LOW AFFINITY AND INCONSISTENT RABIES VIRUS VARIANT RECOGNITION WITH MOST RECENT LOT OF RABIES DIAGNOSTIC CONJUGATE

The direct fluorescent antibody (DFA) test is a highly sensitive and specific test for the *in vitro* detection of rabies in brains and submaxillary glands. The results of the test have clinical and public health impact regarding appropriate and timely rabies post-exposure prophylaxis when it is needed. Due to the critical nature of this testing, the optimal working dilution for each new lot of commercial conjugate should be determined by the end user prior to use. The working dilution of conjugate should demonstrate sparkling apple-green fluorescence (4+ intensity) and detect 100% of antigen compared to a reference conjugate when rabies virus variants endemic to the region are tested. The standard DFA test protocol in the United States, "Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing" requires at least 2 anti-rabies virus conjugates to maximize recognition of different antigenic sites.

Current Situation

The current Fujirebio Diagnostics, Inc. kit lot 308689 (524255) may require a more concentrated titer*.

Variation in affinity and concentration (titer) of this conjugate was noted with different rabies virus variants. More concentrated dilutions of the above conjugate were necessary to obtain 4+ intensity and 100% antigen detection.

The inconsistency of this lot of anti-rabies diagnostic conjugate emphasizes the critical need for thorough evaluation of new lots of rabies conjugates with multiple rabies virus variants prior to use. The manufacturer, Fujirebio Diagnostics, Inc., is aware of the problems with the rabies reagent and is working diligently to resolve the problems as quickly as possible.

Recommendations:

1. - Strict adherence to the national standard protocol, "Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing" (optimize all reagents prior to use).
2. - Repeat (Confirmatory) DFA with 2 diagnostic conjugates and specificity control reagent (Negative Control Conjugate) when inconsistent results occur, especially on weak positive and inconclusive test results.
3. - Prompt submission of samples to a reference laboratory if rabies cannot be ruled-out or confirmed by repeat (Confirmatory) DFA testing. Provide information regarding exposures.
4. - Use of alternate confirmatory testing such as virus isolation and RT-PCR as adjunct test procedures.
5. - The CDC Rabies program should be notified (404-639-1050) when inconsistent results with two or more rabies virus conjugates are recognized.

(*CDC Note: Optimal working dilution may vary depending on the lyssavirus/rabies virus variant, and may require a 2- 10 times or more concentrated working dilution than previous lots. Within the USA, detection of the South Central skunk variant and *Lasiurus cinereus* variant seem to be most problematic.)

SHORTAGE OF ANTI-RABIES CONJUGATES

Due to the delayed release of the current Fujirebio Diagnostics, Inc. kit lot 308689 (524255), and the inability of EMD Millipore Corp. to meet the increase demand for alternative products Cat# 5500 and 6500, an international shortage of anti-rabies conjugates has occurred. These conjugates are essential since the standard DFA protocol requires at least 2 anti-rabies virus conjugates in every test to maximize recognition of different antigenic sites. Fujirebio Diagnostics, Inc. should have product available before the end of June, and EMD Millipore Corp. early July. Some laboratories who are currently using the Fujirebio Diagnostics, Inc. conjugate kit lot 308372 (523486) which will expire at the end of June 2015 may be waiting until July for in-date products.

Recommendations:

1. - Laboratories which have a surplus of in-date conjugate from Fujirebio Diagnostics, Inc. or EMD Millipore Corp. could loan a vial of conjugate to a rabies laboratory within the same state or nearby states. Please ask the supervisor, laboratory director or state epidemiologist to make inquiries, if necessary.

2. - A lab contingency plan may be to send samples for testing to an alternate lab within the state or region.

3. - If there is no available source of in-date anti-rabies conjugate, and no contingency plan to send testing to an alternate lab, an option for this urgent situation only, might be re-titration and revalidation of the expired lot of conjugate. Discontinue the use of the outdated conjugate when an in-date product is available. (See re-validation procedure attached.)

Emergency Use
REVALIDATION OF EXPIRED CONJUGATE
(Discontinue the use of the outdated conjugate when an in-date product is available.)

Revalidation of an expired conjugate (or soon to expire) reagent should include re-titration against the predominant rabies virus variants endemic to the region, and comparison with a reference conjugate to ensure maximum (4+) intensity and 100% of antigen is detected. After titration, a validation test on coded positive and negative slides is performed with the outdated reagent and a second conjugate using the National Standard Protocol.

Recommendation:

1. - Follow the National Standard DFA Protocol for performance of a 2-fold (broad) titration and then a narrow titration to determine a more precise optimal working dilution. Use EMD Millipore 5100 or other non-expired second conjugate as the reference conjugate.

2. - Re-validate the outdated conjugate by performing DFA tests on coded positive and negative slides using the optimal working dilutions of the outdated conjugate and a second conjugate according to the National Standard protocol.

3. - The slides evaluated in the validation should include a representative set of lyssavirus/rabies virus variants found in your area. The number of validation specimens should be approximately 10% of the total number samples expected to be tested in the 2 week emergency period.

4. - All validation tests and controls (positive and negative) should demonstrate the expected values.

5. - Prior to use of the expired reagent in routine testing, the validation documentation (titration worksheets, calculations of the optimal working dilution, validation test worksheets) need to be reviewed and signed by the technical supervisor.

6. - Please consult with the quality manager concerning your lab quality assurance policies, and any further documentation that is required before proceeding.

7. - Any unusual results obtained using the revalidated conjugate need to be confirmed. Samples should be submitted to a reference laboratory, if additional confirmatory testing is needed.

8. Discontinue the emergency use of the outdated reagent as soon as new product is available for use.