#	Question	Asker Name	Answer(s)
1	Is the comparative data derived from samples from clinical trials or during wide scale vaccine roll out?	Vish Nene	Hello, thank you for your question. We have included as many clinical trial data as there is available. The bulk of the results however came from data obtained during vaccine roll-out (from March, 2021 onwards). Given this time frame, we can safely assume that the data were obtained during wide-scale rollout (in most countries). Some of the data included vaccines administered to high-risk groups (for example healthcare workers), which have received the vaccines earlier than the general population.
2	Please post the link in the chat (for the WHO Manual for Secondary Standards)	Manuel Franco	WHO Manual for secondary standards for antibodies against infectious agents - posted on WHO Biological website for public consultation by 30th Nov 2021 https://www.who.int/health-topics/biologicals#tab=tab_1
4	Will you also generate an international standard based on sera from vaccinated subjects (on naive background)?	Börries Brandenburg	We are considering a secondary reagent which is calibrated to the WHO IS which is plasma or serum from vaccinees, no infected, but we have not sourced any material yet
5	This question is for Giada Mattiuzzo - can you comment further on misuse of the WHO IS, in particular the most common misconceptions or misuse of the IS seen in the literature?	Anonymous Attendee	The most common mistake is the use of the WHO IS as a validation tool. I have seen leaflets from kit manufacturers which state they have reproducible results in testing the WHO IS and therefore their assay is validated or standardized. The WHO IS is a calibrant not a validation tool.
7	Does the international standard accommodate for high sensitive antibody detection methodologies?	Adrian McDermott	live answered
8	Can one of the panelists - perhaps Ioannis - comment on the correlation analysis between ELISA binding titers and neuts? I understand the correlation with spike vaccine antigens has been reasonable, however have efforts been made to compare neutralization responses generated by RBD-based vaccines with ELISA data against spike protein? If so, do the correlations still hold? Have correlation analyses been performed using RBD-	Douglas Holtzman	From Ioannis : I am currently working on correlating ELISA and live neutralisation assay results. The work is still ongoing (early stages) and I cannot make a comment about the outcome yet. However, the question was also addressed in David Goldblatt's presentation (slide 184 of the slide deck).
9	If I establish relation of IS to a secondary standard used in my lab to each VOC individually, can I keep using secondary standard for all assays moving forward and converting it to IS for VOC pseudovirus neut assay?	Anonymous Attendee	live answered
10	For Dr. Mattiuzo: The establishment of the IS for neutralization assays seems clear… but for binding assays what antigens will be considered? Are RBD assays being considered?	Manuel Franco	The IS may be used for calibrating RBD assays. But please state that this is what you measured when reporting the data in units. e.g. 150 BAU/ml IgG against RBD

11	I have taken a very different approach to antibody detection by constructing a hybrid double antigen binding assay which measure anti-RBD and predicts/quantifies neutralizing antibody. It produces a good calibration against the WHO standard. The assay is class and species neutral which could allow an immunized animal as a secondary standard, not available to most conventional immunoassays, has anyone done anything similar?	Richard Tedder	live answered
12	We have tested the 21/234 IS (3 times) in our Virus Neutralization test and have obtained an NT50 of 895. In the leaflet of the 21/234 IS, the Neut Ab GM is 1473 IU/ml. So does this mean we have to multiply our NT 50 by 1,65 (1473/895) to have IU ?	Leo Heyndrickx	The intended use is to calibrate the internal control in your assay in IU/mL. The data will be reported relative to the internal control, there is no multiplication factor
13	Do any of the national regulatory agencies make use of the WHO standards?	Wellington Sun	live answered
14	Dr Tedder could you please provide a reference for your assay?	Manuel Franco	
15	Thank you.....Mark - I agree but is this calibrated down to ug or pg/ml...important in pediatrics	Adrian McDermott	We would advocate reporting units (BAU/mL or IU/mL) as this measures the biological activity.
16	Please, how do you use the IS with IC50 of neutralization when using pseudotype neutralization?	Alphonsus Ugwu	The data we have received showed that the WHO IS can be used in the same way in live virus neut assay as in the pseudotype neutralization assays. If I misunderstood your question, please feel free to email me directly giada.mattiuzzo@nibsc.org
17	Giada. Should this standard be used by plate or just to standardize the experiment with many plates (bulk)?	Anonymous Attendee	Hi, we recommend to have your own standard and run it in every experiment. Depending on the reproducibility of your assay, you may not need to have the standard in every plate (I don't).
18	Why not concentrate on a more defined target for antibody, easier for calibration hence use RBD specific antibody? This is also biologically relevant to virus neutralization and relevant to vaccine responses.	Richard Tedder	live answered
22	Mark this is exactly what the hybrid DABA does	Richard Tedder	live answered
21	If secondary standards are not parallel (dose response curve) to the IS standard should they not be used	Mary Matheson	Typically yes, but there are measures you can take with the scope of PLA to help them become "more parallel." The first measure is to log transform your result data and reassess parallelism, the second and final approach is you can remove outlier results at higher-dilutions so long as you have a minimum of 3 results at 3 dilution levels. If you still aren't with 10% parallelism, then you won't be able to confidently convert the secondary standard result into the harmonized BAU of the WHO IS.
23	Thank you Ivana for addressing the issue of NRA use of WHO standards. As we move forward to approvals based on immunogenicity, these international standards are going to be critical.	Wellington Sun	live asnwered

24	Stan Plotkin told us that a correlate is a functional assay that reflects the mechanism of protection and a surrogate is something that just correlates. I think that is a bit back to front but Stan is the boss… https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2897268/	Adam Finn	Comment, no answer needed
26	Can multiplex VNT determine which variants patients are infected with?	Lynn Chen	live answered
27	Did you compare other multiplex platforms before selecting Luminex. Others like MSD don't require biotinylation, and tend to have enhanced precision over Luminex, etc.	Jill Gilmour	No we didn't for two reasons: 1) We have the Luminex system set up in our team already' 2) We did compare coating of RBD with and without biotinylation and the latter increased sensitivity and differentiation reliability.
28	Is the broader Ab response to vaccination following recovery dependent on the severity of COVID?	Vish Nene	As I stated, it was from SARS patients 17 yrs. ago and they were all severe patients
30	for the multiplex VOC assay, how do you determine inherent VOC neut activity in the absence of competitive binding (i.e. situation of infection with a single variant). Is the measured VOC neut activity skewed by competition?	Anonymous Attendee	As I stated briefly, we compared singleplex VOC sVNT vs multiplex VOC sVNT, we found the multiplex version has more differentiation power as it eliminates assay-to-assay or well-to-well variation
31	Why are beta foci larger than all the others?	Adam Finn	Answered live
32	For Dr. hallis, do you perform your neutralization assays in TMPRSS2 + ACE2 cells or parental Veros? We have observed different changes in neutralization changes for variant viruses using different cell lines (Vero vs. Vero TMPRSS2).	Natalie Thornburg (CDC)	Answered live
33	Is there any publication about multiplex VNT could be shared?	Lynn Chen	Here is the paper: Tan CW, Chia WN, Young BE, Zhu F, Lim BL, Sia WR, Thein TL, Chen MI, Leo YS, Lye DC and Wang L-F (2021) Pan-Sarbecovirus Neutralizing Antibodies in BNT162b2-Immunized SARS-CoV-1 Survivors. N Engl J Med doi: 10.1056/NEJMoa2108453. PMID: 34407341.
34	Are there changes in neutralization activity with the live viruses from the time of isolation to the various passages in production cells	James Nyagwange	We have assessed and determined the passage number of cells and only use specific range. For the virus, we always run virus-only controls to ensure the neutralisation activity is as expected
35	To Dr.Shi, are your viruses caring the reporter gene attenuated or they still need to be handled in BSL-3?	Hongquan Wan	live answered
36	Dr Shi - the booster slide was for immunizations and boosters with which vaccine? Pfizer-BNT?	Larry Dumont	Yes. The booster is also Pfizer-BNT vaccine

37	Thanks Dr. Shi for the great presentation. It looks like your PRNT assay is performed under tight control, however, without including the WHO IS, it is hard to compare to other labs and hard to compare to other neut methods. Also, is this assay validated? any cross-reactivity between different chimeric viruses noticed? Thanks.	Branda Hu	The assay has been validated by comparing with the gold standard PRNT assay (Nat Commun. 2020 Aug 13;11(1):4059. doi: 10.1038/s41467-020-17892-0.). The assay has also been shared with many academic labs around the world for research and vaccine development (e.g., N Engl J Med. 2021 Jan 7;384(1):80-82. doi: 10.1056/NEJMc2032195).
39	Wonderful presentation Dr. Shen. In your construction of variant antigenic cartography do you think it can use similar analysis of other viruses, e.g. flu, dengue, based on antigenic distance, to predict emergence of neutralization escape to result in formation of different serotype?	Wellington Sun	Not answered
40	'@Dr. Goldblatt, for your binding / neutralization work, have you specifically looked at just peak titers, or also titers after some waning?	Natalie Thornburg (CDC)	Not answered
41	Dr David, in the graph correlating the binding ab levels for alpha, delta and WT the ab titer was shown as BAU/ml, what was the conversion factor used for the VOCs? As discussed earlier the WHO BAU/ml does not apply for the variants… (We also use the MSD platform for binding ab - panel 7)	Mariana Marmorato	Not answered
42	how confident are you that NRA would accept the Cop in RBD and Neut for conditional licensure? Is Ace2: RBD assay standardized so can be used as RBD for CoP?	Farshad Guirakhoo	Valneva's Phase III met it's co-primary endpoints of superiority in nAb GMT and non-inferior seroconversion vs. AZ in the UK. A CoP has not been established, but this was accepted as an immunological comparative study
43	For anyone / everyone, Does anyone have opinions about what a true "escape" variant might look like? Do we need to just rely on real work VE? What might the lab data look like?	Natalie Thornburg (CDC)	For the time being, there are no actual threshold data for SARS-CoV-2. For influenza, a 4 fold difference in hemagglutination inhibition titers (compared to vaccine seed strains) is considered an indication for immune escape.
45	For next generation of Covid vaccine, would variant specific immunoassay to measure binding antibody be important or extrapolate from binding antibody result against wuhan strain would be sufficient/informative to predict vaccine efficacy?	Lynn Chen	I think it is very like feasible. However the slope will be different for different variants. It may require testing a smaller set of samples first to work up the formula for each variant.
47	Can we discriminate correlates of protection for acquisition of infection based on NC seroconversion or PCR+ swabs vs symptomatic disease.	Anonymous Attendee	Not answered

48	A comment on correlates. It is not necessary to have a very clear separation of antibody levels vaccine breakthrough cases vs. non-cases to be able to have an applicable correlate. The needed output is the relationship between vaccine efficacy and the post-vaccination antibody level. From this curve, one can select the antibody level associated with whatever degree of vaccine efficacy is considered high enough for public health applications. From the Moderna COVE trial correlates analysis, an ID50 titer of 10 IU50/ml was associated with >= 90% vaccine efficacy, and an MSD bAb Spike level of 33 BAU/ml was associated with >= 85% vaccine efficacy. These set benchmark threshold that could potentially be used (which can be put to the test in future studies).	Peter Gilbert	Comment, no answer needed
49	On Question 2, does the Panel agree with the current FDA serologic criteria for demonstrating non-inferiority of neutralizing antibody response, i.e. using GMT ratio of 1.5 and seroconversion rate difference of 10%?	Wellington Sun	Answered live
50	Given the revolution in systems biology and AI, should we also be looking at complimentary and unbiased way to elucidate correlates of protection?	Anonymous Attendee	Not answered
51	I guess though that waning binding or neutralization antibody over time - and trying to gauge a critical threshold - ignores the likelihood that B memory responses will be fast enough in primed individuals to prevent infection or prevent illness given the incubation period of 4+ days. So assays or experiments that evaluate induction of or persistence of memory may be valuable/predicative - ?	Adam Finn	Not answered
52	Great presentations and discussions. When can we get access to the recording of the section?	Anonymous Attendee	Slides and workshop materials will be posted here: https://epi.tghn.org/covax-overview/enabling-sciences/ , but not the a recording.