Supplement file 1

Microneutralization assay (micro-NT)

TBEV strain Neudörfl (National Collection of Pathogenic Viruses, United Kingdom; Cat. No 0201139v) was cultured in monolayer of BHK-21/C13 (BS CL 8, Istituto Zooprofilattico Sperimentale Brescia, Italy) in BSL2+ laboratory of Pasteur Institute Novi Sad. Virus stocks were prepared in concentration of 100 Tissue Culture Infectious Dose (TCID)/100 µl and stored at -80^{O} C until further use.

The micro-NT was performed in 96-well cell culture plate (Thermo Scientific™ , Massachusetts, United States, Cat. no 130338). After sample inactivation at 56 ^{O}C for 30 min, serum and cerebrospinal fluid samples were tested in duplicate, diluted in Glasgow Minimal Essential Medium (Biowest, France; Cat. No P0120) in serial dilutions of 1:5 to 1:640.

In every test run, defined positive and negative control were added together with a cell control and a virus back-titration. A total of 100 TCID of virus stock was added to the respective serum dilutions and incubated for one hour at 37 ^{O}C. Subsequently, serum-virus/CSF-virus mixture was transferred to wells with previously seeded BHK21/C13 2x10^{4} cells and incubated for five days at 37 ^{O}C in atmosphere with 5% CO2. For each sample, cytopathic effect (CPE) in both wells was observed (Figure 1). The sample dilution resulting in virus neutralization in 50% of the replicates (NT50) was calculated using the method of Spearman and Karber. Serum/CSF sample with ≥ 1:10 NT50 for neutralization assay was interpreted as a positive result.

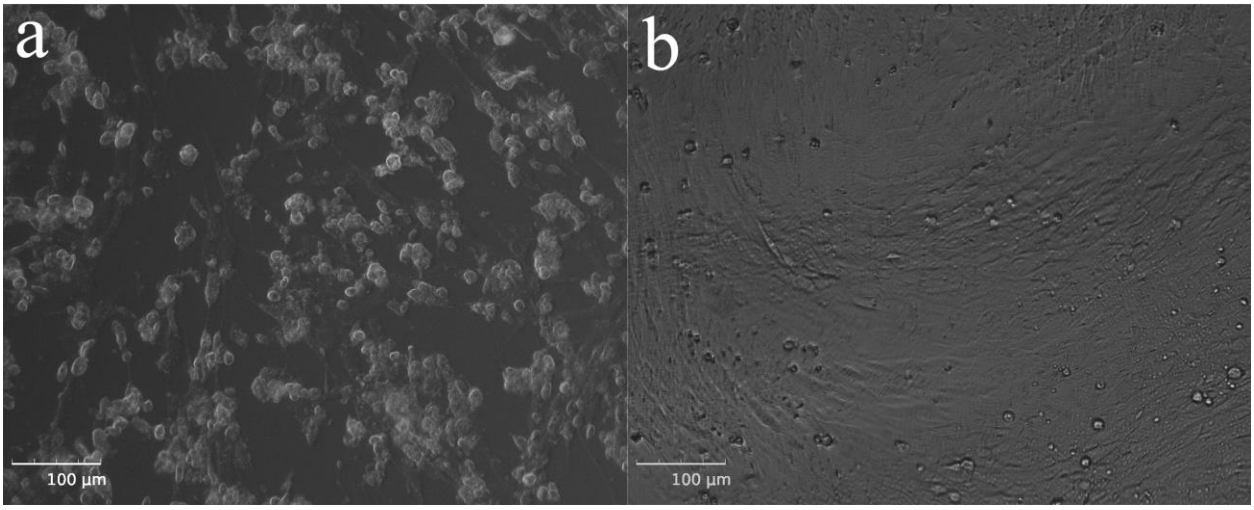

Figure 1.Micro-NT assay results. a) CPE detected in BHK-21/C13 cell line infected with TBEV; b) Absence of CPE in BHK-21/C13 cell line monolayer due to virus neutralization