TRIzol LS Reagent. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30℃ for 2-15 minutes. Centrifuge 12,000×g for 15 minutes at 2 to 8℃.
3. Transfer the aqueous phase to a clean tube,.Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5ml of isopropyl alcohol per 0.75ml of TRIzol LS Reagent used for the initial homogenization. Incubate samples at 15 to 30℃ for 10 minutes and centrifuge 12,000×g for 15minutes at 2 to 8℃.
4. Remove the supernatant, Wash the RNA pellet with 75% ethanol, adding at least 1ml of per 0.75ml of TRIzol LS Reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge 12,000×g for 5 minutes at 2 to 8℃.
5. At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 minutes). It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility.
6. Dissolve RNA in 20ul Nuclease-Free Water and store in -70℃.

One step RT-PCR

1. Combine the reagents below in a thin-wakked 0.5ml reaction tube on ice.

Nuclease-Free Water(to a final volume of 25 ul)	14.5ul
AMV/*Tfl* 5XReaction Buffer	5ul
dNTP Mix(10mM each dNTP)	0.5ul
Downstream primer	0.5ul
Upstream primer	0.5ul
25mM $MgSO_4$	1ul

2. Mix by pipetting. Add the remaining components.

AMV Reverse Transcriptase(5U/ul)	0.5ul
Tfl DNA Polymerase(5U/ul)	0.5ul

3. Gently vortex,Initiate the reaction by adding:

RNA template	2.5ul

4

First Strand cDNA Synthesis

These PCR cycling profiles are only a guideline.
Cycling conditions should be optimized for each RNA template.

1 cycle	45℃ for 45 minutes	reverse transcription
1 cycle	94℃ for 2 minutes	AMV RT inactivation and RNA/cDNA primer denaturation

Second Strand Synthesis and PCR Amplification

35 cycles	94℃ for 30 seconds	denaturation
	52℃ for 45 seconds	annealing
	68℃ for 45 seconds	extension
1 cycle (optional)	68℃ for 7 minutes	final extension
1 cycle	4℃ soak	

Gel Electrophoresis

Analyze 5ul of the reaction products by agarose gel electrphoresis. Store the remainder of the reaction at -20℃.

1. Prepare a 0.5% TAE agarose gel that contains 0.5ug/ml EtBr

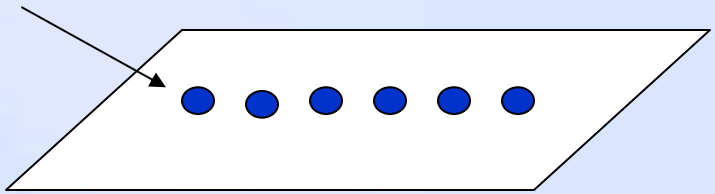
   =Add 2ul EtBr(1mg/ml)/ 40 ml 0.5 × TAE

   Note that EtBr is toxic. Be sure to use groves when you handle it.

2. Place gel in electrophoresis chamber (cover gel with 0.5x TAE)
3. Put drops of gel loading buffer (1-2ul each) onto plastic wrap
4. Mix 5ul of PCR products with the gel loading buffer

Step3 Put 2ul of gel loading buffer on a film.

Step4 Put 5ul of PCR products on drops of the gel loading buffer and mix.



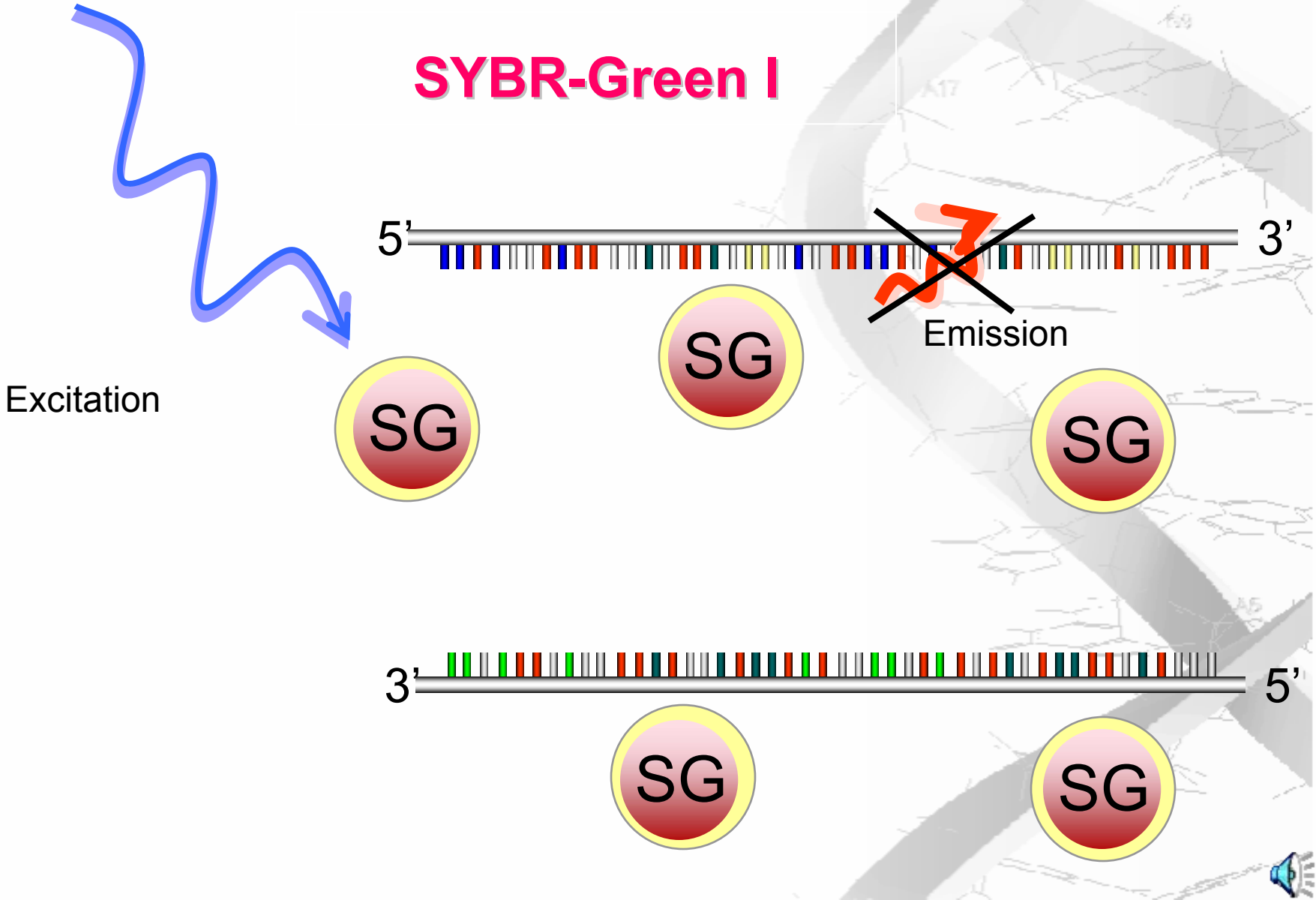
film

5. Load 5ul of the PCR products / gel loading buffer mix to a separate well on the gel
6. Load 4ul MW Marker as same
7. Run gel at 100V for approximately 30 minutes
8. Remove gel from electrophoresis chamber
9. Visualize bands using UV light at 302nm
10. Document with photograph

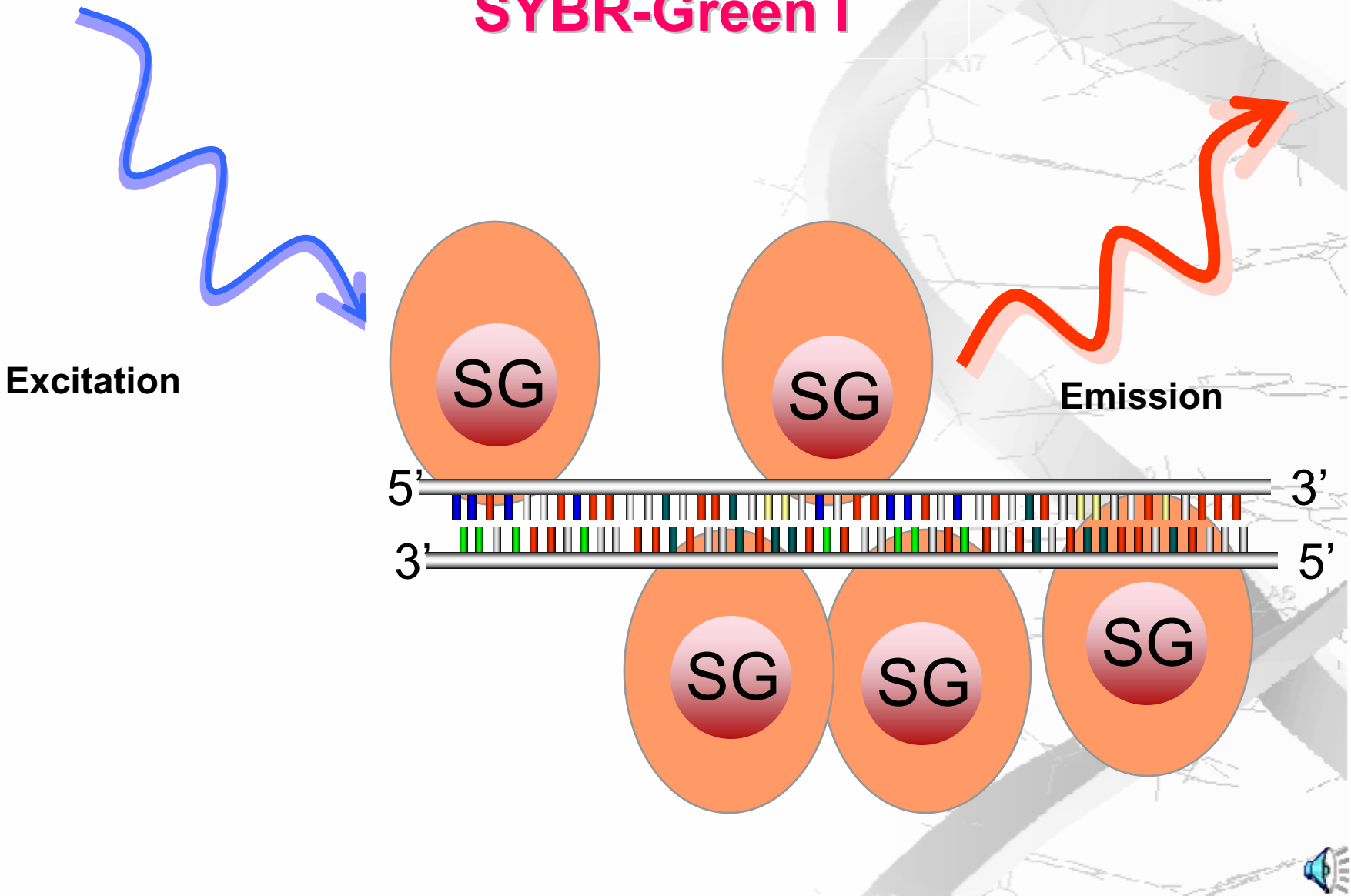
Real-time PCR

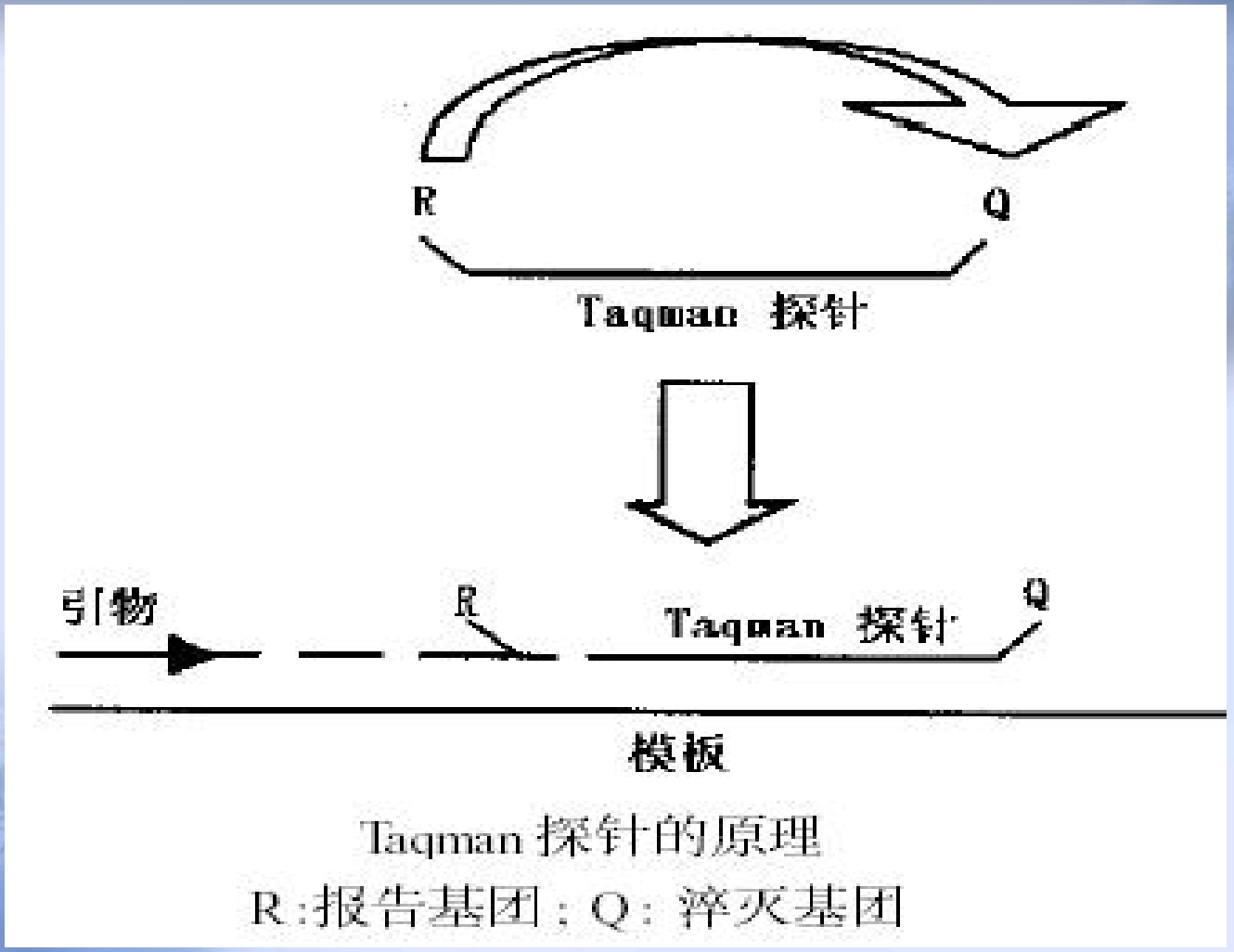
- SYBR Green I
- Taqman
  Molecular Beacons
- Amplifluor (Intergen)

SYBR-Green I



SYBR-Green I



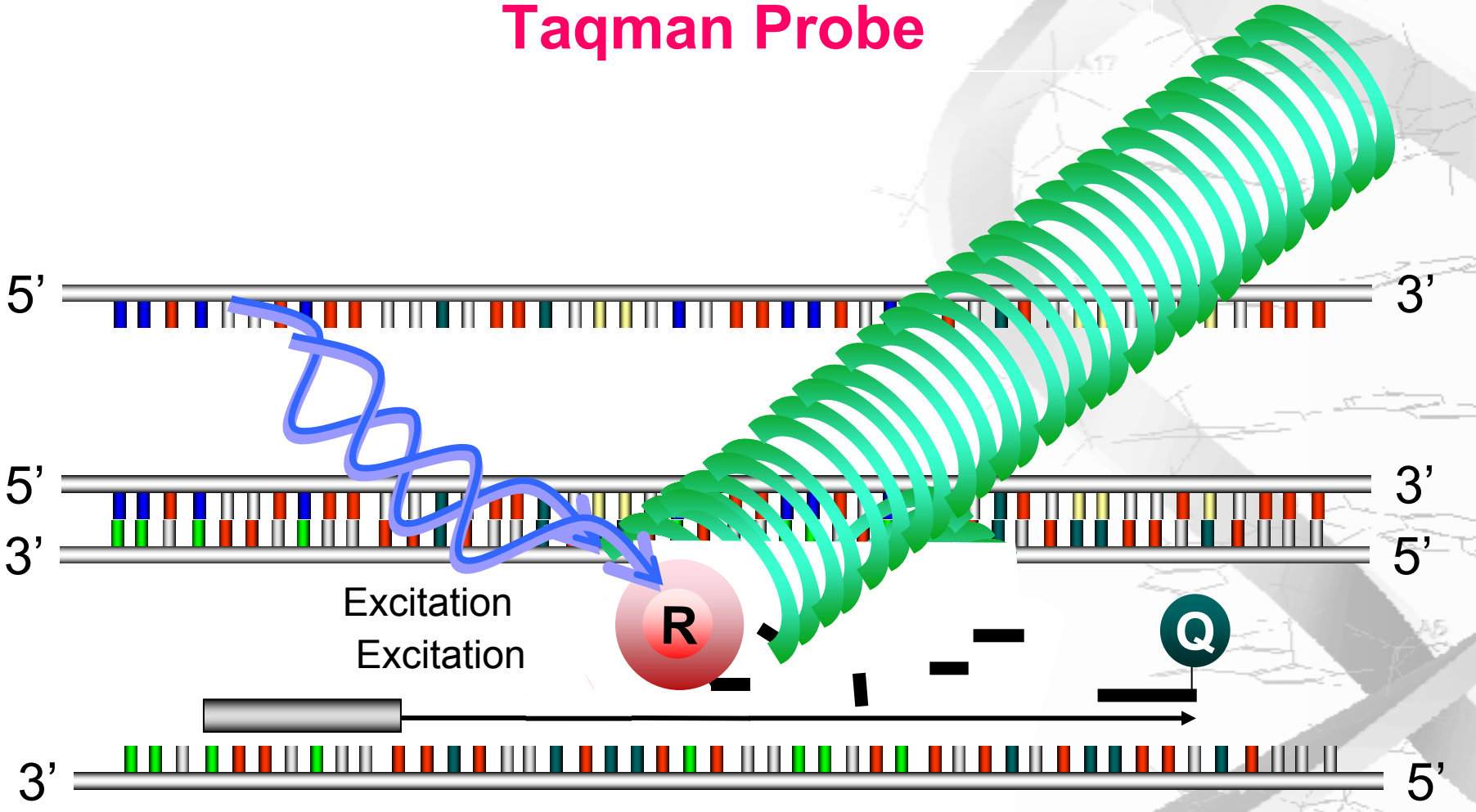


Taqman 探针的原理

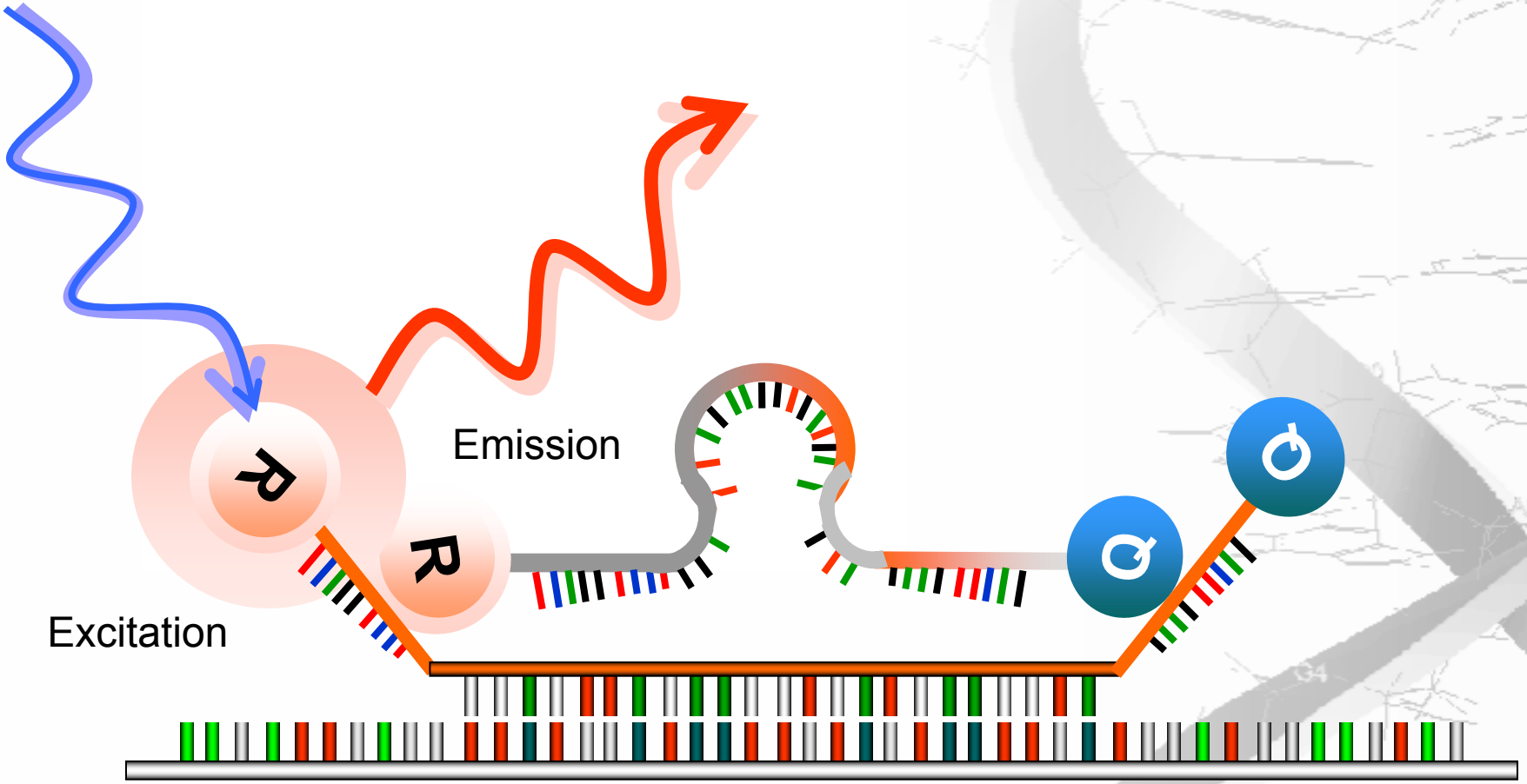
R：报告基团；Q：淬灭基团

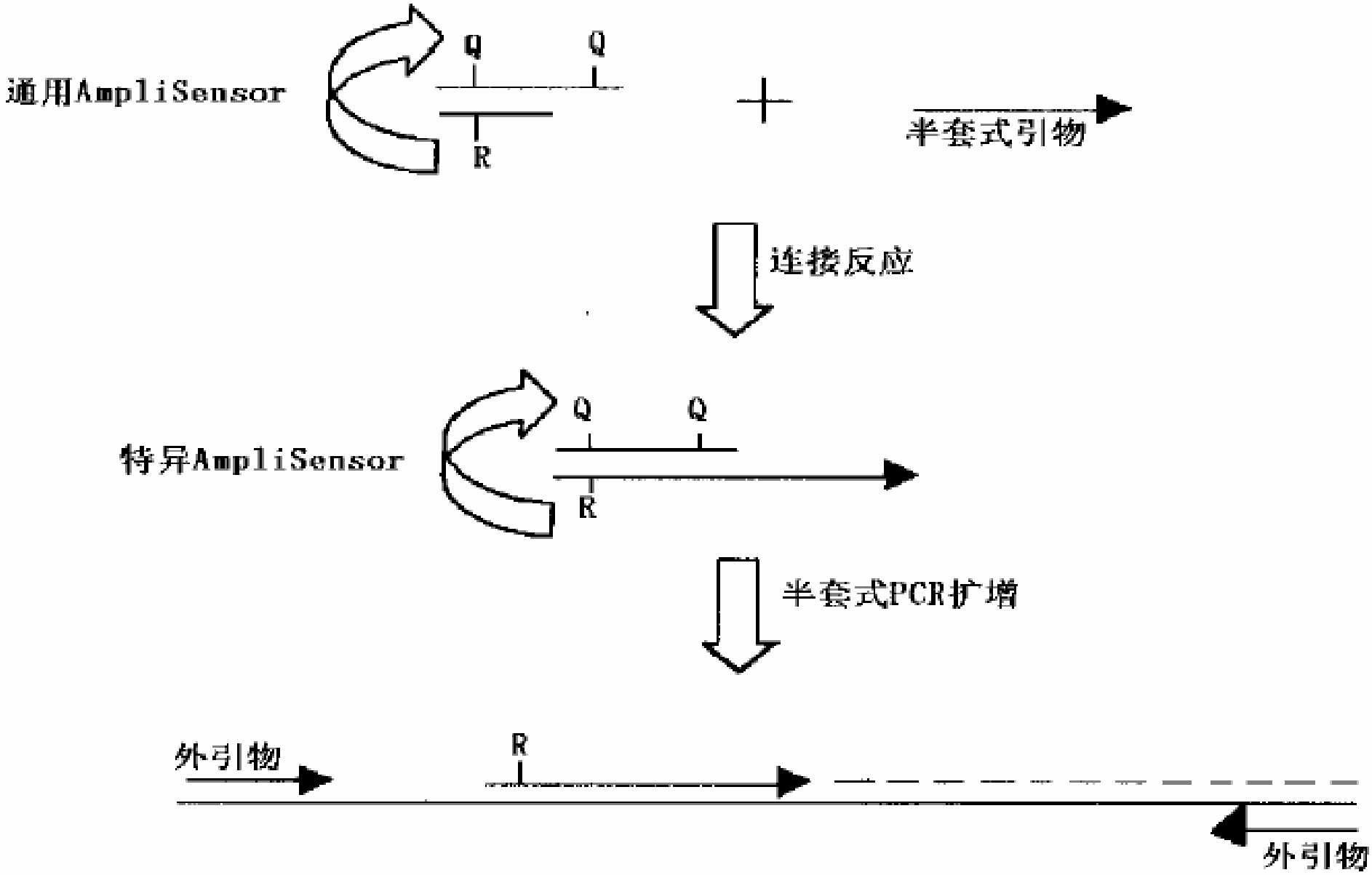
Fluorescence resonance energy transfer , FRET)

Taqman Probe



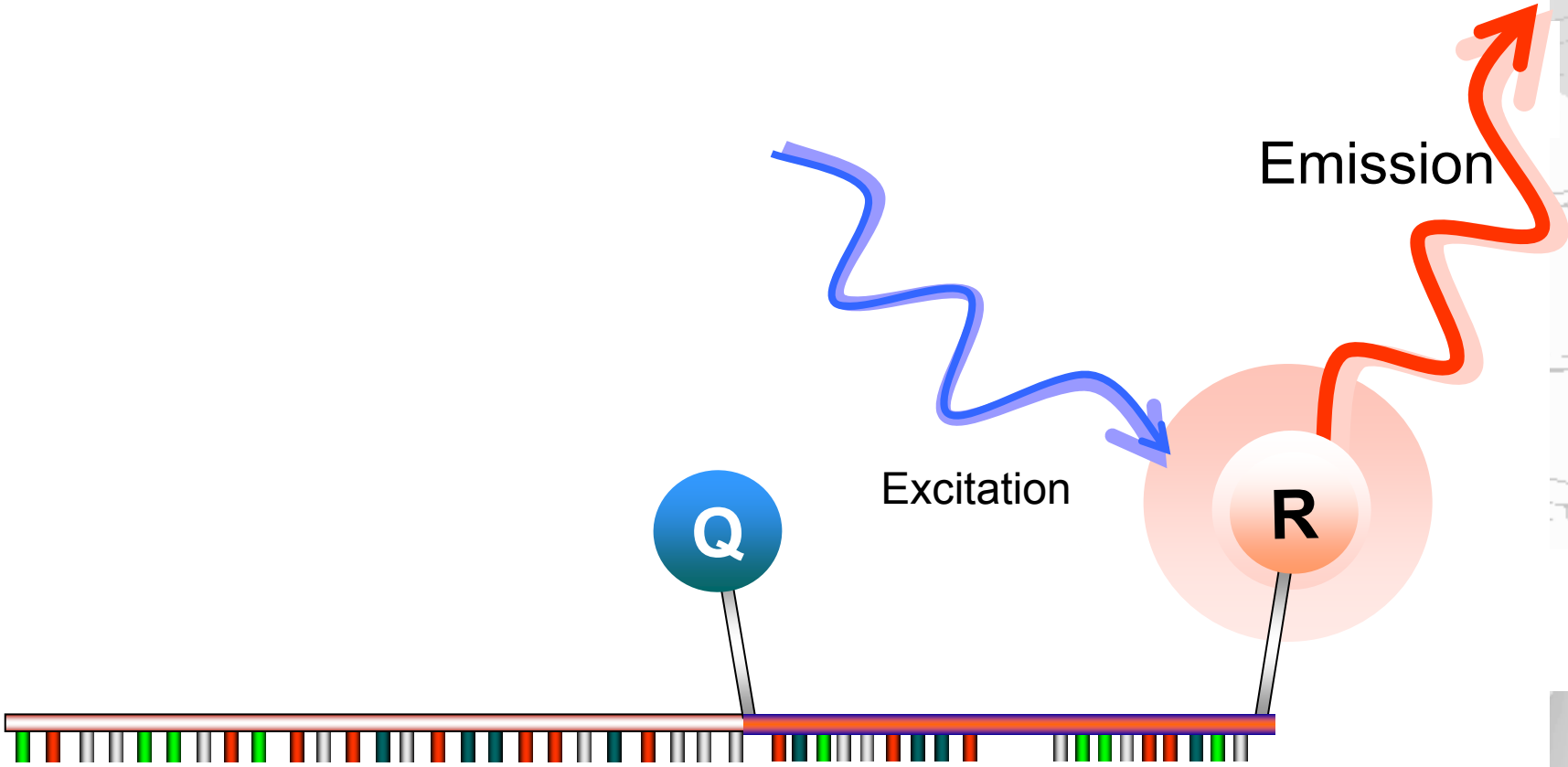
Molecular Beacon Probe





Amplisenor Probe

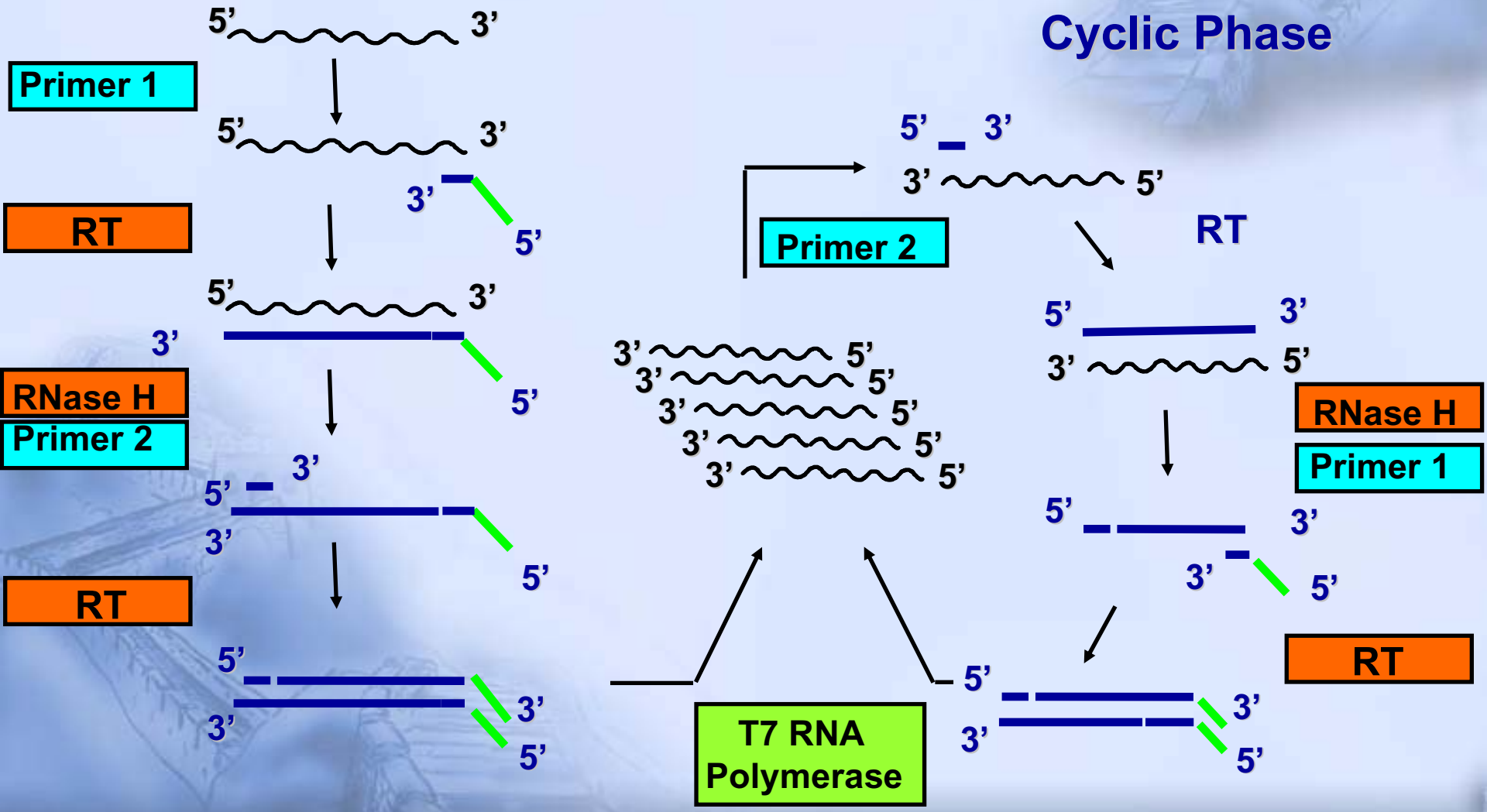
Amplifluor Probe



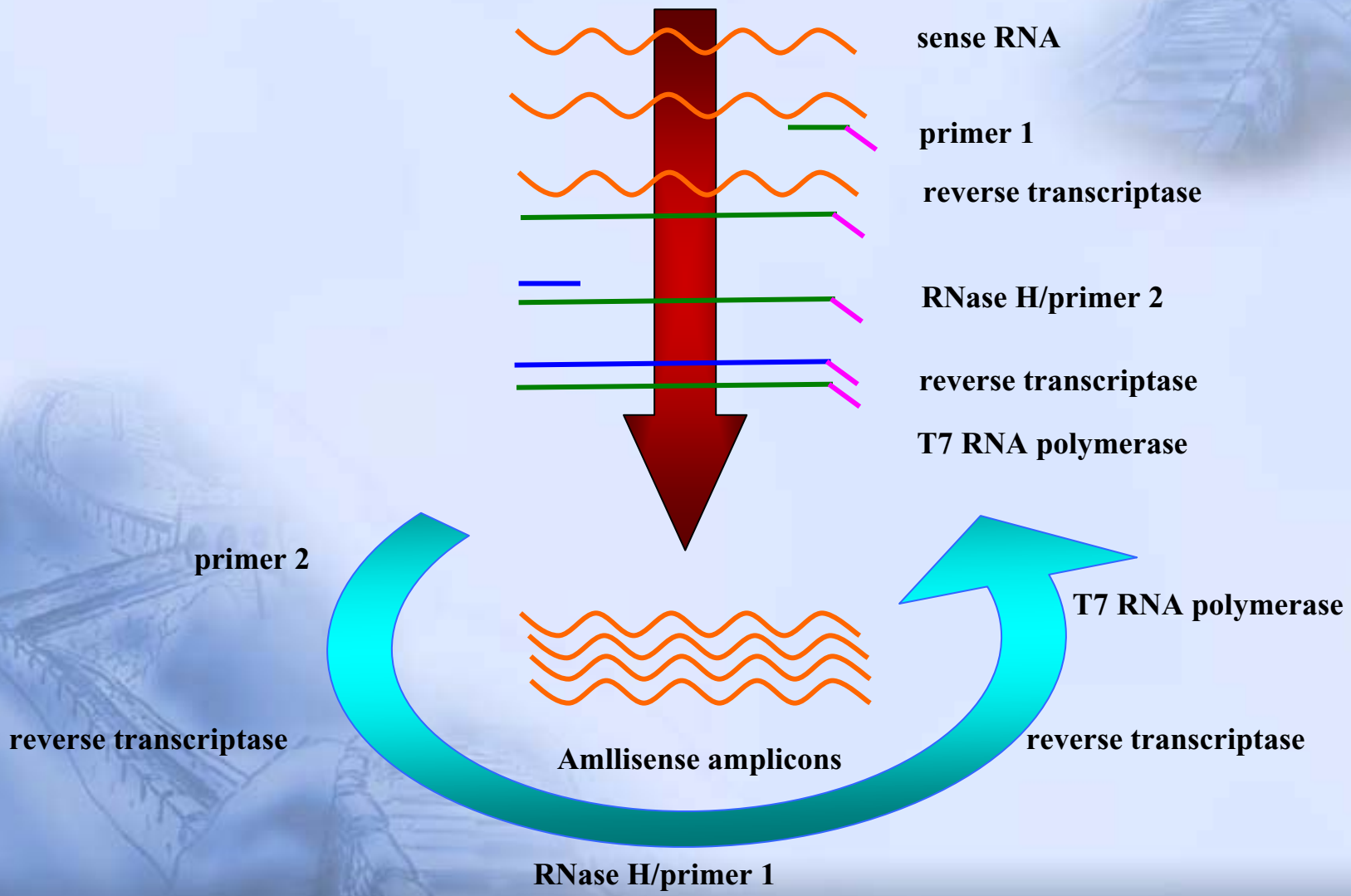
NASBA

NASBA (nucleic acid sequence-based amplification) is a continuous, isothermal, enzyme-based method for the amplification of nucleic acid . The technique employs a mixture of reverse transcriptase, ribonuclease-H, RNA polymerase, and two specially designed DNA oligonucleotide primers. The forward primer has a 5′ extension containing the promoter sequence for bacteriophage T7 DNA-dependent RNA polymerase. The technique is particularly suited for the amplification of single-stranded RNA and has been successfully used in the detection of numerous different RNA viruses.

NASBA


Primer 1
RT
RNase H
Primer 2
RT
Cyclic Phase
Primer 2
RT
RNase H
Primer 1
RT
T7 RNA Polymerase
5'
3'

NABSA


sense RNA
primer 1
reverse transcriptase
RNase H/primer 2
reverse transcriptase
T7 RNA polymerase
primer 2
T7 RNA polymerase
reverse transcriptase
Amllisense amplicons
reverse transcriptase
RNase H/primer 1

Microarray

A DNA-Chip or microarray is a small surface, usually of glass or silica, approximately 1cm2, which is covered by hundreds to hundreds of thousands of different oligonucleotides, each one being located at a specific place of the Chip, called a spot or a feature. These Chips are used in hybridisation reactions to detect nucleic acids generated from a sample by an amplification technique (RT-PCR, PCR, TMA or NASBA). The Microarray technology has several potential applications which are listed below:

- Pathogen identification.
- Pathogen typing or for molecular epidemiology purposes.
- Detection of virulence factors.
- Host–pathogen interaction with gene expression arrays.

Microarray

- Solid support (glass, plastic, metal, silicon)
- Miniaturized array of DNA (genetic material)
- Work on the biochemical principle of DNA/DNA hybridization
- Hybridized probes (DNA molecules) are fluorescently labeled

Thanks for your patient!