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Preparation of nasopharyngeal samples for immunofluorescence assay

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Matsuno AK, Gagliardi TB, Paula FE, Luna LKS, Jesus BLS, Stein RT, Aragon DC, Carlotti APCP, Arruda E (2019) Human coronavirus alone or in co-infection with rhinovirus C is a risk factor for severe respiratory disease and admission to the pediatric intensive care unit: A one-year study in Southeast Brazil. PLoS ONE 14(6): e0217744. doi: 10.1371/journal.pone.0217744

1 Add the nasopharyngeal sample into a conical tube.

2 Only in samples with much mucus, drop N-L-Acetyl-Cysteine (maximum 1ml) into the tube and homogenize by vortex.

3 Centrifuge the tube for 10 minutes at 4oC and 300 x g.

4 Discard the supernatant.

5 Homogenize the pellet of cells with phosphate-buffered saline solution 1x (PBS 1x).

6 Drop 5ul of diluted suspension of cells into a glass slide.

7 Let the slide inside the hood or in front of a hair dryer until the sample dries.

8 Immerge the glass slide into pure and cold acetone (fixing solution).

9 After 5 minutes, wash the slide one in PBS 1x.

10 Store the glass slides at -20oC.