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The EV71 neutralizing antibody test

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1 Works for me dx.doi.org/10.17504/protocols.io.7pwhmpe

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ABSTRACT

This protocol describes the process for the neutralizing antibody test of EV71.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Lee J, Yen T, Shih W, Lu C, Liu D, Huang Y, Chang L, Huang L, Lin T (2019) Enterovirus 71 seroepidemiology in Taiwan in 2017 and comparison of those rates in 1997, 1999 and 2007. PLoS ONE 14(10): e0224110. doi: 10.1371/journal.pone.0224110

GUIDELINES

The serum neutralizing antibody response is the major indicator of EV71 protective immunity. The neutralizing antibody test of EV71 followed the standard protocol of a neutralization test. Serum samples were heat-treated for 30 minutes at 56°C, serially diluted and mixed with 100 50% tissue culture-infective doses (TCID50) of the EV71 TW/2272/98 strain (GenBank accession number AF119795), and incubated for 2 hours at 37°C. Thereafter, rhabdomyosarcoma cells were added into each reaction well and incubated at 37°C in 5% CO_2 incubator. Each plate included a cell control, serum control, and virus back-titration. The cytopathic effect was monitored from 5 to 6days after incubation, and the serotiter was determined when the cytopathic effect was observed in one TCID50 of the virus back-titration. Seropositivity was defined as a serotiter ≥8.

MATERIALS TEXT

Serum or plasma

96 well microtiterplate

1x PBS

DMEM with 2% FBS medium

Multichannel Pipette (8 or 12)

1.5 ml eppendrof

EV71 TW/2272/98 strain (GenBank accession number AF119795)

SAFETY WARNINGS

EV71 needs to operate in BSL-2 lab

BEFORE STARTING

Set water bath to 56℃

Confirm that the RD cells are healthy

1 Inactivate serum (300 µl) at 56℃ for 30 min

Sample volumn (µl)	1x PBS (µl)	Dilute factor
Ex. 75	225	1:4

2 Make 2 fold serial dilution of serum from 1:4 to 1:256 with 2% FBS DMEM

sample	1-1	1-2	2-1	2-2	3-1	3-2	4-1	4-2	5-1	5-2	6-1	6-2
A 1:4	●	●	○	○	○	○	○	○	○	○	○	○
B 1:8	●	●	○	○	○	○	○	○	○	○	○	○
C 1:16	○	○	○	○	○	○	○	○	○	○	○	○
D 1:32	○	○	○	○	○	○	○	○	○	○	○	○
E 1:64	○	○	○	○	○	○	○	○	○	○	○	○
F 1:128	○	○	○	○	○	○	○	○	○	○	○	○
G 1:256	○	○	○	○	○	○	○	○	○	○	○	○
H control	●	○	○	○	○	○	○	○	○	○	○	○

a. Add 50 µl 2% FBS DMEM in well B to H

b. ● means add 50µl serum sample

c. After dilution, every well has 50µl sample

d. ex. sample1-1 well H: serum control; sample 1-2 well H: cell control; 1-1 and 1-2 are duplicate

3 Add 50 µl of adjusted virus suspension (100TCID50EV71) onto the well A to well G of microtiterplate, shake reaction well slightly

4 Prepare control wells including virus control, serum control and cell control.

Virus control well：back titration from 100, 10, 1 to 1/10 100$TCID_{50}$

Serum control：add serum and cell only, no virus

Cell control：add cell only, no serum and no virus

5 Incubate above reaction wells at 37℃for 2 hours in 5% CO_2 incubator

6 Prepare RD cells suspension and adjust cell concentration to 1×10^5/ml

7 Add 100µl of adjusted RD cell into each reaction well after 2 hours in step5

8 Incubate wells at 37°Cin 5% CO_2 incubator for 5-6 days until virus control well reach 100$TCID_{50}$

9 Observe CPE in each well under reverse microscope after 5-6 days incubation

10 Check CPE in control wells

Virus control well：100% CPE in 100$TCID_{50}$ and 10$TCID_{50}$; 50% CPE in 1$TCID_{50}$, < 10% CPE in 1/10$TCID_{50}$

Serum control well：no CPE

Cell control well：no CPE

11 The NT titer was calculated as the reciprocal value of the last dilution of the antiserum that completely inhibited the CPE after incubation.

12 Seropositivity was defined as a reciprocal of the serotiter≧ 8.