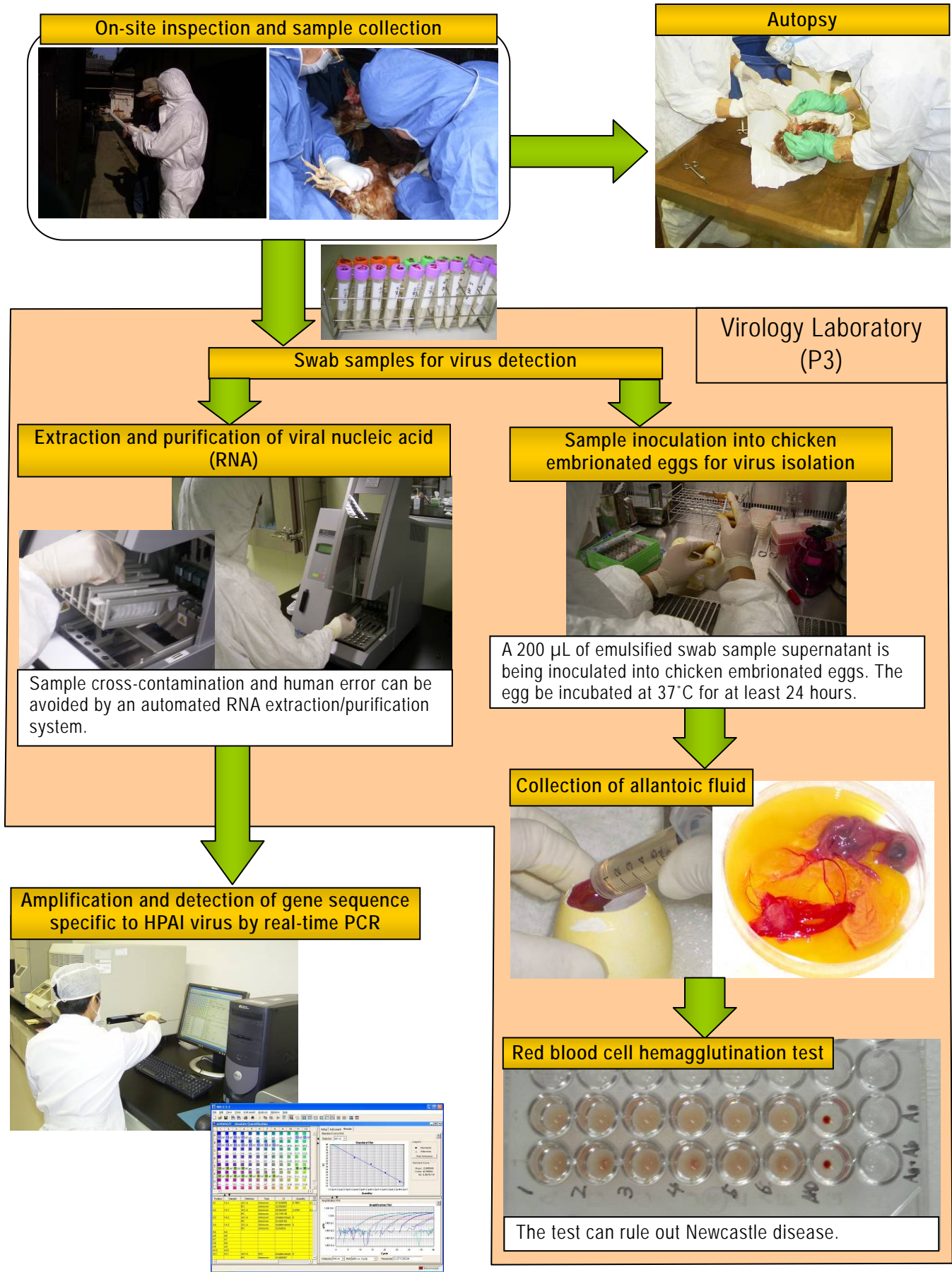


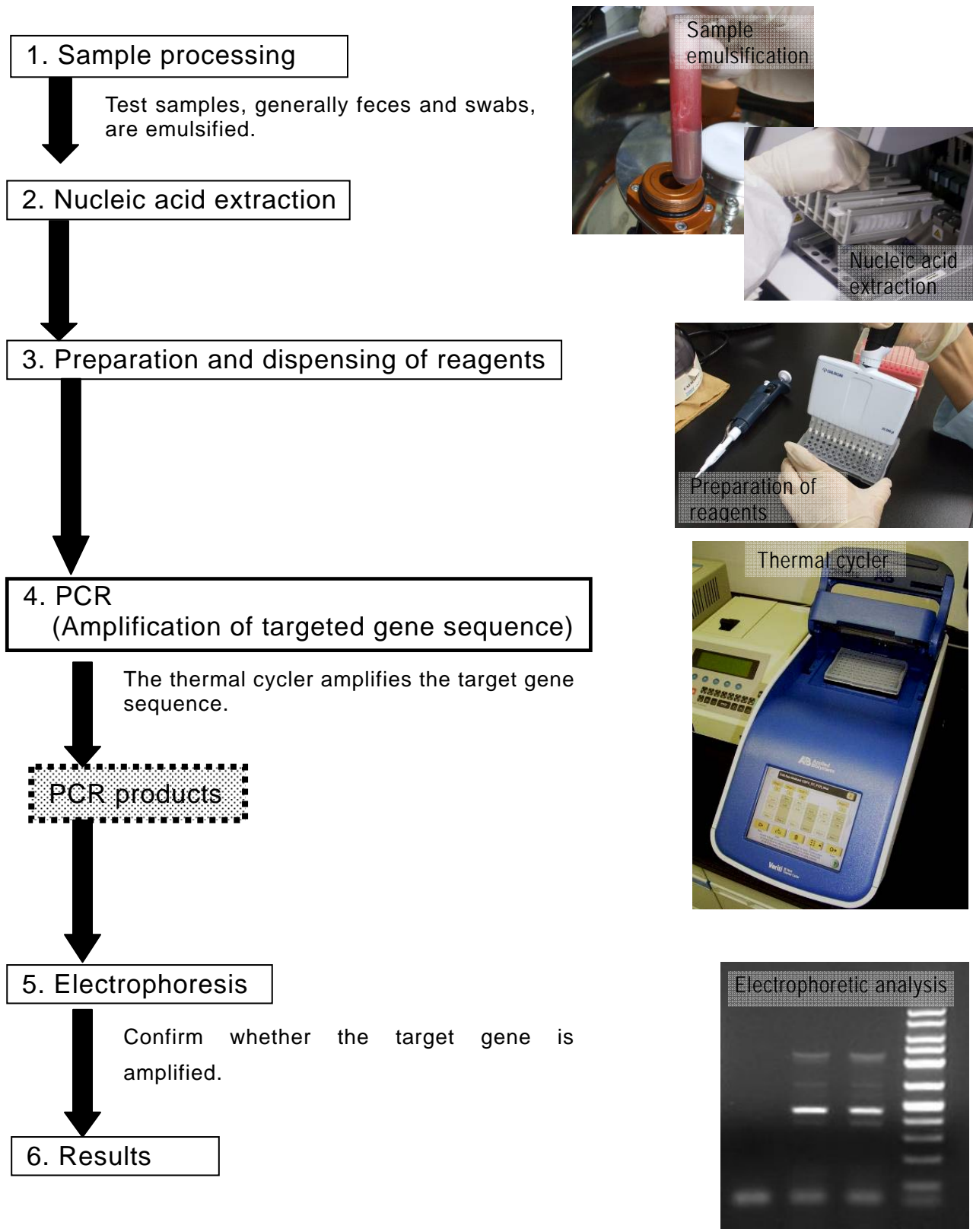
Emergency Diagnosis for Highly Pathogenic Avian Influenza (HPAI)

<Real-Time PCR>



Genetic Analysis Using Polymerase Chain Reaction (PCR)

A specific sequence of the pathogen's genome is amplified for detection of the pathogen.



Western Blot Analysis

Western blotting is a method to detect target protein in samples with high accuracy.

1. Protein extraction from samples



Tissues are cut into small pieces.

2. Purification and concentration of protein

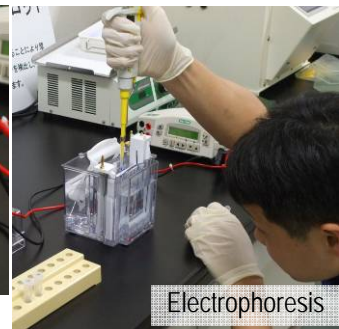
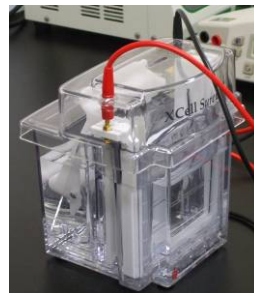


Protein purification and concentration

3. Electrophoresis



Proteins are separated by their size.

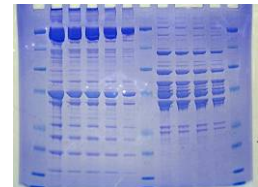


Electrophoresis

4. After separation, proteins are transferred onto a membrane using a blotting system.



Transfer system



5. The target protein is colorimetrically detected.



Blotted membrane



Antibody binding

6. Image capture



Image capturing

Genetic Analysis Using a Sequencer

1. Extraction of genetic materials from samples



2. Amplification of a target gene



A specific sequence is amplified by a thermal cycler.

3. Purification of nucleic acid



Impurities are removed with high-speed refrigerated centrifuge.



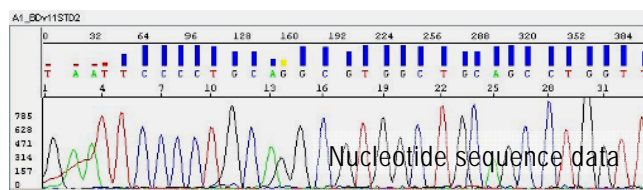
High-speed refrigerated centrifuge

4. Gene sequencing

The nucleic acid sequence is determined by a DNA sequencer, ABI PRISM310.



DNA Sequencer, PRISM 310



Gene Analysis Using Real-Time PCR

Real-Time PCR allows more accurate gene detection and quantification than the conventional PCR/electrophoresis method.

1. Sample processing

Emulsified samples, cell culture supernatants or allantoic fluids from embryonating chicken eggs are centrifuged, and their supernatants are used for analysis.

Nucleic Acid Purification System, BioRobot EZ-1



2. Extraction of nucleic acid

Genetic materials of the pathogen are safely and precisely extracted.



3. Preparation of reagents

4. Real-time PCR

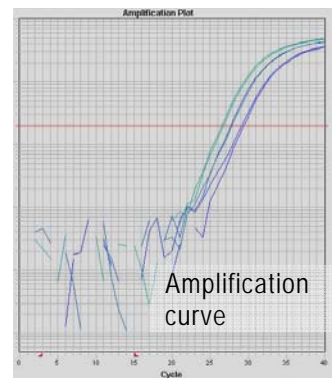
This step allows amplification of a specific sequence and real-time detection of amplification.



Real-time PCR System, Prism 7900HT

5. Results

After the reaction is complete, the obtained data is analyzed and interpreted. This method allows accurate and efficient analysis without using electrophoresis.



At our laboratory, real-time PCR is currently used for diagnostic testing of highly pathogenic avian influenza and for pre-culling surveillance of Johne's disease in accordance with the "Manual of Diagnostic Tests for Johne's Disease."

Handling Infected Animals in a Biological Safety Cabinet: Workflow

A biological safety cabinet (BSC) is used to safely conduct laboratory testing, such as the animal inoculation test with infectious or toxic samples.

Sample inoculation into a mouse

Inoculation is performed inside a BSC to avoid environmental contamination from toxins or bacteria.



Inoculating a sample into a mouse

Observation of animals in the BSC

Animals are kept in the BSC for observation.



Animals in the BSC

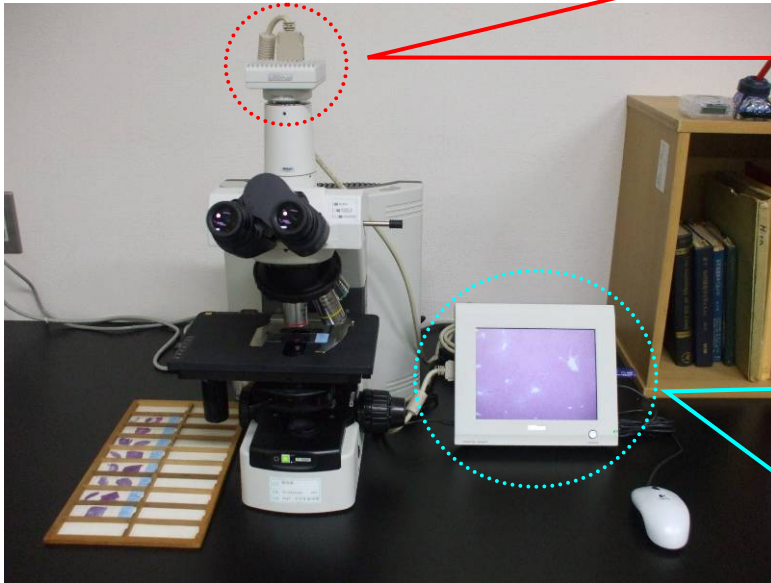
Results



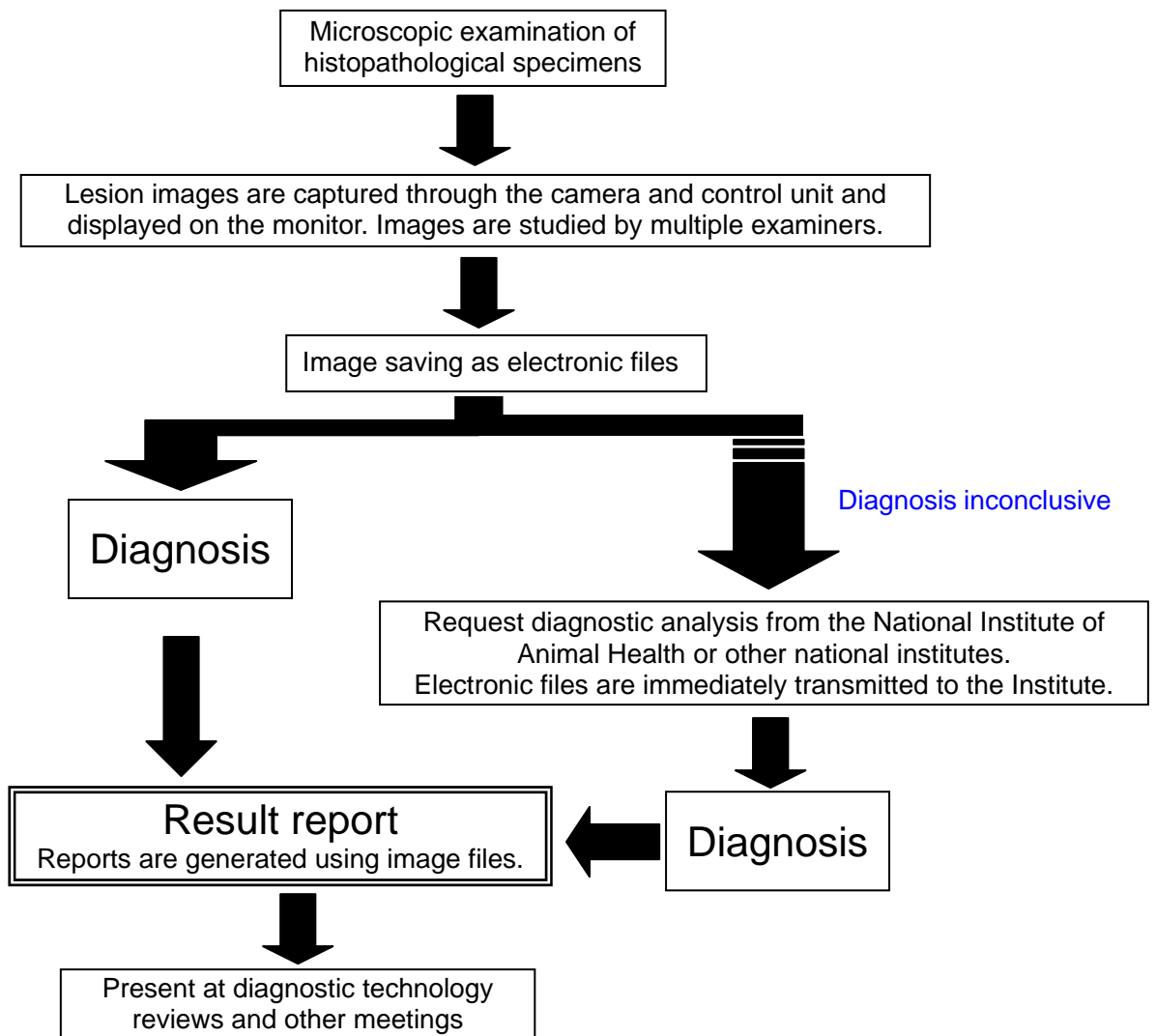
Safe disposal using a dust box



Microscopic Imaging System: Workflow



Through the microscope, an image of histopathological specimen is displayed on a monitor. The image can be saved as a high-resolution electronic file.



Virus Detection Using an Ultracentrifugation System

Ultracentrifugation can concentrate a trace amount of virus in blood, feces, mucus and a small portion of visceral organs taken from animals.

1. Sample processing

Samples are processed and homogenized with Multibeads Shocker[®], which disrupts animal cells containing viruses.



2. Low-speed centrifugation (3,000 rpm, 15 min)

This process removes impurities other than virus particles.

3. High-speed centrifugation (6,000 rpm, 30 min)

This process further removes impurities.

4. Ultrahigh-speed centrifugation (27,000 rpm, 3 hours)

This process concentrates virus particles at the bottom of tube.

Virus isolation

Genetic analysis (PCR)



[Used for high- and ultrahigh-speed centrifugation]



Preparation of Antisera Using an Ultracentrifugation System

Virus particles are concentrated and purified by the use of ultracentrifugation and then inoculated to animals. The animal's serum, which contains antibody against the virus, is used for indirect fluorescence antibody testing for viral detection.

1. Culture of cells infected with virus



2. Virus concentration by ultracentrifugation (40,000 rpm for 2 hours)



3. Virus purification by density gradient ultracentrifugation (40,000 rpm for 2 hours)



Concentrated and purified virus is inactivated and inoculated into animals (2 – 3 times at 3 week intervals). Serum (antiserum or immune serum) is then collected from the animals.



Ultracentrifugation System CP-80WX

[Used for high- and ultrahigh-speed centrifugation]



Histopathological Analysis: Workflow

1. Autopsy

During autopsy, major organs, lesions and other necessary samples are collected from a suspected animal and cut into pieces.

2. Fixation

Excised tissues are immediately immersed in 10% neutral buffered formalin (Mildform® 10N is used at our laboratory) and fixed for at least 1 day. Formalin coagulates proteins and preserves tissue structures in a state similar to the antemortem state.

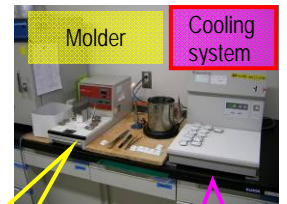
3. Cutting

Fixed tissues are washed with water and cut into a size that fits in a tissue cassette.

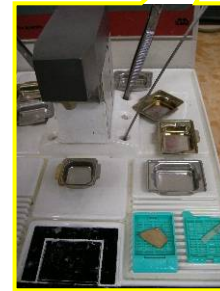


4. Paraffin embedding

1) In the automated embedding machine, tissues are dehydrated with alcohol, removed of alcohol with xylene and then embedded in paraffin.



2) Paraffin is dispensed into the embedding mold and solidified by cooling.

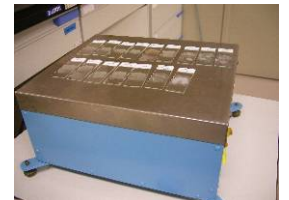


5. Sectioning

The paraffin block is sectioned with 1- to 5- μ m thickness. Sections are mounted on a glass slide.



Microtome



Paraffin heater

6. Staining



7. Sealing

A glass coverslip is mounted on the stained specimen and sealed with a mounting agent.

8. Microscopy

Cellular changes are examined under light microscopy for pathological diagnosis. Specimen images are captured using a microscope imaging system and saved as electronic files. These files can be examined and shared by multiple examiners and electronically sent to the other institutions for rapid and accurate histopathological diagnosis.

*See also "Microscopic Imaging System: Workflow."



Light microscope

Microscope imaging system