

# Genotype differentiation of highly pathogenic avian influenza viruses (HPAIV) of the goose/Guangdong lineage in Germany - Derivation and deployment of reference sequences.

## *Summary*

Highly pathogenic avian influenza viruses (HPAIV) of the H5 goose/Guangdong (gs/GD) lineage have repeatedly emerged in Germany since 2006. Rooted in the respective gs/GD lineages, HPAI viruses in Germany have genetically diversified into a plethora of clades and subclades and evolved into an assortment of sub- and genotypes. This technical note summarizes the genotype differentiation procedure, lists the successfully assigned genotypes and supplies corresponding reference sequences.

## *Methodology*

Genotype differentiation and derivation of reference sequence were established with a combined phylogenetic and similarity-based method. Full genome sequences from clinical samples and virus isolates were continuously screened for new genotypes and compared with the derived reference sequences. For genotype assignment segment-specific multiple alignments were generated using MAFFT (Katoh & Standley, 2013). In addition, alignments of concatenated genomes were generated and included in the analysis. Subsequent maximum likelihood (ML) trees were calculated separately for all segments and for the concatenated genome alignment with RAxML (Stamatakis, 2014) utilizing model GTR GAMMA with rapid bootstrapping and search for the best scoring ML tree supported with 1000 bootstrap replicates. Genotypes were assigned, and new genotypes were differentiated if they clustered separately as a monophyletic group with robust bootstrapping values (>80) and if differences in normalized patristic distances were observed at segment level. The first complete genome sequence of a newly detected genotype is used as a reference sequence, and additional references for new genotypes were derived as needed. Genotype designation here is filed to include locality (ISO 3166-1 alpha-2), date of first discovery (year-month), and NA subtype. When multiple genotypes of the same subtype were assigned within the same locality and date, the names were numbered consecutively. Reference sequences were deployed along HA clades and HPAIV season and are available here. The nomenclature system currently in use here is different from that proposed by the European Reference Laboratory (EURL) for Avian Influenza, Padova, Italy; the corresponding EURL designation is shown in the last column of the table provided if applicable.

## Overview of genotypes

season	year	clade	subtype	genotype	EURL	US
	2006	2.2.1	H5N1	DE-06-03-N1		
		2.2.2	H5N1	DE-06-02-N1		
	2007	2.2	H5N1	DE-07-06-N1		
	2008	2.2.1	H5N1	DE-08-10-N1		
<b>2014/2015</b>	2014	2.3.4.4a	H5N8	DE-14-11-N8		
<b>2016/2017</b>	2016	2.3.4.4b	H5N5	DE-16-12-N5.1		
				DE-16-12-N5.2		
			H5N8	DE-16-11-N8		
				DE-16-12-N8.1		
				DE-16-12-N8.2		
<b>2016/2017</b>	2017	2.3.4.4b	H5N6	DE-17-12-N6		
<b>2019/2020</b>	2020	2.3.4.4b	H5N8	DE-20-01-N8		
<b>Enzootic period</b>		2.3.4.4b	H5N8	DE-20-10-N8	A	
				DE-20-10-N8.1		
				DE-20-10-N8.2		
			H5N5	DE-20-10-N5		
		2.3.4.4b	H5N3	DE-20-12-N3		
	2021	2.3.4.4b	H5N1	DE-21-02-N1	C	
				DE-21-07-N1	C	
				DE-21-10-N1.1	C	A1A2
				DE-21-10-N1.2	C	A5
				DE-21-10-N1.3	C	
				DE-21-10-N1.4		
				DE-21-10-N1.5	AB	
				DE-21-11-N1.1		
				DE-21-11-N1.2		
				DE-21-11-N1.3		
				DE-21-11-N1.4		A3
				DE-21-11-N1.5		
				DE-21-11-N1.6		
				DE-21-12-N1.1		A4
				DE-21-12-N1.2		
				DE-21-12-N1.3		
				DE-21-12-N1.4		
				DE-21-12-N1.5		
				DE-21-12-N1.6		
			H5N4	DE-21-02-N4		
			H5N8	DE-21-02-N8		
				DE-21-03-N8		
				DE-21-08-N8.1		
				DE-21-08-N8.2		
	2022	2.3.4.4b	H5N1	DE-22-01-N1.1		

				DE-22-01-N1.2		
				DE-22-04-N1.1	CA	
				DE-22-09-N1.1	CC	
				DE-22-11-N1.1	CH	
				DE-22-12-N1.1	CH	
			H5N2	DE-22-01-N2.1	AU	
	2023	2.3.4.4b	H5N1	DE-23-02-N1.1	BB	
			H5N1	DE-23-11-N1.1	DB	
			H5N1	DE-23-11-N1.2	AB	
			H5N1	DE-23-11-N1.3	DG	
			H5N1	DE-23-11-N1.4	DA	
	2024	2.3.4.4b	H5N1	DE-24-01-N1.1	DJ	
			H5N1	DE-24-01-N1.2	DO	
			H5N1	DE-24-02-N1.1	DQ	
			H5N1	DE-24-02-N1.2	DR	
			H5N8	DE-24-03-N8.1	DS	
			H5N1	DE-24-03-N1.1	DI.1	
			H5N1	DE-24-10-N1.1	DI.2	
			H5N5	DE-24-02-N5.1	I	A6
			H5N1	DE-24-11-N1.1	EF	
	2025	2.3.4.4b	H5N1	DE-25-01-N1.1	EE	
			H5N1	DE-25-02-N1.1	EJ	
			H5N1	DE-25-04-N1.1	EK	
	2025	2.3.4.4.b	H5N1	DE-25-10-N1.1	DI.2.1	

## References

Katoh K & Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30(4):772-780.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312-1313.

Pohlmann, A. (2023) HPAIV Genotypes in Germany. Zenodo.  
doi.org/10.5281/zenodo.8135769