To: From: Sent: Subject: Received:	5.1.2e 5.1.2e Sun 1/12/2020 Fw: [ext] Initial g Sun	2:21:41 PM genome release of novel co 1/12/2020 12:21:53 PM	l] pronavirus - Novel 2019 co	oronavirus - Virolo	gical	
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From: Date: Su To: Cc: " 5.1.2	5.1.2e unday, 12 Janua 5.1.2e ^{5.1.2e} ^{2e} @erasmu 5.1.2e @who 5.1.2e @who.int>, @who.int>, Re: [ext] Initia	< 5.1.2e @ch ary 2020 at 11:50 5.1.2e @who 5.1.2e @erasmusmo usmc.nl>, 5.1.2e hl b.int>, " 5.1.2e who 5.1.2e @erasmusmo hl b.int>, " 5.1.2e hl b.int>, " 5.1.2e @who 5.1.2e 5.1.2e @who 5.1.2e @who 5.1.2e \$.1.2e \$.1.2e \$.1.2e @who 5.1.2e \$.1.2e \$.1.2e <td< th=""><th>arite.de> .int> c.nl>, 5.1.2 (" < 5.1.2e @hku.hk>, " 1.2e @erasmusmc.nl> .int>, 5.1.2e 5.1 /ho.int>, ' 5.1.2e e @who.int>, " el coronavirus - Novel 2</th><th>e 5.1.2e " < 5.1.2e .2e .2e < 5.1.2e .2e 5.1.2e @v 5.1.2e ? < 019 coronavirus</th><th>5.1.2e @who 5.1.2e @who. < 5.1.2e @who.int>, who.int> 5 < 5.1.2e @ - Virological</th><th>.int>, "b. nl" int>, " 5.1.2e , @hpa.org.uk>, 5.1.2e 1.2e Ocharite.de></th></td<>	arite.de> .int> c.nl>, 5.1.2 (" < 5.1.2e @hku.hk>, " 1.2e @erasmusmc.nl> .int>, 5.1.2e 5.1 /ho.int>, ' 5.1.2e e @who.int>, " el coronavirus - Novel 2	e 5.1.2e " < 5.1.2e .2e .2e < 5.1.2e .2e 5.1.2e @v 5.1.2e ? < 019 coronavirus	5.1.2e @who 5.1.2e @who. < 5.1.2e @who.int>, who.int> 5 < 5.1.2e @ - Virological	.int>, "b. nl" int>, " 5.1.2e , @hpa.org.uk>, 5.1.2e 1.2e Ocharite.de>

855329

Our assays perfectly match all newly released sequences.

5.1.2e		
Institute of Virology		
Charite Campus Mitte		
Berlin		
iPhone		
	5.1.2e	5.1.2e
5.1.2e		
		5.1.2a
540-		
5.1.2e		

On 12 Jan 2020, at 10:46, 5.1.2e < 5.1.2e @erasmusmc.nl> wrote: Yes, 512e we are looking at it. We need to know if there is possible technical issues (sequences generated on different platforms and analysed with different software packages), but if confirmed the diversity of viruses found is remarkable. It could suggest wider circulation or even multiple sources. Discussion is ongoing on virological and twitter.

Btw there also is debate on twitter for a publicly released phylogenetic analysis that gives very little credit to the scientist providing the info.

urgent request for some coordination here

5.1.2e



From	5.	1.2e	<	5.1.2e	@cha	rite.de>					
Sent:	Saturday	, Januar	y 11, 202	20 10:32	PM						
To:	5.1.2e	<	5.1.2e	@erasn	nusmc.nl:	>; malik <	5.1.2e @	hku.hk>	; 5.1.2	e?e	
<	5.1.2e	@erasm	usmc.nl	>;	5.1.2	e	< 5	5.1.2e	@who.int	>;	5.1.2e
 5.1 	l.2e <mark>@w</mark>	ho.int>;	5.1.	2e <	5.1.2e (@who.int	>;	5.1.2e		2	
< 5. ⁻	1.2e <mark>@e</mark> l	asmusn	nc.nl>;	5.1.2e	<	5.1.2e	@HP	A.org.uk	>		
Cc:		5.1.2e		<	5.1.2e	@who.	int>;		5.1.2e		
< 5. ⁻	1.2e <mark>@w</mark>	ho.int>;	5.1.2e	5.1	.2e <	5.1.2e	@who.i	nt>;	5.1.2e		
5.1.	2e <mark>@wh</mark> o	.int>; 5	.1.2e 5.1	.2e <mark><</mark> 5.1.2	e @who.	int>;	5.1	.2e	< 5.1.2	e @wl	no.int>;
	5.1.2e	< 5.1.2e	@who.i	nt>;	5.1.2e	<	5.1.2e	@who	.int>;	5.1.2	e
<	5.1.2e	@charit	te.de>								

Subject: Re: [ext] Initial genome release of novel coronavirus - Novel 2019 coronavirus - Virological

Dear All,

Below are our final oligos. These are three amplicons but to keep it simple, we will focus on two amplicons for further validation and recommendation: those in the RdRp gene and those in the E gene. Note that there is no reason to prefer N gene primers over E or RdRp primers – the transcriptional gradient is not a relevant phenomenon in CoV diagnostics, as has been shown on multiple occasions. We will publish and mention the N assay but not recommend its use for now. It is always good to have a fall back option.

We will most likely recommend the E gene assay as first line assay, RdRp for confirmation. This choice is based on the fact that the RdRp region is where all the PanCoV assays are placed, so labs can easily chose another assay for confirmation (e.g., some PanCoV) and still have confirmation based on two different genome regions as long as they stick to the E gene assay for first line screening. Moreover, lab contaminations tend to be in the RdRp region (PCR products, RNA transcripts) because of the Pan-CoV assays that are widely in use. Better not do a first line screening assay here.

I will give you preliminary validation data tomorrow. We will send off oligos to Rotterdam, London and Hong Kong immediately.

(The oligo concentrations in the table (600nm means: 600 nanomol per liter of final reaction mixture) are results of finetuning but the effects are tiny. Standard reaction conditions will yield full sensitivity.)

All the best,

5.1.2e

Table 1. Primers and probes for assays used to screen for WUHAN-CoV

Assay/ Use	Oligonucleotide ID	Sequence (5'-3')	Comment
WURS-2A (RdRP gene)	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	use 600nm per reaction
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	use 800nm per reaction
			Muhan specific not able to
	E Carbona E1		
	E Sarboso P2	ATATTCCACCACTACCCACACA	use 400pm per regetion
	E Sarbeco P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-	use 200nm per reaction
MUDE 10 (N	N. Carbona E1	CACATTOCCACCOCAATO	use 600pm per reaction
	N Sarbosa P1	CACCAACCACAACACCCTTC	use 800pm per regation
	N Sarbosa D1		use 200pm per regetion





Charité - Universitätsmedizin Berlin Campus Charité Mitte

Chariteplatz 1 D-10117 Berlin Germany

E-Mail: 51.2e @charite.de 5.1.2e https://globalhealth.chante.de/

Von: "	5.1.2e	" <	5.1.2e	@erasm	usmc.nl	>			
Datum: S	amstag, 1	1. Janua	ar 2020 u	m 17:16					
An: 5.1.2e	5.1.2e 🤘	hku.hk>	, "	5.1.2e	" <	5.1.2e	@erasmu	smc.nl>, 5.1.:	2e
5.1.2e <	5.1.2	2e	@charite	e.de>, "		5.1.2e			
< 5.1.2	2e @v	vho.int>	<mark>,"</mark> 5	.1.2e	<mark>" <</mark> 5.1.2	2e <mark>@who</mark>	. <u>int</u> >, "	5.1.2e "	
< 5.1.2e	@who.int	>, "	5.1.2e	" <	5.1.2e	@erasmu	smc.nl>,	5.1.2e	
< 5.1.2	2e <mark>@H</mark>	PA.org	. <u>uk</u> >						
Cc: "	5.1	.2e	"	< 5. ⁻	1.2e	@who.in	<mark>t>,</mark> "	5.1.2e	
5.1.2e	<mark>" <</mark> 5.1	.2e ወ	who.int>	5.1.2e	5	.1.2e ".	< 5.1.2e	@who.int>, "	5.1.2e
5.1.2e <mark>" <</mark>	5.1.2e @v	ho.int>	5.1.2e	5.1.2e " .	< 5.1.2e 🧑	who.int>		5.1.2e	U

< 5.1.2e <u>@who.int</u> >, " 5.1.2e "<5.1.2e <u>@who.int</u> >, " 5.1.2e " 5.1.2e <u>@who.int</u> > Betreff: Re: [ext] Initial genome release of novel coronavirus - Novel 2019 coronavirus - Virological
dear all,
Just received the information from <u>5.1.2e</u> will release their sequence tomorrow morning. According to what she mailed the sequence is somewhat different:
5.1.2i Concept
So maybe it does not affect the primer design, but not sure
best regards
5.1.2e
Van: 51.2e 51.2e 61.2e 61.2e
Hi 5.1.2e
We are also happy to participate.
Best wishes
5.1.2e
From: 5.1.2e 0 5.1.2e 0 <th0< th=""> 0 0</th0<>
Excellent, same here! 5.1.2e as discussed yesterday with 5.1.2e we are in.

5.1.29
5.1.2e
Wytemaweg 80 - room 5.1.2e
3015 CN Rotterdam
Tel: 00 5.1.2e
Fmail: 5.1.2e @erasmusmc.nl
Van: 5.1.2e < 5.1.2e <u>@charite.de</u> >
Verzonden: zaterdag 11 januari 2020 08:43:41
Aan: 5.1.2e ; 5.1.2e ; 5.1.2e ; 5.1.2e ; 5.1.2e ;
5.1.2e ; 5.1.2e ; 5.1.2e
CC: 5.1.2e ;
5.1.2e 5.1.2e 5.1.2e 5.1.2e 5.1.2e
Onderwerp: Re: [ext] Initial genome release of novel coronavirus - Novel 2019 coronavirus - Virological
Dear All
This is great news.
Based on bat-CoV genome alignments, we have made diagnostic PCR candidates last week. They are already in our
iab and are undergoing technical validation over the weekend (signal strength, chemical stability, end point

sensitivity based on preliminary synthetic templates). Several of these candidates match the Wuhan virus perfectly. We will release the best candidates to the group once they have passed our technical pre-evaluation. We will then do a quick but comprehensive clinical evaluation similar to what we did for MERS. We will make Wuhan virus-specific RNA transcripts for use as positive controls and for sensitivity end point studies. Once we have positive controls, we can go public.

Who is interested to participate?

5.1.2e			
5.1.2e	rolomy		
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Charité - Universitätsm	edizin Berlin		
Campus Charité Mitte			
Chariteplatz 1			
D-10117 Berlin			
Germany			
E-Mail: 512e	@charite de		
	(wenance.de		
		5.1.2e	
	5.1.2e	5.1.2e	

6 - 6

5.1.2a