World Health Organization

WHO R&D Blueprint

COVID-19

Reagents, Cross-reactivity and Immune Assays Working Group

March 18 – April 1st, 2020

Geneva, Switzerland

R&DBlueprint

Powering research
to prevent epidemics

18 March 2020 - TC

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Participants

Members of the R&D Blueprint Cross-Reactivity expert group

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Experts invited but unable to attend

Ralph Baric (UNC), Cheryl Bennett (GSAID), Karen Bok (NIAID), Brooke Bozick (NIAID), Christian Berchot (GVN), Darin Carroll (CDC), Monalisa Charrerji (BMGF), Carolyn Clark (CEPI), Kizzmekia Corbett(NIAID), Ian Crozier (NIAID), Inger Damon (CDC), Peter Daszak EcoHealth Alliance), Emmie DeWit (NIAID), Marciela DeGrace (NIAID), Dimiter Dimitrov (U of Pittsburgh), Christian Drosten (Charité - Universitätsmedizin Berlin), Karl Erlandson (BARDA), Darryl Falzarano (VIDO – Intervac), Matthew Frieman (U of Maryland), Luc Gagnon (Nexilis), Susan Gerber (CDC), Volker Gerdts (VIDO-Intervac), Barney Graham (NIAID), Erica Guthrie (CDC), Bart Haagmans (Erasmus), Lisa Hensley (NIAID), Paul Hodgson (VIDO-Intervac), Mike Holbrook (NIAID), Lakshmi Jayashanakar (BARDA), Dan Jernigan (CDC), Reed Johnson (NIAID), Jacqueline Kirchner (BMGF), Marion Koopmans (Erasmus), Joo-Yeon Lee (Korea CDC), Steve Lever (dstl), Hee-Young Lim (Korea CDC), Jim Little (BARDA), John Mellors (Univ of Pittsburgh), Kaitlyn Morabito (NIAID), Vincent Munster (NIAID), Scott Napper (VIDO-Intervac), Jae Ouk Kim (IAVI), Gustavo Palacios (USAMRIID), Jo Prior (dstl), Nicola Rose (NIBSC), Connie Schmaljohn (NIAID), Manki Song (IAVI), Erik Stemmy (NIAID), Natalie Thornburg (CDC), Tracy Thue (VIDO-Intervac), Julia Tree (PHE), Sylvie Van Der Werf (Institut Pasteur), Seshadri Vasan (CSIRO), Linfa Wang (Duke-NUS), Larry Wolfraim (NIAID), Tianlei Ying (Fudan University), Shi Zengli (Wuhan Inst. of Virology)

WHO Secretariat

Pierre Gsell, Ximena Riveros-Balta, Alejandro Costa

Objectives of the call

- To obtain an update of the status of development/availability of critical materials and reagents needed to formally evaluate cross-reactivity between SARS-CoV-2 and other coronaviruses in the laboratory
- To present any new data that has been generated which evaluates cross-reactivity

- Develop and standardize immune assays to support vaccine pre-clinical and clinical development

A link to the WHO sharepoint has been sent out to all members of the group. This contains minutes of previous call, a spreadsheet summarizing reagents available and a folder for protocols.

Updates on Status of Viruses and Reagents

Virus isolates

The only new viral isolate reported was NY-1, which has been sent to BEI, but there was not an idea when that will be available. There were no new isolates reported to be going into the European Archive.

DMID reported that BEI will be working on standardizing conditions including: challenge strain; optimization of cell culture conditions; Plaque assay and TCID(50) (and how the two compare); inactivation conditions for virus stocks and serum. This has not started yet.

Inactivated virus preps : two purposes - a control for nucleic acid extraction and RT-PCR and a control vaccine for studies of disease enhancement. No updates on this topic.

There was a pre-print in the literature (on the sharepoint) showing loss of activity by heat inactivation of sera. Mount Sinai stated that they typically do heat inactivation. They have looked at sera before and after heat inactivation for influenza and see no difference in activity. They will look at this for COVID-19.

Convalescent serum and B cells from SARS-CoV-2 patients and mAbs

NIBSC reported that they are identifying patients, recruiting and collecting large amounts of serum and plasma. Timeline for availability is end of April. The MERS international standard shows cross-reactivity and cross-neutralization, titer of 1:124 in PRNT (standard in MERS PRNT is 1;600) at PHE. There are 1000 ampules of material available. This could be used as an interim standard. The MERS SAB material showed no neutralization titer at PHE (chemically inactivated virus as immunogen). CDC is in the process of testing as well.

VIDO-Intervac used anti-MERS sera from alpacas that had titers between 1:500 and 1:1000 in MERS PRNT assay. There was no activity in a SARS-CoV-2 microneutralization assay.

There was more discussion of the use of the MERS international standard. This was developed in conjunction with CEPI and they will need to be consulted, as the agreement under which it was produced said that it had to be used for MERS.

Assays and cross reactivity

Mount Sinai has developed ELISAs based on the RBD and Full-length stabilized Spike protein. This has been tested with sera from three COVID-19 patients including one with multiple time points. They tested this against a panel of sera from 60 respiratory patients including NL63. They found no reactivity to this panel, no background. They have a manuscript and are trying to upload to the sharepoint. Seroconversion was observed early in infection, as early as day 3, and 6. Question was asked– will they be followed long term? The answer was that these sera are from collaborations from Helsinki and Melbourne, no further access. But they are enrolling patients at Mt Sinai now.

The IgG3 is higher than IgG1, which is unusual.

A question was asked whether institutions have open protocols to collect samples, across the world. What protocol was Mt Sinai using? Is it an open prospective study and is it COVID-19 specific? There are two open protocols . the first is a long-term influenza protocol. The second allows enrollment for any infectious disease.

PHE shared data on mAbs from Mass Biologics. With the anti-SARS-CoV-1 mAb 201, which has been published, there was no cross reactivity observed in a PRNT assay to SARS-CoV-2. A second unpublished mAb was tested and had a titer of 1:27 against SARS-CoV-2; unknown against SARS-CoV-1.

The question was asked whether there is an assay that differentiates between different coronaviruses, for use in pre-screening animals. There was no response.

Establishment of centralized testing labs is still in progress, and BMGF will have more to talk about next week.

Flow cytometry assays: from animal models call, we know some are in use. Proposal to share antibody panels and protocols. Univ of Chicago said they are optimizing assays now.

For T cell assays, peptides have been ordered by several groups but no other updates.

A new working group on serosurveys is starting this week and the group was asked to contact WHO if they are interested in participating.

25 March 2020 - TC

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Participants

Members of the R&D Blueprint Cross-Reactivity expert group

Ralph Baric (UNC), Brooke Bozick (NIAID), Christian Berchot (GVN), Miles Carroll (PHE), Kizzmekia Corbett (NIAID), Marciela DeGrace (NIAID), Rafael Delgado Vazquez (Salud Madrid), William Dowling (CEPI), Darryl Falzarano (VIDO – Intervac), Clint Florence (NIAID), Simon Funnell (PHE), Luc Gagnon (Nexilis), Barney Graham (NIAID), Lisa Hensley (NIAID), Paul Hodgson (VIDO-Intervac), Rachel Ireland (dstl), Jacqueline Kirchner (BMGF), Florian Krammer (Mt. Sinai), Gerald Kovacs (BARDA), Greg Kulnis (Nexilis), Teresa Lambe (Univ of Oxford,) Janet Lathey (NIAID), Karen Makar (BMGF), Giada Mattiuzzo (NIBSC), Kayvon Modjarrad (WRAIR), César Muñoz-Fontela (BNITM), Scott Napper (VIDO-Intervac), Nisreen Okba (Erasmus), Mark Page (NIBSC), Barbara Schnierle (PEI), Amy Shurtleff (CEPI), Ashley Smith (BARDA), Erik Stemmy (NIAID), Natalie Thornburg (CDC), David Vaughn (BMGF), Patrick Wilson (U of Chicago)

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Assay Presentations

There were 4 presentations. Slides that were presented are available on the Who Assay group sharepoint site.

The first presentation was by Natalie Thornburg from CDC on their serological assays.

The ELISA in use at CDC is based on the NIAID VRC recombinant protein (pre-fusion stabilized spike). They coated plates at 0.15 µg/mL and used a goat anti-human pan Ig HRP secondary antibody. They tested 6 COVID-19 patient sera and a panel of US healthy adult sera samples from 2017. The OD cut off for positivity was 0.4. The sensitivity and specificity were 97%.

They analyzed cross-reactivity with other coronaviruses in this assay- NL63, OC43 and HKU, both acute and convalescent. Overall there was no cross-reactivity observed. There were some raises in OD but below the cutoff. MERS and SARS samples were also assayed. Convalescent MERS and SARS-CoV-1 sera reacted , as well as one MERS sera from transchromosomal cows (SAB-301) , although another MERS sera from SAB (SAB-300) did not cross react.

A microneutralization test using USA_WA1-2020 isolate was described. All convalescent sera were at a maximum neutralization titer of 1:640. The level of detection was 1:20. Some cross-neutralization with other coronaviruses (SARS1, MERS and HKU1) was observed, with some other reactivity (229E and NL63) close to the limit of detection. There was no cross neutralization with SAB 300 or SAB 301.the question was asked why there was cross neutralization of HKU1, 229E and NL63 when there was no cross reactivity in ELISA. The reply was that this was a single experiment and repeats were needed. Ralph Baric from UNC indicated he did not see any cross-neutralization with SARS1 using serum samples from the SARS-CoV-1 outbreak. Bart Haagmans from Erasmus also mentioned some papers that showed no cross neutralization between SARS-CoV-1 and 2. A question was also asked whether they had titers against the seasonal coronaviruses to match what was done here against SARS-COV-2 and the answer was that this had not been done. Also an experiment at UMD also revealed no cross reactivity.

The next presentation was by Barney Graham from the NIH Vaccine Research Center.

The VRC assay is a SARS-CoV-2 IgG ELISA based on a stabilized prefusion spike. The paper from Wrapp et al in the March 12, 2020 issue of Science showed that 2P mutations effectively stabilized the SARS-CoV-2 spike. The purpose of the assay is not a diagnostic assay but for quantification of antibody in response to vaccine. They optimized the ELISA parameters including antigen concentration, coating conditions, blocking reagents, secondary antibody HRP concentration, and substrate brand. They used mAbs including and RBD-specific mAb, an S1-NTD specific mAb and an S2 specific mAb. There was no background with healthy adult sera, an HKU1 mAb or a MERS mAb. There was reactivity with a SARS-CoV-1 mAb. There was good reactivity with 4 COVID-19 convalescent human sera and 1 plasma sample, bit no very little with an acute sera. The assay shows humoral immunity in mice vaccinated with the Moderna mRNA vaccine, 2 weeks after a single dose , which was boosted 2 weeks after a second dose (doses .01, .1, 1 and 10 µg). Assays in development include pseudotyped lentivirus and VSV reporter assays, Live virus neutralization and reporter virus neutralization assays. A question was asked about the backbones for the pseduoviruses. For the Lentivirus, it is similar to other VRC constructs for influenza and MERS (Pallesen et al PNAS Aug 29 2017), using Huh7.1 cells. Not sure with one for VSV, adapted from one used by Nancy Sullivan in Vero E6 cells. Another question was asked whether they have tried truncated spike to increase lentivirus yield. Yes, a truncation of the cytoplasmic tail increased expression spike on cell surface.

The third presentation was from Nisreen Okba and Bart Haagmans from Erasmus University.

The SARS-CoV-2 Nucleocapsid protein and Spike protein sequence conservation was analyzed. They tested a commercial Nucleocapsid assay and in house produced Spike, S1, $S1^A$ (N-terminal domain) and RBD proteins. Serum from 3 PCR-confirmed patients from France (1 severe and 2 mild) were tested in the various assays and antibodies were detected against Nucleocapsid. S, S1, $S1^A$. and RBD. Neutralizing antibodies were also detected. In all assays, there were higher titers in the serum from the severe case. Antibodies detected by ELISAs strongly correlated with neutralizing antibody responses.
With the S protein ELISA, there was cross reactivity observed with SARS-CoV-1 and some MERS Sera. The S1 ELISA was more specific, with no MERS cross-reactivity and very little SARS-CoV-1 cross reactivity.

They validated the S1, RBD and N ELISAs. They ran several serum panels – healthy blood donors, non-coronavirus respiratory infections, other human coronaviruses, MERS and SARS-CoV-1. The healthy blood donors, non-coV respiratory infections and other human coronaviruses were negative in all assays. there was one MERS positive in the N protein ELISA but not in the RBD or S1. The SARS-CoV-1 reacted in all assays.
They tried to validate 2 beta version of S1 ELISAs from Euroimmun for IgG and IgA, and had sera from Rotterdam and Berlin. Sensitivity was low and had some cross reactivity from human coronaviruses. As a result, Euroimmun made improvements to their assays.
A question was asked about the cross reactive MERS sera. Was their PRNT as well? there was no neuralization seen. Another was asked about the status of the Euroimmun assay. There is a better version available and is being sent back to Erasmus for evaluation.

The last presentation was from Florian Krammer at Mt. Sinai.

There is a preprint available online in MedXriv. His protocols are being shared with many labs worldwide and are available for download online. The protocol and reagents are in about 150 labs worldwide. There are two versions of the spike (soluble RBD and soluble trimerize spike in prefusion format). Expressed in insect and mammalian cells and see differences in size due to glycosylation.
Samples tested: Collection from 20-65 years of age with acute flu, dengue and others. Serum from NL63 infection. Four COVID19 samples (early after symptom onset). COVID-19 patient sera reacted against RBD and spike. There was no reactivity with NL63 or other sera. The area under the curve show significant differences between COVID19 and all the others. There is higher background with insect cell. There was more IgG3 compared to IgG1. High IgM and IgA as well. They will see more samples soon. Assays have been transferred to the clinical lab at Mt Sinai. There is a 2 step protocol. Step I is screening at 1:100 with RBD then step 2 is a confirmatory Spike ELISA with serum dilutions. 500 samples per week. They are screening for plasma donors. Biomedomics RDT was tried and worked in their hands. There is no signal if heat inactivate the sera. A question was asked about samples. Is there any difference between early and late timepoints – early vs convalescent. There is stronger signal with later timepoints. Found at least one asymptomatic case that seroconverted. There are 800 patients at Mt Sinai. A question was asked whether there was an effort to transfer convalescent plasma to patients? Yes there is an intention to do this. A question was asked about isotype

data , with IgG3. Have any other groups seen this? Are Mt sinai noticing any trends? These studies are in progress at Mt Sinai and Erasmus. The plasmids for RBD and full spike for pseudovirions and a small amount of RBD protein are in BEI.

1st April 2020 - TC

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Updates on Status of Viruses and Reagents

Viral Isolates

BEI has received the Hong Kong isolate. It will be released soon in parallel with QC testing. BEI are in process of getting other isolates from CDC, from California, and also one from NY. One virus is also from a fatal case. Not sure if they are planning to acquire more as the sequence seems to be stable.

European Archive has same four isolates. No updates

Recombinant Proteins

The WHO Serosurvey group (Solidarity II) has identified a critical need for a source for large amounts of full-length, stabilized recombinant Spike protein. There were updates from several sources:

Karen Makar from the Gates Foundation reported that they are producing protein for their grantees. Under the contract with Nexilis they will produce additional 2 P stabilized full length spike with His tag and RBD proteins (both constructs from Barney Graham). There is also production of protein at the Institute of Protein Design at the University of Washington from Neil King. They have made batches of full spike with His tag as early reference material. They have generated antibodies as reagents. If one wants a benchmark immunogen , would want without the His tag, produced in something such as CHO cells. That is still under discussion. There will not be enough made for general usage for all labs around the world. There will be some available for benchmarking studies, but there is no intent to take the place of BEI or others. Not enough for the serosurvey piece.

A point was brought up by Florian Krammer from Mount Sinai that a lot of labs associated with medical centers are starting assays right now, with the goal of

screening plasma for use in plasma therapy, and therefore the need for material is immediate and these assays will set up right away. Mt Sinai has an emergency license for use of their assay in NY state, and discussing with FDA. While there is a need for standard protein for serum serveys, there is an urgent need for a few grams of protein for use in saving lives right now. Six or seven hospitals in NY will need gram amounts of protein in the next week. Mt. Sinai is supplying their lab and other labs elsewhere are using his expression vectors (e.g. Chicago, Penn Stanford). Full length is not expressing well (2 mg/L of culture). They are using 293F cells; 293T or CHO should be ok as well. Their ELISA is different than Erasmus, but not vastly different, and both seem to correlate with neutralization. A question was asked about China - they have surveys up and running and must have protein. There was no information on these from the group nor on lateral flow. (Lateral flow in general was felt to be less useful because not quantitative).

The NIH VRC are providing the plasmid but are not able to produce large amounts.

NIAID DMID have an assay task order, similar to what Gates is doing. They are getting an MTA with NIAID VRC to get their plasmid. This will be used in ELISA for vaccine evaluation. There are no plans to make bulk protein. There is a manufacturing contractor at DMID and it is possible that protein could be made under that.

Florian Krammer indicated that his plasmids are at NCI and they might be able to scale up, ,mammalian and insect cell expression.

Luc Gagnon at Nexilis indicated that their first batch will be available at the end of April at 80L scale.

BEI is distributing the RBD plasmid , full length has been sent to BEI but is not online yet. BEI are ramping up production but don't have capability to make enough protein at very large scale.

A question was asked about the differences between the VRC and Mt Sinai constructs - very similar, if not identical . 2 P, Furin cleavage removed; trimerization domain, 6 HIS. Can this be posted on the shared folder? VRCs is published. And Mt Sinai in pre-print. Action item to pull out the sequences in the shared folder.

Does any group that have the plasmids have capability to make large amounts? Duke groups have VRC construct and a production facility. Others include NCI, Stanford, Portugal, Germany.

A protein quality issue was raised by PHE. "PHE has been conducting assessment of several polyclonal and monoclonal reagents raised against MERS-CoV and would like to update all of a significant finding.

We have found that a certain commercially sourced (ie. not BEI) recombinant COVID-19 spike protein, when used as a coating antigen in an ELISA assay, did not result in ELISA reactivity with a monoclonal antibody and a polyclonal antibody, both of which have significant titres in a PRNT assay with live COVID-19. This suggests that some commercial sources of recombinant spike protein may not be suitable for purpose."

Oxford identified a similar issue. Florian Krammer's RBD works well in their hands and Oxford will work on making full spike

Serum

Mark Page from NIBSC presented slides on their efforts. They are preparing reference reagents. For RNA they have made a research reagent, catalog number 19/304, which will be available end of this week/next week. It is full length SARS-CoV-2 genome RNA packaged into HIV-like particles, in Tris/Human serum albumin and trehalose. It is a volume of 0.5 ML at 10^7 copies/mL. Requires extraction , so controls for that part of the assay. Only characterized in house so not a reference standard yet. It will go through the WHO International standard process. They will need inactivated virus for a control and are in need of a validated inactivation protocol for that. They are recruiting collaborative study participants now for the study starting in June 2020; establishment of International Standard by WHO ECBS expected Q1 2021. Groups can back calibrate to International Standard. Similar path for the serological standard, being done in collaboration with CEPI. COVID-19 human convalescent plasma is being prepared as a research reagent from UK plasma, coming in this week. The material is expected to be available by the end of April. Material is coming from the ISARIC4C consortium and the from the NHSBT. The material will be solvent detergent treated to inactivate, the titer determined to pool several high titer patients together and vial at 0.25 mL /vial. For the International standard, convalescent plasma from several countries are being collected (Norway, Italy, Singapore, USA). Monoclonal antibodies can also be added to study. They are recruiting collaborative study participants now for the study starting in June 2020; establishment of International Standard by WHO ECBS expected Q1 2021. Participants are welcome, contact the NIBSC.

A question was asked about China. This is not on the list, is this because NIBSC unable to acquire convalescent sera from there? NIBSC have been talking with

Chinese colleagues but China is making a national standard. NIBSC has asked for some of this in order to include in the collaborative study.

A question was asked whether information regarding preparation of material can be included on the sharepoint and the answer is yes.

A question was asked whether those interested in participating in the collaborative study should send emails directly to Mark. He replied that those interested should email and they will then receive a questionnaire regarding their capabilities and assays they intend to use.

A question was asked about which mAb will be chosen. There are a few in the UK and elsewhere that might be made available. One mentioned was CR3022 which is well characterized, neutralizes, binds RBD and structure and sequence are known. This is from J&J but many researchers have made this from sequence. Since widely used, talk to J&J? Caveat – don't perform as well as sera in assays. But a useful tool.

Are there any updates from the USG on sera or PBMC collection and distribution? The NIAID VRC is expecting COVID-19 convalescent sera samples. DMID said that samples through VRC are going through USG wide group, prioritized and sent out . To date, very few have come in. There is another effort among diagnostic group for a serum panel.

Florian is putting together a small panel for clinical labs in NY , not for wide distribution.

There is a WHO diagnostics group led by Mark Perkins. They have plans to establish a WHO specimen bank, a collection of acute and convalescent sera from well characterized patients. Know pre-COVID-19 exposure history, some for common coronaviruses. The plan is still in draft form as a document. They are trying to determine whether to do something separate or join with other groups. Question – is this in the planning stage or already collected material? Sites are collecting material under other protocols and documents are in preparation for appropriate agreements, sharing of samples , etc. The question was asked whether there will be enough material to share for other purposes. It depends how broad, how many labs and whether dilution samples (say 1:100) could be used.

A source of convalescent plasma – blood bank screening of COVID-19 for blood supply and screening as part of Plasma studies. Remnants form these purposes could be used . Vitalent has a partnership with Blood Works Northwest, screening of US blood and are putting together COvVD-19 materials for passive immunization . If not used for passive immunization , then material available at large scale potentially. Mark page has gotten good material form Vitalent in the past.

Please post protocols on the share point.

WHO Statement of solidarity has been drafted , that started at the developers call. Members of the call are asked to take a look at this.

A meeting of the immune assays group that involves the vaccine developers is planned , so that the developers can get information . Maybe once a month.

Neutralization assays – the question was asked whether anyone has done live virus vs pseudovirions. Nexillis will do in collaboration with PHE, but none done yet.

Pseudovirions being shared include NIBSC and Mt Sinai's plasmid is in BEI. If others will be widely used, please inform the group.