Supplemental Information— Approach to Estimating Salivary Titers for CoVs

Approach

Find viral titers in saliva for at least one animal + respiratory disease combination. Find viral titers for a more common sampling method (e.g. nasal turbinate) for that same animal + respiratory disease combination. Calculate the conversion from the common sampling method to salivary viral titers. Find peak viral titers from the common sampling method for all other animal + respiratory disease combinations. Calculate associated salivary viral titers.

Step 1. Find viral titers in saliva and nasal turbinates for influenza in mice. Calculate the conversion factor.

From Edenborough 2012, salivary viral titers at 48 hours post-infection are 3.25, 1.25, 3, 3.5, and 1 $\log_{10}$PFU/ml for Udorn, PR8, PR8-Udorn HA + NA, Pr8-Udorn HA, and PR8-Udorn NA, respectively. Data was presented in graphical form and values have been estimated to the nearest 0.25 $\log_{10}$PFU/ml. All values are based on an average over n = 5 animals tested. The corresponding titers in the nasal turbinates, from the same time point and for the same subtypes, are 5.5, 5, 5.5, 5.5, and 4.75 $\log_{10}$PFU/ml. Based on this, the average conversion factor of salivary viral titer per nasal turbinate viral titer is 0.447. If we include only the three transmissible strains, the average conversion factor is 0.591.

When mice were dosed with four different amounts of Udorn, measurements at 24 hours give an average conversion factor (salivary viral titer per nasal turbinate viral titer) of 0.614. When mice were pre-exposed to PBS and PR8, the conversion factors are 0.772 and 0.476, respectively, averaging to 0.624. All data points for these two experiments were based on the average over n = 3 mice and approximations were based on a graph.[1]

Due to the variation in dosing methods, variations in virus type, use of approximations, and variability in calculated conversion factors, a rounded approximation may be used for the conversion factor going forward. The conversion factor is 0.6 $\log_{10}$PFU/ml in saliva per $\log_{10}$PFU/ml in nasal turbinates. {Lower bound = 0.21, Upper bound = 0.77}

It is also assumed that a milliliter of nasal turbinate sample is equal to one gram of the tissue. Values for $\log_{10}$PFU/ml were comparable to literature values for $\log_{10}$PFU/g of influenza in the nasal turbinate tissue.[2] Therefore, the conversion factor can also be viewed as 0.6 $\log_{10}$PFU/ml in saliva per $\log_{10}$PFU/g in nasal turbinates. We can also approximate that 1 pfu = 0.7 $TCID_{50}$, based on agreement of several protocol websites. The error in the nasal turbinate ml to g conversion cannot be precisely quantified due to difference in sampling time, flu subtypes, etc. We will allow an additional +- 0.5 $\log_{10}$PFU/ml to allow for additional uncertainty in the range.

[1] Edenborough KM *et al* (2012) A mouse model for the study of contact-dependent transmission of influenza A virus and the factors that govern transmissibility. *J Virol* 86**:** 12544-12551

[2] Hatta M *et al* (2007) Growth of H5N1 influenza A viruses in the upper respiratory tracts of mice. *PLoS Pathog* 3**:** 1374-1379

Step 2. Find peak viral titers in nasal turbinate tissue for other animals and respiratory diseases.

Table S1. Nasal Turbinate Viral Titer	
Virus and Animal	**Viral Titer**
Influenza in ferrets	7 $\log_{10}$PFU/g[3,4]
SARS in nice	3 to 6 $\log_{10}$TCID$_{50}$/g[5,6] = 2.85 to 5.85 $\log_{10}$PFU/g
SARS in ferrets	2 to 4 $\log_{10}$TCID$_{50}$/g[7]= 1.85 to 3.85 $\log_{10}$PFU/g
MERS in mice	Mice and ferrets are not susceptible to MERS due to their form of the protein DPP4. They can only be used as MERS model organisms if they are transgenic (one mouse paper does this). Primates are the popular choice, including marmoset and macaques.
MERS in ferrets	See above.
Influenza in ferrets	7 $\log_{10}$PFU/g[8,9]

Step 3. Calculate associated salivary viral titers using conversion factor.

Take all values from Table S1 and multiply by 0.6 $\log_{10}$PFU/mL in saliva per $\log_{10}$PFU/g in nasal turbinate. For cells with ranges from multiple sources, the maximum is used to make a conservative estimate.

Table S2. Salivary Viral Titer	
Virus and Animal	**Viral Titer**
Influenza in mice	3.5 $\log_{10}$PFU/ml = $10^{3.5}$ PFU/ml (directly reported)
Influenza in ferrets	4.2 $\log_{10}$PFU/ml= $10^{4.2}$ PFU/ml {1.0 to 5.9 $\log_{10}$PFU/ml}
SARS in mice	3.5 $\log_{10}$PFU/ml = $10^{3.5}$ PFU/ml {0.7 to 5.0 $\log_{10}$PFU/ml}
SARS in ferrets	2.3 $\log_{10}$PFU/ml = $10^{2.3}$ PFU/ml {0.3 to 3.5 $\log_{10}$PFU/ml}

[3] Krammer F *et al* (2014) Assessment of influenza virus hemagglutinin stalk-based immunity in ferrets. *J Virol* 88**:** 3432-3442

[4] Marriott AC *et al* (2014) Low dose influenza virus challenge in the ferret leads to increased virus shedding and greater sensitivity to oseltamivir. *PLoS One* 9**:** e94090

[5] Stadler K *et al* (2005) SARS vaccine protective in mice. *Emerg Infect Dis* 11**:** 1312-1314

[6] Subbarao K *et al* (2004) Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. *J Virol* 78**:** 3572-3577

[7] Chu YK *et al* (2008) The SARS-CoV ferret model in an infection-challenge study. *Virology* 374**:** 151-163

[8] Krammer F *et al* (2014) Assessment of influenza virus hemagglutinin stalk-based immunity in ferrets. *J Virol* 88**:** 3432-3442

[9] Marriott AC *et al* (2014) Low dose influenza virus challenge in the ferret leads to increased virus shedding and greater sensitivity to oseltamivir. *PLoS One* 9**:** e94090

Step 4. Calculated salivary viral titers for MERS.

Neither salivary nor nasal turbinate viral titers are available for MERS in the animals of interest. In camels, the conversion factor is 1.33 $\log_{10}$PFU/g in nasal turbinate per $\log_{10}$PFU/g in lung.[10] Note that the anterior, medial, and posterior turbinate values were averaged to give the overall nasal turbinate titer, while the lung measurement includes right lung upper lobe only. {Lower bound = 1.2 (Medial turbinate only), Upper bound = 1.55 (Posterior turbinate only)}

Now a combined conversion factor is calculated. 0.6 $\log_{10}$PFU/mL in saliva per $\log_{10}$PFU/g in nasal turbinate x 1.33 $\log_{10}$PFU/g in nasal turbinate per $\log_{10}$PFU/g in lung = 0.8 $\log_{10}$PFU/mL in saliva per $\log_{10}$PFU/g in lung. {Lower bound = 0.21 * 1.2 = 0.25, Upper bound = 0.77 * 1.55 = 1.2}

Viral titers in the lung in a transgenic mouse model (containing humanized DPP4) reach up to 7 $\log_{10}$PFU/g with a high dose.[11]

For one rhesus macaque example, viral titers in the lung reached 5 $\log_{10}$TCID$_{50}$/g which is equal to 4.85 $\log_{10}$PFU/g.[12]

Finally, the lung to saliva viral titer conversion factor of 0.8 is multiplied in.

Table S3. Salivary Viral Titer

Virus and Animal	Viral Titer
MERS in mice	5.6 $\log_{10}$PFU/ml = $10^{5.6}$ PFU/ml {1.3 to 8.9 $\log_{10}$PFU/ml}
MERS in Rhesus macaques	3.9 $\log_{10}$PFU/ml= $10^{3.9}$ PFU/ml {0.7 to 6.3 $\log_{10}$PFU/ml}

[10] Adney DR *et al* (2014) Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. *Emerg Infect Dis* 20**:** 1999-2005

[11] Pascal KE *et al* (2015) Pre- and postexposure efficacy of fully human antibodies against Spike protein in a novel humanized mouse model of MERS-CoV infection. *Proc Natl Acad Sci U S A* 112**:** 8738-8743

[12] de Wit E *et al* (2013) Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proc Natl Acad Sci U S A* 110**:** 16598-16603