# Avian Influenza

## Antigen Detection

### Diagnostic Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Specific Equipment / Supplies Needed</th>
<th>Specimen</th>
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</thead>
<tbody>
<tr>
<td><strong>Virus Isolation</strong></td>
<td>- Embryonated chicken eggs from AI/ND antibody free donors (i.e. SPF or SAN) as recommended test by OIE&lt;br&gt;- Incubators, egg candlers, microbiological safety cabinets&lt;br&gt;- Takes 6-14 days to achieve result. This includes serological subtyping of the isolated haemagglutinating agent by HI and NI testing (see below). Proper training required.</td>
<td>Tracheal / oropharyngeal / cloacal swabs in VTM&lt;br&gt;Organ-homogenates in VTM</td>
<td>OIE: Terrestrial Manual, Chapter 2.3.4, 2009: for detailed SOP:&lt;br&gt;- A laboratory manual for the isolation and identification of avian pathogens (2008):&lt;br&gt;- Avian influenza and Newcastle Disease. A Field and Lab manual (eds Capua and Alexander), 2009;&lt;br&gt;- Avian Influenza virus (Ed. Spackman 2008A)&lt;br&gt;- EU AI-diagnostic manual&lt;br&gt;Reference- Labs (see below) will also provide SOP on request</td>
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<tr>
<td><strong>HA/ HI - Test</strong></td>
<td>- U- or V- shaped microtitre plates, red blood cells (RBC) from SPF or SAN-chicken, appropriate reference sera, control antigen, proper training required&lt;br&gt;- Agar-gel preparation, agar-punch, reference antiserum (might be subjective)</td>
<td>Allantoic fluid (AF)</td>
<td>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&lt;br&gt;Takes two days, sometimes difficult to interpret, OIE: Terrestrial Manual, Chapter 2.3.4, 2009; detailed protocols in the books mentioned above&lt;br&gt;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!</td>
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<tr>
<td><strong>AGID Assay</strong></td>
<td>- Multiple, commercially available kits, swabbing material (if not supplied) does not require any additional laboratory equipment&lt;br&gt;- Agar-gel preparation, agar-punch, reference antiserum (might be subjective)</td>
<td>Allantoic fluid (AF)</td>
<td></td>
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<td><strong>Lateral Flow Device</strong></td>
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<td>Oropharyngeal / cloacal swabs, tissue fluids or homogenates</td>
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<td><strong>RNA Extraction</strong></td>
<td>- Numerous commercially available kits or Trizol and a vented biosafety cabinet for manual extractions&lt;br&gt;- Thermocycler, PCR equipment and consumables, disposables (e.g. PCR tubes…), Gel-electrophoresis unit</td>
<td>Swab suspension, organ homogenates</td>
<td>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):&lt;br&gt;- &quot;Field and Lab manual&quot; (eds Capua and Alexander), 2009&lt;br&gt;- &quot;Avian Influenza virus” Ed Spackman, 2008&lt;br&gt;- Southeast Poultry Research Laboratory (SEPRL: contact table)&lt;br&gt;For removal of PCR inhibitors see Das et al., 2009&lt;br&gt;Less sensitive compared to real-time RT PCR Protocols (H5, H7, N1 subtypes) available in:&lt;br&gt;- the “Field and Lab manual” (eds Capua and Alexander), 2009&lt;br&gt;- Starick et al., 2000&lt;br&gt;- Foucher et al., 2000&lt;br&gt;NA-subtyping Fereidouni et al., 2009&lt;br&gt;EU diagnostic Manual, 2006 chapter VI&lt;br&gt;Protocols are available from the reference labs</td>
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<tr>
<td><strong>Conventional RT-PCR</strong></td>
<td></td>
<td>Freshly extracted RNA or RNA properly stored (preferably - 70°C)</td>
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**Nuclear Acid (NA) Detection:**

- Generic and sub-specific

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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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| **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., 2011; Ungar, 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  
- Smiths detection Bio-Seeq Portable Veterinary  
- Optigene: Genie II  
- Portable Real-time system Idaho Technology: R.A.P.I.D.  
- DxA NA LLC  
- Genereach | cDNA | USE FOR RESEARCH PURPOSE ONLY |
| **Portable Late PCR** | | Swab material (Smiths), RNA | |
| **IVPI** | Ten SPF-chicken  
Experimental animal facilities (BSL-3) | Virus isolate to be characterized | 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 |
| **Pathotyping** | Sequencer, various sequencing kits, software for analysis, PCR equipment and consumables/disposables  
Thermo shaker or water bath, restriction enzyme, gel-electrophoresis equipment and documentation system | cDNA amplificate | 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 |
| **Sanger Sequencing** | | DNA-Amplificates of the HA including the cleavage site | 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  
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 Length Pattern** | | | 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) |

**Note:** 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.