Diagnosis of rabies and typing strains of rabies virus

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Summary: Post-mortem diagnosis of rabies is based on the detection of lesions (by means of histological techniques of relatively low sensitivity), viral antigen (direct immunofluorescence, immunoenzymology) or the pathogenicity of the virus in vivo (intracerebral inoculation of mice) or in vitro (inoculation of neuroblastoma).

Differential diagnosis of strains of rabies virus is performed with the indirect immunofluorescence technique using monoclonal antibodies. This technique is helpful in determining both the origin of cases of rabies and appropriate control measures.

KEYWORDS: Differential diagnosis - Laboratory diagnosis - Monoclonal antibodies - Rabies - Viral diseases.

There have been numerous improvements to rabies diagnosis in recent years, both in general procedure and in precision (virus typing).

1. Diagnosis of rabies

Apart from certain unreliable ante-mortem techniques (such as testing for viral antigen in corneal smears), the only reliable diagnosis takes place after death. It relies on detecting histological or cytological lesions, viral antigen and pathogenicity of the virus.

In the first group are histological techniques: staining nerve tissue by Seller’s technique (applied to smears) or Mann’s technique (applied to histological sections). These techniques of relatively low sensitivity can detect 60-95% of positive cases within a few minutes (Sellers) or within 4 days (Mann).

In the second group are immunochemical techniques.

Direct immunofluorescence: antibodies against the viral nucleocapsid are conjugated with fluorescein. They can detect the virus in infected cells (smears of suspect tissue or cultured cells) in 97-99% of cases, within a few hours.

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Immunoenzymology: antibodies are coupled to peroxidase, and their presence is revealed by a colour reaction often visible to the naked eye, with a sensitivity between 56-99%, which varies from laboratory to laboratory. A version of this test available commercially is “RREID” (Rapid Rabies Enzyme Immunodiagnosis).

The last group involves techniques for estimating the pathogenicity of the virus in vivo and in vitro: intracerebral inoculation of mice is as sensitive as the immunofluorescence test, but requires 6-28 days of observation. Inoculation of cells (neuroblastoma) requires only 2-5 days, with similar sensitivity.

The cost of these diagnostic procedures (materials only) is 2 French francs for Sellers, 4 francs for immunofluorescence, 10 francs for Mann and cell culture, 12 francs for RREID and 50 francs for mouse inoculation.

2. Typing rabies virus by monoclonal antibodies

The indirect immunofluorescence technique is used in the differential diagnosis of strains of rabies virus. Suitable samples are obtained directly from infected brain, or after passage in mice, or from cell cultures infected with the virus. The method consists in placing monoclonal antibodies to nucleocapsid in contact with smears of brain or infected cells, followed by counterstaining with mouse antoglobulin conjugated to fluorescein. The presence or absence of fluorescence is determined by the direct immunofluorescence technique described above. Usually the test is performed with a series of monoclonal nucleocapsid antibodies known in advance to react with different strains of the virus. These antibodies are available, upon request, from the WHO Collaborating Centres.

This technique is extremely useful in determining the origin of cases of rabies and the appropriate control measures. For example, rabies cases currently observed among bats in northern Europe have proved to be very similar to the Duvenhage strain, so far found only in South Africa. This technique also made it possible to demonstrate the polar origin of recent rabies cases in Finland, a country free from rabies since 1959. It is thus possible to distinguish cases caused by wild virus (in fox, dog, raccoon-dog, etc.) from cases which might have been induced by live vaccine strains (Flury, ERA, SAD, etc.).