

Institute of Experimental Immunology Department of Pathology Prof. Dr. Christian Münz		
Date 010615	Standard Operating Procedure Influenza virus infection	Pages 1/1

Influenza virus infection via intranasal route using inhalation anesthesia

1. Thaw viral stocks on ice.
2. Adjust volume of planned viral dose to 25µl with cold sterile PBS. 0.1-100 HAU of PR8/34 influenza A (H1N1) virus will be used per infection.
3. Anesthetize animal with isofluorane (see SOP Anesthesia)
4. Fix animals by grabbing in the neck and pulling back the fur.
5. Apply virus suspension with Finnpiquet into nostrils, distribute equal volumes into each of both nostrils.

Influenza virus infection via intranasal route using injection anesthesia

6. Thaw viral stocks on ice.
7. Dilute viral stock in cold sterile PBS to achieve appropriate infectious dose per 25-50ul of volume. Dosages applied vary between different strains but all range between 10 to 100.000 pfu (pfu=plaque forming units). Mouse adapted -strains of different subtype are used, such as H1N1 (e.g. A/Puerto Rico/8/1934, A/Wisconsin/1933, A/Netherlands/602/2009, A/Puerto Rico/8/1934 NS1-GFP), H3N2 (e.g. A/Hongkong/1/1968), and others.
8. Pre-anesthetize animal with isofluorane (see SOP Anesthesia)
9. Intraperitoneal injection of Fentanyl/Midazolam/Medetomidin or Ketamin/Xylazin (see SOP Anesthesia)
10. Place animals on heating pad to prevent hypothermia.
11. Apply virus suspension with onto nostrils. Allow uptake by normal breathing.

12. Check breathing rate for a few minutes. If aberrant, place animal in upright position to aid inhalation of fluid by gravity.
13. Antagonise anesthesia by i.p. injection of Atipamezolin/Naloxon/Flumazenil or Atipamezolin (see SOP Anesthesia) *Comment VetA: Fentanyl und Midazolam müssen zuerst antagonisiert werden (mit Naloxon und Flumazenil). Atipamezol darf erst 10 Minuten danach verabreicht werden, falls die Tiere noch nicht wach sind. Ketamin-Xylazin-Anästhesie: Atipamezol darf nicht früher als 60 Minuten nach initialer Verabreichung von Ketamin gegeben werden, um eine durch einen Ketamin-Hangover verursachte Katalepsie zu verhindern*