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Technology transfer of an oil-in-water vaccine-adjuvant for strengthening pandemic influenza preparedness in Indonesia

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ABSTRACT

With the current enzootic circulation of highly pathogenic avian influenza viruses, the ability to increase global pandemic influenza vaccine production capacity is of paramount importance. This has been highlighted by, and is one of the main pillars of, the WHO Global Action Plan for Influenza Vaccines (GAP). Such capacity expansion is especially relevant in developing countries. The Vaccine Formulation Laboratory at University of Lausanne is engaged in the technology transfer of an antigen-sparing oil-in-water adjuvant in order to empower developing countries vaccine manufacturers to increase pandemic influenza vaccine capacity. In a one-year project funded by United States Department of Health and Human Services, the Vaccine Formulation Laboratory transferred the process know-how and associated equipment for the pilot-scale manufacturing of an oil-in-water adjuvant to Bio Farma, Indonesia's state-owned vaccine manufacturer, for subsequent formulation with H5N1 pandemic influenza vaccines. This paper describes the experience acquired and lessons learnt from this technology transfer project.

1. Introduction

In January 2010 the Vaccine Formulation Laboratory (VFL) was established at the University of Lausanne (UNIL), Switzerland. The VFL is located at UNIL Department of Biochemistry, a World Health Organization (WHO) Collaborating Centre in Immunology Research and Training [1]. WHO promoted the creation of the VFL to assist the vaccine community with gaining wider access to adjuvant technologies and vaccine formulation know-how [1]. On a not-for-profit basis the VFL provides customized adjuvant support to numerous groups worldwide, including academic researchers, biotechnology companies, and vaccine manufacturers. The VFL's facilities include platforms for training, formulation, quality control (QC), and pre-clinical evaluation of adjuvanted vaccines. In support of the WHO Global Action Plan for Influenza Vaccines (GAP) [2], the VFL transfers to influenza vaccine manufacturers, particularly those in developing countries, the technology for the manufacture of oil-in-water adjuvants at laboratory- and pilot-scale. Such adjuvants have been demonstrated to enable antigen-sparing when formulated with pandemic influenza vaccines. Of note, increasing the ability of any country to make influenza vaccines helps every country reduce the spread of influenza since in a pandemic everyone in the world would need a vaccine to be protected. Today, the capability to make vaccine in every country does not exist.

One partner in the VFL's technology transfer programme is PT Bio Farma (Persero), an Indonesian state-owned enterprise that is operated independently of their government. As the only manufacturer of human vaccines in Indonesia, Bio Farma dedicates its material resources and 880 staff to producing vaccines and antisera for both domestic and global markets. Bio Farma's facilities for production, research and development, marketing, and administration are based in Bandung, West Java. It is one of the vaccine manufacturers in a developing country that satisfies WHO pre-qualification criteria and Bio Farma's seasonal influenza vaccine FluBio™ became the first licensed product resulting from the WHO-led influenza vaccine technology transfer initiative in June 2009 [3]. The company produces a wide range of viral and bacterial vaccines and antisera, and manufactures more than one billion vaccine doses annually.

The acquisition of a dose-sparing adjuvant for use with pandemic H5N1 influenza vaccines would be highly beneficial for Indonesia, given the continuing local circulation of H5N1 avian influenza, a population of more than 200 million people, several

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cases of possible human-to-human transmission of H5N1, and a cumulative total of 156 confirmed human deaths (out of 188 cases) from H5N1 influenza in the country since 2005 [4]. Such an adjuvant technology would enable Bio Farma to increase the local pandemic influenza vaccine capacity (currently estimated to be a maximum of 20 million doses annually) and would also provide additional pandemic vaccine capacity at a regional and global level.

In October 2010 the United States Department of Health and Human Services, Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority (HHS-ASPR-BARDA) selected the VFL to transfer the process know-how and equipment to Bio Farma for local manufacturing of an oil-in-water adjuvant at pilot-scale, in order to formulate Bio Farma's pandemic influenza candidate vaccines.

2. Adjuvant technology

Squalene-based oil-in-water adjuvants have demonstrated significant antigen-sparing capabilities when formulated with H5N1 influenza vaccines. For split and subunit H5N1 influenza vaccines, a 90 μg (haemagglutinin content) dose is required to elicit an adequate immune response that meets registration criteria [5]. Following formulation with an oil-in-water adjuvant, this dose can be reduced to between 7.5 and 3.75 μg, whilst still providing an adequate immune response [6,7]. As a consequence, the antigen-sparing capabilities of an oil-in-water adjuvant can increase existing vaccine production capacity significantly. In contrast, aluminium-based adjuvants have not demonstrated such reliable dose-sparing. Oil-in-water emulsions have also been demonstrated to increase and broaden immunogenicity to influenza vaccines [8–10].

Squalene-based oil-in-water adjuvants share common mechanisms of action, but their biological behavior can vary based on their chemical composition and the nature of their excipients [11]. The technology selected for transfer to Bio Farma was an emulsion of similar composition to the MF59® adjuvant (Novartis Vaccine and Diagnostics). This adjuvant comprises a metabolizable oil (squalene 3.9%, w/v), sorbitan trioleate (0.47%, w/v), and polyoxyethylene (80) sorbitan monooleate (0.47%, w/v) dispersed in 10 mM citrate buffer at pH 6.5.

After aluminum salt adjuvants, which have been administered to billions of people, this type of oil-in-water adjuvant is one of the most mature adjuvant technologies currently available. For example, in 1997 a seasonal influenza vaccine containing MF59® (FLUAD®) was approved in Europe and in 2009 a pandemic (H1N1) influenza vaccine formulated with MF59® (Focetria®) was similarly approved. To date, this adjuvant has been administered to more than 80 million people, including children and the elderly, and no increased risk of various adverse events has been reported with MF59-adjuvanted vaccines by analyses of clinical trials and pharmacovigilance databases [12].

The process for manufacturing the transferred oil-in-water adjuvant technology at pilot-scale has already been published [13]. Briefly, an aqueous phase containing polyoxyethylene sorbitan monooleate in citrate buffer at pH 6.5 is added to the oil phase, containing squalene and sorbitan trioleate. The mixture is homogenized with a high-shear mixer (Silverson L5M-A) at 8000 rpm for 2–6 min and immediately microfluidized (Microfluidics M-110EH) with 5–7 passes at 20,000 psi. The final emulsion is sterilized by filtration. QC of the final emulsion consists of assessing visual appearance, measuring pH, determining particle size by dynamic light scattering, and determining squalene concentration by reversed-phase high-pressure liquid chromatography (RP-HPLC). Additional tests on final material include measuring endotoxin content and sterility.

3. Technology transfer process

The VFL provides technology transfer based on the needs of the recipient: classroom and/or laboratory training in laboratory or pilot-scale adjuvant manufacturing and QC, coordination of installation of on-site laboratory or pilot-scale manufacturing capacity, and coordination of installation of QC capacity. Bio Farma requested the transfer of pilot-scale capacity with the associated current good manufacturing practices (cGMP)-compatible manufacturing and QC equipment.

3.1. Customs issues

The handling of customs issues provided complex challenges to the project. For example, an entire HPLC shipment was delayed in customs for several months due to the absence of a valid communication's permit for a single network card that had been installed in the accompanying PC. However, the risk of delayed delivery was mitigated by the expertise of Bio Farma's procurement department. Following initial shipping problems, packing lists, invoices, and air waybills were all reviewed and approved by Bio Farma's procurement department before shipment was initiated.

3.2. Utility requirements and local compatibility

It was of critical importance that the transferred equipment was able to operate safely and effectively at Bio Farma's facility. A detailed description of electricity, clean water, chilled water and compressed air supplies on Bio Farma's site was prepared before selecting and procuring equipment. Following review of these utilities, the chilled-water temperature was too high for effective cooling of the microfluidizer, so the supply from the local water system was replaced by a dedicated recirculating cooling unit that provided a low-temperature and pressure-stable source of coolant.

3.3. Building requirements

Particular attention was paid to pharmaceutical clean room classifications, equipment location for ease of access (for cleaning and decontamination) and to the maximum weight that could be supported by floors and benches.

3.4. Installation of items at Bio Farma

The expertise of Bio Farma's engineering department greatly facilitated equipment set-up and connection to local utilities, particularly when equipment was shipped in multiple parts and when custom-made vessels and connectors were required. Where possible, a representative of the manufacturer was commissioned to travel to Bio Farma to install and qualify equipment. Process qualification of equipment shipped to Bio Farma was performed by Bio Farma staff.

3.5. cGMP support

In order to bring a pilot-scale manufacturing process to Bio Farma, a variety of regulations were taken into account (e.g. quality and safety guidelines) and close attention was paid to cGMP guidelines from the start of the project. cGMP support activities established a project quality documentation system in line with Bio Farma's own quality assurance system. This primarily involved the preparation and tracking of numerous documents in a document

Table 1
Comparison of QC results for Bio Farma-manufactured adjuvant batches analyzed at both Bio Farma (BF) and the Vaccine Formulation Laboratory (VFL).

Batch, QC site	Visual appearance	Squalene conc. (39 mg/mL ± 10%)	pH	Mean diameter (nm)
BF1, Bio Farma	Homogenous milky-white emulsion	Pass	6.49	144.0 ± 1.8
BF1, VFL	Homogenous milky-white emulsion	Pass	6.51	142.5 ± 0.9
BF2, Bio Farma	Homogenous milky-white emulsion	Pass	6.49	140.8 ± 1.7
BF2, VFL	Homogenous milky-white emulsion	Pass	6.47	141.0 ± 1.3
BF3, Bio Farma	Homogenous milky-white emulsion	Pass	6.50	145.4 ± 0.4
BF3, VFL	Homogenous milky-white emulsion	Pass	6.45	142.2 ± 1.4

matrix (e.g. user requirement specifications, design, installation operation and process qualification documents, validation master plan, etc.) to support cGMP and to ensure correct operation of the equipment.

3.6. Training

In the first part of the project, a training workshop for Bio Farma staff was held at the VFL in Lausanne. The aim of this five-day workshop was to introduce Bio Farma staff to the oil-in-water adjuvant preparation process, QC methods, and to discuss these in relation to cGMP. The main topics of the workshop consisted of an introduction to the manufacturing process (including preparation of reagents), equipment (homogenizer and microfluidizer operation, filtration procedure), QC (visual appearance, pH, dynamic light scattering, HPLC analysis) as well as quality assurance and cGMP aspects. After theoretical training via classroom-based lectures, and a practical demonstration of the process and assays, the trainees experienced hands-on preparation and QC testing of two oil-in-water adjuvant batches under close supervision of the trainers. Several months after the training workshop, and following installation of the adjuvant manufacturing and QC facilities at Bio Farma, several site visits were conducted by VFL staff to ensure that the transferred equipment was functioning correctly, and to provide ongoing technical support.

3.7. Translation of protocols

In order to ensure that the transferred manufacturing and QC protocols were useable on a day-to-day basis at Bio Farma, the protocols were first translated from English into Indonesian, and then translated back into English. VFL staff then reviewed the "re-translation" in English in order to assess the accuracy of the initial translation. The re-translation was found to be of a good standard and required only minor amendment.

4. Results

4.1. Autonomous manufacturing of three adjuvant batches at Bio Farma

In order to test whether the technology transfer to Bio Farma had been successful, three batches of emulsion (non-GMP) were autonomously prepared by Bio Farma staff and tested onsite to confirm that manufacturing was of an adequate standard to reach the pre-agreed specifications. QC testing of the same three batches of oil-in-water adjuvant was then repeated at the VFL (Table 1). The duplicate QC analyses indicated that the three batches were within the agreed specifications (Table 2), thus demonstrating that the technology transfer had achieved its initial objectives. Examples of QC results obtained by particle size measurement and RP-HPLC are shown in Figs. 1 and 2, respectively.

4.2. Antigen-adjuvant compatibility

Following the independent manufacture and QC of Bio Farma adjuvant batches, the stability of influenza antigens (whole virus and split H5N1 A/turkey/Turkey/1/2005) formulated with the transferred oil-in-water adjuvant was assessed. Following incubation of the antigen/adjuvant formulation at 25 °C for 24 h and for 2 weeks, single radial immunodiffusion (SRID) was used to assess haemaglutinin (HA) antigen integrity and dynamic light scattering was used to measure any changes in adjuvant particle size. No significant differences in particle size or HA antigen content were observed (Table 3) for either whole or split H5N1 adjuvanted vaccines up to the 2-week timepoint, demonstrating compatibility of Bio Farma's oil-in-water adjuvant with H5N1 influenza antigens.

4.3. Preparation of a preclinical plan

Preparation of a detailed preclinical plan describing the activities and aims of the next steps (second year) of the project involved acquiring input from a wide variety of stakeholders. As established guidelines regarding the preclinical testing of vaccine adjuvants were not available, the details of the preclinical plan were also discussed with a wide range of experts in adjuvants, influenza, animal models, vaccine formulation, immunology, and with regulatory experts from WHO and FDA.

5. Lessons learned

5.1. Regional availability of equipment/supplies/facilities

Several options existed for the acquisition of equipment and raw materials depending on the item in question, usually falling into one of three categories:

- The recipient had an adequate local infrastructure that could acquire the specified item.
- The recipient had a local infrastructure, but this was unable to reliably acquire the item (usually with elevated pricing and extended delivery times).
- The recipient had no local infrastructure capable of acquiring the specified item.

Whenever possible, and with time permitting, local agents were the preferred choice as they usually had effective shipping channels established, as well as an existing relationship with Bio Farma. Where this was not possible, items were purchased by the VFL and shipped directly from Switzerland to the recipient. This negated the need for using local suppliers but occasionally presented additional complexities regarding importation (which were the responsibility of the VFL and Bio Farma).

Table 2
Release assays and specifications for the oil-in-water adjuvant.

Release assay	Specification
Visual appearance	Homogeneous milky-white emulsion
pH	6.4–6.6
Mean particle size (nm)	120–160
Squalene concentration (mg/mL)	39.0 ± 3.9

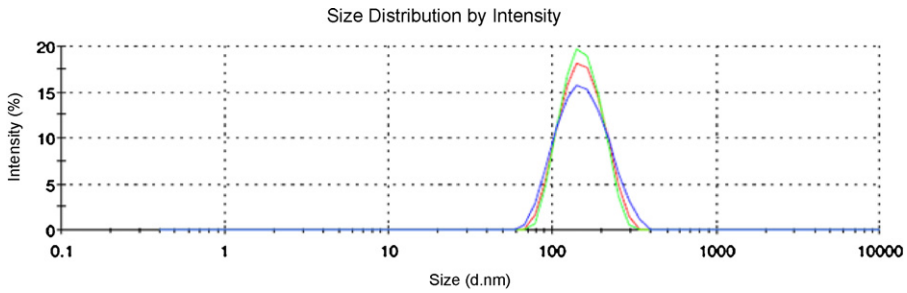

Fig. 1. Particle size measurement of Bio Farma's oil-in-water adjuvant. Average particle size within the emulsion as determined by dynamic light scattering (triplicate measurements of the same sample shown), indicating an average particle diameter of approximately 140 nm.

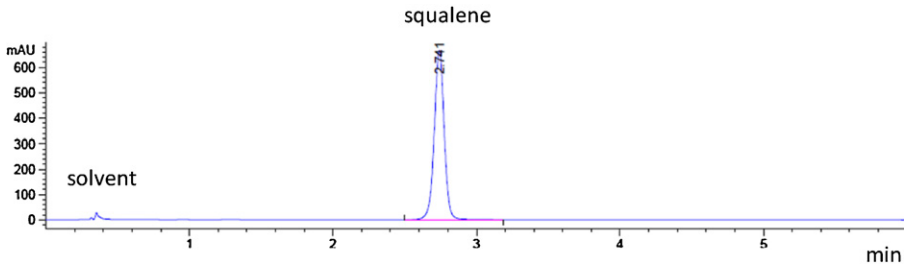

Fig. 2. RP-HPLC chromatogram of squalene in Bio Farma's oil-in-water adjuvant. RP-HPLC analysis is used to determine the squalene concentration in the emulsion; the area of the peak being proportional to the squalene concentration in conjunction with a known set of squalene standards.

Table 3
Antigen/adjuvant compatibility as determined by dynamic light scattering and SRID.

Sample	Particle size (nm)			HA concentration (μg/ml)		
	$T=0$	$T=24$ h	$T=14$ days	$T=0$	$T=24$ h	$T=14$ days
Whole H5N1	NA	NA	NA	34.7 ± 2.2	32.2 ± 2.2	31.4 ± 3.0
Whole H5N1 + BF1	139.9	142.0	143.9	33.9 ± 2.1	32.5 ± 2.4	32.7 ± 3.2
Whole H5N1 + BF2	140.9	140.8	141.4	35.1 ± 2.2	32.2 ± 2.5	33.8 ± 3.4
Whole H5N1 + BF3	141.7	143.2	141.5	31.1 ± 2.2	29.8 ± 2.4	30.6+/_3.1
Split H5N1	NA	NA	NA	29.0 ± 1.3	26.8 ± 3.9	27.7 ± 3.0
Split H5N1 + BF1	141.1	141.1	140.9	29.3 ± 1.3	26.8 ± 3.9	28.0 ± 3.1
Split H5N1 + BF2	140.9	140.9	140.6	29.2 ± 1.3	27.4 ± 4.1	28.6 ± 3.1
Split H5N1 + BF3	142.5	143.1	143.4	27.5 ± 1.3	27.7 ± 4.1	28.2 ± 3.1
PBS + BF1	140.5	140.3	141.2	NA	NA	NA
PBS + BF2	140.0	141.0	140.4	NA	NA	NA
PBS + BF3	141.2	142.1	140.5	NA	NA	NA

5.2. Equipment

The setting up of equipment by qualified engineers, either from the manufacturer or from Bio Farma, ensured that the likelihood of encountering technical issues was minimized. Following installation of the equipment, staff from the VFL visited Bio Farma on several occasions to review the equipment, and the manufacturing and QC process on site. Although operation of the manufacturing equipment was relatively straightforward, setup and operation of the QC equipment was somewhat complex, especially the HPLC system. This was aided by the presence of a highly competent, local HPLC technician but regular visits by VFL experts allowed optimization of the HPLC operation as well as informal onsite instruction in using the complex HPLC software.

6. Conclusions

Successful completion of the project demonstrated the VFL's ability to coordinate the transfer of pilot-scale manufacturing and QC capacity of an oil-in-water adjuvant to a vaccine manufacturer, as well as Bio Farma's ability to assimilate and operate the transferred technology on-site. The selection, ordering, transport, installation, and operation of specialized equipment presented unique project management challenges. Central to overcoming these challenges was a multi-disciplinary team and dedicated project management staff in all parties involved in the technology transfer. Project members who were experienced in managing international projects and with detailed knowledge of equipment, cGMP, and technology transfer greatly increased the chances of successful transfer. Effective dissemination of project information as well as joint, informed decisions to ensure that problems were minimized (and that efficiency was maximized) were central to the project's success. All of the above factors were synergistic in allowing the project to be completed rapidly in nine months. The transfer of this adjuvant technology puts Bio Farma in a position to evaluate and develop oil-in-water adjuvanted pandemic influenza vaccines, and brings Indonesia one step closer towards pandemic preparedness.

Following this successful technology transfer, and in line with the WHO concept of technology transfer hubs to serve multiple technology recipients [14], the VFL is currently working with numerous vaccine manufacturers in developing countries to assist their acquisition of modern adjuvant technologies and know-how.

Conflict of interest statement

The authors state they have no conflict of interest.

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