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# HYPERIMMUNE ANTIRABIES SERA TITRATION BY STANDARD MOUSE NEUTRALIZATION AND COUNTERIMMUNOELECTROPHORESIS TESTS, COMPARING RESULTS OF DIFFERENT LABORATORIES

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## SUMMARY

To determine the rabies antibody level of twenty-four hyperimmune equine sera, Standard Mouse Neutralization (SMN) and Couterimmunoelectrophoresis (CIE) tests were carried out, both at the Instituto Butantan (IB) and Instituto Panamericano de Protecciíon de Alimentos y Zoonosis (INPPAZ). Statistical analysis has shown a correlation (r) of 0.9317 between the SMN and CIE performed at the IB, while at the INPPAZ it scored 0.974. Comparison of CIE data of both laboratories yielded a correlation of 0.845. The CIE technique has shown to be as sensitive and efficient as the SMN in titrating antirabies hyperimmune equine sera. Based on CIE results, a simple, rapid and inexpensive technique, titers of sera antibody can be rellably estimated in SMN test.

**KEYWORDS:** Antirabies sera; Counterimmunoelectrophoresis; Standard Mouse Neutralization Test.

## INTRODUCTION

Rabies represents a serious disease up to now, providing its high rates of mortality[14], due either to the absence or inadequacy of the treatment provided.

The World Health Organization's (WHO)[10] recommended treatment for aggressions made by suspected or rabid animals has been a combination of serum and vaccine. This recommendation is based on experiments carried out by BALTAZARD & BAHMANYAR[3] (1957) at the village of Sahane, Iran, with two groups of persons bitten in the head by a rabid wolf; one of the groups was vaccinated and the other received serovaccination. Human immunoglobulin has been used as a treatment for rabies in the United States of America and Europe; in Brazil, however, hyperimmune serum of equine origin[4,9] is the procedure still employed.

According to the WHO, when using serum treatment, a single intramuscular dose of 40 IU per kg of body-weight should be administered.

In Brazil, antirabies serum is presented in vials of 5.0ml containing, in average, 200 IU/ml. The Ministry of Health of this country recommends the Standard Mouse Neutralization (SMN)[1] method to assess the antibody levels of this serum.

Techniques such as indirect immunofluorescence[13] and the immunoenzymatic method[2] have also been used.

Recently appeared, the counterimmunoelectrophoresis technique is another alternative procedure which has been often employed for diagnosing various

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diseases[8,12], including rabies serodiagnosis[5,6]. This technique, besides providing good sensitivity, does not employ animals and presents its results in approximately 4 hours.

In the present experiment, counterimmunoelectrophoresis (CIE) was the test employed for quantitatively determining antibodies present in rabies hyperimmune equine sera. These sera were comparatively tested by both the SMN and CIE methods at the IB's and INPPAZ's rabies laboratories.

## MATERIALS AND METHODS

### 1. Hyperimmune equine serum

Twenty-four equine serum samples made hyperimmune with rabies antigen obtained from suspensions of $BHK_{21}$ cells (Clone 13), infected with Pasteur fixed virus, PV strain, were used. These samples were collected in various stages of the immunization process.

**TABLE 1**

Titration of hyperimmune antirabies sera. CIE tests carried out at the Instituto Butantan and INPPAZ. Correlations of results.

| SERUM # | CIE TITERS | |
|---|---|---|
| | BUTANTAN | INPPAZ |
| 1 | 20* | 20* |
| 2 | 40 | 48 |
| 3 | 20 | 10 |
| 4 | 80 | 80 |
| 5 | 40 | 160 |
| 6 | 320 | 320 |
| 7 | 320 | 640 |
| 8 | 160 | 160 |
| 9 | 500 | 640 |
| 10 | 320 | 1,000 |
| 11 | 640 | 2,560 |
| 12 | 320 | 640 |
| 13 | 320 | 2,000 |
| 14 | 160 | 640 |
| 15 | 320 | 1,000 |
| 16 | 1,280 | 1,000 |
| 17 | 1,280 | 5,000 |
| 18 | 1,280 | 1,000 |
| 19 | 640 | 2,000 |
| 20 | 640 | 1,280 |
| 21 | 1,280 | 5,120 |
| 22 | 640 | 100 |
| 23 | 1,280 | 1,000 |
| 24 | 1,280 | 1,000 |

**TITER:** Inverse number of maximal serum dilution which does not produce a precipitation band.

**REGRESSION:** $y = 0.369 + 1.035x$

**CORRELATION:** $r = 0.845$

### 2. CIE Antigen

An antigen for counterimmunoelectrophoresis tests was prepared from a suspension of virus CVS/CCL twice-passed in suckling rabbit brains, made inactive with B propiolactone and concentrated with polyethylenoglycol 20,000, according to technique already mentioned[6].

### 3. Serum Indicator

Serum of rabbits immunized with vaccine against rabies produced from brains of suckling rabbits infected with rabies virus and inactivated by B. propiolactone[6], was used as indicator.

### 4. Serological Tests

The equine serum samples were titrated by the Standard Mouse Neutralization[1] and Counterimmunoelectrophoresis[6] techniques at the IB's and the INPPAZ's rabies laboratories.

## RESULTS

Table 1 shows the titres of antirabies antibodies in 24 equine serum samples obtained by the counterimmunoelectrophoresis (CIE) technique at the Instituto Butantan and Instituto Panamericano de Protección de Alimentos y Zoonosis. The linear regression parameters

**TABLE 2**

Titration of 19 hyperimmune antirabies sera. CIE and SMN tests carried out at the Instituto Butantan. Correlation of results.

| CIE | | SMN | | |
|---|---|---|---|---|
| TITER | $\log_2$ | $DE_{50}$ | $\log_5$ | $\overline{X}$ |
| 1:20 | 4.3 | 1:42 | 2.3 | 2.6 |
| | | 1:99 | 2.9 | (2.3-2.9) |
| 1:40 | 5.3 | 1:58 | 2.5 | 3.0 |
| | | 1:255 | 3.4 | (2.5-3.4) |
| 1:80 | 6.3 | 1:221 | 2.3 | 2.3 |
| 1:160 | 7.3 | 1:483 | 3.8 | 4.4 |
| | | 1:2,985 | 5.0 | (3.8-5.0) |
| | | 1:447 | 3.6 | |
| | | 1:451 | 3.8 | |
| | | 1:1,815 | 4.7 | |
| 1:320 | 8.3 | 1:2,285 | 4.8 | 4.5 |
| | | 1:2,957 | 4.9 | (3.6-5.3) |
| | | 1:5,000 | 5.3 | |
| | | 1:2,065 | 4.7 | |
| 1:640 | 9.3 | 1:9,772 | 5.7 | 5.6 |
| | | 1:10,303 | 5.7 | (4.7-6.5) |
| | | 1:37,320 | 6.5 | |
| 1:1,280 | 10.3 | 1:42,750 | 6.6 | 6.8 |
| | | 1:71,769 | 6.9 | (6.6-6.9) |

**TITER:** Maximal serum dilution which does not produce a precipitation band.

**$DE_{50}$:** Serum dilution able to protect 50% of the mice challenged with CVS virus.

**REGRESSION:** $y=1.042 + 0.714x$

**CORRELATION:** $r=0.9317$

were: **y=0.369 + 1.035x** and **r=0.845**. These results of correlation were considered very satisfactory, since the sera have been titrated independently, with the reagents being produced separately by each institution.

Results of CIE and Standard Mouse Neutralization (SMN) tests carried out at the IB, corresponding to 19 of the 24 equine sera above mentioned, can be seen on Table 2. The linear regression parameters found out were: **y=1.042 + 0.714x** and **r=0.9317**. Titers detected by CIE varied from 1:20 to 1:1280, corresponding to variation of 1:42 to 1:71,769 in SMN.

Table 3 shows the values found out for the CIE and SMN tests carried out at the INPPAZ, with 23 samples of the 24 sera above mentioned. The following regression parameters were observed: **y=0.7632 + 0.873x** and **r=0.974**. The CIE titers fell from 1:20 to 1:280, corresponding to values from 1:11 to 1:960,917 in SMN.

**TABLE 3**

Titration of 23 hyperimmune antirabies sera. CIE and SMN tests carried out at INPPAZ. Correlation of results.

| CIE | | SMN | | |
|---|---|---|---|---|
| TITER | $\log_2$ | $DE_{50}$ | $\log_5$ | $\overline{X}$ |
| 1:20 | 4.3 | 1:11 | 1.5 | 2.8 |
| | | 1:625 | 4.0 | (1.5-4.0) |
| 1:40 | 5.3 | 1:280 | 3.5 | 3.9 |
| | | 1:980 | 4.3 | (3.5-4.3) |
| 1:80 | 6.3 | 1:1,190 | 4.4 | 4.4 |
| 1:160 | 7.3 | 1:10,432 | 5.7 | 6.5 |
| | | 1:120,000 | 7.2 | (5.7-7.2) |
| 1:320 | 8.3 | 1:14,142 | 5.9 | 6.6 |
| | | 1:15,625 | 6.0 | (5.9-7.7) |
| | | 1:28,284 | 6.4 | |
| | | 1:40,000 | 6.6 | |
| | | 1:73,363 | 7.0 | |
| | | 1:242,519 | 7.7 | |
| 1:640 | 9.3 | 1:25,576 | 6.3 | 7.0 |
| | | 1:62,176 | 6.9 | (6.3-7.4) |
| | | 1:105,561 | 7.2 | |
| | | 1:143,690 | 7.4 | |
| 1:1,280 | 10.3 | 1:125,076 | 7.3 | 8.1 |
| | | 1:130,491 | 7.3 | (7.3-9.0) |
| | | 1:242,519 | 7.7 | |
| | | 1:934,035 | 8.5 | |
| | | 1:994,809 | 8.6 | |
| | | 1:960,917 | 9.0 | |

**TITER:** Maximal serum dilution which does not produce a precipitation band.

**$DE_{50}$:** Serum dilution able to protect 50% of the mice challenged with CVS virus.

**REGRESSION:** y = 0.7632 + 0.873x

**CORRELATION:** r=0.974

The differences in titers found out in the SMN tests carried out in both laboratories might be attributed to the utilization of different mice strains, since the challenge virus employed (CVC) was obtained from the same viral strain.

## DISCUSSION

Results obtained in this study validate the CIE technique as a procedure to be successfully applied during the production of equine antirabies for controlling the whole process.

Counterimmunoelectrophoresis is an "in vitro" technique which estimates the antigen-antibody link capacity without the need for an indicator system, contrary to the Standard Mouse Neutralization (SMN) or the Rapid Fluorescent Focus-Inhibition (RFFIT) tests where the determination of the antibodies capability of neutralizing the virus infectibility is achieved within a system in which animal or cellular cultures are employed.

Comparing both techniques, the CIE and SMN, as to their antibody antirabies titres, data obtained in this study provided a correlation coefficient superior to the equivalent values reported by DIAZ & MYERS in 1984[7]. Therefore, based on CIE results, titers of these sera can be estimated in SMN, making it possible to reach a simple, rapid and economic decision on how to actuate into the process to be implemented during the immunization process, that is, stopping it, administering additional doses or selecting among the animals those which would produce better antirabies sera.

There are suggestions for the manufacturers of antirabies serum to continue to use the International Standard Serum and the Standard Mouse Neutralization technique to release their final products[11]. Therefore, since SMN requires extensive use of laboratory animals, being as well a time-consuming procedure, the utilization of an "in vitro" technique such as the CIE, which provides good correlation with the SMN, would be a very useful guideline for a rapid and efficient monitoring of titration of antirabies antibodies during the immunization process of horses and at bulk.

## RESUMO

**Titulação de soros anti-rábicos hiperimunes, através da soroneutralização em camundongos e contraimunoeletroforese. Comparação dos resultados obtidos em diferentes laboratórios.**

Para a determinação do nível de anticorpos de vinte e quatro amostras de soros eqüinos hiperimunes contra a Raiva, foram utilizadas as técnicas de soroneutralização em camundongos (SN) e contraimunoeletroforese (CIE). As provas foram realizadas nos Institutos Butantan (IB) e Panamericano de Protección de Alimentos y Zoonosis (INPPAZ). A análise estatística demonstrou que para a SN e CIE feitas no IB, a correlação foi de r=0,9317, enquanto que no INPPAZ foi de r=0,974. Comparando-se os dados obtidos pela CIE nos dois laboratórios observou-se uma correlação de r=0,845. A técnica de CIE demonstrou ser tão sensível e eficiente quanto a SN na titulação de soros anti-rábicos hiperimunes de origem eqüina. Assim, com base nos resultados de CIE, que é uma técnica simples, rápida e econômica, pode-se estimar os títulos de anticorpos dos soros em SN.

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