The Challenge of Laboratory-Based Influenza Surveillance in Indonesia: Cold Chain Application

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ABSTRACT

Since 2006 Indonesia has developed a laboratory-based influenza surveillance system. Confirmation of influenza-like illness (ILI) cases is conducted by PCR examination in regional laboratories and followed by virus isolation on positive PCR samples. The long process ILI samples shipping that in the chain can affect the viability of the virus in the sample to be recovered. The study was aimed to evaluate the initial cold chain application monitoring on ILI specimen shipment and to identify the influencing factors. This cross-sectional study found that in 2014 there were 232 sample shipments from 25 provinces. There were 54% shipment from health centers and only 12% shipment from regional laboratories with temperatures meet 2-8oC delivery standard that contributed to the low number of virus isolates. It also found that some samples were damaged, missing, broken thermometer, poor timeliness, however, the recording forms were not filled properly. The application of cold chain principles to ILI sample shipment in influenza surveillance has various challenges from a less thorough officer such as in incomplete cases or improper packaging and shipping methods. It is necessary to increase understanding of the principles of cold chain application in surveillance shipment to reduce human error and to increase timeliness.

Keywords: cold chain, influenza, surveillance

1. INTRODUCTION

Recently, concerns about detecting and responding to an influenza pandemic have increased in many countries in the world. Thus, influenza surveillance being expanded globally including in Indonesia. Many surveillance in the world sites located in a remote area with a lack of diagnostic capacity, requiring samples to be transported far distance to a central laboratory [1]. Indonesia is an archipelago country that consists of thousand islands spread alongside the equator line. Hence transporting biological specimen become very challenging. National Institute for Health Research and Development (NIHRD) has been designated as National Influenza Center (NIC) since 1976. NIC's form the backbone of the WHO's Global Influenza Surveillance and Response System (GISRS). As a NIC, NIHRD has an obligation to collect virus specimens in the country and perform preliminary analysis. We ship representative influenza viruses to WHO CCs for advanced antigenic and genetic analysis. The results form the basis for WHO recommendations on the composition of influenza vaccine each year, as well as relevant risk assessment activities of WHO. To fulfill this task, NIHRD develops an Influenza-Like Illness (ILI) surveillance system in several sentinels throughout Indonesia with selected regional laboratories to perform the preliminary analysis.

In 2014, there were 26 health center sentinels with 6 regional laboratories participated in the ILI surveillance system. The NIHRD serves as a referral laboratory. This is the first year of general cold chain application with recording form in ILI specimen shipment. At the end of 2013, the surveillance team from NIHRD in collaboration with CDC and USAID began to socialize records on specimen transportation for cold chain monitoring.

Every day, the laboratory technician collected the throat and/or nasal swab from ILI patients. Specimens placed into 1.5mL sterile viral transport media and kept refrigerated (2-8°C) prior to shipping. Every Monday the nurse coordinating with district health officer and cargo to pick the specimens for shipping the specimens to the regional laboratory. The specimen should have arrived at the regional laboratory within 24 hours. The regional laboratory will use 140µL of the specimens and perform real-time Polymerase Chain Reaction (PCR) to detect the subtype of influenza virus circulating. The remaining specimens will be shipped to NIHRD to undergo virus isolation, retaining viable influenza viruses for further antigenic and genetic analysis

Regardless of the detection methods chosen, accurate test results rely on pre-analytical steps, including specimen transport and processing [2]. An adequate biological specimen requires a sufficient amount, a suitable container, well- identified and transported to maintain the integrity and the stability of the material

to be investigated. Obtaining an adequate biological specimen is needed to optimize the validity of the test performed [3]. Many variables can influence the specimen's integrity during the logistic process, including temperature, packaging, courier, specimen's type, export/import requirement, season, cost and transit time/ship days [4].

As mention above, ILI surveillance in Indonesia is conducted in sentinels that are far from the laboratory that processes the specimens and timely transport of specimens is often challenging. While the specimens were temperature- sensitive, the need to monitor the temperature during transport becomes very critical. The World Health Organization (WHO) and United States (US) Center for Disease Control and Prevention (CDC) recommend that a sample should be stored for four days or less at $4^{O}C$ before freezing or diagnostic testing [1]. A study in Kenya found that samples could remain in storage for at least five days without affecting the proportion-positive of samples detected by real-time PCR method for influenza A and B [1]. In virus isolation purposes, specimens should be transported to the laboratory from the collection sites with coolant to maintain refrigerated temperature of approximately $2\text{-}8^{O}C$. Specimens should not be frozen except for those sent from remote locations [5]. Temperature is one of the most important factors affecting virus survival, as it can affect the state of viral protein and the virus genome. The influenza virus is an RNA virus which generally unstable compared to other DNA containing viruses. Generally, as the temperature rises, virus survival decreases [6]. To keep the temperature monitored during transport, each shipment of ILI specimens equipped with shipment monitoring form that contains some information such as shipment temperature (from health centers and regional laboratories), arrival temperature (at regional laboratories and NIHRD), availability of thermometer, number of cold packs used, date of specimens shipment and arrival. This study was aimed to evaluate the initial cold chain application monitoring on ILI specimen shipment and to identify the influencing factors.

2. METHOD

In a cross-sectional study, the data were extracted from 232 shipment monitoring forms from 26 sentinels in 25 provinces between January 2014 and September 2014. The data consist of several parameters such as shipment temperature (^{O}C), number of icepacks used (unit), the availability of analogue thermometer and day of shipment from the health center to NIHRD. The parameters were compared based on the origin and destination of the ILI specimens: health center to regional laboratory and regional laboratory to NIHRD. Additionally, the duration of specimen shipment from the health center to NIHRD were also analyzed.

Three criteria for each parameter were defined. For shipment temperature: acceptable as the temperature at range $2\text{-}8^{O}C$, not acceptable as temperature <2 and >8oC and unknown (no data recorded). For the number of icepacks used: 10-12 pieces, <10 pieces and unknown. For availability of thermometer: yes, no and unknown.

A description of the shipment duration of ILI specimens from the health center to NIHRD was correlated with the success of influenza viral isolation. The shipment duration parameter consists of two criteria: ≤ 3 days which acceptable a n d >3days t h a t not acceptable f o r virus isolation [7].

3. RESULTS AND DISCUSSION

The distribution of ILI specimen shipment listed in Table 1 was not equal in each health center. Most shipment comes from Denpasar Selatan I, Bali and least comes from Kayon, Palangkaraya. Many of the monitoring form did not have a record of the specimen ID sent.

Based on the analysis of shipment monitoring form, the temperature status during shipment from 26 health centers to regional laboratories is shown in Table 2. We can see that the percentage of the shipment arriving at the regional laboratory with an acceptable range of temperature is declining from 54% (125/232) when shipped from health centers to 12% (27/232) when the package arrived at the regional laboratories. Figure 1 also shows that 38% (89/232) shipment from health centers had no temperature data and the temperature that was not acceptable increased by the time the shipment came to the regional laboratory.

Table 1. The Number of Shipment from Health Centers

No	Health Center	Number of shipment (%) N=232
1	Waihaong, Ambon	14 (6.0)
2	Klandasan Ilir,Balikpapan	3 (1.3)
3	Banda Raya, Banda Aceh	3 (1.3)
4	Padasuka, Bandung	4 (1.7)
5	Pemali, Bangka Belitung	20 (8.6)
6	Pekauman, Banjarmasin	8 (3.4)
7	Batu Aji, Batam	3 (1.3)
8	Sukamerindu, Bengkulu	5 (2.2)
9	Denpasar Selatan I, Denpasar	22 (9.5)
10	Sungai Duren, Jambi	16 (6.9)
11	Jayapura Utara, Papua	15 (6.5)
12	Sikumana, Kupang	3 (1.3)
13	Sumur Batu, Lampung	5 (2.2)
14	Sudiang, Makassar	20 (8.6)
15	Dinoyo, Malang	19 (8.2)
16	Tampa Padang, Mamuju	17 (7.3)
17	Karang Taliwang, Mataram	9 (3.9)
18	Teladan, Medan	9 (3.9)
19	Mopah, Merauke	2 (0.9)
20	Lubuk Buaya, Padang	2 (0.9)
21	Kayon, Palangkaraya	1 (0.4)
22	Tujuh Ulu, Palembang	3 (1.3)
23	Birobuli, Palu	6 (2.6)
24	Rumbai, Pekanbaru	3 (1.3)
25	Pandanaran, Semarang	6 (2.6)
26	Kotagede I, Yogyakarta	14 (6.0)

About 84% (194/232) shipment missing temperature record (unknown) during shipment due to incomplete record by the regional laboratory staff. As much as 44% (102/232) shipment arrived at NIHRD in acceptable temperature and 28% (64/232) shipment missing temperature record.

Table 2. Temperature Status during Shipment from Health Centers to Regional Laboratories and NIHRD

	Acceptable (%)	Not acceptable	Unknown (%)
Shipment from health center	54	8	38
Arrival at regional laboratory	12	67	21
Shipment from regional laboratory	14	2	84
Arrival at NIHRD	44	28	28

Each specimen shipment package should be set up with 10-12 pieces of icepacks to maintain the temperature during shipment. Table 3 shows that most shipment (59.5%) only have <10 piece icepacks in its package. While 37.1% (86/232) of it remains no record. We found that, when the package arrives at the regional laboratories, no shipment has 10-12 piece icepacks and most shipment have blank information on icepack status.

Most shipment comes to NIHRD with <10 pieces of icepacks (51,3%). Only 3.4% (8/232) meeting the recommended criteria, and the rest shipment were missing data on icepacks status. The highest unknown status of icepack numbers was found in the regional laboratories whether when it arrived from health centers or when it sent to NIHRD.

The surveillance system supplies the health centers with a thermometer to monitor the temperature of each shipping. However, some shipments still not include the thermometer in its package. The unknown thermometer status also increased in the regional laboratory (Table 4).

Table 3. Ice Packs Status in Shipment from Health Centers to Regional Laboratories and NIHRD

Ice Pack Status	10-12 (%)	< 10 (%)	Unknown (%)
Shipment from health center	3.4	59.5	37.1
Arrival at regional	0.0	12.9	87.1
Shipment from regional laboratory	1.3	29.7	69.0
Arrival at NIHRD	3.4	51.3	45.3

Table 4. Thermometer Status in Shipment from Health Centers to Regional Laboratories and NIHRD

Thermometer Use	Yes (%)	No (%)	Unknown (%)
Shipment from health	63,4	8,6	28,0
Arrival at regional laboratory	31,5	6,0	62,5
Shipment from regional	15,5	3,9	80,6
Arrival at NIHRD	31,5	19,8	48,7

Only 32 of 232 (14%) shipments had duration shipment 0-3 days, and 53% were shipped more than 3 days. Many shipments (33%) were missing data on the date when shipping to the regional laboratories and arriving at NIHRD.

The virus isolation was conducted for 500 specimens with positive influenza result that has been testing before by PCR. The numbers of the influenza viruses that can be isolated were 2 influenza viruses H1N1pdm09 that came from Mataram, and Lampung and 6 influenza B viruses that came from Makassar, Ambon, Padang, Palangkaraya, Denpasar, and Semarang (Table 5).

Table 5. List of Virus Isolate From Indonesia ILI Surveillance In 2014

No	Origin	ID	Shipment duration (health centers to NIHRD)	Influenza Type
1	Waihaong, Ambon	AMB 0232	2	B
2	Denpasar Selatan I, Denpasar	DPS 1144	5	B
3	Sumur Batu, Lampung	LPG 131	NA	A
4	Lubuk Buaya, Padang	PDG 0138	NA	B
5	Sudiang, Makassar	MKS 1084	NA	B
6	Pandanaran, Semarang	SMG 1344	11	B
7	Kayon, Palangkaraya	PLK 0198	2	B
8	Karang Taliwang, Mataram	MTR 1226	NA	A

Based on the shipment monitoring form, we found that most data is unknown. It means that the health center, regional laboratories, and NIHRD staff did not record the shipment monitoring form completely. The regional laboratory staff seems to have a good understanding of the cold chain seen from the shipment practice; however, they have the highest rate of unknown status because mostly they did not record what they did.

Ideally, the health center ships the specimens every week. In our findings, the number of shipments from the health center varies from 1 to 22. In this case, most of the health centers did not send the ILI specimens every week. This situation can indicate that there were no ILI cases in the health center that week and thus the shipment decreased.

On the other hand, the data is very useful for tracking the shipment and quality of the specimen. Whether the specimen still adequate for the testing or not. Refrigerated shipments can be protected by the use of material that can be frozen or refrigerated before shipment and this may vary according to the season as well.4 In this case, we recommend sentinel to use 10-12 pieces of icepacks for each shipment.

Seasonal differences between summer or winter can also negatively affect sample integrity. During the transport, process specimens may undergo extreme heat or cold conditions.4 Indonesia is a warm tropical country. This could be a challenging issue in transporting refrigerated specimens. The application of cold chain principles shipment in influenza surveillance has various challenges from a less thorough officer such as in incomplete cases or improper packaging and shipping methods. All the

staffs should be reminded about the important of filling the shipping form in complete.

Duration time the specimen from the health center to NIHRD is varied depending on how long the transit time in the regional laboratory and the distance of health center to NIHRD. Some of the health centers had a duration time for the shipment of 0-3 days. For these shipments, the specimens did not transit in the regional laboratory because some of the health centers directly shipping the specimens to NIHRD for PCR testing. The other health center shipping the specimen to the regional laboratory, then regional laboratory sent to NIHRD. A plan to minimized transit times of the shipment is important. With a standard courier, it is necessary to avoid weekend shipments and to consider local and national holidays also a proof of delivery [4].

The successful diagnosis of viral infections by culture is enhanced when the specimen contains as much virus as possible upon collection, is protected from thermal inactivation, and is contained in an effective transport system [5,8]. NIHRD received 2497 specimens from ILI surveillance in 2014. There are 57% positive for H3N2, 6% for H1N1pdm09 and 37% for influenza B. Only 1.6% specimens successfully isolated from 500 specimens with influenza- positive results by PCR.

In the same time, WHO monitored a change in the proportion of the subtype that circulating in the northern and southern hemispheres. The H3N2 3C.3a subclade switched to H3N2 3C.2a subclade and they increased in number. The H3N2 3C.2a subclade has some antigenic properties difference from the H3N2 3C.3a subclade. Hemagglutinin (HA) is known to be responsible in the process of attachment to sialic acid on the host cell. Neuraminidase (NA) is liable for removing the terminal sialic acid and destroying receptor for HA. It also accountable for inactivating the inhibitory molecules in the respiratory tract and promote the release of new virus [9].

The dominance of H3N2 3C.2a subclade that circulated in Indonesia made the virus isolation more challenging. In 2014, NIHRD only had regular MDCK to host the virus rather than MDCK SIAT cell lines that known suitable for this clade. The small proportion of shipments duration that ≤ 3 days was also affecting the successful rate of the virus isolation. According to the WHO, specimens that cannot be isolated in
2 days should be kept frozen [5]. In the fact, there was no specimens shipment conducted in frozen.

It is highly desirable to perform viral diagnostic tests within a relatively short time after the collection of clinical specimens. However, in the conduct of daily virological surveillance on a community-wide level, this is not practical because of operational and economic considerations. Based on the results of the virus survival experiment, sampling of mild or asymptomatic cases of influenza might require inoculation of specimens within 48 h after collection or storage at -70°C until diagnostic tests can be performed to overcome the loss of infectivity [10].

4. CONCLUSION

The application of cold chain principles to ILI sample shipment in influenza surveillance has various challenges from a less thorough officer such as in incomplete cases or improper packaging and shipping

methods. Besides, Indonesia's geographical factors also pose challenges such as long-distance sentinels from the airport. Based on the results of the analysis above, it is necessary to increase understanding of the principles of cold chain application in surveillance shipment to reduce human error and to increase timeliness by conducting refresher training.

AUTHOR CONTRIBUTIONS
All authors contributed equally as the main author.

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