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Dr Leon Caly
Senior Medical Scientist
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Doherty Institute

By email: leon.caly@mh.org.au

Dear Dr Caly,

SARS-CoV-2
Virus Isolation Experiment

We are a not-for-profit with the objects of promoting vaccine safety and efficacy, with a membership of over 2,000 concerned Australians.

May I congratulate you on your lead authorship of the March 2020 paper entitled "Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia." That paper broke new ground in the investigation into the causal agent for SARS-CoV-2 virus, in claiming to have isolated the pathogen from a patient using *in vitro* and *in silico* methods.

Our members would be grateful if you could provide further clarification as to certain aspects of the methods and results of the experiment described in the paper.

1. How did you determine the agent you identified caused the patient's symptoms? How did you exclude all other potential aetiological agents?

2. On what basis was the agent identified determined to be of viral origin? How were other possible causes such as bacteria and toxins ruled out?

3. Samples were taken from only one patient. Why was no healthy control subject used in the experiment?

4. Were SARS-CoV-2 viral particles isolated in pure form? Was the supernatant purified of other microbes? In particular, were all metabolic RNA and other contaminants

removed? If not, is the use of the term 'isolation' and 'isolate' intended to convey something other than 'to separate from all other matter'?

5. Did the experiment produce any isolated virus particles purified of all contaminants? If not, how did you exclude the possibility that the identification and characterisation of the virus might be affected by contamination?

6. The Vero/hSLAMs are a hypersensitive and highly reactive pseudo-cell line from African green monkey kidney cells. Should at least 2 other cell lines have also been used for cultivation, especially cell lines more closely resembling human tissue?

7. The electron micrographs do not appear to show significant numbers of coronavirus-like particles, or particles which are identical in size and structure. On what basis did you:
   (a) determine that these particles were indeed virions of the SARS-CoV-2?
   (b) exclude the possibility that these are virus-like particles expressed by the Vero cells?
   (c) exclude the possibility that these are metabolic products?

8. Were different effects observed in cultivation of your isolate on HuH7 cells in a later experiment in which you were involved?

9. Various toxic chemicals were added to the Vero cells during the process. How did you exclude the possibility that this contributed to any cytopathic effect or expression of virus-like particles from the Vero cells? Could treatment of the cells have caused or contributed to the observed effect?

10. Did true cytopathy occur, or was a clumping effect interpreted as cytopathy? How did you exclude the possibility that some agent other than Sars-CoV-2 (whether in the cells or in the specimen) was responsible?

11. Which if any of the PCR primers and probes used were designed by Charite (Drosten)?

12. Kindly provide the genomic assembly software data disclosing number and length of input reads and the abundance, length, coverage and depth of assembled contigs used for the de novo assembly.

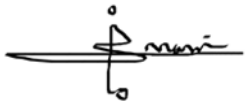
13. Please provide the BLAST top 100 output data showing the basis on which comparative analysis was performed.

14. Which of Koch's Postulates, if any, were satisfied by your methodology?

15. Before shipping the isolate, were the vials labelled for concentration or potency? If not, why not?

Thank you for your prompt attention to these important scientific matters.

Yours sincerely,



Mani Shishineh
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