To: (10)(2e) [(10)(2e)@nhsbt.nhs.uk]; (10)(2e) [(10)(2e)@sanquin.nl]; (10)(2e), (10)(2e) [(10)(2e)@sanquin.nl]; (10)(2e) [(10)(2e)@sanquin.nl]; (10)(2e) [(10)(2e)@rivm.nl]; (10)(2e) (10)(2e) [(10)(2e)@phe.gov.uk]; (10)(2e) [(10)(2e)@phe.gov.uk]
Cc: (10)(2e) [(10)(2e)@ndcls.ox.ac.uk]
From: (10)(2e)
Sent: Sat 10/31/2020 6:44:27 PM
Subject: RE: SCoV-2 virusneutralization in UK and in NL
Received: Sat 10/31/2020 6:44:27 PM
rijkers et al 2020.pdf
jiaa463_suppl_supplementary_method.docx

Hi,

I must have overlooked that request (not so difficult seeing that 60% of my inbox is in a permanent unread situation).

Please find the details here (papers attached):

SARS-CoV-2 Neutralization Assay

Sera were tested by a SARS-CoV-2-specific virus neutralization test (VNT) based on a protocol described previously

with modifications [12]. Two-fold serial dilutions, starting at 1:10, of heat-inactivated serum samples (30 min 56

°C) were incubated in duplicate with 100 TCID50 of SARS-CoV-2 for 1h at 35 °C. In our experience as international reference laboratory for respiratory viruses (including other human coronaviruses) we have come to grow all other respiratory viruses at 35 °C rather than at 37C because they grow much better and too much higher yields at that temperature. For that reason, for SARS-CoV-2 also 35 °C was used. The titers achieved are higher than at 37 °C. African green monkey (Vero-E6) cells were added in a concentration of 2 x 104 cells per well and incubated for three days at 35°C in an incubator with 5% CO2. The 50% and 90% VNT titer was defined as the highest serum dilution that protected more than 50% or 90% of cells from CPE was taken as the neutralization titre.. Samples with titers equal to 10 and higher were defined as SARS-CoV-2 seropositive.
SARS-CoV-2 strain: HCoV-19/Netherlands/ZuidHolland_10004/2020 (EVAg cat.nr. 014V-03968)

Cheers (10)(2e)

From: (10)(2e) <(10)(2e)@nhsbt.nhs.uk>
Sent: zaterdag 31 oktober 2020 19:08
To: (10)(2e) <(10)(2e)@rivm.nl>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@rivm.nl>; (10)(2e) <(10)(2e)@phe.gov.uk>; (10)(2e) <(10)(2e)@phe.gov.uk>
Cc: (10)(2e) <(10)(2e)@ndcls.ox.ac.uk>
Subject: RE: SCoV-2 virusneutralization in UK and in NL

Thanks (10)(2e) for following this up. I am keen to do this but was awaiting to get more information re methods... left it after did not got any responses after 2nd reminder. However, I assume it might be difficult to distribute SOPs (especially when yours is likely in Dutch!), so if you could respond to these questions and provide a reference for your method, I will continue with this work. I have most of these details already for PHE method from (10)(2e)

- Cell line used
- Virus strain used (sequence available/ GenBank number)
- Amount of virus used
- How long incubated
- Overlay used in case of plaque assay

Best wishes (10)(2e)

From: (10)(2e) < (10)(2e) @rivm.nl>
Sent: 31 October 2020 15:05
To: (10)(2e) < (10)(2e) @nhsbt.nhs.uk>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e) < (10)(2e) @rivm.nl>; (10)(2e) < (10)(2e) @phe.gov.uk>; (10)(2e) < (10)(2e) @phe.gov.uk>
Cc: (10)(2e) < (10)(2e) @ndcls.ox.ac.uk>
Subject: RE: SCoV-2 virusneutralization in UK and in NL

Hi (10)(2e)

I was wondering whether you did manage to draft the short note?

Best wishes (10)(2e)

From: (10)(2e) < (10)(2e) @nhsbt.nhs.uk>
Sent: dinsdag 15 september 2020 14:41
To: (10)(2e) < (10)(2e) @rivm.nl>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e) < (10)(2e) @rivm.nl>; (10)(2e) < (10)(2e) @phe.gov.uk>; (10)(2e) < (10)(2e) @phe.gov.uk>
Cc: (10)(2e) < (10)(2e) @ndcls.ox.ac.uk>
Subject: RE: SCoV-2 virusneutralization in UK and in NL

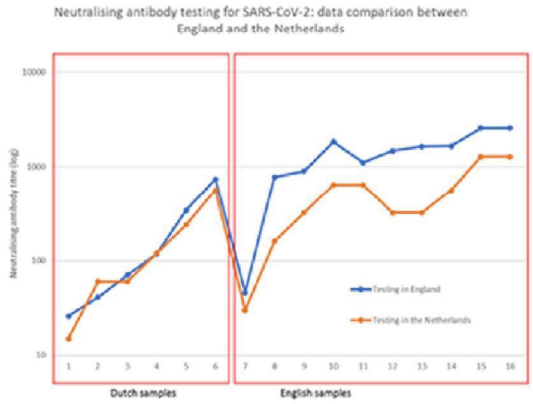
Dear All

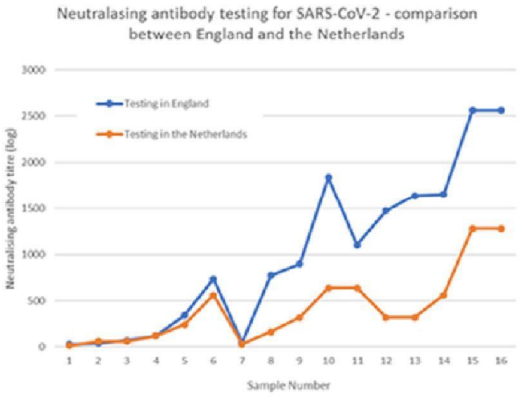
Many thanks for you help with sending samples and testing them for neutralising antibodies. I am finally following up the comparison of neutralising antibody testing between UK and NL. First figure showing nAb titre as in log scale (as it should be) and the second without. Results are amazingly similar, although I think we are using rather different methods. I think it would be really useful to write this up as a very short note, perhaps including some data on nAb titres (with or without epi data) seen in CP donors in UK and NL as that was the reason why we started that (i.e. titres in NL donor lower). There will be more comparative data coming with the EBA/CP/HORIZON2020 project, but this would be a good start.

I am happy to start drafting but it would be useful if could provide the more notes on testing / or perhaps simply share your SOP? Only question I have is whether we should consider repeating the testing of UK panel (using aliquots from our bioarchive, not PHE sample) send to NL in UK – NL results are slightly lower (but always within 1log) and that would help to discuss the potential effect of storing and sending etc..

Let me know what do you think, or if you have any further thoughts on that.

Best wishes (10)(2e)





From: (10)(2e) <(10)(2e)@rivm.nl>
Sent: 21 August 2020 13:53
To: (10)(2e) <(10)(2e)@nhsbt.nhs.uk>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@rivm.nl>
Cc: (10)(2e) <(10)(2e)@ndcls.ox.ac.uk>
Subject: Re: SCoV-2 virusneutralization in UK and in NL

Dear all,

We have tested the PHE samples. Looking forward to get the comparative PHE data so we know where we stand * , The comparison on the sera from the Netherlands showed similar results, hopefully these as well.

Best wishes (10)(2e)

PHE sample nr.	VNT50	VNT90
3000000428	>640	160
3000000429	320	30
3000000430	320	60
3000000431	320	80
3000000432	560	40
3000000433	160	20
3000000434	30	<10
3000000435	640	60
3000000436	>640	40
3000000437	640	40

From: (10)(2e) <(10)(2e)@nhsbt.nhs.uk>
Sent: Friday, 17 July 2020 16:54
To: (10)(2e); (10)(2e); (10)(2e); (10)(2e); (10)(2e)
Cc: (10)(2e)
Subject: RE: SCoV-2 virusneutralization in UK and in NL

Dear All

Many thanks (10)(2e) Apologies for the delay from our end but our samples will be shortly ready for courier. (10)(2e) is organising this and was asking for contact details/address where to send these samples?

Best wishes (10)(2e)

From: (10)(2e) <(10)(2e)@rivm.nl>
Sent: 15 May 2020 07:27
To: (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e)

< (10)(2e) @nhsbt.nhs.uk>; (10)(2e) < (10)(2e) @sanquin.nl>; (10)(2e)
< (10)(2e) @phe.gov.uk>; (10)(2e) < (10)(2e) @rivm.nl>
Cc: (10)(2e) < (10)(2e) @ndcls.ox.ac.uk>
Subject: Re: SCoV-2 virusneutralization in UK and in NL

Hi,

I think it is as informative to exchange protocols. The explanation might be very simple f.i. how you read-out and call titres might be different.

What we see, and others as well, is that neut titres in general are not high espcially in those midly ill.

There are indeed a lot of factors that might, explain the differences, most likely visible when comparing protocols.

We are happy to test some UK plasmas as well.

Cheers (10)(2e)

From: (10)(2e) < (10)(2e) @sanquin.nl>
Sent: Friday, 15 May 2020 08:00
To: (10)(2e); (10)(2e); (10)(2e); (10)(2e); (10)(2e); (10)(2e)
Cc: (10)(2e)
Subject: SCoV-2 virusneutralization in UK and in NL

Dear colleagues,

I contacted (10)(2e) and (10)(2e), who at the RIVM performed the neutralisation of NL donors.

They are happy to join an effort to explain the differences of UK and NL donor titres.

But first let us exchange some samples?

with kind regards from (10)(2e)

Sanquin

Van: (10)(2e)
Verzonden: vrijdag 15 mei 2020 07:44
Aan: (10)(2e); (10)(2e); (10)(2e)
CC: (10)(2e); (10)(2e)

Onderwerp: RE: virusneutralization assay request

Dear (10)(2e)

Thanks for participating in this. It may indeed also be related to lag between onset and donation. The best way is to find out. How much plasma do you need for a neutralization assay? It would be ideal to test the samples that have already been tested at the RIVM because we already have the results from eight serological assays, but the amount of sample is rather limited.

With kind regards,
(10)(2e)

From: (10)(2e) [(10)(2e)@nhsbt.nhs.uk]
Sent: 14 May 2020 22:28
To: (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@phe.gov.uk>
Cc: (10)(2e) <(10)(2e)@ndcls.ox.ac.uk>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>
Subject: RE: virusneutralization assay request

Thanks (10)(2e) and Hello (10)(2e) and (10)(2e)

This is really interesting, especially when you also have the EUROimmun data and that seems more similar to our data… Do I remember correctly that you collect 14 days from onset (or recovery) whereas we are collecting at least 28 days from recovery? If this is true, then in our case bigger proportion of antibodies could be neutralising/higher affinity? But agree, would be good to see that this is not an assay related issue…

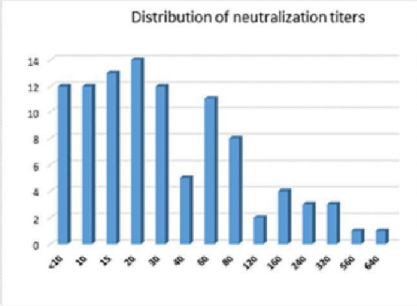
Best wishes, (10)(2e)

From: (10)(2e) <(10)(2e)@sanquin.nl>
Sent: 14 May 2020 21:18
To: (10)(2e) <(10)(2e)@nhsbt.nhs.uk>; (10)(2e) <(10)(2e)@phe.gov.uk>
Cc: (10)(2e) <(10)(2e)@ndcls.ox.ac.uk>; (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@sanquin.nl>
Subject: RE: virusneutralization assay request

Dear (10)(2e)

Thanks for the offer. I have copied (10)(2e) in, he works with (10)(2e) and (10)(2e) Maybe he can directly contact you and (10)(2e) on how to proceed.

Below are our neutralization titers, far lower than yours. (it is a classical plaque reduction test, and this is even the titer giving only 50% reduction) Whereas if we use your Euroimmune algorithm, about 1/3 of our samples would have satisfying titers. Of course, we need standards even when using the same Elisa. But there is no reason to believe that our donors would have lower titers than in the UK.



The neutralization test is done by (10)(2e) and (10)(2e) at the RIVM.

See you Saturday morning

(10)(2e)

From: (10)(2e) [(10)(2e)@nhsbt.nhs.uk]
Sent: 14 May 2020 22:07
To: (10)(2e) <(10)(2e)@sanquin.nl>; (10)(2e) <(10)(2e)@phe.gov.uk>
Cc: (10)(2e) <(10)(2e)@ndcls.ox.ac.uk>
Subject: RE: virusneutralization assay request

Dear (10)(2e)

Many thanks for your email, and it was also good to catch up today. I do agree that it would be really good to get an idea how our convalescent plasma titres compare. It would be also good know what assay RIVM is using for neutralisation assay (or who is doing those for you? I have some long-term collaborators there, but they mostly work on enterovirus field..), and what ELISA you are using?

I discussed this briefly with (10)(2e) yesterday (our virology expert at PHE); she was happy to consider testing of few of your samples and hence I have copied her here. Hope we can get this moving smoothly and looking forward to seeing the comparative data.

Best wishes,(10)(2e)

From: (10)(2e) <(10)(2e)@sanquin.nl>
Sent: 12 May 2020 13:58
To: (10)(2e) <(10)(2e)@nhsbt.nhs.uk>
Cc: (10)(2e) <(10)(2e)@ndcls.ox.ac.uk>
Subject: virusneutralization assay request

Dear (10)(2e)

Congratulations with your nice paper on the comparison between antibody tests and virusneutralising titers.

We are doing similar comparisons, and one of the most striking differences are the virus neutralizing titers we get back from the virology lab (RIVM).

Only about 10% of our convalescent plasma's had titers > 1:100, whereas the far majority showed very high OD's in the different ELISA's. Would it be possible to send you some of our sera, to get neutralizing titers in your microneutralisation test? I think only 3 would already be enough.

Another question: I thought all labs were using the same virus strain for the neutralization. But, I now see you are using SARS-CoV-2 isolate England/02/2020. Do you think that will make huge differences? We also should take that into account in our standardization QA rounds. I told the EQM in Strassbourg that everyone was using the same virus and VeroE6 cell-line. We have to discuss that later this week.

I hope you will find some time to answer my mail. We are all very busy…

Best wishes

(10)(2e)

Prof.dr. C. Ellen van der Schoot, MD, PhD | Dept. Experimental Immunohematology | Sanquin Research, and Landsteiner Laboratory | Academic Medical Center, University of Amsterdam | Plesmanlaan 125 | 1066 CX Amsterdam | The Netherlands | Tel +31 (10)(2e) Fax +31 (0)20 (10)(2e) | Web: http://www.sanquin.nl, http://www.ihe.sanquin.nl

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