Methods for Accelerating the Fluorescent-Antibody Test for Rabies Diagnosis

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The time required to perform the fluorescent-antibody test for rabies was reduced by eliminating acetone fixation of the brain impressions and by incubating the conjugate-impression reaction at room temperature for only 10 min. Elimination of the preliminary acetone fixation had no effect on the diagnosis of impression smears from 246 mammalian brains by immunofluorescence. Staining at 37 C for 30 min and staining at room temperature for 10 min were found to be equally effective in the examination of impression smears from 161 brain samples. The procedure, as modified, shortens the time required for the diagnosis of rabies by immunofluorescence from about 5.5 hr to approximately 45 min.

The fluorescent-antibody (FA) test is sensitive and specific for rabies diagnosis (13). The only inconvenience of the rabies FA test in comparison with the Sellers' stain is the time required for its performance.

Although the use of several fixatives, times of fixation, and times and temperatures of incubation has been reported (4, 6-8, 11, 17), the standard FA test calls for air-drying of the impressions for 30 min, 4 hr of fixation in acetone, and 30 min of incubation for the conjugate-impression reaction (8), a total of approximately 5.5 hr.

Other antigens do not require fixation for use in the FA test (2, 3, 16), and it has been demonstrated that the initial antigen-antibody combination occurs within seconds (14). It seemed, therefore, worthwhile to attempt to apply these principles to the rabies FA test to accelerate the diagnostic process.

In this study, the results obtained with variations in the fixation and incubation schedules of the rabies FA test were compared with those obtained by the standard technique of Goldwasser, Kissling, and Carski (8) and by the mouse inoculation test (10).

MATERIALS AND METHODS

Brain samples. Brain samples were obtained according to the technique described by Tierkel (18)

1 A preliminary report on this study was presented at the II Jornadas Argentinas de Microbiología, 22-26 November 1970, Buenos Aires, Argentina.

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from heads sent to this laboratory for the diagnosis of rabies.

A total of 246 brain samples obtained from 195 dogs, 41 cats, 4 laboratory mice, 2 human beings, 1 cow, 1 goat, 1 monkey, and 1 rabbit were used to study the effect of alterations of the fixation method on the rabies FA test.

Also, 161 brain samples were used for the study of the effect of variations in the incubation schedules on the FA test. These samples were from 128 dogs, 28 cats, 2 laboratory mice, 1 cow, 1 goat, and 1 monkey.

FA test. The conjugate used was prepared according to the technique of Lennette et al. (11) and showed a titer of 1:80. The standard FA technique of Goldwasser et al. (8) was used, and the results were compared with those obtained by FA tests in which the methods of fixation and incubation were modified.

Modified fixation method. Two microscope slides with two impressions each were prepared from the Ammon's horn of each brain sample and were air-dried. One slide was left unfixed and the other was fixed in acetone at -20 C for 4 hr as recommended by Goldwasser et al. (8). Both were then stained, by the standard staining method, at 37 C for 30 min.

Modified incubation method. Two pairs of impressions were obtained from each brain as above and were kept unfixed. One of the slides was stained with conjugate by the standard staining method at 37 C for 30 min and the other was stained by the rapid staining method at room temperature (22 to 25 C) for 10 min.

After staining, the slides were rinsed as usual. The stained slides were coded so that the person observing them under the microscope would not know their sources or the fixation and staining methods. The intensity of the specific stain and the amount of antigen present in the positive smears were graded on a 1 to 4 scale. A monocular Leitz microscope, model SM, was used together with an HBO 200 lamp, exciting filters.
UGI (2 mm) and BG38 (4 mm), and barrier filter K 430.

**Mouse inoculation test.** Suspensions from each brain used in this study were prepared as described by Koprowski (10) and inoculated intracerebrally into 10 mice (3 to 4 weeks old). On one occasion, suckling mice were also used.

**RESULTS**

**Effect of modified fixation.** Complete correlation among the results of mouse inoculation tests and FA tests, with either fixed or unfixed impressions, was found in 245 of 246 brain samples used in this part of the study. In all tests, 105 brains were positive and 140 were negative. The remaining brain sample was negative by adult mouse inoculation and by FA test with the acetone-fixed slide, but was positive by the FA test in which the unfixed slide was used. A suspension of this brain was inoculated into 10 suckling mice, and 2 of these animals subsequently died of rabies, as demonstrated by FA on their brains.

In all but two of the positive cases, the amount of rabies antigen demonstrable in the unfixed slides was equal to or greater than the amount in the acetone-fixed slides.

**Effect of modified incubation.** Of the 161 samples used in this part of the present study, 73 were positive and 88 were negative by both FA staining methods (staining at 37 C for 30 min or at room temperature for 10 min), as well as by mouse inoculation.

The intensity of the stain and the amount of antigen detected with the rapid staining method were somewhat lower than with the standard staining method in 40% of the positive impressions.

**DISCUSSION**

The method described for reducing the performance time of the FA test for rabies by using unfixed brain impressions stained with antibodies conjugate at room temperature for 10 min showed the same sensitivity and specificity as the standard FA and mouse inoculation tests.

In general, more antigen was seen in the unfixed positive impressions than in the fixed ones. Also in one case, a positive result was obtained with the unfixed smear whereas the corresponding fixed one was negative. The specificity of that result was confirmed by inoculating the brain sample into suckling mice, known to be more sensitive to rabies virus than adult mice (1, 15).

Although the reason for finding less antigen with the acetone-fixed impressions is not known, it could be due to damage to the rabies antigen by acetone.

In some cases, less antigen and lower intensity of the stain were observed in the positive smears stained at room temperature for 10 min than in those stained at 37 C for 30 min. Lower intensity of staining could result in erroneous diagnosis on slides with minimal antigen, but we had no problem in differentiating the positive samples from the negative ones. A kinetic study of the rabies antigen-antibody reaction has not been performed, but a situation similar to that found by Mayer and Heidelberger (14) for pneumococcal polysaccharides might occur. In their study, 90% of the reaction was completed in 3 sec, and the remainder “took place with progressively diminishing velocity.”

Fischman and Ward (5) have found infective rabies virus in impressions fixed with acetone, and, because rabies virus is sensitive to organic solvents (9), the occurrence of infective virus is more likely in unfixed than fixed smears. If desired, this problem could be overcome by exposing the smears to ultraviolet light prior to staining and, during air-drying, as described by Lépine and Gamet (12).

The use of the described FA technique would enable the diagnostic laboratory to report the results more quickly to the physician considering rabies treatment for the bitten persons.

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**LITERATURE CITED**


