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A MICROMETHOD FOR MEASURING RABIES-NEUTRALIZING ANTIBODY[1]

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Abstract: Plasma samples collected in micro-hematocrit tubes were shown to have rabies-neutralizing antibody titers comparable to serum samples. Micro-hematocrit tube sampling makes possible the monitoring of antibody levels in very small animals without causing serious injury.

INTRODUCTION

Rabies-neutralizing antibody levels in serum samples have been routinely titrated in our laboratory by an immunofluorescent method previously reported.[3] With a new adaptation of the technique, utilizing plasma samples collected in micro-hematocrit tubes, only 0.01 ml of plasma is necessary for each determination. This technique is useful in rabies serologic surveys of small animals such as bats.

MATERIALS AND METHODS

Tissue culture

The BHK-21 cell line is maintained at the Division of Laboratories and Research, New York State Department of Health. For cell subcultures and challenge virus production, Falcon Tissue Culture flasks[2] (75-cm^2 growth area) were seeded with 20 ml of cell suspension containing approximately 50,000 cells/ml in Eagle medium supplemented with 10% fetal bovine serum (FBS) and 10% tryptose phosphate broth. These cultures formed confluent cell sheets in 48 h at 37 C.

Virus

One dose of the commercially produced, lyophilized ERA vaccine strain of rabies virus[3] was reconstituted to 2 ml with Eagle medium supplemented with 3% FBS (Eagle-FBS). This virus had a mouse intracerebral median lethal dose ($MICLD_{50}$) titer of $10^{3.5}$/0.03 ml in 10-12 g Nya:NYLAR mice.[1]

Challenge virus production

The medium was removed from a confluent 48 h cell culture, and 2 ml of the ERA suspension were added. After 10 min at room temperature, 18 ml of Eagle-FBS were also added. The culture was incubated for 5 days at 34 C, then frozen and thawed once to disrupt the cells and centrifuged for 5 min at 500 g. The supernatant was stored in 1 ml aliquots at -70 C. Challenge virus thus produced has a $MICLD_{50}$ titer between $10^{4.5}$ and $10^{6.5}$. The optimal dilution for tissue culture challenge was determined from box titrations of hyperimmune antirabies serum of known titer against 10-fold dilutions of virus. The dilution with a potency of 100 tissue culture infectious doses (TCID) per 0.01 ml was used for the test.

[1] This research was supported in part by Research Corporation, Grant 70043.
[2] Falcon, Oxnard, California.
[3] Jensen-Salsbury Laboratories, Kansas City, Missouri.

Preparation of slides

Eight-chamber tissue culture slides (Lab-Tek Products, Division of Miles Laboratories, Westmount, IL) were seeded with BHK-21 cells and incubated as previously described.[3] The cultures formed confluent cell monolayers in 24 h.

Plasma-virus dilutions

Blood samples were collected in heparinized micro-hematocrit tubes.[4] The tubes were then centrifuged for 5 min at 4000 g, then broken at the cell-plasma interface. A 1 ml syringe fitted with a 26-gauge needle was used to expel the plasma into the first well of a U-bottom microtiter plate.[5] The plate was covered and placed in a 56 C water bath for 30 min to inactivate the plasma. Serial two-fold dilutions were then made by delivering plasma aliquots of 0.01 ml via an Eppendorf pipette[6] to subsequent wells, which contained 0.01 ml of Eagle-FBS; 0.01 ml was discarded from the last well. An equal volume of challenge virus, pre-titrated and adjusted to 100 TCID/0.01 ml, was added to each well, and allowed to react for 1 h at 22 C.

Chamber-slide assay

The medium was removed from the eight-chamber slides with confluent cell cultures, and 0.01 ml portions of successive plasma-virus mixtures were added to the chambers. After 5 min, 0.4 ml of Eagle's medium (3% FBS) was added. The cultures then were incubated for 48 h at 34 C in a moist, 5% CO_2 atmosphere. After incubation the chambers were detached, and the slides were washed in physiological saline, air-dried, and acetone-fixed for 30 min at -20 C. The fluorescent antibody test then was performed and results interpreted as previously described.[2,3]

RESULTS

The rabies antibody titers of fox sera tested by an established in vitro technique[3] and fox plasmas tested by the procedure described above were in agreement for all samples tested (Table 1).

TABLE 1. Rabies neutralizing antibody titers in foxes, determined from serum samples tested by an established in vitro technique[3] and from plasmas tested by the micro procedure.

Fox number	Serum titer (5-fold dilutions)	Plasma titer (2-fold dilutions)
1	none	none
2	none	none
3	none	none
4	none	none
5	1:25	1:32
6	1:25	1:32
7	1:125	1:128
8	>1:125	>1:128
9	1:125	1:128
10	1:125	1:128
11	1:125	1:128
12	1:125	1:128

[4] Clay Adams, Division of Becton, Dickinson and Co., Parsippany, New Jersey.
[5] Cooke Engineering Co., Alexandria, Virginia.
[6] Bio-Rad Laboratories, Rockville Center, New York.

DISCUSSION

The plasma assay technique has been used routinely in this laboratory to measure neutralizing antibody levels in small laboratory rodents. It also has been used to demonstrate naturally occurring antibodies in populations of the big brown bat, *Eptesicus fuscus*. A small incision in the leg of a bat, or in the tail-tip of a small rodent, provides sufficient blood for collection in micro-hematocrit tubes. This causes minimal trauma to animals that could not survive bleedings of large volumes. As this study with foxes demonstrates, it also is applicable for sampling larger animal species.

The micro-hematocrit tube is quite convenient for storage. Since the plasma/cell interface is not disrupted by a freeze-thaw cycle, the plasma samples can be stored in the tubes at -20 C after centrifugation.

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