## *Special Report*

◆◆◆

# Technical Analysis of the State of DTP Vaccine Production in Latin America and the Caribbean[1]

AKIRA HOMMA,[2] JOSÉ LUIS DI FABIO,[3]
DARIO P. MIRANDA,[4] & OTAVIO F. P. OLIVA[5]

Most of the laboratories that manufacture vaccines for human use in the region of Latin America and the Caribbean were established at the beginning of this century and have already earned their place in history through their work in controlling and eradicating diseases of public health importance. Nevertheless, vaccination has existed in this region since the early 19th century, when the variolization technique (whereby healthy individuals were inoculated with material from smallpox vesicles from the benign form of the disease) was introduced, this being followed by vaccination against smallpox based on Edward Jenner's development of a smallpox vaccine derived from cowpox lesions (*1*).

By the early 20th century, a technique developed to propagate virus vaccines on the skin of cattle or sheep was already being used by several laboratories in the Region. Also, around this time Dr. Vital Brasil showed the importance of the specificity of antivenom sera for the treatment of snakebites—a technology disseminated throughout the world that represented significant progress in this field.

During the first half of the century, in the 1930s, the vaccine against yellow fever (strain 17D) was developed by the Rockefeller Foundation in collaboration with researchers in the Region (*2*); and some years later, in the mid-1950s, a method for producing rabies vaccine in newborn mouse brains was developed by E. Fuenzalida and R. Palacios (*3*). This latter method is used to this day by laboratories in the region because of its simplicity, high degree of efficacy, low levels of adverse reactions, and manufacture based on readily available raw materials.

In recent years Cuban scientists have developed methods for manufacturing vaccines against serogroup B meningococcal meningitis (*4*) and also against hepatitis B, the latter employing recombinant DNA (*5*); Argentine researchers, in collaboration with the Walter Reed Army Research Institute in the United States, have developed a live attenuated

[1]This report will also be published in Spanish in the *Boletín de la Oficina Sanitaria Panamericana*, Vol. 117, No. 3, September 1994.

[2]Regional Adviser in Biologics, PAHO/WHO.
[3]Bureau of Biologics, Drug Directorate, Canada.
[4]Consultant in Biologics, PAHO/WHO, Brazil.
[5]Bio-Manguinhos, FIOCRUZ, Brazil.

vaccine against Argentine hemorrhagic fever (*6*); and a Colombian research group led by M.E. Patarroyo has developed a synthetic antimalarial vaccine that is now in the last stages of clinical evaluation (*7*).

## CURRENT TECHNOLOGIC CHALLENGES

In recent decades vaccine manufacturing technology has made major strides in a number of areas. On the one hand, a series of standards and strict specific procedures have been established as "Good Manufacturing Practices" (GMP) (*8–12*) to guarantee the quality of vaccines produced. On the other hand, manufacturing technology has been improved so as to provide better vaccine purity and greater yields.

To keep up with these advances, laboratories in developed countries, as well as a few in the developing world, have invested large sums of money to upgrade existing installations, build new ones, and acquire more sophisticated modern equipment. However, the sizable investments required, the degree of technical complexity involved, and stiff competition have tended to concentrate vaccine manufacture in the hands of a few very large laboratories. In addition, there has been a trend toward globalizing technologic activities and forming strategic alliances to share technology, products, and markets in order to reduce development costs, financial risks, and monetary losses.

With respect to meeting today's quality control standards, a need has arisen for modern techniques capable of detecting impurities and characterizing various vaccine components with increased accuracy. This demand, in turn, has created a need for up-to-date specialized equipment.

Virtually all the laboratories manufacturing vaccines for human use in Latin America and the Caribbean are public laboratories that are directly or indirectly under the jurisdiction of the State. In varying quantities, they manufacture all the vaccines used in the Expanded Program on Immunization (BCG, DTP, DT, Td, oral poliomyelitis, and measles vaccines), as well as the Fuenzalida-Palacios rabies vaccine, the recombinant hepatitis B vaccine, and vaccines against yellow fever and meningococcal meningitis serogroups A, B, C, and W. The few private laboratories manufacturing vaccines for human use are very small and have hardly any impact on the market.

One point convincingly driven home at the September 1990 World Summit for Children was that millions of children's lives could be saved by improving vaccine quality and developing new low-cost vaccines for use in public health programs. Accordingly, development agencies and the scientific and technologic community were specifically asked to redouble their efforts to this end.

As part of this drive, the World Health Organization, United Nations Development Program (UNDP), United Nations Children's Fund (UNICEF), and Rockefeller Foundation organized what has become known as the "Children's Vaccine Initiative" (*13*). In connection with this work, a number of desired technologic developments have been identified and are currently being pursued. One of these is the production of an improved DTP vaccine and utilization of DTP as a basis for new vaccine combinations. DTP vaccine was singled out for attention partly because it is administered to very young children and partly because it will probably be needed for many years.

These new vaccines, improved and/or combined with other antigens, are already being produced by several laboratories in the developed countries. Therefore, the questions are whether laboratories in Latin America and the Caribbean area are technologically prepared

to participate in this new phase involving the manufacture of improved vaccines, what principal obstacles are currently being encountered in their manufacturing operations, what their technologic potential is, and what new mechanisms are available for exchanging technology. Above all, it is important to establish viable procedures for immunizing the 11 million children born each year in Latin America and the Caribbean against diseases preventable by vaccination.

## ANALYSIS OF REGIONAL LABORATORIES' TECHNICAL CAPACITY

To obtain appropriate technical information and assess the technical capacity of the 13 laboratories in the region presently producing DTP vaccine (Table 1), PAHO's Regional Vaccine System (SIREVA) recently conducted a survey based on visits by technical experts to the above-mentioned labs. The results of these visits were presented at the First Regional Meeting on Improved DTP and DTP-based Combination Vaccines, a conference sponsored by PAHO/SIREVA in collaboration with the Children's Vaccine Initiative that was held in Washington, D.C., in September 1993. Data from this survey and the corresponding technical assessments are presented on the pages that follow.

**Table 1.** A list of countries and institutions with laboratories producing DTP vaccine or its components in Latin America and the Caribbean.

| | |
|---|---|
| ARGENTINA: | Malbran Institute |
| | Central Laboratory of Public Health of La Plata |
| BRAZIL: | Butantan Institute |
| | Institute of Technology in Immunobiologicals (Bio-Manguinhos/FIOCRUZ) |
| | Technology Institute of Paraná (TECPAR) |
| | Vital Brasil Institute |
| CHILE: | Institute of Public Health |
| COLOMBIA: | National Institute of Health |
| CUBA: | Finlay Institute |
| ECUADOR: | Institute of Hygiene and Tropical Medicine "Leopoldo Izquieta Pérez" |
| MEXICO: | General Biologicals and Reagents Administration and the National Institute of Hygiene |
| URUGUAY: | Institute of Health "Dr. Arnaldo Berta," University of the Republic |
| VENEZUELA: | National Institute of Health "Rafael Rangel" |

### DTP Production and Demand: Current Status and Future Projections

If the nine DTP producing countries are grouped according to subregions (the Southern Cone, the Caribbean, the Andean area, and Mexico and Central America), it may be seen that the Southern Cone subregion (Argentina, Brazil, Chile, and Uruguay) has the highest demand and the highest projected capacity. Cuba has projected production well above its own needs and has plans to export DTP vaccine. Production and demand in the other subregions will be fairly well balanced once the producing countries reach their projected production capacities.

The figures for installed capacity were calculated taking into account the current installations and existing equipment, as well as present possibilities for improving production yields. Laboratory production volume was found to vary from 0.4 million doses per year to 9 million. Even though the overall reported production capacity was said to approach 39 million doses a year, total 1992 production came to only 22.3 million doses, corresponding to 57.3% of stated production capacity or 24.7% of the annual demand in all the region's countries, which was estimated at 90.2 million doses.

**Table 2.** The status of DTP vaccine in the nine producing countries of Latin America and the Caribbean, showing theoretically installed capacity and production as of 1992, projected future production, and demand.

| Country | Current installed capacity ($\times 10^6$ doses) | Current production (1992) ($\times 10^6$ doses) | Projected production | Demand* |
|---|---|---|---|---|
| Argentina† | 2.0 | 0.4 | 3.0 | 5.8 |
| Brazil‡ | 3.5 | 3.5 | 60.0§ | 32.0 |
| Chile | 6.0 | 2.5 | 12.0 | 2.4 |
| Colombia | 3.5 | 2.8 | 3.5 | 6.5 |
| Cuba | — | — | 60.0§ | 1.4 |
| Ecuador | 2.5 | 0.9 | 8.0 | 2.4 |
| Mexico | 9.0 | 9.0 | 15.0§ | 17.0 |
| Uruguay | 0.4 | — | 1.0 | 0.5 |
| Venezuela‖ | 12.0 | 3.2 | 15.0§ | 4.1 |
| Total | 38.9 | 22.3 | 177.5 | 72.1¶ |

*Calculated on the basis of 3 doses in the first year plus a booster at 15–18 months and a loss of 50%, for 1993.

†Argentina has two producers.

‡Brazil has four producers, only one of which is producing DTP at present.

§The specific project and financial support for it have been approved, and vaccine production is scheduled to begin in 1994 or early 1995. The other laboratories listed are still working on proposals.

‖Information presented at the First Regional Meeting on Improved DTP and DTP-based Combination Vaccines indicated that Venezuela had achieved self-sufficiency in DTP vaccine.

¶Total demand of the DTP-producing countries (the total demand of the entire Latin American and Caribbean region is estimated at 90.2 $\times 10^6$ doses).

As of 1992, only 7 of the 13 regional laboratories surveyed were producing all three components of DTP vaccine (see Tables 1 and 3). Two others, one in Argentina and one in Uruguay, were producing diphtheria and tetanus components and formulating bivalent DT or Td vaccines. These laboratories had suspended production of the pertussis component because of technical problems. One Brazilian laboratory was producing a single component, tetanus toxoid. As noted above, three laboratories with large planned production capacities were not yet operating at the time of the survey.

Only Chile and recently Venezuela have reported having sufficient production capacity to meet their own demands. For this reason, almost all the region's operating laboratories have plans to expand production. Some countries, such as Brazil and Cuba, are currently building modern plants for large-scale manufacture of bacterial vaccines. These facilities, supplemented by other planned expansions, would raise present capacity 4.6 times, to a level of 177.5 million doses per year. In the case of Cuba, the vaccine production plant (which was in the final stages of being set up in September 1993) commenced production at the end of 1993. This plant has a projected production capacity of roughly 60 million doses of DTP per year. In Brazil, one laboratory recently inaugurated a new production installation. In addition, investment in the construction of two other laboratories will raise the country's current production capacity from 3.5 million doses per year to approximately 60 million. These two latter laboratories are scheduled to begin start-up operations in 1994.

It is important to point out that all of these new plants are designed to produce

**Table 3.** Production of diphtheria toxoid, tetanus toxoid, pertussis vaccine, and complete DTP vaccine in 1992 at each of the 13 laboratories surveyed, together with their theoretical installed capacities for producing the components and DTP and their projected future production of DTP as of the dates indicated in Table 2.

| | Diphtheria toxoid (× $10^6$ Lf/year) | | Tetanus toxoid (× $10^6$ Lf/year) | | Pertussis vaccine (× $10^6$ OU/year) | | DTP (× $10^6$ doses/year) | | |
|---|---|---|---|---|---|---|---|---|---|
| Lab | Installed capacity | 1992 production | Installed capacity | 1992 production | Installed capacity | 1992 production | Installed capacity | 1992 production | Projected production |
| 1 | 10 | 2 | 28 | 20 | — | — | 0.5 | — | 0.5 |
| 2 | 30 | 25 | 24 | 24 | 15 | 9 | 1.5 | 0.4 | 2.5 |
| 3 | 350 | 250 | 540 | 350 | 150 | 64 | 3.5 | 3.5 | 20.0 |
| 4 | —* | — | 500 | 350 | — | — | — | — | — |
| 5 | — | — | — | — | — | — | — | — | 20.0 |
| 6 | — | — | — | — | — | — | — | — | 20.0 |
| 7 | 120 | 60 | 60 | 60 | 48 | 32 | 3.5 | 2.8 | 3.5 |
| 8 | 200 | 100 | 400 | 205 | 256 | 240 | 6.0 | 2.5 | 12.0 |
| 9 | — | — | — | — | — | — | — | — | 60.0 |
| 10 | 68 | 40 | 40 | 30 | 15 | 11 | 2.5 | 0.9 | 8.0 |
| 11 | 800 | 340 | 900 | 690 | 600 | 375 | 9.0 | 9.0 | 15.0 |
| 12 | 16 | 12 | 12 | 9 | — | — | 0.4 | — | 1.0 |
| 13 | 180 | 90 | 200 | 80 | 320 | 128 | 12.0 | 3.2 | 15.0 |
| Total | 1 774 | 919 | 2 704 | 1 818 | 1 404 | 859 | 38.9 | 22.3 | 177.5 |

*— = no production

a variety of antigens, based on the need for vaccines in either the country or the region. In other words, in addition to the components of DTP vaccine, they can manufacture other bacterial vaccines produced in fermentors.

Assuming all goes well, once the new and expanded plants are operating at capacity, Brazil, Chile, Cuba, Mexico, and Venezuela will have the ability to meet not only their own national needs but also the needs of the entire region. It should be noted that the adoption of the acellular pertussis (since it utilizes only a few antigenic components of bacteria) will require a much larger production capacity, thus changing the production situation in the region.

## Current and Projected Capacity to Produce DTP Vaccine and its Components in the Region

Table 3 presents figures on the installed capacity and 1992 production of DTP components by the 13 laboratories surveyed. The data shown indicate the diphtheria component was being produced at 51.8% of installed capacity in 1992, within a range for the individual producing laboratories of 20–83%. Similarly, the tetanus component was being produced at 67.2% of capacity within a range of 40–100%, and the pertussis component was being produced at 61.2% of capacity within a range of 40–94%. (As of 1992 the pertussis component was being manufactured by only seven laboratories, another two having suspended production of it due to technical problems.) Overall, total DTP production stood at only 57.3% of the region's installed capacity.

These differences between actual output and installed capacity appear due to a broad range of technical problems. In some laboratories, for example, there were no technical production backups, while in others the production levels were so low as to suggest inconsistencies in the production process, raising questions about vaccine quality.

## Formulation of DTP Vaccines

Differences were found in the antigen concentrations formulated for DTP vaccines manufactured at different regional laboratories. The diphtheria component ranged from 7.5 to 30 flocculation units (Lf) per dose; the tetanus component ranged from 2.5 to 20 Lf per dose; and the pertussis component ranged from 8 to 16 optical density units (OU) per dose.

While these formulations met the minimum requirements of the World Health Organization (concentrations of diphtheria toxoid <30 Lf/dose, tetanus toxoid <25 Lf/dose, and pertussis component <40 OU/dose) (*14*), it is nevertheless important to know the technical and scientific criteria used to define the various antigen concentrations. The antigen concentrations in a DTP vaccine should be sufficient to ensure that 100% of the lots formulated will be able to pass potency tests for each of the antigens in question. The different concentrations of diphtheria and tetanus antigens may indeed be related to use of different potency tests adopted in the region, such as those of WHO and the U.S. Food and Drug Administration (FDA), and to the absence of a reference vaccine for use in these tests. Only one laboratory produced 1 ml doses of DTP vaccine; all the others produced 0.5 ml doses. The concentrations of aluminum and thimerosal employed in making these vaccines were found similar in all the laboratories visited.

## Diphtheria Toxoid Production (*15*)

In 1992, the nine laboratories involved produced 919 million Lf units of diphtheria toxoid, as compared to their the-

oretical installed capacity of 1 774 million Lf units per year. The individual outputs of the laboratories ranged from 2 to 340 million Lf units, as compared to an installed capacity of 10 to 800 million Lf units per year.

All the laboratories were using the Parke-Williams strain 8 (PW-8) of *Corynebacterium diphtheriae*, but this came from different sources, often without any reliable record regarding its origin or toxin-producing capacity. There was also a difference in the ways lots were preserved during the production process: three of the laboratories lyophilized the lots while the others froze them. These potential source and preservation variations could partly explain the performance variations observed for diphtheria toxoid produced by different laboratories. Those laboratories with low production levels might wish to consider employing a *C. diphtheriae* strain of known origin with high toxin-producing capacity for the culture method used.

All the laboratories visited were using the seed lot concept, but the strains employed could have originated from working lots of the laboratories providing them. Within this context, it is important to underline the need to establish microbiologic and biochemical control and define other characteristics of the seed strain before it is introduced into the regular production process.

Most of the laboratories (seven of them) were still using the static culture method for production of the diphtheria component. The culture media varied according to whether the static culture or fermentor approach was being used. Production volumes with the static culture method ranged from 20 to 70 l per operation and yielded an antigen concentration of 70–120 Lf/ml. The volume of culture medium processed in the fermentor was considerably larger (250–700 l), but the concentration of the diphtheria antigen produced by this method was very low (50–85 Lf/ml) compared with fermentor yields (around 200 Lf/ml) obtained by large private laboratories. To improve the region's output, measures including the following need to be considered:

- Using a strain better suited to the deep culture process.
- Selecting the culture medium and its components so as to produce a large biomass quantity without inhibiting the production of toxin.
- Effectively managing the conditions and parameters of fermentation procedures—including the geometry of the fermentor, the volume of the culture medium, the degree of agitation and the speed of propeller rotation, the amount of dissolved oxygen incorporated in the medium, the quantity and quality of the antifoaming agent, the quantity and quality of the inoculum, the pH of the medium, and the fermentation time.

Differences were also observed in the processes used to separate the biomass from the culture medium. Only two laboratories had adopted the closed system, one employing continuous-flow centrifugation and the other tangential filtration. Classical centrifuges were used by two others, the remainder using filtration, either by membrane filter presses (in three cases), or gravity filtration through filter paper (in two cases). (In general, it is desirable that closed systems be used for processing the cultures and biomass, as this avoids exposure of the product to the environment and also facilitates fulfillment of GMP standards.)

Detoxification was performed in seven of the laboratories after separation of the bacterial mass. One of the other two laboratories detoxified the concentrated toxin, while the last detoxified with the cells

present. The latter procedure, although much safer, could account for this laboratory's relatively low production yields.

All nine laboratories concentrated their diphtheria toxoid by ultrafiltration, eight using 10 000-dalton membranes and one using 30 000-dalton membranes. All the laboratories purified their products by precipitation using ammonium sulfate, but some performed only two precipitations while others performed three.

A technologic alternative would be to filter the culture, then do the concentration using sterile 30 000-dalton ultrafilters, and then proceed to detoxify, adding an amino acid (glycine or lysine) together with formaldehyde to prevent toxin reversion.

The percentage of diphtheria toxoid obtained through these production processes by the different laboratories varied from 25% to 77% of the toxoid originally present in the cultures. The low end of this range (25%) clearly reflected the use of inadequate and obsolete technology at various stages of the production process, since the yield should always exceed 70%.

The purity of the toxoid produced ranged from 930 to 1 780 Lf/mg protein nitrogen (PN). Although only three of the nine laboratories' products had the minimum purity of 1 500 Lf/mg PN required by WHO standards (*14*), the vaccines prepared with this toxoid were nevertheless released for routine use in vaccination programs. No registers were available for noting the occurrence of diphtheria toxoid reversion.

## Tetanus Toxoid Production (*16*)

In 1992 a total of 10 laboratories produced 1 818 million Lf units, as compared to their theoretical installed capacity of 2 704 million Lf units per year. The individual outputs of the laboratories ranged from 9 to 690 million Lf units, as compared to an installed capacity of 12 to 900 million Lf units per year.

The same *Clostridium tetani* strain was used in all the laboratories, although there were differences with respect to its origin and the manner in which it was maintained. The observations made with regard to diphtheria strain procurement and maintenance also apply to the *C. tetani* used to produce tetanus toxoid.

Six laboratories were using the static culture method, with production volumes ranging from 40 to 150 l. Four of these laboratories produced yields with relatively low titers (in the range of 40–60 Lf/ml), whereas the two others obtained somewhat better titers (≥90 Lf/ml).

The four other laboratories used fermentors processing volumes of culture medium ranging from 300 to 600 l per operation. The tetanus toxoid titers obtained were very low for this procedure, ranging from 40 to 90 Lf/ml.

In order to improve the production conditions for this toxin, it will be necessary to consider the manufacturing method being used together with a broad range of factors including the following:

- The bacterial strain and the method used to maintain the seed lot.
- The culture medium and components used in production; the source of nitrogen, carbon, etc.; and the method of sterilization.
- Culture conditions, agitation, pH, temperature, etc.

As in the production of diphtheria toxoid, several methods were used to separate the biomass. Only one laboratory had adopted closed-circuit tangential filtration, the most modern methodology and the one that allows the best production yields.

Only two of the laboratories were performing detoxification on the concen-

trate; the others were doing this step with the cells present. To curtail losses in the detoxification of tetanus toxoid, it is advisable to filter it before placing it in the oven for detoxification; but this would require that the producing laboratory have closed-circuit systems for separating the biomass as a safety precaution.

In performing ultrafiltration of the tetanus toxoid, five of the laboratories used 10 000-dalton membranes and five used 30 000-dalton membranes. In nine laboratories, the final stage of purification was carried out with precipitation using ammonium sulfate and subsequent dialysis. Only one facility performed chromatographic purification.

A technologic alternative would be to centrifuge or filter the cultures in a closed-circuit system, then provide ultrafiltration with a 30 000-dalton membrane to concentrate the toxin, then perform sterile filtration, and finally detoxify the product, adding glycine along with formaldehyde.

Overall, the percentage of tetanus toxoid recovered by means of the existing production processes ranged from 26% at one laboratory to more than 60% (considered the minimum acceptable) at six facilities.

The purity of the toxoid ranged from 700 to 2 000 Lf/mg PN per dose; seven laboratories achieved a purity exceeding 1 000 Lf/mg PN per dose, which is the minimum level specified in the WHO requirements (*14*). As in the case of diphtheria toxoid, no registers were available for noting the occurrence of toxoid reversion.

## Pertussis Vaccine Production (*17*)

In 1992 seven laboratories produced a total of 859 million opacity units (OU) of the pertussis component (versus an installed capacity of 1 404 million OU/year). The individual outputs of the seven producing laboratories ranged from 9 to 375 million OU, relative to an installed capacity of 15 to 600 million OU/year.

Several strains of *Bordetella pertussis* of varying origins were used. The observations made previously regarding the diphtheria and tetanus strains also apply to these pertussis strains. In cellular vaccines, the agglutinogens in the strains used must meet the criteria specified by Preston (*18*); and since these are not easily determined, only strains from laboratories that can certify the presence of these surface agglutinogens should be employed. Immediately after the arrival of the strain in the production laboratory, it should be propagated with the fewest possible transfers and made into a lyophilized seed lot. It is then desirable to again assay for the presence of agglutinogens. In the case of one producing laboratory, which was employing a single strain, the need to determine whether this strain possessed all the agglutinogens required for an effective pertussis vaccine appeared especially critical.

The culture media varied, depending on whether the static process or fermentors were employed. In the three laboratories that used the static method, the volume produced in one operation ranged from 20 to 40.5 l. Although the antigen concentrations obtained were relatively high, ranging from 150 to 400 OU/ml, the use of bottles greatly limited production volume. In the four laboratories utilizing fermentors, the volume of culture medium processed in one operation ranged from 30 to 950 l and the resulting antigen concentrations ranged from 35 to 90 OU/ml. This last concentration is above average for the fermentor process, and if consistency of this result is confirmed, it would be important that the other laboratories using fermentors adopt the same methodology and technology in order to obtain this level of bacterial mass concentration.

In three of the four laboratories using fermentors, separation of the biomass was accomplished by acid precipitation (using citric acid), a technique that some say increases toxicity. Of all the producing laboratories, only two were employing a closed-circuit system using continuous centrifugation or tangential filtration, the system that should be adopted in order to obtain a better-quality product.

Several different detoxification processes were used. Three laboratories used a combination of heat and thimerosal; two others used thimerosal alone; and the remaining two used formaldehyde. Generally speaking, it is important that the detoxification method used be one that has been validated, that gives reproducible results, that does not increase toxicity, and that preserves the product's immunizing capacity.

Regarding the percentage yield obtained from the entire production process, one laboratory had a yield of only 45%. However, four others had yields exceeding 80%, which may be considered good results. Nevertheless, production of this pertussis component was found to present numerous technical problems for the facilities seeking to manufacture DTP, which is why two of these laboratories had suspended production of this component at the time of our visit.

## Formulation of DTP Vaccine

Only three of the eight laboratories actively formulating DTP vaccine were found to have an independent area specifically dedicated to formulation that was also bioclean in accordance with GMP standards (*8, 9*). (These standards require that entry to the area be restricted to the personnel directly involved in these formulation activities, that there be protective barriers, that the air be filtered by a High Efficiency Particulate Air (HEPA) filter, that positive air pressure be employed, and that the personnel involved wear sterile clothing.)

Aluminum phosphate was used as an adjuvant in four of the producing laboratories (see Table 4), while the other five used aluminum hydroxide. Four of the latter five imported an aluminum hydroxide gel from Denmark.

The concepts of lot and sublot were interpreted differently by different laboratories. Only four were found to use the concept of "lot" established for vaccine formulation in the WHO requirements (*14*), and only three were using the WHO concept of "sublot."

According to WHO, all the vaccine components and the adjuvant should be mixed in a single formulation tank. The vaccine should then be homogenized immediately and dispensed into sublots. However, most of the producing laboratories surveyed did not have formulation tanks for large-volume lots. Instead, most of them formulated a "lot" in a series of 20-liter or 30-liter containers, adding a concentrate of each of the respective components and the adjuvant to each container, and this series of containers was considered a single "lot."

According to the WHO requirements, each of these containers should be considered a single lot (thereby creating a very large number of lots) because formulation is a complex operation, and each of the several necessary steps entails risk of contamination and adds considerably to the challenge of quality control.

According to the definitions of the producing laboratories, a total of 157 lots of DTP were produced in 1992, but according to the WHO definition the actual total was 776. Therefore, instead of performing 157 potency tests for the diphtheria component, 157 for the tetanus component, and 157 for the pertussis component, it would have been necessary to conduct 776 tests of each. As all this suggests, there is a clear need to employ

better technologies in the formulation process.

## Filling of DTP Vaccine Vials

Table 4 presents 1992 data on the nine actively producing laboratories' filling installations and systems. Eight (all except one) had an independent area dedicated to filling vaccine vials, although only four of these areas were bio-clean according to GMP standards.

Only one laboratory was using a manual filling system. All the others had semiautomated or fully automated systems. While the capacity of the equipment ranged from 1 000 to 12 000 vials per hour, the percentage utilization of this capacity varied considerably. This percentage was calculated on the basis of 800 hours per year, a total derived from 4 hours of use a day for 5 days a week and 40 weeks a year). The specific utilization percentages (shown in Table 4) suggest underutilization of the equipment at some or even most of the laboratories, though the degree of underutilization could be changed by plans for expanded output.

Most of the laboratories were producing multidose vials, with one lab having a 40-dose presentation. Such a presentation is inadequate because its use entails a relatively high risk of contamination and wastage. Two laboratories were producing single-dose vials, one of which was sealed in plastic.

## Installation Conditions at the 13 Planned or Producing Laboratories (*19, 20*)

### *Storage Rooms*

The storage room is an essential part of the vaccine production process, without which it is impossible to manage or control flows in and out, quarantined products, releases, and the shelf life of various materials and supplies. At four of the 10 operating laboratories the storage room was specifically built for that purpose and was well organized. Four of the others had storage rooms that had been adapted for the purpose and were in adequate condition. One laboratory had a storage room that was adapted for the purpose but was inadequate—being too small and not well situated. And finally, one laboratory had no storage room—the supplies, equipment, and materials being scattered about various laboratory spaces and corridors. In sum, eight laboratories had storage rooms in good condition, whereas two had poor storage arrangements.

### *Production Area*

Of the 10 laboratories operating at the time of the survey, only two had an antigen production area that was specifically designed for production and met the GMP requirements (*8*) (by being bioclean and having adequate traffic flows and protection barriers). One of the eight remaining laboratories was producing in an area adapted for the purpose where the working conditions were adequate, but the other seven had producing areas adapted for the purpose that did not offer adequate working conditions. Specifically, they were inadequate in terms of traffic flows, the mixing together of sterile and contaminated materials, lack of protective barriers, and several other technical problems that would be difficult to overcome. Of the 10 operating laboratories visited, five were judged to be in good working condition.

The three laboratories in the process of being set up were planned with production areas specifically designed to provide good working conditions and meet all the international requirements for installations producing antigens for use in human vaccines.

**Table 4.** Arrangements at the nine producing laboratories for filling DTP vaccine vials, showing the type of filling area (independent and/or bio-clean), the type of filling system (automatic or manual) and capacity (in thousands of vials per hour), installed capacity for annual production (in millions of vials per year, assuming operation at the hourly rate for 800 hours per year), DTP produced in 1992 (in millions of doses per year), the amount of filling capacity used, and presentation of the final product as multidose or single dose vials.

| Lab | Independent area | Bio-clean area | Type of filling system and capacity | Installed capacity for filling vials (million/year) | DTP doses produced in 1992 (million/year) | % use of filling capacity† | Presentation of vials |
|---|---|---|---|---|---|---|---|
| 1 | Yes | No | Automatic, $3 \times 10^3$ vials/h | 2.4 | 0 | 0 | Multidose |
| 2 | Yes | No | Automatic, $1 \times 10^3$ vials/h | 0.8 | 0.4 | 5 | Multidose |
| 3 | Yes | Yes | Automatic, $12/4.4 \times 10^3$ vials/h | 9.6/3.5 | 3.5 | 4/100‡ | Multi/single dose |
| 7 | Yes | No | Automatic, $3 \times 10^3$ vials/h | 2.4 | 2.8 | 120‡ | Single dose |
| 8 | Yes | Yes | Automatic, $2 \times 10^3$ vials/h | 1.6 | 2.5 | 16 | Multidose |
| 10 | No | No | Manual, $0.2 \times 10^3$ vials/h | 0.16 | 0.9 | 14§ | Multidose |
| 11* | Yes | Yes | Automatic, $5 \times 10^3$ vials/h | 4.0 | 9.0 | 23 | Multidose |
| 12 | Yes | No | Automatic, $1.6 \times 10^3$ vials/h | 1.3 | 0 | 0 | Multidose |
| 13 | Yes | Yes | Automatic, $5 \times 10^3$ vials/h | 4.0 | 3.2 | 8 | Multidose |

*Filling system being reorganized.
†Assuming 10 doses of DTP per vial, except where indicated.
‡One dose per vial. The 120% use of filling capacity at laboratory 7 was due to operation of the filling equipment for more than the 800 hours used to estimate the annual installed capacity for filling vials.
§40 doses per vial.

### *Formulation Area*

One of the nine producing laboratories was designed with a dedicated vaccine formulation area. One laboratory had adapted its area, which was adequate for the purpose; and another did the same thing but obtained unsatisfactory results. Six of the laboratories did not have a specific area for vaccine formulation, creating a potentially hazardous situation. In two of the laboratories the formulation area was well maintained. The three laboratories in the process of being set up had areas that were specifically designed and adequate for formulation.

### *Filling Area*

The filling area in two of the eight actively producing laboratories with such an area was specifically designed for the purpose, but in one of them the area was inadequate. Two laboratories had areas adapted for this purpose that met the technical requirements, while four others were in the process of adapting filling areas that did not meet all the established requirements. Five of the 10 operating laboratories had well-maintained filling areas, while the three laboratories in the setup stage were being planned with areas specifically designed to serve this purpose and meet all specifications.

### *Cold Room*

In general, the cold rooms (used for stocking supplies, vaccines in bulk, quarantined products, and released vaccines) were designed specifically for their purpose and were well maintained. Only two were not specifically designed to serve the intended purpose; these were very small, were located in unsuitable areas, and were used for stocking a variety of materials.

### *Laboratory Animal Facilities*

Five of the 10 operating laboratories had a technically adequate animal area built specifically for the purpose. One had an especially designed animal area that was not technically adequate. The remaining four had animal areas suitably adapted for the purpose, but in two cases these areas were not in adequate condition. Overall, seven of the operating laboratories had animal colonies that were well maintained. However, in general there was a serious shortage of laboratory animals, especially of the genetically and microbiologically controlled animals essential for quality control testing of vaccines and their components.

## Personnel

The GMP standards determine that an institution dedicated to vaccine production must have a health unit for monitoring the staff's health conditions. As indicated in Table 5, all but two of the laboratories had a specific health program for the staff. In general, they used a routine medical service provided for the entire institute.

Technical training was provided regularly and routinely at all but two of the laboratories visited. Training in biosafety (*21*) was provided at eight. Only one laboratory reported that it had never provided a course in GMP for its production staff. In some cases, however, the knowledge provided by the training was not being effectively utilized. For example, in several of the laboratories offering courses in GMP, the GMP standards were not being applied. This demonstrates the need to change the attitudes of laboratory personnel in order to have them apply their knowledge to concrete situations.

The number of staff members assigned to the production area of a given laboratory tended to depend on the com-

**Table 5.** Health programs and training provided for personnel by the 13 laboratories visited.

| Lab | Health program | Type of training | | | Numbers of staff members assigned | | | |
|---|---|---|---|---|---|---|---|---|
| | | Technical | Biosafety | GMP | Production | R & D* | Quality control | Total† |
| 1 | No | Yes | Yes | Yes | 44 | — | 5‡ | 68 |
| 2 | Yes | Yes | No | Yes | 20 | — | 17‡ | 59 |
| 3 | Yes | Yes | No | Yes | 142 | 45 | 36 | 760 |
| 4 | Yes | No | No | Yes | 15 | 3 | 22 | — |
| 5 | Yes | Yes | Yes | Yes | 164 | 11 | 33 | 253 |
| 6 | Yes | Yes | Yes | Yes | 156 | 8 | 12 | 436 |
| 7 | Yes | No | No | Yes | 100 | — | 10‡ | 500 |
| 8 | Yes | Yes | Yes | Yes | 82 | 2 | 23 | 503 |
| 9 | Yes | Yes | Yes | Yes | 165 | 176 | 47 | 503 |
| 10 | No | Yes | Yes | Yes | 51 | — | — | — |
| 11 | Yes | Yes | Yes | Yes | 197 | 8 | 50 | 342 |
| 12 | Yes | Yes | Yes | No | 20 | — | 4‡ | — |
| 13 | Yes | Yes | No | Yes | 122 | — | 3 | 148 |
| | | | | Total | 1 278 | 253 | 262 | 3 649 |

*Research and development specific for vaccines.
†Total in the institution.
‡Not organized as internal control.

plexity of the production process at that facility. As noted above, some laboratories were manufacturing a single component, others two components, and others complete DTP vaccine as well as several other vaccines for human use. As Table 5 indicates, those laboratories that were still organizing for DTP production had already assigned staff members for this purpose; overall, three laboratories had 15 to 20 people assigned to production; three others had 44 to 82; and seven had 100 to 197.

Seven laboratories had a research and development section, the number of staff members assigned to this section's activity ranging from 2 to 176. However, only a few of the laboratories had specific vaccine research and development projects, mainly because each of the laboratory facilities was attached to a national institute or agency with responsibilities in all areas of health.

## Quality Control

Table 5 also shows that the 12 laboratories exercising quality control assigned anywhere from 3 to 50 staff members to this task. Indeed, one laboratory (which indicated no technical or biosafety training) reported having more personnel assigned to quality control than to production. In general, it appears that the number of staff members assigned may be affected by the manner in which quality control is carried out and also by other functions of the staff members at the institution responsible for the laboratory, since the institutions involved carry out other public health activities.

Of the nine operating laboratories that provided data on quality control activities, only four were found to exercise internal quality control outside the production area, as recommended by the international standards and WHO requirements (*22, 23*). At these nine laboratories, the production staff also performed the quality control tests. Although none of these tests for controlling the production process (e.g., testing of pH or application of the Gram stain for a microscopic test of purity) could be eliminated because of pressure to reach a quick decision, final responsibility for the quality of the intermediary and final products rested with the internal quality control staff of the production laboratory.

With regard to quality standards followed, eight of the nine laboratories reported following either the WHO requirements, standards of other countries, standards of the U.S. Food and Drug Administration, or standards of the *Pharmacopoeia Europeia*. Only one laboratory said it simply followed its own country's national standards.

Only two laboratories applied quality control to all the inputs used in the production process. Four other laboratories exercised partial control over these inputs.

In six of the nine operating laboratories that provided data on quality control activities, documentation of a given vaccine lot was being kept in a single file. In contrast, at the remaining three laboratories the file records were incomplete or were found scattered in different places.

Of the three new laboratories in the process of being set up, two had already established internal quality control at the time of the survey—in order to implement quality control in the design of production parameters. However, in one of the production laboratories there was no internal quality control or control of intermediate products, while testing of the final product was done by the country's National Quality Control Laboratory. Also, of the nine countries listed in Table 1, three had no national quality control laboratory. Only one of the laboratories visited was found to systematically perform external reference quality control of DTP vaccine.

It is important to emphasize that the manufacturer's responsibility does not end with production and distribution of the vaccine. The manufacturer is responsible for any problems that may occur in connection with utilization of a poor quality, low potency, or contaminated vaccine, as well as for any adverse reactions that may occur in response to inappropriate vaccines. It is thus essential that the production process meet the required standards in order to ensure the quality of the final product.

## CONCLUSIONS

The long tradition of vaccine production in the region is an integral part of the area's historic public health development, a development paralleling that of the more industrialized nations. However, in recent years only Chile's National Institute of Public Health has consistently produced enough DTP vaccine to meet national demand, though information presented in 1993 indicated that Venezuela's "Rafael Rangel" National Institute of Health had also achieved a production level consonant with national self-sufficiency.

It is encouraging to note that the laboratories in Brazil, Cuba, and Mexico are receiving large government investments and making effective strides toward establishment of modern plants specifically designed for human vaccine production that will satisfy all the technical requirements set forth by prevailing international standards and WHO. At the same time, some of the currently operating laboratories making DTP or its components are considering how they might modernize their facilities, while others are in need of finding ways to overcome pronounced problems affecting current production activities. Within this context, whatever the precise goals involved, the following are some of the matters that need to be considered by all laboratories desiring to change the present situation:

(1) Since virtually all the vaccine production laboratories of the region are directly or indirectly dependent on the State, all of these laboratories should demand that their respective governments make a political commitment to support their work. Such a commitment should be accompanied by a broad overall plan that includes short-, medium-, and long-term planning; targets specific products; and provides for the investment needed to attain the proposed goals.

Governments in some of the vaccine-producing countries have already made political commitments along these lines, and a few have established programs directed at achieving national self-sufficiency in production of essential vaccines—programs with short- and medium-term plans for modernizing production and maintaining effective quality control laboratories.

(2) Each country engaged in vaccine production should establish a National Control Authority with regulatory functions such as product registration and laboratory licensing, and if possible should possess or create a National Quality Control Laboratory. If the latter is not feasible because of insufficient funding or because very few lots are used, the National Control Authority should establish alternate ways of testing vaccines before they are released for use. (Development and use of a quality control laboratory network is a valid alternative for countries that have not yet organized a quality control system.)

(3) It is worrisome that some operating vaccine laboratories actually do not

have internal control mechanisms. Such mechanisms constitute an essential part of any vaccine production scheme and should be established as soon as possible in any producing laboratory not having them in place. In addition, quality assurance activities, together with biosafety and GMP standards, should be effectively incorporated into the regular laboratory routine.

(4) Most of the institutions engaged in vaccine manufacture in the region do not have the organizational, administrative, or managerial characteristics needed to ensure economically and financially self-sustaining production of biologicals. To begin with, only three of the 13 laboratories surveyed had an appropriate administrative structure encompassing the study of cost-benefit aspects of their various production activities.

In addition, the production activity in a vaccine laboratory requires a special organizational support structure, as follows:

(a) An administrative unit with managerial mechanisms that are flexible and permit quick decision-making. It is essential to have specific sections assigned to deal with personnel matters, the management of expendable materials and supplies, finances, and various other concerns depending on each laboratory's level of complexity. Among other things, it is very important for each laboratory to establish a personnel policy that offers wages both compatible and competitive with the private sector in order to attract, encourage, and motivate new talent.

(b) An internal quality control unit and quality assurance system that is independent of production operations and technically capable of providing professional advisory services to the production unit.

(c) A technical unit to maintain the highly specialized equipment that is usually found in the production area.

(d) A technologic development unit capable of supporting both the production and quality control activities.

(5) As suggested above, like those engaged in any productive activity, it is important for vaccine-producing institutions to study cost-benefit relationships and the economic viability of their work. The information gained from studies of this type can serve to correct or define the course of the activity.

In general, the production activity should be self-sustaining and capable not only of recovering the cost of operations and the investments made, but also of using the income generated to regularly update its installations and equipment and to invest in research and development. Thus, it is a *sine qua non* for laboratories that plan to expand their operations or invest in new installations to carry out this type of study, as well as others, in order to assess the quality of the technology they are going to use, the equipment to be selected, the technical support requirements of the equipment, the availability of locally produced or imported supplies and raw materials, the nature of the vaccine product market, etc.

(6) Today shared production is quite routine among private laboratories. The idea is to make the most of each facility's technical capabilities in a given area and to seek out complementary capabilities at other laboratories in order to make all the

activities more effective. In Latin America and the Caribbean there are laboratories that produce only one or two DTP components; shared production would seem the most logical option for dealing with this situation.

(7) In order to implement item 6 above, it is important to establish a system for certifying laboratories that have adequate technologic conditions for vaccine production; in this way, a laboratory obtaining a vaccine component from another laboratory that is certified will be able to expect that the product will possess adequate quality.

(8) Although laboratories in the region are receptive to the idea of exchanging information, this option is not being fully explored. Hence, it is important to find ways of strengthening technical exchanges—including exchanges of experiences, production strains, training activities, methodologies, etc.

(9) To judge from the intense technologic development taking place at several vaccine laboratories in the developed countries, it can be assumed that new and better vaccines will be appearing in the very near future. The process for manufacturing these vaccines will also be more technologically complex. Therefore, laboratories wishing to participate in this new technologic "revolution" will need to have the technical and scientific capacity to incorporate the new technologies required. Among other things, they should have installations and equipment that meet the international GMP standards and WHO requirements for existing technologies, as well as appropriate specialized human resources. In addition, since projects taking advantage of the new technologies will be complex and will generally need substantial time to mature, it can be expected that an enormous financial investment will be needed.

Considering all this, it seems both appropriate and important to organize a regional strategic plan for development and production of improved DTP vaccine and other DTP vaccine combinations. Such a plan should make provisions for various types of technical cooperation, development and sharing of production, and selection criteria for participating laboratories.

## REFERENCES


1. Horsfall FL Jr, Tamm I, eds. The poxvirus. In: Horsfall FL Jr, Tamm I, eds. *Viral and rickettsial infections of man.* Philadelphia: Lippincott; 1965:932–934.
2. Homma A, ed. *Simpósio Internacional sobre Febre Amarela e Dengue: Cinqüentenário da Introduçao da Cepa 17D no Brasil, Rio de Janeiro, 15 a 19 de maio de 1988.* Rio de Janeiro: Bio-Manguinhos, Fundação Oswaldo Cruz; 1988. 470p.
3. Fuenzalida E, Palacios R. Un método mejorado para la preparación de la vacuna antirrábica. *Bol Inst Bacteriol Chil* 1955;8:3.
4. Sierra GVG, Campa HC, Varcacel NM, et al. Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba. *Ann Natl Inst Public Health (Oslo)* 1991;14:195–207.
5. Julião O, González A, Ramírez V, et al. Estudio de inmunogenicidad para dos vacunas recombinantes contra hepatitis B comparando dos esquemas. *Biomédica* 1991;11:71–83.
6. Barrera Oro JG, McKee KT Jr. Toward a vaccine against Argentine hemorrhagic fever. *Bull Pan Am Health Organ* 1991; 25(2):118–126.
7. Patarroyo ME, Amador R, Clavijo P, et al. A synthetic vaccine protects humans against challenge with asexual blood stages of *P. falciparum* malaria. *Nature* 1988; 332:158–161.
8. WHO Expert Committee on Biological

Standardization. *Forty-second report.* Geneva: WHO; 1992:20–30. (Technical report series, 822; Annex 1, good manufacturing practices for biological products).

9. WHO Expert Committee on Specifications for Pharmaceutical Preparations. *Good manufacturing practices for pharmaceutical products.* Geneva: WHO; 1992:Annex 1, 14–79. (Technical report series, 823).
10. Willig SH, Stoker JR, eds. *Good manufacturing practices for pharmaceuticals: a plan for total quality control.* 3rd ed. New York: Marcell Deker; 1992. 268p.
11. United States Government Printing Office. *Code of federal regulations, title 21, parts 200–211, subchapter C, drugs: general.* Washington, DC: USGPO; 1993.
12. Dent NJ, ed. *Implementing international good practices.* Buffalo Grove: Interpharm Press; 1993. 284p.
13. Mitchell VS, Philipose NM, Sanford JP, eds. *The children's vaccine initiative: achieving the vision.* Washington, DC: National Academy Press; 1993. 221p.
14. WHO Expert Committee on Biological Standardization. *Requirements for diphtheria, tetanus, pertussis and combined vaccines.* Geneva: WHO; 1990:Annex 2, 87–179. (Technical report series, 800).
15. World Health Organization. *Manual for the production and control of vaccines: diphtheria toxoid.* Geneva: WHO; 1977. 150p. (Unpublished document BLG/UNDP/77.1, rev.1).
16. World Health Organization. *Manual for the production and control of vaccines: tetanus toxoid.* Geneva: WHO; 1977. 139p. (Unpublished document BLG/UNDP/77.2, rev.1).
17. World Health Organization. *Manual for the production and control of vaccines: pertussis vaccine.* Geneva: WHO; 1977. 136p. (Unpublished document BLG/UNDP/77.3, rev.1).
18. Preston NW. Prevalent serotypes of *Bordetella pertussis* in nonvaccinated communities. *J Hyg* 1976;77:85–91.
19. World Health Organization. *Requirements for biological substances: requirements for manufacturing establishments and control laboratories.* Geneva: WHO; 1966:Annex 1, 11–22. (Technical report series, 323).
20. World Health Organization. *Manual for the design, equipping and staffing of facilities for production and quality control of bacterial vaccines.* Geneva: WHO; 1978. 59p. (Unpublished document BLG/UNDP/78.1).
21. World Health Organization. *Laboratory biosafety manual.* 2nd ed. Geneva: WHO; 1993. 133p.
22. WHO Expert Committee on Biological Standardization. *Forty-second report.* Geneva: WHO; 1992:31–46. (Technical report series, 822; Annex 2, guidelines for national authorities on quality assurance for biological products).
23. WHO Expert Committee on Biological Standardization. *The national control of vaccines and sera (a guide to the provision of technical facilities).* Geneva: WHO; 1981:Annex 11, 299–313. (Technical report series, 658).