



Influenza A Cleavage Sites

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Background:

As specified by the OIE **Terrestrial Animal Health Code**, H5 and H7 avian influenza viruses classified as:

- high pathogenicity avian influenza viruses have an IVPI in six-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in four-to eight-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin molecule (HA₀); if the amino acid motif is similar to that observed for other high pathogenicity avian influenza isolates, the isolate being tested should be considered as high pathogenicity avian influenza virus;
- low pathogenicity avian influenza viruses are all influenza A viruses of H5 and H7 subtypes that are not high pathogenicity avian influenza viruses

Avian influenza viruses attach to host cells using the viral hemagglutinin (HA) protein. Progeny influenza viruses contain an HA₀ protein, which has to be cleaved to form HA₁ and HA₂ to become functional. This cleavage occurs at the HA₀ cleavage site by cellular proteases of the host. The type of cellular proteases that can cleave HA₀ depends on the type of cleavage site in the HA₀ protein:

- **Mono-basic cleavage sites** contain one basic amino acid in the critical position (-1; e.g. **PEKQTR/GLF**) of the cleavage site and are cleaved by few cellular proteases. Therefore these viruses can grow only in limited areas of the poultry host: generally the intestinal and the respiratory systems.
- **Multi-basic cleavage sites** contain several basic amino acids in the critical position (-1 and immediately preceding; e.g. **PQRESRRKK/GLF**) of the cleavage site, some having insertions of 1 or more amino acids that lengthen the cleavage site. They are cleaved by several common cellular proteases. Therefore these viruses have the potential to grow systemically (throughout the body) of the host.

How to use this cleavage site information:

Use **Table 1 (H5 HPAI)** for previously reported multi-basic cleavage sites from outbreaks throughout the world and for an overview of the very consistent multi-basic cleavage sites of H5 HPAI viruses of the Goose/Guangdong lineage (Asia, Africa, Europe and North America). This currently includes subtypes H5N1, H5N2, H5N3, H5N5, H5N6 and H5N8 derived from the H5 Gs/GD lineage by reassortment.

Use **Table 2 (H7 HPAI)** for previously reported multi-basic cleavage sites from outbreaks throughout the world.

- ✚ The presence of a previously reported multi-basic cleavage site strongly indicates that the new virus is HPAI.
- ✚ The presence of any insertion in the cleavage site (even if not previously reported) should be discussed with your regional reference center.

Table 3 lists **unusual, 2-3 residue multi-basic cleavage sites**.

- ✚ Please **contact** your **FAO** or **OIE** reference laboratory (<http://www.offlu.net/index.php?id=78>) **for further advice**.

How to determine whether a cleavage site is HP using molecular methods:

1. Determine whether your new cleavage site exactly matches a previously reported HPAI virus cleavage site
 - a. Table 1 for HPAI H5Nx
 - b. Table 2 for HPAI H7
 - c. Table 3 for unusual, 2-3 residue multi-basic cleavage sites

If your cleavage site has previously been reported, you should report the new virus as HPAI and/or seek advice from your FAO or OIE reference laboratory.

(Remember all H5 and H7 viruses are reportable regardless of cleavage site)

2. If your cleavage site shows **more than one basic amino acid** compared to the LPAI cleavage site (first row of Tables 1 and 2), **contact** your **FAO or OIE** reference laboratory (<http://www.offlu.net/index.php?id=78>) **for further advice.**
3. If your cleavage site shows **any insertions of amino acids** compared to the LPAI cleavage site (first row of Tables 1 and 2), **contact** your **FAO or OIE** reference laboratory (<http://www.offlu.net/index.php?id=78>) **for further advice.**

Table 1: Multi-basic cleavage sites of H5Nx HPAI viruses.

Sub-type	Clade ¹ /type virus	Cleavage site consensus ²	critical basic aa ³	Size of insert
H5	LP	PQRETR/GLF	1	0
H5N1	Gs/Gd-lineage	PQRE RRRKKR /GLF	6	4
H5N1	Clade 1	PQRE RRRKKR /GLF PQREG RRRKKR /GLF PQRVG RRRKKR /GLF	5	4
H5N1	Clade 2.1	PQRES RRKK /GLF PQKEG RRRKKR /GLF PQIE RRRKKR /GLF PQRE RRREKR /GLF	4-6	3-4
H5N1	Clade 2.2	PQGE KRRKKR /GLF PQGE RRRKKR /GLF PQGE GRRKKR /GLF PQGD RRRKKR /GLF	5-6	4
H5N1	Clade 2.3.1	PQRE RRRKR /GLF	5	3
H5N1	Clade 2.3.2	PQRE RRRKR /GLF PRRE RRRKR /GLF PQRE KRRKR /GLF PQKE RRRKR /GLF PQIE RRRRKR /GLF PQRES RRRKR /GLF	5-6	3-4
H5N1	Clade 2.3.3	PQRE RRRKR /GLF	5	3
H5N1, H5N2, H5N3, H5N5, H5N6, H5N8	Clade 2.3.4	PLRE RRRKR /GLF PLRE KRRKR /GLF PPRE KRRKR /GLF PLRE KRRRKR /GLF PLRE RRRKKR /GLF PLRER IRKKR /GLF PLGE KRRKR /GLF PLIE KRRKR /GLF PLRD KRRKR /GLF ⁴	4-6	3-4
H5N1	Clade 2-like	PQRE RRRKKR /GLF PQRE RRRKR /GLF	5-6	3-4
H5N1	Clade 3	PQRE RRRKKR /GLF	6	4
H5N1	Clade 4	PQRE RRRKKR /GLF	6	4
H5N1	Clade 5	PQRE IRRKKR /GLF	5	4
H5N1	Clade 6	PQRE RRRKKR /GLF	6	4
H5N1	Clade 7	PQIEG RRRKR /GLF PQRG RRRKR /GLF	4-5	3-4

		PQR RRRKKR /GLF PQREGG RKKR /GLF PQREGG RRRKR /GLF PQREREGG RRRKR /GLF		
H5N1	Clade 9	PQRE RRRKKR /GLF	6	4
H5N2	A/ostrich/SA/AI2114/11 ^[18]	PQR RKKR /GLF	4	1
	A/ostrich/SA/AI2887/11	PQR RRKR /GLF	4	1
H5N2	A/ostrich/SA/AI1091/06 ^[1, 16]	PQRE KRRKKR /GLF	6	4
H5N1	A/gull/Germany/R882/06 ^[29]	PQGE RRRKKR /GLF	6	4
H5N2	A/chicken/Italy/1485/97 ^[6, 30]	PQR RRKKR /GLF	5	2
H5N2	A/chicken/Puebla/8623-607/94 ^[4-6]	PQR RKKR <u>TR</u> /GLF PQR KRKR <u>TR</u> /GLF	5 6	3 4
H5N2	A/chicken/Puebla/8624-602/94 ^[5]	PQR KRKR <u>TR</u> /GLF	4	2
H5N1	A/turkey/England/50-92/91 ^[10]	PQR KRRK <u>TR</u> /GLF	5	3
H5N8	A/turkey/Ireland/1378/83 ^[6, 31]	PQR KRKR /GLF	5	2
H5N9	A/turkey/Ontario/7732/66 ^[6, 32]	PQR RRKKR /GLF	5	2
H5N3	A/tern/South Africa/61 ^[6, 10]	PQRE TRRQKR /GLF	4	4

¹ LP - low pathogenic. Gs/Gd = A/goose/Guangdong/1/1996-lineage highly pathogenic avian influenza H5N1; clades include all higher order subclades (i.e clade 7 includes clade 7, 7-like, 7.1, 7.2) unless otherwise specified; numbers in parentheses are references.

² Consensus sequence generated from H5 HA sequences available in public databases; red color indicates critical basic residues; / indicates cleavage position; residue insertions are underlined (38).

³ Basic residue at the -1 position and basic amino acids immediately preceding this position; excludes the -4 position found in the LPAI cleavage site consensus motif.

⁴APHA-Weybridge

Table 2: Multi-basic cleavage sites of sporadic H7 HPAI avian influenza A viruses

Sub-type	Pheno-type ¹	location	year	Type virus	cleavage site ²	Critical basic aa ³	Size of insert	Accession number ⁴	Ref.
H7	LP				PEIPKGR/GLF, PELPKGK/GLF, PEPPKGR/GLF, PENPKTR/GLF, PESPKTR/GLF	1	0		38
H7N3	HP	USA	2020	A/turkey/South_Carolina/20-010561-003/2020	PENPKTD RKSRHRRIR /GLF ⁸	6	9	MT444387	NVSL
H7N9,	HP	China	2017	A/chicken/Guangdong/GD4/2017	PEVPKG KRTAR /GLF	3	4	KY855518	43-46
				A/chicken/Guangdong/GD15/2016	PEVPK RKRTAR /GLF	4	4	EP1960361	
				A/Guangdong/Th005/2017	PEVPKG KRIAR /GLF ⁵	3	4	EP1926825	
H7N3	HP	Japan ⁶	2018	A/duck/Japan/AQ-HE30-1/2018	PEVPK RRRTAR /GLF	4	4	LC416566	47
H7N9	HP	USA	2017	A/chicken/Tennessee/17-007147-2/2017	PENPKTD RKSRHRRIR /GLF ⁸	6	9	KY818811	42
H7N7	HP	Italy	2016	A/chicken/Italy/16VIR-1873/2016	PELPKG RKRR /GLF	4	3	EPI220955	IZSVenezie
H7N8	HP	USA	2016	A/turkey/Indiana/16-001403-1/2016	PENPK KRKR /GLF	4	3	KU558906.1	41
H7N7	HP	UK	2015	A/chicken/England/26352/2015	PEIPRH RKGR /GLF	4	3	EP1623939	APHA
H7N7	HP	Germany	2015	A/chicken/Germany/AR1386/2015	PEIPK RKRR /GLF	5	3	TBA	FLI
H7N7	HP	Italy	2013	A/chicken/Italy/13VIR4527_11/13	PETPK RRERR /GLF	4	3	KF569186.1	39
H7N3	HP	Mexico	2012-2018	A/chicken/Jalisco/CPA1/12	PENPKD RKSRHRRTR /GLF	6	8	JX397993.1	23
				A/chicken/Puebla/CPA-04451/16	PENPKD RKNRHRRTR /GLF	6	8	KX351916.1	
				A/chicken/Jalisco/CPA-01859/16	PENPKG KKSRHRRTR /GLF	6	8	KX351892.1	
H7N7	HP	Spain	2009	A/chicken/Spain/6279-2/2009	PELPKGT KPRPR /GLF	4	6	GU121458.1	24
H7N7	HP	UK	2008	A/chicken/England/1158-11406/08	PEIPK RKKR /GLF	4	2	FJ476173.1	25,26
H7N3	HP	Canada	2007	A/chicken/Saskatchewan/HR-00011/07	PENPK TKPRPR /GLF	4	6	EU500860.1	17
H7N7	HP	North Korea	2005	A/chicken/North Korea/1/2005	PEIPKG RHRRPKR /GLF	5	6		13
H7N3	HP	Canada	2004	A/chicken/Canada/rv504/04	PENPKQ AYRKRMT /GLF	4	7	CY015006.1	13
					PENPKQ AYOKRMT /GLF	3	7		
					PENPKQ AYKKRMT /GLF	4	7		
					PENPKQ AYHKRMT /GLF	3	7		
					PENPKQ AHQKRMT /GLF	3	7		
					PENPKQ AYRKRMT /GLF	4	7		
					PENPKQ ACQKRMT /GLF	3	7		
H7N7	HP	Netherlands	2003	A/chicken/Netherlands/219/03	PEIPK RRRR /GLF	4	2	AY338459.1	9,27
H7N3	HP	Chile	2002	A/chicken/Chile/4322/02	PEPKP TCSPS RCRETR/GLF	3	10	AY303631.1	7,28
					PEPKP TCSPS RCRKT/GLF	4	10		
H7N1	HP	Italy	1999	A/chicken/Italy/444/99	PEIPK GSRVRR /GLF	3	4	AJ704810.1	12
					PEIPK GSRMRR /GLF	3	4		
					PEIPK RSRVRR /GLF	4	4		
H7N4	HP	Australia	1997	A/chicken/New South Wales/2/97	PEIPR RRKR /GLF	4	2	CY022693- CY022700	19,20
					PEIPR KRKR /GLF	4	2		
H7N3	HP	Pakistan	1995	A/chicken/Pakistan/447/95	PETPK RKRRK /GLF	5	3	AF202226	2
				A/chicken/Pakistan/CR2/95	PETPK RRKR /GLF	4	2		
				A/chicken/Pakistan/16/99/95	PETPK RRNR /GLF	3	2		
H7N3	HP	Australia	1994	A/chicken/Queensland/94	PEIPR KRKR /GLF	4	2	CY022685	11,20
H7N1	HP	USA ⁷	1994	A/Pekin_robin/California/30412/94	PEIPK RRRR /GLF	4	1	GU052922	47
H7N3	HP	Australia	1992	A/chicken/Victoria/224/92	PEIPK KKKR /GLF	4	2	CY025077- CY025084	20
					PEIPK RKKR /GLF	4	2		
					PEIPK KRKR /GLF	5	3		
					PEIPK KKKKR /GLF	6	4		
H7N7	HP	Australia	1985	A/chicken/Victoria/85	PEIPK KREKR /GLF	4	3	CY025069	10,20
H7N7	HP	Germany	1979	A/chicken/Leipzig/79	PEIPK KKKR /GLF	4	2	U20459.1	21
				A/goose/Leipzig/137-8/79	PEIPK RKKR /GLF	4	2	L43913.1	
				A/goose/Leipzig/192-7/79	PEIPK KRKR /GLF	5	3	L43915.1	
				A/goose/Leipzig/187-7/79	PEIPK KKKKR /GLF	6	4	L43914.1	
H7N7	HP	Australia	1976	A/chicken/Victoria/76	PEIPK KREKR /GLF	4	3	CY024786	10,20
H7N3	HP	England	1963	A/turkey/England/63	PETPK RRRR /GLF	4	2	AF202238	1,10,14

¹ LP = low pathogenic, HP = highly pathogenic as determined by IVPI.

² Cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.

³ Number of basic residue at immediately preceding and including the -1 position; excludes the -4 position found in the LPAI cleavage site consensus motif.

⁴ GenBank or GISAID record number

⁵ This cleavage site is presumed HP, but awaits confirmation by IVPI testing in chickens

⁶ Virus isolated from Muscovy duck meat from a passenger that originated in China. Sample was confiscated in Japan by authorities.

⁷ Virus isolated from Pekin Robin (*Leiothrix lutea*) imported into a USA quarantine station from Asia. The entire lot of quarantined birds were euthanized.

⁸ HA is from similar North American wild bird lineage; same source (host cellular 28s rRNA), sequence and mechanism for insertion into the HA cleavage site; but the insertion events occurred at different times (2017 vs 2020)

Table 3. Unusual, 2-3 basic residue multi-basic cleavage sites with variable LP and HP phenotypes

Sub-type	Pheno-type ¹	location	year	Type virus	cleavage site ²	Critical basic aa ³	Size of insert	Accession number ⁴	Ref.
H5	LP				PQRE <u>TR</u> /GLF	1	0		39
H5N1	HP	France	2015	A/chicken/France/150169a/15	HQR <u>RRK</u> /GLF	3	0	KU310447.1	
H5N2				A/duck/France/150233/15				KX014878.1	
H5N9				A/duck/France/150236/15				KX014886.1	
H5N2	HP	Taiwan	2012	A/chicken/Taiwan/A1997/12	PQR <u>RRK</u> /GLF	3	0	KF193394.1	40
H5N2	HP	Taiwan	2008	A/chicken/Taiwan/K703-1/08	PQR <u>RRK</u> /GLF ⁵	3	0	AB507264.1	34,35
H5N2	LP	USA	2004	A/chicken/Texas/298313/04	PQR <u>RRK</u> /GLF ⁶	3	0	AY849793.1	6
	LP	Taiwan	2003	A/chicken/Taiwan/1209/03	PQR <u>RRK</u> /GLF	2	0	AY573917.1	34,35
H5N2	LP/HP	USA	1983	A/chicken/PA/1370/83	PQK <u>RRK</u> /GLF ⁷	3	0	CY107848.1	6,10,37
H5N1	HP	Scotland	1959	A/chicken/Scotland/59	PQR <u>RRK</u> /GLF ⁸	3	0	GU052518.1	6
H7	LP				PEIPK <u>GR</u> /GLF	1	0		38
H7N4	LP	China	2018	A/chicken/Jiangsu/1/2018	PELP <u>KGR</u> /GLF	2	0	EPI_ISL_332358	HVRI, 48
H7N1	LP	UAE	2004	A/houbara bustard/UAE/2004	PELP <u>KRR</u> /GLF	2	0		APHA-UK
H7N3	HP	Pakistan	1995	A/chicken/Pakistan/16/99/95	PETPK <u>RRNR</u> /GLF	3	2	AF202233	2
H7N7	HP	England	1979	A/turkey/England/199/79	PEIPK <u>KREK</u> /GLF	3	2		1,9,14
H7Nx	LP	Australia	1976	A/duck/Victoria/76	PEIPK <u>RR</u> /GLF	2	0	U20463.1	33

¹ LP = low pathogenic, HP = highly pathogenic as determined by IVPI. Where both are indicated, age of chickens affected IVPI score or presence/absence of a glycosylation site.

² Cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.

³ Number of basic residue at immediately preceding and including the -1 position; excludes the -4 position found in the LPAI cleavage site consensus motif.

⁴ GenBank record number

⁵ Based on OIE mandated 6 week-old chickens, the IVPI = 1.86 and virus was declared HP. However, in 8 week-old chickens the IVPI = 0.89.

⁶ LP in 4 week-old chickens (0/8). Parent virus had putative glycosylation site at position 11-13 (NST). Was declare HP based on HA cleavage site sequence similarity to A/chicken/Scotland/1959

⁷ Required loss of putative glycosylation at position 11-13 (NSK) for highly pathogenic phenotype

⁸ Lack of putative glycosylation site at position 11-13 (KST)

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