EUROPEAN COMMISSION

Advice provided by the IVD Expert Panel/Influenza Sub-group

Background Information
In general influenza viruses, as many respiratory viruses, can be defined as transmissible agents that may have the potential to cause a life-threatening disease with a high or suspected high risk of propagation. To what extend one influenza virus strain might be more or less likely to fulfil one of those characteristics depends upon many factors, among which some are virus specific (e.g., virulence, pathogenicity factors), host specific (such as susceptibility to the agent, age, gender, health status, vaccinations/ infection history) and environmental factors (e.g., geographic, ecologic, demographic, travel, social distancing and occupational measures). The interaction between these factors will eventually define whether the pathogen indeed will be able to show a more severe course of infection and/or disease or mainly lead to self-limiting conditions. For influenza viruses, these characteristics also depend upon the type and subtype, i.e., mainly influenza A and B viruses causing seasonal epidemics, of which only the A subtypes have also a zoonotic background, and through that, a higher pandemic potential. Whereas the pandemic strains are associated with increased attributable excess in morbidity and mortality, in between the pandemics the seasonal circulation of influenza strains may also lead to significant numbers of infections, severe disease, and death. The seasonal changes in influenza viruses are generally referred to as "antigenic drift", small changes (or mutations) in the genes of influenza viruses that can lead to changes in the surface proteins of the virus, hemagglutinin (HA) and neuraminidase (NA). Major changes are referred to as "antigenic shift" and occur only in influenza A viruses, resulting in new HA and/or new HA and NA proteins in the viruses that infect humans. Antigenic shift can result in a new influenza A subtype, for example when an animal population gains the ability to infect humans as was the case for A(H1N1)pdm09 [1]. The assessment of risk classification should therefore consider the influenza types and the epidemiological context. For the risk classification within the scope of *In Vitro* Diagnostic Regulation (IVDR) it is important that transmissibility and severity are put into the perspective of the individual patient and the public health risk. Within the context of the present request, it is important to realise that influenza viruses are transmitted between humans but also from animals to humans and from humans to animals. Influenza viruses from animal reservoirs may be not very efficiently transmitted between humans (e.g. H5N1, H7N7) and high pathogenicity in an animal reservoir is not identical to high pathogenicity in a human reservoir. But still influenza virus with a highly pathogenic potential in humans can cause significant public health consequences.

[1] Centers for Disease Control and Prevention (CDC). How Flu Viruses Can Change: "Drift" and "Shift". 2021. https://www.cdc.gov/flu/about/viruses/change.htm.

Transmissibility

Transmissibility is the defining characteristic of infectious diseases. Quantifying transmission matters for understanding infectious disease epidemiology and designing evidence-based disease control programs [2].

Following the 2009 H1N1 pandemic, WHO published a pandemic severity assessment [3]. Here the transmissibility indicator is defined as "*the ease of movement of the influenza virus between individuals and communities".*

Factors affecting this transmissibility include the ability of the virus to spread from person to person, the dynamics of transmission and the susceptibility of the exposed population. Transmissibility will be influenced by social and climatic factors. During seasonal influenza epidemics, transmissibility is usually measured by routine surveillance systems using a proxy (e.g., how many people are seeking healthcare for influenza-like illness). The actual dynamics of the spread (the reproductive number) and the susceptibility of the exposed population would be measured by *ad hoc* special studies during a pandemic [3].

Transmissibility of influenza viruses from birds to humans depends on the nature of the exposure to infected birds or their products and the susceptibility to infection with the influenza virus concerned [4]. Subsequent human-to-human transmission may lead to "gain of function" resulting in potential pandemic risk. Transmission from pigs to humans may also occur and has been shown relatively frequently in the US during agricultural fairs. This has never led to efficient human-to-human transmission, unlike A(H1N1)pdm09, which was most likely also introduced into the human population through pig-to-human transmission. Transmission from human to pigs in these events is also described.

Disease severity

The life-threatening characteristic and high (or suspected high) risk of propagation represents the patient specific and the public health risk, respectively. The seriousness of disease or severity of infection indicator describes the extent to which individual people get sick when infected with the influenza virus, i.e., the frequency of clinical symptoms, complications of influenza illness and outcomes following influenza infection depends on the virus, the host and the environment and is likely to show different severity in different groups in the population. During seasonal influenza, seriousness of disease is measured with routine, hospital-based surveillance [3].

[2] Woolhouse M. Quantifying Transmission. Microbiol Spectr. 2017 Jul;5(4). doi: 10.1128/microbiolspec.MTBP-0005-2016. PMID: 28812540.

[3] World Health Organization (WHO). Pandemic Influenza Severity Assessment (PISA). 2017. https://apps.who.int/iris/bitstream/handle/10665/259392/WHO-WHE-IHM-GIP-2017.2-eng.pdf

[4] European Scientific Working group on Influenza and other Respiratory Viruses (ESWI). Important factors for flu virus transmission - Knowledge center (eswi.org).

In an effort to classify risks of influenza virus after (unintentional) professional exposure to influenza viruses (e.g., laboratory employees, health care personnel), a European Union risk classification has been published [5], where influenza viruses as a whole are listed as class 2 pathogens, which is defined as *"one [pathogen] that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available".* Although it is acknowledged that this reflects a different risk assessment, it may be helpful in addressing the current risk assessment.

1) Can the influenza virus strain A (H1N1) pdm09 be considered, today, as a transmissible agent that causes a life-threatening disease with a high or suspected high risk of propagation?

In April 2009, a new virus labelled A(H1N1)pdm09 appeared in Mexico and California (USA) and was responsible for the first pandemic of the 21st century, which spread rapidly from person to person and was not related to any circulating inter-pandemic virus. The virus turned out to be a quadruple reassortant virus, consisting of two swine-origin viruses, one avian-origin virus and one human-origin virus. Molecular studies identified the North American A(H3N2) triple reassortant virus circulating among swine in Europe and Asia, comprising genes from a classic swine H1N1 virus, and an "avian-like" swine H1N1 virus [6]. This virus proved to be remarkably different from the classic seasonal influenza H1N1 viruses and the viruses used to prepare vaccines [7], resulting in largely immunologically naïve populations with a high probability of transmission and rapid global spread.

Compared to the seasonal A(H1N1) and A(H3N2) viruses, A(H1N1)pdm09 showed a high fatality rate and higher incidence among younger people. Since such data come mainly from laboratory-confirmed cases, it was suggested that this was an underestimation of the magnitude of the event. Several studies since then have addressed the disease severity of infection with the influenza A(H1N1)pdm09 virus. A standardised case-based surveillance by the European Centre for Disease Prevention and Control (ECDC) of hospitalised patients with severe influenza infections in nine European countries over the epidemic seasons 2010-2011, showed a significant association of disease severity with age, underlying diseases and obesity [8].

[5] Directive 2000/54/EC of the European Parliament and of the Council of 18 September2000 on the protection of workers from risks related to exposure to biological agents at work [2000] OJEC L 262/21. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000L0054&from=EN

[6] Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghwani J, Bhatt S, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic . Nature. 2009;459:1122–1125.

[7] Gatherer D. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2009. 45;174:178.

[8] Snacken R, Quinten C, Devaux I, Plata F, Broberg E, Zucs P, Amato-Gauci A. Surveillance of hospitalised severe cases of influenza A(H1N1)pdm09 and related fatalities in nine EU countries in 2010-2011. Influenza Other Respir Viruses. 2012 Nov;6(6):e93-6. doi: 10.1111/j.1750-2659.2012.00406.x. Epub 2012 Jul 13. PMID: 22788875; PMCID: PMC4941705.

The A(H1N1)pdm09 virus continued to circulate after the pandemic of 2009. Co-circulation of A and B influenza viruses is frequently observed, resulting in the need for combined A(H3N2), A(H1N1)pdm09 and B/Victoria, B/Yamagata influenza virus vaccines. For example, it was estimated that both the A (84%) and the B (16%) influenza viruses were circulating simultaneously during the 2014-2015 seasonal influenza. Specifically, the A(H1N1)pdm09 virus accounted for 52% of all laboratory-confirmed cases and for 76% of all severe clinical manifestations. This was the epidemic with the highest number of severe cases reported since the 2009 pandemic [9].

The Centers for Disease Control and Prevention (CDC) calculated the disease burden attributable to A(H1N1)pdm09 since the 2009 pandemic until 2018 and estimated that in the US the virus strain has resulted in approximately 100.5 million illness cases, 936.000 hospitalizations and 75.000 deaths [10]. Comparable reporting data for Europe was not found, although ECDC provides detailed annual reports [11]. These reports indicate that European figures are unlikely to be significantly different.

[9] V. Baldo, C. Bertoncello, S. Cocchio, M. Fonzo, P. Pillon, A. Buja, and T. Baldovin (2016) The new pandemic influenza A/(H1N1)pdm09 virus: is it really "new"? J Prev Med Hyg. 2016 Mar; 57(1): E19–E22.
PMCID: PMC4910438 PMID: 27346935.

[10] Centers for Disease Control and Prevention (CDC). The burden of the influenza A H1N1pdm09 virus since the 2009 pandemic. 2019. https://www.cdc.gov/flu/pandemic-resources/burden-of-h1n1.html.

[11] European Centre for Disease Prevention and Control (ECDC). Influenza season summaries https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/seasonal-overviews.

Based on several reports from the pandemic and post-pandemic period there is no doubt that influenza virus A(H1N1)pdm09 has to be classified as easily transmissible virus causing life-threatening disease in unprotected population. These findings are supported by frequent outbreaks of influenza virus A(H1N1)pdm09 in different countries like Pakistan, Saudi Arabia, Cameroon, Israel and Denmark [12, 13,14, 15] and its predominance in different epidemic seasons (e.g., 2018-2019) [16]. Also, in 2022, A(H1N1)pdm09 virus is still recommended by WHO as part of the seasonal vaccine composition [17].

Although vaccines are available, of concern is a low to moderate vaccine effectiveness of influenza vaccines as reported by CDC and ECDC [18, 19], generally estimated to be between 30-60%. Another concern is the finding of oseltamivir-resistant variant of A(H1N1)pdm09 influenza viruses [20 21]. Both findings support continued virus evolution with possible risks of treatment failure and immune escape and subsequent risks for susceptible persons with different pathogenic potential depending on pre-existing immunity and fitness of the immune system. This implies that global surveillance of influenza viruses and the analysis of antiviral susceptibility remains a continued task for public health and patient care.

12 N. Badar, M. Salman, U. B. Aamir, et al. (2020) Evolutionary analysis of influenza A(H1N1)pdm09 during the pandemic and post-pandemic period in Pakistan.
J Infect Public Health, 2020 Mar;13(3):407-413; doi: 10.1016/j.jiph.2019.03.008. Epub 2019 Apr 15; PMID: 31000492; DOI: 10.1016/j.jiph.2019.03.008.

13 A. A. Rabaan, S. A. Alshaikh, A. M. Bazzi, et al. (2018) Influenza A(H1N1)pdm09 epidemiology in the Eastern Province of Saudi Arabia. J Infect Public Health 2018 Sep-Oct;11(5):636-639; doi: 10.1016/j.jiph.2018.05.014. Epub 2018 Jun 21; PMID: 29937408; PMCID: PMC7102725; DOI: 10.1016/j.jiph.2018.05.014.

14 C. G. Monamele, H. L. M. Njifon, M.-A. Vernet et al. (2019) Molecular characterization of influenza A(H1N1)pdm09 in Cameroon during the 2014-2016 influenza seasons.
PLoS One, 2019 Jan 14;14(1): e0210119; doi: 10.1371/journal.pone.0210119. eCollection 2019

15 J. N. Nissen, S. J. George, C. K. Hjulsager, et. al. (2021) Reassortant Influenza A(H1N1)pdm09 Virus in Elderly Woman, Denmark, January 2021 Emerg Infect Dis, Case Reports, 2021 Dec;27(12):3202-3205.; doi: 10.3201/eid2712.211361. PMID: 34808097; PMCID: PMC8632190; DOI: 10.3201/eid2712.211361.

16 European Centre for Disease Prevention and Control (ECDC). Infographic: Influenza in Europe, Season 2018-2019. 2019. https://www.ecdc.europa.eu/en/publications-data/infographic-influenza-europe-season-2018-2019.

17 World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2022-2023 northern hemisphere influenza season. 2022. https://www.who.int/publications/m/item/recommended-composition-of-influenza-virus-vaccines-for-use-in-the-2022-2023-northern-hemisphere-influenza-season.

18 Centers for Disease Control and Prevention (CDC). Past Seasons Vaccine Effectiveness Estimates. 2021. https://www.cdc.gov/flu/vaccines-work/past-seasons-estimates.html.

19 European Centre for Disease Prevention and Control (ECDC). Influenza vaccine effectiveness. https://www.ecdc.europa.eu/en/seasonal-influenza/prevention-and-control/vaccine-effectiveness.

20 Govorkova EA, Takashita E, Daniels RS, Fujisaki S, Presser LD, Patel MC, Huang W, Lackenby A, Nguyen HT, Pereyaslov D, Rattigan A, Brown SK, Samaan M, Subbarao K, Wong S, Wang D, Webby RJ, Yen HL, Zhang W, Meijer A, Gubareva LV. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2018-2020. Antiviral Res. 2022 Apr;200: 105281. doi: 10.1016/j.antiviral.2022.105281. Epub 2022 Mar 12. PMID: 35292289; PMCID: PMC9254721.

21 S. M. K. Win, R. Saito, N. C. Win, et al., (2020) Epidemic of influenza A(H1N1)pdm09 analyzed by full genome sequences and the first case of oseltamivir-resistant strain in Myanmar 2017. PLoS One 2020 Mar 4;15(3): e0229601.;doi: 10.1371/journal.pone.0229601.; eCollection 2020.

2) Are there any other currently circulating influenza virus strains that meet both of these conditions?

Influenza viruses show a continuous virus evolution with a potential for immune escape, resulting in potentially more virulent and transmissible viruses [22,23]. Although more severe epidemics and disease burden are generally associated with influenza A viruses, the 2017-2018 epidemic was unusually long and severe and predominated by influenza B virus [24]. During this season, severe cases were observed in hospital settings due to influenza B virus, but also due to influenza A(H3N2) and A(H1N1)pdm09 viruses in adults older than 40 years. Although most hospitalisations were due to influenza virus type B infection, patients in intensive care units were mostly infected by influenza type A virus. Different subtypes of influenza A viruses may have different attack rates (transmissibility) or disease severity, which makes a general conclusion on fulfilment of conditions not unequivocal [25].

An increase in the zoonotic risk of transmission of novel influenza strains other than A(H1N1) has been reported across different countries in the last years, regarding subtypes H3N2 [26], H7N9 [27], and H5N6 [28], among others. The potential transmission of a novel influenza A virus between animals and humans and the risk of pandemic emergence are well known [29]. Other environmental factors such as global warming and migration movements may also contribute to escalating the zoonotic risk of influenza spread [30]

22 A. V. Danilenko, N. P. Kolosova, A. N. Shvalov, et al., (2021) Evaluation of HA-D222G/N polymorphism using targeted NGS analysis in A(H1N1)pdm09 influenza virus in Russia in 2018-2019. PLoS One; 2021 Apr 29;16(4): e0251019.; doi: 10.1371/journal.pone.0251019. eCollection 2021. PMID: 33914831; PMCID: PMC8084186; DOI: 10.1371/journal.pone.0251019.

23 L. Xing, Y. Chen, B. Chen, et al., (2021) Antigenic Drift of the Hemagglutinin from an Influenza A (H1N1) pdm09 Clinical Isolate Increases its Pathogenicity In Vitro. Virol Sin; 2021 Oct;36(5):1220-1227.; doi: 10.1007/s12250-021-00401-y. Epub 2021 Jun 9. PMID: 34106413; PMCID: PMC8188537; DOI: 10.1007/s12250-021-00401-y.

24 European Centre for Disease Prevention and Control (ECDC). Influenza in Europe, summary of the season 2017–18. 2018. https://www.ecdc.europa.eu/en/seasonal-influenza/season-2017-18.

25 Park JE, Ryu Y. Transmissibility and severity of influenza virus by subtype. Infect Genet Evol. 2018 Nov;65: 288-292. doi: 10.1016/j.meegid.2018.08.007. Epub 2018 Aug 10. PMID: 30103034.

26 Bowman, et al. Influenza A(H3N2) Virus in Swine at Agricultural Fairs and Transmission to Humans, Michigan and Ohio, USA, 2016. Emerg Infect Dis 23, 1551–1555.

27 Iuliano, et al. Increase in human infections with avian influenza A(H7N9) virus during the fifth epidemic—China, October 2016–February 2017. MMWR Morb Mortal Wkly Rep. 2017; 66:254–5.

28 Chen, et al. A study of the relationship between human infection with avian influenza a (H5N6) and environmental avian influenza viruses in Fujian, China. BMC Infect Dis. 2019;19(1):762.

29 Short KR, et al. One health, multiple challenges: The inter-species transmission of influenza A virus. One Health. 2015; 1:1-13.

30 Canavan BC. Opening Pandora's Box at the roof of the world: Landscape, climate and avian influenza (H5N1). Acta Trop. 2019; 196:93-101.

In summary, there are different circulating seasonal influenza viruses that have the potential of high transmissibility and high risk of severe disease. Active global surveillance is critical as well as early warning system to detect such circulating or emerging strains, require sensitive and specific detection methods and international collaborations and sharing of data. This is globally done by the World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS) in a network of national influenza centres and specialized reference laboratories and in Europe also by ECDC.

3) Taking into account possible emergence of future influenza strains that would meet both of the above conditions, is it scientifically appropriate and feasible to develop general common specifications with minimum performance requirements and modalities of carrying out the performance studies for the corresponding class D devices?

Based on the above-mentioned unpredictable changes of influenza strains regarding their pandemic potential, it is not advised to develop general common specifications with minimum performance requirements and modalities of carrying out the performance studies for the corresponding class D devices.

As most assays used are indicated for detection of infection in individuals, whereby the outcome is presence or absence of influenza virus, often for type A or B level only, it would be useful to have common specifications. However, it is uncertain whether this will be a class D device in all cases. The risk for the individual patient or population of a specific new subtypes or strains of the type A virus can generally not be predicted as this information will not be generated through the used diagnostic assays. Several of these molecular assays are designed as multiplex assays, targeting other respiratory viruses which are not classified as class D devices.

Subtyping of influenza strains is usually done for surveillance purposes or outbreak management only, in specialised laboratories such as the WHO National Influenza Centres (NIC). These laboratories have the capacity to quickly adapt their assays to emerging subtypes that are a threat to humans. Moreover, specimens where the subtyping is inconclusive, raise suspicion of an unusual influenza A subtype composition indicative of a reassortant or a possible zoonotic event and trigger in-depth investigation including sequencing at the NIC or WHO Collaborating Centre for Reference and Research on Influenza (WHO CC).

Furthermore, the definition of "state of the art" performance of diagnostic devices depends also on the status of technological specifications which are continuously improving. Defining performance requirements independently of rapid technological development may be outdated at a certain point in time. As demonstrated in the scientific investigations, the effect of mutations, antigenic drift, recombination and reassortments can result in completely new influenza variants with a concurrent risk on increased transmissibility and disease severity. The pathogenicity of new strains, however, depends furthermore on the immunological background in the target population depending on previous influenza infections and/or vaccinations, the age, gender, and pre-existing health conditions.

Since many of these parameters are not known, the development of "general" common specifications with minimum performance requirements and modalities is challenging. Establishing virus characteristics of strains with pandemic potential or from non-human origin requires a different approach compared to infection detection in epidemic seasons, with different associated risks for individual or population that may be assessed differently from the seasonal detection assays. Respective assays may be conducted under different safety requirements, e.g., because of unknown/novel clinical or epidemiological features of the virus (e.g., pandemic potential, or viruses from non-human origin), the need for virus propagation through culture or testing in virus neutralisation assays, or anticipated risk for those handling the virus materials.

Such assays intended to detect and/or characterize strains with pandemic potential or from non-human origin could be classified as class D devices. Based on the above, it is however, recommended to classify the assays used for detection of seasonal influenza viruses as class C devices.

To be able to carry out proper performance evaluation studies for the corresponding class D devices at least the basic parameters like age, gender, infection/vaccination history and pre-existing health conditions should be considered. For clinical performance studies plans or protocols should be in place to rapidly confirm technical performance results in real-life settings.

If it is agreed to develop "general" common specifications for all influenza strains, irrespective of the anticipated risks and test objectives, then the possibility should be provided for fast-track adaption of the requirements based on the available performance studies and device minimal performance (e. g. sensitivity, specificity) characteristics taking into consideration the latest scientific findings to get the most robust evaluation studies for those class D devices.