To: [5.1.2e] [5.1.2e @erasmusmc.nl]
From: 5.1.2e
Sent: Tue 1/14/2020 6:43:15 AM
Subject: Antw: Fwd: OFFICIAL: RE: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?
Received: Tue 1/14/2020 6:43:15 AM

Ok helder!

Ik mail ze geoon beiden in neutrale bewoordingen.

-:) gr 5.1.2e

Van: 5.1.2e < 5.1.2e @erasmusmc.nl>
Datum: 13 januari 2020 om 21:38:26 CET
Aan: 5.1.2e < 5.1.2e @rivm.nl>
Onderwerp: Fwd: OFFICIAL: RE: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Confidential maar dit zijn de docs die ik bedoel.
Gaat in erg detail over geschiktheid bestaande primers.
Voor ECDC zouden dus beide approaches van belang kunnen zijn

Van: 5.1.2e < 5.1.2e @erasmusmc.nl>
Verzonden: maandag, januari 13, 2020 9:58 AM
Aan: malik; 5.1.2e
CC: 5.1.2e 5.1.2e ; 5.1.2e ; 5.1.2e ; 5.1.2e
Onderwerp: Re: OFFICIAL: RE: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Dear 5.1.2e

Please find attached the latest version of the primer evaluation, including the primer set 5.1.2e send us.

Best regards,

5.1.2e

Van: 5.1.2e 5.1.2e @hku.hk>
Datum: vrijdag 10 januari 2020 om 12:16
Aan: " 5.1.2e " < 5.1.2e @erasmusmc.nl>, 5.1.2e < 5.1.2e @phe.gov.uk>
CC: 5.1.2e 5.1.2e @hku.hk>, " 5.1.2e " < 5.1.2e @charite.de>, 5.1.2e
< 5.1.2e @hkucc.hku.hk>, " 5.1.2e " < 5.1.2e @erasmusmc.nl>, " 5.1.2e "
< 5.1.2e @erasmusmc.nl>
Onderwerp: Re: OFFICIAL: RE: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Dear 5.1.2e

I just want to calrify that the primer set 5.1.2e sent you has sucessfully detected both alpha and beta coronviruses in bats as well as HKU23, 229E like camel virus and MERSs-CoV in camels as well as the known endemic human CoVs. So I am confident it picks up most coronaviruses, including the Wihan virus.

5.1.2e

From: 5.1.2e < 5.1.2e @erasmusmc.nl>
Sent: Friday, January 10, 2020 18:59
To: 5.1.2e
Cc: dkwchu; malik; 5.1.2e ; 5.1.2e 5.1.2e
Subject: Re: OFFICIAL: RE: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Yes, and we will not. If anything, we caution that there may be some assays out there, that have specifically been developed for broader capture, and that need to be looked at by specialists.

On 10 Jan 2020, at 11:58, 5.1.2e < 5.1.2e @phe.gov.uk> wrote:

OFFICIAL

5.1.2e

Apologies, have been fully occupied...will feed back shortly. I agree that important at this stage not to recommend any specific assay

5.1.2e

From: 5.1.2e [5.1.2e @erasmusmc.nl]
Sent: 10 January 2020 10:54
To: 5.1.2e < 5.1.2e @hku.hk>
Cc: malik < 5.1.2e @hku.hk>; 5.1.2e < 5.1.2e @charite.de>; 5.1.2e < 5.1.2e @hkucc.hku.hk>; 5.1.2e < 5.1.2e @phe.gov.uk>; 5.1.2e < 5.1.2e @erasmusmc.nl>; 5.1.2e < 5.1.2e @erasmusmc.nl>
Subject: Re: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Thanks, we will add it and send you the update

I discussion with 5.1.2e we will be careful not to specifically recommend, and keep each other posted on views as things evolve

5.1.2e

On 10 Jan 2020, at 11:51, 5.1.2e < 5.1.2e @hku.hk> wrote:

Dear 5.1.2e

Please find the pan-CoV protocol 5.1.2e mentioned in the attachment. Primer sequences can be found in the document and they are:

pan-CoV 1st forward 5'-GGKTGGGAYTAYCCKAARTG-3'
pan-CoV 1st reverse 5'-TGYTGTSWRCARAAYTCRTG-3'
pan-CoV 2nd forward 5'-GGTTGGGACTATCCTAAGTGTGA-3'
pan-CoV 2nd reverse 5'-CCATCATCAGATAGAATCATCAT-3'

This assay was published in paper: Chu et al. Avian coronavirus in wild aquatic birds. J Virol. 2011 Dec;85(23):12815-20

Best regards
5.1.2e

On 10 Jan 2020, at 17:42, `5.1.2e` wrote:
Thanks `5.1.2e`

From `5.1.2e` `5.1.2e` @hku.hk>
Sent: Friday, January 10, 2020 10:30 AM
To: `5.1.2e` ; `5.1.2e` ; `5.1.2e` `5.1.2e` ; `5.1.2e`
Cc: `5.1.2e` ; dkwchu
Subject: Re: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Dear `5.1.2e`

I did not have e mail access yesterday and I missed our message.

I am copying my colleague `5.1.2e` to send your our PanCoV primer set that has been used sucessfully for CoV discovery. We are sure it will work also on the new Wuhan virus, but it is a heminested RT-PCR
I am not sure if it has changed since the original Poon primers you have listed .
Anyway, `5.1.2e` can send you the primers and protocol

`5.1.2e`

From: `5.1.2e` < `5.1.2e` @erasmusmc.nl>
Sent: Friday, January 10, 2020 5:44
To: `5.1.2e` ; `5.1.2e` `5.1.2e`
Cc: `5.1.2e`
Subject: URGENT REQUEST IN PREPARATION OF WHO TC, CAN YOU REPLY ASAP?

Dear colleagues
Please find some information that was compiled today by our team (`5.1.2e` with input from `5.1.2e` `5.1.2e` `5.1.2e` , `5.1.2e` `5.1.2e` `5.1.2a` `5.1.2a` `5.1.2a`
Would you please have a look to see if you would endorse this/ sign on to this? If we can present this as consensus among us that would be a strong signal and we could share this with `5.1.2e`

Look forward for your comments!

What it is:
`5.1.2e` ran a software algorithm that allows checking of a large set of genomes against whatever primersets you would like to test, to check for mismatches. He did this to get a feel for the potential suitability of some available PCR protocols for testing as long as we do not have more specific information. The software has been developed through collaboration with colleagues from DTU, in a EU project (www.compare-europe.eu), and is freely available at Bitbucket (https://bitbucket.org/jszarvas/oligo/src/master/) but people need to know UNIX code to use it. Note that it uses a reference database that is automatically getting new coronavirus genomes when they appear in the public domain. We developed this as a tool to help our diagnostic department in keeping up if the primers that they use still are adequate.
Of course, we are still guessing, but based on what was shared, `5.1.2e` selected all available sequences with taxid 694009 (Severe acute respiratory syndrome-related coronavirus, so leaving out the seasonal viruses) and ran the algorithm. Results are listed in the files attached:
1. A document describing the primersets (Annex 1)
2. A document showing the alignment . The strains in red are SARS (and viruses mislabeled as human viruses from an animal experiment, Annex 2).
3. A document showing the results of the screening of all the primers against all the viruses (Annex 3)
4. A document providing more detail, showing for each of the primersets where the mismatches for all the viruses are (Annex 4)
5. A (very long) document showing all the allignments for primers that have mismatches with any of the sequences. (we need to work on making this easier)(Annex 5).
Conclusion:
Based on this analysis, we would recommend including the SARS NP primerset and the pancoronavirus primersets in screening, while waiting for details from China:
Kuiken T, et al, Newly disc.coronavirus as the primary cause of SARS, lancet 2003;362(9380);263-70
    SARS NP fwd1   caaacattggccgcaaatt
    SARS NP probe   cacaatttgctccaagtgcctctgca
    SARS NP rev1   caatgcgtgacattccaaaga
Luna et al. Journal of clinical microbiology, 2007, p 1049-1052
    panCoV_fwd1   TTATGGGTTGGGATTATC
    panCoV_fwd2   TGATGGGATGGGACTATC
    panCoV_rev1   TCATCACTCAGAATCATCA

```
panCoV_rev2        TCATCAGAAAGAATCATCA
panCoV_rev3        TCGTCGGACAAGATCATCA
```

DISCLAIMER:
In silico analyses are only indicative, and do not guarantee success. However, we do think this narrows down the choice of primersets to use for testing at this stage. Obviously, the best way forward is to share the sequence data. Alternatively, we can share this with the colleagues in China that have the sequence and ask them to check the primer fit.

5.1.2e , 5.1.2e 5.1.2e 5.1.2e
ErasmusMC, Rotterdam, The Netherlands

<pan-CoV protocol.docx>