ORTHOMYXOVIRUSES

- HA - hemagglutinin
- NA - neuraminidase
- helical nucleocapsid (RNA PLUS NP protein)
- lipid bilayer membrane
- polymerase (3 PROTEINS)
- M1 protein

type A, B, C: NP, M protein
sub-types: HA or NA protein

HA (H5 AIV kit Marker)

NA

MA

Nucleoprotein (AIV kit Marker)
Migratory Birds

Poultry

Swine (Mixing Vessel)

Human Transmission
### Influenza A Type

<table>
<thead>
<tr>
<th>Haemagglutinin subtypes</th>
<th>Neuraminidase subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>N1</td>
</tr>
<tr>
<td>H2</td>
<td>N2</td>
</tr>
<tr>
<td>H3</td>
<td>N3</td>
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<tr>
<td>H4</td>
<td>N4</td>
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<tr>
<td>H5</td>
<td>N5</td>
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<tr>
<td>H6</td>
<td>N6</td>
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<tr>
<td>H7</td>
<td>N7</td>
</tr>
<tr>
<td>H8</td>
<td>N8</td>
</tr>
<tr>
<td>H9</td>
<td>N9</td>
</tr>
<tr>
<td>H10</td>
<td></td>
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<tr>
<td>H11</td>
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<tr>
<td>H12</td>
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<tr>
<td>H13</td>
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<tr>
<td>H14</td>
<td></td>
</tr>
<tr>
<td>H15</td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis of AIV

• Antigen Detection
  - PCR: takes 5 hours and needs some special equipments
  - Virus Isolation: takes 2 or 3 days in embryonic egg
  - Immunocromatography test: takes 20 minutes and does not need any equipment

• Antibody Detection
  - AGP: takes 2 days
    • Detect the NP and M antibodies
    • Not recommend in the early stage of HPAI and not suitable to turkey and Pheasant
  - Hemagglutination Inhibition test
    • Specific for each serotype
    • Not recommend in the early stage of HPAI
  - ELISA
    • Detect the antibody against NP
    • More specific than AGP and HI
    • Not recommend in the early stage of HPAI
Due to the virulence and fast spreading of the AIV virus

◆ A rapid ANTIGEN test should be used since ANTIBODY test would not detect the disease before the animal is dead and potentially infect human and other animals.

◆ A SIMPLE and FAST diagnostic method that could be used by veterinarians in the field could be critical for the early detection.
Virus Isolation

Inoculation

Agglutination
Virus Identification

Identified as H5N1
Virus Virulence

Amino Acid Sequencing at HA cleavage site

CPE confirm in Chicken Embryo Fibroblast

Control

Isolated virus
AIV Antigen Rapid Test Kits

It is most optimal AIV Antigen screening method

World First & Sole H5 AIV Antigen Differential Test
Test Procedures

1. Sample Collection
2. Dilution
3. Dropping
4. Result

20~30 minutes
[ Cloacal Swab method ]
[Environmental feces Swab method]

1. Insert the sample collection swab to the wet feces

2. Pull out the sample collection swab
Test Procedures - Dropping

4 ~ 5 drops
Test Procedures - Results

**AIV Ag kit**
- Positive result
- Negative result

**H5 AIV Ag kit**
- Positive result
- Negative result
Advantages of Rapid Kit

- **Accurate**
  - High sensitive and good correlation with other test methods
- **Anywhere**
  - Laboratory, Farm, Clinic, Home: No equipment needed
- **Simple**
  - One Step after collection a sample
- **Fast**
  - Reliable Result within 20 min.
- **Immediate action**
  - Treatment and management (quarantine or slaughter) after identifying a cause
- **Self**
  - Self testing by Farmer themselves or Gov. officials
- **Low cost**
  - Time, Labor, Money saving
Anigen Rapid AIV Ag Test Kit

World First
• Principle: Immunochromatographic assay using Direct Sandwich Method
  – Monoclonal anti-AIV (Capture)– AIV Type A –Monoclonal anti-AIV (Detector)
  – Monoclonal antibody against Type A specific nucleoprotein of Avian influenza virus
• Specimen: Avian cloacal swab or feces (trachea)
• Sensitivity : 100% by farm (n=19), 77.3% by feces (n=150) compare to virus isolation
• Specificity: 100% by virus isolation (n=1,402)
• Detection limit: 10^4.6 EID50/ml or 0.13 HAU
Principles AIV KIT

*MoAb : Monoclonal antibody

AIV (H1N1~H1SNY)

Feces/Organs

N/C membrane

Control line  Test line

AIV MoAb

Gold conjugate

AIV kit

Principles AIV KIT
H1N1 : A/PR/8/34
H2N2 : A/Singapore/1/57
H3N8 : A/Duck/Ukraine/1/63
H4N6 : A/Duck/Czechoslovakia/56
H5N3 : A/Duck/Hong Kong/820/80
H6N5 : A/Shearwater/Australia/1/72
H7N1 : A/Duck/Hong Kong/301/78
H8N4 : A/Turkey/Ontario/6118/68
H9N2 : A/Turkey/Wisconsin/1/66
H10N7 : A/Chick/Germany/N/49
H11N6 : A/Duck/England/56
H12N5 : A/Duck/Alberta/60/76
H13N6 : A/Gull/Maryland/704/77
H14N5 : A/Mallard/Gurjev/263/82
H15N9 : A/Shearwater/West Australia/2576/79
Detection

Result
Virus

Control line
Test line

H1   H2    H3    H4   H5    H6    H7    H8   H9   H10  H11  H12 H13 H14 H15  –ve
N1   N2    N8 N6   N3    N5    N1     N4   N2    N7    N6   N5    N6   N5   N6  Control
## Cross Reactivity

<table>
<thead>
<tr>
<th>Result</th>
<th>Virus</th>
<th>Control line</th>
<th>Test line</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>1. AIV (<strong>ES/03, H5N1</strong>)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>2. AIV (<strong>MS96, H9N2</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>3. NDV (<strong>Kyojungwon</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4. IBV (<strong>KS96</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>5. ILTV (<strong>Serva</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>6. Pneumovirus (<strong>TRTV</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>7. Reovirus (<strong>IVR-1133</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>8. IBDV (<strong>Winterfield2510</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>9. MDV (<strong>CVI988+HVT</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>10. REV (<strong>REV-T</strong>)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Legend:**
- **AIV kit**
- **Control line**
- **Test line**
- **Result**
- **Virus**
- Virus Titer: $2^7$ HA unit, $10^{7.6}$ EID$_{50}$/ml
- Detection limit: $0.13$ HA unit, $10^{4.6}$ EID$_{50}$/ml
Summary of AIV kit

- First commercialized Avian Influenza Antigen Rapid Kit in the world
- All AIV (H1N1~H15N9) can be detected in the farm, market, or laboratory by use of feces or cloacal swab
- High Sensitivity and Specificity
- Easy assay procedure: No technical expert is required
- Labor & Time saving because of fast results
Anigen Rapid H5 AIV Ag Test Kit

World First
Properties

- Principle: Immunochromatographic assay using Direct Sandwich Method
  - Monoclonal anti-AIV H5 (Capture) – AIV H5
  - Monoclonal anti-AIV H5 (Detector)
  - Monoclonal antibody against subtype H5 specific protein of avian influenza virus
- Purpose: Detection of Avian influenza type A subtype H5 virus (H5N1 ~H5N9)
- Specimen: Avian cloacae swab or feces
- Sensitivity: 100% by farm (n=13), 76.6% by feces (n=115) compare to virus isolation
- Specificity: 100% compare to virus isolation (n=1,402)
- Detection limit: 10^6.5 EID50/ml or 1 HAU
Principles

Feces/Organs

*MoAb: Monoclonal antibody

AIV (H5Nx)

H5 AIV MoAb

N/C membrane

Control line Test line

Gold conjugate

National Veterinary Research & Quarantine Service

H5 AIV Ag

Anigen
Animal Genetics Inc.

Cultek
Specific Detection

H5 AIV Kit

Control line  ➔
H5 Test line  ➔

Result

AIV subtype

- - - - + - - - - - - - - - - -

H1  H2  H3  H4  H5  H6  H7  H8  H9  H10  H11  H12  H13  H14  H15  –ve
N1  N2  N8  N6  N3  N5  N1  N4  N2  N7  N6  N5  N6  N5  N6  Control
Cross Reactivity

1. AIV (ES/03, H5N1)  
2. AIV (MS96, H9N2)  
3. NDV (Kyojungwon)  
4. IBV (KS96)  
5. ILTV (Serva)  
6. Pneumovirus (TRTV)  
7. Reovirus (IVR-1133)  
8. IBDV (Winterfield2510)  
9. MDV (CVI988+HVT)  
10. REV (REV-T)
- Virus Titer: $2^7$ HA unit, $10^{7.6}$ EID$_{50}$/ml
- Detection Limit: 1 HA unit, $10^{5.5}$ EID$_{50}$/ml

**Detection Limit**

**Control line**

**H5 Test line**

<table>
<thead>
<tr>
<th>HA titer</th>
<th>64</th>
<th>32</th>
<th>16</th>
<th>8</th>
<th>4</th>
<th>2</th>
<th>1</th>
<th>0.5</th>
<th>0.25</th>
<th>0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Summary of H5 AIV kit

- The Sole H5 Avian Influenza Antigen Rapid Kit in the world

- HPAI(H5N1~H5N9) can be detected in the farm, market, or laboratory by use of feces or cloacal swab

- High Sensitivity and Specificity

- Easy assay procedure: No technical expert is required

- Labor & Time saving because of fast results
Conclusion

- **Detection of the Viruses**
  - AIV Ag Test Kit: from H1N1 to H15N9
  - H5 AIV Ag Test Kit: from H5N1 to H5N9

- **Detection Limit**
  - AIV Ag Test Kit: 0.13 HAU, 104.6 EID50/mL
  - H5 AIV Ag Test Kit: 1 HAU, 105.5 EID50/mL

- **Sensitivity and Specificity**
  - AIV Ag Test Kit
    - Sensitivity: 100% by farm or flock, 77.3% by feces
    - Specificity: 100% by feces
  - H5 AIV Ag Test Kit
    - Sensitivity: 100% by farm or flock, 76.6% by feces
    - Specificity: 100% by feces
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