UDC 619:578.231.31:636.22/.28:57.082.26

DOI: 10.29326/2304-196X-2019-4-31-31-36

# CULTURAL PROPERTIES
## OF BOVINE RESPIRATORY SYNCYTIAL VIRUS STRAIN AB 1908

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**SUMMARY**

Cattle respiratory diseases are some of the most spread pathologies that can cause economic damage, resulting from financial losses and costs of treatment and diagnostics. One of the major factors contributing to respiratory pathology development is bovine respiratory syncytial infection. The analysis of serological testing, performed by the FGBI "ARRIAH" Reference Laboratory for Cattle Diseases in 2017–2018, showed that respiratory syncytial virus seroprevalence in animals of dairy farms is 60%. Herewith, it was noted that the most susceptible animals to this infection are calves under one year of age. The effectiveness of bovine respiratory syncytial infection control measures depends on timely diagnosis; that is why reliable and accurate diagnostic tools are needed, including optimal techniques of virus isolation from pathological material. For successful virus isolation from clinical samples, it is necessary to adhere strictly to optimal parameters of this agent cultivation. This paper presents data on study of bovine respiratory syncytial virus strain AB 1908 cultural properties. The tests performed showed that a continuous bovine turbinate (BT) cell line, continuous bovine fetal trachea (FBT) cell line and continuous bovine calf kidney (RBT) cell line are sensitive for cultivation of this agent and can be used to prepare viral suspension, needed for further research. Virus titre in BT cell culture was $4.33 \pm 0.16 - 4.66 \pm 0.12$ lg $TCID_{50}/cm^3$, in RBT cell culture – $4.33 \pm 0.33 - 4.70 \pm 0.36$ lg $TCID_{50}/cm^3$ and in FBT cell culture – $4.13 \pm 0.20 - 4.78 \pm 0.17$ lg $TCID_{50}/cm^3$. The following virus cultivation optimal parameters were also determined during this study: the age of the culture for virus inoculation should be 1–2 days and multiplicity of inoculation should be 0.1 $TCID_{50}$/cell.

Key words: bovine respiratory syncytial virus, AB 1908 strain, cell culture, cultivation, virus titre, infectivity.

## INTRODUCTION

Cattle respiratory diseases form the major part of economic damage associated with the diseased animals' growth rate reduction and costs of treatment, diagnostic and prevention measures [4]. One of the leading positions is taken by bovine respiratory syncytial virus infection.

Infection with bovine respiratory syncytial virus (BRSV) is a contagious respiratory disease causing great damages to the livestock industry all over the world, including Russia. BRSV infection manifests as catarrhal bronchopneumonia, mucoid nasal discharge, general depression and loss of appetite. Calves during their first year of live are the most susceptible to the disease [1, 6, 7]. According to the official Rosselkhoznadzor web-publications BRSV seroprevalence in animals amounted to 78.9% of the total number of tested samples. In 2017–2018, BRSV circulation was reported on those dairy farms, where vaccination is not practiced. Test results obtained by the Reference Laboratory for Cattle Diseases of the FGBI "ARRIAH" demonstrated that on such a farm the number of positives amounted to 60%. In view of the above it may be concluded that of topical significance are examination of BRSV biological properties and development of the disease diagnostic and prevention tools.

Effectiveness of BRSV control measures depends on diagnosis accuracy and expediency. The diagnosis is set basing on the epidemic data, clinical signs and post-mortem examination, and it is confirmed by the laboratory tests. During BRSV diagnosis such bovine disorders as infectious rhinotracheitis (IR), bovine viral diarrhea (BVD), parainfluenza-3 (PI-3), rotavirus and coronavirus infections should be excluded [6, 7]. Enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and virus isolation in cell cultures are used for BRSV diagnosis.

Until now, the key method for BRSV diagnosis in Russia has involved the virus isolation from biological samples in cell culture and its subsequent identification using

virus neutralization test (VNT). According to the domestic publications, such cell cultures as lung diploid cells ($L_5E$) and bovine fetal kidney ($K_5E$), continuous fetal lamb kidney (FLK), calf kidney (C-1, CK) and primarily trypsinized bovine fetal lung cell cultures (BFL) are believed to be BRSV-sensitive [3, 5]. BRSV is highly difficult to isolate due to its extreme lability, and this is possible only during the early stage of the infection; the chance of the virus isolation decreases with the onset of the disease signs. Due to complicated isolation and cultivation, production of sufficient amount of the highly infective virus-containing material is also problematic.

Therefore, of topical significance is examination of cultural properties of the agent for further selection of the most advanced approaches to the design of the disease diagnostic and prevention tools.

**Table 1**
**Replication of BRSV strain AB 1908 in cell cultures** ($n = 3$)

| Cell culture | Passage No. | Cultivation period, days | Infectivity titre, lg $TCID_{50}/cm^3$ | Virus genome determined using RT-PCR |
|---|---|---|---|---|
| BT | 1 | 10 | 4.33 ± 0.16 | + |
| | 2 | 7 | 4.63 ± 0.13 | + |
| | 3 | 5–6 | 4.66 ± 0.12 | + |
| | 4 | 5 | 4.66 ± 0.12 | + |
| | 5 | 5 | 4.50 ± 0.25 | + |
| RBT | 1 | 10 | 4.33 ± 0.33 | + |
| | 2 | 7 | 4.36 ± 0.11 | + |
| | 3 | 5–6 | 4.66 ± 0.33 | + |
| | 4 | 5 | 4.70 ± 0.36 | + |
| | 5 | 6 | 4.66 ± 0.33 | + |
| FBT | 1 | 10 | 4.25 ± 0.10 | + |
| | 2 | 7 | 4.33 ± 0.80 | + |
| | 3 | 5–6 | 4.78 ± 0.05 | + |
| | 4 | 5 | 4.13 ± 0.20 | + |
| | 5 | 6 | 4.78 ± 0.17 | + |
| PB | 1 | 7 | 2.68 ± 0.13 | + |
| | 2 | 7 | 2.05 ± 0.14 | + |
| | 3 | 7 | 1.67 ± 0.10 | + |
| | 4 | 7 | n/d | + |
| | 5 | 7 | n/d | – |
| FBN | 1 | 7 | 1.26 ± 0.03 | + |
| | 2 | 7 | 1.13 ± 0.08 | + |
| | 3 | 7 | n/d | – |
| MA-104 | 1 | 7 | 1.05 ± 0.30 | + |
| | 2 | 7 | n/d | – |

n/d – not detected.

The work was aimed at the examination of cultural properties of BRSV strain AB 1908 and degree of its accumulation in different cultivation systems.

## MATERIALS AND METHODS

Strain AB 1908 adapted to the continuous bovine turbinate cell line (BT, ATCC, USA) was used in the experiment.

The following cell cultures were chosen for the experiment: continuous fetal bovine nasal turbinate epithelial cell line (FBN), continuous bovine turbinate epithelial cell line (BT), continuous African green monkey kidney cell line (MA-104), continuous bovine calf kidney cell line (RBT) [2], continuous fetal bovine trachea cell line (FBT), primarily trypsinized ovine kidney cell line (PB). The cell line sensitivity to BRSV was determined by successive passages. To this effect, the degeneration-free and Hanks' solution pre-washed monolayer cell culture was inoculated with cell-adsorbed BRSV. After 60-minute contact of the virus with the cells the maintenance semi synthetic medium supplemented with 10% bovine serum was added and cultivated at (37 ± 0.5) °C. The infected culture was daily microscopically examined for typical morphological changes in the cells.

BRSV infectivity was determined in BT cell culture by routine microtitration test. Final reading of the virus titration results was performed after 6–7 days of incubation provided the monolayer integrity in the control wells was preserved.

The infectivity was calculated according to Reed and Muench method and expressed as lg $TCID_{50}/cm^3$. BRSV genome was determined using reverse transcription polymerase chain reaction (RT-PCR) and RiboSorb test-kit (AmpliSens, Russia).

## RESULTS AND DISCUSSION

Five successive passages of BRSV strain AB 1908 in various cell cultures were performed for the examination of its cultural properties. The results are demonstrated in Table 1.

The data shown in Table 1 indicate that levels of BRSV strain AB 1908 accumulation in different cell cultures varied.

The virus cultivation in PB and FBN cell cultures resulted in gradual decrease of the virus activity and after 2–3 passages the virus titre ranged from 2.68 ± 0.13 to 1.13 ± 0.08 lg $TCID_{50}/cm^3$. The cultivation period amounted to 7 days.

During the virus cultivation in MA-104 cell culture the minimal virus accumulation was observed only during the first passage (1.05 ± 0.30 lg $TCID_{50}/cm^3$). Hereafter, the virus was not detected.

High accumulation of BRSV strain AB 1908 was reported in BT, RBT and FBT cells starting for the first passage. The virus titre in the cultures ranged within 4.33 ± 0.16 – 4.66 ± 0.12, 4.33 ± 0.33 – 4.7 ± 0.36 and 4.13 ± 0.20 – 4.78 ± 0.17 lg $TCID_{50}/cm^3$, respectively.

During the examinations, the BRSV cytopathic effect (CPE) was noted to be different in different cell cultures (Fig. 1–6). So far, for example, in BT cell-culture by passage 5 in 2–3 days of cultivation the cell rounding was observed, by day 4–5 – cellular membrane fusion and by day 5–6 – syncytium formation, fragmentation, monolayer disruption and cell detaching from the glass (Fig. 1–2).

Similar virus CPEs were reported in FBT and BT cell cultures: i.e. formation of indistinct symplasts and conglomerates (Fig. 3–6). In these cell lines, by passage 5 in

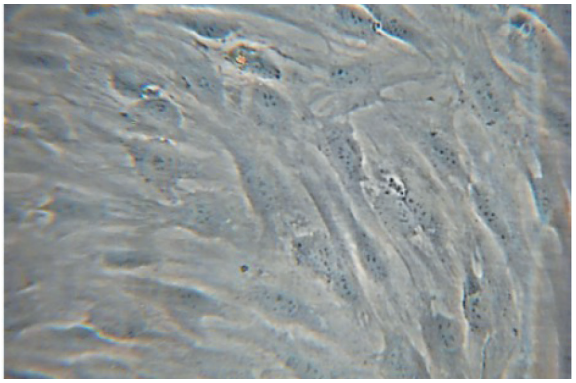

*Fig. 1. Monolayer of non-infected BT cell-culture on day 6 (×400)*

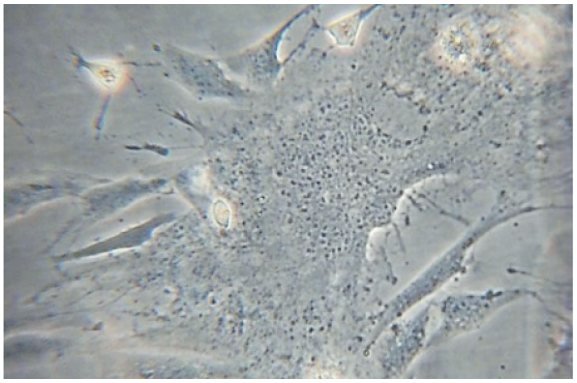

*Fig. 2. Cells of BT monolayer after BSRV infection, 6 dpi (×400)*

2–3 days of cultivation deformation of individual cells was reported, by day 3–5 – formation of well-expressed localized cell lesions, by day 6–7 – formation of symplasts and conglomerates.

It should be noted that out of all tested cell cultures the most suitable ones for BRSV replication turned out to be BT, FBT and RBT cell systems, which are of homologous origin. BT and FBT cell cultures originate from respiratory system tissues and they are clearly permissive. RBT cell cultures produced from bovine kidney were also found to be sensitive to the virus under study.

The next stage involved determination of BT, FBT and RBT seeding density most appropriate for the maximal virus replication (Table 2). The studied cell seeding density ranged from 100 to 400 ths/cm$^3$. The cell cultures were cultivated for 1 day at (37 ± 0.5) °C and multiplicity of infection (MOI) amounted to 0.1 $TCID_{50}$/cell. The virus activity was determined by the cell lysis in the test culture suspension and by titration in BT cell culture. The results are demonstrated in Table 2.

The data in Table 2 demonstrate that the maximal virus accumulation in BT cell culture was reported at seeding density 100–200 ths/cm$^3$. The virus titre in this culture amounted to 4.25 ± 0.44 lg $TCID_{50}$/cm$^3$. The highest virus activity in RBT and FBT was reported at seeding density 200–300 ths/cm$^3$: 3.78 ± 0.13–4.25 ± 0.25 and 3.62 ± 0.13–4.54 ± 0.24 lg $TCID_{50}$/cm$^3$, respectively.

Therefore, optimal seeding density for BT cells is 100 ths/cm$^3$, for RBT and FBT – 200–300 ths/cm$^3$.

During the next stage, effect of the cell culture age on BRSV AB 1908 infectivity was determined. Cell cultures demonstrating the highest virus accumulation levels were

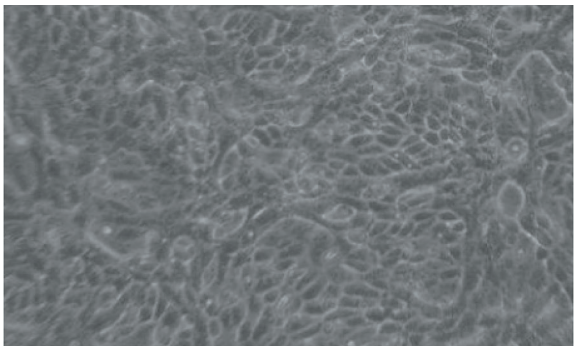

*Fig. 3. Monolayer of non-infected RBT cell culture on day 6 (×400)*

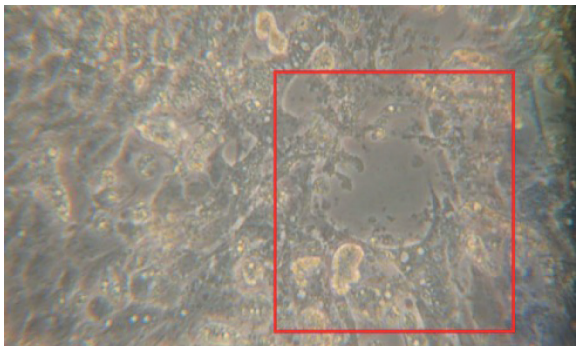

*Fig. 4. RBT cell monolayer after BSRV inoculation, 6 dpi (×400)*

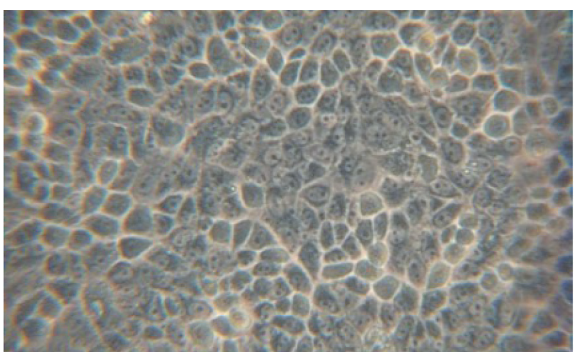

*Fig. 5. Monolayer of non-infected FBT-cell culture on day 6 (×400)*

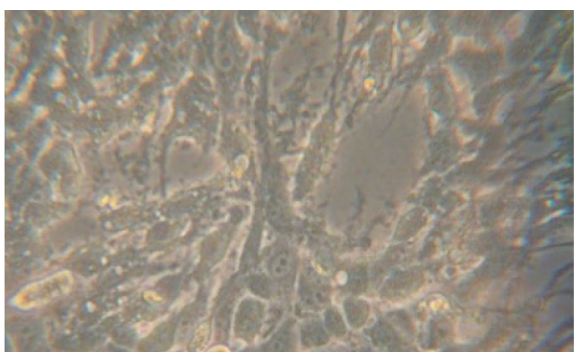

*Fig. 6. FBT cell monolayer after BSRV inoculation, 6 dpi (×400)*

**Table 2**
**Effect of BT, FBT and RBT seeding density on BRSV AB 1908 replication** ($n = 3$)

| Cell seeding density, ths/cm³ | Cultivation period, day | Virus titre, lg $TCID_{50}$/cm³ | | |
|---|---|---|---|---|
| | | BT | FBT | RBT |
| 100 | 6 | 4.25 ± 0.44 | 3.33 ± 0.23 | 3.33 ± 0.33 |
| 200 | 5 | 4.25 ± 0.25 | 3.62 ± 0.13 | 3.78 ± 0.13 |
| 300 | 5 | 3.78 ± 0.33 | 4.54 ± 0.24 | 4.25 ± 0.25 |
| 400 | 6 | 2.70 ± 0.10 | 3.33 ± 0.30 | 3.16 ± 0.16 |

selected (BT, RBT, FBT). Results of the studies are demonstrated in Table 3.

Table 3 demonstrates that in 1–2-day-old BT, RBT and FBT cell monolayer BRSV AB 1908 replicated at 4.75 ± 0.06; 4.50 ± 0.25 and 4.63 ± 0.05 lg $TCID_{50}$/cm³, respectively. Moreover, in case the virus was inoculated in 3–4-day-old cell cultures the virus replication decreased and averaged to 3.66 ± 0.29 (BT); 3.60 ± 0.16 (RBT) and 3.53 ± 0.13 lg $TCID_{50}$/cm³ (FBT). Decrease of the virus infectivity can be associated with slower metabolic processes between the cells and environment, which are more active at the initial stage of the monolayer growth. Thus, the most optimal BT, RBT and FBT cell age for the virus inoculation is 1–2 days.

While studying the virus cultural properties, determination of the virus MOI is of great importance. Therefore, the next stage included determination of the MOI impact on the cytopathic effect of the virus in BT, RBT and FBT cell cultures. Infection doses of 0.001; 0.01 and 0.1 $TCID_{50}$/cell were used for that purpose. The virus incubation was stopped at 90% cell destruction and detachment from the slide. Cytopathic effect was tested in BT cell culture using microtitration method. Results are demonstrated in Table 4.

Table 4 demonstrates that at MOI 0.1 $TCID_{50}$/cell the virus's cytopathic effect of the virus amounted to 4.33 ± 0.10 $TCID_{50}$/cell in BT cell culture; 4.54 ± 0.10 $TCID_{50}$/cell in RBT cell culture; 4.33 ± 0.33 $TCID_{50}$/cell in FBT cell culture. With lower infection doses (0.01 and 0.001 $TCID_{50}$/cell) the virus infectivity decreased and amounted to 3.16 ± 0.16 – 4.16 ± 0.16 lg $TCID_{50}$/cm³ and time point of CPE development delayed from 6 to 10 days.

Therefore, optimal dose of the virus for the cells' infection amounted to 0.1 $TCID_{50}$/cell. Cell infection with low virus concentration (0.001 and 0.01 $TCID_{50}$/cell) slowed down the virus replication process and reduced its cytopathic effect.

During the next stage, effect of the period of cultivation on BRSV replication was studied. Day-old BT, FBT and RBT cell cultures were used in the study. The virus was cultivated for 1–6 days. The virus replication rate was determined by its CPE on the cells; its activity was assessed in BT cell culture using titration method.

It was determined that the highest virus harvest in BT, FBT and RBT cell cultures was reported following its replication for 4–6 days as evidenced by the data demonstrated in Table 5. Maximal virus accumulation level, however, ranged from 3.33 ± 0.23 to 4.75 ± 0.25 lg $TCID_{50}$/cm³.

**Table 3**
**Effect of cell culture age on BRSV strain AB 1908 infectivity** ($n = 3$)

| Cell culture age, days | Period of cultivation, days | Virus titre, lg $NCID_{50}$/cm³ | | |
|---|---|---|---|---|
| | | BT | RBT | FBT |
| 1 | 6 | 4.75 ± 0.06 | 4.25 ± 0.11 | 4.50 ± 0.17 |
| 2 | 7 | 4.25 ± 0.11 | 4.50 ± 0.25 | 4.63 ± 0.05 |
| 3 | 10 | 4.00 ± 0.25 | 4.00 ± 0.22 | 3.83 ± 0.15 |
| 4 | 10 | 3.33 ± 0.33 | 3.21 ± 0.11 | 3.23 ± 0.11 |

**Table 4**
**MOI effect on BRSV strain AB 1908 activity** ($n = 3$)

| Infection dose, $TCID_{50}$/cell | Period of cultivation, days | Virus titre, lg $TCID_{50}$/cm³ | | |
|---|---|---|---|---|
| | | BT | RBT | FBT |
| 0.001 | 7–10 | 3.63 ± 0.20 | 3.33 ± 0.30 | 3.16 ± 0.16 |
| 0.01 | 6–8 | 4.16 ± 0.16 | 4.00 ± 0.25 | 4.00 ± 0.19 |
| 0.1 | 5–6 | 4.33 ± 0.10 | 4.54 ± 0.10 | 4.33 ± 0.33 |

**Table 5**
**Effect of cultivation period on BRSV strain AB 1908 replication** ($n = 3$)

| Cultivation period, days | Age of culture, days | Virus titre, lg $TCID_{50}/cm^3$ | | |
|---|---|---|---|---|
| | | BT | FBT | RBT |
| 1 | 1 | 2.23 ± 0.10 | 2.00 ± 0.16 | 2.16 ± 0.16 |
| 2 | | 2.54 ± 0.08 | 2.23 ± 0.10 | 2.22 ± 0.11 |
| 3 | | 2.70 ± 0.10 | 2.33 ± 0.33 | 2.54 ± 0.24 |
| 4 | | 3.78 ± 0.33 | 3.33 ± 0.23 | 3.33 ± 0.33 |
| 5 | | 4.25 ± 0.44 | 3.62 ± 0.13 | 3.78 ± 0.13 |
| 6 | | 4.75 ± 0.25 | 4.54 ± 0.24 | 4.25 ± 0.25 |

After virus cultivation for 1–3 days its titer varied from 2.00 ± 0.16 to 2.70 ± 0.10 lg $TCID_{50}/cm^3$.

To produce the test samples of BRSV cultural virus three successive passages of experimental BRSV samples were made according to the optimized cultivation procedure: cell culture – BT, FBT or RBT, age of culture – 1 day; inoculation concentration – 100 ths cells/$cm^3$ for BT, 200–300 ths cells/$cm^3$ for FBT and RBT; MOI – 0.1 $TCID_{50}$/cell; time point of the virus harvesting – day 6.

During passage 1 in BT cell culture, the virus CPE was manifested as cell rounding by day 3–4 of cultivation, membrane fusion along with symplast formation were reported by day 4–6 and conglomerate and syncytium formation was observed by day 6–7. By day 6, the monolayer disintegration and partial cell flaking with suspension formation were reported in FBT and RBT cell cultures. Herewith, infectivity titre amounted to 3.78 ± 0.33; 3.33 ± 0.13 and 3.63 ± 0.33 lg $TCID_{50}/см^3$ for BT, FBT and RBT cell cultures, respectively. During passage 2, the virus CPE remained nearly the same. By passage 3, however, the formation of conglomerates and syncytia was reported in all tested cell cultures by day 4–6. The results are demonstrated in Table 6.

Therefore, the studied cell cultures are biological systems sensitive to BRSV strain AB 1908; the virus titre in BT cell culture amounted to 3.78 ± 0.33 – 4.75 ± 0.06 lg $TCID_{50}/cm^3$, in RBT cell culture – 3.63 ± 0.33 – 4.65 ± 0.25 lg $TCID_{50}/cm^3$ and in FBT cell culture – 3.33 ± 0.13 – 4.67 ± 0.17 lg $TCID_{50}/cm^3$.

## CONCLUSION

Studies of the cultural properties of BRSV strain AB 1908 demonstrated that sensitive biological systems for its replication included BT, RBT and FBT cell cultures being of homologous origin. MA-104, PB and FBN cell cultures proved unsuitable for BRSV strain AB 1908 replication. In BT cell culture the virus accumulation ranged from 4.33 ± 0.16 to 4.66 ± 0.12 lg $TCID_{50}/cm^3$, in RBT cell culture – from 4.33 ± 0.33 to 4.70 ± 0.36 lg $TCID_{50}/cm^3$ and in FBT cell culture – from 4.13 ± 0.20 to 4.78 ± 0.17 lg $TCID_{50}/cm^3$. Optimal conditions for BRSV strain AB 1908 cultivation in the studied cell cultures included the following: inoculation concentration 100 ths/$cm^3$ – for BT cell culture, 200–300 ths/$cm^3$ – for FBT and RBT cell cultures; cell culture age – 1–2 days, multiplicity of infection – 0.1 $TCID_{50}$/cell, virus material collection time – day 6–7 of cultivation.

***Conflict of interest.*** The authors declare no conflict of interest.

**Table 6**
**Infectivity of BRSV strain AB 1908 during various passages** ($n = 3$)

| Passage | Cell culture | CPE time point, day | Infectivity, lg $TCID_{50}/cm^3$ |
|---|---|---|---|
| 1 | BT | 4 | 3.78 ± 0.33 |
| | FBT | 5 | 3.33 ± 0.13 |
| | RBT | 5 | 3.63 ± 0.33 |
| 2 | BT | 4 | 4.00 ± 0.25 |
| | FBT | 5 | 3.63 ± 0.12 |
| | RBT | 5 | 3.75 ± 0.25 |
| 3 | BT | 4 | 4.75 ± 0.06 |
| | FBT | 6 | 4.67 ± 0.17 |
| | RBT | 6 | 4.65 ± 0.25 |

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*Submitted on 22.08.19*
*Approved for publication on 09.12.19*