Influenza A Cleavage Sites

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Background:

As specified by the OIE **Terrestrial Manual**, for determining pathogenicity of an influenza A virus, the following criteria have been adopted.

- A high pathogenicity influenza A virus is any influenza A virus that is lethal for six, seven or eight of eight 4- to 8-week-old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid or any influenza A virus that has an intravenous pathogenicity index (IVPI) greater than 1.2;

- For all H5 and H7 viruses of low pathogenicity in chickens, the amino acid sequence of the connecting peptide of the haemagglutinin molecule (HA0) (i.e. the cleavage site) must be determined. The presence of several basic amino acids, inserts of cellular or viral nucleic acids or loss of specific glycosylation sites in the HA0 cleavage site is the genotypic standard for HPAI strains; therefore, if the isolate being tested has an HA0 cleavage site motif identical to previous HPAI viruses, it should be designated as HPAI irrespective of a low or high pathogenicity determined by pathotyping in chickens (see the table that lists all the reported haemagglutinin proteolytic cleavage sites of HA0 protein for H5 and H7 LPAI and HPAI viruses based on deduced amino acid sequence, which can be found on the OFFLU site (see footnote 2). Furthermore any isolate with a new motif must be tested in vivo by IVPI. In case of difficulties in the interpretation of the cleavage site motif, OIE and/or FAO reference laboratories should be consulted

The occurrence of H5 and H7 low pathogenicity avian influenza viruses should be monitored as some have the potential to mutate into high pathogenicity avian influenza viruses.

Avian influenza viruses attach to host cells using the viral hemagglutinin (HA) protein. Progeny influenza viruses contain an HA_0 protein, which has to be cleaved to form HA_1 and HA_2 to become functional. This cleavage occurs at the HA_0 cleavage site by cellular proteases of the host. The type of cellular proteases that can cleave HA_0 depends on the type of cleavage site in the HA_0 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, H5N4, 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 (https://www.offlu.org/index.php/oie-fao-reference-laboratories-and-experts-for-highly-pathogenic-avian-influenza-and-low-pathogenic-avian-influenza-poultry/) **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 H5
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.

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 (https://www.offlu.org/index.php/oie-fao-reference-laboratories-and-experts-for-highly-pathogenic-avian-influenza-and-low-pathogenic-avian-influenza-poultry/) **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 (https://www.offlu.org/index.php/oie-fao-reference-laboratories-and-experts-for-highly-pathogenic-avian-influenza-and-low-pathogenic-avian-influenza-poultry/) **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	**PQRERRRKKR/GLF**	6	4
H5N1	Clade 1	**PQREERRKKR/GLF** **PQREGRRKKR/GLF** **PQRVGRRKKR/GLF**	5	4
H5N1	Clade 2.1	**PQRESRRKK/GLF** **PQKEGRRKKR/GLF** **PQIERRRKKR/GLF** **PQRERRREKR/GLF**	4-6	3-4
H5N1	Clade 2.2	**PQGEKRRKKR/GLF** **PQGERRRKKR/GLF** **PQGEGRRKKR/GLF** **PQGDRRRKKR/GLF**	5-6	4
H5N1	Clade 2.3.1	**PQRERRRKR/GLF**	5	3

H5N1	Clade 2.3.2	PQRERRRKR/GLF PRRERRRKR/GLF PQREKRRKR/GLF PQKERRRKR/GLF PQIERRRRKR/GLF PQRESRRRKR/GLF	5-6	3-4
H5N1	Clade 2.3.3	PQRERRRKR/GLF	5	3
H5N1, H5N2, H5N3, H5N4, H5N5, H5N6, H5N8	Clade 2.3.4	PLRERRRKR/GLF PLREKRRKR/GLF PPREKRRKR/GLF PLREKRRRKR/GLF PLRERRRKKR/GLF PLRERIRKKR/GLF PLGEKRRKR/GLF PLIEKRRKR/GLF PLRDKRRKR/GLF[4] PLRGKRRKR/GLF[5]	4-6	3-4
H5N1	Clade 2-like	PQRERRRKKR/GLF PQRERRKKR/GLF	5-6	3-4
H5N1	Clade 3	PQRERRRKKR/GLF	6	4
H5N1	Clade 4	PQRERRRKKR/GLF	6	4
H5N1	Clade 5	PQREIRRKKR/GLF	5	4
H5N1	Clade 6	PQRERRRKKR/GLF	6	4
H5N1	Clade 7	PQIEGRRRKR/GLF PQRGRRRKR/GLF PQRGRRRKKR/GLF PQREGGRKKR/GLF PQREGGRRRKR/GLF PQREREGGRRRKR/GLF	4-5	3-4
H5N1	Clade 9	PQRERRRKKR/GLF	6	4
H5N2	A/ostrich/SA/AI2114/11[18] A/ostrich/SA/AI2887/11	PQRRKKR/GLF PQRRRKR/GLF	4 4	1 1
H5N2	A/ostrich/SA/AI1091/06[1, 16]	PQREKRRKKR/GLF	6	4
H5N1	A/gull/Germany/R882/06[29]	PQGERRRKKR/GLF	6	4
H5N2	A/chicken/Italy/1485/97[6, 30]	PQRRRKKR/GLF	5	2
H5N2	A/chicken/Puebla/8623-607/94[4-6]	PQRRKRKTR/GLF PQRKRKRKTR/GLF	5 6	3 4
H5N2	A/chicken/Puebla/8624-602/94[5]	PQRKRKTR/GLF	4	2
H5N1	A/turkey/England/50-92/91[10]	PQRKRRKTR/GLF	5	3
H5N8	A/turkey/Ireland/1378/83[6, 31]	PQRKRKKR/GLF	5	2
H5N9	A/turkey/Ontario/7732/66[6, 32]	PQRRRKKR/GLF	5	2
H5N3	A/tern/South Africa/61[6, 10]	PQRETRRQKR/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.

[4] APHA-Weybridge

[5] Siencsano-Belgium

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
H7N7	HP	Australia	2020	A/chicken/Victoria/20-02865-8/2020	**PEIPGKREKR/GLF**	4	4	Pending	CSIRO
H7N3	HP	USA	2020	A/turkey/South_Carolina/20-010561-003/2020	**PENPKTDRKSRHRRIR/GLF[8]**	6	9	MT444387	50
H7N9,	HP	China	2017	A/chicken/Guangdong/GD4/2017 A/chicken/Guangdong/GD15/2016 A/Guangdong/Th005/2017	**PEVPKGKRTAR/GLF PEVPKRKRTAR/GLF PEVPKGKRIAR/GLF[5]**	3 4 3	4 4 4	KY855518 EPI960361 EPI926825	43-46
H7N3	HP	Japan[6]	2018	A/duck/Japan/AQ-HE30-1/2018	**PEVPKRRRTAR/GLF**	4	4	LC416566	47
H7N9	HP	USA	2017	A/chicken/Tennessee/17-007147-2/2017	**PENPKTDRKSRHRRIR/GLF[8]**	6	9	KY818811	42
H7N7	HP	Italy	2016	A/chicken/Italy/16VIR-1873/2016	**PELPKGRKRR/GLF**	4	3	EPI220955	51
H7N8	HP	USA	2016	A/turkey/Indiana/16-001403-1/2016	**PENPKKRKTR/GLF**	4	3	KU558906.1	41
H7N7	HP	UK	2015	A/chicken/England/26352/2015	**PEIPRHRKGR/GLF**	4	3	EPI623939	APHA
H7N7	HP	Germany	2015	A/chicken/Germany/AR1386/2015	**PEIPKRKRR/GLF**	5	3	EPI634885	52
H7N7	HP	Italy	2013	A/chicken/Italy/13VIR4527_11/13	**PETPKRRERR/GLF**	4	3	KF569186.1	39
H7N3	HP	Mexico	2012-2018	A/chicken/Jalisco/CPA1/12 A/chicken/Puebla/CPA-04451/16 A/chicken/Jalisco/CPA-01859/16	**PENPKDRKSRHRRTR/GLF PENPKDRKNRHRRTR/GLF PENPKGKKSRHRKTR/GLF**	6 6 6	8 8 8	JX397993.1 KX351916.1 KX351892.1	23
H7N7	HP	Spain	2009	A/chicken/Spain/6279-2/2009	**PELPKGTKPRPRR/GLF**	4	6	GU121458.1	24
H7N7	HP	UK	2008	A/chicken/England/1158-11406/08	**PEIPKRKKR/GLF**	4	2	FJ476173.1	25,26
H7N3	HP	Canada	2007	A/chicken/Saskatchewan/HR-00011/07	**PENPKTTKPRPRR/GLF**	4	6	EU500860.1	17
H7N7	HP	North Korea	2005	A/chicken/North Korea/1/2005	**PEIPKGRHRRPKR/GLF**	5	6		13
H7N3	HP	Canada	2004	A/chicken/Canada/rv504/04	**PENPKQAYRKRMTR/GLF PENPKQAYQKRMTR/GLF PENPKQAYKKRMTR/GLF PENPKQAYHKRMTR/GLF PENPKQAHQKRMTR/GLF PENPRQAYRKRMTR/GLF PENPKQACQKRMTR/GLF**	4 3 4 3 3 4 3	7 7 7 7 7 7 7	CY015006.1	13
H7N7	HP	Netherlands	2003	A/chicken/Netherlands/219/03	**PEIPKRRRR/GLF**	4	2	AY338459.1	9,27
H7N3	HP	Chile	2002	A/chicken/Chile/4322/02	**PEKPKTCSPLSRCRETR/GLF PEKPKTCSPLSRCRKTR/GLF**	3 4	10 10	AY303631.1	7,28
H7N1	HP	Italy	1999	A/chicken/Italy/444/99	**PEIPKGSRVRR/GLF PEIPKGSRMRR/GLF PEIPKRSRVRR/GLF**	3 3 4	4 4 4	AJ704810.1	12
H7N4	HP	Australia	1997	A/chicken/New South Wales/2/97	**PEIPRRRKR/GLF PEIPRKRKR/GLF**	4 4	2 2	CY022693-CY022700	19,20
H7N3	HP	Pakistan	1995	A/chicken/Pakistan/447/95 A/chicken/Pakistan/CR2/95 A/chicken/Pakistan/16/99/95	**PETPKRKRKR/GLF PETPKRRKR/GLF PETPKRRNR/GLF**	5 4 3	3 2 2	AF202226 AF202230 AF202233	2
H7N3	HP	Australia	1994	A/chicken/Queensland/94	**PEIPRKRKR/GLF**	4	2	CY022685	11,20
H7N1	HP	USA[7]	1994	A/Pekin_robin/California/30412/94	**PEIPKRRR/GLF**	4	1	GU052922	47
H7N3	HP	Australia	1992	A/chicken/Victoria/224/92	**PEIPKKKKR/GLF PEIPKRKKR/GLF PEIPKKRKKR/GLF PEIPKKKKKKR/GLF**	4 4 5 6	2 2 3 4	CY025077-CY025084	20
H7N7	HP	Australia	1985	A/chicken/Victoria/85	**PEIPKKREKR/GLF**	4	3	CY025069	10,20
H7N7	HP	Germany	1979	A/chicken/Leipzig/79 A/goose/Leipzig/137-8/79 A/goose/Leipzig/192-7/79 A/goose/Leipzig/187-7/79	**PEIPKKKKR/GLF PEIPKRKKR/GLF PEIPKKRKKR/GLF PEIPKKKKKKR/GLF**	4 4 5 6	2 2 3 4	U20459.1 L43913.1 L43915.1 L43914.1	21
H7N7	HP	Australia	1976	A/chicken/Victoria/76	**PEIPKKREKR/GLF**	4	3	CY024786	10,20
H7N3	HP	England	1963	A/turkey/England/63	**PETPKRRRR/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 euthanatized.
[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				**PQRETR/GLF**	1	0		39
H5N1 **H5N2** **H5N9**	HP	France	2015	A/chicken/France/150169a/15 A/duck/France/150233/15 A/duck/France/150236/15	**HQRRKR/GLF**	3	0	KU310447.1 KX014878.1 KX014886.1	53
H5N2	HP	Taiwan	2012	A/chicken/Taiwan/A1997/12	**PQRRKR/GLF**	3	0	KF193394.1	40
H5N2	HP	Taiwan	2008	A/chicken/Taiwan/K703-1/08	**PQRKKR/GLF**[5]	3	0	AB507264.1	34,35
H5N2	LP	USA	2004	A/chicken/Texas/298313/04	**PQRKKR/GLF**[6]	3	0	AY849793.1	6
	LP	Taiwan	2003	A/chicken/Taiwan/1209/03	**PQREKR/GLF**	2	0	AY573917.1	34,35
H5N2	LP/HP	USA	1983	A/chicken/PA/1370/83	**PQKKKR/GLF**[7]	3	0	CY107848.1	6,10,37
H5N1	HP	Scotland	1959	A/chicken/Scotland/59	**PQRKKR/GLF**[8]	3	0	GU052518.1	6
H7	LP				**PEIPKGR/GLF**	1	0		38
H7N4	LP	China	2018	A/chicken/Jiangsu/1/2018	**PELPKGR/GLF**	2	0	EPI_ISL_332358	HVRI, 48
H7N1	LP	UAE	2004	A/houbara bustard/UAE/2004	**PELPKRR/GLF**	2	0		APHA-UK
H7N3	HP	Pakistan	1995	A/chicken/Pakistan/16/99/95	**PETPKRRNR/GLF**	3	2	AF202233	2
H7N7	HP	England	1979	A/turkey/England/199/79	**PEIPKKREK/GLF**	3	2		1,9,14
H7Nx	LP	Australia	1976	A/duck/Victoria/76	**PEIPKKR/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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