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An international technology platform for influenza vaccines

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ABSTRACT

Since 2008, the World Health Organization has provided seed grants to 11 manufacturers in low- and middle-income countries to establish or improve their pandemic influenza vaccine production capacity. To facilitate this ambitious project, an influenza vaccine technology platform (or "hub") was established at the Netherlands Vaccine Institute for training and technology transfer to developing countries. During its first two years of operation, a robust and transferable monovalent pilot process for egg-based inactivated whole virus influenza A vaccine production was established under international Good Manufacturing Practice standards, as well as in-process and release assays. A course curriculum was designed, including a two-volume practical handbook on production and quality control. Four generic hands-on training courses were successfully realized for over 40 employees from 15 developing country manufacturers. Planned extensions to the curriculum include cell-culture based technology for viral vaccine production, split virion influenza production, and generic adjuvant formulation. We conclude that technology transfer through the hub model works well, significantly builds vaccine manufacturing capacity in developing countries, and thereby increases global and equitable access to vaccines of high public health relevance.

1. Introduction

Until recently, international efforts to boost capacity in low- and middle-income countries along the vaccinology value chain have been limited to quality control, regulatory support and clinical trials. The direct transfer of knowledge and technology for vaccine manufacturing itself has received very little attention. This trend mirrors a decline in the number of domestic and regional vaccine manufacturers in all parts of the world.

The (re)emergence of infectious diseases such as highly pathogenic avian influenza changed this picture. Governments saw investment in health security and pandemic influenza preparedness to be of increasing strategic importance. In several countries, this has resulted in significant national investment in manufacturing capacity. At the global level, the threat of an influenza pandemic has led to an acknowledged need for technical know-how and vaccine production capacity in developing countries.

In 2006, in response to the human-to-human transmission of A(H5N1), the World Health Organization (WHO) took steps to enhance global access to influenza vaccine as part of its Global Pandemic Influenza Action Plan [1]. This included a pioneering project to strengthen the capacity of developing countries to produce influenza vaccine. WHO has to date provided seed grants for this purpose to 11 manufacturers that belong to the Developing Countries Vaccine Manufacturers Network (DCVMN), a voluntary, public health driven network supported by international organizations and vaccinology resource institutions such as the Netherlands Vaccine Institute (NVI) [2–4]. As the national vaccine agency of the Ministry of Health, NVI is tasked with the supply of vaccines for the Netherlands Immunization Programme, either through production or procurement. Over the last decades, NVI has carried out a number of technology transfer projects to developing country manufacturers in various settings (Table 1) [3,5].

2. "Hub-based" transfer of technical know-how

In early 2007, to address numerous requests from countries for support to their pandemic influenza vaccine production capacity, WHO developed the concept of a centralized technology and training platform (a "hub"). The objective of the hub was to pool public sector knowledge and expertise on a generic pilot process for influenza vaccine production that could be transferred to and easily scaled up in developing countries. Following a transparent bidding process, WHO selected NVI to fulfil this role, and an International Technology Platform for Influenza Vaccines was thus created in Bilthoven, the Netherlands [6]. A collaborative agreement between WHO and NVI was signed with the aim to establish an egg-based production process for inactivated whole

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Table 1
Netherlands Vaccine Institute technology transfer projects.

Project	Vaccine	Approach	Recipient	Developing country
World Bank (1990–1998)	Diphtheria-tetanus-pertussis, measles, oral polio	Turnkey	SIBP, LIBP, KIMB,	China
Haemophilus influenza type b (1999–now)	*Haemophilus influenza* type b conjugate	Development and transfer of pilot process	Bio Farma, SIIL, BE, Glovax/SIBP	Indonesia India Republic of Korea/China
WHO Sabin-IPV (2008–now)	New safer polio	1) Generic, hub 2) Bilateral technology transfer agreements with royalties	Potentially several	To be determined
WHO/NVI (2007–now)	Egg-based inactivated influenza	1) Generic, hub 2) Bilateral technology transfer agreements	1) 15 developing country manufacturers* 2) Vacsera, IVAC	1) 12 countries 2) Egypt, Viet Nam

SIBP, Shanghai Institute of Biological Products; LIBP, Lanzhou Institute of Biological Products; KIMB, Kunming Institute of Medical and Biological Products; SIIL, Serum Institute of India Limited; BE, Biological E Limited; WHO, World Health Organization; IPV, inactivated polio vaccine; NVI, Netherlands Vaccine Institute; IVAC, Institute of Vaccines and Medical Biologicals.

virion influenza vaccine and relevant documentation (standard operating procedures, batch process records, validation methods, analytical methods and release criteria). The choice of technology was based on its simple and robust production process, and therefore its feasibility for transfer to developing countries to produce pandemic influenza vaccine. In addition, whole virus vaccines evoke the broadest immune responses, are largely exempt from intellectual property hurdles and can be produced without using licensed adjuvants [7]. This said, the ability to produce rapidly a pandemic vaccine invariably depends on the existence of annual seasonal influenza vaccine production; since split-virion vaccine is by far the most widely used technology in seasonal influenza programmes, NVI has added a process for split vaccine to its curriculum.

3. Establishment and validation of the basic process

The process established at pilot scale (10,000 eggs) follows the international quality and safety regulations of WHO [8] and the European Pharmacopoeia [9] (Fig. 1).

To determine robustness, we used one monovalent seasonal strain to set up and test a classical egg-based process in our facilities. The main steps outlined in Fig. 1 can be summarized as follows. The primary seed virus obtained from the National Institute for Biological Standards and Control (NYMC X-175C reassortant derived from A/Uruguay/716/2007) was processed to working seed on specific pathogen-free eggs before propagating the bulk virus at pilot scale for 48–72 h in fertilized hen eggs at 35 °C. The virus-containing fluid was harvested semi-automatically and clarified by centrifugation and depth filtration. The virus was purified and concentrated by sucrose gradient zonal ultracentrifugation and then inactivated by ß-propriolactone, filtrated using depth filters and further purified by subsequent ultrafiltration/diafiltration. Finally, the product was formulated and filtrated at 0.22 μm to obtain monovalent vaccine.

After producing 12 monovalent batches, the final production settings were defined and consistency runs performed. The average recovery from zonal ultracentrifugation to monovalent vaccine was 53% and the average yield 1.1 dose/egg. The sucrose density gradient purification method – the international standard for influenza virus purification – resulted in the purification profile shown in Fig. 2. The performance per process step and the impurity profile for the consistency runs are shown in Tables 2 and 3, respectively. The ovalbumin, total protein and endotoxin content meet the specifications set by WHO and the European Pharmacopoeia.

Comparison with other industrial processes is difficult, as most international manufacturers do not publish their process results. We found one publication on density gradient yields [10] and another comparing six European influenza vaccines for impurities [11]. Our data on haemagglutinin antigen yield (Table 2) and impurity profile for ovalbumin and endotoxin (Table 3) fitted well within the ranges reported in these two studies.

4. Hands-on training courses

In the preparatory phase, a suitable production training facility meeting international Good Manufacturing Practice standards within NVI was fitted with all necessary equipment. Process steps and test assays were set up and validated, and a two-volume coursebook written. Extensive documentation on the entire process was generated including all standard operating procedures for manufacturing and testing, and a Bill of Testing.

Participants for the training courses were selected in collaboration with WHO. Of the 15 public and private entities trained to date, 11 have represented manufacturers or regulatory agencies supported by the WHO influenza technology transfer project.

In June 2009, the first one-week interactive workshop was held on quality assurance and GMP aspects, including biosafety risk analysis and management, for 13 participants. This was followed in late 2009 and early 2010 by three courses of three weeks each on influenza production and quality control for a total of 29 participants. These courses addressed the production process in general, as well as specific quality control and release assays of each individual process such as 50% of the egg infectious dose (EID_{50}) and single radial immunodiffusion (SRID). Regulatory issues related to influenza vaccines were covered, as well as the insights and skills needed to work safely and securely. Each course included a demonstration run at 10,000 egg pilot scale, and excursions to external suppliers such as a private egg-breeding facility. Invited international experts complemented the course faculty of NVI scientists and researchers. Participants who successfully completed the course were awarded a WHO certificate.

In addition to the training courses, bilateral technology transfer agreements have been signed with two WHO grantees to ensure further technical support to their vaccine manufacturing projects. Additional staff from both institutions attended tailor-made training programmes at NVI in 2010. The surge of interest in these courses from many countries and regions across the world, created by the 2009 H1N1 pandemic, has led to a waiting list for the next course which is scheduled for early 2011. The International Technology Platform for Influenza Vaccines has a dedicated web site as a communication tool for interested parties (www.itpiv.nl).

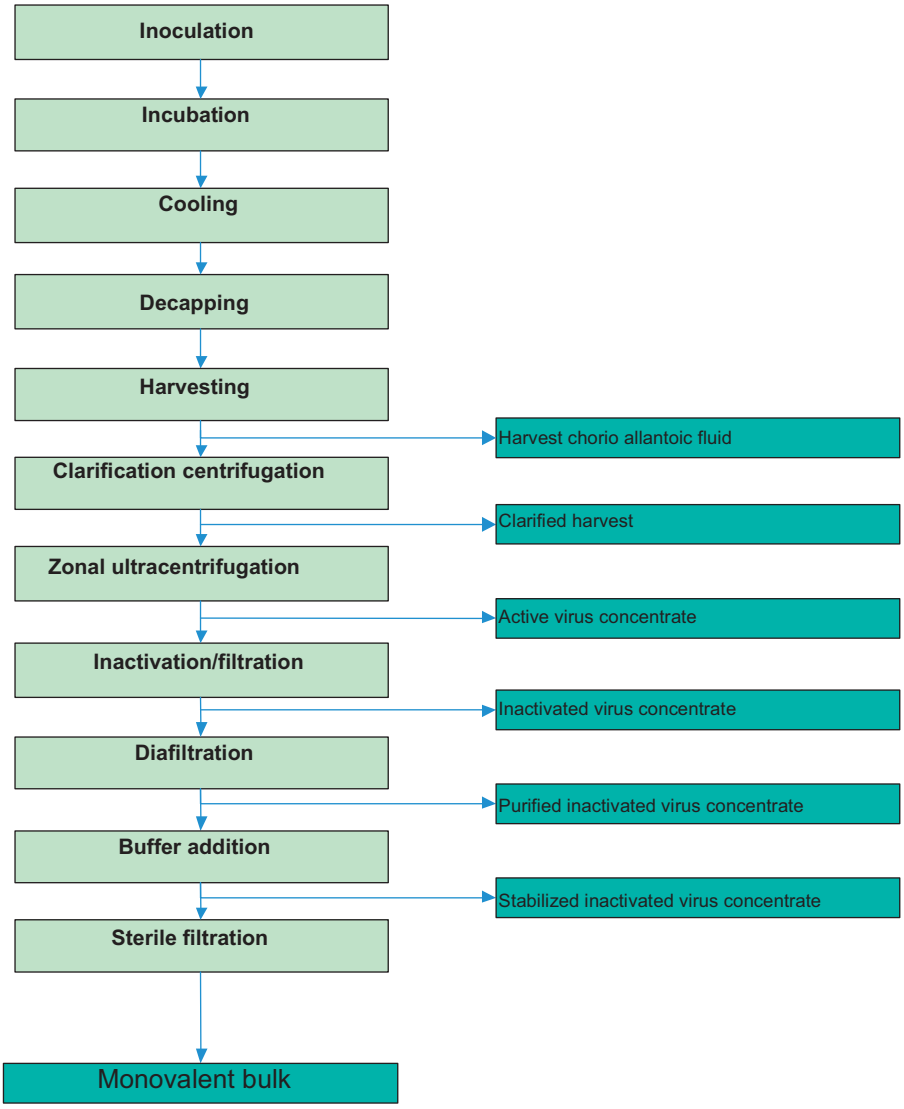

Fig. 1. Egg-based pilot influenza vaccine production process.

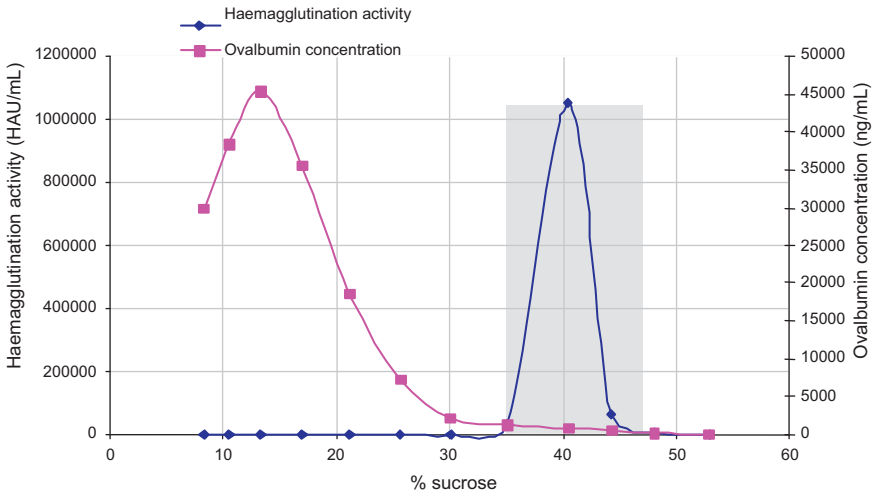

Fig. 2. Ovalbumin and haemagglutinin profile in zonal sucrose gradient.

Table 2
Performance per process step.

Process step	Haemagglutinin recovery	Bioburden removal	Total protein removal	Ovalbumin removal	Sucrose removal
Unit	(%)	Log	(%)	(%)	(%)
Clarification	–	Up to 1.3	–	–	–
Zonal ultracentrifugation	–	Up to 1.2	92	99.993	–
Inactivation	89	Complete	26	–	–
Ultrafiltration/diafiltration	101	–	1	93.4	99.9
Formulation	98	–	–	–	–
Sterile filtration	66	Complete		–	–

Average values obtained in three consistency runs. Bioburden reduction values were the maximum values achieved (removal depends on initial bioburden load of a batch).

Table 3
Impurity profile of three consistency runs.

		Min.	Max.	Spec [8;9]
Ovalbumin/HA	ng/100 μg HA	0.7	3.6	≤2000
Total protein/HA	ug/100 μg HA	225	245	≤600
Endotoxin/HA	IU/100 μg HA	0.09	4.19	≤200
Sucrose/HA	mg/100 μg HA	0.09	0.18	≤0.4

HA, haemagglutinin.

5. Discussion

On the basis of evaluations held after our courses, and in order to serve a broader range of developing countries interested in influenza manufacturing, we are now extending the knowledge base of our Centre. The basic process established for monovalent seasonal strains will be used for pandemic strains, allowing practical training in BSL2+ conditions. To validate the processes developed and immunogenicity of the NVI vaccine, clinical batches of a candidate pandemic H5N1 strain (NIBRG23 A/turkey/Turkey/1/2005) are being produced under GMP for clinical studies in early 2011. We will also extend the process to include a step to serve parties that prefer split over whole virus pandemic vaccine and those interested in seasonal vaccine production.

A major challenge of the hub model is its sustainability. The need to secure NVI's international role in building capacity for common public goods such as those described here have led to other initiatives and innovative approaches that will be introduced into the curriculum. For instance, we plan to develop and introduce cell-culture based technology modules for viral vaccine production. Developing countries may thereby enhance their capacity to manufacture not only influenza, but also other vaccines of high public health relevance, such as rabies or rotavirus. In addition, we serve as a training partner within the recently launched project for the technology transfer of an oil-in-water adjuvant for pandemic influenza vaccines in developing countries.

6. Summary and concluding remarks

The first years of operation have shown the International Technology Platform for Influenza Vaccines to be a highly successful capacity-building tool. The egg-based pilot-scale process established is robust, consistent and meets all international specifications. The technology is easy to scale up and has proven suitable for transfer to developing country manufacturers. The training and technology transfer objectives have been met, since participants at the fully booked generic courses are successfully using the technology and know-how gained in their facilities, and two bilateral consultancy agreements for follow-up activities have been signed. The generic hub approach to technology transfer can thus be seen as complementary to the bilateral partnerships for domestic influenza vaccine production reported by the International Federation of Pharmaceutical Manufacturers & Associations, which usually focus on fill/finish activities.[1]

In conclusion, technology transfer from the public domain to emerging developing country manufacturers and regulators will increase global and equitable access to vaccines of high public health relevance. The hub approach is thus meeting a critical international need, and may be worth considering for other vaccines needed in low- and middle-income countries [12].

Conflict of Interest Statement: The authors state they have no conflict of interest.

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