Bustin to Coppolino - Feb 2, 2021 via email

(1) your referring to pre/asymptomatic transmission (which may be two different things) has made me think again and I am looking into this now. Clearly, the crux of the matter is what does a molecular test result actually mean in a clinical context. I said this to you and I am saying this in my papers, there is an urgent need for certified standards as well as large scale studies to investigate what Cqs actually mean in terms of infectiousness. Given that we are facing variants that will no longer be neutralised by the immune response from the Mk.1 vaccine, this is doubly urgent. It is a mammoth task, and I am starting from the first report (Rothe et al) and shall work my way through some of the key papers. I had an inkling that many of the reports dealing with SARS-CoV-2 would be unclear and conclusions not necessarily supported by the data and so it is. One issue that is immediately apparent, and is one I have bemoaned for years, is that the experimental protocols are incomplete and not transparent. This means that much of the information I would need to assess the validity of a result and how a Cq relates to that, is unavailable. This will make it very difficult to compare different definitions of pre/asymptomatic as well as assess what Cq cutoffs were used. For example, the Rothe paper provides no detail at all. I shall prepare a manuscript based on my analyses, because as far as I can tell no one has looked at this from the molecular biologist's point of view. Once I am happy with it, I shall let you have a look at it.

(2) Critique of the Corman paper: as I said, there are some valid criticisms, but they do not significantly impinge on the initial results obtained by people using the primers. A correction was published promptly, and the assay targeting the RdRp has not been in use for a long time now. I expect Eurosurveillance will release our comments on both the Corman paper and its critique any time now.

I think you should remember that the primers were designed a day or so after the SARS-CoV-2 sequence had been published, so the authors' aim was speed. Expedited publication is not unheard and whatever one might think about the RdRp primer design details, the E-gene assay

(F:ACAGGTACGTTAATAGTTAATAGCGT R:ATATTGCAGCAGTACGCACACA) is specific for SARS-CoV-2, the amplicon is of a reasonable length (113bp) and neither target regions of secondary structure. Certainly, the melting temperatures for the primers are a little high and a little low for the probe and I would have designed them a little differently, they are perfectly serviceable and will detect SARS-CoV-2. They may not generate the most sensitive of assays, but then analytical sensitivity of RT-qPCR assays is exactly the question we need to address.

These, and other comments can be made in a perfectly civilised tone.

SB

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Bustin, Stephen February 2, 2021 at 10:41 AM

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Re: No asymptomatic spread - NATURE

To: Eric F Coppolino

Hi,

(1) your referring to pre/asymptomatic transmission (which may be two different things) has made me think again and I am looking into this now. Clearly, the crux of the matter is what