Dear Professor Zambon

Thank you for your reply received April 28, 2020 [see below].

Based on your reply I have further questions to which I would be grateful for answers.

I've highlighted in red the sections of your reply to which I have twelve questions

"i) RT-PCR tests –

- *the gold standard for PCR tests is not virus isolation"*

Q1. Why is isolation of the COVID-19 virus [SARS-CoV-2] not the gold standard in the PCR test for the virus?

Q2. What gold standard does PHE use to evaluate the RT-PCR test for SARS-Cov-2 infection?

- *"Typically specificity exceeds 95%"*

Q3. Which specificity? Analytical or clinical? As per the MIQE guidelines [1].

"*Analytical sensitivity* refers to the minimum number of copies in a sample that can be measured accurately with an assay, whereas *clinical sensitivity* is the percentage of individuals with a given disorder whom the assay identifies as positive for that condition".

Q4. Do you agree that in the case of the test under discussion, the "assay" is RT-PCR and the "given disorder" is SARS-CoV-2 infection?

Q5. What gold standard does PHE use to calculate clinical specificity?

The UK population is approximately 67 million and the prevalence of SARS-Cov-2 infection unknown. Estimates based on ELISA have been published but the specificity of ELISA is based on a PCR gold standard (as per Professor Crook's paper you supplied). The gold standard for the ELISA cannot be any better than the gold standard for the RT-PCR. However, PHE has yet to say what this is.

It is elementary that the amount by which "the specificity exceeds 95%" is critical for calculating the probability that a positive test (positive predictive value, PPV), is proof of infection. Applying a 95.1% specific test to a 1/1000 prevalence population for example, results in a PPV of 2% with 98% false positives. The PPV for a prevalence of 1/100 is better but still far short of desirable: 17% with 83% false-positives.

My table below shows this here:

Sensitivity 100%			**Sensitivity 100%**		
Specificity 95.1%			**Specificity 99%**		
Prevalence	1/1000	1/100	Prevalence	1/1000	1/100
PPV	2%	17.00%	PPV	9%	50.00%
Prob. false positive	98%	83%	Prob. false positive	91%	50%

Q6. Are RT-PCR tests reported PCR positive/negative or SARS-CoV-2 positive/negative?

Q7. Who, PHE or the ordering physician, interprets a positive RT-PCR as proof of virus infection?

Q8. Is the caveat of PPVs reflected in reports PHE and other laboratories issue to physicians?

In the table below I contend that whatever gold standard PHE employs, that GS is, by definition, what the RT-PCR procedure tests for.

Q9. Do you agree?

Test result	**PHE GS +**	**PHE GS -**	**Totals**
RT- PCR test +	A	B	A+B
RT-PRC test -	C	D	C+D

In my previous e-mail, I requested data proving the sensitivity and specificity of the RT-PCR test for SARS-CoV-2 infection.

Test result	**?? pos**	**?? neg**	**Totals**
RT- PCR test +	A	B	A+B
RT-PRC test -	C	D	C+D

Q10. Would you please send me these data and indicate PHE's column titles?

ii) antibody tests –

- *"There is a pre-publication manuscript available at https://www.medrxiv.org/content/10.1101/2020.04.15.20066407v1.full.pdf in which the authors describe testing plasma for SARS-Cov-2 IgM and IgG antibodies by ELISA and using nine different commercially available lateral*

flow immunoassay (LFIA) devices. There findings were that "Point estimates for the sensitivity of LFIA devices ranged from 55-70% versus RT-PCR and 65-85% versus ELISA, with specificity 95-100% and 93-100% respectively. Within the limits of the study size, the performance of most LFIA devices was similar."

- *No commercial kits have yet been validated for use in the UK – work is ongoing."*

Q11. Are gold standards other than RT-PCR used to evaluate antibody tests for infection with SARS-CoV-2?

Q12. If so, what is this gold standard?

Online calculator http://vassarstats.net/clin2.html

1. Bustin, S. A., V. Benes, et al. (2009). "The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments." Clinical Chemistry **55**(4): 611-622.