# **Double the Antibiotics and Antifungals**

A review of A pneumonia outbreak associated with a new coronavirus of probable bat origin



Monky Science 21 hr ago



In 2021, I started to investigate the experimental controls for the original alleged SARS-COV-2 isolation papers. Because most of the papers do not include control information or only briefly mention control information, I had to request the control information from the authors.

As you may have read, I am involved in a court case requesting the control information from the <u>HSA</u> (you can read about it <u>here</u> and <u>here</u>). This is using FOIA requests for the organization to provide such records. However, sometimes just a friendly email to the authors of the paper works.

I reviewed <u>A pneumonia outbreak associated with a new coronavirus of probable bat</u> <u>origin</u> and had many questions for the authors.

# **Experimental Components**



### Purified

The SARS-COV-2 virus separated from other organisms (generally in a vial).

Not specified in the paper so we can assume based on their experiments that purification did not occur.

X Our use of cookies

U We use necessary cookies to make our site work. We also set performance and functionality cookies that help us make improvements by measuring traffic on our site. For more detailed information about the cookies we use, please see our privacy policy.

Universal Transport Medium also known as Viral Transport Medium. Contains the following ingredients: salt solution, FBS, Antibiotics and Antifungals.

The <u>paper</u> includes the following:

The supernatant from swabs or BALF (no pre-treatment) was added to either lysis buffer for RNA extraction or to viral transport medium for isolation of the virus

And

The viral transport medium was composed of Hank's balanced salt solution (pH 7.4) containing BSA (1%), amphotericin (15  $\mu$ g ml<sup>-1</sup>), penicillin G (100 units ml<sup>-1</sup>) and streptomycin (50  $\mu$ g ml<sup>-1</sup>)

### Antibiotics

A poison targeted at killing bacteria which can cause damage to kidneys and other tissues known as Nephrotoxicity.

From the protocol provided by Zhengli Shi (download here):

Anti-anti (Gbico, REF15240-062)

### Antifungals

A poison targeted at killing fungi that has known adverse reactions in animals.

From the protocol provided by Zhengli Shi (download <u>here</u>):

Anti-anti (Gbico, REF15240-062)

### Trypsin

#### *Trypsin is a proteolytic enzymne that breaks down proteins. The possible problem when using*

trypsin is that cell walls can be compromised distorting the physiology of cells and cellular **Our use of cookies** 

mative dise necessary cookies to make our site work. We also set performance and functionality cookies that help us make improvements by measuring traffic on our site. For more detailed information about the cookies we use,

The please see our privacy policy llowing:

The PCR-positive BALF sample from ICU-06 patient was spun at 8,000g for 15 min, filtered and diluted 1:2 with DMEM supplemented with 16  $\mu$ g ml<sup>-1</sup> trypsin before it was added to the cells. After incubation at 37 °C for 1 h, the inoculum was removed and replaced with fresh culture medium containing antibiotics (see below) and 16  $\mu$ g ml<sup>-1</sup> trypsin.

### FBS

Fetal Bovine Serum is the liquid fraction of clotted blood from fetal calves.

The <u>paper</u> includes the following:

Vero E6 and Huh7 cells, which were cultured in DMEM containing 10% FBS.

From the protocol provided by Zhengli Shi (download here):

FBS with 2% final concentration

The concentration of FBS was reduced from 10% to 2% during experimentation.

### **MNS**

*M*inimal Nutrient Solution is a liquid growth medium where the volume of the medium has been reduced below normal levels.

The <u>paper</u> includes the following:

The PCR-positive BALF sample from ICU-06 patient was spun at 8,000g for 15 min, filtered and diluted 1:2 with DMEM supplemented with 16  $\mu$ g ml<sup>-1</sup> trypsin before it was added to the cells.

From the protocol provided by Zhengli Shi (download here):

#### DMEM Medium

X Our use of cookies

Frowe use necessary cookies to make our site work. We also set performance and functionality cookies that help us make improvements by measuring traffic on our site. For more detailed information about the cookies we use, please see our privacy policy.

Samples were originally stored in 1.5ml of DMEM but both the experimental and controls's DMEM was reduced to 0.5ml during the experiment.

### Controls

Control groups are an important part of any scientific experiment. A control group provides a reference for the experimental group to be compared against. Control groups must contain the exact components as the experimental group sans the component being tested known as the independent variable.

If for instance an experimental group contains Antibiotics, Antifungals, Minimal Nutrient solution, and FBS, than the experimental groups should contain all of those components at the same volume.

For this group to receive a check mark, the control must contain the same composition sans the viral sample (note, if Viral Transport Medium were used and viruses NOT purified from that medium, than this experiment will be rejected as the antibotics, antifungals, and FBS found in VTM could containment the experiment). Since the experimental groups use an unpurified sample from a sick person, the control must use an unpurified sample from a healthy person to be considered valid.

From the protocol provided by Zhengli Shi (download <u>here</u>):

#### **Vero E6 Experimental Groups**

- 2% anti-anti (Gbico,REF15240-062) per well
  - Reduce to 1% anti-anti after CPE detected

#### **Huh7 Experimental Groups**

- 2% anti-anti (Gbico,REF15240-062) per well
  - Reduce to 1% anti-anti after CPE detected

#### Vero E6 Control Groups ≫ Our use of cookies

We use necessary cookies to make our site work. We also set performance and functionality cookies that help us 1% anti-anti (Gbico REF15240-062) make improvements by measuring traffic on our site. For more detailed information about the cookies we use, please see our privacy policy.

#### **Huh7 Control Groups**

1% anti-anti (Gbico,REF15240-062)

# **Control Clarification**

Reading the glaring issue with the control groups only using 1% anti-anti while the experimental groups using 2% and then reducing to 1%, I thought this might be an error. So during our email conversation I asked the following:

If Cytopathic Effects were not observed in the 1st gen, then would you continue to use 2% anti-anti? And then once Cytopathic effects were observed, the Anti-Anti's would be reduced to 1%. Can you explain why the Controls are only using 1% Anti-Anti when the experimental group used 2% until Cytopathic Effect was observed?

Zhengli Shi passed on my inquires to a colleague Xinglou Yang. Xinglou Yang responded:

The intention of Anti-Anti is to prevent contamination from bacteria or fungi during virus isolation, so 1% or 2% concentration did not affect the cell growth.

2% in 1st gen was just to prevent contamination from samples.

If you could make sure that you could prevent contamination from bac or fungi, you do not need to use the Anti-Anti.

I was still surprised by the response and inquired further:

Can you run the controls again with 2% anti-anti to confirm?

Zhengli Shi responded:

A if you want, it is OK. 1% or 2% has no effect for cell growth.

🗴) the vie of cookies ave admitted they don't believe 1% or 2% makes any difference

bet We use necessary cookies to make our site work. We also set performance and functionality cookies that help us

do exactly the same procedure with the controls? They are already making assumptions please see our privacy policy.

like there are no bacteria or fungus in the controls so no need to turn up the anti-antis. That step alone disqualifies their results as their controls are invalid.

I also wondered about the 1st gen comment. Zhengli Shi wrote that during the 1st gen, they used 2% anti-antis. And my understanding is that they will wait for CPE to occur to move from 1st gen to 2nd gen. They also typically 'wash' the tissue culture between generations which is a step that never happens in the controls either. Disturbing the tissue culture in any way could result in CPE as well.

# **My Analysis**

In 2021, I decided to write an analysis paper that included the information I learned from the protocols and the email exchange. I asked for input from Zhengli Shi but declined my request.

Download my paper here.

I have also picked out another interesting find is that only 1 or 2 wells experienced CPE. I tried asking for confirmation but I never received a response from Zhengli Shi. There are copious amounts of questions we could ask virologist, like what is the length of time before the controls experience CPE?

The paper "A review of A pneumonia outbreak associated with a new coronavirus of probable bat origin" is a typical virology paper that never isolated and purified any virus particles, never demonstrated that these particles cause disease, nor never proved that these particles are contagious. The control experiment is invalid as it does not provide the same environment as the experimental groups nor is there any attempt to create a control that is the same as the experimental group except for the independent variable.

### 7 Comments



X Our use of cookies

We use necessary cookies to make our site work. We also set performance and functionality cookies that help us make improvements by measuring traffic on our site. For more detailed information about the cookies we use, please see our privacy policy.



Liked by Monky Science

Great job Michael, thank you!!



**BB\_DAVID** Writes BB\_DAVID's Newsletter 16 hr ago Viked by Monky Science

The bat lady was busy telling the CCTV: "Yes, we can never prove something that doesn't exist."

https://www.youtube.com/watch?v=bppR6UQNJO0&t=880s

 $\heartsuit$  LIKE (1)  $\bigcirc$  REPLY ...

4 replies by Monky Science and others

5 more comments...

 $\bigcirc$  2023 Monky Science  $\cdot$  <u>Privacy</u>  $\cdot$  <u>Terms</u>  $\cdot$  <u>Collection notice</u> <u>Substack</u> is the home for great writing

X Our use of cookies

We use necessary cookies to make our site work. We also set performance and functionality cookies that help us make improvements by measuring traffic on our site. For more detailed information about the cookies we use, please see our privacy policy.