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TECHNICAL REPORT

Survey report on laboratory capacity for molecular diagnosis and characterisation of zoonotic influenza viruses in human specimens in the EU/EEA and the Western Balkans

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Survey report on laboratory capacity for molecular diagnosis and characterisation of zoonotic influenza viruses in human specimens in the EU/EEA and the Western Balkans

This report of the European Centre for Disease Prevention and Control (ECDC) was coordinated by Angeliki Melidou.

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Abbreviations

CDC	Centers for Disease Control and Prevention
CVV	Candidate vaccine viruses
EFSA	European Food Safety Authority
EQA	External quality assessment
EQAP	External quality assessment panels
ERLI-Net	European Reference Laboratories for influenza
EU/EEA	European Union/European Economic Area
EURL	European Union Reference Laboratory for Avian Influenza
GISAID	WHO Global Influenza Surveillance and Response System
RT_PCR	Reverse transcription polymerase chain reaction
WGS	Whole genome sequencing
WHO CC	World Health Organization Collaborating Centre

Executive summary

The aim of this survey was to review the expertise, capabilities and capacities for detection and characterisation of avian and other zoonotic influenza viruses in national influenza reference laboratories in the European Union/European Economic Area (EU/EEA), in members of the European Reference Laboratories for influenza (ERLI-Net), and in EU Enlargement policy countries. Thirty-three ERLI-net laboratories from all EU/EEA countries and three laboratories from the Western Balkan region (i.e. Albania, Kosovo[i], Montenegro) participated in the survey. Timely detection and characterisation of zoonotic influenza infection human cases are crucial to interrupt the spread of the viruses causing them.

Overall, all but one of the participating laboratories reported a substantial level of expertise, capability and capacity to detect avian and other zoonotic influenza viruses in human specimens, and the majority (69%) can also genetically characterise the viruses. In addition, more than half of the laboratories (63%) can isolate the zoonotic influenza viruses and one third of the laboratories can further antigenically characterise those viruses.

The key findings are summarised below:

- The vast majority of participating ERLI-Net laboratories in EU/EEA countries (93%) and two out of three laboratories from the participating Western Balkan countries are receiving specimens for testing or for confirmation of positive tests performed elsewhere from exposed people when there is suspicion of zoonotic influenza virus infection.
- Importantly, most participating laboratories (78%) reported that there is collaboration between the human and animal health sectors that is crucial for an effective response if there are human cases of zoonotic influenza virus infections.
- All but one participating laboratory from EU/EEA and Western Balkans reported that they are able to perform RT-PCR for the detection and identification of A(H5) and A(H7) avian influenza viruses, while 21 are also able to detect A(H9) or other subtypes. More than half of the reporting laboratories (56%) are performing NA subtyping. Overall, there is a variability in the assays/methods used for detection of zoonotic influenza viruses in human specimens in the different laboratories. The availability of positive control material for the RT-PCR assays does not seem to be an issue.
- The majority of the laboratories (69%) indicated that they have the capabiliy and capacity to perform whole genome sequencing for unsubtypable influenza A viruses, H5 influenza A viruses or other zoonotic influenza viruses.
- All laboratories that are performing sequencing stated they are uploading sequences in real time in a public repository. Nine laboratories indicated that they have restrictions with data sharing due to national regulations, GDPR or other institutional data protection practices. As sharing of data is crucial for an effective response to outbreaks and epidemics of zoonotic influenza viruses, it is important to explore ways to overcome potential GDPR issues or other institutional data protection practices that may prohibit timely data sharing.
- Importantly, a high proportion of participating laboratories (72%) reported that they are equipped with BSL3 facilities for virus isolation and handling of highly pathogenic viruses.
- The majority of laboratories that are receiving human specimens with suspicion of zoonotic influenza (76%) are using dedicated physical facilities. This is in order to separate the handling of zoonotic and seasonal influenza clinical specimens for molecular diagnostics and/or storage. Other laboratories implement other precautions, such as separating laboratories into different sections, adding decontamination/disinfection steps, using other facilities in veterinary laboratories or inactivating clinical specimens. There appears however to be limited harmonisation in the selection of facility types and/or other precautions used for the different methods (e.g. BSL3, BSL2 with additional precautions, BSL2). Guidelines for the development and sharing of laboratory techniques and standard operating procedures could be useful for harmonisation and for laboratories to further develop their capabilities.
- Twenty-two laboratories (63%) are performing isolation of avian influenza viruses, and half of those are able to assess the antigenic relatedness to candidate vaccine viruses/reference viruses. Lack of ferret antisera and reference strains is a significant impediment to expanding the capacity to performing antigenic characterisation in additional EU/EEA laboratories, as only three laboratories mentioned they have access to such materials. There is also a lack of access to reference material for population immunity studies. Extended collaborations with international organisations and existing influenza networks could further facilitate the exchange and distribution of reference material.
- Twenty-three laboratories (64%) indicated they have the capability and capaciy to perform genotypic antiviral susceptibility testing and out of those, the majority (n=18, 78%) have the capability and capacity to also perform phenotypic antiviral resistance testing for neuraminidase inhibitors.

[i] This designation is without prejudice to positions on status and is in line with UNSCR 1244/1999 and the ICJ Opinion on the Kosovo declaration of independence.

- As the requirement and awareness level of the regulations related to import/export permits and other restrictions for working with human specimens with a suspicion of zoonotic influenza viruses are diverse, awareness training organised both at the national and international levels would be needed to facilitate international collaboration.
- Overall, external quality assessment (EQA) and bioinformatic ring trials to prepare for diagnosis of zoonotic influenza viruses from human specimens are well appreciated and sought-after by the countries. ECDC and the WHO Regional Office for Europe are providing laboratory support and planning for EQAs and other zoonotic influenza-related activities. National reference laboratories for influenza are encouraged to participate in those activities.
- Harmonisation of methods and good laboratory practices can be facilitated via the laboratory support that ECDC and the WHO Regional Office for Europe provide to the national reference influenza laboratories, that include training and technical support.

Background

Influenza A viruses circulate in a diverse range of host animal species. In recent years, there has been a significant global increase in the detection of avian influenza infections among wild birds, that led to an increase in infections of domestic poultry and a variety of wild, domestic, and farmed mammals [1-3]. Zoonotic transmissions of the avian influenza virus, from animals to humans, can happen through direct contact with infected animals or exposure to their contaminated environment, leading to infection with symptoms varying from mild illness to death. Although, these infections rarely result in effective human-to-human transmission, they have the potential to mutate or undergo reassortment. They therefore pose a pandemic threat and need to be monitored. Timely detection and characterisation of zoonotic influenza infection cases is important to interrupt the transmission and the spread of such viruses with pandemic potential.

In the EU/EEA, surveillance for avian influenza is compulsory according to the Regulation (EU) 2020/690 [4]. Human infections with avian influenza viruses are notifiable through the Early Warning and Response System in accordance with Regulation (EU) 2022/2371 [4] and through the World Health Organization International Health Regulations (IHR) notification system, which requires immediate reporting of any laboratory-confirmed case of a zoonotic influenza A human infection, irrespective of symptoms [5].

ECDC has published a guidance on 'Testing and detection of zoonotic influenza virus infections in humans in the EU/EEA, and occupational safety and health measures for those exposed at work' in collaboration with the European Food Safety Authority (EFSA), European Union Reference Laboratory for Avian Influenza (EURL) and European Agency for Safety and Health at Work. This guidance document offers recommendations aimed at public health and laboratory professionals for the identification of human infections resulting from animal influenza viruses. A protocol for investigation of potential human cases has also been developed to aid countries in planning their response to human cases of avian influenza infections [6]

In November 2023, ECDC published a survey report of EU/EEA Member States to evaluate preparedness planning and risk mitigation initiatives implemented at the country level for people exposed to highly pathogenic avian influenza [7]. Previous ECDC surveys on the same topic were published in 2016 and 2018 [8, 9]. Laboratory preparedness is key for an effective response to human cases. During the COVID-19 pandemic, a number surveys were conducted to assess the laboratory capabilities and capacities for detection, characterisation and antiviral susceptibility monitoring. The most recent survey aimed to assess the capability and capacity to detect and characterise zoonotic influenza viruses.

Scope of this survey

With this survey, we aimed to assess:

- The existing expertise, capability and capacity for molecular detection of avian and other zoonotic influenza viruses in human specimens in national influenza reference laboratories in the European Union/European Economic Area (EU/EEA), in members of the European Reference Laboratories for influenza (ERLI-Net), and in the Western Balkans.
- The capability and capacity to isolate and perform further antigenic and genetic characterisation of avian and other zoonotic influenza viruses detected in human specimens, including Whole Genome Sequencing (WGS) and analyses of WGS data.

The survey results will be used to assess the support needs for strengthening capacity and capability in the EU/EEA and in the Western Balkans.

Methods

A survey was developed by ECDC experts and shared for review with external experts. The online EUSurvey platform[ii] survey link was distributed to ECDC's Operational Contact Points (OCPs) and the National Focal Points (NFPs) for influenza and ECDC contacts in the Western Balkans. The invitation was sent on 15 May 2023 and the survey was open until 8 June 2023.

The survey contained 41 questions in nine different categories: specimen sources, molecular diagnostics, genetic characterisation using Sanger/Whole Genome Sequencing (WGS), data sharing, virus isolation and antigenic characterisation, antiviral susceptibility testing, special studies, biosafety measures, restrictions and relevant permits and needs for laboratory support.

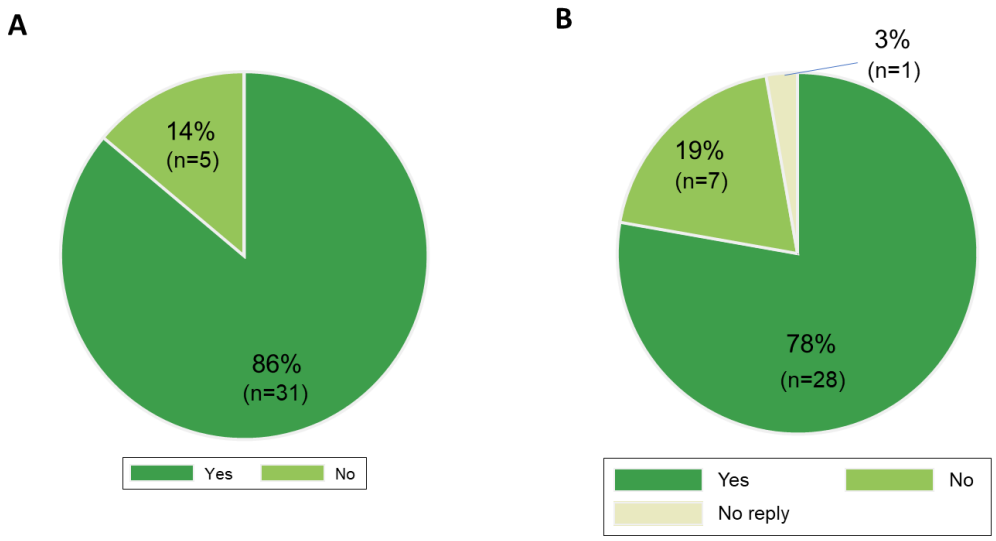
Results

Thirty-three laboratories from all EU/EEA Member States and three laboratories from the Western Balkans (Albania, Kosovo and Montenegro) replied to the survey.

Specimen sources

Out of 36 responding laboratories, 31 (86%) responded that they receive specimens from exposed people when there is suspicion of zoonotic influenza virus infection, although three of those indicated that there hasn't been a need so far. Of the five laboratories that reported 'No' to this question (14%), two reported that they are only receiving specimens for confirmation of zoonotic influenza infection (initial testing is performed at the local/regional level) and three laboratories are not receiving such specimens (Figure 1A). We can therefore deduct that in total, 33 laboratories (92% of the responders) are receiving specimens either for initial testing or confirmation.

Figure 1. (A) Number of laboratories that receive human specimens to test for zoonotic influenza viruses (B) Number of laboratories that collaborate with the animal health sector



Twenty-eight out of 36 laboratories (78%) reported that there is a laboratory collaboration between animal and public health/national reference laboratories, mostly exchanging materials/reagents/protocols (e.g. RT-PCR protocols, primers/probes, controls, sera), data and information. Seven laboratories (19%) reported they have no collaboration with animal health laboratories; two of those commented they are planning to share data and build a collaboration in the future (Figure 1B). One laboratory did not answer the question.

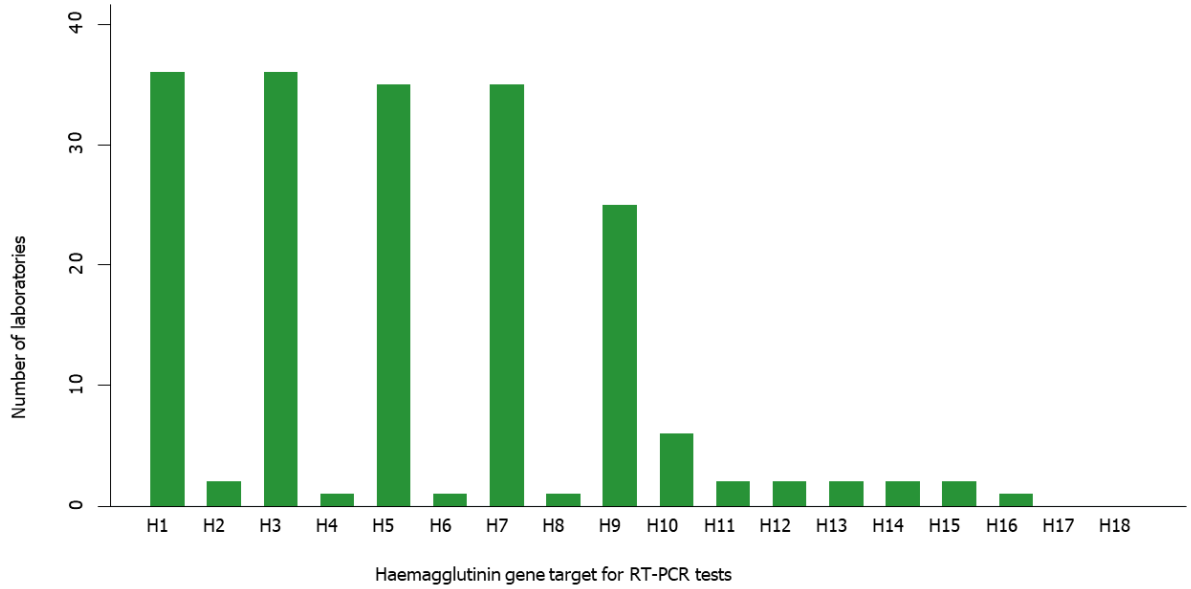
[ii] https://ec.europa.eu/eusurvey/home/welcome

Molecular diagnostics

Thirty-four out of 36 laboratories (94%) reported they are testing for avian influenza viruses in their laboratory. All but one participating laboratory has the capacity and capability to perform RT-PCR testing for avian influenza viruses and the single laboratory that is not performing such tests, sends the specimens to another laboratory for testing (also indicated above). Thirteen laboratories use multiplex assays, while the remaining are using single-plex assays. A wide variety of tests/assays are being used, as indicated below.

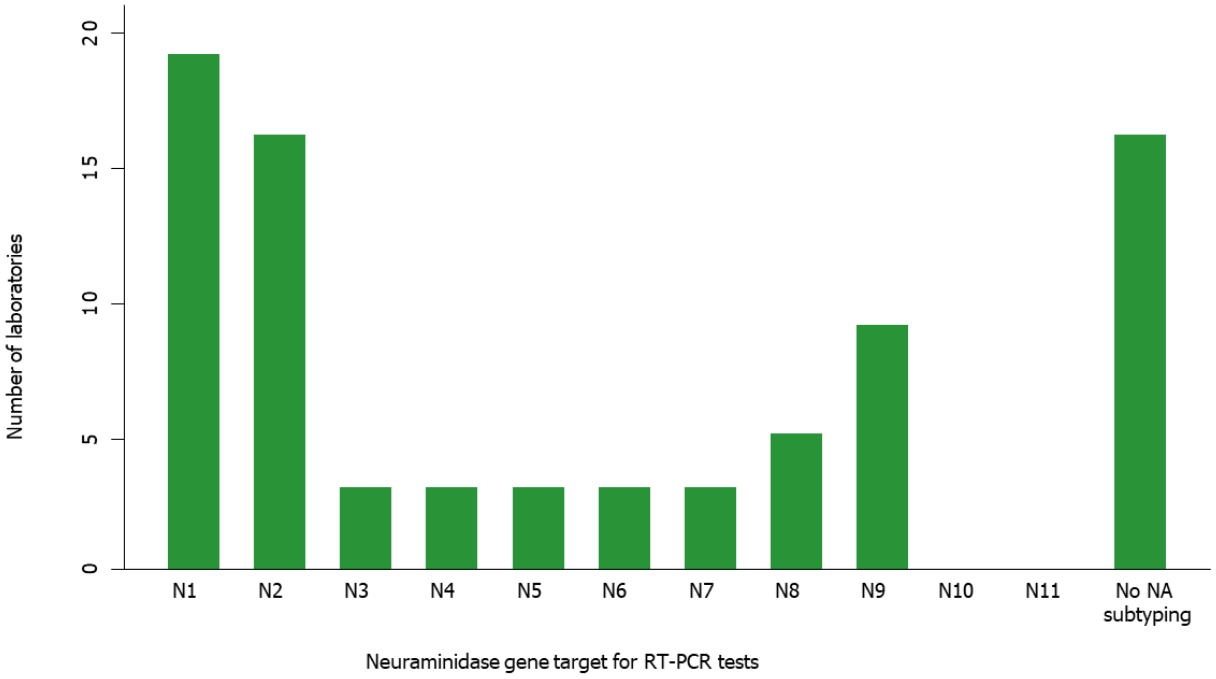
The 35 laboratories that have the capability and capacity to perform RT-PCR assays for avian influenza are using RT-PCR assays that cover H5 and H7 haemagglutinins (Figure 2). Twenty-five laboratories (69%) are also testing for H9, seven (19%) are also testing for H10. One is also testing for subtypes H11- 15, while one laboratory is testing for all HA subtypes. Examples of methods used for HA subtyping are in house RT-PCR assays (real time or conventional), Seegene Allplex kit, WHO protocols, CDC/Influenza Reagent Resource (IRR) assays, Sacacce, TimMolBiol and WGS. All three Western Balkan laboratories that participated in the survey and 16 EU/EEA laboratories are using the Influenza Reagent Resource (IRR) assays.

Figure 2. Number of countries that perform HA subtyping by HA subtype H1-H18



Twenty laboratories (57%) are performing NA subtyping (Figure 3). Of those, all but one laboratory is performing N1 typing, all but four laboratories are performing N2 typing. Examples of methods used for NA subtyping are in house RT-PCR assays (real time or conventional), NRC protocol, Sanger sequencing/WGS, Seegene assay, China CDC protocol, CDC protocol.

Figure 3. Number of countries that perform NA subtyping by NA subtype N1-N11



Thirty-two out of 35 laboratories that can perform RT-PCR specifically for detecting and identifying avian influenza viruses indicated that they have positive control material for avian influenza viruses, but the remaining three do not. Examples of RT-PCR positive control material that are used by these laboratories are: synthetic RNA and RNA extracted from cell cultures, inactivated virus or RNA provided by the WHO Collaborating Centre (WHO CC) or veterinary institutes, RNA extracted from external quality assessment panels (e.g. EQAP), positive controls supplied by commercial RT-PCR kits, and reference viruses shared by WHO CC London, ordered via IRR (CDC material). Twenty-three laboratories indicated that they don't have positive control material of the currently circulating H5 viruses of clade 2.3.4.4b for RT-PCR testing and are therefore using other H5 viruses. Laboratories that indicated they have access to such material obtained it from circulating viruses in animals in the country, usually via the veterinary institutes.

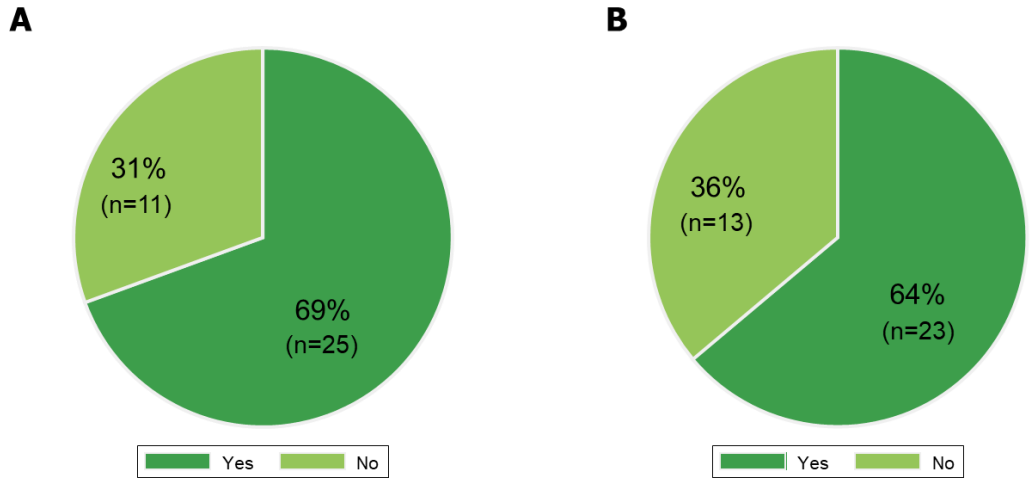
Overall, 19 laboratories are using IRR/CDC material: 17 order reagents, 13 order control material and two also order other material.

Genetic characterisation using Sanger/Whole Genome Sequencing

Almost 70% (n=25) of the 36 laboratories indicated that they have the capability and capacity to perform WGS for un-subtypable influenza A viruses (Figure 4 A). Thirteen of these 25 laboratories are using either Illumina or Nanopore technology or both, and two laboratories are using the Ion Torrent platform. Three laboratories indicated that they are currently in the implementation phase to use WGS for influenza viruses. The remaining laboratories did not provide any information about the sequencing platform they use.

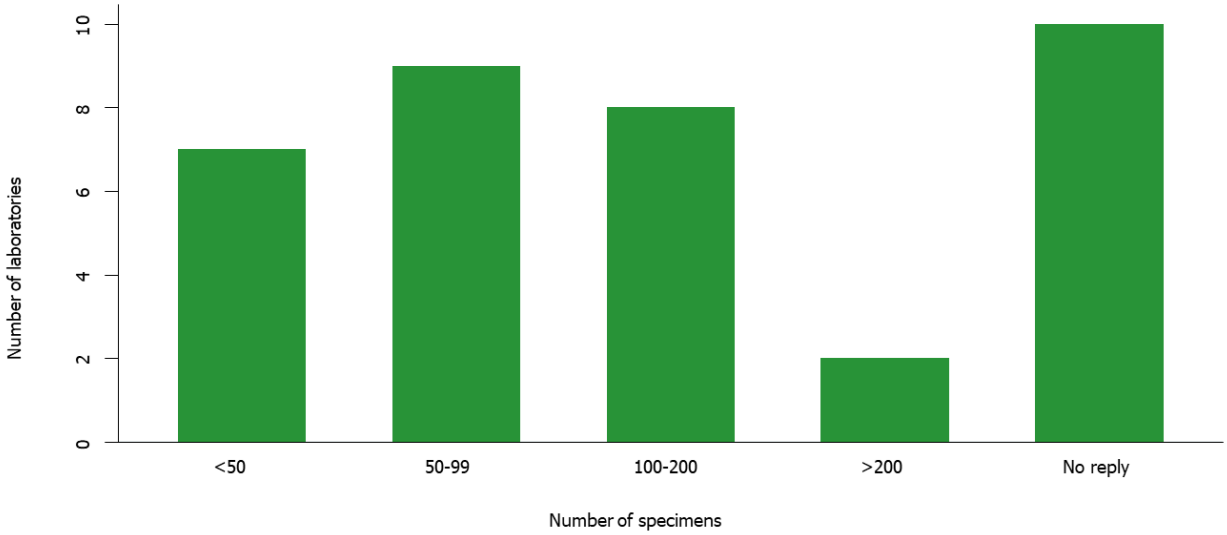
Sixty-four per cent (n=23) of laboratories can perform WGS for H5 influenza A viruses and 36% (n=13) replied that they do not have the capability and capacity to do this (Figure 4 B). Fourteen laboratories use Ilumina or Nanopore technology or both, and two laboratories use the Ion Torrent platform. Similar to the previous question, three laboratories indicated that they are currently in the implementation phase to use WGS for influenza viruses and the remaining laboratories did not provide any information about the sequencing platform they use.

Figure 4. Capabilities and capacities to perform WGS of (A) un-subtypable and (B) H5 influenza A viruses



Twenty-six laboratories responded to the question about the number of virus specimens that can be sequenced (Sanger or WGS) in their laboratory per week. Seven laboratories indicated that they can sequence less than 50 specimens per week, nine laboratories are able to sequence between 50-99 specimen, eight laboratories between 100-200 specimen and two laboratories are able to sequence more than 200 specimen per week (Figure 5).

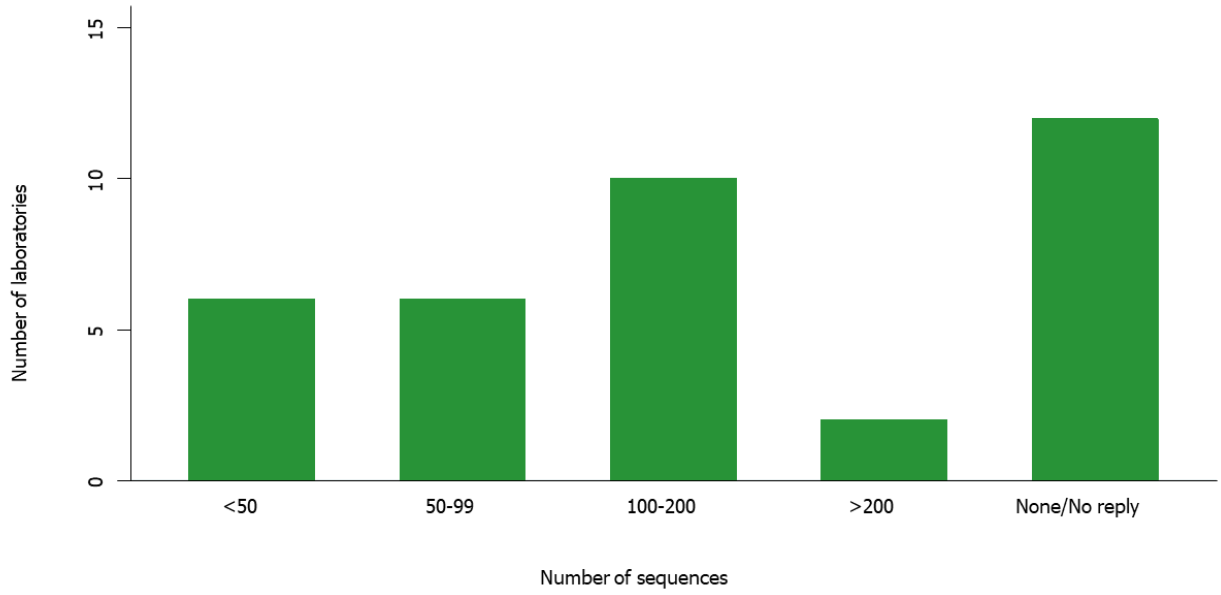
Figure 5. Number of virus specimens that can be sequenced (Sanger or WGS) in laboratories per week



Twenty-four out of 36 laboratories (67%) indicated they have the capability and capacity to analyse WGS data generated from zoonotic influenza viruses. Twelve of those 24 laboratories reported that they use the WGS data for clade assignment and to analyse antiviral resistance, mammalian adaptation, virulence and vaccine strain match. The other 12 laboratories use WGS data for clade assignment and/or antiviral resistance only, or are currently establishing further WGS analysis for zoonotic influenza viruses. Twelve laboratories (33%) do not have capability and capacity to analyse WGS data generated from zoonotic influenza viruses, but five of them have plans to implement it soon.

Of the 24 laboratories which have capability and capacity to analyse WGS data generated from zoonotic influenza viruses, six of them can analyse less than 50 sequences per week, six between 50 and 99, 10 between 100 and 200 and two laboratories are able to analyse more than 200 sequences per week. Twelve laboratories indicated they cannot analyse any sequences (yet) or did not provide any reply (Figure 6).

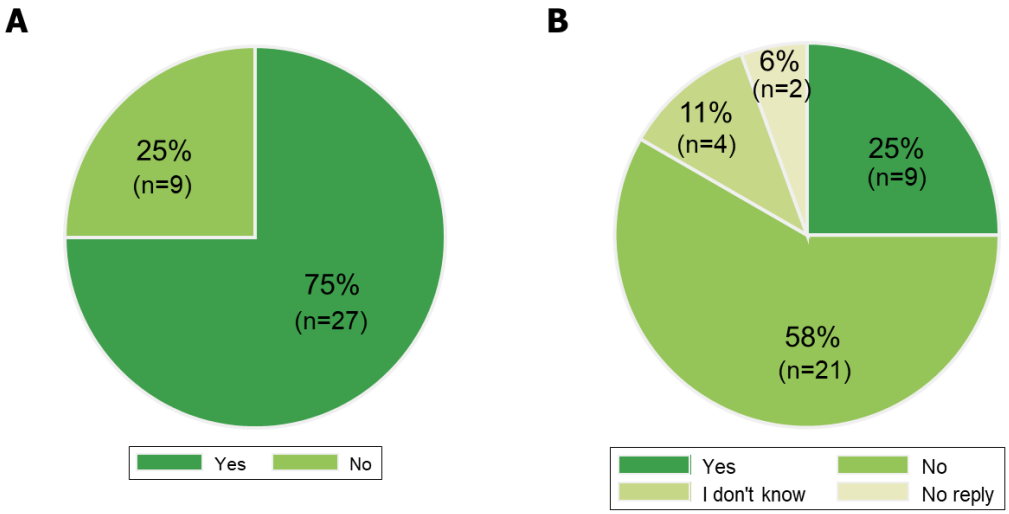
Figure 6. Number of sequences that can be analysed in laboratories per week



Data sharing

Seventy-five percent of the laboratories (n=27) upload sequences in real time in a public repository and 23 of them upload their sequences to the WHO Global Influenza Surveillance and Response System (GISAID). The remaining four laboratories did not provide the respective repository. Nine laboratories (25%) reported they do not upload sequences in real time in a public repository (Figure 7A). Reasons provided were that there is no developed protocol or that WHO-CC in London uploads the data in GISAID for the laboratory. Two laboratories indicatedthey are planning to upload their sequences to GISAID after the respective implementations of WGS analyses.

Figure 7. Number of laboratories that upload sequences in a public repository (e.g. GISAID, Genbank) (A), and number of laboratories with national regulations for sharing metadata databases (B)



Regarding metadata, 25% of the laboratories (n=9) indicated that they cannot share them in databases due to national regulations. All nine laboratories mentioned GDPR and institutional data protection practices as being the reason for this. However, the majority of laboratories (58%; n=21) reported no restrictions to share their metadata in public repositories. Four laboratories (11%) indicated that they do not know if there are any restrictions in place and two laboratories (6%) did not provide a reply (Figure 7B).

A single laboratory (3%) is uploading raw reads to a public database, while the vast majority (86%, n=31) of the laboratories do not do so. Three laboratories (8%) did not know if they upload raw reads, and one laboratory (3%) did not provide a reply to this question. The reasons provided for not uploading raw reads to a public database were:

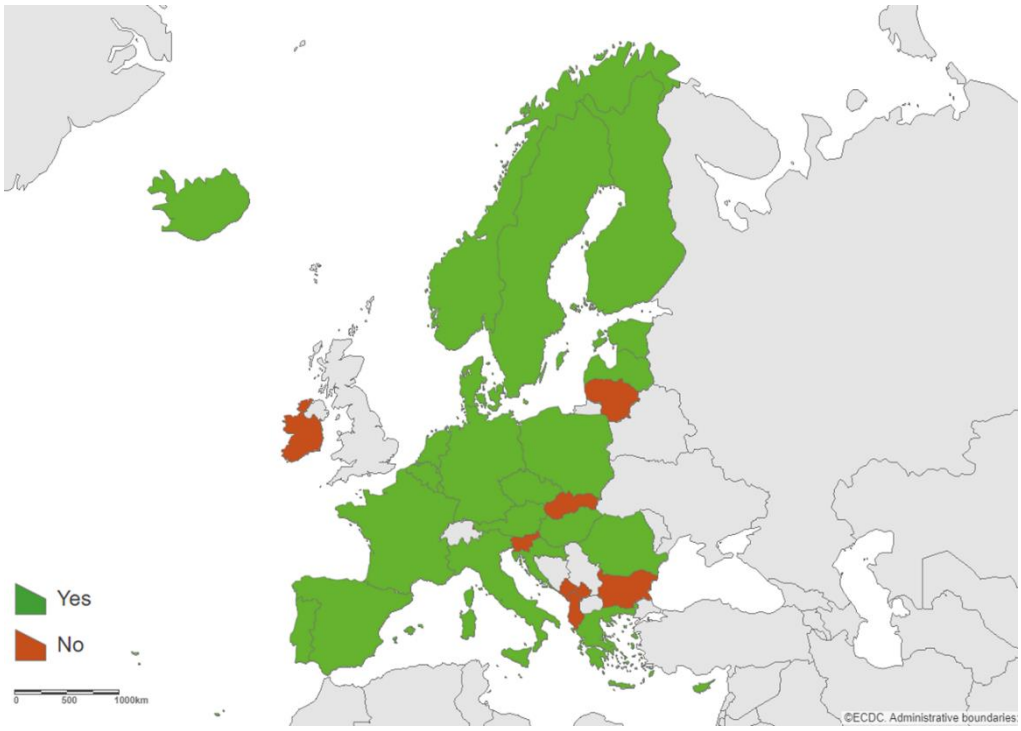
- No added value given the efforts/work involved to upload the data;
- We did not know about it;
- ENA is not the first option for uploading sequences; there are no analytic tools available (as opposed to GISAID);
- The files requested for uploading are too big;
- Limited human resources/capacity/time.

Laboratories were asked if they need to obtain formal permission to upload zoonotic virus sequences (e.g. H5Nx) to a public sequence database. Two of the 36 laboratories (6%) said permission is needed, 25 laboratories (69%) do not need formal permission, eight laboratories (22%) did not know if permission is needed and one laboratory (3%) did not reply to the question.

Virus isolation and antigenic characterisation

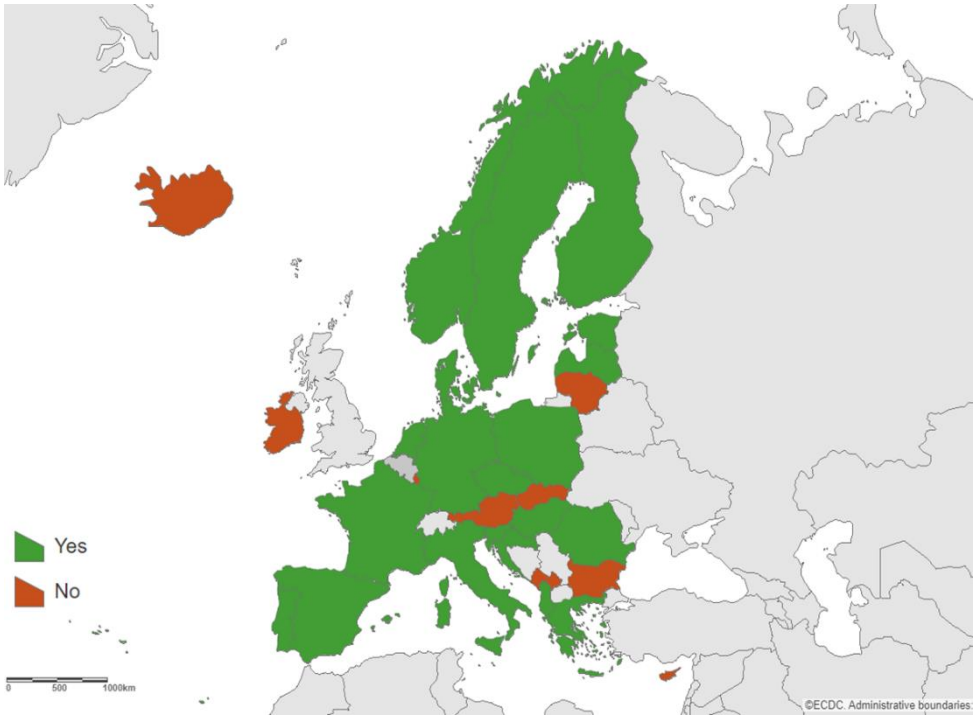
Twenty-six laboratories (72%) stated that they have BSL3 facilities for virus isolation and handling of highly pathogenic viruses from human specimens. Six (60%) of the 10 laboratories, who responded 'No', indicated that they have plans for establishing a BSL3 facility or have a BSL3 facility available in a nearby institute.

Map 1. Biosafety level 3 facilities available in the EU/EEA and Western Balkan countries, 2023



Twenty-two (63%) of 35 laboratories indicated they have capability and capaciy to isolate avian influenza viruses from human specimens, seven of the laboratories can also cell culture in hen's eggs. The capacity spans from less than five to up to 100 isolation attempts a week.

Map 2. Capability and capacity to isolate avian influenza viruses, EU/EEA and Western Balkan countries, 2023



Eleven (31%) of 36 laboratories reported capability and capacity to further antigenically characterise avian influenza viruses to assess their antigenic relatedness to candidate vaccine viruses/reference viruses. The methods include HI-assay with ferret antisera and if needed virus neutralisation (VN) or microneutralisation (MN) assays were mentioned. Alack of reference H5 strains and reference antisera was mentioned as a challenge. The capacity for antigenic characterisation varied from upon request (e.g. once a month), to up to a 100 viruses per week.

Out of the 36 responding laboratories, only three (<10%) indicated they have reference antisera for the currently circulating H5 virus candidate vaccine viruses (CVVs) and/or clade 2.3.4.4b viruses for antigenic characterisation. The specifics of those reference materials were:

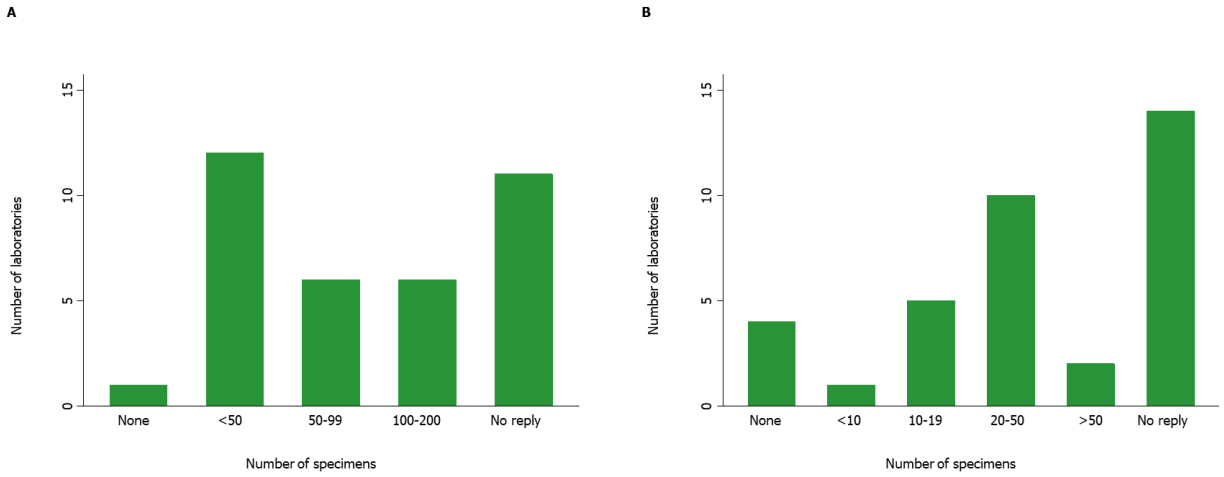
- In-house generated ferret antisera against CVVs and all major clades (including 2.3.4.4.b);
- Ferret antisera raised against CVV distributed by the CDC Influenza division;
- In-house ferret antisera raised against other 2.3.4.4b clade viruses (not CVV) through the Food and Agriculture Association/WHO funding and used to expand the antigenic coverage;
- The homologous inactivated antigens for each of these sera.

Two laboratories indicated they would be able to share antisera with other EU/EEA laboratories. The same laboratories mentioned they produce all ferret antisera on their own and one can also outsource some of the production. However, there is not much capacity to share antisera beyond H5 CC and WHO CC London due to limited supplies. Thirteen laboratories reported that they would need ferret antisera for circulating virus strains to be better prepared for human cases. EU reference material was requested by 25 laboratories.

Antiviral susceptibility testing

Of the 36 data reporters, 23 (64%) laboratories indicated they have the capability and capacity to perform genotypic antiviral susceptibility testing. Of those laboratories ,18 (78%) have capability and capacity to perform phenotypic antiviral resistance testing for neuraminidase inhibitors and four laboratories for baloxavir marboxil in addition. The weekly capacity spanned from 10 to 200 specimens for genotypic and five to 60 specimens for phenotypic antiviral susceptibility testing.

Figure 8. Number of specimens that can be genotypically (A) or phenotypically (B) analysed for influenza antiviral susceptibility in the human influenza reference laboratories, EU/EEA and Western Balkan countries, 2023



Special studies

In order to understand the capability and capacity to analyse serum samples for population immunity studies, laboratories were asked if they can perform such analyses and to describe the methods used. Twenty-one (58%) of the 36 responding laboratories indicated they have capacity to perform such special studies. Additionally, to the traditional hemagglutination inhibition assay and (micro)neutralisation studies, ELISA and protein array methods were also mentioned as methods of choice for analysing sera.

Only two laboratories indicated they have been involved in influenza A(H5) seroprevalence studies through national investigations of animal-human transmission or staff collecting dead birds. Five laboratories responded that they have access to a serum panel to use as control material for serological assays. The listed materials included: NIBSC H5 international standard 07/150, serum from avian species, and sera from healthcare workers with no history of poultry exposure.

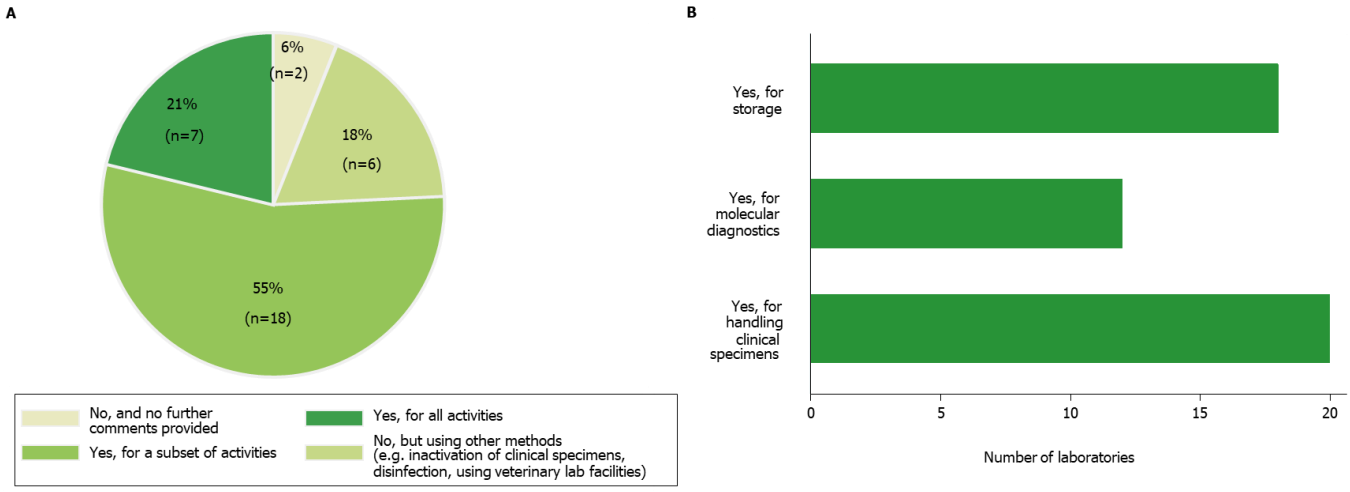
Biosafety measures, restrictions and relevant permits

In this session, the aim was to better understand the various biosafety measures, the restriction implemented and permits required in the context of working with zoonotic influenza viruses from human specimens in the EU/EEA.

Laboratory facilities

Firstly, countries were asked if they have separate physical facilities for handling seasonal and zoonotic influenza viruses from human specimens. They were also asked to list the specific activities that are performed in such separate facilities (should they exist), namely storage or isolation of virus, molecular diagnostics or handling of clinical isolates. Twenty-five out of 33 laboratories that receive human specimens for zoonotic influenza testing (76%) reported they have dedicated physical facilities to separate seasonal and zoonotic influenza specimens for handling clinical specimens and/or molecular diagnostics and/or storage, for either all activities (seven laboratories; 21%) or a subset of activities (18 laboratories; 55%) (Figure 9A). Laboratories reported using separate facilities to handle clinical specimens (n=20), storing clinical specimens/viruses (n=18) and/or perform molecular diagnostics (n=12) (Figure 9B). Six of the remaining laboratories said that while they do not physically separate seasonal and zoonotic influenza clinical specimens, they apply other precautions, such as processing seasonal or zoonotic influenza viruses in separate sessions with decontamination/disinfection steps in between, using different facilities (e.g. in veterinary laboratory) or inactivating the clinical specimens. Two laboratories did not provide further details.

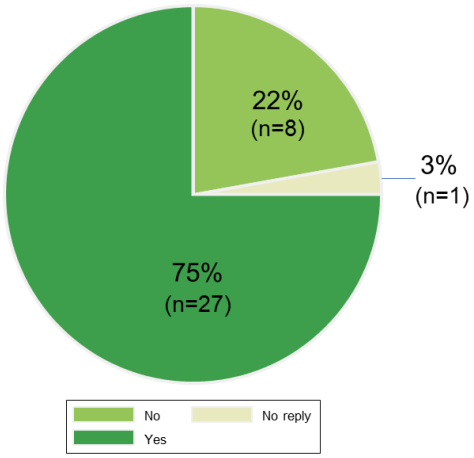
Figure 9. (A) Number of laboratories that reported having separate physical facilities for seasonal and zoonotic influenza virus (B) Number of laboratories that have separate physical facilities by activity (storage, molecular diagnostics, handling of clinical specimens)



Twenty-one laboratories are inactivating clinical specimens with suspected zoonotic virus content prior to RNA extraction and RT-PCR testing; 11 use a dedicated BSL3 laboratory, seven institutes work in a dedicated BSL2+ laboratory and three institutes process the specimen in a BSL2 facility. Regarding the method for inactivation of clinical specimens with suspected zoonotic influenza virus content, most laboratories inactivate them with lysis buffer (n=16), while another three add a heat inactivation step to the inactivation procedure.

Twenty-seven countries reported their influenza laboratory is accredited according to e.g. ISO15189 or ISO17025, while influenza laboratories in eight countries do not have accreditation and one country chose to not answer this question. When looking further into which kind of accreditation these 26 laboratories have, 11 laboratories reported that they operate under ISO15189 and five carry an ISO17025 accreditation. Three laboratories have both ISO15189 and ISO17025 accreditations, eight laboratories do not further specify which accreditation/s they carry.

Figure 10. Number of laboratories that have accreditation (e.g. ISO15189 or ISO17025)



Restrictions and regulations

When it comes to import permits of H5-containing materials, a rather diverse situation was presented. Nine laboratories (25%) require import permits for at least three of the four mentioned materials, i.e. H5 clinical specimens, virus isolates, reference antisera or RNA. Twelve laboratories (33%) do not require any import permits for such materials. One laboratory needs an import permit for clinical isolates only, the national public health institute is, however, exempt from that requirement and consequently does not need any import permit either. Fourteen national contact points could not provide an answer to this question.

For exporting clinical specimens with H5 virus from humans, virus isolates, reference antisera or RNA outside the EU/EEA, 17 laboratories (47%) reported that they do not require an official export permit. Again, many national contact points (n=13) could not provide an answer to this question. Consequently, only six laboratories could confirm they generally need export permits, three require such a permit for some, and another three require a permit for all of the mentioned materials.

Notably, the situation looks similar for exporting clinical H5 virus specimen, isolates, reference antisera and genetic material within the EU/EEA when compared to outside the EU/EEA. Nineteen laboratories (53%) reported that export permits are not required, while national contact points of 14 countries were not aware of required export permits. Three laboratories reported requirement of export permits for at least some of the above-mentioned materials.

In general, the processing time for obtaining any permit, either for export or import, seem to vary greatly between countries and span a range from one day to two months.

Regarding national restrictions or regulations in place, only a few countries could define the framework under which laboratory work with human specimens of avian influenza H5 virus can be performed. Twenty-one laboratories (58%) did not know about additional regulations or restrictions, while 10 confirmed that no such restrictions or regulations apply. Five laboratories (14%) reported a variety of additional regulations, ranging from restrictions for staff to work with avian influenza viruses under certain circumstances, to the necessity to declare such specimens as infectious agents under biosafety category B or to apply to additional shipping regulations when shipping specimens.

Needs for laboratory support

The following set of questions aimed to explore the need for laboratory support in the human influenza reference laboratories. Most of the thirty-three laboratories (92%) confirmed participating in the WHO external quality assessment programme (EQAP) which includes avian influenza strains and only three laboratories (8%) do not take participate. Similarly, 29 (81%) laboratories would be interested in participating in a bioinformatic ring-trial on zoonotic influenza WGS data analyses, while seven laboratories (19%) reported they have no interest in such an activity for now.

When going into further detail of a possible EQA and which activities it should entail, 20 laboratories (56%) would be interested in participating in a more complex EQA including molecular diagnostics, genetic characterisation and antiviral susceptibility monitoring of zoonotic influenza viruses. Some laboratories would reduce the complexity of an EQA to only two pillars, i.e. molecular diagnostics and genetic characterisation. Another five laboratories would opt for an EQA on molecular diagnostics only and one laboratory would be interested in adding even antiviral susceptibility monitoring.

In an open question, countries could submit suggestions for ECDC on how to improve and better support public health laboratories in the EU/EEA countries for zoonotic influenza monitoring. Only six laboratories responded with specific suggestions. Generally, countries would value sharing of protocols, including sequencing support, and reference reagents. Overall, closer collaborations between countries is desired and ongoing exchange with institutions such as EFSA are also well appreciated.

Discussion

Most participating ERLI-Net laboratories in EU/EEA countries (31/33) and two out of three laboratories from the participating Western Balkan countries are receiving specimens from exposed people when there is suspicion of zoonotic influenza virus infection for testing or for confirmation of positive tests performed elsewhere. Importantly, many participating laboratories (78%, 28/36) reported there is collaboration between the human and animal health sectors.

Overall, all but one of the participating laboratories have reported a substantial level of expertise, capability and capacity to detect avian and other zoonotic influenza viruses in human specimens, and the majority (69%) can also genetically characterise the viruses. In addition, more than half of the laboratories (63%) can isolate the zoonotic influenza viruses from human specimens and one third of the laboratories can further antigenically characterise those viruses.

The vast majority (97%, 35/36) of the participating laboratories have the capacity to detect zoonotic influenza virus A(H1), A(H3), A(H5) and A(H7), while 25 (69%) have the capacity to detect A(H9) or other subtypes or test for the neuraminidase type (56%, n=20). Overall, there is a variety of assays/methods used for detection of zoonotic influenza in different laboratories. Most of the laboratories (91%, n=32) have positive control material to use for RT-PCR for the detection of the respective subtypes, although usually not specifically a positive control using the currently circulating 2.3.4.4b A(H5) avian influenza strain.

The majority of countries have capacities to isolate and characterise avian influenza viruses and those capacities are available across EU/EEA countries [10]. Countries who do not have capacities on their own, have access to reference laboratory support from other countries and/or WHO CC.

In accordance with the results of a previous survey, a high proportion of laboratories are equipped with BSL3 facilities for isolation of highly pathogenic viruses [10]. Although 72% (26/36) laboratories reported that have BSL3 facilities for virus isolation and handling of highly pathogenic viruses and many laboratories handle clinical specimens with suspected zoonotic virus content in dedicated facilities, there seems to be little harmonisation in what kind of facility is being used for the different clinical specimen/virus/RNA handling steps (BSL3, BSL2 with additional precautions, BSL2). Guidelines for the development and sharing of laboratory techniques and standard operating procedures could be useful for harmonisation and for laboratories to further develop their capabilities.

Overall, there is a variability in the assays/methods used for detection of zoonotic influenza viruses in human specimens in the different laboratories. Guidance, harmonisation of methods and good laboratory practices can be facilitated via the laboratory support that ECDC and WHO Regional Office for Europe are providing to the national reference laboratories, that include trainings and provision of technical support. For more information, please contact ECDC.influenza@ecdc.europa.eu. Exchange of information, protocols and reagents (e.g. control material) for detection and characterisation of zoonotic influenza viruses from human specimens can be facilitated through the existing international laboratory influenza networks.

Similar to our previous survey in 2022 [11], more than half of the participating laboratories (64%, 23/36) reported they have the capability and capacity to perform antiviral susceptibility testing. Of those, 78% (18/23) can also perform phenotypic testing for antiviral susceptibility which is morecountries compared to the last survey [11].

Again, similar to a previous survey on capacity to perform antigenic characterisation of SARS-CoV-2, lack of reference material for virus isolation, antigenic characterisation and antiviral susceptibility hampers performance of these techniques at a practical level and therefore the true availability of e.g. virus neutralisation techniques could be lower [8]. Access to reference viruses and ferret antisera for avian influenza viruses would be crucial to increase the virus characterisation capabilities in the EU/EEA. The same lack of reference materials also applies to performance of serological assays to investigate human immunity to circulating viruses. It should be ensured that appropriate reference control and material are distributed in a timely way to the national reference laboratories.

Most of the participating laboratories indicated they have the capability and capacity to perform WGS analysis for un-subtypable influenza A viruses (69%), for H5 influenza A viruses (64%) and/or for zoonotic influenza viruses (67%). Laboratories upload the generated consensus sequences in real time in a public repository, often in GISAID. Only a single laboratory uploads raw reads to a public database.

The majority of laboratories (58%) reported no restrictions to share their metadata in public repositories and nine laboratories indicated they cannot share them in databases due to national regulations, GDPR and institutional data protection practices. As sharing of data is crucial for an effective response to outbreaks and epidemics of zoonotic influenza viruses, it is important to explore ways to overcome potential GDPR or other institutional data protection issues that may prohibit timely data sharing.

Although 21 of the 36 responding laboratories (58%) have the capacity and capability to perform serological studies for assessing population immunity, only five laboratories have control sera and only two laboratories have already participated in such studies at the national level.

When it comes to importing and exporting H5-containing materials, either within or outside the EU/EEA, national legislation is diverse. Fourteen laboratories report they are not aware of national permit requirements or other regulations and restrictions that may apply when working with avian influenza viruses from human specimens. This could delay and complicate sharing of material, e.g. in the context of international research collaborations or capacity building activities. Further awareness training organised both at the national and international levels would be needed as well as international collaboration.

An important outcome of the survey is the continued appreciation by the countries of laboratory support in the form of EQAs. Almost all national public health laboratories in the EU/EEA participate in such activities and are will continue to do so. In addition, many laboratories expressed an interest in bioinformatic ring trials. ECDC provides laboratory support and organises EQAs on influenza. Information is regularly shared by ECDC and by the laboratory support provider to inform the network about the offered services, including training activities, EQAs and bioinformatic ring trials and virus characterisation services. The WHO Regional Office for Europe also regularly organises EQAs for influenza virus detection and other laboratory support activities. National reference laboratories for influenza are encouraged to participate in those activities.

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Annex 1. Survey on laboratory capacity survey for molecular diagnosis and characterisation of zoonotic influenza viruses in the EU/EEA

To: Operational Contact Points for Influenza and COVID-19 (Microbiology)
Cc: Operational Contact Points for Influenza and COVID-19 (Surveillance)
Cc: National Focal Points for Viral Respiratory Diseases, National Coordinators

Reporting laboratory:

Country:

Contact detail of the reporting expert:

Can we contact you if we have additional questions? Y/N

Do you agree to share the raw data of this survey with WHO Euro? Y/N

Questions:

Specimen sources

1) Is your laboratory receiving specimens from exposed people when there is suspicion of zoonotic influenza virus infection? Yes/No

   If no, please describe where the specimens are sent:

2) Is there a laboratory collaboration between animal and public health/national reference laboratories e.g. in receiving specimens/isolates at the NIC or send human specimens/isolates to the animal national laboratory, exchange of other reagents/protocols (e.g. RT-PCR protocols, primers/probes, controls, sera)? Yes/No

   Please describe further:

Molecular diagnostics

3) Do you perform testing for avian influenza viruses using RT-PCR in your laboratory? Yes/No

4) Do you use multiplex assays for avian influenza virus detection? Yes/No

5) Which haemagglutinins (HAs) are covered by your RT-PCR tests? Tick box H1-H18 Free comment field, please describe method used

6) Which neuraminidases (NAs) are included in your detection systems? Tick box N/A/ N1-N11 Free comment field, please describe method used

7) Do you have RT-PCR positive control material for avian influenza viruses? Yes/No

   If yes, please comment

8) Do you have positive control material for the currently circulating H5 viruses of clade 2.3.4.4b for RT-PCR testing? Yes/No

   If yes, please comment

9) Are you using the Influenza Reagent Resource (IRR) RT-PCR reagents, controls and protocols for avian influenza virus detection? No/Yes reagents/Yes controls/Yes antisera/Yes other/I don't know.

   If yes, please specify here which ones:

Genetic characterisation using Sanger/Whole Genome Sequencing (WGS)

10) Do you have the capabilities and capacities to perform WGS for unsubtypable influenza A viruses? Yes/No

    If yes, please describe method:

11) Do you have the capabilities and capacities to perform WGS for H5 influenza A viruses? Yes/No

If yes, please describe method:

12) How many virus specimens can be sequenced (Sanger or WGS) in your lab per week? Free comment field

13) Do you have the capabilities and capacities to analyse WGS data generated from zoonotic influenza viruses? Yes/No

If yes, please comment on analyses performed (e.g. clade assignment, antiviral resistance, mammalian adaptation, virulence, vaccine strain match):
If no, please comment:

14) How many virus sequences can be analysed in your lab per week? Free comment field

Data sharing

15) Do you upload sequences in real time in a public repository (e.g. GISAID, Genbank)? Yes/No/I don't know

If yes, please describe which one. (free comment field)
If no, please describe the constraints. (free comment field)

16) Is there any metadata that cannot be shared in this database due to national regulations? Yes/No/I don't know

If yes, please describe (free comment field)

17) Do you upload raw reads in a public database (e.g. European Nucleotide Archive ENA)? Yes/No/I don't know

If no, please describe the constraints. (free comment field)

18) Do you need to obtain formal permission to upload zoonotic virus sequences (e.g. H5Nx) to a public sequence database? Yes/No/I don't know

Virus isolation and antigenic characterisation

19) Do you have BSL3 facilities for virus isolation and handling of highly pathogenic viruses? Yes/No

20) Do you have the capabilities and capacities to isolate avian influenza viruses? Yes/No

a. If yes, please describe here the capabilities and methods (e.g. cell lines, trypsin use, other supplements):

b. If yes, please describe here capacities per week:

21) Do you have the capabilities and capacities to further antigenically characterise avian influenza viruses to assess their antigenic relatedness to candidate vaccine viruses/reference viruses? Yes/No

a. If yes, please describe here the capabilities (including method):
b. If yes, please describe here capacities per week:

22) Do you have reference antisera for the currently circulating H5 virus CVVs and/or clade 2.3.4.4b viruses for antigenic characterisation? Yes/No

a. If yes, please specify (free comment field)
b. If yes, would you be able to share with other EU/EEA laboratories? Yes/No
c. If yes, can you make such sera yourself, in which species? (free comment field)
d. If no, would you need such material? Please describe needs (free comment field)

23) Would you be interested to have EU reference material? Yes/No

Antiviral susceptibility testing

24) Do you have the capabilities and capacities to perform genotypic antiviral susceptibility testing? Yes/No

25) Do you have the capabilities and capacities to perform phenotypic antiviral resistance testing? Yes for neuraminidase inhibitors/Yes for baloxavir/Yes for M2 blockers/ No (Multiple choice)

26) How many virus specimens can be analysed for antiviral susceptibility in your lab per week? Free comment field

Special studies

27) Has your laboratory the capabilities and capacities to perform the analysis of serum samples for population immunity studies (using hemagglutination inhibition, neutralisation studies and/or ELISA) to assess the level of cross-reactive immunity? Yes/No – Please describe method, free comment field

28) Has your laboratory been involved in H5 seroprevalence studies? Yes/No – Please describe the study and study period

If yes, please describe

29) Do you have access to a sera panel as control material for serological assays?

If yes, please describe

Biosafety measures, restrictions and relevant permits

30) Do you have separate physical facilities for handling seasonal influenza and zoonotic influenza viruses? Yes for storage/Yes for isolation/Yes for handling clinical specimens/Yes for molecular diagnostics/No – Multiple choice possible

31) Do you inactivate the zoonotic virus in clinical specimens before RNA extraction using a dedicated facility? Yes/No

If yes, do you use BSL2, BSL2+ (with enhanced precautionary measures) or BSL3 facility for inactivation of clinical specimens? BSL2/BSL2+/BSL3

32) Does your laboratory have an official permit to work with agricultural pathogens e.g. avian influenza viruses detected in avian species (not from human cases) and/or swine influenza viruses? No only working with human avian influenza specimens/No only working with human swine influenza specimens/Yes working also with veterinary avian influenza strains/Yes working also with veterinary swine influenza strains/An official permit is not needed to work with both human and veterinary avian influenza specimens – Mutiple Choice

33) Is an import permit needed in your country for importing H5 clinical specimens, virus isolates, reference antisera, RNA (incl. e.g. for EQA purposes)?Yes for clinical specimens/Yes for reference antisera/Yes for virus isolates/Yes for genetic material/No import permits needed/I don't know – Multiple choice possible

If yes, please describe (incl. how long do you anticipate it will take to issue such a permit)

34) Is an export permit needed in your country for the laboratory to send out clinical specimens positive for H5 from humans, virus isolates, RNA to WHO CC in London (outside EU/EEA)? Yes for clinical specimens/Yes for reference antisera/Yes for virus isolates/Yes for genetic material/No import permits needed/I don't know – Multiple choice possible

If yes, please describe (incl. how long do you anticipate it will take to issue such a permit)

35) Is an export permit needed in your country for the laboratory to send out clinical specimens positive for H5 from humans, virus isolates, RNA to another laboratory within EU/EEA? Yes for clinical specimens/Yes for reference antisera/Yes for virus isolates/Yes for genetic material/No import permits needed/I don't know – Multiple choice possible

If yes, please describe (incl. how long do you anticipate it will take to issue such a permit)

36) Do any other restrictions/regulations apply in your country based on the national regulatory authority related to the laboratory work with a human specimen of avian influenza H5 virus? Please describe (free comment field)

37) Is your influenza laboratory accredited (e.g. ISO)?

If yes, please indicate which facilities and/or methods and what accreditation (free comment field)

Needs for laboratory support

38) Is your laboratory participating to the WHO EQAP that includes avian influenza virus strains? Yes/No

39) Would your laboratory be interested in participating to an EQA that will include molecular diagnostics and genetic characterisation and antiviral susceptibility monitoring of zoonotic influenza viruses? Yes to molecular diagnostics/Yes to genetic characterisation/Yes to antiviral susceptibility monitoring/No (Multiple choice)

40) Would your laboratory be interested in participating to a bioinformatics ring-trial on zoonotic influenza WGS data analyses? Yes/No

41) Do you have any suggestions for ECDC on how to improve and better support public health laboratories in the EU/EEA countries for zoonotic influenza monitoring – Free comment field

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