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# CERTIFICATE OF ANALYSIS

Product: **Biocan Novel DHPPi/L4R vakcina A.U.V.**

Batch No.: **02 52 22**
Expiry: 19.11.2016
Date of production: 20.11.2014

**Results of analysis:**

| Tests | Release limits | Shelf-life limits | Results |
|---|---|---|---|
| **Freeze-dried fraction** | | | |
| Appearance | Spongy matter, white colour. | | conforms |
| Residual humidity | 0.5 - 3 % | | 2.20 % |
| Vacuum | Residual pressure must be lower than 2.66 Pa. | | conforms |
| Sterility | Vaccine must be sterile. | | sterile |
| Mycoplasma | Vaccine must be free from *mycoplasma*. | | conforms |
| Extraneous agents | Free from extraneous agents. | | conforms |
| Virus titre | Function: To determine the virus titre (potency) of each CAV-2, CDV, CPiV-2 and CPV-2b antigen in the finished product. | | conforms |
| CDV | min. $10^{4.0}$ $TCID_{50}$ - max. $10^{8.1}$ $TCID_{50}$ | min. $10^{4.3}$ $TCID_{50}$ - max. $10^{8.1}$ $TCID_{50}$ | $10^{7.1}$ $TCID_{50}$ |
| CPV | min. $10^{5.5}$ $TCID_{50}$ - max. $10^{8.6}$ $TCID_{50}$ | min. $10^{6.3}$ $TCID_{50}$ - max. $10^{8.6}$ $TCID_{50}$ | $10^{8.1}$ $TCID_{50}$ |
| CAV-2 | min. $10^{4.7}$ $TCID_{50}$ - max. $10^{7.1}$ $TCID_{50}$ | min. $10^{5.8}$ $TCID_{50}$ - max. $10^{8.1}$ $TCID_{50}$ | $10^{7.1}$ $TCID_{50}$ |
| CPiV-2 | min. $10^{3.8}$ $TCID_{50}$ - max. $10^{8.1}$ $TCID_{50}$ | min. $10^{5.1}$ $TCID_{50}$ - max. $10^{8.1}$ $TCID_{50}$ | $10^{7.8}$ $TCID_{50}$ |
| Identity | Function: The test is performed to confirm presence of each CAV-2, CDV, CPiV-2 and CPV antigen in the finished product. | | conforms |
| CDV | Neutralization index has to be equal or higher than 2 $log_{10}$ and/or specific immunofluorescence has to be seen in the cell culture infected with CDV. | | 4.1 |
| CAV-2 | Neutralization index has to be equal or higher than 2 $log_{10}$ and/or specific immunofluorescence has to be seen in the cell culture infected with CAV-2. | | 4.3 |
| CPV-2b | The CPV type must be CPV 2a, b, c group. | | conforms |
| CPiV-2 | Neutralization index has to be equal or higher than 2 $log_{10}$ and/or specific immunofluorescence has to be seen in the cell culture infected with CPiV. | | 4.8 |
| **Liquid fraction** | | | |
| Appearance | Pink liquid with easily shakeable sediment. | | conforms |
| Extractable volume | Minimum 1 ml | | 1.08 ml |
| Airtightness | The glass vial must be hermetically sealed. | | conforms |
| Aluminium content * | 2 mg/ml (1.8 – 2.2 mg/ml) | | 2.04 mg/ml |
| Sterility | Vaccine must be sterile. | | sterile |
| *Leptospira* inactivation * | No live *Leptospira*. | | conforms |
| Rabies inactivation * | No live rabies virus must be detected. | | conforms |
| Formaldehyde detection * | The formaldehyde detected has to be below 0.01%. | | 0.00 % |
| Leptospira identity | Vaccine stimulates the production of specific antibodies against each *Leptospira* serovar. | | conforms |
| L. Icterohaemorrhagiae | Minimum of 1:64 ALR geometric mean titre, for each *Leptospira* serovar. | Minimum of 1:51 ALR geometric mean titre for L. Icterohaemorrhagiae. | 1:72 |
| L. Canicola | | Minimum of 1:51 ALR geometric mean titre for L. Canicola. | 1:144 |
| L. Bratislava | | Minimum of 1:51 ALR geometric mean titre for L. Bratislava. | 1:228 |
| L. Grippotyphosa | | Minimum of 1:40 ALR geometric mean titre for L. Grippotyphosa. | 1:72 |
| Rabies identity / potency (serology in mice) | The vaccine stimulates the production of specific neutralizing antibodies. The serology response after vaccination has to be significantly higher in comparison to a serology response of standard 2 IU vaccine. | | conforms |
| **Reconstituted Vaccine** | | | |
| Appearance | Pink-red or yellowish colour with light opalescence. | | conforms |
| pH | 6.8 – 7.8 | | 7.57 |

* tests carried out during manufacturing proces of liquid fraction

I certify that this product has been tested in the laboratories of Bioveta a.s. and has been found to meet specification requirements.
Preparation is suitable with control regulations of the valid PN No. 464.

12/05/2015
Mgr. Hana Nezvalová
HEAD of QC, QP