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Technology transfer of oil-in-water emulsion adjuvant manufacturing for pandemic influenza vaccine production in Romania

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ABSTRACT

Many developing countries lack or have inadequate pandemic influenza vaccine manufacturing capacity. In the 2009 H1N1 pandemic, this led to delayed and inadequate vaccine coverage in the developing world. Thus, bolstering developing country influenza vaccine manufacturing capacity is urgently needed. The Cantacuzino Institute in Bucharest, Romania has been producing seasonal influenza vaccine since the 1970s, and has the capacity to produce ~5 million doses of monovalent vaccine in the event of an influenza pandemic. Inclusion of an adjuvant in the vaccine could enable antigen dose sparing, expanding vaccine coverage and potentially allowing universal vaccination of the Romanian population and possibly neighboring countries. However, adjuvant formulation and manufacturing know-how are difficult to access. This manuscript describes the successful transfer of oil-in-water emulsion adjuvant manufacturing and quality control technologies from the Infectious Disease Research Institute in Seattle, USA to the Cantacuzino Institute. By describing the challenges and accomplishments of the project, it is hoped that the knowledge and experience gained will benefit other institutes involved in similar technology transfer projects designed to facilitate increased vaccine manufacturing capacity in developing countries.

1. Introduction

The Infectious Disease Research Institute (IDRI) in Seattle, USA is a non-profit biotechnology institute dedicated to developing vaccines, diagnostics, and drugs for diseases that affect developing countries. IDRI has employed stable oil-in-water emulsions as adjuvants in influenza vaccines to facilitate antigen dose sparing, enhanced immunogenicity, and broadened pathogen protection [1–3]. Since access to adjuvants and formulation expertise is difficult to obtain, IDRI's main objectives include transferring the manufacturing and quality control (QC) know-how of adjuvant production to developing countries.

The National Institute for Research and Development in Microbiology and Immunology "Cantacuzino" (designated as CI hereafter) was founded in 1921 by Professor Ion Cantacuzino, under the original name of Institute for Serums and Vaccines "Dr. I. Cantacuzino". Built as an integrated structure, the Institute conducts research in areas of microbiology, immunology and related sciences, produces vaccines, performs public health activities (technical and methodological support for microbiology laboratories in Romania) and trains medical and scientific personnel. CI has produced seasonal influenza vaccine for over 40 years, including an H1N1 vaccine during the 2009 pandemic.

CI currently has the capacity to produce ~5 million doses of monovalent pandemic influenza vaccine antigen (sufficient for vaccinating 2.5 million people assuming 2 doses of vaccine are needed). With a population of ~21 million, Romania would therefore need to look for an external supplemental source of vaccine in the event of a pandemic in order to provide universal coverage. However, inclusion of a vaccine adjuvant could facilitate several-fold dose sparing, potentially enabling vaccine coverage for the Romanian population. For example, compared to the non-adjuvanted split inactivated H5N1 vaccine dose licensed in the US, the literature indicates that squalene-based oil-in-water emulsions may provide ~24 fold dose sparing capacity [4,5]. 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. In 2010 the US Biomedical Advanced Research and Development Authority (BARDA) awarded a cooperative agreement to IDRI to transfer adjuvant manufacturing technology to CI to facilitate pandemic influenza preparedness. The cooperative agreement consisted of two phases, with funding for the second phase (preclinical evaluation of adjuvanted vaccine, US$640,234) contingent upon the success of the first phase (technology transfer of adjuvant

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Table 1
SE release test methods and specifications (4%, v/v oil).

Test	Specification
Appearance (visual)	Milky-white liquid
Squalene quantitation (GC)	27.5–41.2 mg/ml
Osmolality (vapor pressure)	250–350 mmol/kg
pH	5.0–6.5
Particle size (dynamic light scattering)	120 ± 40 nm

manufacturing technology, $US790,200). IDRI was selected as a partner based on its extensive development and clinical experience with adjuvant formulations. CI was selected based on its history of influenza vaccine production and because it is one of fourteen WHO-supported developing country influenza vaccine manufacturing partners. This manuscript describes the accomplishment of the goals for phase I of the cooperative agreement: the successful technology transfer of vaccine adjuvant manufacturing and quality control from IDRI to CI. In order for the technology transfer project to be successful, it was determined *a priori* that three batches of adjuvant, independently manufactured by CI within 10 months of the initiation of the cooperative agreement, would need to pass the QC specifications listed in Table 1 and described below. In addition, the following criteria were established regarding adjuvant–antigen mixtures for each of the three adjuvant batches as measured up to 24 h after mixing: equivalent single radial immunodiffusion (SRID) hemagglutinin (HA) concentration compared to antigen without adjuvant, <40 nm change in particle size compared to adjuvant without antigen, and <1 unit change in pH compared to antigen without adjuvant, with all mixtures stored at 2–8 °C.

2. Adjuvant technology

Oil-in-water (o/w) emulsions developed at IDRI consist of metabolizable oils emulsified with biocompatible emulsifiers in an aqueous bulk phase [6–10]. Emulsion droplets are ~100 nm in diameter and batches manufactured at IDRI with research-grade materials show long-term stability at 5 °C (Fig. 1). The IDRI stable emulsion (SE) is similar to a formulation already approved in Europe for influenza vaccines, MF59®, although the squalene dose in SE is 2% (v/v) compared to the 2.3% (v/v) in MF59®. It is important to note that no association between narcolepsy and MF59®-containing vaccines has been found [11] and MF59® has been shown to have an excellent safety record with millions of doses administered to all age groups [12]. In contrast, clinical research conducted by the Finnish National Institute of Health and Welfare has led them to hypothesize that rare cases of narcolepsy in young people in Finland may be associated with a pandemic influenza vaccine containing the emulsion adjuvant AS03 [13]. AS03 differs from SE and MF59® in that it contains 2.5% (v/v) squalene as well as 2.5% (v/v) of an additional oil, α-tocopherol. In addition, the primary emulsifier in SE is phospholipid, whereas AS03 and MF59® contain non-ionic surfactant. Oil-in-water emulsions have been shown to effectively and safely induce immune responses to influenza antigens, including enabling antigen sparing and cross-clade neutralizing antibody responses [1,14–17]. Extensive preclinical animal testing in various animal models has not revealed any safety concerns with the SE formulation transferred to CI [18] or SE variations [2]. Likewise, clinical testing of SE-based formulations such as GLA-SE or MPL-SE has revealed no safety issues [19,20]. Since the purpose of the project was to transfer a royalty-free adjuvant technology, it is important to note that the composition of SE described herein is free of intellectual property infringements.

The SE manufacturing procedure for a final batch size of 550–600 ml is diagrammed in Fig. 2. The SE is prepared by first mixing squalene oil (27.4 g) and egg phosphatidylcholine (6.1 g) in a 1-l glass media bottle by water bath sonication at 70 °C for 1–2 h until the phosphatidylcholine is dissolved. Next, 1.5 l of aqueous phase is prepared by dissolving poloxamer 188 (0.037%, w/v) and glycerol (2.37%, w/v) into ammonium phosphate buffer (24.3 mM ammonium phosphate monobasic and 1.3 mM ammonium phosphate dibasic) with final aqueous phase pH of 5.6 ± 0.2. Then, 768 ml of aqueous phase is added to the oil phase prepared earlier as described above. The crude emulsion is then high speed mixed (L5M-A Laboratory Mixer, Silverson Machines Ltd., with ¾ in. tubular mixing assembly and square hole high shear screen mixing head) at ~10,000 rpm for a minimum of 10 min and then transferred to a high pressure homogenizer (M-110EH-30 Pilot/Production Processor, Microfluidics Corp.), operating at 30,000 psi and water-cooled by a recirculating chiller set to 10 °C. The emulsion is recirculated in the homogenizer for approximately 16 cycles based on the measured flow rate of the M110EH and the emulsion volume (*i.e.* 800 ml). Prior to and immediately after processing, the microfluidizer is cleaned by processing 1.5 l of Contrad 70 (Decon Labs Inc.) and 0.5 M NaOH, with water-for-injection flushing of the cleaning agents before and after processing. In a laminar flow hood, the final formulation is filter-sterilized through a 0.2 μm filter (Millipore Millipak-40) under constant flow *via* peristaltic pump. Final emulsion yield is generally 550–600 ml after dead volume losses in the homogenization and filtration processes.

QC of the adjuvant is ensured through assessment of physical appearance, particle size, squalene content, pH and osmolality (Fig. 1 and Table 1). Visual appearance is performed manually under adequate lighting by gently swirling a sample of the emulsion in a glass vial and assessing whether the appearance is milky-white and homogeneous. Particle size is measured by dynamic light scattering (Zetasizer Nano-S, Malvern Instruments Ltd.) following system suitability assessment using dispersions of 60 nm and 220 nm polystyrene beads. Triplicate emulsion samples are prepared for particle size measurement by diluting them 100-fold into sterile water for injection in a disposable polystyrene cuvette. Particle size is reported as *Z*-avg, which is the average droplet size of the intensity-based distribution profile. Squalene content is quantified by flame ionization gas chromatography (7890A GC System, Agilent Inc.) by first preparing squalene standards in 2:1 (v:v) chloroform:methanol in volumetric flasks and preparing triplicate emulsion samples by diluting them 80-fold into 2:1 chloroform:methanol. System suitability is monitored by evaluating six injections of a squalene standard for retention time (≤5% CV), peak area (≤5% CV), tailing factor (between 0.7 and 2), and capacity factor ($k' \geq 2.5$). The standard curve must have a correlation coefficient of ≥0.995 and an in-process standard injected at the end of each sample set must be ≤5% different from the actual concentration. Squalene concentration of the emulsion sample is then calculated from the squalene standard curve. Measurement of pH on a sample of emulsion is conducted by a standard electrode device calibrated previous to measurement. Osmolality is assessed by vapor pressure osmometry (Vapro Vapor Pressure 5520 or 5600, Wescor Inc.) with the instrument calibrated with vendor-supplied standards prior to measurement of triplicate samples, which are pipetted to the sample holder as undiluted 10-μl aliquots. Emulsion stability over time is monitored by periodic assessments of visual appearance, particle size, and pH. In addition, emulsions manufactured under cGMP conditions undergo further release testing for bioburden, sterility, safety and pyrogenicity. However, the goal of this technology transfer project was the manufacture of three engineering batches meeting the specifications in Table 1, which did not necessitate cGMP conditions or further release testing.

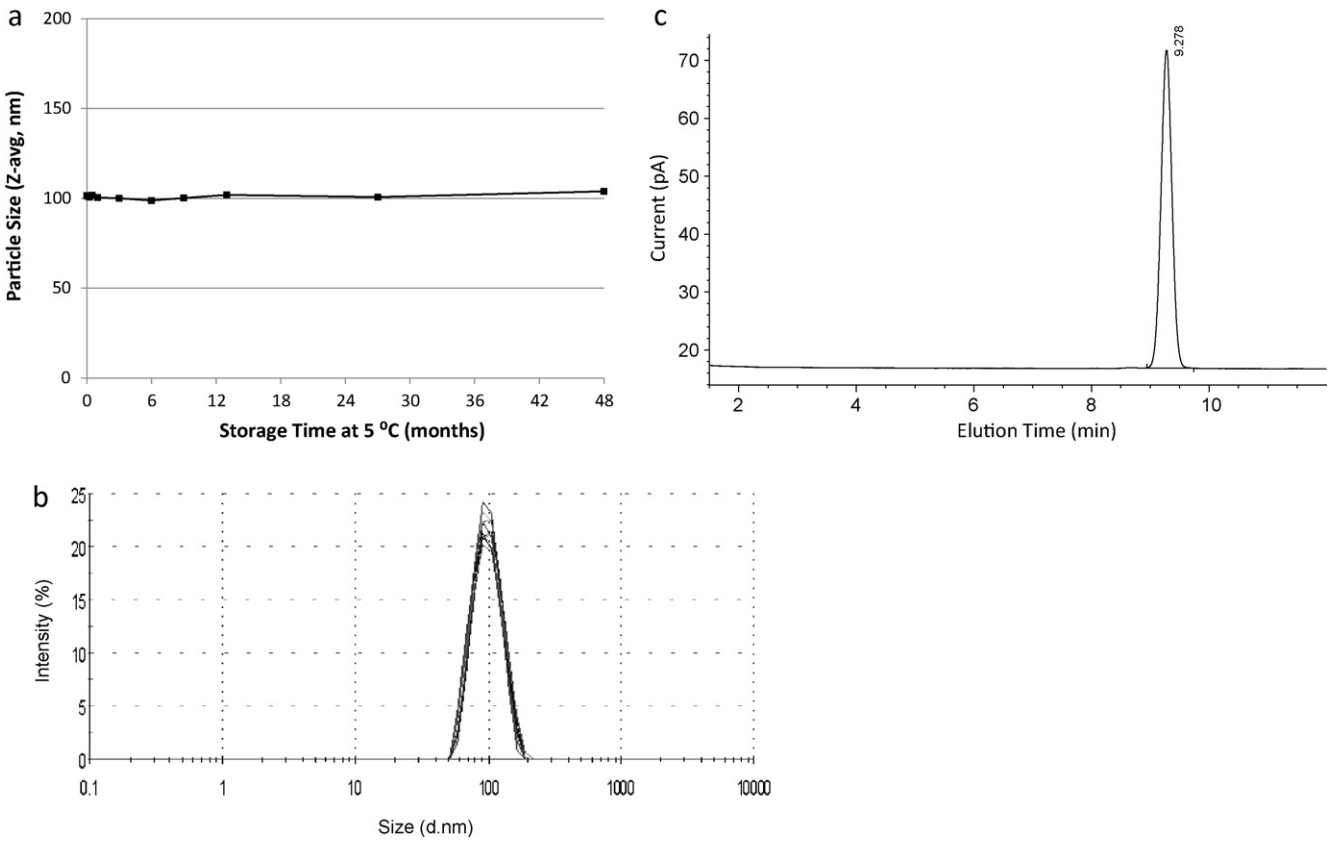

Fig. 1. Physicochemical characterization of SE. (a) Particle size as measured by dynamic light scattering of SE adjuvant stored at 5 °C (manufactured at IDRI and employing research-grade squalene). Average particle size values of a single batch of SE are shown. Error bars representing standard deviation of nine total measurements on three separate aliquots at each timepoint are plotted but not visible due to very low magnitude. (b) SE particle size distribution by intensity as measured by dynamic light scattering. Nine total measurements were made on three separate aliquots from the same batch of SE. (c) Gas chromatography-flame ionization detection of squalene content in SE adjuvant.

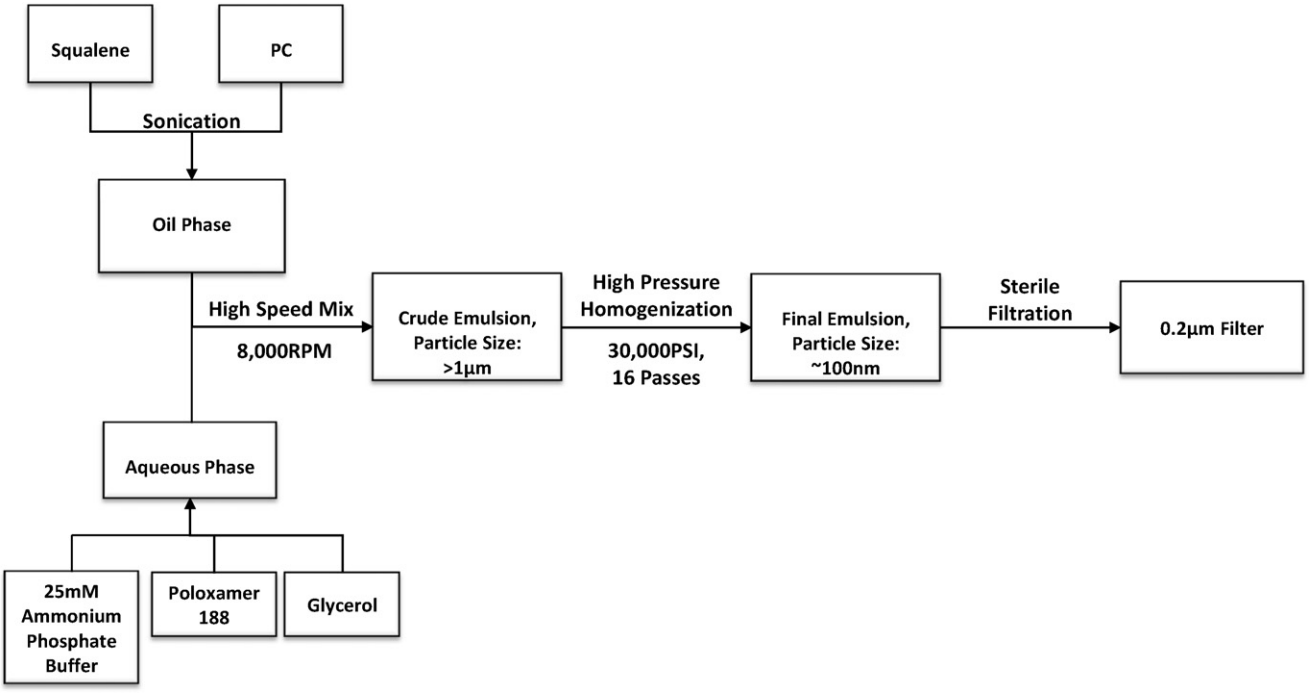

Fig. 2. Process flow diagram of SE manufacturing procedure.

3. Influenza antigen

The Cantacuzino Institute (CI) in Bucharest, Romania has produced seasonal influenza vaccine since 1971. Recently, CI has developed whole and split pandemic virus antigens, including an inactivated split virus H5N1 antigen (NIBRG-14 reassortant) that has been evaluated preclinically. Batches of H5N1 (A/Vietnam/1194/2004 – NIBRG-14) split virion have been produced in CI's vaccine facility following the same technological flow used for production of seasonal influenza vaccines. Influenza vaccines are manufactured at CI according to the seasonal WHO recommendation for the Northern Hemisphere. Specific vaccine production details and quality control specifications are described in the Supplementary Information. After the quality is checked by the Quality Control Laboratory of CI, the vaccine receives approval for marketing release from the National Agency for Medicines and Medical Devices. The main goal of this adjuvant technology transfer project was to enable the combination of SE adjuvant developed by IDRI with the inactivated split virus H5N1 antigen developed by CI and by so doing to significantly reduce the antigen dose and/or number of immunizations required to elicit a seroprotective response to the antigen.

4. Technology transfer process

4.1. Communication

A communication strategy was established at the kick-off teleconference which included establishing biweekly teleconferences and three face-to-face meetings between CI, IDRI, and BARDA. Prior to each meeting, an agenda was sent out with highlights and action items from the previous meeting. In addition to meetings, emails were constantly exchanged and quarterly grant progress reports were prepared and submitted.

4.2. Equipment and supplies acquisition

Dedicated process and QC equipment was purchased directly by the Cantacuzino Institute from various suppliers. Production equipment was mentioned above and included a high pressure homogenizer, also called a microfluidizer (Microfluidics M-110EH-30), a high speed mixer (Silverson L5M-A) and some smaller items, all of which were placed in the newly established pilot production area for influenza vaccine development at CI. The QC equipment included a gas chromatograph (Agilent 7890A), a dynamic light scattering particle sizer (Malvern Zetasizer Nano-S), and a vapor pressure osmometer (Wescor Vapro Vapor Pressure 5600). A small dedicated area for QC instrumentation was set up at CI. The equipment purchasing was guided by the national public purchasing regulations and also the US federal code. Most of the reagents were purchased by normal purchase channels. Exceptions were particular GMP grade reagents such as phosphatidylcholine (Avanti Polar Lipids) and squalene (Sigma-Aldrich Fine Chemicals), where the purchasing required special attention and involved specific suppliers and locale.

4.3. Translation of SOPs

About 100 pages were provided by IDRI to CI for translation into Romanian. First they were translated faithfully in Romanian, keeping the original IDRI format, to be afterwards adapted to the SOP format used in CI. The initial faithful translations were retained to ensure the traceability of documents and their modifications under this grant.

4.4. Visit of CI trainees to IDRI

The initial training of CI scientists was carried out through a site visit to IDRI in Seattle, WA, USA. During the visit, which lasted five working days, the CI scientists first observed the full manufacturing and characterization process of one batch of adjuvant. Then, the CI participants manufactured and characterized two more batches of adjuvant with guidance from IDRI personnel as necessary. From CI's perspective, the visit was informative not only with respect to the adjuvant manufacturing technology but also for related aspects such as the type of analytical laboratory required for this type of product, since traditional biological process laboratories differ slightly in technology.

4.5. IDRI visits to CI

While awaiting the completion of the equipment acquisition process, IDRI personnel visited CI in Bucharest, Romania for two days. This face-to-face meeting facilitated consideration of appropriate equipment installation locations and contingency planning. A more extensive, five-day visit of an IDRI team to CI occurred after critical equipment and supplies were installed. Two pilot lots of adjuvant were manufactured and characterized under IDRI supervision using the newly installed equipment at CI. These pilot lots were distinct from the three additional lots independently produced by CI following the site visit. Critical product specification testing was successfully conducted by the CI staff. The two pilot adjuvant lots were also included in SRID assays at CI during the week of the visit and test results indicated that no detrimental effects on HA content were caused by mixing the antigen with the adjuvant.

Besides the manufacture of the two pilot lots of adjuvant, an extensive preclinical study planning session was held, including a tour of the animal facilities at CI where most of the preclinical studies will be conducted in the second phase of the project. Subsequent to the 2nd site visit to CI, a consultation with the European Medicines Agency was carried out to discuss the development and planned use of the adjuvant technology in Romania, and to receive useful guidance regarding the preclinical development of the adjuvanted vaccine. In addition, CI is undertaking efforts to be established as a pre-qualified WHO influenza vaccine manufacturing site.

Finally, on the last day of the visit, all interested CI personnel as well as local university students and faculty were invited to attend a one-day adjuvant training course provided by the IDRI team. Covered were adjuvant history and a current overview of the field, adjuvant mechanisms of action, TLR agonist design and activity, formulation and characterization, emulsion manufacturing and QC, adjuvant *in vivo* activity assays, antigen process development, and future prospects in adjuvant science.

From the perspective of CI, this training visit was a good opportunity for an evaluation of the facilities and for performance verification. The manufacturing of the two pilot batches increased the degree of confidence for the CI team and allowed CI to expand the number of persons trained, thus providing better sustainability of the manufacturing process. The adjuvant lectures enabled CI scientists to extend their knowledge regarding the new generation of adjuvants, and were a perfect means to obtain an overview of the field.

5. Results

5.1. Independent production of three adjuvant batches at CI

This project required evidence that, within 10 months of the cooperative agreement award notification, CI produce and

Table 2
Emulsion manufacturing process performance.

Batch	Volume (ml, post-high speed mixing)	Volume (ml, post-microfluidization)	Yield (%)	Volume (ml, pre-filtration)	Volume (ml, post-filtration)	Yield (%)
1	797	672	84	643	591	92
2	799	670	84	645	588	90
3	801	650	81	626	556	89

successfully test three engineering lots of oil-in-water stable emulsion adjuvant (SE). Accordingly, three lots of SE were manufactured at CI according to the method described above. Process parameters were reproducible as shown in Table 2. The expected small losses in product yields were largely due to homogenizer and filtration device dead volume.

QC testing of the product was performed post-sterile filtration at CI according to the defined SOPs of the methods in Table 1. All three batches of SE produced at CI met each required specification (Table 3). Aliquots from each batch were shipped to IDRI for confirmation of QC results. The high reproducibility of the characterization results between the two labs is striking (Table 3).

5.2. Antigen–adjuvant compatibility

Besides adjuvant QC, an important consideration for the final vaccine product is to find modalities to assess the compatibility and stability of the antigen–adjuvant mixture. Unfavorable interactions could impair the stability of the hemagglutinin structure itself, which changes conformation at slightly acidic pH. Therefore, it is important that the virus preparation be kept at a pH greater than 6.5. Since the pH of the adjuvant is approximately 5.6, a good buffering system is needed to maintain the HA conformation. Therefore the buffer of the vaccine diluent was designed with a high buffering capacity in the neutral range (7.2). As seen in Table 4, no major drop in pH occurred after mixing the antigen with adjuvant. The pH was checked up to 24 h after mixing with little change. In addition, visual appearance and osmolality were evaluated on selected samples and, as expected, showed little change since antigen and adjuvant had similar osmolality values before mixing (data not shown). Moreover, the stability of the antigen–adjuvant mixture was assessed by monitoring changes in particle size and HA content. If antigen conformational change occurs, the virus exposes hydrophobic regions, leading to aggregate formation and antigen degradation. As can be seen in Table 4, no significant differences in particle size or SRID results are apparent.

To quantify the expected lower doses of antigen, earlier work at CI validated a modified SRID assay (to account for adjuvant inclusion) for a larger range of antigen concentrations. The differences noticed are below the statistical significance of the test and therefore we can assume the antigen compatibility is good for the three batches. The intra-assay coefficient of variation (CV) obtained during the method validation for the HA concentration of 30 μg/ml was, on average, 3.3% with a maximum value of 4.3%. For the compatibility assay the CV ranged between 4.7 and 6.1%. Also, regarding the apparent particle size, the values were stable during 24 h incubation at 2–8 °C. Therefore, all three lots met QC specifications and are compatible with a test antigen preparation. The presence of the oil-in-water adjuvant did not influence the antigen concentration determined by SRID at a ~15 μg/0.5 ml dose during 24 h at 2–8 °C.

Since the three batches of adjuvant were manufactured within 10 months from the initiation of the cooperative agreement and passed all established specifications for quality control and antigen compatibility, the technology transfer process was considered successful. Another outcome of the efforts in phase I of the project was a preclinical plan for the *in vivo* evaluation of the adjuvanted vaccine. The plan was designed to demonstrate the immunogenicity, protective efficacy, dose sparing capacity, and safety of the adjuvanted vaccine in small animal models following applicable EMA regulatory guidelines. This plan will be implemented during phase II of the project (2011–2012). Longer-term plans for further development, such as a Phase I clinical trial, and sustainability are under discussion. This includes development of appropriate fill/finish procedures, with initial stability evaluations indicating that inert gas overlay may be required. The initial vaccine presentation is designed as a two-vial system with bedside mixing of adjuvant and antigen, as with current pandemic vaccine configurations involving AS03 [21]. However, ease of use considerations may favor a single vial system or a pre-filled syringe. For this reason, ongoing studies are evaluating long term stability of single vial preparations, including methods of improving stability.

6. Lessons learned

6.1. Facility considerations and regional availability of equipment/supplies

Romania is ideally located within the European Union so it would be amenable to technology transfer. Unlike most European countries, Romania is a developing country still requiring technological and economic assistance for development, but is required to strictly obey the EU laws and regulations including for manufacturing, research, environmental, and taxation; thus, medicinal products produced in Romania such as vaccines are regulated by the European Medicines Agency (new products) as well as the local national authority (already registered products). Other regulations can also have an impact on the technology transfer. For example, the microfluidizer cleaning detergent Contrad 70 had to be replaced with a different biodegradable version in the Romanian market as required by EU environmental regulations. The EU market provided access to a diversity of equipment and supplies not readily available in the Romanian regional market, or at greater costs. Available equipment and supplies were purchased directly by CI from regional suppliers, which avoided potential delays from customs and import regulations requirements.

6.2. Flexibility for time delays

Some delays were experienced due to the public tender process and the regional unavailability of basic lab supplies. The accelerated timeline of the project required forward thinking to arrange for suitable alternatives in such cases. Overall the lesson learned is that timing of such complex projects should be carefully estimated, with extra time included for unexpected delays.

6.3. Ambient effects

One unexpected issue was the influence of the humidity in the manufacturing area. Although relative humidity is not usually defined as a critical parameter in the bioprocess pilot area, this

Table 3
Emulsion quality control.

Batch	Cl visual appearance	IDRI visual appearance	Cl particle size (*Z*-avg, nm)	IDRI particle size (*Z*-avg, nm)	Cl squalene (mg/ml)	IDRI squalene (mg/ml)	Cl osmolality (mmol/kg)	IDRI osmolality (mmol/kg)	CI pH	IDRI pH	Cl pass/fail	IDRI pass/fail
1	Milky-white liquid	Milky-white liquid	101.1	101.1	34.3	33.6	310	306	5.6	5.7	Pass	Pass
2	Milky-white liquid	Milky-white liquid	100.6	100.6	35.4	34.1	299	299	5.6	5.7	Pass	Pass
3	Milky-white liquid	Milky-white liquid	101.2	102.5	35.3	34.9	309	310	5.6	5.7	Pass	Pass

Table 4
Adjuvant–antigen compatibility.

Batch	Particle size $T=0$ (*Z*-avg, nm)	Particle size $T=8$ h (*Z*-avg, nm)	Particle size $T=24$ h (*Z*-avg, nm)	HA content $T=0$ (mg/ml)	HA content $T=8$ h (mg/ml)	HA content $T=24$ h (mg/ml)	pH antigen	pH antigen-adjuvant mix
Antigen alone	–	–	–	36.4	35.5	37.7	7.2	–
1	106.2	105.7	105.9	37.9	36.4	40.3	7.2	7.0
2	109.7	105.7	105.4	37.5	34.2	38.5	7.2	7.1
3	107.2	107.4	108.4	33.3	36.6	35.5	7.2	7.0

particular process was found to be sensitive to the humidity since phosphatidylcholine will not disperse well into the squalene oil if it is not mixed immediately after combining. As a pilot production facility at CI, some environmental controls were not designed for extreme environmental conditions (*i.e.* the cooling system was not powerful enough to assure dehumidification in the production room when outside temperatures were very high as they were in the Bucharest summer of 2011). As a result the average room temperature was 24–26 °C and the relative humidity was ~70%, compared to estimated conditions in Seattle of room temperature ~16 °C with a similar relative humidity (~70%), meaning less air moisture than in the facility at CI in Romania. Therefore the environmental parameters of the area should be taken into consideration.

6.4. Analytical testing

Particle sizing (by dynamic light scattering) can be a challenge for emulsions. Higher concentrations can produce false size information due to interference of multiple light scattering particles. Our previous experience with a related particle sizing instrument helped us to avoid these mistakes. In addition, we could compare the performance of the backscattering-based detection mechanism in the Zetasizer Nano-S with the 90° detection mechanism in a related instrument; we found the backscattering detection to be more robust, especially at higher particle concentrations. Another QC issue of particular importance was the osmolality determination. The Pharmacopoeia recommends the freezing point method and this was adopted by CI, whereas the technology transfer called for the vapor pressure method; thus, CI performed both methods. Glycerol and macromolecules behave anomalously with the freezing point method, and therefore the results were dramatically affected. In summary, QC methods and equipment specifications must be followed exactly for successful performance.

7. Conclusions and recommendations

This project was successfully executed with a cross-cultural team. Working in two countries in two different types of institutes necessitates understanding the laws and regulations of each. IDRI and CI have developed a working model for the transfer of stable emulsion adjuvant technology from one institution to another. Although this project was specifically intended to provide dose sparing for a pandemic flu vaccine in Romania, the same technology could be useful in other countries or in dose-sparing other vaccines where there is a critical need and the vaccine is in short supply. The cost of this program is relatively modest (*e.g.* $790,200 for the adjuvant technology transfer phase) for the tremendous potential return on investment expected through antigen dose-sparing and pandemic influenza preparedness. Of course, long-term follow-up and development will be necessary to ensure consistent results, including manufacturing scale-up and development of additional excipient characterization assays (*e.g.* emulsifier quantitation) in order to ensure adequate adjuvant supply and comprehensive quality control. These efforts are ongoing. We propose that other technology transfer efforts can benefit from the lessons learned during the present project.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2012.10.048.

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