Avian Influenza — Antigen Detection LAB CARD

	Diagnostic methods	Test specific equipment / Supplies needed	Specimen	Comments / Reference
Virus Isolation	**Virus Isolation** GOLD STANDARD	Embryonated chicken eggs from AI/ND antibody free donors (i.e. SPF or SAN) as recommended test by OIE Incubators, egg candlers, microbiological safety cabinets Takes 6-14 days to achieve result. This includes serolgocal subtyping of the isolated haemagglutinating agent by HI and NI testing (see below). Proper training required.	Tracheal / oropharyngeal / cloacal swabs in VTM Organ-homogenates in VTM BSL 3	OIE: Terrestrial Manual, Chapter 2.3.4, 2009; for detailed SOP: - A laboratory manual for the isolation and identification of avian pathogens (2008): - Avian influenza and Newcastle Disease. A Field and Lab manual (eds Capua and Alexander), 2009; - Avian Influenza virus (Ed. Spackman 2008A) - EU AI-diagnostic manual Reference- Labs (see below) will also provide SOP on request
Ag detection/Typing	**HA/HI -Test**	U- or V- shaped microtitre plates, red blood cells (RBC) from SPF or SAN-chicken, appropriate reference sera, control antigen, proper training required	Allantoic fluid (AF)	The HA-test detects haemagglutinating activity for AI and ND viruses, for subtyping of AIV, HI-test is necessary, OIE: Terrestrial Manual, Chapter 2.3.4 detailed protocols in the books mentioned above or from reference labs
	AGID assay	Agar-gel preparation, agar-punch, reference antiserum (might be subjective)	Allantoic fluid (AF)	Takes two days, sometimes difficult to interpret , OIE: Terrestrial Manual, Chapter 2.3.4, 2009; detailed protocols in the books mentioned above
	Lateral Flow Device	Multiple, commercially available kits, swabbing material (if not supplied) does not require any additional laboratory equipment	Oropharyngeal /cloacal swabs, tissue fluids or homogenates	Because of the low sensitivity only recommended for use with dead poultry on a flock base, not for regular use, not for waterfowl and not for surveillance purpose. All results (neg and pos) need to be confirmed by a validated (OIE) method!
Nucleic Acid (NA) detection : (I) generic and (II) subtype-specific	**RNA Extraction**	Numerous commercially available kits or Trizol and a vented biosafety cabinet for manual extractions	Swab suspension, organ homogenates	The type of kit used depends on the sample type, use of Trizol is cheaper but laborious and hazardous (contact Reference Labs for Trizol- Protocol) Protocols available from (among many others): -"Field and Lab manual" (eds Capua and Alexander), 2009 - "Avian Influenza virus" Ed Spackman, 2008 - Southeast Poultry Research Laboratory (SEPRL: contact table) For removal of PCR inhibitors see Das et al., 2009
	Conventional RT-PCR	Thermocycler, PCR equipment and consumables, disposables (e.g. PCR tubes…), Gel-electrophoresis unit	Freshly extracted RNA or RNA properly stored (preferably -70°C)	Less sensitive compared to real-time RT PCR Protocols (H5, H7, N1 subtypes) available in: - the "Field and Lab manual" (eds Capua and Alexander), 2009 - Starick et al., 2000 - Fouchier et al., 2000. NA-subtyping Fereidouni et al., 2009 EU diagnostic Manual, 2006 chapter VI Protocols are available from the reference labs

Bold-printed methods are fully validated methods and are already recommended elsewhere (OIE-manual),
other methods have been developed recently and are in progress of evaluation and are here called "investigative methods".
HPAI H5N1 is classified as Risk Group 3 agent

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Nucleic Acid (NA) detection: (i) generic and (ii) subtype-specific	**Real Time RT-PCR**	Real-time PCR cycler and software; PCR equipment and consumables/disposables (e.g. PCR tubes or plates)	Freshly extracted or properly stored RNA (preferably -70°C)	Various protocols available for different subtypes including (i) generic PCR for M (Spackman et al., 2002); (ii) subtype-specific PCRs HA-2 (Phipps et al., 2004; Gall et al., 2008) H5 (Spackman et al., 2002, modified by Slomka et al., 2007; Hoffmann et al., 2007); H7 (Monne et al., 2008; Slomka et al., 2009) H9 (not yet fully evaluated, Monne et al., 2008) N1 (Validated protocols are available from AH-VLA)
	Sanger Sequencing (investigative test)	Sequencer, various sequencing kits, software for analysis, PCR equipment and consumables/disposables	cDNA (amplificates)	For advanced labs: Various protocols for HA and NA subtyping and for molecular pathotyping available from the reference lab and SEPRL. Hoffmann et al., 2001; Gall et al., 2009; Phipps et al., 2004 (evaluated on egg fluids and not clinical specimens)
	LAMP (investigative test)	Loopamp H5, EIKEN Chemical Co. Ltd (H5), Loopamp M, EIKEN Chemical Co.Ltd (M) IAEA LAMP (H5) currently under validation Waterbath or LAMP machine or real-time PCR cycler for isothermal reaction	Freshly extracted or properly stored RNA	less sensitive than rRT-PCR; latter should always be preferred where available; Postel et al., 2009; Yoshida et al., 201; Unger, IAEA; Unpublished kits and assays still require evaluation! USE FOR RESEARCH PURPOSE ONLY
	Microarray (investigative test)	Several commercial kits available, differentiation of all 16 HA-subtypes, 9 NA-subtypes, highly and low pathogenic H5/H7-strains	cDNA	Very expensive and only for advanced labs validated by Gall et al., 2009. Metzgar et al., 2010; Townsend et al., 2006; Wang et al., 2008; Sun et al., 2011; Yueqing et al., 2008; Huang et al., 2009 USE FOR RESEARCH PURPOSE ONLY
	Portable LATE PCR	- Smiths detection Bio-Seeq Portable Veterinary - Optigene: Genie II - Portable Real-time system Idaho Technology: R.A.P.I.D. - DxNA LLC - Genereach	Swab material (Smiths), RNA	Some evaluation (e.g. for FMD) has been performed but technology not yet recommended by any Animal Health organisation or Reference Laboratory USE FOR RESEARCH PURPOSE ONLY
PathoTyping	**IVPI**	Ten SPF-chicken Experimental animal facilities (BSL-3)	Virus isolate to be characterized	This method requires BSL-3 experimental animal facilities with proper good management practices, cleaning and disinfection procedures and appropriate disposal of carcasses. Protocol available in OIE manual
	Sanger Sequencing	Sequencer, various sequencing kits, software for analysis, PCR equipment and consumables/disposables	cDNA amplificate	OIE and EU manual (Eurasian strains), Slomka et al., 2007; Gall et al., 2008; for primer sequences and protocols of contact Reference Labs
	Restriction fragment lengthpattern	Thermo shaker or water bath, restriction enzyme, gel-electrophoresis equipment and documentation system	DNA-Amplificates of the HA including the cleavage site	A simple method that has been validated by Fereidouni et al., 2008; (But restricted to HPAIV H5N1 clade 2.2) protocol available from FLI, needs reevaluation in the labs and confirmation necessary by IVPI or sequencing (shipment to International Reference Lab)

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