# IMMUNOMORPHOLOGICAL METHOD OF TESTING CELL CULTURES FOR CONTAMINATION BY VIRUSES

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A method of detecting contamination of green guenon kidney cell cultures by viruses using labeled serum globulins of healthy monkeys of the same species is described.

Successful results of the use of the fluorescent antibody method (FAM) for the diagnosis of contamination of monkey kidney cell cultures with virus SV 40 and with measles-like virus were described previously [1, 3]. Specific antisera were used in this work.

The sera of adult animals used to obtain cell cultures may contain antibodies against all viruses of animals of these species. A mixture of sera of dogs, monkeys, and other animals has been used successfully [4, 8, 9] for the indirect FAM with the object of securing the early and fullest possible detection of spontaneous contamination of cell cultures.

The object of the present investigation was to study the effectiveness of use of a mixture of monkey sera in the direct FAM for testing cell cultures for spontaneous contamination and to compare the results with those of cytological and cytochemical analysis.

The sera of 15 clinically healthy green guenons, kept for at least 2-3 months in the animal house after being caught, were mixed and the $\gamma$-globulins isolated from them and tagged with fluorescein isothiocyanate by the method described previously [2]. The results described below were obtained during tests of cultures of kidney cells from 218 green guenons. The cultures were grown on narrow slides in flasks or tubes, fixed in Bouin's and Carnoy's mixtures and with acetone for the FAM on the 7th, 14th, and 21st days of growth, stained with hematoxylin and eosin and with acridine orange, and then treated with a mixture of labeled monkey $\gamma$-globulins with or without the addition of albumin-rhodamine sulfonyl fluoride.

During the study of the specimens after different periods of incubation, changes characteristic of contamination by foamy agent and by rheo-, adeno-, and cytomegaloviruses were detected in some of the cells from individual batches. The proportion of infected cultures was 30.2%, a result attributable to a seasonal increase in the number of cultures contaminated by symplast-forming agents (27.5%). Cytopathological changes under the influence of the foamy agent took the form of the development of symplasts without intranuclear inclusions, with fluorescence of granules in the nuclei and cytoplasm. RNA was clearly visible in the granules. In cultures contaminated by rheovirus, cytoplasmic inclusions containing RNA were found, and these structures gave strong fluorescence. The presence of adenoviruses and cytomegaloviruses in the cultures was characterized by the appearance of intranuclear DNA-containing inclusions with bright fluroescence. Positive findings were obtained soonest by means of the FAM (Fig. 1).

The addition of rhodamine-labeled albumin reduced the nonspecific fluorescence of the cultures and gave good contrast of the specific green fluorescence against the red color of the cells. As a control of the specificity of fluorescence, labeled antisera against the viruses of tick-borne encephalitis, Chikungunya, and measles were used; after treatment with these sera the fluorescence of the contaminated cultures disappeared.

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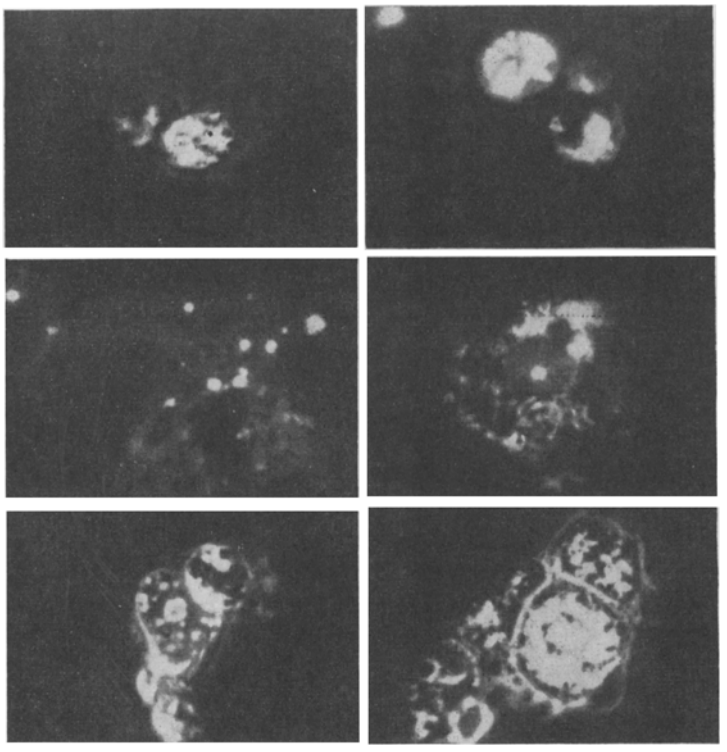

Fig. 1. Specific fluorescence in contaminated cultures of green guenon kidney cells. Direct fluorescent antibody method. Labeled mixture of sera from 15 green guenons used. Objective 90×, Homal 3×.

The quality of the cultures and the possibility of their further use can be assessed by comparing all the results obtained. The final decision regarding specificity of infection of the primary cultures can be reached only as the result of combined virological, serological, and morphological investigations, but simultaneous use of the FAM and of cytological and cytochemical methods proved to be sufficient for an "early" diagnosis.

The use of a mixture of labeled γ-globulins from the sera of 15 green guenons in the present investigation enabled contaminants to be detected on the 7th day after transplantation (the first time of observation). The increase in intensity of the changes on the subsequent days of observation indicated the progressive nature of the process, and in the case of adenovirus infection and of considerable contamination with the foamy agent, degeneration of the culture was observed by the 21st day. Subsequent passages led to the accumulation of the isolated contaminants.

A problem of considerable interest is that of the advantages of using autologous or isologous sera. By analogy with the avian leukemias, Rowe [8] considers that a donor's serum will not react with viruses in its own cell cultures because of immunological tolerance; however, a mixture of sera of adult animals does not possess this feature. On the other hand, by using sera of the animal from which the culture was prepared, no reactions occur with isoantigens which can appear in tests with sera from animals of other isoantigenic groups.

However, the detection of group antigens by the FAM usually presents considerable difficulty and, as a rule, requires the use of special antisera [7, 10]. As Franks [5] points out, isoantibodies for antigens of human leukocytes are found mainly in multiparous women or patients who have received frequent transfusions. These antibodies reacted only with certain human cell lines. Difficulties may arise through the presence of rheumatoid factor, when autoantibodies will be found [5, 6].

In the present tests no difficulties were found during the study of specimens treated with a mixture of labeled sera from adult monkeys which could have been attributable to isoantigens, whereas the need for

using labeled antiglobulins against proteins of the donors of the cells and sera in the indirect FAM increases the background fluorescence of the culture specimens because of binding of labeled antibodies with cell globulins. The opinion of Smith [9] that a mixture of $\gamma$-globulins is a "universal reagent" can be accepted.

The method described in this paper can be recommended for practical use in testing for spontaneous contamination of cell cultures, for it enables contaminated cultures to be discarded at an early stage.

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