

June 2014 CONSIDE Newsletter

Dear CONSIDE Members,

We're happy to share the second newsletter of CONSIDE updating you on recent progress of CONSIDE.

CONSIDE Work Plan and Recent Achievements

Epidemiology working group

The epidemiology working group has continued to make progress on the development of a comprehensive set of influenza seroepidemiology protocols. Several protocols are available here (<https://consise.tghn.org/articles/available-consise-influenza-protocols/>) and ready to be used. If any members are planning influenza seroepidemiology studies, please consider using one of our protocol templates.

Several of the CONSIDE protocols have been adapted by the World Health Organization for MERS-CoV. Currently, the protocols to evaluate risk factors for infection among MERS-CoV patients, MERS-CoV infection among health care workers and MERS-CoV infection among risk groups are in use in Qatar and Saudi Arabia. The protocols are available here: http://www.who.int/csr/disease/coronavirus_infections/en/.

CONSIDE is actively engaging research institutions and public health agencies in several countries to support implementation and field validation of CONSIDE protocols. The feedback received will be used to improve the protocol templates.

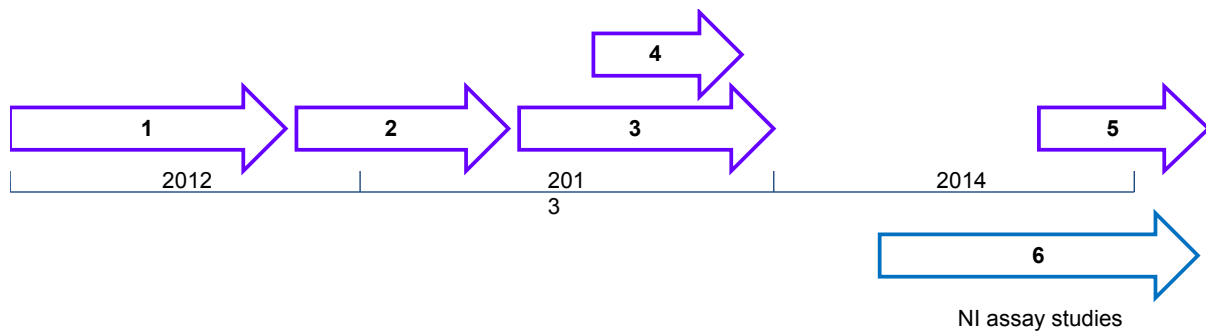
The question bank is still under development. One of the challenges of developing questionnaires to accompany each of CONSIDE's protocol templates is that it is impossible to anticipate all questions in advance as the exact epidemiologic situation and the context of the outbreak will be unknown and unique. To address these uncertainties, the working group is developing a question bank, which holds a collection of questions under major headings such as background information, medical and vaccination history, exposures (animal, environmental, occupational, etc), travel history, signs and symptoms, healthcare worker-specific questions, etc. The question bank is designed to facilitate the rapid development of questionnaires in conjunction with the study protocols. We plan to develop an online interface for users to download specific questions for their own questionnaires.

Laboratory working group

The main focus of the laboratory working group is to coordinate and standardize the international serology laboratory response to new emerging influenza viruses. The approach taken by CONSIDE is as follows:

- Develop a consensus protocol for each of the two main serology assay methods - haemagglutination inhibition assay (HI) and microneutralization assay (MN).
- Compare these consensus assay protocols with local assay protocols in international inter-laboratory studies.
- Evaluate sources of antibody for use as an antibody standard.
- Evaluate new serology assays within the CONSIDE laboratory network

The timetable for these studies is shown in the figure below. As reported in the first CONSISE newsletter, we have developed two consensus protocols for MN; the 2-day ELISA-based and the 3-day haemagglutination (HA) assay-based methods (Figure arrow 1). A comparison of these two assay formats in 11 laboratories using the A(H1N1)pdm09 strain demonstrated that they are comparable and therefore either of these CONSISE consensus protocols could be recommended (Figure arrow 2). MN assay comparison was then extended to H3N2 and H5N1 viruses and a preliminary analysis of the H3N2 results indicate generally good correlation between the two consensus protocols (Figure arrow 3). Analysis of the H5N1 results is still in progress. A major outcome from this exercise is that laboratories within the CONSISE network have now aligned all the parameters used in their assays with the CONSISE protocols established for this study. We hope to complete and prepare these studies for publication in the next few months.



The CONSISE laboratory group strongly supports the use of the HI assay as a primary serology assay for seasonal, pandemic and some novel influenza subtypes, but will assess how it can be better standardized. During the period leading to the September 2013 CONSISE meeting in Cape Town fourteen CONSISE laboratories submitted their HI assay protocols for comparison. Although there were significant differences between the protocols, it was possible to develop and agree on a consensus protocol, largely based on that recommended by the WHO¹ (Figure arrow 4). A comparative study using the HI consensus protocol and the MN assay protocols will be started in the last few months of 2014 (Figure arrow 5). This study will examine laboratory-to-laboratory variability using A(H1N1)pdm09 virus(es) and a small study group will be established to develop the detailed study protocol. A panel of human A(H1N1)pdm09 positive sera will be distributed to participating laboratories. At the same time, various sources of potential antibody standards will be evaluated as described in the first CONSISE newsletter.

At the Cape Town CONSISE meeting, it was agreed to evaluate the enzyme-linked lectin assay (ELLA) for detection of neuraminidase (NA) antibodies. This assay had been developed by Dr Maryna Eichelberger at the US FDA (profiled in this newsletter) and although several laboratories have some experience of ELLA, there is a need to evaluate how robust and reproducible the assay is on wider use. A small group headed by Dr Eichelberger has made plans for a collaborative study of the ELLA assay.

- The study will be open to all CONSISE members
- A common panel of sera, and if necessary, antigen, will be shared between laboratories
- The study is scheduled to commence in June 2014, with results due in August 2014.

There has been a very enthusiastic response to the invitation to take part in the study with thirty eight confirmed laboratories taking part at the time of writing. Approximately half of these laboratories have not used ELLA so this will be a great opportunity to learn the assay at the same time as helping to standardize the assay internationally. However the large number of newcomers

may need a re-adjustment to the study timelines, with the experienced laboratories completing the study first and newcomers later on (Figure arrow 6). This will be an opportunity to establish international harmonization to reduce inter-laboratory variability as this new assay becomes established.

CONSIDE Website and Upcoming Meetings

The Global Health Network website has recently undergone an upgrade and there are now new landing pages for all of the pages on the site. Please have a look at CONSIDE's new landing page (<https://consise.tghn.org/>) and send feedback to Maria Van Kerkhove (m.vankerkhove@imperial.ac.uk). As this is our main mechanism for sharing information, we hope to utilize the website more.

There are several upcoming meetings where we hope to present CONSIDE ongoing work. These include: Respiratory Viruses 8 September 2014 in Oxford, UK (<http://lpmhealthcare.com/respiratory-viruses-2014/>); Influenza 2014, 9-11 September 2014 <http://lpmhealthcare.com/influenza-2014/>; and ESWI 14-17 September 2014 in Riga, Latvia (<http://www.eswiconference.org/core/>).

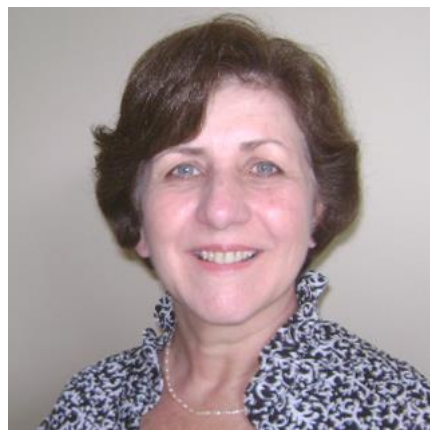
Please let us know if you are planning on attending an influenza conference in the near future and if it is appropriate for CONSIDE to submit an abstract.

CONSIDE Member Highlight

We will be highlighting one CONSIDE member in each newsletter. For our second newsletter we have interviewed Maryna Eichelberger, Principal Investigator from the Center for Biologics Evaluation and Research, US Food and Drug Administration.

Can you tell us a little about yourself?

It's been fascinating to look back and see how my past experiences have shaped my ability to address some of the gaps we need to address in order to develop better influenza vaccines. I was born and raised in South Africa where I didn't recognize flu as a public health issue – influenza infections of ostriches or horses were occasional headline news because of their impact on farming and racing revenues, but discussions of flu in humans were rare. It wasn't until my grandfather died at the age of 93 as a consequence of an influenza infection that I realized the importance of influenza vaccination.



I graduated from the University of Natal with a bachelor's degree in Biochemistry and training at the Natal Institute of Immunology made me aware of infectious diseases and methods used to screen for them. I set off to pursue a PhD at the University of Alabama at Birmingham where I was fortunate to do a lab rotation in Gillian Air's laboratory – Gillian of course is "the" expert in neuraminidase and she has always had a great interest in its antigenic structure. She was hands-on in the lab so with her guidance, my project went smoothly and resulted in a first author publication after only a few months. This experience was the impetus to continue working with Gillian as my Ph.D. advisor where one of my projects focused on identifying antigenic epitopes of NA by sequencing monoclonal antibody escape variants. I had hoped to get some training in cellular immunology and fortunately for me, Peter Doherty was setting up his lab at St Judes Children's'

Research Hospital, right at the time when I was looking for a post-doc. Working in Peter's lab on flu-specific T cell responses was awesome – apart from being brilliant, he's incredibly creative and enthusiastic.

I worked on a variety of interesting projects that gave me an opportunity to learn methods that I have continued to use throughout my career. Peter received the Nobel Prize a few years after I left his lab – what an honor to have worked with him! As I started my own lab at Johns Hopkins School of Public Health, I decided to investigate the mechanisms by which influenza-specific adaptive responses are initiated. Interestingly, we demonstrated that the enzyme activity of NA was responsible for enhancing the interaction of T cells with antigen-presenting dendritic cells, thus merging my interest in NA and T cells. At Hopkins, I was responsible for the lab team that supported clinical vaccine studies conducted by MaryLou Clements-Mann and Ruth Karron. This provided a great foundation for my more recent work in developing methods used to evaluate vaccine immunogenicity. Human studies are really difficult and of course expensive. The mouse model has disadvantages and so when I left Hopkins to join a small biotech company, Virion Systems, it gave me an opportunity to begin to use cotton rats as a model for flu – cotton rats are an excellent small animal model for RSV and we were encouraged by the ability of most human influenza viruses to replicate in the nose and lungs of these rodents. I used this model quite a bit because it was easy to quantify disease by measuring breathing rate – cotton rats become tachypneic when infected with influenza and so the efficacy of vaccines or antivirals could be measured by using respiratory rate as an end-point. I continued to use this model when I started as a researcher/regulator at the Food and Drug Administration in 2006, however, I've since completed these experiments and no longer use this model because my goals have changed. I have both research and regulatory responsibilities within the Office of Vaccines here at the Center of Biologics Evaluation and Research (CBER). My research goal is to establish methods to facilitate the evaluation of new types of influenza vaccines, including universal vaccines that target the induction of flu-specific T cell responses. As you know, we optimized a practical assay to measure NA inhibition titers and have used this assay to examine antigenic drift of NA and to evaluate NA-specific antibody responses to both inactivated and live, attenuated vaccines. I'm fortunate that my lab members share my enthusiasm for NA as a vaccine antigen – they have worked hard to identify antigenic domains of the NA of H1N1 viruses. It's particularly gratifying to have identified antigenic domains of NA that are conserved in seasonal and pandemic H1N1 viruses as well as H5N1 viruses. Antibodies to these conserved domains explain why responses against the NA of seasonal influenza viruses provide some protection against 2009 H1N1 pandemic and even H5N1 challenge. We'd like to identify the NA inhibition titer that correlates with this protection so that the contribution of NA immunity to vaccine efficacy can accurately be evaluated. Since NA-specific antibodies are often cross-reactive with the NA of new emerging viruses, this correlate is also important for us to be able to assess the potential susceptibility of individuals during a pandemic. It is very exciting to think that our small advances have potential to help development of influenza vaccines that provide broad immunity. My hope is that vaccines using new technologies will benefit all age groups, particularly the elderly, who like my grandfather are particularly susceptible to infection.

What is your involvement in CONSIDE?

I didn't get involved with CONSIDE until attending the meeting in Cape Town this past September. I participated in the lab group discussion of HAI and microneut assays and was impressed by the practical outcomes of the studies that had been conducted. I presented the work that we had done to optimize the enzyme-linked lectin assay (ELLA) to measure NA inhibition titers. A number of CONSIDE members were interested in setting up the assay and it seemed like an interlab study would provide results that would give us a mechanism to implement a single standard method and to evaluate its variability across labs. We formed a steering committee to discuss how to conduct the study and earlier this year, sent out an invitation to all CONSIDE members, asking those that were

interested in participating in the ELLA study to reply. We were overwhelmed with responses! Many labs had not previously run this assay and so we are providing reagents to get them started. Antigen and serum samples have been sent to a number of experienced labs – the goal is to receive the data from the experienced labs by the end of June so that the statistician can analyse the results in July.

How do you see CONSIDE adding to the standardization of serologic assays for influenza?

There is a huge need to standardize assays as it is currently difficult to compare seroepidemiologic studies conducted in different countries and to have confidence in results generated in different clinical vaccine trials. CONSIDE had provided a mechanism to make this possible – when assay results from many different laboratories are compared, potential problems can be identified and a consensus protocol can be established. I envision CONSIDE members identifying assays that need to be standardized in the future – clearly HAI and microneut assays were a priority to begin with; once the ELLA study is complete, there will be additional assays that are worthwhile standardizing. One example of an assay that I think is worthwhile standardizing is the pseudotype neutralization assay – it is currently not widely used but is a practical way to measure antibody titers to viruses that currently can only be handled in BSL3. One strength of CONSIDE is the combined expertise of the epidemiology and laboratory groups: it would be great for the 2 groups to discuss common goals and establish collaborative projects that address specific needs. I think it would be particularly valuable to design a study to identify antibody titers that correlate with protection against influenza – although we have some idea of HAI and even microneut titers that contribute to protection; we currently do not know the NA inhibition titer that is associated with immunity. Another strength of CONSIDE is its international membership – having expertise spread around the globe is extremely important when tackling a pathogen like flu that spreads so quickly worldwide. There are some very practical benefits - the lessons learnt by one laboratory can quickly be communicated to other labs so that we're not wasting time re-inventing the wheel. In addition, we have forged relationships with each other so that its easy to share information and if needed, reagents and new protocols. I look forward to continuing to interact with many of the CONSIDE members even after the ELLA study is complete!

With best wishes,

Maria Van Kerkhove, Othmar Engelhardt and John Wood for the CONSIDE Steering Committee

References

1. WHO Global Influenza Surveillance Network. *Manual for the laboratory diagnosis and virological surveillance of influenza*. WHO 2011.

http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf pages 43-59