Evaluation of the Interference Filter for Use in Rabies Diagnosis by the Fluorescent Antibody Test

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When compared with primary filters widely used for rabies diagnosis by the fluorescent antibody test, an interference filter markedly increased specific staining intensity and contrast.

Primary filters for immunofluorescence microscopy with fluorescein conjugates should allow strong transmission at 495 nm, the maximal excitation wavelength for this fluorochrome, without transmission at the 523-nm emission maximum (2). Unfortunately, commonly used glass filters do not completely separate these wavelengths (5). Interference filters now available, however, reportedly (1) achieve the separation and transmit more effectively at 495 nm than do the filters in general use. Because experience with these newer filters in diagnostic laboratories is limited, we evaluated an interference filter by the rabies fluorescent antibody (FA) test, the immunofluorescence procedure most widely employed in diagnostic virology.

Routine specimens were tested from throughout the United States and elsewhere and included frozen animal heads, brain impressions on microscope slides, and brain tissue either preserved in 50% glycerol saline or frozen. Impressions prepared on glass slides from the specimens were acetone fixed and stained by the procedure of Goldwasser et al. (3) with immunofluorescence reagents obtained from the Biological Reagents Section, Center for Disease Control.

The stained impressions were examined with a Zeiss RA microscope equipped with an ×40 objective, ×8 binoculars, and a dark-field condenser. Each impression was observed with the filter combinations A, B, and C listed in Table 1. As shown, the primary filter for combination A was an interference filter, the Zeiss model KP500; primary filters for combinations B and C were the glass filters usually used in the rabies FA test. An Osram HBO-200 mercury arc lamp was the light source with filter systems B and C. As is routinely done in this laboratory, the lamp was replaced when the specific staining fluorescence of the daily prepared rabies-positive control impression appeared unacceptably weak. A tungsten lamp (6 V, 15 W, 2.5 A) was used with combination A, because preliminary studies indicated that this convenient, inexpensive light source was entirely satisfactory for the interference filter. Each impression was examined first with combinations B and C, after which an individual unaware of the results of the first examinations used the interference filter system.

Rabies antigen was observed with each filter system in the stained brain impressions from 23 specimens: one each from a human, chimpanzee, cat, cow, dingo, horse, and skunk; three from bears; three from foxes; and 10 from dogs. More than 70 specimens representing 16 species were negative. No disagreement in results from any specimen was found with the three filter systems listed.

| Filter combination | Primary filter | Secondary filter |
|--------------------|---------------|------------------|
| A                  | KP 500<sup>a</sup> | 50° (1 mm)       |
| B                  | BG-12° (3 mm) with BG-38° (2.5 mm) | 41° (1 mm) with 65° (1 mm) |
| C                  | UG-1° (1.5 mm) with BG-38 (2.5 mm) | 41 (1 mm) with 65 (1 mm) |

<sup>a</sup> Zeiss filter transmitting from 400 to 500 nm.
<sup>b</sup> Zeiss filter transmitting from 400 to 500 nm.
<sup>c</sup> Schott filter transmitting from 320 to 500 nm.
<sup>d</sup> Zeiss filter transmitting above 410 nm.
<sup>e</sup> Zeiss filter transmitting above 650 nm.
<sup>f</sup> Schott filter transmitting 300 to 700 nm.
<sup>g</sup> Schott filter transmitting from 280 to 410 nm.
TABLE 2. Immunofluorescence intensities of impressions from a deteriorating brain examined with routinely used primary filters and with an interference filter

| Brain storage day (25 C) | Impression specific staining intensity |        |        |
|--------------------------|---------------------------------------|--------|--------|
|                          |                                       | UG-1 filter | BG-12 filter | Interference filter |
| 1-3                      |                                       | 4*      | 4       | 4       |
| 4-6                      |                                       | 3       | 3       | 4       |
| 7                        |                                       | 2       | 3       | 4       |
| 8-10                     |                                       | 1       | 2       | 3       |
| 11                       |                                       | 1       | 2       | 2       |
| 12-15                    |                                       | 1       | 1       | 2       |
| 16-27                    |                                       | Negative | 1       | 2       |
| 28-29                    |                                       | Negative | Negative | 2       |

* Fluorescence intensity is graded from 4 (maximal) through 1 (barely discernable).

systems. The specimens were tested also by mouse inoculation (4); the results were in complete agreement with the FA test results.

Several positive specimens, especially those appearing partially decomposed when received, exhibited brighter specific staining and less background autofluorescence with the interference filter system. To verify this observation with a decomposed specimen, we stored a rabies-positive dog brain without preservative at 25 C for daily preparation and examination of stained impressions. As recorded in Table 2, the impressions from the progressively deteriorating brain clearly demonstrated the superiority of the interference filter system. By brain storage day 29, stained impressions appeared positive only with that system.

Results of this study indicate that interference filters merit consideration for use in the rabies FA test and possibility in other immunofluorescence diagnostic procedures.

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