

OFFLU

Avian Influenza Matching (AIM)

Technical Report

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Table of Contents

1. Guiding Information	3
2. Executive Summary	4
3. Background	5
3.1. Vaccine Seed Strains	7
3.2. Panel of Chicken Antisera	8
3.3. Phylogenetic Relationships	9
4. Main Findings	12
4.1. Antigenic Distances.....	12
4.2 Antigenic Cartography	16
5. Conclusions and Future Directions.....	18
Links to Key AIM Documents	19
References.....	19

1. Guiding Information

Acknowledgements

This report was produced by OFFLU network, the joint World Organisation for Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO) network of expertise in animal influenza. This work was led by OFFLU's Avian Influenza Vaccination technical activity, with contributions from Ian Brown (The Pirbright Institute, United Kingdom), Ashley Banyard, Joe James, and Joshua Lynton-Jenkins (Animal and Plant Health Agency, United Kingdom), Francesco Bonfante (Istituto Zooprofilattico Sperimentale delle Venezie, Italy), Frank Wong and Joanne Grimsey (CSIRO Australian Centre for Disease Preparedness, Australia), Amelia Coggon and Nicola Lewis (Royal Veterinary College, United Kingdom), Lorcan Carnegie (OFFLU Scientist, FAO), Amelia Coggon, and Fabien Filaire (FAO). Antigenic cartography was undertaken by Amelia Coggon and Nicola Lewis (Royal Veterinary College, United Kingdom). Hemagglutination inhibition (HI) data was generated by the International Reference Laboratory for Avian Influenza team, led by Joshua Lynton-Jenkins at the Animal and Plant Health Agency (United Kingdom), and by Francesco Bonfante and Silvia Maniero at the Istituto Zooprofilattico Sperimentale delle Venezie (Italy). We gratefully acknowledge Leslie Sims (OFFLU) for their role in initiating this project and providing strategic guidance. Additional HI data were contributed by Frank Wong and Joanne Grimsey at the CSIRO Australian Centre for Disease Preparedness (Australia). Viral isolates were kindly shared with the International Reference Laboratories for Avian Influenza from the following countries: Bangladesh, Belgium, Benin, Bulgaria, Burkina Faso, Canada, Cambodia, Cameroon, Croatia, Czech Republic, Falkland Islands, Finland, Georgia, Ghana, Guinea, Republic of Indonesia, Iran, Ireland, Israel, Italy, Ivory Coast, Kosovo, Latvia, Moldova, Nepal, Niger, Nigeria, Norway, Philippines, Poland, Romania, Saint Helena, Ascension and Tristan da Cunha, Slovakia, Slovenia, South Georgia and the South Sandwich Islands, Spain, Switzerland, Togo, Turkey, Ukraine, Viet Nam and the United Kingdom. We would like to thank all the OFFLU network experts, FAO and WOAH for their support with this project.

Benefits Sharing

The OFFLU AIM project operates on a principle of mutual benefit, ensuring that contributors of virus samples and associated data receive timely, meaningful support. Participating laboratories and submitting institutions can request technical assistance, including data analysis, interpretation of antigenic and genetic findings, and context-specific recommendations. This support is particularly valuable where vaccination is being considered or implemented. In such cases, testing representative local isolates against multiple within-clade antisera is recommended to detect antigenic drift which might impact vaccine match. In settings where viruses are enzootic in poultry or where multiple clades or subclades co-circulate, vaccine challenge studies using local strains are advised to evaluate vaccine effectiveness. Reagents (such as antisera and inactivated viruses) produced under the OFFLU AIM framework by APHA and IZSve can also be shared, upon request, with selected WOAH and FAO reference laboratories. This enables generation of comparative antigenic data using standardized reagents and protocols under a defined quality framework. However, appropriate inter-laboratory standardization and interpretation are essential before data from additional sources can be reliably integrated into analyses. For further information or technical dialogue, please contact: secretariat@offlu.org.

Disclaimers

This report provides the point of view of independent OFFLU experts and does not necessarily reflect the position of the parent organisations FAO and WOAH. Additionally, there has been a recent update to the H5 nomenclature by the WHO/FAO/WOAH H5 Nomenclature Working Group to reflect the genetic diversification of the A(H5) viruses, particularly clade 2.3.2.1c, to add 2.3.2.1d, e, f, and g (Ort *et al.*, 2025). Where relevant, updated clade nomenclatures have been adopted in this report.

2. Executive Summary

OFFLU has established a system to guide countries in how well their vaccine seed strains are antigenically matched to contemporary H5Nx Goose/Guangdong/1/96 (Gs/Gd) lineage high pathogenicity avian influenza viruses (HPAIVs). Reference antisera are raised in chickens using either clade-representative field isolates or isolates that genetically mirror existing vaccine seed strains (surrogate vaccine seed strains). Contemporary field viruses are then tested against these antisera with haemagglutination-inhibition (HI) assays, and the resulting titres are analysed and visualised through antigenic cartography to quantify the distance between current viral antigens and those used to generate the reference antisera. Larger antigenic distances indicate antigenic drift, which means the ability of antibodies to neutralise strains may be reduced, that in turn may compromise vaccine efficacy. This may necessitate updating vaccine seed strains. Background and methods appear in [Module 1 \(Background Report\)](#)¹ and [Module 2 \(Guidance Report\)](#)², while this document contains the technical findings.

As of July 2025, clade 2.3.4.4b H5N1 viruses account for most poultry outbreaks globally, but H5Nx viruses from clades such as 2.3.4.4h, 2.3.2.1a, 2.3.2.1e, and 2.3.2.1g continue to widely circulate in parts of South and Southeast Asia. To capture their combined impact, this OFFLU AIM assessment applies HI testing and antigenic cartography to quantify and visualise antigenic relationships between field isolates from these clades and within-clade vaccine seed strains. For these analyses, the post-infection chicken antisera panel was expanded to include three representatives from previously untested clades and two additional clade 2.3.4.4b viruses selected to better reflect contemporary H5 diversity in Africa. The test virus dataset comprises new HI data from 85 H5N1 viruses representing clades 2.3.2.1a, 2.3.2.1c, 2.3.2.1e, 2.3.4.4b, 2.3.4.4e, and 2.3.4.4h, covering with multiple neuraminidase subtypes (N1, N2, N5, N8, and others). These viruses were collected between 2016 and 2025 from Europe, North America, South and Southeast Asia, East Asia, and Antarctica, with testing conducted by international reference laboratories at APHA (United Kingdom), IZSVe (Italy), and ACDP (Australia).

Analyses conducted during this period indicate that the most recent H5 viruses from clade 2.3.4.4b, collected in 2024 and 2025, are expected to be suitable candidate antigens for inactivated whole virus adjuvanted vaccines targeting these strains, although some subtype-specific heterogeneity was observed, consistent with previous reports. In contrast, vaccine antigens from outside clade 2.3.4.4, including contemporary 2.3.2.1a viruses and earlier ancestral clades such as clades 1, 2.2.1, and 2.3.4 showed limited antigenic cross-reactivity and are unlikely to offer effective protection against currently circulating clade 2.3.4.4b viruses. Notably, H5N1 viruses from clade 2.3.2.1a, particularly those detected in South Asia in 2024, exhibited reduced reactivity to both clade 2.3.4.4 and 2.3.2.1 antisera, continuing a trend of increasing antigenic divergence observed since 2021. This data demonstrates that continued monitoring of genetic and antigenic changes, particularly in countries where the virus remains enzootic in poultry, as well as regions that periodically become free of circulation but are at risk of re-introduction from nearby endemic areas, is essential for the early detection of antigenic drift and the timely updating of vaccine compositions.

¹ <https://www.offlu.org/index.php/offlu-avian-influenza-matching-offlu-aim-introduction-and-background/>

² <https://www.offlu.org/index.php/offlu-avian-influenza-matching-offlu-aim-guide-to-assessing-antigenic-characteristics-of-avian-influenza-viruses-june-2024/>

3. Background

Since 2020, H5Nx A/Goose/Guangdong/1996 (Gs/Gd) lineage high pathogenicity avian influenza viruses (HPAIVs) have shifted from being a seasonal threat to poultry populations to a persistent global threat to both poultry production and wild bird populations (EFSA, 2025). Wild bird migration has facilitated their spread to most regions, triggering unprecedented outbreaks in domestic poultry and causing large-scale mortality events in wild birds. These events have had substantial financial impacts on poultry production and adversely affected avian biodiversity, including several species of conservation concern. In areas experiencing high environmental viral pressure due to large-scale bird mortality caused by HPAIV, the virus has also spilled over sporadically into terrestrial and aquatic mammalian species causing mass die-off events. The primary clade driving current global outbreaks is 2.3.4.4b H5Nx, but viruses from clades 2.3.2.1a, 2.3.2.1e, 2.3.2.1g, and 2.3.4.4h continue to circulate in countries in Asia (Figure 1). In response, global calls for the use of vaccination have spurred a series of initiatives highlighting the importance of monitoring haemagglutinin (HA) evolution to inform optimal vaccine strain selection.

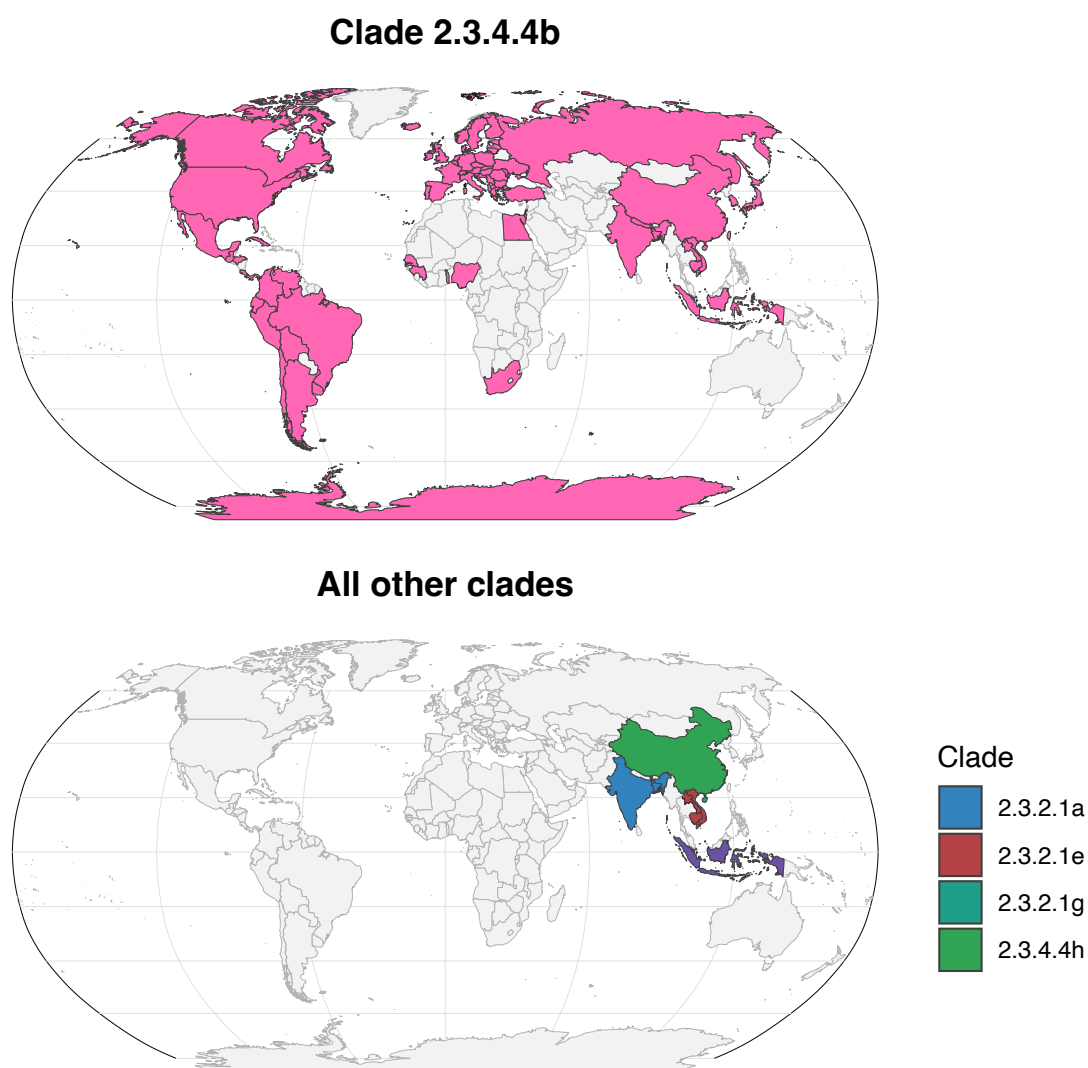


Figure 1. Global distribution of H5 clades according to the updated nomenclature between September 2022-February 2025, based on information from WHO reports on ‘Genetic and antigenic characteristics of zoonotic influenza A viruses and development of candidate vaccine viruses for pandemic preparedness’ from February 2023-February 2025. Geographic locations of detections are shown based on publicly available sequence data and information.

One key component of effective vaccination is maintaining close antigenic relatedness between circulating viruses and vaccine strains, which can significantly reduce the impact of infection in vaccinated flocks. Vaccination has been used in some countries for many years, and in light of the current situation, many more countries are now considering or implementing vaccination against Gs/Gd-lineage viruses. Vaccination is supported and actively recommended in certain contexts to control HPAI (see

[FAO/WOAH Global strategy for the prevention and control of high pathogenicity avian influenza \(2024–2033\)](#))³. However, the continued evolution of HPAIVs poses a persistent risk of antigenic drift and escape from antibody-mediated neutralisation (Wang *et al.*, 2025), underscoring the need for ongoing surveillance and timely phenotypic characterisation to inform vaccine effectiveness.

The OFFLU Avian Influenza Matching (AIM) project is a collaborative initiative led by the Animal and Plant Health Agency (United Kingdom) with support from partners in Italy (IZSve), the USA (USDA Southeast Poultry Research Laboratory), and Australia (CSIRO ACDP). Its primary goal is to generate and regularly update a standardised panel of chicken sera and homologous antigens, serving as an FAO–WOAH international standard for the antigenic characterisation of circulating avian influenza viruses to monitor viral evolution. The group operates reactively, continuously monitoring emerging strains and obtaining relevant isolates through global partnerships to ensure antigenic relevance. Antigenic diversity is assessed using standardised HI testing (see protocols [here](#)), with results visualised through antigenic cartography to support vaccine strain evaluation in a constantly evolving landscape.

The aims of the overall and ongoing project are to:

- I. Generate a continually updated panel of reagents (antisera and inactivated viruses), produced by APHA and IZSve using local and internationally requested avian influenza viruses (AIVs).
- II. Evaluate these reagents at APHA and IZSve, alongside emerging viral strains, to generate preliminary haemagglutination inhibition (HI) data using standardised HI assays.
- III. Collaborate with other WOA and FAO international reference laboratories in strategically selected locations by sharing protocols and reagents to broaden antigenic assessment coverage.
- IV. Produce quality assured comparative HI data across laboratories and merge these into antigenic distance data to assess the antigenic characteristics of contemporary viruses as they emerge globally.
- V. Standardise laboratory procedures by sharing protocols and generating harmonised reagents and methodologies within defined quality frameworks, enabling global multi-centre comparisons.

In addition to establishing harmonised methods across designated laboratories, antigenic data generated through AIM complements data from FAO and WOA member countries, OFFLU partners, and regional surveillance networks. This includes epidemiological data, evidence of vaccine breakthrough, and experimental studies. Together, these data could help to inform need to update vaccine antigens and may support alignment among vaccine manufacturers undertaking similar analyses. By identifying areas of increasing antigenic diversity, this work helps position responses to existing vaccines (**Table 1**) within antigenic maps of HA glycoprotein variation. While AIM does not directly assess vaccine efficacy, the combined data provide a robust basis for conducting challenge studies in vaccinated birds to assess protection, updating poultry vaccine antigens, and refining vaccination strategies.

³ <https://openknowledge.fao.org/items/4c4f67c1-c8c7-401b-9d04-720493406c19>

3.1. Vaccine Seed Strains

Table 1. Seed strains of H5 vaccines which are either currently understood to be in use or used previously. Where available clade information and references have been included. An asterisk (*) indicates instances where the clade nomenclature may differ from the latest designation of the WHO/FAO/WOAH H5 Nomenclature Working Group.

Seed Strain / HA Gene Source	Clade	Reference	Represented in AIM
A/duck/Potsdam/1402/86	non-Gs/Gd	DEFRA Vaccination Taskforce ⁴	No
A/Goose/Guangdong/96	0	(Shi et al., 2023)	No
A/chicken/Vietnam/C58/04	1	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Yes
A/Vietnam/1194/2004	1	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Yes
A/chicken/Legok/2003	2.1.1	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Pending
A/Swan/Hungary/4999/2006	2.2	DEFRA Vaccination Taskforce	Yes
A/CK/Egypt/ME1010/2016	2.2.1.1	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Pending
A/Chicken/Egypt/Q1995D/2010	2.2.1.2	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	No
A/Chicken/Egypt/RG-173 CAL/2017	2.2.1.2	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Pending
A/Duck/EGYPT/M2583D/2010	2.2.1.2	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Yes
A/chicken/West-Java/Pwt-Wij/2006	2.3.1.2	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	No
A/duck/China/E319-2/2003	2.3.2	DEFRA Vaccination Taskforce	No
A/duck/Anhui/SI246/2014	2.3.2.1	(Shi et al., 2023)	No
A/Hubei/1/2010	2.3.2.1a	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Yes
A/duck/Guangdong/S1322/2010	2.3.2.1b	(Shi et al., 2023)	Yes
A/chicken/Tanggamus/031711076-65/2017	2.3.2.1c*	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	No
A/chicken/Liaoning/SD007/2017	2.3.2.1d	(Shi et al., 2023)	Pending
rgCA2/2.3.2.1d	2.3.2.1d	(Kang et al., 2022)	No
A/chicken/Vietnam/NCVD-KA435/13	2.3.2.1e*	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Pending
A/duck/Sukoharjo/BBVW-1428-	2.3.2.1g*	(Indriani and Dharmayanti, 2014)	Yes
A/Duck/Anhui/1/2006	2.3.4	(Shi et al., 2023)	Yes
A/Waterfowl/Korea/S57/2016	2.3.4.4	Kuruppuarachchi et al., 2022	No
A/chicken/Egypt/ME-2018/2018	2.3.4.4b	(Ibrahim et al., 2021)	Yes
A/green-winged teal/Egypt/877/2016	2.3.4.4b	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Yes
A/whooper swan/Shanxi/4-1/2020	2.3.4.4b	(Shi et al., 2023)	Yes
A/turkey/Indiana/22-003707-003/2022	2.3.4.4b	DEFRA Vaccination Taskforce	No
A/gyrfalcon/Washington/41088-6/2014	2.3.4.4c	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Yes
A/duck/Korea/ES2/2016	2.3.4.4e	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	Pending
A/chicken/Guizhou/4/2013	2.3.4.4g	(Shi et al., 2023)	No
A/duck/Guizhou/S4184/2017	2.3.4.4h	(Shi et al., 2023)	Yes
A/duck/Fujian/S1424/2020	2.3.4.4h	(Shi et al., 2023)	Pending
rgES3/2.3.4.4h	2.3.4.4h	(Kang et al., 2022)	No
A/duck/Guanzhou/S4184/2017	2.3.4.4h	(Shi et al., 2023)	Pending
A/Duck/VietNam/QB7412	unknown	(EFSA Panel on Animal Health and Animal Welfare (AHAW) et al., 2023)	No
A/Chicken/Shanxi/2/2006	7.2	(Shi et al., 2023)	No
A/Chicken/Liaoning/S4092/2011	7.2	(Shi et al., 2023)	No

⁴ <https://www.gov.uk/government/publications/vaccination-of-poultry-against-highly-pathogenic-avian-influenza/vaccination-of-poultry-against-highly-pathogenic-avian-influenza-hpai-joint-industry-and-cross-government-vaccination-taskforce>

3.2. Panel of Chicken Antisera

The AIM post infection chicken antisera panel has been expanded to include three representatives from previously untested clades and two additional 2.3.4.4b viruses that more accurately reflect the current H5 diversity (Table 2).

Table 2. Viruses used in OFFLU AIM to generate post-infection chicken antisera, including those in the original core panel and those selected for the expanded panel. A double-dagger (‡) indicates an antigen used as a surrogate for a vaccine strain. Clade information is provided.

Strain	Subtype	Clade	Relevant vaccine seed strain
A/Vietnam/1194/2004/1‡	H5N1	1	A/chicken/Vietnam/C58/04
A/turkey/turkey/2005*	H5N1	2.2	A/swan/Hungary/4999/2006
A/duck/Egypt/MS2583D/2010‡	H5N1	2.2.1.1	A/Chicken/Egypt/Q1995D/2010
A/chicken/Nepal/T360/2014‡	H5N1	2.3.2.1a	A/duck/Guangdong/S1322/2010
A/Hubei/1/2010‡	H5N1	2.3.2.1a	A/Hubei/1/2010
A/duck/Bangladesh/17D1012/2018	H5N1	2.3.2.1a	
A/mynah/Indonesia(AustriaQ)/13064792-010/2013‡	H5N1	"2.3.2.1c" like	A/duck/Sukoharjo/BBW-1428- 9/2012
A/Anhui/1/2005* (RG)	HN51	2.3.4	A/Duck/Anhui/1/2006
A/Guizhou/1/2013	H5N1	2.3.4.2	
A/chicken/Czech Republic/1175-1-20VIR465-1/2020	H5N8	2.3.4.4b	
A/chicken/Bulgaria/722-1-22VIR778-1/2021	H5N1	2.3.4.4b	
A/duck/Cambodia/f4k241D3/2021‡	H5N8	2.3.4.4b	A/whooper swan/Shanxi/4–1/2020
A/great skua/Scotland/B07779/2021	H5N1	2.3.4.4b	
A/mallard/Georgia/DT09382/2017‡	H5N8	2.3.4.4b	A/chicken/ME-2018
A/mute swan/Croatia/102/2016*	H5N5	2.3.4.4b	A/green-winged teal/Egypt/877/2016
A/chicken/Nigeria/VRD-21-338/21VIR7423-29/2021	H5N1	2.3.4.4b	
A/chicken/Nigeria/VRD-011_23VIR8254-67/2023	H5N1	2.3.4.4b	
A/gyrfalcon/Washington/41088-6/2014‡	H5N8	2.3.4.4c	A/Gyrfalcon/WA/41088-6/2014
A/Guangdong/18SF020/2018	H5N6	2.3.4.4h	

3.3. Phylogenetic Relationships

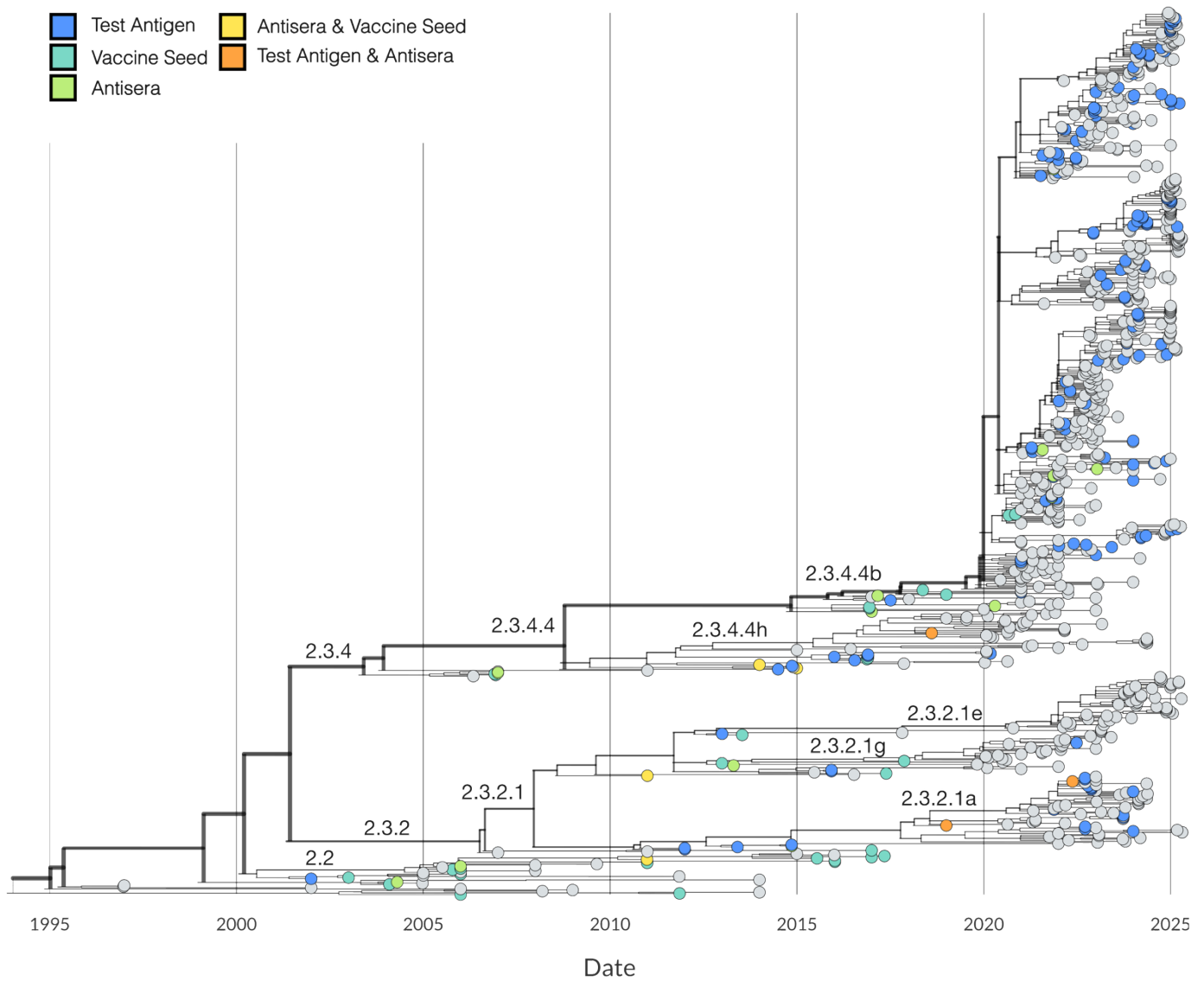


Figure 2. Maximum likelihood phylogenetic trees of the HA1 of H5 Gs/Gd lineage viruses included in this project and vaccine seed strains where sequences were available. Tips are coloured by test antigens, vaccine seed strains, antisera used in this study. Major clades are annotated along the branches according to nomenclature.

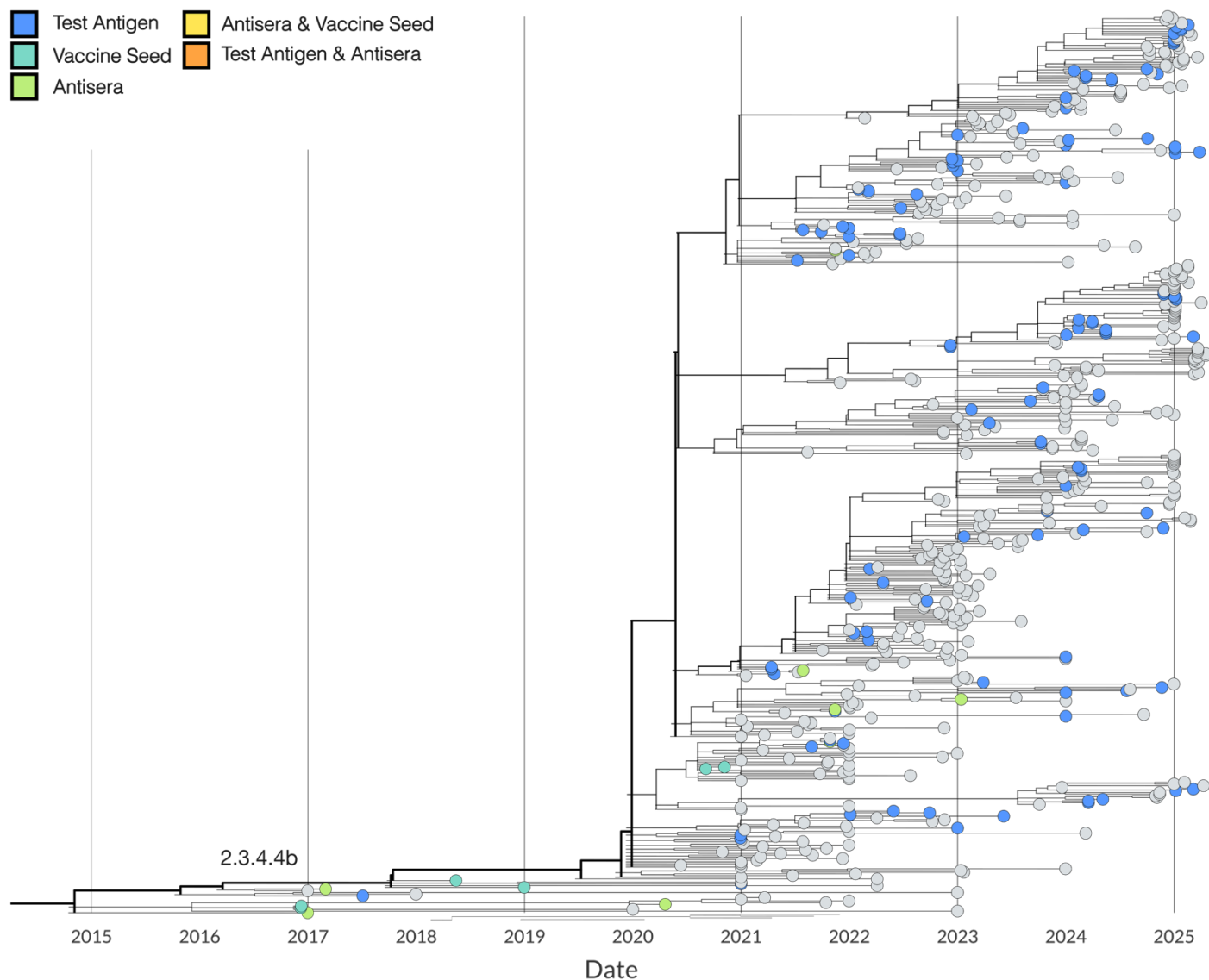


Figure 3. Maximum likelihood phylogenetic trees of the HA1 of H5 Gs/Gd lineage clade 2.3.4.4b viruses included in this project and vaccine seed strains where sequences were available. Tips are coloured by test antigens, vaccine seed strains, antisera used in this study.

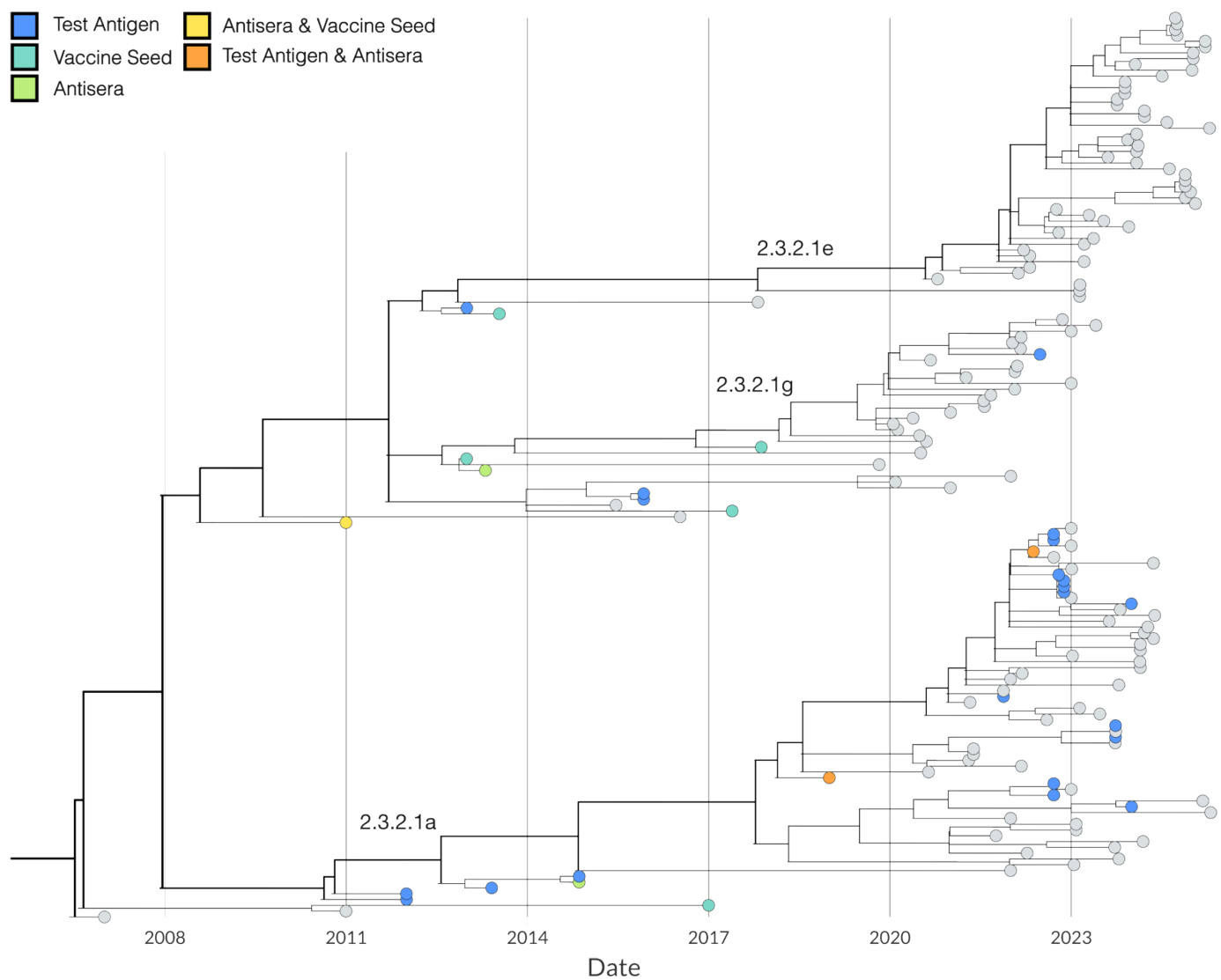


Figure 4. Maximum likelihood phylogenetic trees of the HA1 of H5 Gs/Gd lineage clade 2.3.2.1(a/e) viruses included in this project and vaccine seed strains where sequences were available. Tips are coloured by test antigens, vaccine seed strains, antisera used in this study.

4. Main Findings

During this testing period, APHA, IZSVE, and ACDP have applied the panel using haemagglutinin inhibition (HI) assays against a further 88 test antigens representing a diversity of H5 HA. At APHA, 55 isolates were tested, with a focus on current diversity within clade 2.3.4.4b (44 isolates). These included viruses from Europe (12), North America (21), and South Asia or associated regions (8). Additionally, three viruses were from Antarctica (including the Antarctic Peninsula and sub-Antarctic islands). While not directly relevant for poultry vaccination, these Antarctic isolates provide valuable surrogate antigens for Central and South America, as they capture many antigenic and genetic features shared with viruses circulating more widely in the region (Banyard *et al.*, 2024; Kuiken *et al.*, 2025), making them important for vaccine development considerations. In addition, four clade 2.3.2.1a viruses from Asia spanning 2022–2025 and five clade 2.3.2.1a viruses from South Asia or associated regions (2023–2024) were also tested, along with two 2016 viruses in clade 2.3.4.4e from East Asia. At IZSVE, an additional 25 isolates were tested, focusing on contemporary H5 clade 2.3.4.4b strains, the majority of which were collected from 2024 onwards. These included viruses from Europe (15), North America (5), and Africa (5). At ACDP an additional eight isolates were tested with a focus on contemporary strains from Southeast Asia, including clades 2.3.4.4b, 2.3.2.1g, and 2.3.4.4e collected since 2020.

4.1. Antigenic Distances

The following text describes the distance in antigenic units of tested antigens and sera (see Table 3 for 2024–2025 virus results). Antigenic units (AUs) are a standardised measure of antigenic distance in antigenic cartography, calculated as the $\log_2$ difference in haemagglutination inhibition (HI) titres between two viruses (i.e., a difference of 1 AU corresponds to a two-fold difference in HI titre, 2 AUs to a four-fold difference, 3 AUs to an eight-fold difference). Although no single threshold can definitively predict vaccine protection, and thresholds may vary between hosts, virus subtypes and vaccine technology relative cut-offs are often used to guide interpretation. In evaluations of traditionally inactivated whole virus adjuvanted poultry AIV vaccines, antigenic differences of around 4 AU have typically been used pragmatically to exclude strains as vaccine candidates due to markedly reduced immunological cross-reactivity⁵. Such values should be regarded as guiding benchmarks rather than absolute predictors of vaccine efficacy, and interpreted alongside epidemiological, virological, and field data (EFSA 2023).

Conclusions around antigenic diversity observed are detailed for each of the clades below:

Clade 2.3.4.4b

- **H5N1** clade 2.3.4.4b viruses from 2025 from Europe (n=10) and North America (n=3) were between 1.1 and 4.5 antigenic units (AU) from antisera raised against clade 2.3.4.4b surrogate vaccine seed strains. These viruses were also 1.0–2.5 AU from clade 2.3.4.4c, 5.1–6.3 AU from clade 2.3.4.4h, 3.1–5.4 AU from clade 2.3.2.1x, and 3.3–5.8 AU from antisera raised against ancestral clades 1, 2.2.1, 2.2.1.2, and 2.3.4 vaccine seed strains.
- **H5N1** clade 2.3.4.4b viruses from 2024, originating from Africa (n=5), Europe (n=13), North America (n=8), South Asia (n=1), and the sub-Antarctic (n=3), showed antigenic distances of 1.2–5.0 AU to clade 2.3.4.4b antisera, 0.9–3.5 AU to clade 2.3.4.4c, >3.9 AU to clade 2.3.4.4h, and 2.3–6.1 AU to surrogate vaccine seed strain antisera from clades 1, 2.2.1, 2.2.1.2, 2.3.4, and 2.3.2.1x.
- **H5N5** clade 2.3.4.4b viruses from 2025 from Europe (n=2) were between 2.1 and 4.5 AU from antisera raised against clade 2.3.4.4b surrogate vaccine seed strains. These viruses were also 1.9–2.1 AU from clade 2.3.4.4c, 4.8–6.2 AU from clade 2.3.4.4h, and 5.0–6.5 AU from antisera raised against clades 1, 2.2.1, 2.2.1.2, 2.3.4, and 2.3.2.1x vaccine seed strains.

⁵ <https://edepot.wur.nl/649465>

- **H5N5** clade 2.3.4.4b viruses from **2024** from Europe (n=1) and North America (n=2) were 2.0–4.5 AU from clade 2.3.4.4b, 1.6–2.6 AU from clade 2.3.4.4c, 4.5–6.7 AU from clade 2.3.4.4h, and 4.4–7.3 AU from clades 1, 2.2.1, 2.2.1.2, 2.3.4, and 2.3.2.1x surrogate vaccine seed strain antisera.
- **H5N2** clade 2.3.4.4b viruses from **2024** from North America (n=2) were between 2.2 and 4.3 AU from antisera raised against clade 2.3.4.4b putative vaccine strains, between 1.7 and 2.3 AU from antisera raised against clade 2.3.4.4c and between 4.4 and 6.3 AU from antisera raised against clade 2.3.4.4h putative vaccine strains.

Clade 2.3.2.1a

- A **H5N1** clade 2.3.2.1a virus tested from South Asia from **2024** was ≥ 3.4 AU from chicken antisera raised against clade 2.3.4.4 strains, 2.3 - 4.9 AU from clades 1, 2.2.1, 2.2.1.2, 2.3.4, and 2.3.2.1x surrogate vaccine seed strain antisera.
- This follows the trend from viruses tested from 2021 onwards to increasing antigenic distances over time against clade 2.3.2.1 sera.
- Other clade viruses were tested, however strains were prior to 2024 and so they are not included in these results. For more information on the antigenic properties of these viruses please contact OFFLU (secretariat@offlu.org).

Table 3. Antigenic distances derived from antigenic maps, limited to antigens from viruses collected between 2024 and 2025. One antigenic unit is equal to a two-fold decrease in HA titer. Distances were coloured using a heat map. Names of the closest vaccine antigen are labelled in red italics next to their surrogate chicken antisera. Antigens are ordered by clade, subtype and region.

Subtype	Year	Clade	Region	A/Vietnam/1194/2004/1 <i>A/chicken/Vietnam/C58/04</i>	A/Turkey/turkey/2005 <i>A/swan/Hungary/4999/2006</i>	A/duck/Egypt/M2583/2010 <i>A/chicken/Egypt/Q1995D/2010</i>	A/Anhui/1/2005 <i>A/Duck/Anhui/1/2006</i>	A/chicken/Nepal/T360/2014 <i>A/duck/Guangdong/S1322/2010</i>	A/Hubei/1/2010 <i>A/Hubei/1/2010</i>	A/mynah/Indonesia/AustriaQ/13064792-010/201 <i>A/duck/Sukoharjo/BBWW-1428-9/2012</i>	A/mallard/Georgia/D109382/2017 <i>A/chicken/NE-2018</i>	A/Mute_Swan/Croatia/1102/2016 <i>A/green-winged teal/Egypt/877/2016</i>	A/duck/Cambodia/14k241D3/2021 <i>A/whooper swan/Shanxi/4-1/2020</i>	A/gyr/falcon/Washington/41088/6/2014 <i>A/Gyr/falcon/WA/41088-6/2014</i>	A/Guangdong/18SF020/2018 <i>A/duck/Guizhou/S4184/2017</i>
Subtype	Year	Clade	Region	1 H5N1	2.2.1 H5N1	2.2.1.2 H5N1	2.3.4 H5N1	2.3.2.1a H5N1	2.3.2.1a H5N1	2.3.2.1g H5N1	2.3.4.4b H5N8	2.3.4.4b H5N5	2.3.4.4b H5N8	2.3.4.4c H5N8	2.3.4.4h H5N6
N1	2024	2.3.2.1a	South Asia	4.2	3.5	4.0	4.9	4.2	2.3	3.5	3.8	5.5	4.6	3.4	5.0
N1	2024	2.3.4.4b	Africa	3.5	3.3	3.2	4.1	4.2	2.8	3.4	2.1	3.9	3.0	1.7	4.8
N1	2024	2.3.4.4b	Africa	4.9	4.7	4.9	5.4	5.9	4.2	5.1	2.5	4.8	3.1	2.1	4.1
N1	2024	2.3.4.4b	Africa	4.5	4.4	4.5	4.9	5.7	4.1	4.9	2.1	4.4	2.8	1.7	3.9
N1	2024	2.3.4.4b	Africa	4.5	4.4	4.6	5.0	5.6	4.0	4.8	2.4	4.7	3.1	2.0	3.9
N1	2024	2.3.4.4b	Africa	4.7	4.4	4.5	5.2	5.4	3.8	4.6	2.4	4.5	3.0	2.0	4.7
N1	2024	2.3.4.4b	Europe	4.6	4.4	4.2	5.2	5.1	3.7	4.3	2.2	4.1	2.9	1.9	5.4
N1	2024	2.3.4.4b	Europe	4.2	3.9	3.9	4.8	4.7	3.2	3.9	2.3	4.2	3.1	2.0	5.2
N1	2024	2.3.4.4b	Europe	4.8	4.5	4.7	5.4	5.5	3.8	4.7	2.8	4.9	3.4	2.4	4.7
N1	2024	2.3.4.4b	Europe	4.3	4.1	4.0	4.9	4.9	3.4	4.1	2.1	4.1	2.9	1.8	5.1
N1	2024	2.3.4.4b	Europe	4.3	4.1	3.9	4.8	5.0	3.6	4.2	1.9	3.9	2.7	1.6	5.2
N1	2024	2.3.4.4b	Europe	4.1	4.0	3.8	4.6	4.8	3.5	4.1	1.8	3.7	2.6	1.4	5.1
N1	2024	2.3.4.4b	Europe	4.2	4.0	3.9	4.8	4.8	3.4	4.1	2.1	4.0	2.8	1.7	5.1
N1	2024	2.3.4.4b	Europe	3.7	3.5	3.4	4.2	4.5	3.1	3.7	1.9	3.8	2.7	1.5	4.8
N1	2024	2.3.4.4b	Europe	4.6	4.3	4.4	5.2	5.1	3.5	4.4	2.7	4.7	3.4	2.3	5.0
N1	2024	2.3.4.4b	Europe	3.7	3.5	3.3	4.3	4.3	3.0	3.5	2.1	3.7	2.9	1.7	5.2
N1	2024	2.3.4.4b	Europe	4.3	4.1	4.0	4.8	5.0	3.5	4.2	2.0	4.0	2.7	1.6	5.1
N1	2024	2.3.4.4b	Europe	4.5	4.3	4.4	5.1	5.4	3.8	4.6	2.2	4.4	2.9	1.8	4.5
N1	2024	2.3.4.4b	Europe	4.4	4.1	4.2	4.9	5.1	3.6	4.3	2.3	4.4	3.0	1.9	4.6
N1	2024	2.3.4.4b	North America	4.1	4.1	3.6	4.5	4.8	3.8	4.1	1.3	2.9	2.1	1.2	5.7
N1	2024	2.3.4.4b	North America	5.5	5.3	4.9	6.1	5.6	4.4	4.9	2.9	4.2	3.4	2.8	6.8
N1	2024	2.3.4.4b	North America	5.0	4.8	4.4	5.5	5.4	4.2	4.7	2.1	3.8	2.6	1.9	6.0
N1	2024	2.3.4.4b	North America	4.4	4.3	3.9	4.9	5.0	3.8	4.3	1.6	3.3	2.3	1.4	5.7
N1	2024	2.3.4.4b	North America	2.9	2.7	2.6	3.5	3.6	2.3	2.8	2.4	3.7	3.2	2.0	5.1
N1	2024	2.3.4.4b	North America	4.6	4.6	4.0	5.2	5.0	3.9	4.3	2.1	3.4	2.7	2.0	6.2
N1	2024	2.3.4.4b	North America	4.6	4.3	4.3	5.2	5.0	3.5	4.3	2.6	4.4	3.3	2.3	5.4
N1	2024	2.3.4.4b	North America	4.6	4.5	4.2	5.1	5.2	3.9	4.5	1.9	3.7	2.5	1.6	5.5
N1	2024	2.3.4.4b	South Asia	4.8	4.7	4.4	5.4	5.3	4.0	4.6	2.1	4.0	2.8	1.9	5.7
N1	2024	2.3.4.4b	Subantarctic	4.2	4.3	3.9	4.7	5.2	3.9	4.4	1.2	3.3	2.0	0.9	5.2
N1	2024	2.3.4.4b	Subantarctic	5.0	4.5	4.3	5.7	4.5	3.2	3.9	3.8	5.0	4.5	3.5	6.6
N1	2024	2.3.4.4b	Subantarctic	3.3	3.3	2.7	3.8	4.0	3.1	3.3	1.8	2.9	2.6	1.6	5.7
N2	2024	2.3.4.4b	North America	4.2	4.0	4.1	4.7	5.1	3.5	4.3	2.2	4.3	2.9	1.7	4.4
N2	2024	2.3.4.4b	North America	5.8	5.7	5.3	6.3	6.3	5.0	5.6	2.4	4.1	2.7	2.3	6.3
N5	2024	2.3.4.4b	Europe	6.5	6.6	6.1	6.9	7.3	6.1	6.6	2.5	4.1	2.4	2.6	6.7
N5	2024	2.3.4.4b	North America	4.8	4.7	4.7	5.3	5.9	4.4	5.1	2.0	4.3	2.5	1.6	4.5
N5	2024	2.3.4.4b	North America	5.4	5.3	5.2	5.8	6.4	4.8	5.6	2.2	4.5	2.6	1.9	4.8
N1	2025	2.3.4.4b	Europe	4.6	4.7	4.1	5.1	5.3	4.2	4.6	1.5	3.0	2.0	1.4	6.0
N1	2025	2.3.4.4b	Europe	4.5	4.3	3.9	5.0	4.8	3.7	4.1	2.0	3.5	2.7	1.9	6.0
N1	2025	2.3.4.4b	Europe	4.2	4.3	3.7	4.6	5.0	4.0	4.3	1.1	2.9	1.9	1.0	5.7
N1	2025	2.3.4.4b	Europe	4.9	4.7	4.4	5.4	5.3	4.0	4.5	2.2	4.0	2.8	2.0	5.9
N1	2025	2.3.4.4b	Europe	4.9	4.7	4.4	5.5	5.2	3.9	4.5	2.4	4.1	3.0	2.2	5.8
N1	2025	2.3.4.4b	Europe	4.1	3.9	3.4	4.7	4.1	3.1	3.5	2.5	3.7	3.2	2.3	6.2
N1	2025	2.3.4.4b	Europe	4.0	3.9	3.5	4.6	4.4	3.2	3.7	2.1	3.5	2.9	1.9	5.9
N1	2025	2.3.4.4b	Europe	4.0	4.0	3.3	4.5	4.4	3.5	3.7	2.0	3.0	2.7	1.9	6.3
N1	2025	2.3.4.4b	Europe	4.4	4.2	4.1	4.9	5.1	3.6	4.3	2.0	4.0	2.7	1.7	5.1
N1	2025	2.3.4.4b	Europe	4.1	4.0	3.8	4.7	4.8	3.4	4.0	2.0	3.9	2.7	1.6	5.1
N1	2025	2.3.4.4b	North America	5.2	4.9	4.7	5.8	5.5	4.1	4.7	2.7	4.5	3.3	2.5	5.9
N1	2025	2.3.4.4b	North America	4.9	4.8	4.4	5.4	5.4	4.2	4.7	2.0	3.8	2.6	1.8	5.8
N1	2025	2.3.4.4b	North America	4.8	4.5	4.2	5.4	4.9	3.6	4.2	2.7	4.2	3.4	2.5	6.1
N5	2025	2.3.4.4b	Europe	5.8	5.8	5.4	6.3	6.5	5.3	5.8	2.1	3.9	2.3	2.1	6.2
N5	2025	2.3.4.4b	Europe	5.5	5.4	5.4	5.9	6.5	5.0	5.7	2.2	4.5	2.5	1.9	4.8

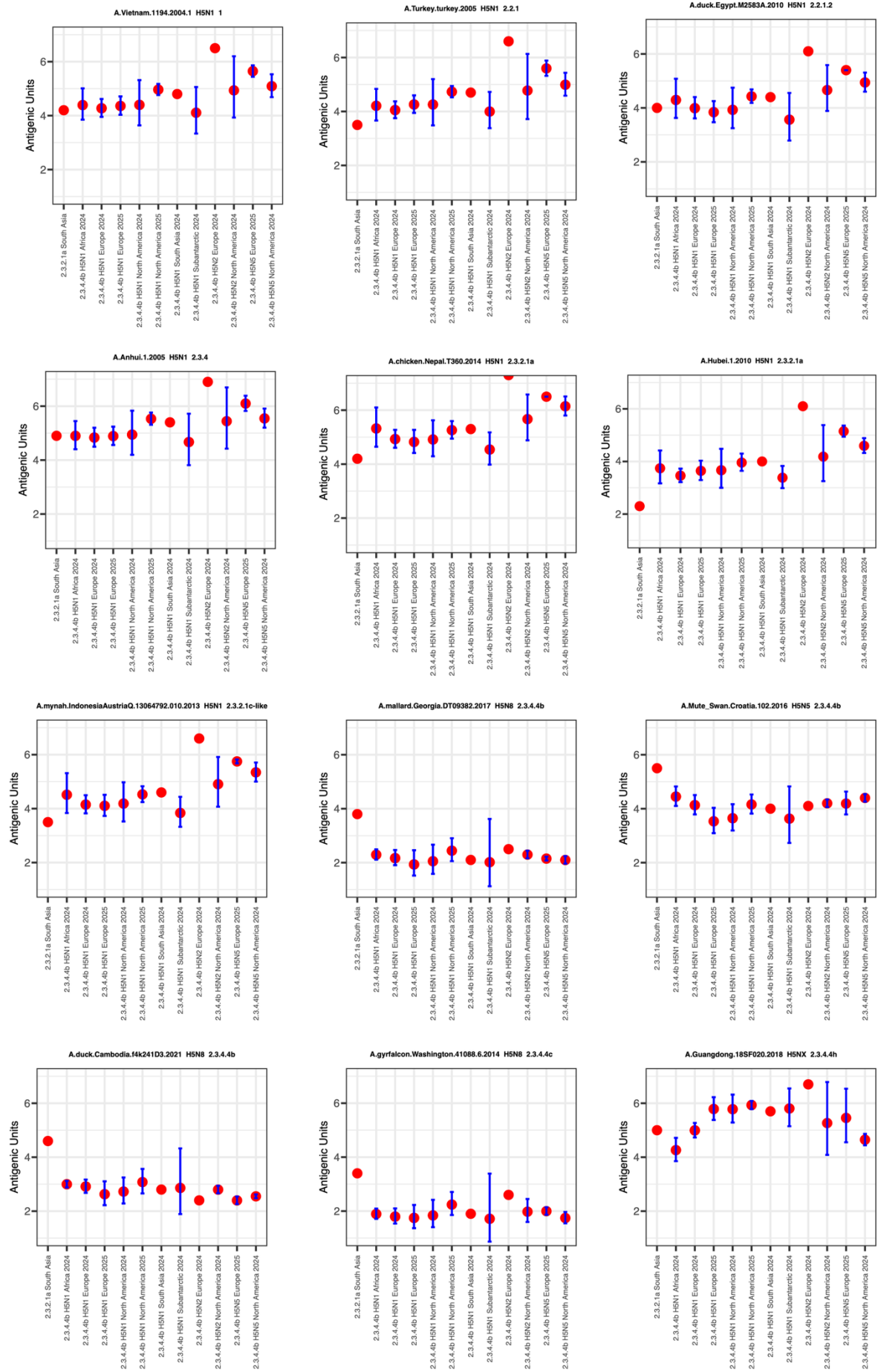


Figure 5. Box-and-whisker plots of antigenic distances for H5 clade 2.3.4.4 viruses, grouped by country, subtype, and year. Each box represents an antiserum raised against a vaccine seed strain or surrogate antigen.

4.2 Antigenic Cartography

Antigenic cartography was undertaken to further quantify and visualise the antigenic distances between virus's representative of vaccine seed strains and contemporary circulating viruses as described in the AIM pilot project. Maps were analysed and visualised using R Studio version 2023.03.1+446 and the package 'Racmacs'⁶ v1.2.9 built under R v4.3.1 as described by (Smith *et al.*, 2004) and previously used in (Lewis *et al.*, 2021). For information regarding map generation and interpretation please contact OFFLU experts via the secretariat email (secretariat@offlu.org). Amino acid changes in the HA1 were visualised by reconstructing ancestry using 'TreeTime' v0.11.1 (Sagulenko, Puller and Neher, 2018) and were compared between within-clade and within-subtype test antigens. Antigenic maps were coloured according to unpublished viral clade nomenclature and subtype using the H5Nx dataset in Nextclade (Ort *et al.*, 2025).

These results described here and above (section 4.1) suggest that of the vaccine seed strains and surrogates tested, we would expect the currently used H5N8 clade 2.3.4.4 antigens to remain suitable candidates for inactivated whole-virus adjuvanted vaccines for most contemporary clade 2.3.4.4b viruses. Antigens which exhibit higher antigenic distance from the sera should be prioritised for testing in vaccine efficacy challenge studies and work is required to determine for each vaccine technology the cut-off antigenic distance associated with loss of protection. Furthermore, subtype-specific heterogeneity was observed, consistent with previous reports and this should be considered further. In contrast, vaccine antigens from outside clade 2.3.4.4 - including contemporary 2.3.2.1a viruses and earlier ancestral clades such as 1, 2.2.1, and 2.3.4 - showed limited cross-reactivity and are unlikely to provide effective protection against currently circulating 2.3.4.4b viruses. Notably, H5N1 viruses from clade 2.3.2.1a, particularly those detected in South Asia in 2024, exhibited reduced reactivity to antisera from both clades 2.3.4.4 and 2.3.2.1, continuing a trend of increasing antigenic divergence observed since 2020. These results underscore the value of analysing virus relationships at a regional level, particularly in South and South-East Asia, where vaccine requirements may need to address both clade 2.3.4.4b viruses and other circulating clades such as 2.3.2.1a, 2.3.2.1e, and 2.3.2.1g.

⁶ <https://github.com/acorg/Racmacs/>

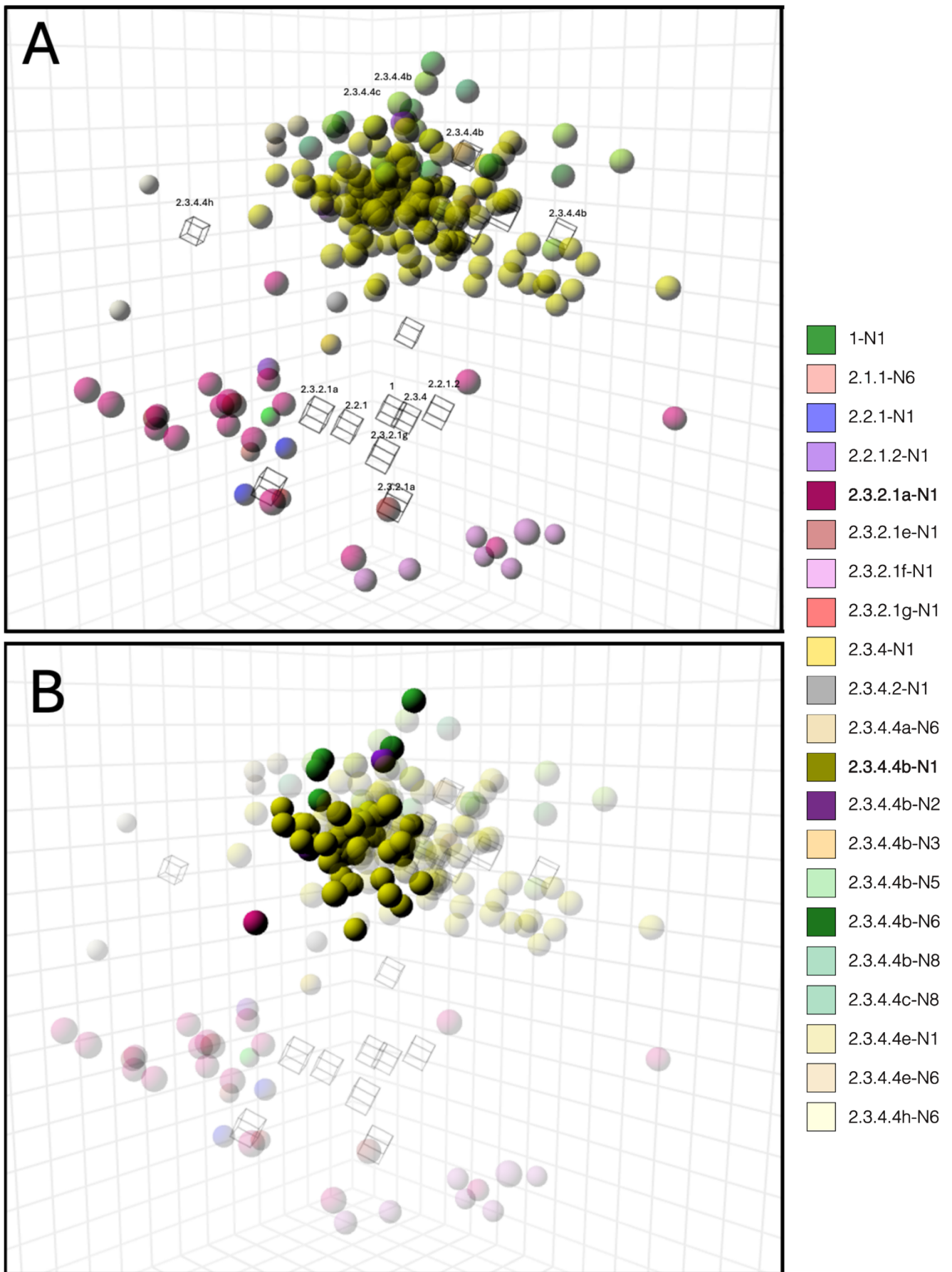


Figure 6. Three-dimensional antigenic map illustrating the relationships among Gs/Gd-lineage avian influenza viruses characterized in this study. Each sphere represents a virus (antigen), coloured by subtype and clade; squares represent post-vaccination chicken antisera. Clade labels are placed on representative vaccine seed strains. **Panel A:** Overview of the full antigenic map, without highlights. **Panel B:** Antigens characterized from 2024 and 2025 are highlighted, including multiple clade 2.3.4.4b viruses and one clade 2.3.2.1a virus.

5. Conclusions and Future Directions

In summary, the OFFLU AIM project continues to provide a critical foundation for the global monitoring of antigenic evolution in circulating H5 viruses. Results from this reporting period highlight the increasing divergence of H5 viruses over time, the rapid evolutionary dynamics that underscore the need for ongoing reassessment, and the importance of examining relationships within a clade to assess potential regional implications of antigenic drift. We believe this data is vital for the international community and especially those concerned with decision making over whether to vaccinate and what vaccine to use. This programme provides trusted impartial science-based evidence that we hope will shape and inform all those concerned with either maintaining or newly implementing vaccination programmes.

Continued Surveillance and Test Virus Selection

We will continue to monitor and characterise newly emerging H5Nx viruses from the Gs/GD lineage, with a focus on expanding representation of underrepresented clades in the test virus panel, particularly 2.3.2.1a, 2.3.2.1c, 2.3.2.1g, 2.3.2.1e, and 2.3.4.4h, to better capture the diversity of circulating viruses. Outputs will be delivered through the annual technical report, executive report, and a webinar in late 2025 or early 2026 for interested stakeholders. Gaps in test virus representation will be addressed through both classical and reverse genetics approaches. Classical approaches will include the ongoing acquisition and sharing of live virus isolates and associated reagents with global partners. For viruses where isolation is unsuccessful or not routinely performed, reverse genetics (RG) will be applied. This process will be conducted transparently, with sequence database contributors systematically contacted to request permission for sequence use, ensuring they are acknowledged and kept informed.

Expansion and Refinement of the Reference Antisera Panel

Concurrently, the OFFLU reference antisera panel will be expanded and refined to remain globally representative and relevant. Antisera production targeting clades 2.3.4.4h and 2.3.2.1a/c/g is underway, alongside production for additional putative vaccine seed strains not yet represented in the AIM panel (see **Table 2**). Priority will be given to a clade 2.3.4.4b H5N1 sub-Antarctic isolate identified as an antigenic outlier, which is genetically related to viruses detected in Chile, Uruguay, Brazil, and several sub-Antarctic island populations. Harmonisation and ring testing across participating laboratories will remain central to ensuring consistent production and application of reference antisera.

Broadening the Scope of the AIM Framework

To meet evolving needs, the AIM project will expand both in scope and functionality. Planned developments include evaluating novel vaccine concepts such as computationally optimised broadly reactive antigens (COBRA) (Giles and Ross, 2011) to determine their capacity to induce antibody responses against contemporary circulating strains; extending the AIM framework to additional virus subtypes, beginning with H9, using comparable methodologies to assess global antigenic diversity and guide vaccine strain selection; and enhancing reporting and communication through the introduction of a meeting-report format for regular outputs. Laboratories and interested parties will be invited to contribute data to support reviews of antigenic diversity, particularly of surface proteins, and to assess implications for vaccine performance. Epidemiological developments that may necessitate vaccine updates (e.g., the emergence of a novel virus in a region with existing vaccine use) will also be identified. These outputs will complement technical reports and aim to strengthen transparency and information sharing across the network.

Network Growth and Capacity Building

Expanding the AIM network remains a priority, with a focus on engaging additional global reference laboratories, particularly in regions of strategic importance. Targeted capacity-building efforts will be undertaken to support laboratory participation and enable broader evaluation of antigenic diversity using harmonised protocols. Simultaneously, we will focus on addressing the technical challenges of assimilating data from multiple sources into a single cartographic read-out, even when common standards are applied.

Links to Key AIM Documents

[Concept Note](#)

[Module 1: OFFLU AIM report background updated 2024](#)

[Module 2: OFFLU AIM Annex 1- sub-national and national level guidance for countries on assessing the antigenic characteristics](#)

[OFFLU AIM Webinar \(English\)](#)

[OFFLU AIM Webinar \(Spanish\)](#)

[OFFLU AIM Webinar Summary and FAQs](#)

[OFFLU AIM Pilot Report \(October 2023\)](#)

[OFFLU AIM Technical Report \(July 2024\)](#)

[OFFLU AIM Executive Summary \(October 2024\)](#)

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