Application of Fluorescent Antibody Technique For Diagnosis of Infectious Diseases Of Domestic Animals in Japan

By RYOTARO ISHIZAKI

Researcher, Equine Infectious Anemia Division,
National Institute of Animal Health

Fluorescent antibody technique was originally developed by Coons et al. (1942)[1] and established by his group (1950).[2] As the principle of the technique, proteins such as serum antizodies were labelled by chemical combination with fluorescent dyes without their effect on the biological or immunological properties of the proteins.

The serum antibodies labelled with fluorescent dyes which are called fluorescent antibody can be used for detection of specific antigen in the preparation for examination by the fluorescent microscopy which is illuminated by ultraviolet light source and equipped with some combined filters.

For the purpose of detecting some infectious agents as the antigents, the fluorescent microscopic preparations can be ready for examination in a few hours. It has been used, then, for the visualization and identification of bacterial, viral, protozoic, helminthic, fungal antigens, and also used for the detection of serum antibody.

In the diagnostic laboratory, the fluorescent antibody technique occupies recently an important place in routine immunological examinations for preventive medicine and also for veterinary field. In this report, two examples using the technique for diagnosis of infectious diseases of domestic animals are introduced. These techniques have been recently developed and practically prevailed into the diagnostic laboratory or animal health laboratory distributed in local areas of Japan.

Application for Toxoplasmosis in swine

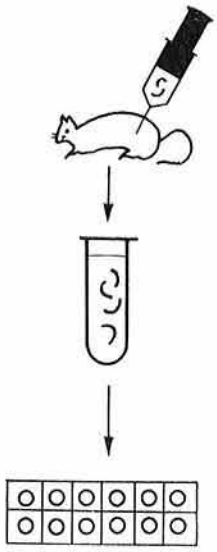
Recently, swine toxoplasmosis has become an important problem both from an economic and a public health point of view. As the toxoplasmosis, a more reliable method than the serological technique such as dye tests, complement fixation test, and hemagglutionation test, has been desired.

For the detection of the etiological organism in the infected tissues, Giemsa staining and hematoxylien-osin staining are generally employed for the purpose. These staining methods, however, are sometimes unsatisfactory for the detection when applied to a sample of tissue infected with a few organisms.

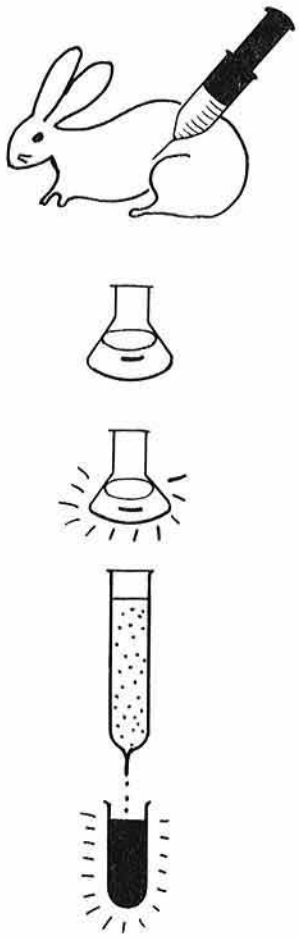
To the detection of toxoplasma, the fluorescent antibody technique was first applied by Goldman.[3][4] Under the method, the assay of antibody titer against toxoplasma can be performed as the dye test and the direct detection of the organisms in infected material can also be revealed more suitably and specifically in comparison with the staining of Giemsa or hematoxilin-eosin.

1) Antibody assay against Toxoplasma by the indirect fluorescent antibody staining.

The dye test can only be applied to pigs with clinical symptoms of toxoplasmosis, since it is capable of demonstrating antibodies at



A



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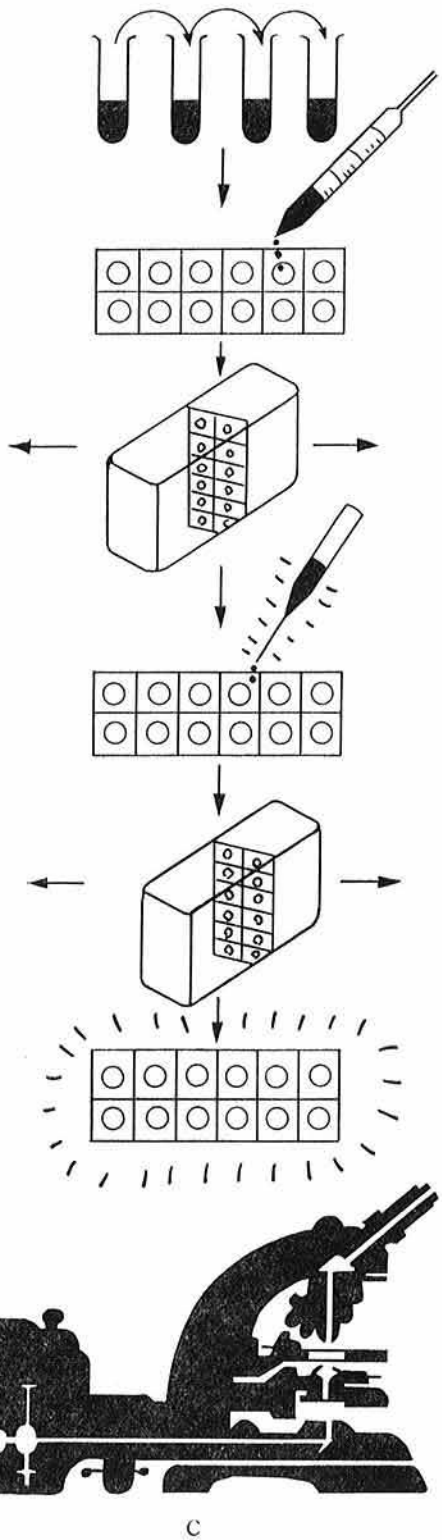


C

A. Antigen preparation.
1. Inoculate toxoplasma into mouse by i.p. route.
2. Harvest from the ascites of a mouse inoculated.
3. Wash toxoplasma with saline and fixed in the formalin solution.
4. Drop toxoplasma suspension on the slide glass and dry.

B. Fluorescent antibody preparation.
1. Immune rabbit with swine serum globulin for the preparation of anti-swine globulin rabbit serum.
2. Preparation of the globulin fraction of the anti-swine globulin rabbit serum by the ammonium sulfate semi-saturation fractionation method.
3. Conjugate the anti-swine globulin rabbit serum with fluorescein isothiocyanate.
4. Pass the conjugate serum through a coarse Sephadex G-25 column and then fractionate

the conjugate serum on DEAE-cellulose column to eliminate the properties of non-specific staining contained in the conjugates.

C. Indirect fluorescent antibody staining.

1. Dilute serially the serum to be tested and drop them on the antigen.
2. Incubate the slide at 37°C for 40 min. for the reaction.
3. Wash the slide in phosphate buffered saline on magnetic shaker, then dry it to some extent.
4. Drop the fluorescent antibody purified (Fluorescent labelled anti-swine globulin rabbit serum solution) on the antigen-antibody complex and then incubate 37°C for 20 min. for staining.
5. Wash again in phosphate buffered saline on magnetic shaker and then dry it.
6. Mount the glycerine-saline solution and seal it with cover glass.
7. Examine the slide by fluorescent microscopy.

Fig. 1. Procedure of antibody assay against to oplasma by the indirect fluorescent antibody staining.

the early stage of infection on account of its high sensitivity. This test, however, has some disadvantageous points. As it is performed with living toxoplasma organisms as antigen, the antigen cannot be preserved nor be free from danger.

In addition, it requires a large amount of so-called accessory factor (fresh human serum) which is obtained from some human with difficulty. Reading the results of the dye test can be made only by a skilled person.

Using the fluorescent antibody staining, it is easy to detect protozoa in smear preparations as well as histological sections and also to titrate antibody by the one-step inhibition test according by Goldman's report.[4]

Suzuki et al.[12] carried out the antibody survey among the swine in our country, using the indirect fluorescent antibody staining method. Principle and procedure of the method are explained in Fig. 1.

Comparative antibody titers determined by the indirect fluorescent antibody staining with those by the dye test for 78 serum samples collected from pigs experimentally infected with toxoplasma and from pigs in the field, are shown in Fig. 2.

The presence of correlation between the antibody titers by both tests is clearly observed. In addition, it is very convenient that the antigen prepared from the ascites of a mouse inoculated with toxoplasma and fixed in formalin solution, dropped on a slide, and dried, could be used for the test up to six months after preparation when stored at

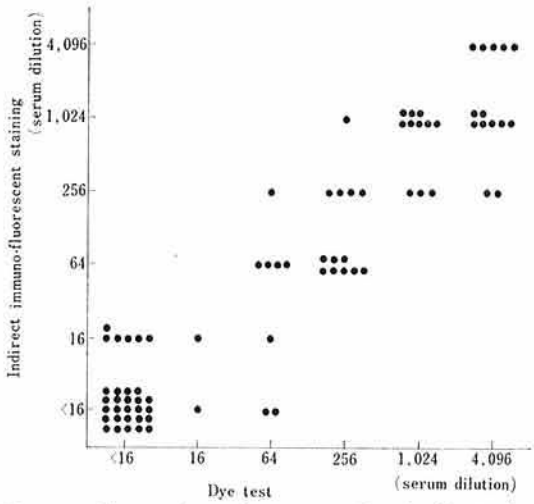


Fig. 2. Comparison between the indirect immunofluorescent staining and the dye test on field swine sera.

−20°C.

2) The detection of the antigen in infected tissue.

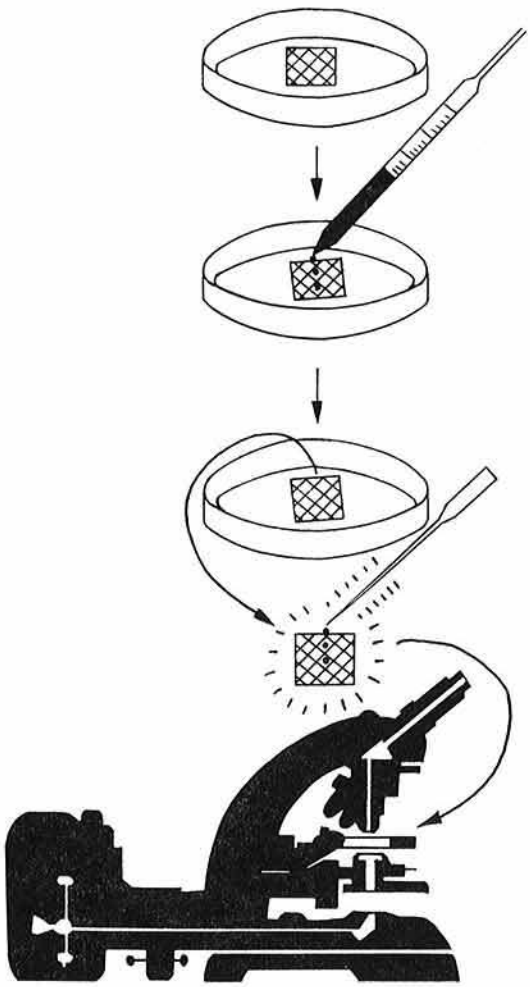
On the other hand, the detection of the organism in infected tissue using the fluorescent antibody staining is a very easy and specific test. The staining gives usually a higher rate of detection of the organisms than any other routine staining methods. When this technique was applied to some cases of swine toxoplasmosis, the organism was stained clearly in the lung, liver, various lymph nodes, and spleen on the back ground without brightness (Ito et al. 1964).[5]

Application of the fluorescent antibody staining for detection of hog cholera virus

For the detection and titration of hog cholera virus, the reliable method *in vitro* was first developed by Kumagai et al. (1961)[6] such as the phenomenon of the Exaltation of Newcastle Disease (END) virus caused by preinfection of hog cholera virus in swine testicle tissue culture.

In the following year, Solorzano reported that the fluorescent antibody technique was successfully applied to the detection of hog cholera virus from pig materials. This technique has been improved and evaluated mainly for the purpose of the hog cholera diagnosis.

The most beautiful and satisfactory method of the fluorescent antibody for the purpose of hog cholera diagnosis was developed by Mengeling et al.[9][10] The method was called the Fluorescent Antibody Cell Culture Test (FACCT) using the PK-15 established cell line. FACCT was confirmed as reliable as the END method for hog cholera virus detection in our country (Lin et al. 1969a, 1969b).[7][8] The procedure is explained in Fig. 3.



Make PK-15 cell monolayer on coverslip.
Inoculate suspected material to be tested.
Incubate the petri-dish containing coverslip for 48 hours at 37°C in CO_2 incubator.
Stain coverslip with fluorescent antibody.
Examine coverslip by fluorescent microscopy.

Fig. 3. Procedure of FACCT.

In this case, satisfactory specific immune serum against hog cholera virus for the test was prepared by the use of Specific-Pathogen-Free (SPE) pig immunized with the virus.

The results of the comparison of the virus titers determined by the FACCT and END methods are shown in Table 1. Almost the same value of infective titer was revealed by these two methods. The FACCT and END methods were also compared for virus detection and titration in various organs of pig infected with virulent hog cholera virus.

In an experiment, the seven pigs inoculated with virulent hog cholera virus were sacrified by exsanguination at 24-hour-intervals after infection, one pig at each time, to harvest materials. Good correlation of virus detection and titration of the materials conducted by

Table 1. Comparison of infective titer of hog cholera virus determined between the FACCT and END methods.

Experiment No.	END method (log $TCID_{50}$)	FACCT (log PFU)	Difference
1	4.6	4.3	0.3
2	4.4	4.2	0.2
3	6.9	6.9	6.5
Average	5.3	5.0	0.3

Difference: log $TCID_{50}$—log PFU

the FACCT and END methods were obtained in the 77 samples with the exception of one urine sample which was positive by the FACCT but negative by the END method.

The FACCT method is, however, somewhat complicated since the PK-15 cells have to be maintained for the test. Then, the method can only be carried out in the laboratory equipped with the instruments for tissue culture.

The other technique for the diagnosis of hog cholera in the field trial using the fluorescent antibody staining was also developed by Sato et al. in our country.[11] The method is very simple and useful for the diagnosis of hog cholera practically.

The principle for diagnosis is based on the pathogenesis of hog cholera virus infection in pigs. The virus is found most consistently and continuously in a high titer in the tonsils of all the pigs examined. Then, the tonsil of the suspected pig for hog cholera is taken out by biopsy with the special tool made for this purpose. The tool is called "Ton-drill".

The preparation of the stamp smear or tissue section is made from the tonsil removed by biopsy. The preparation is then fixed with acetone and stained with the fluorescent antibody against hog cholera virus. This test is called the Fluorescent Antibody Staining of Tonsillar (FAST) smear method. The test can be done only in two hours. The FAST method was tried for the diagnosis in the experimentally infected pigs and field cases. Satisfactory results using the FAST method have been accumulated for the diagnosis in the field trial in Japan.

Before closing the report, two important points of the technique should be mentioned. The first consideration must be for the antiserum. It is, then, often best served by serum of the highest possible titer for preparation of the fluorescent antibody.

The labelled serum should be examined for specific staining properties by some standard method before it is applied experimentally.

In the second, the microscopic equipment used for the technique requires the use of a more powerful light source than would otherwise be necessary. The high pressure mercury lamp such as the HBO 200-watt lamp (Manufactured by the German Osram Company) is a satisfactory light source for the purpose.

The fluorescent microscope is currently available for the technique from Nikon Inc. or Chiyoda Optical Co. & Ltd. in Japan. These microscopes are equipped with appropriate filter system and illuminated with HBO 200 lamp.

Refereces

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Introduction of Research Institutes for Agriculture, Forestry and Fisheries in Japan

Brief introduction of research and experiment stations of the Ministry of Agriculture and Forestry will be made in series hereafter

NATIONAL INSTITUTE OF AGRICULTURAL SCIENCES

Established: 1950 (Started as Agricultural Experiment Station in 1893)
Location: Nishigahara, Kita-ku, Tokyo
Director: ISAMU BABA
Total Number of Employees: 449 (Research personnel: 283)
Outline of Work:

This institute performs fundamental studies on agricultural techniques for the improvement of crop plant varieties, fertilizer application, soil survey, the control of insect pests and diseases, and the betterment of farmers' home living and farm management as well as studies on climatic conditions for farming and the application of agricultural statistics, maintaining close contact with national and prefectural experiment stations all over the country.

Recent Principal Research Findings:

Gibberelline (a new plant growth hormone).
Utilization of zeolitic tuff for agricultural purpose.
OED (evaporation suppressant).
Blasticidin S (new antibiotic for the control of blast disease).
Hemmagglutionation test for the detection and determination of plant virus.
Utilization of activation analysis by atomic reactor for agricultural purposes.
Methodology of the programming and diagnosis of family farm management.
Development of another culture of rice plants.

Publications:

Bulletin of the National Institute of Agricultural Sciences. (In series, in English or Japanese with English summary, annual or semi-annual, free exchange.)
Series A (Physics and Statistics).
Series B (Soils and Fertilizers).
Series C (Plant Pathology and Entomology).
Series D (Plant Physiology, Genetics and Crops in General).
Series H (Farm Management and Land Utilization).
Materials of the National Institute of Agricultural Sciences. (Irregular).
Annual Report of the National Institute of Agricultural Sciences.

Organization and Main Research Themes:

- Director
  - Department of General Administration (3 sections)
  - Department of Physics and Statistics
    - Division of Meteorology (3 laboratories) — Agricultural meteology, especially local climate and micro-meteorology; Mechanism of weather hazard on crop plants.
    - Division of Statistical Research (4 laboratories)—Statistical studies on experiment programming and investigation methods; Crop condition survey methods.
  - Department of Soils and Fertilizers
    - Division of Chemical Fertilizers (3 laboratories)—Fertilizer analysis and evaluation; Improvement of fertilizer quality.
    - Division of Plant Nutrition (4 laboratories)—Nutrient uptake, metabolic processes and role of trace elements in crop plants; Survey of radioactive contamination in soil and crop.
    - First Division of Soils (5 laboratories)—Soil chemistry, micro-organisms, and physical properties of soils: Soil-plant relation.
    - Second Division of Soils (3 laboratories)—Interpretative classification of soils and soil conservation.
    - Third Division of Soils (4 laboratories)—Soil classification and survey.
  - Department of Plant Pathology and Entomology
    - Division of Plant Pathology (5 laboratories) — Taxonomic, physiological and ecological studies on plant pathogenic bacteria, and fungi. Biochemical studies on plant diseases; Control of plant diseases.
    - Division of Entomology (6 laboratories)—Taxonomic, physiological and ecological studies on injurious insects; Phyto-parasitic nematodes Control of insect pests.
    - Division of Agricultural Chemicals (7 laboratories)—Chemical and physical studies on agricultural chemicals, methods of analysis residue study of pesticides; Development of new antibiotics for agricultural use.
  - Department of Physiology and Genetics
    - Division of Genetics (8 laboratories)—Genetical studies on crop plants and pathogens Breeding method by artificial mutation; Seed preservation and variety characteristics.
    - First Division of Physiology (6 laboratories)—Physiological and ecological studies on crop plants, especially on rice: Chemical control of plant growth and development.
    - Second Division of Physiology (3 laboratories)—Physiological and ecological studies on upland crops.
  - Department of Farm Management and Land Utilization
    - First Division of Farm Management (3 laboratories)—Farm economies.
    - Second Division of Farm Management (3 laboratories) —Farm management system and organization.
    - Third Division of Farm Management (3 laboratories) —External relationships and land utilization.
    - Division of Rural Life (4 laboratories)—Economical and sociological studies on the environment of farm families; Fundamental studies on the betterment of farmers' home living.