



## Influenza A Cleavage Sites

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### **Background:**

By **The OIE Terrestrial Animal Health Code**, all avian influenza viruses of subtypes **H5 and H7** are reportable. Avian influenza viruses are classified as **highly pathogenic (HP)** for poultry if the:

- **IVPI** in six-week-old chickens is **greater than 1.2** or causes at least **75% mortality** in four-to eight-week-old chickens infected intravenously;
- or
- H5 and H7 viruses with **multiple basic amino acids at the cleavage site** of the haemagglutinin molecule (HA<sub>0</sub>): if the amino acid motif is similar to that **observed previously** in highly pathogenic avian influenza isolates, the isolate being tested should be considered as highly pathogenic.

Avian influenza viruses attach to host cells using the viral hemagglutinin (HA) protein. Progeny influenza viruses contain an HA<sub>0</sub> protein, which has to be cleaved to form HA<sub>1</sub> and HA<sub>2</sub> to become functional. This cleavage occurs at the HA<sub>0</sub> cleavage site by cellular proteases of the host. The type of cellular proteases that can cleave HA<sub>0</sub> depends on the type of cleavage site in the HA<sub>0</sub> 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 (HPAI H5N1)** for an overview of the very consistent multi-basic cleavage sites of HPAI H5N1 viruses of the Goose/Guangdong lineage (Asia, Africa, previously also Europe).

Use **Tables 2 (other HPAI H5) and 3 (HPAI H7)** 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 4** 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 H5N1
  - b. Table 2 for other HPAI H5
  - c. Table 3 for HPAI H7
  - d. Table 4 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.

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, 2, 3), **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, 2, 3), **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 recently circulating Goose/Guangdong-lineage HPAI H5 viruses.**

Sub-type	Clade <sup>1</sup>	no. of sequences 2009-2016	Cleavage site consensus <sup>2</sup>	critical basic aa <sup>3</sup>	Size of insert
H5	LP	251	PQRETR/GLF	1	0
H5N1	Gs/Gd-lineage	83 <sup>4</sup>	PQRE <b>RRRKR</b> /GLF	6	4
	Clade 1	122	PQRE <b>RRRKR</b> /GLF PQREG <b>RRRKR</b> /GLF	5	4
	Clade 2.1	61	PQRES <b>RRKK</b> /GLF	4	3
	Clade 2.2	471	PQGE <b>KRRKKR</b> /GLF PQGE <b>RRRKR</b> /GLF PQGE <b>GRRKKR</b> /GLF	5-6	4
	Clade 2.3.1	20 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	5	3
	Clade 2.3.2	735	PQRE <b>RRRKR</b> /GLF	5	3
	Clade 2.3.3	30 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	5	3
	Clade 2.3.4	551	PLRE <b>RRRKR</b> /GLF PLRE <b>KRRKR</b> /GLF	5	3
	Clade 2-like	8 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	6	4
	Clade 3	18 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	6	4
	Clade 4	6 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	6	4
	Clade 5	9 <sup>5</sup>	PQRE <b>IRRKR</b> /GLF	5	4
	Clade 6	8 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	6	4
	Clade 7	23	PQIE <b>GRRKR</b> /GLF	5	4
	Clade 9	24 <sup>5</sup>	PQRE <b>RRRKR</b> /GLF	6	4

<sup>1</sup> 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. <sup>2</sup> consensus sequence generated from all complete segment of H5 HA sequences in IRD clade-search tool 2009-2016, recovered December 2016, using MUSCLE alignment and Geneious® 8.1.7; variants with a population frequency greater than 10% were included; red color indicates critical basic residues; / indicates cleavage position (38). <sup>3</sup> basic residue at the -1 position and basic amino acids immediately preceding this position. <sup>4</sup> no sequences available for 2009-2016 therefore the consensus sequences were generated on data from 1996-2008. <sup>5</sup> no sequences available for 2009-2016 therefore the consensus sequences were generated on data from 2000-2008.

**Table 2: Multi-basic cleavage sites of sporadic HPAI avian influenza A viruses of subtype H5Nx**

Sub-type	Pheno-type <sup>1</sup>	location	year	Type virus	cleavage site <sup>2</sup>	Critical basic aa <sup>3</sup>	Size of insert	Accession number <sup>4</sup>	Ref.
H5	LP				PQRE <u>TR</u> /GLF	1	0		38
H5N2	HP	South Africa	2011	A/ostrich/SA/AI2114/11	PQR <u>RRKR</u> /GLF	4	1	JX069081.1	18
				A/ostrich/SA/AI2887/11	PQR <u>RRKR</u> /GLF	4	1	JX069097.1	
H5N2	HP	South Africa	2006	A/ostrich/SA/AI1091/06	PQRE <u>KRRKR</u> /GLF	6	4	EF591749.1	1,16
H5N1	HP	Germany	2006	A/gull/Germany/R882/06	PQGE <u>RRRKR</u> /GLF	6	4	AM408215.1	29
H5N2	HP	Italy	1997	A/chicken/Italy/1485/97	PQR <u>RRKR</u> /GLF	5	2	GU052473.1	6,30
H5N2	HP	Mexico	1994	A/chicken/Puebla/8623-607/94	PQR <u>RRKR</u> <u>TR</u> /GLF	5	3	AB558473.1	4,5,6
					PQR <u>KRRK</u> <u>TR</u> /GLF	6	4		
H5N2	HP	Mexico	1994	A/chicken/Puebla/8624-602/94	PQR <u>KRRK</u> <u>TR</u> /GLF	4	2		5
H5N1	HP	England	1991	A/turkey/England/50-92/91	PQR <u>KRRK</u> <u>TR</u> /GLF	5	3	EU636692.1	10
H5N8	HP	Ireland	1983	A/turkey/Ireland/1378/83	PQR <u>KRRK</u> /GLF	5	2	CY015089.1	6,31
H5N9	HP	Canada	1966	A/turkey/Ontario/7732/66	PQR <u>RRKR</u> /GLF	5	2	CY107859.1	6,32
H5N3	HP	South Africa	1961	A/tern/South Africa/61	PQRE <u>RRQKR</u> /GLF	4	4	CY107861.1	6,10

<sup>1</sup> LP = low pathogenic, HP = highly pathogenic as determined by IVPI.

<sup>2</sup> cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.

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

<sup>4</sup> GenBank record number

**Table 3: Multi-basic cleavage sites of sporadic HPAI avian influenza A viruses of subtype H7Nx**

Sub-type	Pheno-type <sup>1</sup>	location	year	Type virus	cleavage site <sup>2</sup>	Critical basic aa <sup>3</sup>	Size of insert	Accession number <sup>4</sup>	Ref.
H7	LP				PEIPKGR/GLF PELPKGR/GLF PEPPKGR/GLF PENPKTR/GLF PESPKTR/GLF	1 1 1 1 1	0 0 0 0 0		38
H7N7	HP	Italy	2016	A/chicken/Italy/16VIR-1873/2016	PELPKGRKR/GLF	4	3	EPI_ISL_220955	IZSVenezie
H7N8	HP	USA	2016	A/turkey/Indiana/16-001403-1/2016	PENPKKRKR/GLF	5	3	KU558906.1	41
H7N7	HP	UK	2015	A/chicken/England/26352/2015	PEIPRRKRGLF	4	3	EP1623939	APHA
H7N7	HP	Germany	2015	A/chicken/Germany/AR1386/2015	PEIPKRKRGLF	5	3	TBA	FLI
H7N7	HP	Italy	2013	A/chicken/Italy/13VIR4527_11/13	PETPKRRERGLF	4	3	KF569186.1	39
H7N3	HP	Mexico	2012	A/chicken/Jalisco/CPA1/12 A/chicken/Puebla/CPA-04451/16 A/chicken/Jalisco/CPA-01859/16	PENPKDRKRHRRT/GLF PENPKDRKNHRRT/GLF PENPKGKSRHRKT/GLF	6 6 6	8 8 8	JX397993.1 KX351916.1 KX351892.1	23
H7N7	HP	Spain	2009	A/chicken/Spain/6279-2/2009	PELPKGTKPRPR/GLF	4	6	GU121458.1	24
H7N7	HP	UK	2008	A/chicken/England/1158-11406/08	PEIPKRKR/GLF	4	2	FJ476173.1	25,26
H7N3	HP	Canada	2007	A/chicken/Saskatchewan/HR-00011/07	PENPKTKPRPR/GLF	4	6	EU500860.1	17
H7N7	HP	North Korea	2005	A/chicken/North Korea/1/2005	PEIPKRHRPRK/GLF	5	6		13
H7N3	HP	Canada	2004	A/chicken/Canada/rv504/04	PENPKQAYRKRMT/GLF PENPKQAYQKRMT/GLF PENPKQAYKKRMT/GLF PENPKQAYHKRMT/GLF PENPKQAHQKRMT/GLF PENPROAYRKRMT/GLF PENPKQACOKRMT/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	PEIPKRKR/GLF	4	2	AY338459.1	9,27
H7N3	HP	Chile	2002	A/chicken/Chile/4322/02	PEKPKTCSPLSRCRET/GLF PEKPKTCSPLSRCKTR/GLF	3 4	10 10	AY303631.1	7,28
H7N1	HP	Italy	1999	A/chicken/Italy/444/99	PEIPKGSRVRR/GLF PEIPKGSRMRR/GLF PEIPKBSRVRR/GLF	3 3 4	4 4 4	AJ704810.1	12
H7N4	HP	Australia	1997	A/chicken/New South Wales/2/97	PEIPRRKR/GLF PEIPKRKR/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	PETPKRRKR/GLF PETPKRRKR/GLF PETPKRRNR/GLF	5 4 3	3 2 2	AF202226 AF202230 AF202233	2
H7N3	HP	Australia	1994	A/chicken/Queensland/94	PEIPKRKR/GLF	4	2	CY022685	11,20
H7N3	HP	Australia	1992	A/chicken/Victoria/224/92	PEIPKKKR/GLF PEIPKRKR/GLF PEIPKKKR/GLF PEIPKKKKR/GLF	4 4 5 6	2 2 3 4	CY025077- CY025084	20
H7N7	HP	Australia	1985	A/chicken/Victoria/85	PEIPKREKR/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	PEIPKKKR/GLF PEIPKRKR/GLF PEIPKRKR/GLF PEIPKKKKR/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	PEIPKREKR/GLF	4	3	CY024786	10,20
H7N3	HP	England	1963	A/turkey/England/63	PETPKRRR/GLF	4	2	AF202238	1,10,14

<sup>1</sup> LP = low pathogenic, HP = highly pathogenic as determined by IVPI.

<sup>2</sup> cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.

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

<sup>4</sup> GenBank or GISAID record number

**Table 4. Unusual, 2-3 basic residue multi-basic cleavage sites**

Sub-type	Pheno-type <sup>1</sup>	location	year	Type virus	cleavage site <sup>2</sup>	Critical basic aa <sup>3</sup>	Size of insert	Accession number <sup>4</sup>	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	LP/HP	Taiwan	2008	A/chicken/Taiwan/K703-1/08	PQR <u>KKR</u> /GLF	3	0	AB507264.1	34,35
H5N2	LP/HP	USA	2004	A/chicken/Texas/298313/04	PQR <u>KKR</u> /GLF <sup>5</sup>	3	0	AY849793.1	
		Taiwan	2003	A/chicken/Taiwan/1209/03	PQR <u>EKR</u> /GLF	2	0	AY573917.1	34,35
H5N2	LP/HP	USA	1983	A/chicken/PA/1370/83	PQR <u>KKR</u> /GLF <sup>5</sup>	3	0	CY107848.1	6,10,37
H5N1	HP	Scotland	1959	A/chicken/Scotland/59	PQR <u>RRK</u> /GLF	3	0	GU052518.1	6
H7	LP				PEIPKGR/GLF	1	0		38
H7N1		UAE	2004	A/houbara bustard/UAE/2004	PELP <u>KRR</u> /GLF	2	0		
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>KR</u> /GLF	2	0	U20463.1	33

<sup>1</sup> 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.

<sup>2</sup> cleavage site between HA1 and HA2; red color indicates critical basic residues; residue insertions are underlined.

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

<sup>4</sup> GenBank record number. <sup>5</sup> requires loss of glycosylation for highly pathogenic phenotype.

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