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Review

A need for standardized rabies-virus diagnostic procedures: Effect of cover-glass mountant on the reliability of antigen detection by the fluorescent antibody test

Robert J. Rudd[a,*], Jean S. Smith[b,2], Pamela A. Yager[b,3], Lillian A. Orciari[b,4], Charles V. Trimarchi[a,1]

[a] *Wadsworth Center, New York State Department of Health, Box 509, Albany, NY 12201-0509, USA*
[b] *Division of Viral and Rickettsial Diseases, Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention (CDC) Atlanta, GA 30333, USA*

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Abstract

The direct fluorescent antibody test is a sensitive and specific procedure used in the routine diagnosis of rabies. However, given the critical role of the rabies diagnostic laboratory in patient management and public health decision-making, the use of a standardized national rabies diagnostic procedure is highly recommended. Seemingly small variations in test procedures may have dramatic effects on sensitivity. For example, two independent reports of diminished staining performance of two lots of a commercial anti-rabies conjugate were investigated in this study. The diminished staining occurred only with a single rabies-virus variant, associated with big brown bats, *Eptesicus fuscus*, in the southwestern United States. Similarly diluted and prepared diagnostic reagents provided bright staining on all other variants of rabies-virus tested. Subsequent evaluation disclosed that the phenomenon was associated with the relative concentrations of glycerol used in the mounting media by the reporting laboratories. These findings, related to the proper selection of an optimal cover-glass mountant for use in the immunofluorescence procedure, demonstrate the potential for erroneous results with severe implications for patient health, when uncontrolled variations in protocol occur. This paper underscores the necessity for all rabies diagnostic laboratories to follow one standard protocol. Such a protocol has been placed on the websites maintained by the Centers for Disease Control and Prevention: http://www.cdc.gov/ncidod/dvrd/rabies/professional/publications/DFA_diagnosis/DFA_protocol-b.htm.

Keywords: Rabies; Fluorescent antibody test; Microscope slide cover-slip mountant; Standard protocol

Contents

* Corresponding author. Fax: +1 518 869 6540.
E-mail addresses: rjr06@health.state.ny.us (R.J. Rudd), jss2@cdc.gov (J.S. Smith), pay2@cdc.gov (P.A. Yager), lao0@cdc.gov (L.A. Orciari), trimarch@wadsworth.org (C.V. Trimarchi).
[1] Fax: +1 518 869 6487.
[2] Fax: +1 404 639 1058.
[3] Fax: +1 404 639 1058.
[4] Fax: +1 404 639 1058.

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1. Introduction

Institution of modern surveillance systems for infectious diseases assumes the existence of a reliable, laboratory-based diagnostic method. After its introduction in the 1950s, the direct fluorescent antibody test (FAT) (Coons et al., 1941) became a widely applied method for the detection of rabies-virus infection, and is considered the preferred diagnostic procedure on a global scale. Because the diagnosis of rabies in animals can now be completed in a reliable manner in less than 1 day, the physician's decision to initiate or withhold post-exposure prophylaxis is often based on post-mortem examination of the biting animal. This practice imposes the highest standards of sensitivity on the performance of the test, as false-negative reports can be expected to include human mortality (Trimarchi and Smith, 2002). Nevertheless, the post-mortem examination of animals for evidence of rabies infection is not regulated, as is human clinical testing.

2. Fluorescent antibody test

As is the case with any diagnostic test, reliability of the FAT depends upon the adherence to a standard, validated operating procedure. Clearly, critical variables in the test are the nature of the diagnostic conjugate and its operational environment. The primary reagent in the direct FAT is a fluorescein isothiocyanate (FITC)-labeled globulin conjugate specific for the rabies-virus nucleocapsid protein. The FAT is rapid and accurate, but its specificity and sensitivity depends, in part, upon the affinity, titer, and optimal fluorochrome labeling of the rabies-virus nucleocapsid-specific antibodies contained in the conjugate. Originally the globulin fraction purified from the blood of hyper-immunized animals was the source of these antibodies (Trimarchi and Debbie, 1974). Introduction of hybridoma technology (Kohler and Milstein, 1975) permitted the production of monoclonal antibodies for the utilization of consistent, specific, and pure conjugates constructed from a cocktail of such antibodies. However, monoclonal antibody-derived conjugates have attributes requiring strict adherence to the established protocols (Zola, 1985; Durham et al., 1986; Steward and Lew, 1985; Mosmann et al., 1980). Due to the distinct specificities and variable avidities of the limited number of individual monoclonal antibodies in these reagents, it must be verified that the chosen monoclonals have the ability, either individually or in combination, to bind to all antigenic variants of the virus. Furthermore, all testing conditions must be evaluated, and it must be determined that none of them deleteriously affects the stability and affinity of the labeled antibodies, both before and after specimen staining. The objective of this study was to elucidate the effects, upon the FAT, of alteration of the mounting medium. This investigation revealed that the glycerol concentration in the microscope slide cover-glass mounting medium is critical to the stability of the specific staining in the rabies FAT. In addition, we determined that this effect applies to antibody conjugates prepared from both monoclonal antibody and serum-derived conjugates.

3. Evaluation of reagent quality and formulation

The New York State (NYS) Rabies Diagnostic Laboratory provides pre-market evaluation and quality control testing of the Light Diagnostics Rabies Reagent (DFA 1) produced by Chemicon International Inc.[1] During 1998 and 1999, the NYS lab was notified of problems relating to the DFA 1 reagent in two state rabies laboratories in the southwestern United States. Neither of the laboratories was able to arrive at a satisfactory working dilution, when titrating the diagnostic reagent. Two lots of conjugate were involved. Initially, the Centers for Disease Control (CDC) investigation determined that the two laboratories were using positive control antigens composed of a rabies-virus of close genetic relatedness. This particular virus variant is associated with the big brown bat (*Eptesicus fuscus)* found in the southwestern United States. The variant has been identified occasionally in terrestrial mammals. Further tests completed at the CDC duplicated the staining deficiency, using a virus with the same molecular characterization.

When the CDC laboratory applied the DFA 1 reagent to the tissue containing this rabies-virus variant, the original bright staining of the viral antigen faded rapidly, and was extinguished sometimes in as little as 5 min. When identical staining was performed on other rabies-virus variants, bright staining was seen that did not fade for up to 12 h at room temperature. An alert was issued by the manufacturer to all users of the DFA 1 product, indicating its diminished reaction with one identified variant of rabies-virus. Efforts to duplicate this finding at the NYS laboratory by direct fluorescent antibody (DFA) testing on the original and other tissues containing this variant resulted in strong staining and no observed fading. A close comparison of the details of the staining process at the CDC and NYS labs identified a difference in the composition of the cover-glass mounting medium as a possible cause of

[1] Chemicon International, Temecula, CA, USA. The monoclonal antibodies included in, and the DFA I conjugate were developed in the Wadsworth Center rabies laboratory, and are licensed (through Health Research Inc.) for manufacture and sale by Chemicon.