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Meeting Report

Influenza serological studies to inform public health action: best practices to optimise timing, quality and reporting

Karen L. Laurie,[a] Patricia Huston,[b] Steven Riley,[c] Jacqueline M. Katz,[d] Donald J. Willison,[e] John S. Tam,[f] Anthony W. Mounts,[g] Katja Hoschler,[h] Elizabeth Miller,[i] Kaat Vandemaele,[g] Eeva Broberg,[j] Maria D. Van Kerkhove,[c] Angus Nicoll[j]

[a]WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, North Melbourne, Vic., Australia. [b]Pandemic Preparedness Division, Centre for Immunization and Respiratory Infectious Diseases, Public Health Agency of Canada, Ottawa, Ontario, Canada. [c]MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, UK. [d]Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, USA. [e]Surveillance and Epidemiology, Public Health Ontario, Toronto, Canada. [f]Initiative for Vaccine Research, World Health Organization, Geneva, Switzerland. [g]Epidemiology and Surveillance, Global Influenza Programme, World Health Organization, Geneva, Switzerland. [h]Respiratory Virus Unit, Microbiological Services Division (Colindale), Virus Reference Department, Health Protection Agency, London, UK. [i]Immunisation Department, Health Protection Agency, Health Protection Services (Colindale), London, UK. [j]European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden.
Correspondence: Karen Laurie, Senior Virologist, WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, 10 Wreckyn Street, North Melbourne, Vic. 3051, Australia. E-mail: karen.laurie@influenzacentre.org

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Background Serological studies can detect infection with a novel influenza virus in the absence of symptoms or positive virology, providing useful information on infection that goes beyond the estimates from epidemiological, clinical and virological data. During the 2009 A(H1N1) pandemic, an impressive number of detailed serological studies were performed, yet the majority of serological data were available only after the first wave of infection. This limited the ability to estimate the transmissibility and severity of this novel infection, and the variability in methodology and reporting limited the ability to compare and combine the serological data.

Objectives To identify best practices for conduct and standardisation of serological studies on outbreak and pandemic influenza to inform public policy.

Methods/Setting An international meeting was held in February 2011 in Ottawa, Canada, to foster the consensus for greater standardisation of influenza serological studies.

Results Best practices for serological investigations of influenza epidemiology include the following: classification of studies as pre-pandemic, outbreak, pandemic or inter-pandemic with a clearly identified objective; use of international serum standards for laboratory assays; cohort and cross-sectional study designs with common standards for data collection; use of serum banks to improve sampling capacity; and potential for linkage of serological, clinical and epidemiological data. Advance planning for outbreak studies would enable a rapid and coordinated response; inclusion of serological studies in pandemic plans should be considered.

Conclusions Optimising the quality, comparability and combinability of influenza serological studies will provide important data upon emergence of a novel or variant influenza virus to inform public health action.

Keywords Antibodies, influenza, pandemic, public health response, serological studies.

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Introduction

The value of serological studies to inform the public health response was apparent in the 2009 influenza A(H1N1) pandemic (the 2009 pandemic) despite the major challenges in conducting them. Serological studies are able to measure the antibodies that most people develop in response to infection with an antigenically novel influenza virus. This antibody response develops within 2–3 weeks of infection[1–3] and may persist at a detectable level for months, providing protection against further challenge with the same virus. Influenza serological assays, such as haemagglutination inhibition (HI) or microneutralisation (MN), quantify these virus-specific antibodies, as an indicator of infection. Serological assays can confirm past infection in the absence of positive virological testing[4,5] and regardless of clinical

presentation. Serology may detect both symptomatic and asymptomatic infection. This provides the most reliable measure of total infection with a novel pathogen in a population. Combining serological, epidemiological and clinical data improves clinical disease definitions, enables estimation of the severity and transmissibility of an emerging virus and can identify population groups that have been infected versus those that remain susceptible. Furthermore, with samples obtained prior to widespread transmission, previous infection or vaccination to antigenically similar viruses can be identified and the level of serological reactivity to the new virus inferred. These data can be used directly and within transmission models to predict the impact of infection and disease, and assess mitigation strategies, thereby informing public health policy and allowing for the adjustment of planning assumptions and countermeasures (Table 1).[6–8]

A large number of serological studies were conducted for the 2009 pandemic providing a comprehensive picture for many parts of the world. The extensive use of serology to ascertain population infection rates to the 2009 pandemic virus was particularly impressive given the inclusion of serology in only a limited number of pandemic preparedness plans.[9–11] Shortly after the onset of the 2009 pandemic, standard virus strains and sera were made available and serological correlates of infection were established.[1,12,13] Early studies demonstrated the prevalence of pre-existing cross-reactive antibodies was age and location dependent,[12–14] and rapid assessment suggested a lack of protection through seasonal A(H1N1) vaccination.[12,13] However, despite the efforts to gather and analyse samples in many countries[5,15–17] technical and logistical challenges (Table 2) meant there were no early publications using sera from close contacts to identify symptomatic and asymptomatic infections. As a result, essential information to aid in calculating the transmissibility and severity of this emerging infection was greatly limited. Rather, severity and transmissibility were inferred from non-laboratory confirmed symptomatic studies from early outbreaks[18–21] and animal infection studies (Figure 1).[14,22–23] Although the basic reproductive number can be estimated accurately without good knowledge of asymptomatic infection, variation in infection rates between age groups, for example (i.e. children and adults) cannot be estimated. The degree of variation in infection rates between infectious subgroups greatly influences the size of an epidemic. Risk factors associated with severe outcomes were primarily identified by clinical and epidemiological studies.[24–27] The majority of serological studies were published after widespread virus transmission and measured the cumulative age-specific incidence rates in populations.[1,28–37] Later studies also assessed attack rates in vulnerable populations and healthcare workers,[30,38–40] risk factors for infection,[30,37,39–41] the effectiveness of mitigation strategies,[42,43] transmission dynamics within populations[5,16,44] the severity of infection in different age groups[37] and the extent of asymptomatic disease[5,28,45] (Figure 1). These studies informed public health policy by addressing some of the 'known unknowns' of the novel virus. Although an international antibody standard was developed in a effort to standardise laboratory results,[46] it was not widely used for standardisation of seroprevalence studies, in part due to limited knowledge of its availability. Therefore, variability in data collection, analysis and reporting (Table 2) meant it was not always possible to ascertain whether differences between study results were population and location specific or reflected assay and other methodological distinctions.[47]

In the light of the challenges encountered (Table 2) and the potential value of influenza serological studies, the World Health Organisation (WHO) called for greater standardisation of such studies.[47] To facilitate this goal, an international meeting led by the Public Health Agency of

Table 1. Potential contribution of early serological data to mitigating the impact of influenza pandemics

'Known Unknown' of influenza pandemics	Rationale for knowing – the actions that may follow
Whether current seasonal vaccines are likely to be effective against the new pandemic virus	Whether or not to offer existing seasonal vaccine before new pandemic influenza-specific vaccine becomes available
Susceptibility of a population to the novel infection and then incidence of infection and disease by age group	Target interventions and refine countermeasures, for example, who to give antiviral drugs and/or pandemic influenza-specific vaccines
Key parameters for modelling and estimations: proportions of infections that are symptomatic and asymptomatic, effective reproductive number *R* (transmissibility)	Modelling of current and future scenarios to allow rapid re-casting of planning assumptions and resource deployment (now-casting and fore-casting)
The severity of the pandemic as inferred from a number of parameters including cumulative attack rate and case or infection fatality rate	The level of public health response proportionate to the threat

Adapted from ECDC's concept of pandemic *known unknowns* (81) and (8).

Table 2. Common challenges faced by researchers performing influenza serological studies in 2009 and 2010

Overall challenge	Key difficulties and concerns
Timeliness in responding to initial outbreaks	Necessity for rapid ethics approval and funding decisions, unless study was based on pre-existing surveillance (1) Availability of clinical and laboratory staff Rapid training of staff to collect and process samples Development of consistent sampling procedures Patient access and retention low, particularly when serial sampling required Laboratory capacity overwhelmed and difficult to prioritise virological and serological work
Assay development, standardisation and up-scaling in testing laboratories	Handling virus of unknown infectivity and severity Time constraints for assay development: virus-specific HI and MN assays require up to 6 weeks development (82, 83) Laboratory capacity and storage for expected surge activity Variability in laboratory protocols resulting in variability in titres across laboratories (52–54) – Laboratory variability minimised by use of an international 2009 pandemic antibody standard (09/194) (46), but awareness of standard limited
Data gathering for samples: accompanying information may inform risk factors	Majority of studies used residual sera with sample data restricted by ethical requirements Most studies insufficiently powered to detect risk factors in real time
Sampling at the most appropriate time during the first wave and performing assays with age-stratified background controls	Prospective collection of pre-pandemic samples limited by the narrow timing between identification of the novel virus and subsequent widespread infection (28, 30–33, 41) Window of collection of samples for post-first wave infection analysis limited by the imminent availability of matched vaccine Asynchrony of pandemic waves meant sampling at the end of the pandemic wave was difficult for some studies (1, 34)
Variability in data collection and reporting practises	Variability in pandemic waves made timing of post-first wave studies difficult to compare Data were shared but common protocols and methodology was not established
Ethical issues when designing/ conducting studies	Need for approvals from several ethics committees for multi-site studies resulted in delays and duplication of effort Risk that the validity of studies would be compromised when different sites placed alternate conditions on investigators

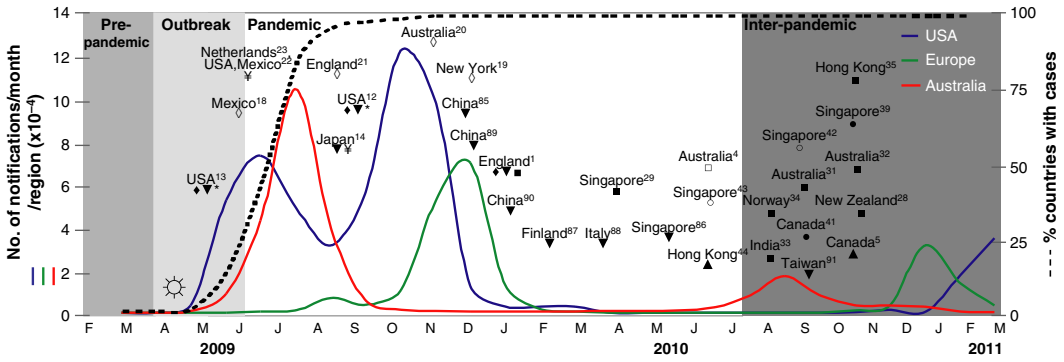

Figure 1. The time course of the initial pandemic wave in the Northern and Southern Hemispheres and proportion of countries with reported cases, after the identification of the novel virus in mid-April 2009 (☼). The working classification for serological studies according to time of sample collection and study objective is shown in this context, by shaded regions (pre-pandemic, outbreak, pandemic, inter-pandemic). Location and reference (superscript) of studies over time are shown. The earliest published serological studies in 2009 described assay sensitivity and specificity (♦), the cross-reactive pre-pandemic antibody responses (▼) and demonstrated a lack of protection from seasonal vaccines (*). In 2010, serological studies describing the first wave of infection (■), clinical identifiers of infection (□), household transmission (▲), risk factors for infection (●) and mitigation strategies (○) were published (shown are known serological studies until October 2010). The earliest published epidemiological outbreak studies (◇) and animal infection and transmission studies (¥) are shown for comparison.

Table 3. Serological studies for influenza. The study classification (pre-pandemic, outbreak, pandemic, inter-pandemic) is based on the timing of serum sample collection

Classification & Overall Objective	Key Serological Study Aims	Published examples of 2009 pandemic serological studies	Study Design Options	Requirements for Study*	Enabling strategies*
PRE-PANDEMIC –to develop assays and assess population susceptibility	Develop assays – provide sensitivity and specificity controls	(1–3, 13)	Longitudinal study – at least two bleeds col lected at acute and convalescent timepoints from individuals with virologically-confirmed infection	Access to patients High patient retention Timely swab, sera and clinical data collection	Prior ethics approval Outbreak response team to collect patient samples and clinical data
	Identify the magnitude and kinetics of the sero logical response in con firmed cases	(1–3)		Higher containment laboratory facilities Potential for surge capacity in testing laboratory	Cross-training of laboratory staff
	Identify the prevalence of pre-existing cross-reactive antibodies by age group	(1, 12, 14, 84–91)	Age-stratified cohort or cross-sectional Sample size depends on estimated attack rate and precision requirements	Age-stratified serological samples collected before emergence of novel influenza virus	National serum bank
OUTBREAK – to characterise a novel or variant virus and identify the potential for infection	Identify potential protective effect of current seasonal vaccine	(12, 13)	Age-stratified cohort or cross-sectional – with vaccine history	Appropriate pre-vaccination serological baseline control collected before emergence of novel influenza virus	Sera collected for vaccine cross-reactivity studies as part of routine influenza surveillance
	Establish transmission dynamics of novel strain	(5, 15–17, 44)	Age-stratified cohort or cross-sectional	Timely swab, sera and clinical data collection from individuals and	Prior ethics approval
	Provide the denominator for severity assessment	(5, 28, 37, 45)	Sample size dictated by location of outbreak	contacts in early outbreaks, that is, schools, households, military facilities	Established prospective representative cohort
				Appropriate pre-outbreak, age-stratified serological baseline control. A pre-established cohort may have pre-outbreak serum samples, whilst a reactive cohort would require col lection of acute blood samples	Outbreak response team to collect patient and contact samples and clinical data
	Reveal the extent of asymptomatic illness				National serum bank for cross-sectional studies
	Inform development of clinical and subclinical case definitions	(4)		Assay development	

Table 3. (Continued)

Classification & Overall Objective	Key Serological Study Aims	Published examples of 2009 pandemic serological studies	Study Design Options	Requirements for Study*	Enabling strategies*
	Assist in identifying risk factors for, and multipliers of, infection or severity	(30, 37, 39–41)	Age-stratified cohort or cross-sectional Sample size depends on estimated attack rate and precision requirements	Control group with same exposure to infection Detailed data collection to eliminate confounders Appropriate pre-outbreak, age-stratified serological baseline control.	Prior ethics approval Established prospective representative cohort
PANDEMIC-to determine the impact on the population after widespread transmission and inform useful mitigation factors	Determine cumulative incidence of infection by age	(1, 28–37) reviewed in (55)	Age-stratified cohort or cross-sectional	Age-stratified samples collected before emergence of novel influenza virus	Prior ethics approval
	Determine likelihood of further infection by age		Sample size depends on estimated attack rate and precision requirements	Appropriate timing of collection at end of initial pandemic wave	National serum bank for cross-sectional studies with potential for monthly rolling sample collection throughout pandemic wave to ascertain end point accurately
	Identify groups to be targeted for priority vaccination Assess vaccine effectiveness and need for subsequent boosting vaccinations	(1, 28–37, 92) reviewed in (55)	Age-stratified cohort or cross-sectional	Appropriate pre-vaccination/intervention serological baseline control	
	Evaluate public health interventions and mitigation strategies	(42, 43)		Appropriate control group with same exposure to infection	
	Assess decline of immunity		Age-stratified longitudinal study	Access to patients High patient retention	Prior ethics approval Established prospective representative cohort
				Timely swab, sera and clinical data collection, including vaccination records and influenza-like illness reporting	Outbreak response team to collect patient and contact samples and clinical data

Table 3. (Continued)

Classification & Overall Objective	Key Serological Study Aims	Published examples of 2009 pandemic serological studies	Study Design Options	Requirements for Study*	Enabling strategies*
INTERPANDEMIC-to monitor infection and vaccination throughout typical influenza seasons	Assess protection against antigenically drifted variants		Age-stratified cohort or cross-sectional	Appropriate collection of samples at end of first pandemic wave Access to emerging influenza virus drift variants	National serum bank with potential for monthly rolling sample collection
	Develop novel immunological assays		Age-stratified longitudinal study	Timely swab, sera and clinical data collection, including vaccination records and influenza-like illness reporting	Prior ethics approval Established prospective representative cohort
				Potential for access to peripheral blood mononuclear cells	Outbreak response team to collect patient and contact samples and clinical data

The overall objective for each classification is defined and the aims of key serological studies are identified. The most appropriate design options, essential requirements and enabling strategies to perform informative serological studies in a timely manner are described. *All studies require adequate funding.

Canada was held in Ottawa in February 2011 with participation from public health authorities and researchers from the disciplines of epidemiology, virology, immunology, mathematical modelling and ethics (web address pending). The goal of the meeting was to foster consensus-building and standardisation on how to improve the timing, quality, and reporting of outbreak and pandemic influenza serological studies. An Expert Committee was formed following the meeting to build on the consensus forged during the meeting and develop guidance for advancing future influenza serological studies.

Guidance for best practices

Adopt a common framework for serological studies and standardise methodology and reporting practices

Serological studies may address a number of different objectives depending on their timing. Because of the many different types of studies that are possible, it is useful to have a common framework (Table 3). We recommend a working classification for presentation of serological studies according to the time that serum specimens were obtained: pre-pandemic studies evaluate the level of antibodies or reactivity to the pandemic strain in samples collected prior to the onset of the pandemic, outbreak studies evaluate the sera of early cases and their close contacts, pandemic studies assess the degree of infection in a larger population once widespread transmission is known to have occurred and inter-pandemic studies assess the extent of serological immunity when infection incidence has fallen to levels more typical of seasonal influenza. This is preferable to identifying studies according to local 'epidemiological waves', which may be difficult to define and are typically asynchronous across the world, making comparisons by waves difficult (Figure 1). Because of the plethora of information that can be obtained, the experimental and public health objective of each serological study needs to be clearly identified (Table 3).

Standardised methodology and data sets would greatly facilitate the ability to compare and combine serological data for each study type[47] which would improve global tracking of a novel influenza virus. Identifying key elements that may be incorporated into common data collection forms, designed to accompany any blood sample intended for serological use, would enable this. Standardised reporting can be achieved by adherence to reporting guidelines of observational epidemiological studies as identified in the STROBE statement.[48]

Agreements to facilitate the rapid sharing of data and analyses are needed.[49] Reporting of aggregated data in a standardised way will inform early decisions and facilitate the meta-analyses and substudies to further inform advisories and alerts on the development of the disease globally.[50] Investigator teams should make anonymised individual-level data available freely at the time of publication, especially when important epidemiological claims are made, and, where possible, via well-managed bilateral relationships, share individual-level data with relevant health authorities as soon as those data are available. Any public health organisation in receipt of unpublished data must ensure that the interests of all stakeholders are protected. Statements from key journals that data published in surveillance bulletins and ministry health reports will not preclude later research publications, which target different audiences, would also encourage data sharing.

Coordinate and standardise the international laboratory serological response

Identification of a novel influenza virus in future outbreaks will require the rapid development of virus-specific HI and MN assays. A large quantity of the novel influenza strain isolated directly from infected individuals will need to be grown, and sera from experimental animals inoculated with the emerging virus will need to be collected for controls for assays. Optimal assay development will require panels of sera from non-exposed persons across a broad age range to determine specificity criteria and access to convalescent sera from virologically confirmed cases to define 'true positive' serological responses and determine sensitivity criteria.[2] This may necessitate the support of clinical and epidemiological partners. It is anticipated that small scale serological testing could be performed within 6 weeks of identification of a novel influenza virus. As the severity of a novel infection remains unknown in an outbreak, virus culture and the development and expansion of early serological assays will likely include the need for BSL-3 facilities.

To expand the capacity for widespread and high-volume serological studies, in the inter-pandemic period, laboratories would need to cross-train staff in serological methods, establish quality assurance procedures and plan for surge capacity in specimen management, testing and data management. The potential for expansion of BSL-3 serological studies may be explored in laboratories currently conducting A(H5N1) serological studies. Development of a network of laboratories conducting serological studies would provide a forum for rapid distribution of methodological updates, announcements of availability of reagents and standards and discussion to achieve common seropositivity criteria. An updated Global Influenza Surveillance and Response System (GISRS) manual of influenza protocols, including serological assay protocols, is now available.[51] Certain GISRS laboratories (formally GISN) with the necessary resources, especially BSL-3 facilities, should they be needed, could produce and globally distribute reagents

(e.g. http://www.influenzareagentresource.org). This capacity building may require additional funding and support.

To develop data that can be combined and compared, rapid development and use of an international antibody standard and potentially common serum panels is necessary.[52–54] This may be facilitated by prior commitment from (i) vaccine manufacturers to provide large volumes of sera from early clinical trials, although timeliness in vaccine development may impact this; (ii) a dedicated laboratory to produce and distribute the product and organise collaborative assignment of unitage, and (iii) global laboratories to use the standard and report normalised titres. Finally, there would be great value in the development of new high-throughput antibody-based assays to distinguish between recent infection and vaccination, and alternate specimen collection processes that overcome the limitations of venous blood collection.

Plan for outbreak studies to identify asymptomatic and symptomatic infection, enabling severity and transmissibility calculations

To calculate the strain-specific severity parameters (case or infection fatality ratio (IFR), cumulative attack rate, infection hospitalisation ratio) and transmissibility (particularly the incidence of infection for different age groups), identification of asymptomatic and symptomatic infection is needed.[8] Determination of the total burden of infection in an outbreak in a structured population (such as a household or school) can provide a denominator to explain otherwise seemingly large and incompatible observed differences in severity (i.e. case fatality rate of 1:100 compared to IFR of 1:10000) and transmissibility, which greatly affect the likely later public health impact of disease. Any studies that can reliably provide early estimates of these parameters prior to large populations being infected would have great public health value.

At the time of early outbreaks, it is proposed that sample collection be enabled in multiple locations and among individuals of all age groups, providing paired sera from virologically confirmed cases for assay development and from all cases in initial small outbreak groups including contacts, to inform strain-specific parameters. The necessary sample collection may be integrated with outbreak investigation plans or routine clinical specimen collection and established epidemiological surveillance systems.

For a more structured approach, a small number of globally well-connected cities could implement prospective representative cohorts as soon as a novel strain of influenza emerges. The cohorts may include those directed to potential risk factors for severe outcomes or increased infection rates.

Early serological studies would be facilitated by their inclusion in pandemic preparedness plans. Specifically, protocols for rapid and detailed serum and data collection and processing, and ethical approvals, may be incorporated into pandemic preparedness planning, enabling a rapid response. Rapid funding strategies, such as a 'sleeping grants', may also be developed. Collections could be the responsibility of pre-specified healthcare professionals (outbreak response team), allowing laboratory researchers to focus on assay development. Protocols for serial sample collections from virologically confirmed individuals and collection of peripheral blood mononuclear cells may be included. As some serum samples will be collected *before* laboratory assays are fully developed, adequate facilities for storage and recording of samples are needed at testing laboratories or hospitals to ensure sample integrity is maintained. These proposals require advanced planning and infrastructure development that may be tested and optimised in normal influenza seasons.

Use either a cohort or a serial cross-sectional design and link serological with epidemiological and clinical data

Serological study design options are dictated by the study objectives and timing (Table 3). Serial cross-sectional studies collect samples from different individuals before and after an event, allowing for population-level analyses (e.g. cumulative incidence of infection). Anonymised discarded diagnostic or blood donations (residual sera) are often used for this, as well as sampling from individuals, although these may not be entirely representative of source populations. Cohort studies collect serial samples from the same individuals over time, enabling the analysis of changes in the immune response (e.g. generation or waning of antibody levels) following recorded events, such as infection or vaccination. Comparison of serological study designs has demonstrated little variation in the age-specific cumulative incidence of infection in the 2009 pandemic.[37,55,56]

Personal data and information describing potential exposure to infection with a novel strain of influenza would be of great value when collecting serological samples in defining risk factors for infection and severe outcomes. Sample date of collection, location of collection (postal code) and diagnostic test is already typically available. Personal data may encompass recent history of vaccination, socio-economic status, ethnicity, sex, residence in a group setting, date of birth, pre-disposing conditions, profession and household composition. Exposure to infection would include recent travel history, use of personal protective equipment (PPE), occupational/social contact with known-infected person(s) and antiviral drug use. For cohort studies, data collection could be specifically tailored to identify risk factors for infection or severity. The approach for data collection involving anonymised residual serum samples for cross-sectional studies may benefit from encouraging

patients, clinicians and diagnostic centres to use a common data form at the time of sample donation. This approach is expensive and may only be enabled by targeting specific collection centres or constraining the requested data. A critical element would be the ability to link serum samples with these data, as well as other epidemiological and clinical data, preferably in a central database, with appropriate measures to maintain confidentiality. This would enable retrospective study whilst retaining anonymity. Unlinked anonymous (blinded) surveillance is routinely performed in HIV and vaccine preventable disease seroprevalence programmes in the UK,[7,10] using a similar process.

Use national serum banks to ensure baseline serum availability and enable rolling convenience serum collections during an outbreak

The improved availability of serological samples could be addressed by annual, or biannual, collection of age-stratified, geographically representative sera, within countries. Single serum collections are currently undertaken to assess the susceptibility of a population to vaccine preventable infectious diseases[11,57,58] and other infections,[59,60] and where resources permit, ongoing collections are also performed.[1,7,10,60–61] These current systems could be expanded to encompass more regular collections to form national serum banks, which may be used for multiple vaccine preventable diseases and other infections (e.g. HIV, dengue). Access to existing national and routine serum banks established for other purposes may also be possible with approval and support.[62] Ideally, samples held in national serum banks would be complemented with vaccination history and other health information and comprise sera from children and adults, and healthy and patient donors, from a variety of sources. Crucially, the procedure for collection could be exploited during future pandemics to measure population infection at more regular intervals (monthly), ensuring key events are not missed. The collection of peripheral blood mononuclear cells may be considered to assess the complementary role of cell-mediated cross-reactive immunity; though, much larger blood volumes and immediate processing are required, which poses considerable logistic difficulties and costs.

Conduct prior research ethics review and promote public transparency on the secondary use of residual blood samples

In many countries, formal ethics review is not required when using serum samples for surveillance when investigating outbreaks and during potential public health emergencies.[63,64] However, it is often difficult to distinguish when this surveillance falls into the realm of research, requiring formal ethics review. When dealing with multi-site studies, it is especially important to secure collaboration when formal ethics review is secured. Rapid response capacity would be greatly facilitated if generic protocols for investigations were developed and approved by relevant ethics review boards in advance of an outbreak. In Cambodia, for example, where serological studies are part of the national outbreak investigation plans, it is possible to seek ethical 'pre-approval in principle', for A(H5N1) outbreaks.[65] In the UK, a pre-existing approved serosurveillance mechanism is used.[1,7,10,69] Should the specifics of a particular outbreak call for changes to the sampling protocol, only the relevant changes and rationale would need to be reviewed.

Studies involving multiple institutions also benefit from streamlined ethics review processes, with centralised submission and/or review. Denmark has a system for a single ethics review of multi-site studies.[66] In Australia, the National Health and Medical Research Council (NHMRC) is developing a similar system nationally where a single ethical review would be recognised by all institutions participating in a collaborative research project.[67] In some countries, such as Hong Kong (China SAR), a different distinction is made and research such as seroepidemiology that cannot harm the individual is subject to a more rapid and simple review than potentially invasive studies. (Cowling B, The University of Hong Kong, Hong Kong, personal communication)

A key ethical issue concerning serological studies is whether or not consent is required for the secondary use of blood samples. For surveillance and research serological studies, use of leftover samples from blood drawn for diagnostic purposes or blood donations is common, usually with the need for individual consent or notification waived by ethics committees.[1,7,64] This is generally considered ethical when it involves unlinked anonymous testing including collection of only a limited number of variables (such as age group, sex and broad locality).[10,64,68,69] Whilst this approach addresses ethical and legal concerns over secondary use of samples, it restricts what can be done scientifically with the data.[70] Countries with more stringent ethical restraint, especially those needing multiple local decisions, may simply not be suitable for sero-epidemiological studies in an emergency.

It has been argued that patients have a right to know that their records or specimens may be used for research[64] and questionnaire-based surveys in the UK demonstrated little public knowledge of the use of residual samples,[71,72] but high public support.[71] Greater public transparency and discussion on the use of residual samples for research and surveillance processes may secure public confidence. Knowledge of the public health impacts of information gained from serological studies may also improve participant retention in longitudinal studies. However, individual consent-based systems pose both logistical challenges and may compromise scientific validity, depending upon response rate.[7,10]

It is important for researchers to maintain an ongoing relationship with their ethics committees and work with them to create solutions to address the conflicting needs for greater detail to increase the usefulness and validity of scientific evidence, and for the protection of research participants.[10]

Discussion and limitations

Serological studies complement the epidemiological and clinical studies to inform public health actions in a pandemic response. Performed early in an outbreak, they may identify both symptomatic and asymptomatic disease, providing an important denominator to calculate the severity and transmissibility of a novel virus infection. Serological studies can ascertain the population infection rates and determine the presence of pre-existing immunity generated from previous infection with influenza virus and vaccination. They can assist in identifying risk factors for infection and enable assessment of the effectiveness of vaccine and other mitigation strategies. Impressively during the 2009 pandemic and afterwards, serological studies successfully fulfilled many of these roles. We anticipate that the value of serological studies will be greatly increased by the standardisation of methods, laboratory assays, data collection, analysis and reporting. Advance planning will enable rapid and coordinated early outbreak studies that will help determine the severity of a novel influenza virus and inform subsequent action; inclusion of serological studies in national pandemic plans should be considered.

Serological studies are not without weaknesses. A minority of people did not seroconvert following virologically confirmed infection with the 2009 pandemic virus,[1–3,44] antibody titres to the 2009 pandemic virus may be reduced in patients undergoing antiviral treatment[44] and antibody levels may wane over time,[73,74] limiting the window of detection following infection with a novel virus. Furthermore, interpretation of the results of HI and MN assays may be complicated by vaccination and ongoing circulation of identical or antigenically related strains.

Further research is needed (see Table 4). More specific and simplified assays to measure the population infection and immunity are required. Historical challenge studies suggest a correlation between higher HI titre and

Table 4. Priorities for further work

Timing for Further Work	Priorities
Immediate preparation	Promote inclusion of key serological studies in principle in pandemic preparedness plans
	Identify pre-existing serum banks, current collections and unlinked anonymous residual sera suitable for influenza studies
	Explore the potential to obtain national ethical 'pre-approvals in principle' for undertaking pandemic serological studies in an emergency or outbreak
	Develop national or international protocols for rapid serum sample collection, preparation and transport to laboratories in influenza outbreaks. Include the potential for dedicated outbreak response teams
	Identify key elements for inclusion in data collection forms for anonymised residual sera and for samples from cohort studies
	Explore the potential for clinical, serological and epidemiological data to be linked in real time
Longer term preparation and consideration	Use national serum banks and unlinked anonymous residual sera and/or targeted cohorts for studies on influenza and other vaccine preventable diseases
	Develop outbreak serological investigation plans (i.e. prospective cohorts) for globally well-connected cities where early transmission is likely (such as cities with major airport hubs, for example, New York, London, Hong Kong, Singapore)
	Develop an international network of laboratories for conducting serological studies. Key responsibilities may include establishing commitment for production of international antibody standard(s) and control panels, and ensuring a common approach to generating comparable seroepidemiological data
	Undertake relevant national seroepidemiological studies for seasonal influenza, to improve understanding of epidemiology and the impact of recent seasonal vaccination on seasonal infection and disease, and to ensure laboratory capacity for serological studies is maintained
Research gaps	Investigate potential for alternate specimen collection processes than venous blood
	Develop antibody-based assays that can distinguish between recent infection and vaccination; potential for recent infection to be detected in a single acute sample
	Develop less labour-intensive, but rapid, serological test methods for infection
	Measure kinetics of influenza antibody decay after natural infection and vaccination in different age groups
	Develop assays to measure cellular immunity and heterosubtypic antibody immunity
	Explore potential to establish a correlate of protection from antibody or cellular immunity assays
	Assess antigenic drift in the 2009 pandemic virus and its impact on population susceptibility

protection from infection,[75–77] yet the contribution of other components of the immune response in mediating protection has not been assessed, nor has any age bias. Heterosubtypic antibodies may contribute to reducing influenza infection and have been shown to prevent infection in animal models.[78,79] Similarly, cross-reactive T-cell immunity may also limit infection by novel influenza A strains.[80] Further understanding of the contribution of the quality and quantity of the antibody and cellular response to protection from subsequent influenza infection is needed. Finally, considerations need to be given to stratified, modular study designs to allow database linkages and varying degrees of participation by different countries. To the extent possible, the creation of linkable national systems would facilitate real-time knowledge acquisition at the global level.

Conclusions and next steps

In summary, we have identified the following best practices:

1. Classify serological studies as pre-pandemic, outbreak, pandemic or inter-pandemic studies, based on time of sample collection

2. Standardise methodologies, prepare and agree protocols, data collection forms and reporting guidelines and make agreements for rapid data sharing

3. Coordinate and standardise the international laboratory serological response

4. Plan for early outbreak studies of novel influenza viruses to identify asymptomatic infection, enabling estimation of severity and transmissibility

5. Determine the best application of either cohort or serial cross-sectional designs for serological studies and link serological with epidemiological and clinical data

6. Use national serum banks to ensure baseline serum and rolling convenience serum collections during an outbreak

7. Conduct prior research ethics review and promote public transparency on the secondary use of residual blood samples

A second international meeting organised by member of the Expert Committee and supported by the European Centre for Disease Prevention and Control was planned for December 2011 in Stockholm. The goal was to initiate some of the recommendations for improving capacity for serological studies (Table 4). The potential for this guidance to encompass multiple vaccine preventable diseases and infections was also explored. However, it is important to recognise that much of our guidance for best practice requires capacity building that can only be achieved with support from public health authorities and funding bodies. To truly increase our ability to conduct serological studies will require resources as well as ongoing national and international collaboration.

Addendum

ICMJE criteria for authorship met: KLL developed first outline of issues, PH developed first draft of article. KLL, PH, SR, JMK, DJW, JST, AWM, KH, EM, KV, EB, MDVK and AN contributed to the writing of the article, and after critical review, all authors finalised the text of the manuscript. EB and AN drafted initial Tables 1, 3 and 4, KLL drafted initial Table 2 and Figure 1. PH chaired the Planning Committee for the International Influenza Seroprevalence Meeting held in February 2011, EB, AN, JMK, JST, EM and KH were the members of the Planning Committee and participated in regular conference calls.

Competing interests

All authors have completed the ICMJE uniform disclosure form at http://www.icmje.org/coi_disclosure.pdf (available on request from KLL) and declare that (i) SR has support from Wellcome Trust; KH has support from National Institute of Health Research (NIHR), UK; MDVK has support from the UK Medical Research Centre and the Bill and Melinda Gates Foundation, for the submitted work; (ii) KH, on behalf of HPA, was involved in projects outside the scope of this article, which were supported by funding from Novartis, Sanofi Pasteur, Baxter and CSL Limited in the previous 3 years; JMK received support for work not submitted in this manuscript from Juvaris Inc, Nobilon-Merck Sharp and Dohme, and GlaxoSmithKline in the previous 3 years; KLL, PH, SR, DJW, JST, AWM, EM, KV, EB, MDVK and AN have no relationships that might have an interest in the submitted work in the previous 3 years; (iii) KLL, PH, SR, JMK, DJW, JST, AWM, KH, EM, KV, EB, MDVK and AN their spouses, partners, or children have no financial relationships that may be relevant to the submitted work, and (iv) KLL, PH, SR, JMK, DJW, JST, AWM, KH, EM, KV, EB, MDVK and AN have no non-financial interests that may be relevant to the submitted work.

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