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To: Laboratory Directors, All State Public Health Laboratories and Rabies Diagnostic Laboratories

Subject: Problems with Interpretation of Rabies Direct Fluorescent Antibody (DFA) Tests Due to Non-specific Fluorescence

Human contact with wildlife is more frequent in summer, and there is a subsequent increase of rabies test submissions, especially bats. During the past month, there has been an increase in non-diagnostic or indeterminate results reported by state laboratories due to non-specific fluorescence. This atypical fluorescence is most frequently seen in the *Eptesicus fuscus* (big brown bats), however, it has been noted in other species. The fluorescence is apple-green in color, 3-4+ intensity, uniform in size, and appears to be on the surface of the tissue. However, in thick impressions or smears it may give the appearance that it is within the tissue. Sometimes the problem can be easily resolved by repeat DFA testing using additional rabies conjugates and specificity control (non-rabies antibody) conjugate. In other cases, reactions occur with all the currently available commercial rabies reagents and specificity control reagents. In these cases, adjunct testing protocols such as the direct rapid immunohistochemistry test (DRIT), RT-PCR and virus isolation, available at reference laboratories, are necessary to confirm or rule-out rabies. False negative and false positive results have a direct and critical public health impact in informing decisions regarding provision of appropriate

and timely post-exposure prophylaxis. The Centers for Disease Control and Prevention (CDC) Rabies Team is available for consultation and technical assistance (404-639-1050)

**Recommendations:**

1. Strict Adherence to the national standard protocol for DFA testing.  
[http://www.cdc.gov/rabies/docs/standard\\_dfa\\_protocol\\_rabies.pdf](http://www.cdc.gov/rabies/docs/standard_dfa_protocol_rabies.pdf)
2. Repeat (Confirmatory) DFA with 2 diagnostic conjugates and specificity control reagent (Negative Control Conjugate) on all weak positive and inconclusive test results.
3. Prompt submission of samples to a reference laboratory if rabies can not be ruled-out or confirmed by repeat (Confirmatory) DFA testing. Provide information regarding exposures.
4. Use of alternate confirmatory testing such as DRIT, RT-PCR and virus isolation as adjunct test procedures.
5. Follow laboratory practices which minimize cross-contamination of samples
6. Avoid multiple freezing and thawing of conjugates to reduce aggregate production. Filter rabies conjugates through 0.45 µm low protein binding filter onto rabies slides as recommended by the national standard protocol for DFA.