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Chemistry, manufacturing and control (CMC) and clinical trial technical support for influenza vaccine manufacturers

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ABSTRACT

With the support of the Biomedical Advanced Research and Development Authority (BARDA) of the US Department of Health and Human Services, PATH has contributed to the World Health Organization's (WHO's) Global Action Plan for Influenza Vaccines (GAP) by providing technical and clinical assistance to several developing country vaccine manufacturers (DCVMs). GAP builds regionally based independent and sustainable influenza vaccine production capacity to mitigate the overall global shortage of influenza vaccines. The program also ensures adequate influenza vaccine manufacturing capacity in the event of an influenza pandemic.

Since 2009, PATH has worked closely with two DCVMs in Vietnam: the Institute of Vaccines and Medical Biologicals (IVAC) and VABIOTECH. Beginning in 2013, PATH also began working with Torlak Institute in Serbia; Instituto Butantan in Brazil; Serum Institute of India Private Ltd. in India; and Changchun BCHT Biotechnology Co. (BCHT) in China.

The DCVMs supported under the GAP program all had existing influenza vaccine manufacturing capability and required technical support from PATH to improve vaccine yield, process efficiency, and product formulation. PATH has provided customized technical support for the manufacturing process to each DCVM based on their respective requirements.

Additionally, PATH, working with BARDA and WHO, supported several DCVMs in the clinical development of influenza vaccine candidates progressing toward national licensure or WHO prequalification. As a result of the activities outlined in this review, several companies were able to make excellent progress in developing state-of-the-art manufacturing processes and completing early phase clinical trials. Licensure trials are currently ongoing or planned for several DCVMs.

1. Introduction

Since 2009, with the support of the Biomedical Advanced Research and Development Authority (BARDA) of the US Department of Health and Human Services, PATH has contributed to the World Health Organization's (WHO's) Global Action Plan for Influenza Vaccines (GAP) by providing technical and clinical assistance to several developing country vaccine manufacturers (DCVMs). Technical assistance included direct support in manufacturing process development, including process yield optimization, process validation, analytical method development and validation, quality control, and quality management systems (implementing and strengthening good manufacturing practices (GMP)). Clinical assistance included clinical trial design, implementation, laboratory testing, and regulatory support. Moreover, PATH country offices managed technical and clinical development and facilitated daily regulatory interactions. PATH also facilitated interactions with the US Centers for Disease Control and Prevention (CDC) for serology testing and reagents, the National Institutes for Biological Standards and Control (NIBSC) for vaccine potency and standards, and academic institutions in Europe and the United States for training on analytical methods. PATH also provided DCVMs with introductions to the relevant WHO personnel and meetings necessary for influenza strain selection.

The DCVMs varied in their capacity to develop new products. Some companies had vaccines on the market but were only experienced with legacy processes and had limited in-house development capacity. Other companies had the technical skills to develop new manufacturing processes but needed to improve quality standards or lacked clinical development expertise. Therefore, PATH provided customized support, initiated by due diligence

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visits to the sites to understand the capacity, processes in place, and existing gaps. Relevant PATH staff or consultants then worked with each DCVM to develop a tailored technical assistance plan beginning with a series of on-site visits and as-needed trainings followed by email and teleconference communications.

Vaccine manufacturers that received assistance for national product licensure followed pharmacopeias and national guidelines (when available) in product development and testing, and in clinical trial design and implementation. For WHO pre-qualification, manufacturers observed WHO and European Medicines Agency or US Food and Drug Administration (FDA) guidelines, which frequently proved to be more stringent than local guidelines. PATH's core functions and experts, including chemistry, manufacturing, and controls (CMC), clinical, regulatory, and business development, were involved as part of the provided technical assistance.

This review outlines the most frequent observations found during PATH's CMC, regulatory, and clinical trial support activities, and briefly outlines the remediation, improvements, and progress made in the development of regional capacities for influenza vaccine production.

2. CMC technical assistance

This section discusses the most common challenges experienced by DCVMs developing and producing influenza vaccines. This section also details how PATH's technical staff and consultants provided training, expert advice, and strategies to prevent and remediate such challenges.

Most manufacturers supported under the GAP program were developing a conventional egg-based process for seasonal split vaccines, and a whole-virion approach for pandemic vaccines (see Fig. 1). Low-resource country manufacturers frequently use egg-based influenza vaccines because the technology is already well-established, and quality eggs are readily available. In the DCVMs PATH was supporting, however, vaccine yields were often low because the manufacturers were not using mass balance with single radial immunodiffusion (SRID) (or high performance liquid chromatography (HPLC)) to determine the hemagglutinin (HA) yields of each step in the process. When instituted, however, the steps responsible for such large losses in yield became obvious and remediation efforts could be implemented to improve yield.

2.1. Seed development

Seasonal influenza vaccine formulations can vary year-to-year. Vaccine manufacturers procure the seed viruses from laboratories that generate reassortants. These viruses combine the surface immunological antigenic properties of the circulating viruses and the growth properties of strains that have proven to grow well in eggs. Frequently, the DCVMs were unaware that differences in yield can occur between the various reassortants and genetically engineered viruses. Using the optimal seed virus, however, can sometimes improve production yields by two- to three-fold. PATH invited the DCVMs to participate in the yearly WHO technical calls where seed information is discussed, and provided assistance in choosing the best seed viruses. The laboratories began using ideal seed optimization methods, such as serial passaging in eggs with relatively high concentration of virus that allows the rare better-growing mutants to outgrow the average-yielders. Additionally, short incubation periods allow the faster-growing mutants to prevail, and those mutants often result in the highest yields after the full incubation period. Finally, many RNA viruses tend to cycle in their yields. Sequential passaging of virus affects the ratio of defective interfering particles to live virus, so it is necessary to choose the correct passaging conditions as well as the correct number of passages for optimal seed virus. Appropriate understanding of these concepts led to significant improvements in the yield.

It is important to produce a seed that, when inoculated for the pass in large scale production, will give the best HA yields. This can be done by tracking the yields for small-scale passage and replicating the relevant passage to produce large quantities of working seed. Once a working seed had been made, however, none of the DCVMs were doing the additional work required to identify optimal incubation time and dilution for production. Small-scale time/dilution studies can provide guidance to improve production-scale yields. Inclusion of these studies also resulted in significant improvement in viral titer at harvest and overall process for the various DCVMs.

Finally, it is important (especially for pre-pandemic strains) to connect with authorities who will confirm the working seed has not changed with regard to antigenicity or sequence of the polybasic region of the HA. Changes in some of the amino acids in the hemagglutinin are often associated with increased yield versus changes in antigenicity. Rarely, some seeds can change their antigenicity after as few as one or two passages. Change impact can be assessed using ferret antiserum panels and reference viruses. Since ferret panels are difficult to produce, it is important for DCVMs to connect with a WHO reference laboratory to outsource product testing.

2.2. Egg handling and fluid clarification

Egg contamination is a major concern in vaccine production. PATH educated DCVMs on the best way to handle eggs in order to protect the cuticle (the outer coating on the shell that makes eggs less porous and reduces contamination). PATH also impressed upon manufacturers the importance of routine monitoring, as many were unaware that water used for incubator humidification can be a prime source of bioburden contamination. PATH also taught manufacturers that egg fumigation is much more effective in reducing egg-shell contamination because it kills bacteria before they have a chance to enter the egg at day 0 instead of day 11. Finally, PATH encouraged manufacturers to discard grossly contaminated eggs rather than wash them and risk losing the protective cuticle.

Some of the facilities that received technical assistance had access to modern automatic or semi-automatic egg inoculation and harvesting equipment. Typically, DCVM were aware of and routinely implemented methods to reduce egg contamination by frequently changing or cleaning the inoculation needles and visually inspecting eggs after decapping before harvesting. Fluid clarification, however, was often performed using filtration through membranes that were too tight, resulting in excessive loss of virus yields. The main purpose of the clarification step is to remove large debris and red cells; filters with a pore size small enough to remove bacteria are likely to remove virus as well, depending on the quality of each particular batch of allantoic fluid. PATH recommended use of larger pore filters and, where possible, a filter cascade from large to small pores or low speed centrifugation, which greatly improved yields. Using excessive speed was a common error for the low speed centrifugation process, particularly as influenza virus tends to clump loosely and relatively low speeds can pellet some of the virus. However, the centrifugation speed and fluid flow rate can be adjusted to apply only the minimum *g* force needed to pellet red cells without pelleting the virus.

2.3. Purification, inactivation, and filtration

The typical downstream process for both split and whole virion vaccines includes a step to purify the virus from the infected

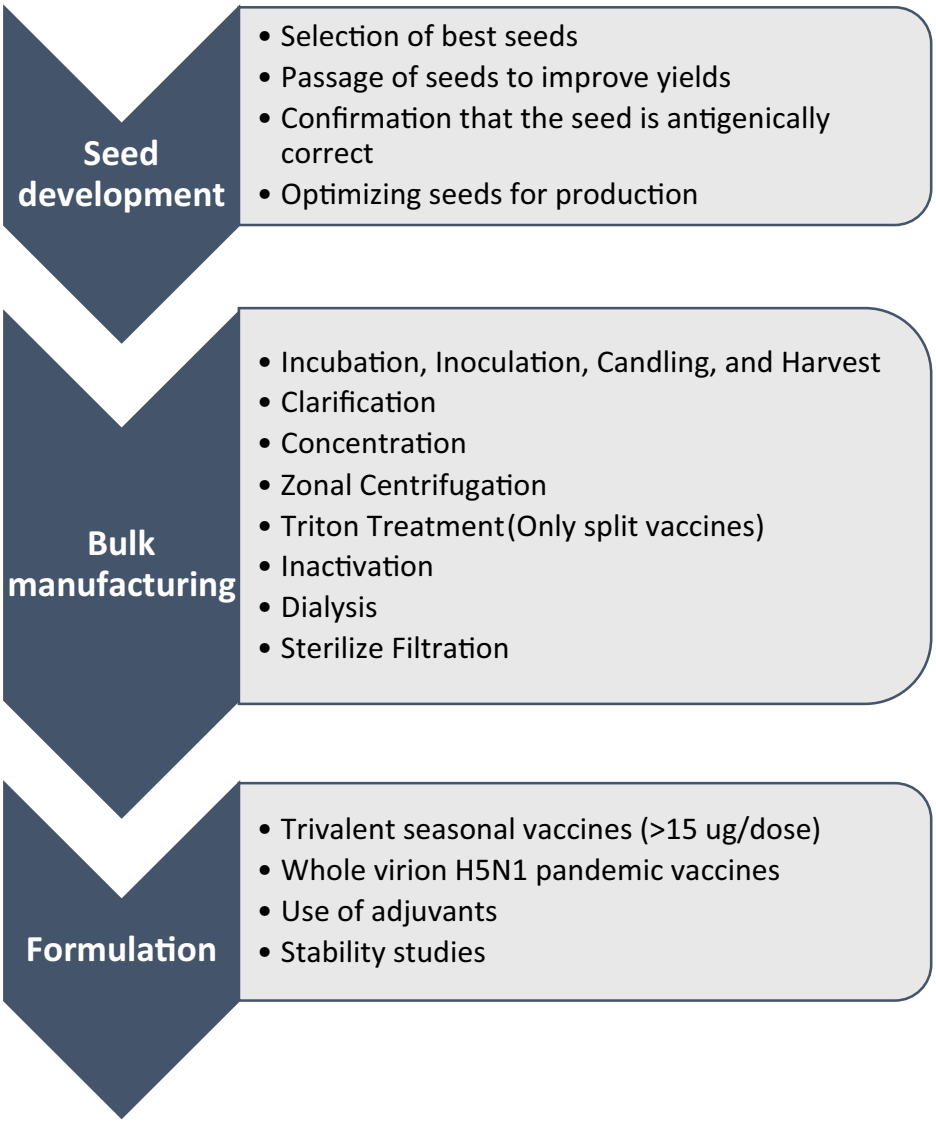

Fig. 1. Diagram of the manufacturing process and development activities for egg-based vaccine manufacturing.

allantoic fluid, which is subsequently split (for split vaccines only) and chemically inactivated.

I. **Purification:** All facilities were using standard zonal centrifugation to purify the virus. PATH experts recommended studies that determined which fractions needed to be included in the retained virus band in order to reduce contaminants and optimize viral recovery.
II. **Viral inactivation:** Some facilities were using more formalin or Beta-propiolactone (BPL) than was needed for viral inactivation, resulting in unnecessary losses of HA. PATH experts helped identify the correct amount of formalin or BPL and recommended use of a standard two vessel procedure during inactivation to ensure complete viral inactivation.

Other occasional problems included:

- Inadequate temperature control.
- Inadequate amounts of detergent (Triton X-100®) following dialysis (of split vaccines), which led to excess aggregation of the HA, thus causing losses both in-process and during stability monitoring.
- Inadequate membrane cleaning, either before or after zonal centrifugation, which led to the addition of bioburden in subsequent lots.
- Increased pressure during the dialysis and final filtration steps, for both whole and split vaccines.
- Inadequate membrane surface area.

III. **Filtration:** Introducing controlled cross filtration parameters such as Liters/Meter/Hour and Trans-Membrane-Pressure improved processes and resulted in higher recovery and better filtration efficiency and contaminant removal. Despite this improvement, the filtration steps were sometimes performed with vaccine at too high a concentration so that aggregation and subsequent losses were more likely. This could easily be corrected by defining appropriate ranges for the tangential flow filtration steps to reduce losses and ensure lot-to-lot consistency.

2.4. Formulation

Blending monovalent bulks to formulate the final product has been a major focus. Inactivated trivalent seasonal influenza vaccines are blended so each strain is present in the final product in an

amount exceeding 15 μg HA per dose in PBS. Vaccine stability impacts the final formulation because loss of potency over time must be factored in the initial product formulation. The overage required can be experimentally determined and must account for product loss during process and storage. One important factor is that, once influenza virus is split with detergent, disulfide bonds start to form and cause aggregation of HA. The cross-linked proteins will not diffuse properly in the SRID test and will result in a lower amount of measured immunoreactive HA [1]. This cross-linking tends to stabilize after about a month of monovalent concentrate storage. Understanding the concentrate stability curve is important in order to adjust formulation targets. Trivalent bulk stability data is also important and for a new strain, stability should be anticipated from data from previous related strains. Most of these elements are unique to influenza vaccines. Several DCVMs developing influenza vaccine for the first time did not have the experience to efficiently anticipate and resolve these challenges. External support was necessary to ensure the final product would remain potent during long-term stability testing and clinical trials.

Another important consideration was the use of adjuvants. PATH recommended against using adjuvants in the seasonal vaccine formulation based on limited efficacy of aluminum-based adjuvants, and the complexity and risks involved in developing more potent adjuvants (such as oil-in-water squalene-based ones). Split pandemic vaccines have been shown to be poorly immunogenic for influenza A/H5N1 and require either very high doses (90 μg hemagglutinin per dose) or potent proprietary adjuvants to be effective. Existing alum-adjuvanted whole-virion A/H5N1 vaccines have shown acceptable safety profiles and good immunogenicity in clinical trials [2,3]. Based on these data, PATH recommended DCVMs pursue development of an alum-adjuvanted whole-virion A/H5N1 vaccine.

2.5. Manufacturing process yields

The typical influenza vaccine yield is usually estimated to be about one 45 μg trivalent dose per egg harvested. This standard does not take into account formulation and filling losses, though it does include an average yield of all three strains in a trivalent vaccine (when possible). By implementing the manufacturing improvements described above in Sections 2.1–2.3, manufacturers were able to improve the overall yield. For example, in one case yield increased from 0.25 dose/egg to 1.30 dose/egg. Another manufacturer increased from about 0.13 dose/egg to about 1.00 dose/egg.

2.6. Process validation

Once a manufacturing process is deemed satisfactory, a developer should determine acceptable operating ranges to ensure production batch consistency (known as process validation (PV)). This needs to be completed before the vaccine enters late-stage clinical development, to guarantee the material used in development will be representative of the commercial product.

Occasionally, there were an inadequate number of developmental runs prior to PV. These runs are essential to determine critical quality attributes and process parameters and should be well planned and documented. Manufacturers should consider the biological variability of influenza virus strains and eggs to determine ranges of the study parameters to understand the inherent variability of the process.

At times, assay validation, cleaning validation, hold time determination, and mixing validation were not completed before PV. Inexperienced laboratories had a tendency to set acceptance criteria and specifications too tightly, increasing the likelihood of failure during PV or routine production. PATH helped these facilities draft PV plans before initiating PV activities.

2.7. Analytical development

Analytical testing development or quality control laboratories had variable levels of resources available which affected in-process and product release testing. Some laboratories were limited by types of equipment and others by limited experience in appropriate application of methodologies.

As an example, in place of the HPLC-based method typically used in high resource pharmaceutical laboratories, PATH introduced for one manufacturer a colorimetric assay to measure vaccine Triton levels.

The SRID potency assay, also used during in-process testing to determine yield at various steps, presented problems for laboratories that did not dilute the samples. For samples with low HA, such as allantoic fluid, a full dilution SRID was not possible so PATH recommended a modified single point SRID. HA measurement at harvest can be confounded by the presence of HA not bound to virus particles, yet an approximation of hemagglutinin concentration at harvest was instrumental to study and optimize downstream process recovery.

HPLC can be a simple and efficient substitute for SRID when measuring total HA for in-process monitoring [4]. While HPLC only indicates total HA mass and cannot be used to measure stability, it can be a useful tool to measure and optimize recovery at specific process steps. For laboratories that had HPLC equipment, PATH provided the required support to develop the assay and sample preparation methods.

3. Quality management systems and common challenges

Good manufacturing practices (GMP), quality, production, facilities and equipment, laboratory controls, materials, and packaging and labeling sit under the broad umbrella of a quality management system (QMS). All manufacturers need appropriate QMS in place to adequately support these various systems and ensure product safety and efficacy. PATH QMS experts worked with several DCVMs to assess existing quality systems, mitigate gaps, and strengthen overall quality systems. Most efforts were focused in three areas: deviation identification, investigation, and risk management; method validation and documentation; and data integrity management.

3.1. Deviation identification, investigation, and risk management

New standard operating procedures (SOPs) had to be developed to delineate the deviation identification, investigation, and corrective processes. PATH experts worked with Quality Assurance (QA) staff to implement the SOPs under GMP. One of the primary goals was to foster a culture change in which deviations could be accepted as normal instead of as an admittance of failure. SOP implementation and appropriate deviation management led to new reporting systems that are routinely presented to the upper management teams.

3.2. Quality control: Method validation

Method validation is the process used to confirm that an analytical test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability, and consistency of analytical results; it is an integral part of any good analytical practice [5]. Some DCVMs did not have the time and resources to develop, optimize, and validate methods for critical assays. In some

cases, while manufacturers understood the value and need for method validation, the process and parameters by which to do so were not well understood.

Much of PATH's work emphasized method optimization, validation, and implementation. Again, PATH worked with DCVM analytical teams and QA staff to develop SOPs that guide method development, documentation, and validation. To provide on the job training, PATH quality and analytical method experts then worked with testing teams to develop new methods and work through method validation of several new and existing methods using the newly developed guidance SOPs. It is important to note that while the validation SOP was derived from FDA guidelines [5], the manufacturers followed national pharmacopeias for individual method validation or verification. Establishing formal procedures allowed for a more seamless transition into the overall QMS, because deviations could be identified, tracked, and managed as needed.

3.3. Quality control: Documentation and data integrity management

Perhaps the area that has required the most focus is documentation. From process or method development to final manufacturing process and conduct of process and method validation, in-process and release testing, all steps have to be meticulously recorded. Most importantly, the records have to be traceable and data integrity has to be proven and maintained. This applies to everything from appropriate documentation and storage of raw data, to notebooks and test records. PATH QMS experts worked with DCVM staff to develop appropriate documentation and systems that allowed manufacturing and process oversight by the Quality Assurance staff.

4. Common challenges in clinical trial implementation

Even under the best of circumstances and in countries with clear laws and policies on vaccine research, scientific, operational, and regulatory challenges inevitably arise when conducting clinical trials. This section highlights some of the challenges associated with planning and executing influenza vaccine candidate trials in low- to middle-resource countries that import most of their vaccines, including interpretations of the regulatory framework; the ethical review process; lack of effective community engagement; and laboratory issues.

4.1. Regulatory framework

The regulatory framework refers to the regulations and guidance documents that identify proper practices for drug or biologic manufacturing, testing, and product approval in a country. A proper regulatory framework provides a clear path for licensing approval.

Many countries have underdeveloped regulatory frameworks for clinical trial conduct, particularly on how to advance vaccines through the process [6]. In one country, where there are no existing regulations for influenza vaccine candidates, PATH and an experienced contract research organization provided technical assistance that led to implementation of the country's first-ever vaccine trial in healthy adults with a seasonal influenza vaccine candidate. This required PATH to provide training to governmental agencies on both vaccine clinical trials and research in healthy adults.

In countries where regulators are inexperienced, the default impulse is—understandably—one of excessive caution and unwillingness to deviate from familiar country guidelines. For instance, when a Phase 1 seasonal influenza study shows a favorable safety and immunogenicity profile and the dose selection has already been well established (as in the case of seasonal influenza vaccines), it can be reasonable to proceed directly to a Phase 3 licensure trial. If the guidelines lack this mechanism explicitly, however, it can be difficult to advance the approach in countries with inexperienced regulators. As a result, clinical trial planning for seasonal influenza vaccine may include an unnecessary Phase 2 component, which requires regulatory authority evaluation before proceeding to the Phase 3 licensure trial. This additional phase in the clinical development of a widely accepted vaccine adds complexity, leads to licensure delays, and further constrains resources that may be better used elsewhere.

4.2. Ethical review process

The independent ethical review process is a critical function that ensures the protection of research subjects in clinical trials [7]. Generally, review is satisfied by a committee composed of scientists, non-scientists, and at least one person who is not affiliated with the principal investigator's institution. Though single research proposals are frequently reviewed by multiple ethics committees, this is typically limited to multicenter trials. Sometimes, however, even though only one institution is interacting with research participants the ethics committees of other organizations providing financial support, technical assistance, etc. require review of the trial dossier. Several DCVM influenza vaccine trials, even though conducted at a single site in one country, were subject to three or four ethics committee reviews. This creates an undue burden on the research team and results in only marginal improvement, if any, of the research plan. Moreover, in PATH's case, the need for multiple translations of the documents further complicated the review. Some of the burden can be mitigated by use of an authorization agreement, a method that allows one institutional review board (IRB) to rely on the review of another, but this is only a partial solution [7]. Significant effort is needed to harmonize the multiple reviews, ensure committees are aware of the changes required by one another, and finally produce one version of the protocol and consent that achieves approval from all committees in the necessary languages. Additional challenges arise when ethics committees have opposing views and the research team has to develop a compromise acceptable to all committees.

4.3. Laboratory challenges

In early phase vaccine research, clinical laboratory screening can be an important factor in determining eligibility and detecting any abnormalities that may be elicited by the vaccine. Laboratory results help establish that a person is in good health and able to participate in the vaccine research. In addition, clinical laboratory tests conducted during the trial can establish adverse events or better explain the clinical picture of a participant who may have physical signs of illness. Endpoint laboratory testing can help determine whether or not a vaccine candidate should progress to later phase testing.

Inexperienced laboratories lacking in resources posed a challenge for influenza vaccine trials with DCVMs. In general, a lack of resources creates a lack of items that would be considered standard practice for clinical trials (such as temperature monitoring equipment). Laboratories may also lack standard certifications of properly functioning equipment, such as centrifuges—critical to preparing specimens for aliquot and storage.

Frequently PATH helped build laboratory capacity, train staff, and facilitate the receipt of appropriate control reagents for the assays. For example, some laboratories were trained to perform hemagglutination inhibition and microneutralization testing to support clinical development of both seasonal and pandemic

vaccines. PATH also provided support to develop and strengthen quality systems in these laboratories.

4.4. Lack of effective community engagement/recruitment

Community engagement is increasingly regarded as an important ethical practice in clinical research [8]. Though it is a key requirement for some areas of study, it is not widely practiced around the world due to the additional time and resources required before research begins.

At the most basic level, community engagement informs the community about planned research, the disease under study, and the aim of the study. At the highest level, community engagement includes community representatives in the research design, implementation, and dissemination of results.

The DCVMs that initiated influenza vaccine research experienced confusion between community engagement and recruitment. Recruitment is a process of inviting potential trial participants to join a study. Recruitment activities must be reviewed and approved by an IRB/ethics committee before the activities can begin, because recruitment is the start of the informed consent process. If community engagement is to be fully effective, it should begin before the ethics committee approves the research, so community attitudes and feedback can help guide materials developed for trial participants. Community input can also help frame issues critical to recruitment and retention, such as how participants should be contacted by the study team and how the study team can make participants comfortable. Good community engagement should inform the community about the health issue being studied and the upcoming vaccine trial (without recruiting), and ask about community concerns.

A lack of community engagement can create an information gap that negative attitudes and rumors might fill—which sometimes make their way to the media, fostering concern and distrust over clinical trial operations. Rumors surrounding one influenza trial claimed, in the absence of any evidence, that the vaccine was harmful and volunteers would not be compensated in the case of adverse effects. Such rumors made volunteer enrollment a challenge, and eventually required study staff to conduct home visits and host community meetings to build trust and reverse the damage rumors had done. Engaging the community before the trial began and providing education on the influenza virus, the vaccine trial, and the importance of the research would have better prepared the community and potentially prevented rumors from starting.

Similarly, some GAP countries opposed recruitment by advertisement. Subsequently, recruitment was limited to word-of-mouth and, in some countries, physician referral. This further hampered trial recruitment, since word-of-mouth can lead to misinformation about the research or the vaccine.

5. Conclusion

Since 2009, PATH has worked with several DCVMs on various aspects of manufacturing, testing, quality, and clinical development. Each DCVM has unique strengths and areas of expertise, which have allowed successful partnerships with international organizations such as BARDA, WHO, and PATH to develop and advance new products.

Under the GAP, and with direct support from PATH, DCVMs have made considerable progress in influenza vaccine development capacity. In particular, DCVMs have achieved optimized manufacturing conditions, increased yield, and vaccine capacity. DCVMs that initially lacked internal clinical teams, did assign staff to learn the clinical development process and are now working to develop clinical teams in-house. Certainly, DCVMs now have more capacity to implement clinical trials in compliance with international standards. And because QMS has been strengthened overall, the DCVMs are poised to produce high quality, low cost, efficacious vaccines to reduce the global burden of influenza. Already several manufacturers have advanced both seasonal and pandemic vaccine candidates that are in early clinical development, while others have candidates that are on the pathway to licensure.

Conflict of interest

Authors declare no conflicts of interest.

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