

# IMMONLINE

INFLUENZA VIRUS MONITORING



**USAID** | INDONESIA

## FOREWORD



### DIRECTOR GENERAL OF LIVESTOCK AND ANIMAL HEALTH SERVICES

By the grace of God almighty, we are grateful that the Influenza Virus Monitoring (IVM) platform **IVM Online** could be realized. The IVM programme was established through collaboration between the Government of Indonesia and the Food and Agriculture Organization. This programme is implemented by all technical implementing units across the Directorate General of Livestock and Animal Health Services and will be further developed, where in the future it could involve the private sector or universities. It is expected that through the **IVM Online** platform the mapping of avian influenza viruses in Indonesia can be done to allow policy making in the control and prevention of avian influenza.

Thank you to all parties who gave contributions, ideas, or played active roles in the development of the **IVM Online** programme. It is expected that this programme can be utilized optimally for the control and prevention of avian influenza and other epidemic and zoonotic influenzas in Indonesia.

**Ir. Syukur Iwantoro**

Director General of Livestock and Animal Health Services



### DIRECTOR OF ANIMAL HEALTH

**IVM Online** is a new system in animal disease monitoring. The existence of influenza viruses, in particular HPAI virus in Indonesia, raises concerns on the development of zoonoses in humans. Therefore, **IVM Online** is one of the systems used to manage and monitor AI virus in an integrated manner. The **IVM Online** programme is implemented within a network to allow data generation anywhere, in a faster and real-time manner.

**IVM Online** is significantly useful in the improvement of laboratory capacity to support animal health. It is expected that this programme could support the establishment of a veterinary laboratory network across Indonesia. In the future, it is expected that private laboratories as well as universities could join and participate in **IVM Online**.

**Dr. Pudjiatmoko**

Director of Animal Health



### FAO REPRESENTATIVE - INDONESIA

The launch of the Influenza Virus Monitoring (IVM) platform **IVM Online** marks a milestone in the continuing collaboration between the Directorate General of Livestock and Animal Health Services (DGLAHS), Ministry of Agriculture and FAO Indonesia. This partnership has flourished since 2006 through a series of technical projects supporting the control of highly pathogenic avian influenza (HPAI). In efforts to control HPAI and reduce the threat to human health, as well as the economic losses to the national economy and to the poultry sector, Indonesia has used vaccination as the principal means of controlling the disease. During this time, the virus has undergone gradual antigenic drift, which has necessitated changes in seed strains for vaccine production and associated modifications to diagnostic reagents. In order to improve the system of monitoring such viral evolution, the Government of Indonesia, with the assistance of FAO/OFFLU and regional reference laboratories such as the Australian Animal Health Laboratory, has developed an innovative IVM network whereby H5N1 isolates are antigenically and genetically characterized.

The benefits of the IVM network approach to HPAI surveillance was demonstrated by the detection of a new clade ("2.3.2.1") H5N1 virus in Indonesia in 2012. The new virus, which caused high mortality in domestic ducks, was isolated by the Wates DIC, and then sequenced by the IVM partner laboratories. The resulting antigenic and genetic characterization of this new clade virus lead to the successful and timely development (by Pusvetma) of a clade 2.3.2.1 vaccine ("Afluvet"), based on the Sukoharjo strain.

The example of the Indonesian IVM network, and the development of the **IVM Online** platform have relevance for other countries in the Asia region seeking to establish laboratory networks for the surveillance of avian influenza and other pathogens. FAO looks forward to a continuing partnership with the DGLAHS in the prevention and control of emerging infectious diseases of veterinary and public health significance.

**Dr. Mustafa Imir**

FAO Representative  
Indonesia  
20 May 2014

## THE EFFORT TO FIGHT INFLUENZA VIRUS IN INDONESIA

The poultry industry makes up 1% of Indonesia’s gross domestic product and provides the protein needs of 232 million Indonesians. The complex poultry industry structure, starting from intensive poultry farms, semi-intensive broiler and layer farms, and small scale backyard poultry farms provide meat and eggs through traditional markets all over Indonesia. The introduction of the H5N1 Highly Pathogenic Avian Influenza (HPAI) virus in 2003 has disrupted the productivity of the poultry industry. Vaccination is used as one of the 8 strategies to fight the influenza virus in poultry.

Since 2009, the Government of Indonesia, supported by the influenza expert network of OIE/FAO (OFFLU) and FAO – ECTAD Indonesia have tried to increase the capacity of eight (8) animal health diagnostic laboratories, PUSVETMA, BBLITVET and BBPMSOH to detect and monitor the presence of the avian influenza virus circulating in Indonesia. The laboratory network was then developed to identify potential virus variants; determining the candidate strains for challenge virus and monitoring the efficacy of AI vaccines used. The diagnostic methods are harmonized with standard biological reagents for high quality results. The characterization of clade 2.1.3 H5N1 AI in the laboratory provided the following results:

A. Determine 4 vaccine strains:

1. A/Chicken/West Java/PWT-WIJ/2006;
2. A/Chicken/Pekalongan/BBVW-208/2007;
3. A/Chicken/Garut/BBVW-223/2007;
4. A/Chicken/West Java (Nagrak) 30/2007

and 2 challenge strains:

1. A/Chicken/West Java-Subang/29/2007 dan
2. A/Chicken/West Java/Smi-Pat/2006 through the Director General of Livestock and Animal Health Service’s decision letter on the use of local vaccine that matches the virus epidemiologically.

B. Detect the new clade 2.3.2.1 H5N1 HPAI virus in mid-August 2012 that infects ducks and has spread to islands outside of Java (*see Figure 1*). With the presence of two H5N1 HPAI clades, the HPAI situation in Indonesia becomes more complex and requires a rapid communication system.

In relation to this matter, a web-based communication system has been developed to accelerate the reporting of HPAI H5N1 virus monitoring to decision makers at the National level. Communication methods between laboratories have been implemented within the **Influenza Virus Monitoring (IVM) Online** system, developed since 2011 and launched on 20<sup>th</sup> of May 2014.

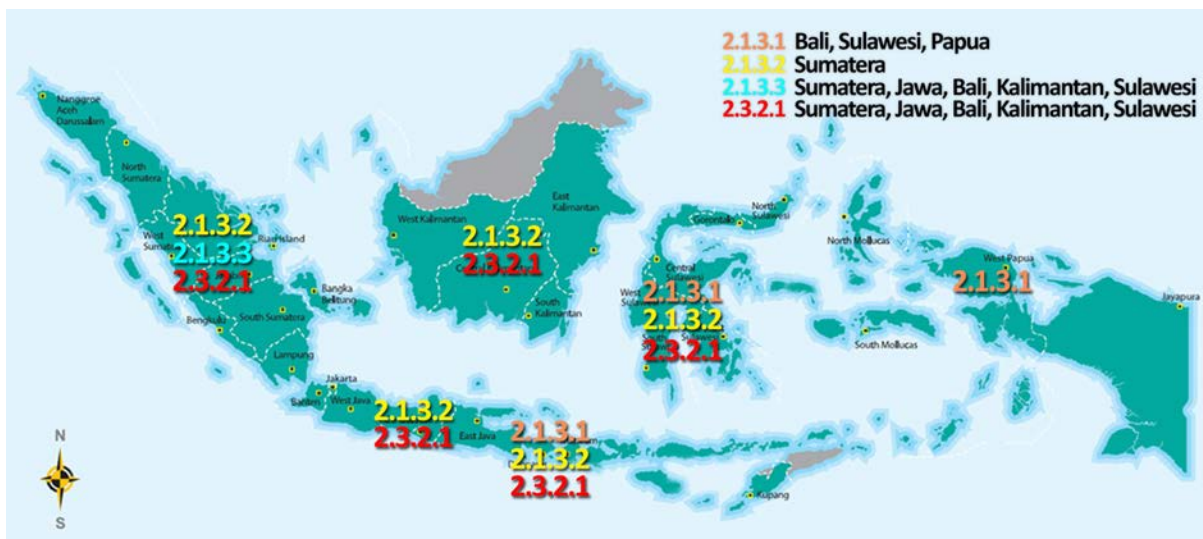


Figure 1

**IVM Online is:**

A web-based animal health laboratory network system in Indonesia that manages antigenic and genetic data of HPAI viruses circulating in Indonesia.

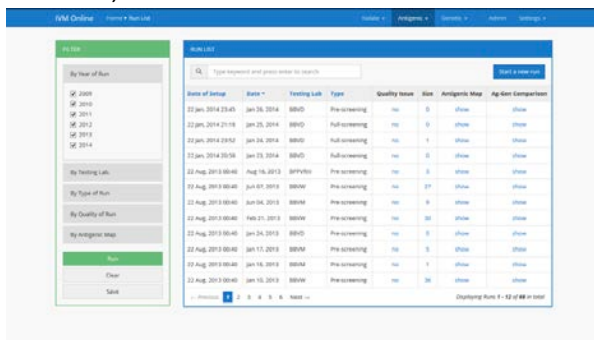
The implementation of **IVM Online** aims to:

1. Identify potential virus variants;
2. Identify and determine the strain for challenge virus, and
3. Monitor the efficacy of the vaccines used.

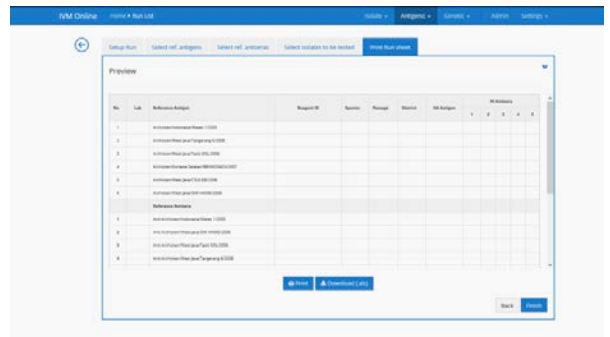
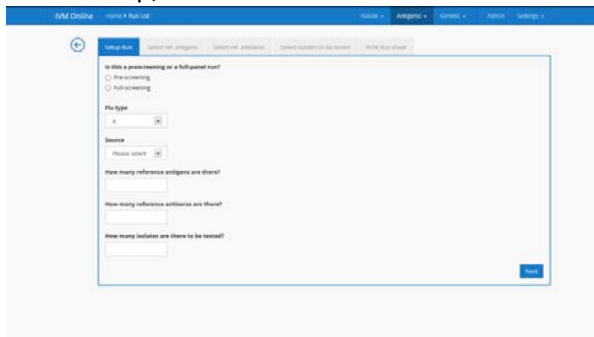
**The main features of IVM Online**

Within the **IVM Online** Software, there are many features that are useful to map out the HPAI virus situation in Indonesia. The main features are:

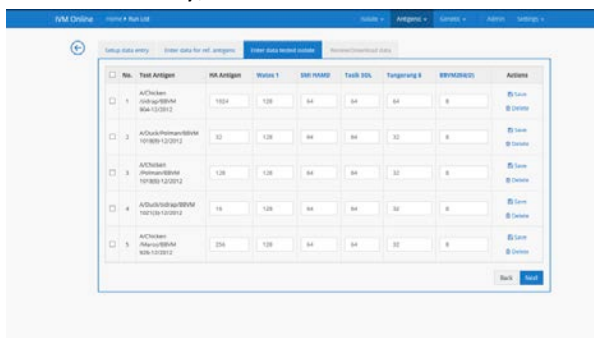
1. Antigenic Modules consisting of :
  - a. Run list;



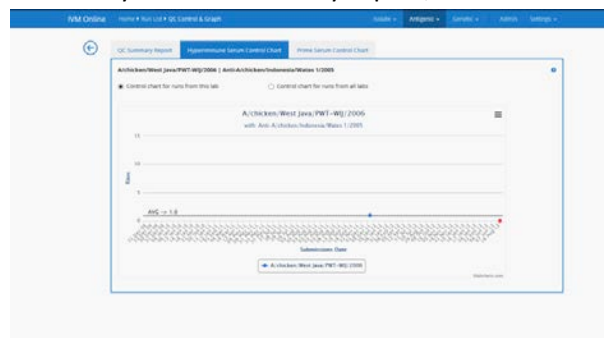
b. Run set up;



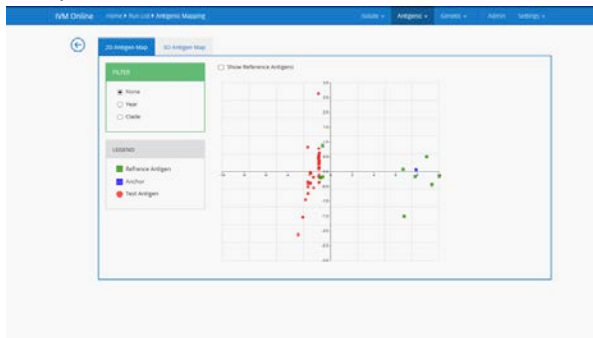
c. Run data entry;



d. Quality control summary report;



e. Antigenic mapping (displaying an antigenic map of a run);



f. Antigenic and genetic comparison.



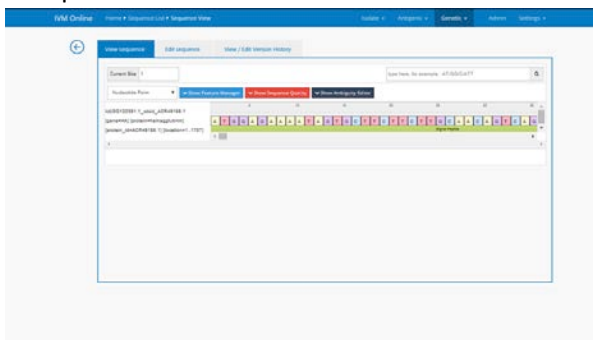
## 2. Genetic Modules consisting of:

a. List of sequences;

isolate name	Segment	Year of Sequencing	Sequencing Lab	GenBank	Complete Segment?
A/Chickens/Bandung/99/01/2008 / upload more sequences...	4	2012	AHHL	Yes	Yes
A/Chickens/Bandung/99/01/2008 / upload more sequences...	4	2012	AHHL	Yes	Yes
A/Chickens/Bandung/99/01/2008 / upload more sequences...	4	2012	AHHL	Yes	Yes

b. Sequence uploader;

c. Sequence viewer.



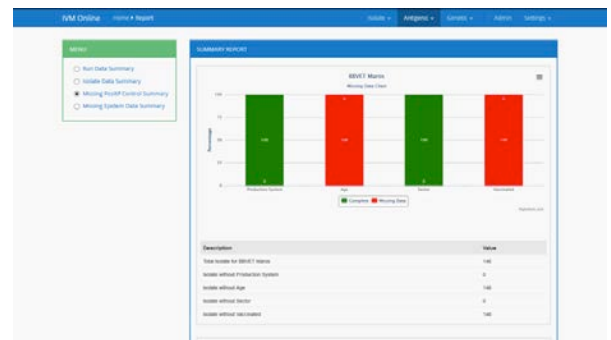
## 3. Isolate module

isolate name	Province	Year	Species	Lab	Antigenic	Genetic
A/Chickens/Hulu Seluas/01/03/2011	Bali	2011	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/24/2014	Bali	2014	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/23/2014	Bali	2014	Chicken	BBVU	0	0
A/Chickens/Bandung/01/09/2011	Bali	2011	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/03/2011	Sumatra Utara	2011	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/03/2011	Riau	2011	Chicken	BBVU	0	0
A/Chickens/Bandung/01/09/2011	Bali	2011	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/03/2011	DI Yogyakarta	2011	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/03/2011	DI Yogyakarta	2011	Chicken	BBVU	0	0
A/Chickens/Hulu Seluas/01/03/2011	DI Yogyakarta	2011	Chicken	BBVU	0	0

4. Reporting Module  
a. Result conclusion ;



b. Report of a false result to guarantee data quality.



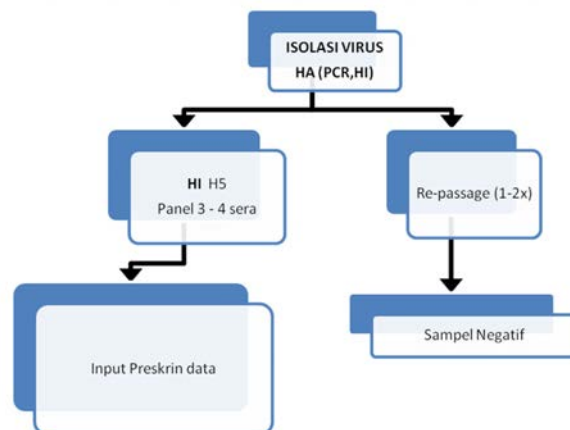
**Members of IVM ONLINE**

The members of the **IVM ONLINE** network are 8 diagnostic laboratories (DIC), BBPMSOH, PUSVETMA and BBLITVET. DIC Wates has been chosen as the focal point to control the activities of IVM Online.



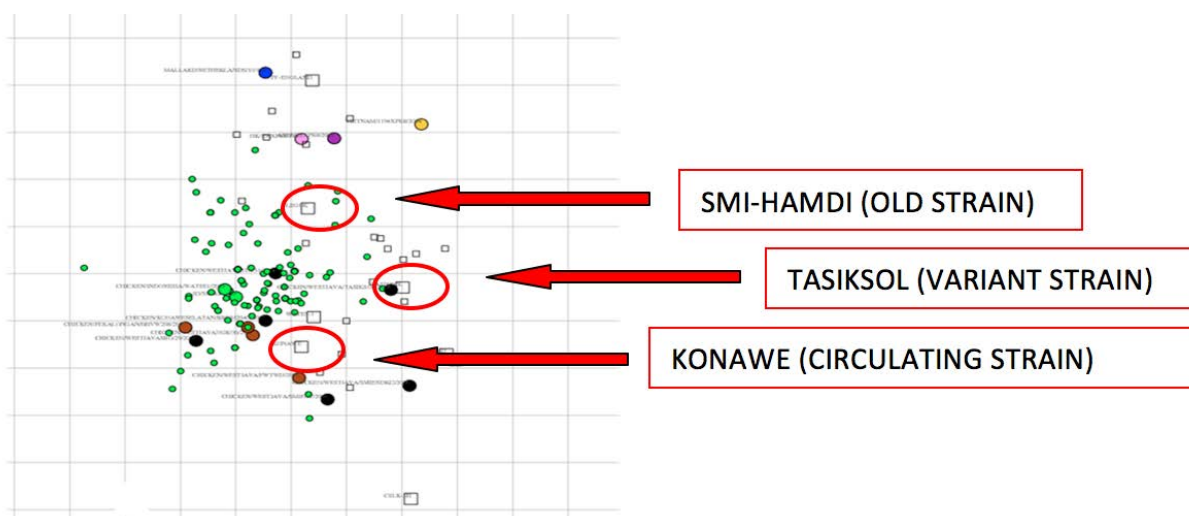
### ACTIVITY PHASE

1. Members of **IVM ONLINE** routinely accept and process all AI samples from the field and only H5 viruses that have been through the **virus isolation process** will go to the next phase.
2. Those allantoic H5 HPAI viruses are identified by using hyper-immune sera like NDV and H5.
3. Positive H5 HPAI viruses are then **PRE-SCREENED** using the **PRE-SCREEN KIT** containing 3 antigens, 3-4 prime sera and H5 hyper-immune antiserum. The data of the HI titres along with epidemiological data of every isolate are then uploaded to **IVM ONLINE**.

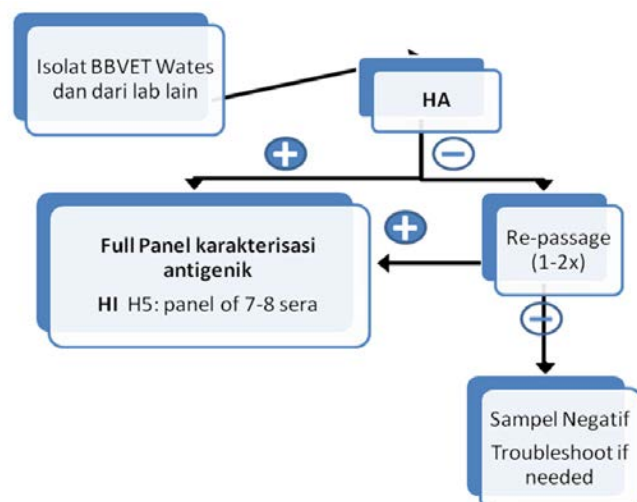


### PRESCREEN PROCESS

The reference antigens and antisera in each Pre-screen Kit used are always representative of viruses in circulation. The Pre-screen Kit constantly changes according to development of virus in the field. The image below is an example of Pre-screen Kit 1 used in 2010. To match the current situation in 2014, the Pre-screen Kit used is Pre-screen Kit 5, where the SMI-HAMDI strain has been removed and more antigenically relevant strains have been added, namely A/Chicken/West Java/Tangerang 6/2008, A/Chicken/West Java-Sbg/29/2007 and the A /VN 2.3.2.1 strain, which is genetically the same as clade 2.3.2.1 Indonesia H5N1 virus strains.



4. This pre-screen data is then analyzed by the **FOCAL POINT (DIC WATES)** by using an analysis module in **IVM ONLINE** to evaluate the selected isolate. All members have been requested to submit their selected isolates to DIC WATES to be propagated, and antigenically characterized using a pre-screen and a **FULL PANEL KIT** containing 7-8 antigens and antisera (prime and hyper-immune). The HI titres from the full panel antigenic characterization are uploaded to **IVM Online** and re-analyzed.



- Identified variant strains are then sequenced by sequencing partners (DIC BUKITTINGGI, PUSVETMA, BBLITVET, and BBPMSOH). The results of the HA gene sequencing in the form of a **fasta file** are uploaded to **IVM ONLINE**.

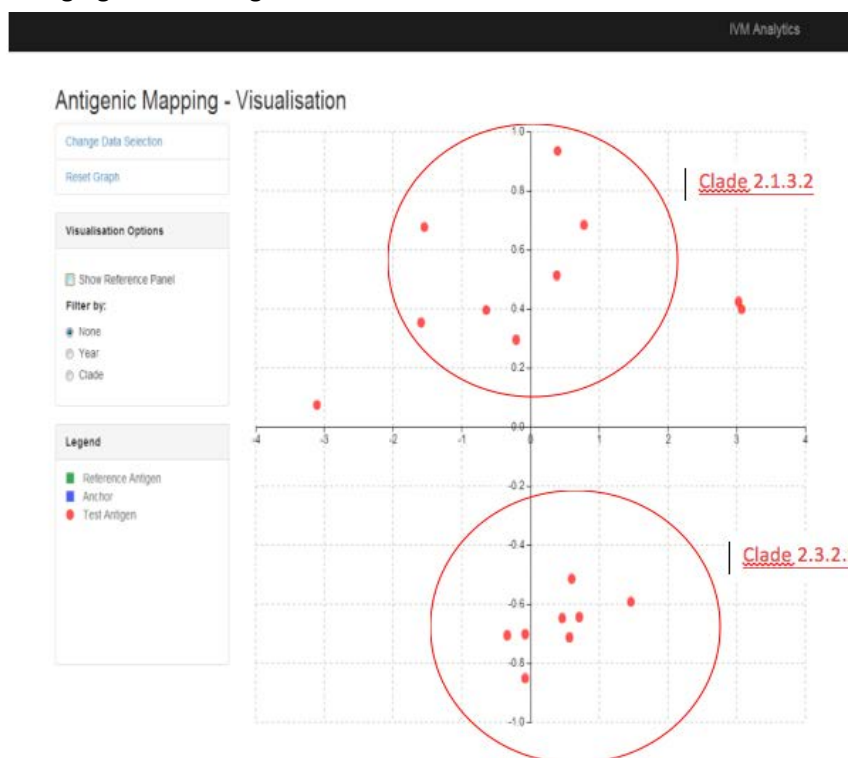


- Bioinformatics analysis of the sequencing and antigenic characterization results is conducted and mapped by the FOCAL POINT, and reported to the **Management Team**.





- If the management team finds the presence of **ANTIGENIC SHIFT** in variant strains, then the management team reviews the data completely, including epidemiological and phylogenetic tree data of the variant strain; if necessary, the management team will immediately **recommend** changing the challenge virus strain to the Director of Animal Health.



- All of the activity flow above can be monitored by the Directorate General of Livestock and Animal Health Services online.



## RESULTS

- IVM ONLINE** is a system to select challenge and vaccine seed strains objectively.
- Faster antigenic and genetic characterization which allows decision making for production of AI vaccine against the new clade (Afluvet) that matches the type of HPAI in circulation.
- Established pre-screening and full panel screening methods using HI protocols with standard panel reagents for antigenic characterization.
- Established a web-based database (**IVM ONLINE**) which contains bioinformatics modules for antigenic and genetic characterization, as well as visualization.
- Update the molecular diagnostic method (PCR) for Type A, H5N1 clade 2.1.3 and 2.3.2.

## IMPACT

- Increases the capabilities of animal health laboratories in Indonesia in performing antigenic and genetic characterization of influenza virus.
- Improves knowledge about circulating AI viruses and informs vaccination policies.
- IVM ONLINE** can provide recommendations for determination of a candidate challenge strain or vaccine seed strain.

- IVM ONLINE** activities conducted by competent officers/staff.
- IVM ONLINE** staff/officers competence is developed through **Competency Based Training**.
- Training is determined and performed both internally by focal points and externally.
- For **QUALITY ASSURANCE** and **QUALITY CONTROL**, the implementation of **IVM ONLINE** activity follows **TECHNICAL GUIDELINES** and **STANDARD OPERATIONAL PROCEDURES – SOP**.
- Meetings between **IVM ONLINE** members are held every year to ensure the continuation of the **IVM ONLINE** system.

## FUTURE PLANS

- Expanding the bioinformatics module within the **IVM Online** system to detect other HPAI viruses such as H7N9, H7N7 etc, related to epidemiological data.
- Developing a linkage mechanism between **IVM Online** and **iSIKHNAS**.
- Expanding the membership of **IVM Online** to include laboratories from universities and the private sector.
- Strengthening the regional and global laboratory network to face the emergence of many different zoonotic pathogens.
- Forming an Influenza Virus Monitoring (IVM) Team determined by the Director General of Livestock and Animal Health Services to ensure the continuation of **IVM Online**.

## PUBLICATION

### A SUSTAINABLE APPROACH TO INFLUENZA VIRUS MONITORING FOR ANIMAL HEALTH IN INDONESIA

**HISTORY** The first outbreak of HPAI H5N1 in Indonesia was reported in Legok, Banten, in 2003. Vaccination using homologous and heterologous strains was implemented with no specific guidelines. In 2007-2008, in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the OIE/FAO Network of Expertise on Animal Influenza (OFFLU), a national animal health influenza virus monitoring system was set up to monitor circulating viruses and identify potential virus variants, to monitor efficacy of vaccines in use and to identify potential candidates for challenge/vaccine strains.

**OUTPUTS** Increased capacity of Indonesian laboratories to diagnose and characterize avian influenza viruses. Network established to monitor circulating influenza viruses, and share biological specimens and data, including data analysis. The Animal Health Influenza Virus Monitoring (IVM) Network established.

Approximately 40 technical officers are engaged long term under the network and attend regular network meetings and receive refresher training and reagents.

Sharing of over 300 virus isolates with international reference laboratories for advanced characterization, and approval in principal granted for release of sequence data to public domain.

**IMPACT** Improved knowledge of circulating H5N1 viruses to directly inform vaccine policy through the characterization and analysis of circulating virus in village-based poultry and sector 3.

Pioneered application of antigenic cartography, a technique to characterize human influenza viruses since 2002, using avian sera.

**SUSTAINABILITY** Establishment of harmonized HI protocol and standardized antigen/antiserum panels across all eight Disease Investigation Centres (DIC) plus the three national sequencing laboratories.

1. All DIC routinely receive and process samples for avian influenza. Only samples for virus isolation will be sented using proper...
- 2a. Routine typing of HA-positive fluids by: 1) HI, 2) RT-PCR, 3) Sequencing 2b. Identify HI positive avian influenza fluid
3. For HI positive isolates DIC use pre-established standard data panel for cartography and submit raw HI data to DIC Wates for analysis
4. Select potential antigenic variants. Home practices data analysis for further testing and request to Forward to DIC Wates
5. DIC Wates to conduct further antigenic analysis to select the candidates for sequencing
6. DIC Wates to submit material for HI gene sequencing to sequencing partners
7. Review of data analysis to ensure challenge strains are adequate to assess vaccine protection
8. Expert group to analyze data and update challenge strains when needed

Disease Investigation Centres INDONESIA

**Development of a data platform (Alat Preskrin)** to facilitate the rapid sharing, analysis, and reporting of results.

**FUTURE** Institutionalize the IVM network laboratories and support additional partner laboratories (e.g. poultry industry and university laboratories).

Develop mechanism to apply data from the field to decision making instruments at the central government level.

POSTER

### Surveilans pada tingkat molekuler: Mengembangkan jaringan yang terintegrasi untuk mendeteksi varian virus flu burung di Indonesia.

Hendra Wibawa<sup>1</sup>, Padjatmoko<sup>2</sup>, Fajri Semping Tjara Kasa<sup>3</sup>, Sri Handayani Irtaningah<sup>4</sup>, Rama Dhanarwan<sup>5</sup>, Muhammad Ashari<sup>6</sup>, Eddy Soewito Soewito<sup>7</sup>, Niang Hartaningah<sup>8</sup>, James McGroun<sup>9</sup>, Frank Wang<sup>9</sup>, Paul Schick<sup>9</sup>, John Allen<sup>9</sup>, Ivano Bilo<sup>9</sup>, Mia Kim Touchette<sup>9</sup>, Gwenevaelle Daughe<sup>9</sup>, Filip Chao<sup>9</sup>, Wiyadi Santianang<sup>9</sup> dan Peter Durr<sup>9</sup>

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**Kata Kunci:** kartografi antigenik, flu burung, bioinformatika, H5N1 HPAI, jaringan laboratorium, OFFLU

**Abstrak**

Sejak 2006, Indonesia telah menggunakan vaksinasi sebagai metode untuk mengendalikan H5N1 HPAI. Dalam kasus vakuis tersebut, virus telah menunjukkan perubahan antigenik secara bertahap, yang menimbulkan ketidakpastian perubahan serotipe untuk vaksin dan antigen untuk diagnosis. Untuk meningkatkan sistem monitoring evolusi virus, Pemerintah Indonesia dengan dukungan dari FAO/OIE/ILU, telah mengembangkan jaringan inovatif dimana tes H5N1 dikarakterisasi dengan antigenik dan genetik. Jaringan surveilans molekuler ("Influenza Virus Monitoring" atau Monitoring Virus H5 Hanyang atau "IVM") dibentuk pada jaringan regional laboratorium kesehatan hewan diagnosis, yang didukung dengan sistem pengolahan data berbasis web ("IVM Online"). Jaringan IVM Indonesia dapat menjadi referensi untuk negara lain yang ingin mengembangkan jaringan laboratorium untuk surveilans flu burung dan unggas lainnya.

**Pendahuluan**

Salah satu H5N1 flu burung dengan patogenitas tinggi (HPAI) menyebar ke Indonesia di 2005, dan pemerintah mengambil kebijakan dengan menggunakan peternakan vaksinasi untuk pencegahan penyakit pada tahun 2004 [1]. Indonesia adalah satu diantara empat negara, selain Cina, Mesir dan Vietnam, yang secara komisioner menggunakan vaksinasi sebagai strategi pengendalian H5N1, dalam 10 tahun terakhir [2].

Seperti telah diketahui bahwa virus H5N1 mengalami evolusi yang cepat dan konstan, melalui proses perubahan genetik (mutasi, drift) dan reassortment (reassortment). Masalah spesifik yang dihadapi oleh negara-negara adalah meningkatkan kemampuan deteksi varian virus saat adanya (tidak baru) telah terdeteksi. Pada praktiknya hal ini akan berakibat pada perubahan kebijakan penggunaan vaksin, yaitu vaksin dan tipe perubahan ini vaksin dari vaksin yang berisi satu antigen ke vaksin yang memiliki 2 atau lebih antigen. Selain itu, prosedur diagnosis kemungkinan juga akan mengalami perubahan.

Gambar 1. Anggotanya jaringan laboratorium dengan pengolahan data untuk masing-masing anggota dalam jaringan monitoring.

**Materi dan Metode**

Pengembangan Sistem Monitoring Virus Influenza (IVM) dimulai pada akhir 2007 dibawah proyek FAO dan Dukungan Jendral Peternakan dan Kesehatan Hewan (DJKPH) Kementerian Pertanian Indonesia. Kegiatan ini merujuk di implementasikan di Indonesia dengan bantuan OFFLU (<http://www.offlu.net>), yang merupakan jaringan para ahli influenza hewan dari FAO dan OIE [3]. Para ahli dari proyek ini berasal dari Australia, Animal Health Laboratory (AHL) atau Laboratorium Kesehatan Hewan Australia, Swedia, Profound Research Laboratory (SPEFL) atau Laboratorium Penelitian Unggas Asia Tenggara dan

PAPER