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A collaborative study of an experimental kit for rapid rabies enzyme immunodiagnosis (RREID)

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Six laboratories took part in a study to assess an experimental kit for the diagnosis of rabies using the rapid rabies enzyme immunodiagnosis (RREID) technique. The test is based on the immunocapture of rabies antigens present in homogenized brain specimens, followed by enzyme immunoassay. A total of 1253 specimens from various geographical locations and 27 animal species were tested with the RREID technique, and also with the fluorescent antibody test (FAT), which was used as a reference method. For 1220 specimens the results in RREID and FAT were the same (651 positive and 569 negative — concordance: 97.4%). However, the RREID technique appeared to be less sensitive, since 22 (3%) of the 673 specimens that were positive with FAT were negative with RREID. The RREID test is therefore specific and convenient and is a useful tool for epidemiological studies and for laboratories not equipped with an ultraviolet microscope.

Since 1958 when Goldwasser & Kissling (*1*) identified rabies antigens in the tissue of infected animals, the fluorescent antibody test (FAT), as described by Dean & Abelseth (*2*), has become the recommended procedure for routine diagnosis of the disease. Nevertheless, to carry out the test a microscope equipped with well-maintained ultraviolet (UV) accessories as well as considerable experience are prerequisites. Epidemiological studies under field conditions are therefore not easily carried out using FAT. However, many convenient enzyme immunoassays have been described (*3–5*), and we have recently reported a new method for diagnosing rabies in which rabies antigens in infected brain specimens are detected by enzyme immunoassay (rapid rabies enzyme immunodiagnosis, RREID) (*6*). Since the method appeared to offer promise for the routine diagnosis of rabies, we have developed an experimental kit for RREID. Six laboratories agreed to evaluate the test, and we report here the results of the study.

MATERIALS AND METHODS

Principle of the RREID technique

The RREID technique is an enzyme-linked immunosorbent assay (ELISA), based on the following principle (sandwich technique): rabies antigens that have been solubilized from brain specimens are specifically bound to immobilized rabies antinucleocapsid antibodies and subsequently revealed using antinucleocapsid antibodies conjugated to horseradish peroxidase.

Description and use of the RREID experimental kit

The RREID experimental kit[a] contains all the reagents necessary for the diagnosis of rabies in brain specimens:

— ELISA plates sensitized with rabbit antirabies nucleocapsid IgGs.
— washing solution (phosphate-buffered saline, pH = 7.2);
— peroxidase conjugate (rabbit antirabies nucleocapsid IgGs conjugated with horseradish peroxidase);
— buffer containing the substrate (hydrogen peroxide) for the enzymatic reaction;
— tablets of *o*-phenylenediamine (chromogen);
— stopping solution (2 mol/l sulfuric acid);
— positive control antigen (inactivated and lyophilized brain suspension collected from mice infected with the CVS-strain of the rabies virus); and
— a negative control antigen (suspension of inactivated and lyophilized brain from uninfected mice).

The immunoassay using sensitized ELISA plates was performed following the procedure described in

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the kit instructions and as previously reported (*6*), except that the enzymatic coloration was allowed to develop for 30 minutes instead of 5 minutes.

Preparation of specimens

Specimens of brain were homogenized in four volumes of washing solution and centrifuged (800 *g* for 30 minutes) to eliminate particles of debris. The clear supernatant was then analysed for the presence of rabies antigen using the experimental kit.

Participating laboratories

Six laboratories (see Annex) participated in the collaborative study and these were coded A–F (the letters do not correspond to the order in the list reported in the Annex).

Assay methods

Each laboratory received experimental kits and staff were requested to carry out the RREID test using the instructions supplied with the kits. The results were compared with those obtained using the reference technique (FAT).

Although the RREID kit permits detection of rabies antigens with the naked eye, for the purposes of the study the absorbance of solutions was determined quantitatively using a spectrophotometer. The absorbance of specimens at $\lambda = 492$ nm was compared with that of a negative control antigen; samples were considered positive if their absorbance was greater than or equal to 0 05 absorbance units above that of the control.

RESULTS AND DISCUSSION

The results obtained by the six laboratories are summarized in Table 1. Of the 1253 specimens analysed in the trial, 651 were positive in both the FAT and the RREID tests, 569 were negative in both tests, 22 were positive in the FAT but negative in the RREID test, while 11 were negative in the FAT but positive in the RREID test. The same results were obtained for the FAT and RREID tests for 1220 specimens (concordance: 97.4%).

Of the 684 specimens that were positive in one or other of the tests, 651 (95.2%) were positive in both, 22 (3.2%) were positive in only the FAT, and 11 (1.6%) were positive in only the RREID test. Furthermore, of the 602 specimens that were negative in one or other of the tests, 569 (94.5%) were negative in both, 11 (1.8%) were negative in only the FAT, while 22 (3.6%) were negative in only the RREID test.

The concordance varied from 89.7% to 99.2%, depending on the laboratory. For laboratories B, C, D, and F, where 84.5% of the total number of specimens were analysed, the concordance was greater than 96%. Laboratories B, C, and D reported that, of the 11 specimens that were negative in the FAT but

Table 1 Correlation between the results of the fluorescent antibody test (FAT) and rapid rabies enzyme immunodiagnosis (RREID) for the six laboratories in the study

Laboratory	No of specimens[a]					Concordance[b] (%)
	Total	+FAT/+RREID	−FAT/−RREID	+FAT/−RREID	−FAT/+RREID	
A	155	79	66	10	0	93 3
B	130	39	89	1	1	98 5
C	474	115	352	4	3	98.5
D	199	150	41	1	7	96.0
E	39	34	1	4	0	89.7
F	256	234	20	2	0	99 2
Total	1253	651	569	22	11	97 4

[a] Results are expressed as the number of specimens that were positive in the FAT and RREID tests (+FAT/+RREID), negative in both (−FAT/−RREID); positive in FAT but negative in RREID (+FAT/−RREID), and negative in FAT but positive in RREID (−FAT/+RREID).

[b] Defined as $\frac{\text{(No positive in both FAT and RREID + No negative in both FAT and RREID)}}{\text{No of specimens tested}} \times 100$

Table 2. Correlation between the results of the fluorescent antibody test (FAT) and rapid rabies enzyme immunodiagnosis (RREID) for five laboratories according to type of animal

Type of animal	No. of specimens[a]					
	Total	+FAT/ +RREID	−FAT/ −RREID	+FAT/ −RREID	−FAT/ +RREID	Concordance[b] (%)
Cat	197	30	164	0	3	98.5
Dog	130	23	104	2	1	97.7
Cattle	85	48	35	1	1	97.6
Sheep	50	30	19	1	0	98 0
Goat	3	1	2	0	0	100
Horse	11	5	5	1	0	91.0
Pig	3	2	1	0	0	100
Fox	367	331	28	6	2	97 8
Skunk	18	17	1	0	0	100
Coyote	5	5	0	0	0	100
Raccoon	2	2	0	0	0	100
Groundhog	2	2	0	0	0	100
Badger	16	12	2	0	2	87.5
Marten	19	7	12	0	0	100
Weasel	3	1	2	0	0	100
Ferret	1	0	0	0	1	0
Rat	9	0	9	0	0	100
Muskrat	2	0	2	0	0	100
Dormouse	3	0	3	0	0	100
Wild mouse	2	0	2	0	0	100
Laboratory mouse[c]	7	6	1	0	0	100
Squirrel	6	0	6	0	0	100
Rabbit	4	0	4	0	0	100
Hedgehog	2	0	2	0	0	100
Deer	11	5	6	0	0	100
Bat	7	5	2	0	0	100
Bird	1	0	1	0	0	100
Total	966	531	414	11	10	97.8

[a] See footnote *a* to Table 1

[b] See footnote *b* to Table 1

[c] Inoculated with wild rabies isolates

positive in the RREID test, some (if not all) were decomposed upon receipt. Studies are currently under way to determine the specificity of this positive RREID response: in two cases, the RREID response was inhibited by a previous incubation with antirabies nucleocapsid serum, but no inhibition occurred with normal rabbit serum. On the other hand, the 22 specimens that were positive in the FAT but negative in the RREID test indicate that the latter technique may be less sensitive. Nevertheless, if the high concordance between the two methods is taken into account, the findings indicate that the RREID test is specific and sensitive.

The results obtained from five of the laboratories (A, C, D, E, and F) were also analysed according to animal species (Table 2). In general, for large samples, there was no difference between the results of the RREID and FAT tests for the various animal

species in the study. For example, for species for which more than 18 animals were tested (cat, cattle, dog, fox, marten, sheep, and skunk), in 866 out of a total of 966 (90%) specimens the concordance was greater than 97.6%.

The purified antinucleocapsid immunoglobulins used in the RREID test kits were obtained from rabbits that had been immunized with antigens prepared with the Pasteur strain of rabies virus, and the test is recommended for diagnosis of lyssaviruses of serotype 1. The specificity of the antibodies used in the kit was the same as that of those prepared in the laboratory and marketed by Diagnostics Pasteur for the diagnosis of rabies by direct immunofluorescence. Use of these antibodies in the FAT test permitted detection also of antigens of other lyssaviruses (Lagos Bat, Mokola, and Duvenhage) but the sensitivity was lower than for the serotype 1. Nevertheless, with the RREID experimental kit several of the laboratories observed a weak reaction with Mokola, Lagos Bat, and Duvenhage viruses. In view of this, we have prepared a new batch of horseradish peroxidase conjugate using the same antinucleocapsid immunoglobulins as before, which appears to be more sensitive. An improved RREID experimental kit for the detection of the various serotypes of lyssaviruses will soon be available

In conclusion, if a laboratory has a good UV microscope and the number of specimens to be analysed for rabies antigens is not too large, use of the RREID test instead of the FAT is not recommended. However, for laboratories that have no fluorescence microscope or for epidemiological studies that involve a large number of specimens, the RREID technique is a useful tool for the rapid and specific diagnosis of rabies.

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RÉSUMÉ

ÉTUDE CONCERTÉE D'UNE TROUSSE DE RÉACTIFS DESTINÉE À L'IMMUNODIAGNOSTIC ENZYMATIQUE RAPIDE DE LA RAGE (RREID)

A la demande de laboratoires de diagnostic de la rage nous avons récemment mis au point une méthode permettant de détecter les antigènes rabiques dans des broyats de substances cérébrales. Cette méthode est fondée sur le principe d'une immuno-capture des antigènes rabiques (nucléocapsides) dans des microplaques sensibilisées (anticorps antinucléocapsides), suivie d'une révélation de l'antigène fixé spécifiquement par le même anticorps (antinucléocapside) conjugué à la peroxydase et d'une réaction enzymatique en présence d'un chromogène. Dans ces conditions, les prélèvements rabiques génèrent une coloration jaune-brun après mise en œuvre du RREID.

Cette nouvelle technique s'étant révélée sensible et spécifique, nous en avons poursuivi le développement et proposé une trousse de réactifs prêts à l'emploi pour le dépistage des antigènes rabiques. Plusieurs trousses ont été fournies à six laboratoires différents ayant accepté de participer à une étude concertée sur la faisabilité et l'intérêt de l'utilisation du RREID comme technique de diagnostic de la rage. Nous rapportons ici les résultats de cette étude.

Six laboratoires ont testé 1253 prélèvements provenant de 27 espèces animales et de 10 origines géographiques différentes. Les résultats obtenus avec le RREID ont été comparés à ceux obtenus par immunofluorescence (FAT) Les deux techniques ont donné le même résultat pour 1220 prélèvements (651 positifs et 569 négatifs), ce qui correspond à 97,4% du total des prélèvements testés. Le RREID semble cependant légèrement moins sensible que le FAT puisque, sur l'ensemble des prélèvements positifs avec le FAT, 22 soit 3% se sont révélés négatifs avec le RREID.

Toutefois, l'étude concertée confirme que le RREID est une technique facile à mettre en œuvre, rapide et spécifique, qui peut être considérée comme très utile pour des études épidémiologiques et pour des laboratoires non équipés en immunofluorescence.

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Annex

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