Gmail

virus isolation protocol

24 messages

石正丽 <zlshi@wh.iov.cn> Mon, Aug 2, 2021 at 3:12 PM
[redacted]
Cc: yangxl <yangxl@wh.iov.cn>

Dear Dr. Speth,

Thank you for your interest. Please see the attached protocol we used for virus isolation. For the detailed information, please contact my colleague Xinglou Yang (yangxl@wh.iov.cn).

Best rgards,

Zhengli Shi

[redacted] Mon, Aug 2, 2021 at 3:14 PM
To: 石正丽 <zlshi@wh.iov.cn>
Cc: M[redacted] yangxl <yangxl@wh.iov.cn>

你好，
对不起，I didn't receive the attached protocol. Can you please resend?

谢谢
[Quoted text hidden]
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石正丽 <zlshi@wh.iov.cn> Mon, Aug 2, 2021 at 3:15 PM
[redacted]
Cc: yangxl <yangxl@wh.iov.cn>

Sorry.

> -----原始邮件-----
> **发件人**[redacted]
> **发送时间:**2021-08-02 11:14:04 (星期一)
> **收件人:** "石正丽" <zlshi@wh.iov.cn>
> **抄送:**[redacted], yangxl <yangxl@wh.iov.cn>
> **主题:** Re: virus isolation protocol
> [Quoted text hidden]

virus isolation-yxl.pdf
79K

[redacted] Mon, Aug 2, 2021 at 3:48 PM
To: yangxl <yangxl@wh.iov.cn>

Greetings and 你好，
I am researching the SARS-COV-2 virus and am seeking additional information about the paper "A pneumonia outbreak associated with a new coronavirus of probable bat origin". You colleague provided the Isolation Protocol which is much appricated.

Kindly, can you provide the following information?

- What is the volume of the DMEM used for the experimental groups?
- What is the volume of the DMEM used for the control groups?
- Was the Anti-anti (Gbico,REF15240-062) added to the controls, and if so, at what volume?
- Was trypsin added to the controls, if so what volume?

谢谢

[Quoted text hidden]

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yangxl@wh.iov.cn <yangxl@wh.iov.cn> Wed, Aug 4, 2021 at 12:38 AM
[redacted]
Cc: zlshi <zlshi@wh.iov.cn>

Dear [redacted]
It is nice to receive your email that interested with our paper.
The information are as follows:
For easy observation, it is better to treat controls using the same way as the infection wells.
For virus isolation in this paper, we used 12 wells cell culrue plate, so we added 1ml medium for each well, the volume was same in the control group. But after this, we used 24 wells plate for virus isolation, each well was added 0.5ml medium.
For the antibiotics, we used two times for the first generation, so the dilution was 1:50. For the following generation, the dilution ratio was 1:100 as recomended by the manufacture.
And we did not use trypsin for virus passage and other samples isolation later, meanwhile we only use Vero E6 cell line (not Huh7 cell line) and added 2% FBS to the DMEM for maintaining.
Hope these information could answer your question.
Best regards.

yangxl@wh.iov.cn

[Quoted text hidden]

[redacted] Wed, Aug 4, 2021 at 8:06 AM
To: "yangxl@wh.iov.cn" <yangxl@wh.iov.cn>
Cc: [redacted], zlshi <zlshi@wh.iov.cn>

Greetings Mr Yang,

Thank you for your response. I have created the following groups based on the paper plus your feedback. Can you please have a look and correct me where I am mistaken?

Virus Isolation Experiment

Control Group Vero E6

- Cell Culture Plate: 12 wells
- Cell Lines: Vero E6
- DMEM: 1ml per well
- FBS: 10%
- Anti-Anti: ???
- Trypsin: None

Control Group Huh7

- Cell Culture Plate: 12 wells
- Cell Lines: Huh7
- DMEM: 1ml per well
- FBS: 10%
- Anti-Anti: ???
- Trypsin: None

Experimental Group Vero E6

- Cell Culture Plate: 24 wells
- Cell Lines: Vero E6
- DMEM: 0.5ml per well
- FBS: 2%
- Anti-Anti: 1:50 (1st gen), 1:100 (next gen)
- Trypsin: 16 μg ml−1 trypsin

Experimental Group Huh7

- Cell Culture Plate: 24 wells
- Cell Lines: Huh7
- DMEM: 0.5ml per well
- FBS: 2%
- Anti-Anti: 1:50 (1st gen), 1:100 (next gen)
- Trypsin: 16 μg ml−1 trypsin

Kind Regards
[Quoted text hidden]

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yangxl@wh.iov.cn <yangxl@wh.iov.cn> Thu, Aug 5, 2021 at 12:25 AM
To: [redacted]
Cc: zlshi <zlshi@wh.iov.cn>

Dear [redacted]
I have made minor change to your proposol. Fresh samples and high virus concentration are the key to successful isolation.

Control Group Vero E6

- Cell Culture Plate: 24 wells
- Cell Lines: Vero E6
- DMEM: 0.5 ml per well
- FBS: 2%
- Anti-Anti: 1%
- Trypsin: None

Control Group Huh7

- Cell Culture Plate: 24 wells
- Cell Lines: Huh7
- DMEM: 0.5 ml per well
- FBS: 2%
- Anti-Anti: 1%
- Trypsin: None

Experimental Group Vero E6

- Cell Culture Plate: 24 wells
- Cell Lines: Vero E6
- DMEM: 0.5ml per well
- FBS: 2%
- Anti-Anti: 2%(1st gen), 1% (next gen)
- Trypsin: None

Experimental Group Huh7

- Cell Culture Plate: 24 wells
- Cell Lines: Huh7
- DMEM: 0.5ml per well
- FBS: 2%
- Anti-Anti: 2% (1st gen), 1% (next gen)
- Trypsin: None

[Quoted text hidden]

[redacted] Thu, Aug 5, 2021 at 8:48 AM
To: "yangxl@wh.iov.cn" <yangxl@wh.iov.cn>

Greetings,

Thank you for the clarifications. I have a few additional questions.

DMEM & FBS
Before the Isolation Experiment, what was the DMEM Volume and FBS Percentage used for the cell cultures?

> The following cell lines were used for virus isolation in this study: Vero E6 and Huh7 cells, which were cultured in DMEM containing 10% FBS.

Trypsin
The paper describes Trypsin as being used. How does this relate to the isolation experiment?

> 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 μg ml−1 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 μg ml−1 trypsin.

Anti-Anti
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?

Thank you for your time!

Kind Regards
[Quoted text hidden]

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yangxl@wh.iov.cn <yangxl@wh.iov.cn> Thu, Aug 5, 2021 at 12:06 PM
To: [redacted]

Greetings,
DMEM & FBS
Before the Isolation Experiment, what was the DMEM Volume and FBS Percentage used for the cell cultures?
DMEM containing 10% FBS.
For nornal cell culture

Trypsin
The paper describes Trypsin as being used. How does this relate to the isolation experiment?
The trypisn could help to digest and cut the spike protein for some coronavirus isolation,, such as SADS-CoV. For SARS-CoV-2, we didnot use it later for isolation because of this virus could grow well without trppsin during the following experiment.

Anti-Anti
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?

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.

kind regards
[Quoted text hidden]

[redacted] Thu, Aug 5, 2021 at 12:27 PM
To: "yangxl@wh.iov.cn" <yangxl@wh.iov.cn>
Cc: [redacted]

Greetings,

DMEM
For the Isolation experiment, was the stock culture DMEM concentration reduced for both ctrl and exp groups? If so, do you have an estimate of the reduction of DMEM from the stock cell culture?

Trypsin
Where did you use Trypsin in your experiments as it is listed in the paper?

Anti-Anti

> 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.

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

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

Have you considered filtering out bacteria and fungi through centrigation, filtration, immunoaffinity, and/or precipitation?

Thank you

[Quoted text hidden]

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yangxl@wh.iov.cn <yangxl@wh.iov.cn> Thu, Aug 5, 2021 at 12:41 PM
To: [redacted]

Greetings
DMEM
For the Isolation experiment, was the stock culture DMEM concentration reduced for both ctrl and exp groups? If so, do you have an estimate of the reduction of DMEM from the stock cell culture?
A:Reduced for both.

Trypsin
Where did you use Trypsin in your experiments as it is listed in the paper?
A: It is used in the begining. If you want try, it is OK.

Anti-Anti

> 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.

Can you run the controls again with 2% anti-anti to confirm?
A：if you want, it is OK. 1% or 2% has no effect for cell growth.

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

Have you considered filtering out bacteria and fungi through centrigation, filtration, immunoaffinity, and/or precipitation?
A: yes, you are right. During the past decades, I have tried different methods. It is difficult to say which is better depends on your sample type.
[Quoted text hidden]

[redacted] Thu, Aug 5, 2021 at 1:11 PM
To: "yangxl@wh.iov.cn" <yangxl@wh.iov.cn>
Cc: [redacted]

Greetings,

Thank you again for answering all my questions and for your patients!

DMEM
Why do you reduce the DMEM concentration and FBS for the isolation experiment? Would you get a different result if the DMEM and FBS concentrations were the same as the stock cell culture?

Trypsin
Is Trypsin added to the sample before the sample is added to the experimental group? And added again during 1st gen processing?

Photos
Do you have photographs of the control and experimental groups at the end of the experiment?

CPE
How many wells demonstrated CPE from each group?

Control Group Vero E6
Number of Wells with CPE:
Time of First CPE:
Time of Last CPE:

Control Group Vero Huh7
Number of Wells with CPE:
Time of First CPE:
Time of Last CPE:

Experimental Group Vero E6
Number of Wells with CPE:
Time of First CPE:
Time of Last CPE:

Experimental Group Vero Huh7
Number of Wells with CPE:
Time of First CPE:
Time of Last CPE:

Thank you!
[Quoted text hidden]

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yangxl@wh.iov.cn <yangxl@wh.iov.cn> Thu, Aug 5, 2021 at 2:03 PM
To: [redacted]

Greetings,

Thank you again for answering all my questions and for your patients!

DMEM
Why do you reduce the DMEM concentration and FBS for the isolation experiment? Would you get a different result if the DMEM and FBS concentrations were the same as the stock cell culture?

You can read some paper, this is the normal choice for cell maintaining during virus isolation

Trypsin
Is Trypsin added to the sample before the sample is added to the experimental group? And added again during 1st gen processing?
You can choose one way depending on the virus, both is OK

Photos
Do you have photographs of the control and experimental groups at the end of the experiment?
Please see our paper

CPE
How many wells demonstrated CPE from each group?
For control, cell grew well.
For the samples, only one CPE.
[Quoted text hidden]

[redacted] Thu, Aug 5, 2021 at 2:15 PM
To: "yangxl@wh.iov.cn" <yangxl@wh.iov.cn>
[redacted]

Greetings,

Thank you again for answering my questions.

> For the samples, only one CPE.

Can you please clarify this?

Each Group had 24 wells.

Control Group Vero E6

- Detected CPE: 0 Wells

Control Group Huh7
- Detected CPE: 0 Wells

Experimental Group Vero E6
- Detected CPE: 1 Well

Experimental Group Huh7
- Detected CPE: 1 Well

Photos
My apologies, are these the photos that you were referring to?
https://www.nature.com/articles/s41586-020-2012-7/figures/9

Do you have comparison photos between the controls and the experimentals? I am most interested in the last photo of each well taken at the end of the experiment (so 96 photos in total).

Thank you

[Quoted text hidden]

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yangxl <yangxl@wh.iov.cn> Thu, Aug 5, 2021 at 2:18 PM
To: [redacted]

我晕，一个样本只感染1-2个孔

---- Replied Message ----

From	[redacted]
Date	08/05/2021 10:15
To	yangxl@wh.iov.cn<yangxl@wh.iov.cn>
Cc	[redacted]
Subject	Re: Re: virus isolation protocol

[Quoted text hidden]

[redacted] Thu, Aug 5, 2021 at 2:30 PM
To: yangxl <yangxl@wh.iov.cn>

你好
Just to confirm, you are saying in the samples (not the control group), only 1 or 2 wells for each cell line (24 wells) demonstrates CPE?
谢谢
[Quoted text hidden]

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yangxl <yangxl@wh.iov.cn> Thu, Aug 5, 2021 at 2:38 PM
To: [redacted]

是的

---- Replied Message ----

From	[redacted]
Date	08/05/2021 10:30
To	yangxl<yangxl@wh.iov.cn>
Subject	Re: virus isolation protocol

[Quoted text hidden]

[redacted] Thu, Aug 5, 2021 at 3:34 PM
To: yangxl <yangxl@wh.iov.cn>
Cc: [redacted]

Greetings,

Sorry again for the inconvenience, but I want to make sure I understand.

Was the sample added to each well in the experimental group the same? Was it a BALF sample from ICU-06 patient added to all 48 wells in the experimental group?
Or were different samples added to each of the 48 wells in the experimental group?

Thank you
[Quoted text hidden]

[redacted] Fri, Aug 6, 2021 at 12:53 PM
To: yangxl <yangxl@wh.iov.cn>
Cc: [redacted]

Greetings,

I have incorporated the responses that I have received from you about the paper into a supplemental document. Can you please have a read? There are a few questions in the document that I hope you can clear up.

Thank you
[Quoted text hidden]

[redacted] **Supplamental Virus Isolation Protocol.pdf**
56K

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yangxl <yangxl@wh.iov.cn> Fri, Aug 6, 2021 at 4:00 PM
To: [redacted]

Could you tell me your purpose of this protocol? There are some minor errors in your file.

---- Replied Message ----

From	[redacted]
Date	08/06/2021 08:54
To	yangxl<yangxl@wh.iov.cn>
Cc	[redacted]
Subject	Re: virus isolation protocol

[Quoted text hidden]

[redacted] Fri, Aug 6, 2021 at 4:03 PM
To: yangxl <yangxl@wh.iov.cn>
Cc: [redacted]

Thank you for having a look! I would like to write a comparison report with other papers on their isolation protocols.

Thank you
[Quoted text hidden]

[redacted] Sat, Aug 7, 2021 at 9:57 AM
To: yangxl <yangxl@wh.iov.cn>
Cc: [redacted]

My apologies, but can you please point out the errors in the document?

Thank you
[Quoted text hidden]

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yangxl@wh.iov.cn <yangxl@wh.iov.cn> Sun, Aug 8, 2021 at 5:28 PM
To: [redacted]

Sorry, I have answered all the questions you asked and I have told you all the details. But you do not have the biology background, so I don't trust the rigorous and accurate of your manuscript, I don't have the responsibility to help you to publish your manuscript.
[Quoted text hidden]

[redacted] Mon, Aug 9, 2021 at 10:30 AM
To: "yangxl@wh.iov.cn" <yangxl@wh.iov.cn>

Greetings & 你好,
My apologies, I was not intended on asking you to co-author my paper. I am only seeking clarification of your paper. I still have a few questions if you don't mind answering.

- Each Experimental group (Vero E6 & Huh7) uses 24 well cell culture plates
  - How many of those wells were used for the experiment?
  - Where there 7 patient samples used in this experiment? (ICU-01,ICU-04,ICU-05,ICU-06,ICU-08,ICU-09,ICU-10)
  - Was each of the patient samples used in 2 wells per cell culture plate?

- Was CPE detected in in any of the wells for the Vero E6 Group?
- Was CPE Detected in any of the wells for the Huh7 Group?

Thank you & 谢谢

[Quoted text hidden]