PT.103
VALORACIÓN DE UN TEST RÁPIDO DE INMUNOCROMATOGRAFÍA EN PLACA PARA LA DETECCIÓN DE RABIA EN MUESTRAS FRESCAS Y EN AVANZADA DESCOMPOSICIÓN DE ARGENTINA

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En el presente estudio se evaluó un test de inmunocromatografía (RIDT) para rabia (Rabies Ag Test Kit, Bionote Inc., Korea) de las principales cepas virales circulantes en la República Argentina, en muestras frescas que incluyeron a los reservorios más frecuentes, como así también en cerebros en avanzado estado de descomposición bajo condiciones de laboratorio. Para ello se realizó el diagnóstico sobre un lote de 50 de muestras clínicas frescas por las técnicas de IFD, RIDT y EBRL entre los años 2011 y 2012. Para evaluar las cepas de *Lasiurus sp* (V6) y terrestre silvestre (V2) se utilizaron aislamientos en cerebro de ratón, con la intención de incluir en el ensayo las variantes de mayor circulación del país. Posteriormente se descongelaron 5 cerebros guardados a -70°C de un brote de rabia canina (V1) 2002-2008. La sensibilidad del test se valoró con una cepa de virus fijo utilizado en la producción de vacuna CRL, previamente titulado en ratones de 21 días y RT-PCR en forma paralela. La concordancia del RIDT con la IFD y EBRL fue del 100% y pudo detectarse hasta la dilución 10-4 del virus fijo, que correspondió a 100 DL50 en 0,03ml. Existen regiones de explotación ganadera en el norte del país con rabia paresiante y otras con antecedentes de rabia de ciclo terrestre de difícil acceso cerca de la frontera con Bolivia en donde el veterinario del municipio podría realizar un primer diagnóstico diferencial a fin de dar parte a las autoridades sanitarias y así aplicar rápidamente las acciones de profilaxis correspondientes a los mordidos y luego remitir la muestra a los laboratorios de referencia para confirmación y caracterización de la cepa responsable del brote. En nuestra conclusión el RIDT es de uso muy simple y podría ser considerado de suma utilidad en muestras post-mortem bien conservadas o descompuestas que hayan completado el período de estado de la enfermedad.

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USE OF PROPIDIUM IODIDE LIKE A CELULAR CONTRAST STAINING IN THE DIRECT FLUORESCENT ANTIBODY (DFA) TEST FOR THE RABIES VIRUSES DIAGNOSTIC.

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Introduction: Rabies remains one of the most important zoonosis worldwide and represents a serious problem in many countries. Into the diagnostic tools the direct fluorescent antibody (DFA) test is a fast and sensitive method to diagnosis rabies infection in animals and in humans (1, 3). The test is based on microscopic examination, under ultraviolet light, impressions, smear samples of tissue of the hippocampus (flagpole of Ammon), the cerebellum and the medulla or tissue sections; antibodies (IgG) used in the conjugate monoclonal allow specific and uniform coloring without interference from the fund.

In cell biology, propidium iodide is used as a dye contrast that differentiates the nucleus from the cytoplasm (intercalates in DNA) (4), there is evidences of its use in stains for identification of Herpes Simplex Type 1 (5).

Goal: Implement the use of propidium iodide in the diagnosis of the rabies virus by direct fluorescent antibody (DFA) test **Materials and methods:** 70 Samples were selected: 50 brains: (25 positive samples of varying degrees of positivity (1 + to 4 +) and 25 samples negative) and 20 isolates of the rabies virus in mouse neuroblastoma cell (15 positive samples of varying degrees of positivity (1 + to 4 +) and 5 negative samples) of the rabies laboratory samples bank.

The direct fluorescent antibody (DFA) test was applied as indicated by the supplier Anti-Rabies Monoclonal Globulin (IDF, FUJIREBIO.) (DIAGNOSTICS, Inc.); to the end of the test, added 20 µL of the propidium iodide solution to a final concentration of 0.3 µg/mL (in each imprint of brain or well cell culture) and it was incubated at room temperature for 5 minutes, it was eliminated by rinsing with PBS pH 7.4, let air dry and was added a drop of buffered glycerin pH 8.4; reading was conducted on an magnification lens fluorescence microscope 10 X and 40 X. The reading was evaluated for 4 people; 2 experts in rabies diagnosis and 2 people in the learning process.

Results and conclusion: Of the 50 samples brains were obtained the following results; 25 positive samples of varying degrees of positivity (1 + to 4 +) and 25 negative samples and all 20 isolates of the rabies virus of in mouse neuroblastoma cell (15 positive samples of varying degrees of positivity (1 + to 4 +) and 5 negative samples; all samples coincided with the previously reported results. All personnel involved in reading coincided in the ease of identify the nucleus of the cell in the brain imprints as well as in cell culture slides. Because of the propidium iodide is used to staining the DNA, were watched the cells nucleus of red-orange color; likewise facilitated the identification of infection in the cytoplasm of the cell (in positive cases) by the fluorescent apple green contrast of the fluorescein isothiocyanate (FITC) fluorochrome, that is conjugated to the monoclonal antibodies targeting the protein of rabies virus.

Therefore, it is concluded that the use of propidium iodide does not interfere in the DFA test it is concluded that the use of propidium iodide does not interfere in the DFA test, since all results were identical to the reported in the samples tested before; the use of propidium iodide is helpful mainly for technical staff who do not have experience in identifying cells in imprints and cell culture; the use of propidium iodide allows a contrast which facilitates the identification of the fluorescence of the rabies virus.