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From: (b) (6)
Sent: Wed, 11 Mar 2020 06:19:13 -0400
To: NIAID Public Inquiries
Subject: Fwd: Coronavirus bioweapon production method

Sent from my iPhone

Begin forwarded message:

From: Adam Gaertner (b) (6)
Date: March 11, 2020 at 6:16:40 AM EDT
To: "Fauci, Anthony (NIH/NIAID) [E]" (b) (6) >
Subject: Coronavirus bioweapon production method

Hello Anthony,

This is how the virus was created.

Intervirion Fusion. HIV-luc(ACE2) (500 ng of p24) was mixed with 1,000 ng of p24 of HIV-gfp particles incorporating ASLV-A envelope, SARS-CoV S protein, or both envelopes in PBS at 4°C for 30 min to allow binding. Samples were raised to 37°C for 15 min to allow for conformational rearrangements. Virions were adjusted to the desired pH with 0.1 M citric acid. PBS, TPCK-trypsin (final concentration 10 µg/ml), CTSL, cathepsin B (CTSB) (final concentrations 2 µg/ml) or CTSL buffer alone was then added. Recombinant CTSL (R & D Systems) was preactivated by incubation for 15 min at 10 µg/ml in 50 mM Mes, pH 6.0, on ice. Recombinant CTSB (R & D Systems) was preactivated in 25 mM Mes, 5 mM DTT, pH 5.0, for 30 min at 25°C. After a 10-min incubation at 25°C, proteolysis was halted by the addition of 300 µl of DMEM10 containing leupeptin (25 µg/ml) and STI (75 µg/ml). Virions were then incubated at 37°C for 30 min to allow membrane fusion. 100 µl of the virion mixture was added in quadruplicate to HeLa-Tva cells pretreated for 1 h with leupeptin (20 µg/ml). The cells were spin-infected and incubated at 37°C for 5 h