Report prepared for the Board of the

Global Alliance for Vaccines and Immunization (GAVI)

by

the New Technologies Working Group (NTWG)

of

the GAVI Research and Development Task Force (R&D T F)

Draft for approval by NTWG and R&D T F members

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A. Overall Executive Summary

The Research and Development Task Force (R&D T F) of the Global Alliance for Vaccines and Immunization (GAVI) appointed a New Technologies Working Group (NTWG) to examine how recently developed technologies could be applied to improve the effectiveness and efficiency of immunization programs in developing countries. The R&D T F had beforehand, through extensive consultations, identified three technologies that had the potential of having a significant positive impact in a ten-year timeframe. The NTWG was asked to prepare a report on these technologies and make suggestions on how they could be implemented in an efficient manner. This report - which follows- was meant to be submitted for consideration by the GAVI Board who would decide whether to fund the development and introduction of the three technologies.

The three technologies prioritized for rapid development and introduction were:

- glassification of sugars that can render vaccines thermostable (i.e. resistant to freezing and exposure to a wide range - on the high and low sides - of environmental temperatures). Thermostable vaccines can be distributed without the need of maintaining the current onerous “cold chain” which is required to preserve the potency of vaccines until they are administered
- a rapid, inexpensive assay to detect in oral fluid - an analyte that can be obtained by a simple non-invasive method - a concentration of tetanus antitoxins that is accepted as being protective. Ideally this assay should be done in the field, thus allowing an objective and direct measurement of the performance of immunization services since antibodies to tetanus are only found in individuals who have been properly vaccinated with a potent vaccine
- a device to “defang” syringes of their attached needle before disposal. This operation if properly carried out - has the potential of reducing the risk of iatrogenic transmission of blood-borne diseases.

The NTWG started work in October 2002. It divided itself into three sub-groups to each produce a report on their assigned technology. These reports, each prefaced by an Executive Summary, can be found in Sections C, D and E, respectively, of the present document. The sub-groups on “glassification” and “defanging” also briefly - due to time constraints - examined alternative technologies that could address these issues(see Sections C.4. and E.15.) In this “Overall Executive Summary” only the main conclusions of the Working Group will be recorded. The method of work and activities of the NTWG, as well as the names of its members, are described in the “Introduction”(Section B).

A.1. GLASSIFICATION

Laboratory and pre-clinical studies have established that a variety of vaccines of interest to GAVI can be stabilized by the sugar glass technology. However, no company has advanced a stabilized vaccine beyond pre-clinical testing. This is because multiple barriers to commercial availability exist. These include a perceived lack of demand, little incentive to

invest in low-margin vaccine products, opportunity costs, a complex intellectual property landscape and the loss of market volume if the new products reduce wastage. They must be dealt with if sugar glass stabilized vaccines are to become a reality for public sector immunization. On the other hand, positive incentives, such as production efficiencies, reduction in distribution costs, longer shelf lives and competitive advantages also exist. Sharing of risks between the public and private sectors will be necessary to make thermostable vaccines a reality.

The proposed strategy aims to:
- Ensure that research, development and commercialization efforts are focused on vaccine stabilization projects that will yield maximum public health impact
-Facilitate collaborative private/public sector research and development with priority vaccines
-Streamline supply by engaging policy makers and purchasers early on, thereby preparing for introduction and targeted distribution
-Maximize impact by preparing immunization programs for the introduction of thermostable vaccines

In conclusion, R&D on vaccine stabilization by sugar glass glassification is likely to prove complex, expensive, risky and time-consuming but it could, in the long run, yield great rewards for improving the ease, effectiveness and efficiency of immunization programs. Initial efforts should focus on risk sharing with the private sector in order to achieve completion of clinical trials with at least one priority vaccine.
Success in a forerunner project would demonstrate the advantages of this technology to other industry partners who might be motivated to scale-up production of other vaccines according to more stringent standards of stability.

A.2. NON-INVASIVE FIELD ASSAY FOR TETANUS ANTITOXINS

Programmes to immunize individuals, particularly those to immunize children in developing countries are essential for global health against infectious diseases. Methods to actively monitor the effectiveness of vaccination efforts are few, and the extent to which programmes meet their expectations is unknown. The Working Group was tasked with identifying technology that would offer a non-invasive means to assess the effectiveness of immunization programmes, while being appropriate for field use as well as in laboratories that possess only basic capabilities.

Crevicular fluid is a filtrate of plasma that is produced at the interface of gums and teeth and which, when diluted with saliva and other oral secretions and excretions (effluents) forms what can be described as “oral fluid”. Crevicular fluid contains IgG antibodies of different specificities that are in the same proportions as found in plasma. However, because of the dilution caused by oral effluents the absolute concentration of any particular antibody, for example, tetanus antitoxin will be, on average, 400 times lower than in plasma. With currently available sensitive assay methods, the presence and even concentration of specific antibodies can be assessed. In fact, commercial diagnostic kits, for example for HIV, using oral fluid (which is collected with specially designed devices) as analyte have

been developed. In principle, and based on existing experience and technologies, there is no reason to believe that a rapid field assay to detect “protective” levels of tetanus antitoxin in oral fluid could not be developed. The sensitivity of the assay could be set at a level that would predict that the plasma concentration of tetanus antitoxin is above that known to protect against tetanus. The NTWG therefore determined that “the development of an oral fluid test to effectively measure protection to tetanus”, and thus assess the performance of immunization programmes, “is feasible”. The group agreed that such a test could be developed and validated - assuming adequate funding – within 1-2 years if commercially available devices for collecting oral fluid were exploited. The recommendation is to make the test in a format that allows both on-site testing with a rapid (less than 30 minutes) assay kit and testing in a laboratory of collected samples - that should be easily transportable at ambient temperatures – by individuals other than those making the collections.

The project is envisioned to require:
(1) Collection of appropriate oral fluid/serum sample pairs to be used as calibrators for test development
(2) assessment of different oral fluid collection devices for their ability to effectively collect samples rich in crevicular fluid with adequate antibody levels
(3) modification of existing tetanus antibody tests to perform with oral fluids (e.g., increased sensitivity) and assessment
(4) replacement of this method with a rapid test format and re-assessment
(5) extensive validation of the rapid oral fluid test using large numbers of sample pairs
(6) standardization of the product for manufacturing

Groups to be considered to undertake test development include industry and academia. Industrial partners offer the advantages of experience in development and bringing products to market, but may be limited by the expected low return of profits because of relatively low numbers of kits required according to a preliminary forecast. Academic support is generally faster, less expensive, and intellectual property issues may be less complicated. Ideally, collaboration between industry and academia may result in speed of test development, rapid standardization, and at more reasonable costs. A “roadmap” (page .....) for the proposed development project was generated.

A.3 DEFANGERS

Improper reuse of unsterile injection equipment is a frequent cause of iatrogenic disease in curative medicine in the developing world, and a concern for immunization programs, which are estimated to account for ten percent of all injections worldwide. Needlestick injuries threaten health care workers, ancillary hospital personnel, and the general community exposed to “sharps” waste that is improperly discarded in the environment.

Defangers, which are devices that destroy or segregate the needle of needle-syringes used for injections from other components of the medical waste stream, offer the potential, when used as intended, to reduce an increasing volume of “sharps” as a result of the growing uptake of auto-disable (A-D) needle-syringes. If defangers are actuated by the health

worker immediately after administering each injection, they may also decrease the likelihood of accidental needlestick injuries if “sharps boxes” are unavailable or overfilled. Defangers capable of destroying both the needle and the syringe of conventional (non-A-D) needle-syringes might also reduce the scandal of their improper pilferage, “recycling”, resale, and unsterile reuse.

The advantages just described for defangers, however, remain theoretical. To date, there have been no independent, well-designed, controlled, scientific studies to support such claims. It is entirely possible that the additional defanging step might lead to an increased frequency of needlestick injuries from the extra manipulation involved. This is particularly possible if defangers are not available within one or two paces of every locus where patients are injected, requiring health workers to walk elsewhere with an exposed needle, or to “batch” the used needle-syringes for later defanging. The latter would require someone to reach dangerously into a container of used needle-syringes in order to insert each into a defanging device. Defangers will not obviate the need for universal availability of standard needle-syringe disposal boxes.

Existing commercial defangers range in cost from approximately US$100 to $800 (for the electric models) and approximately US$50 to $300, depending of features, for the manually operated models. Investigational manual defangers are targeted to sell for less than $20 each. Assuming proper defanger use requires one to be located within reach of every health care worker administering a vaccination, the theoretical global demand for defangers is estimated at 0.5 to 1.0 million defangers needed for the 75 Vaccine Fund-eligible countries, or 1.0 to 1.25 million for all 165 developing countries.

The working group proposed a number of performance and design specifications for defangers whose research, development, promotion, and distribution would be funded or facilitated by the Global Alliance for Vaccines and Immunization (GAVI) for use in developing countries. The major specifications, inter alia, were that the defanger should:

- not require electricity or batteries
- defang all currently available A-D syringes as well as all non-A-D plastic syringes of nominal capacity sizes from 1 mL up to 25 mL
- defang syringes with either Luer cone (slip), Luer lock, or snap-on type interfaces with their needle hubs
- render unreusable non-A-D syringes defanged by the device
- leave no needle stub on the defanged syringe that could produce a percutaneous injury
- provide a clearance (or a safety shield) for the operating hand of at least 15 cm
- demonstrate a lifetime of at least 25,000 “defangs”
- use disposable, fill-level-marked, needle containers with visible content level and permanently locking closures.
- minimize splatter of needle-syringe contents
- be easy to clean and maintain

Given the unproven claims for defangers, it is recommended that defanger developers and manufacturers be given approximately 4 months to modify their products or prototypes in

order to satisfy the proposed specifications. These should then be tested at the bench by independent testing laboratory(ies). Defangers found to meet the specifications should then be evaluated in field trials in a variety of settings in representative developing countries of Africa, Asia, and the Americas.

Competent institutions or agencies that do not have any compromising relationships to defanger developers, intellectual property holders, or manufacturers should conduct the field evaluations. The evaluations should include passive (uncontrolled) studies, as well as controlled trials (and head-to-head, if multiple devices are submitted) in both routine immunization settings and special (mass) vaccination campaigns. They should use objective, quantitative research endpoints such as rates of needlestick injury, proportions of needle-syringes defanged, and frequency of defanger breakdown and other causes of non-use, such as loss, theft, disappearance, or deliberate intention to “recycle” non-A-D needle-syringes. Subjective health care worker experience and preferences towards the devices, as well as economic analyses should also be studied. The suggested research agenda might require from 12 to 24 months to conclude, and cost from US$0.5 to $1.5 million.

Upon review of the results of such trials, GAVI would decide whether and to what extent defangers should be purchased, promoted, distributed, and/or bundled with donated vaccine in eligible Vaccine Fund countries. It would then select an implementing organization or entity to carry out the programme, including design and management of training syllabi and other rollout materials and ancillary needs. Based on estimates mentioned above, full rollout of defanger technology in all Vaccine Fund eligible countries might cost US$20 million over several years.

B. Introduction

In November 2000,during its meeting in the Netherlands, the GAVI Board decided that immunization programmes, particularly in developing countries,
should be improved by applying recently developed technologies that could make them more effective and efficient. The Task Force (T F) on Research and Development (R&D) was requested to recommend, in addition to the three R&D priorities that were approved for "accelerated development" at that meeting, up to three research projects that each, separately and/or together, had the potential to achieve this objective within 10 years. The T F undertook an extensive process of information gathering, evaluation, rationalization and prioritization to identify key technologies (or improved managerial and operational strategies) that could have a favorable impact upon immunization services. Using the criteria of potential programmatic impact, technical feasibility, probability of successful introduction, and cost-benefit ratio, three priority research "agendas" were identified. Within each of the three "agendas", a specific promising technology - among the many possible technologies that were examined - was then prioritized for consideration as a candidate for "accelerated development".

The "agendas" and the corresponding specific technology recommended by a panel of international vaccinology experts were:

- Decreased dependence upon and ultimate elimination of the cold chain
  >Sugar glass preservation to make vaccines "temperature-stable" (i.e. resistant to high environmental temperatures and to freezing)

- Improved tools to measure immunization services performance
  >Non-invasive (oral fluid) field assays to measure protective levels of tetanus antitoxin (and other antibodies) in infants and toddlers as an objective measurement of the performance of immunization services

- Reducing infectious wastes and ultimately eliminating the use of sharps (needles and syringes)
  >Devices to "defang" syringes

The prioritization process that was undertaken to identify these "agendas" and "technologies" was outlined in a document named "Paper # 4" that is available on request to interested individuals.

The R&D T F then formed a New Technologies Working Group (NTWG) that was tasked to produce a report on the prioritized technologies. In order to avoid any conflict of interest situation, the members of this group were not involved in the prioritization exercise. The NTWG met for the first time at WHO headquarters in Geneva, under the chairmanship of Dr. Francis André, on October 28th 2002.By the end of the meeting it was agreed that three sub-groups, each headed by one person, would be formed to produce a report on the prioritized technologies.

The sub-groups and the elected leaders for each technology, were:

- Sugar glass technology

Ms. Debra Kristensen (leader), Mr. Peter Carrasco and Mr. Mogens Munck

- Non-invasive field assay

Dr. Helen Lee(leader), Ms. Patricia Cricenti and Dr.Aldo Tagliabue
(Note:Dr.Lee resigned on January 9th 2003 and was replaced by Prof.Niel Constantine)

- Devices to “defang” syringes

Dr.Bruce Weniger (leader) and Mr.Souleymane Kone.
Dr. GordonLarsen (not a permanent member of the NTWG) as well as Mr. Mogens Munck agreed to help this sub-group.

After the first meeting, many telephone conversations took place between sub-group leaders, members, the chairman and others to clarify the nature and scope of the task to be accomplished. On December 16th 2002, a conference call between members (except Mr. Kone who could not be reached) took place when progress made and future plans, including a proposed visit to PATH in Seattle and the next NTWG meeting, were discussed. The meeting at PATH on January 9-10,2003,attended by the three sub-group leaders and the chairman was very informative and provided an occasion to prepare the next meeting in Geneva scheduled for February 11-12,2003. That meeting, attended by several external consultants and WHO experts, gave access to much information and set the scene for the preparation of draft reports by the three sub-group leaders. Comments by the concerned NTWG sub-group members were invited. On April 23-24, 2003, during a meeting in Baltimore, these drafts were edited by the chairman, sub-group leaders and Dr. Myron Levine. On May 9th 2003,a telephone conference call between R&D T F members and NTWG leaders to review progress and plans to finalize the report for submission to the GAVI Board, was held. After this, the NTWG sub-group leaders and its chairman edited the report and incorporated the comments received on the drafts circulated to various individuals as agreed during the May 9th conference call (see the Acknowledgements section at the end of the report for the persons involved).

Reports on the NTWG meetings of October 28th 2002, January 9th 2003,February 11-12, 2003 and April 23-24,2003,as well as the telephone conference calls of December 16th 2002 and May 9th 2003 are available if required for information.

The “final” draft report was assembled by the NTWG chairman from the reports prepared by each sub-group leader with the help of sub-group members. The report consists of an “Overall Executive Summary” and an ”Introduction” written by the chairman, followed by the three sub-group reports, each with a more detailed “Executive Summary” followed by descriptions of the three prioritized new technologies and a proposed research plan for each. There is, as well, a brief discussion of possible alternative technologies for dealing with the “cold chain” and “sharps” issues at the end of the reports of these two sub-groups. After the final draft was prepared, it was sent to all NTWG and R&D T F members for final approval before its submission to the GAVI Board.

C. Reducing dependency upon - and ultimate elimination of - the “cold chain” by developing heat-and freeze-resistant (thermostable) vaccines using the sugar glass preservation technology

C.1. Executive Summary

Vaccine stabilization through the use of sugar- or composite-glassification offers a means to improve both the effectiveness and efficiency of immunization programs in developing countries. Improved effectiveness is achieved through prevention of heat- and freeze-damage to vaccines. Efficiency gains are possible via reduction of wastage due to temperature damage to vaccines, lower shipping and storage costs, decreased logistical and equipment requirements, and longer shelf lives.

To date, laboratory and pre-clinical studies have validated stabilization of a variety of vaccines of interest to the Global Alliance for Vaccines and Immunization (GAVI). However, few data have been published and no company has advanced a stabilized vaccine beyond pre-clinical testing. Sources of expertise include stabilization technology and drug delivery companies and vaccine producers.

While there is optimism that thermostable vaccines can be achieved, multiple barriers to commercial availability exist and must be dealt with if these products are to become a reality for public sector immunization programs. From the perspective of a vaccine producer, barriers are likely to include lack of demand, lack of incentive to invest in low-margin vaccine products, substantial investment requirements, opportunity costs, perceived excessive risk due to the complexity of the intellectual property landscape, and potential loss of market if resulting products reduce wastage during use.

Potential incentives also exist for vaccine producers pursuing thermostable vaccine products including production efficiencies, reduction in shipping and storage logistics and costs during production and initial distribution, longer shelf lives for bulk and final product, and competitive advantages.

Advancement of thermostable vaccines is unlikely to proceed without some measure of public- and private-sector risk sharing.

The strategy proposed aims to:

C.1.1.Ensure that research, development and commercialization efforts are focused on vaccine stabilization projects that will yield maximum public health impact

Initial prioritization of vaccines currently used in developing country immunization programs was performed based on the potential public health impact of converting these vaccines to thermostable formats. The opinion of the working group is that *multivalent*

vaccines[1] and vaccines currently in development, and not yet in clinical trials, are prime candidates for stabilization.

In addition, guidelines for selection of vaccine formats and delivery devices are offered. Specifically, the thermostable vaccine should not increase logistical complexity for national immunization programs and should not decrease safety for vaccinators or recipients. For example, it is unacceptable to convert an existing liquid vaccine to a thermostable dry vaccine in a vial format. The resulting product would decrease safety because of the reconstitution step and would not obviate the need for ice at the point of delivery if presented in a multi-dose vial as the thermostability characteristics of the vaccine may be lost after reconstitution.

C.1.2.Facilitate collaborative private/public sector research and development with priority vaccines

Projects should be selected based on potential public health impact, technical feasibility, and commercial viability. Preliminary economic analyses should be conducted and updated throughout the project to validate (or invalidate) the worthiness of further investment as major milestones are reached. Ideally, projects will be undertaken with multiple stabilization technologies and multiple vaccine producers to spread risks and encourage competition. It is essential that selected vaccine producers be United Nations pre-qualified or capable of such pre-qualification.

Organizations implementing or coordinating selected projects will need to identify and reduce commercialization barriers, such as demand and intellectual property uncertainty and regulatory complexity, to encourage adoption by vaccine producers.

Potential public sector roles include involvement as:

A development partner and facilitator that can "*push*" the technology forward by directly conducting research, providing financial support, creating initial specifications, assisting with regulatory issues, or securing access to key component technologies for thermostable vaccines

A purchaser or influencer of demand that can assist with characterization and development of the market for thermostable vaccine products to "*pull*" the technology forward

Product development for new or reformulated vaccines will be a lengthy process and is expected to require between seven to twelve years to reach the point of availability for use in developing country immunization programs.

[1] Such as Diptheria-Tetanus-Pertussis-Hepatitis B- *haemophilus influenzae* type B (DTP-Hep B-Hib) vaccine and DTP-Hep B vaccine.

C.1.3. Streamline supply by engaging policy makers and purchasers early on, thereby preparing for introduction and targeted distribution

Close coordination and planning will need to occur between implementing groups, the World Health Organization, and vaccine purchasers including the Supply Division of the United Nations Children's Fund and the Pan American Health Organization. Pre-qualification, pricing, and other procurement issues will need to be addressed. In addition, mechanisms for targeted distribution of thermostable vaccines to countries that can utilize them to their full potential should be established, in anticipation of the availability of thermostable products.

C.1.4. Maximize impact by preparing immunization programs for introduction of thermostable vaccines

It is likely that thermostable vaccines can be handled according to the existing "good storage practices for pharmaceuticals". Such products do not require refrigeration, but simply storage in dry, well-ventilated premises at temperatures up to 30 degrees C. A higher upper temperature limit should be possible with thermostable vaccines. As product development progresses, policy development, advocacy, training materials development, and plans for pilot and final introduction should also progress. Management of a mix of vaccines that are freeze-sensitive, heat-sensitive, and thermostable will be required until such time that thermostable vaccines are the norm in developing country immunization programs.

In conclusion, the advancement of vaccine stabilization research and development is likely to be a complex, expensive, risky, and time-consuming venture that can yield great rewards in terms of improving the ease and effectiveness of immunization programs. Initial efforts should focus on risk sharing with the private sector to achieve completion of clinical trials and, if successful, subsequent production scale-up with one or two priority thermostable vaccines. Ideally the efforts of such "forerunner" projects will demonstrate the possibilities to other industry partners and serve to create new standards for vaccine stability.

C.2. A STRATEGIC APPROACH TO VACCINE STABILIZATION RESEARCH AND DEVELOPMENT USING SUGAR GLASS PRESERVATION TECHNOLOGIES

C.2.1.PURPOSE

The Global Alliance for Vaccines and Immunization (GAVI) Research and Development Task Force identified vaccine stabilization as the highest priority area for future research to reduce reliance on the cold chain. Accordingly, the Task Force appointed a New Technologies Working Group (NTWG) to develop a strategy to accelerate vaccine stabilization research and development [GAVI2002] The purpose of this resulting document is multifold:

- To define a process and roles for public sector stakeholders that maximizes the likelihood of success and helps to ensure appropriate allocation of resources
- To identify barriers to implementation that may affect key stakeholders and to provide potential remedies
- To educate GAVI partners about the potential challenges and opportunities of pursuing relevant vaccine stabilization technologies

C.2.2. VISION

The availability of thermostable vaccines will improve the effectiveness and efficiency of immunizations. Improved effectiveness is achieved through the prevention of heat- and freeze-damage to vaccines. Gains in efficiency are achieved through reduction of wastage due to temperature damage to vaccines, lower shipping and storage costs, decreased logistical and equipment requirements, and longer shelf lives. Additional benefits, such as improved safety, are also possible depending upon the format of the stabilized product. The products envisaged can be stored without refrigeration for their entire shelf life.

C.2.3. BACKGROUND INFORMATION

C.2.3.1.Glassification

The focus of this strategy is on vaccines stabilized by processes known as glassification. Glass-forming sugars (such as trehalose and sucrose) and potentially other excipients are added to vaccine formulations. As the vaccines are dried, glassy solids are formed that restrict molecular mobility and protect the actives. The higher the glass transition temperature of the sugars used the higher the temperature at which the dried products can be stored. [Colaço 1992] [Roser 1991]

C.2.3.2.Drying Techniques

Drying methods to produce glassified vaccines include foam drying with lyophilizers (without freezing and with vacuum) and spray drying. The characteristics of each drying method are outlined in Table C1. Both foam and spray drying have strong, but slightly different, advantages over traditional freeze-drying. The preferred method in a given vaccine production facility will be dependent upon the capacity of existing freeze-dryers; required particle size; ability to purchase, install, and validate new production and processing equipment; overall cost-effectiveness; and intellectual property constraints.

Table C1: Characteristics of Drying Processes

Freeze drying	Foam drying	Spray drying
Uses standard freeze dryers	Uses standard freeze dryers	Requires spray-drying and processing equipment
Batch limited	Batch limited	Continuous process
Lengthy drying time (2-3 days)	Slightly reduced drying time	Markedly reduced drying time
Diverse particle sizes	Particle size control (via milling and screening)**	Greater particle size control (via nozzle)
Aluminum hydroxide adjuvants can lose activity*	Aluminum hydroxide adjuvants do not lose activity	Aluminum hydroxide adjuvants do not lose activity

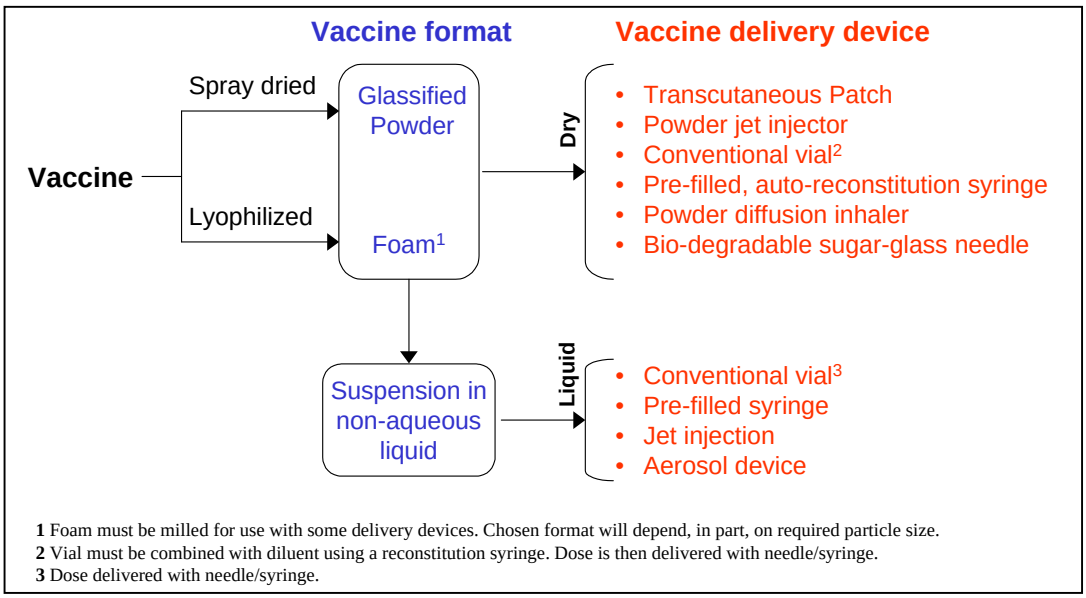
* Successful freeze-drying of alum-containing vaccines has been performed by industrial manufacturers (M.Friede personal communication) however the procedure has not been published.

** In theory lyophilized vaccine could also be subject to milling and screening to obtain a defined particle size

C.2.3.3.Potential Thermostable Vaccine Products

Dry glassified vaccine can be further developed into a variety of vaccine products with different routes of administration (see Figure C1). The pros and cons of various formats will be discussed later in this document.

Figure C1: Potential Thermostable Vaccine Formats and Delivery Devices



C.2.3.4.Status of Research

To date, both killed and live vaccines have been stabilized with sugar-glass technology. Killed vaccines include diphtheria-tetanus, diphtheria-tetanus-pertussis, pertussis, tetanus toxoid, hepatitis B, and Influenza (A/PR/8/34) with and without adjuvant. Live vaccines include measles. Aluminum hydroxide gel has also been stabilized. Attempts to stabilize Polio Sabin 1 vaccine have been unsuccessful thus far due to inability to penetrate the tight capsid with disaccharides. In addition, stabilizing live bacteria can be challenging, as growth conditions must be manipulated to ensure a high level of stabilizing sugars prior to drying.

Most research with vaccines has been conducted by stabilization and drug delivery companies and vaccine producers, and little has been published. To our knowledge, no glass-stabilized vaccine has advanced beyond preclinical testing. Immunogenicity in animal models has been demonstrated with some vaccines, but studies in humans have not been undertaken. Appendix A contains a more detailed overview of existing data on sugar-glass stabilization of vaccines.

C.2.3.5. Sources of Stabilization Technical Expertise

A number of companies[2] hold expertise in the area of sugar and composite glass stabilization. These include, but are not limited to, Avant Immunotherapeutics, Inc.[3] (U.S.), Cambridge Biostability Limited (U.K.), Elan Corporation[4] (Ireland), and Nektar[5] (U.S.). In addition, a number of vaccine producers have conducted research in this area both independently and in collaboration with companies specializing in stabilization techniques.

C.2.3.6 .Intellectual Property Situation

The sugar-glass stabilization patent landscape is complex, dynamic, and has been rife with conflict. Relevant patents are primarily pending and issued in developed countries, with sparse coverage in developing countries. Some basic techniques underlying sugar-glass stabilization are considered to be in the public domain. The seminal idea that carbohydrates, alone or in combination with other compounds like proteins, can be mixed with actives to form stable dried compounds is known from documents dating to the early- and mid-1900s.[Epstein1948][Wasllerstein1932] However, other significant aspects of sugar glass stabilization are not in the public domain. These include, for example, patents focused on specific actives; composite formulations; drying systems and methods; and processing materials, systems, and methods. When exploring a specific thermostable vaccine product, care must be taken to assess “freedom to practice” in all relevant areas.

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[2] Mention of specific companies is for information and identification only and does not imply endorsement.
[3] Avant acquired the technology portfolio of Universal Preservation Technology in 2002.
[4] Elan acquired Quadrant Healthcare, plc in 2000.
[5] Formerly Inhale Therapeutic Systems, Shearwater, and Bradford Particle Design.

C.2.3.7. Barriers to Commercial Availability of Thermostable Vaccines

There is optimism that thermostable vaccines can be achieved. Multiple barriers to commercial availability exist, however, and must be addressed in order to make thermostable vaccines a reality for developing-country immunization programs. Because it is likely that most vaccine producers will obtain intellectual property and know-how from stabilization technology or other companies, stabilization technologies can be characterized as “value-added” technologies for vaccines in which *vaccine producers play the critical role for success*.

Barriers to pursuing thermostable vaccine products were identified through confidential interviews conducted by a consultant with representatives of the vaccine industry and included:

- *Lack of demand for thermostable vaccines*. The interest for thermostable vaccines is focused on developing-country markets and does not seem to apply to developed-country markets.
- *Lack of incentive to invest in low-margin vaccine products*.
- *Substantial required investment*s in capital equipment (e.g., filling lines), research and development, scale-up, licensing, preclinical and clinical trials, and regulatory approvals.
- *Concerns about the feasibility of scale-up*.
- *Opportunity costs*—other areas of research are more profitable.
- *Perceived excessive risk due to the complexity of the intellectual property landscape*.
- *Potential loss of market* if products are more thermostable and have longer shelf lives.
- *Risk when changing formulations due to the current environment of skepticism about vaccine safety.*

C.2.3.8.Incentives for Pursuing Thermostable Vaccines

Potential *incentives* for pursuing thermostable vaccine products were also identified through interviews with vaccine producers and included:

- *Facilitation of combination vaccines*. In theory, individual vaccine components can be glassified via spray drying or foaming/milling and then combined with other glassified components. These techniques should reduce or eliminate chemical interactions between components that often confound formulation of multivalent vaccines.

- *Longer shelf lives* allow for production of larger batches of vaccine, where cost-effective, and longer-term storage of bulk and final product by the vaccine producer.
- *Production efficiencies*, such as decreased lyophilization time and increased yield with spray dryers, may lower costs of goods.
- *Decreases in internal losses* due to temperature maintenance problems during storage or initial transit are likely with thermostable vaccines.
- *Reduction in shipping and storage logistics and costs* as bulk and final products will be more resistant to variations in temperature.
- *Research gains* with vaccines may have potential application to more profitable products.
- *Competitive advantage* via product differentiation and proprietary access to stabilization methods can also be strong drivers for vaccine producers considering new technologies. [Note: Such objectives are likely to be in conflict, however, with public-sector interest in equal access and wide-scale adoption of stabilization methods by multiple vaccine producers so that thermostable vaccines can be broadly available at affordable prices.]

C.2.4. MAJOR AIMS OF STRATEGY

The strategy for advancement of thermostable vaccines was developed with the following major aims:

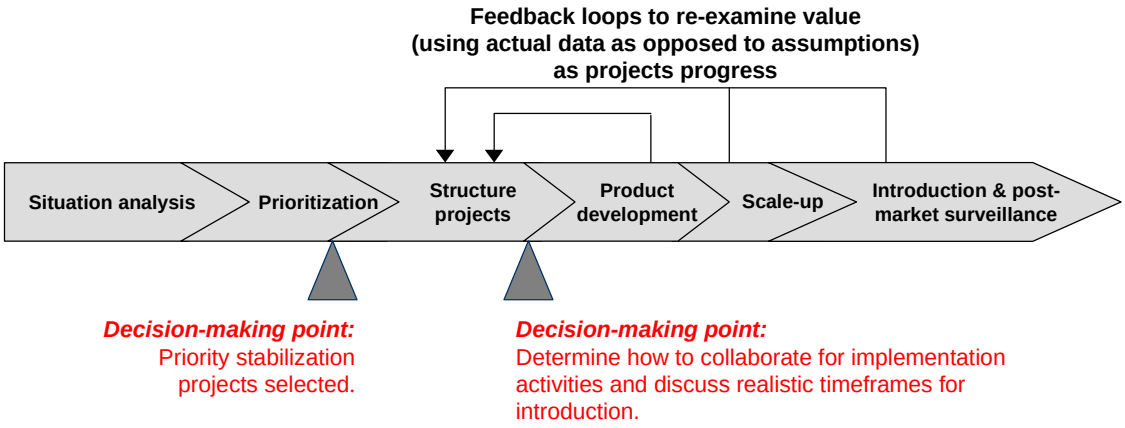
- Ensure that research, development, and commercialization efforts are focused on vaccine stabilization projects that will yield maximum public health impact.
- Facilitate collaborative private/public-sector research and development with priority vaccines.
- Identify and reduce commercialization risks, such as demand and intellectual property uncertainty and regulatory complexity, to encourage adoption by vaccine producers.
- Streamline supply by engaging policy makers and purchasers early on, thereby preparing for introduction and targeted distribution.
- Maximize impact by preparing immunization programs for introduction of thermostable vaccines.

C.2.5. KEY COMPONENTS OF STRATEGY

The strategy is divided into six phases—situation analysis, prioritization, structuring projects, product development, scale-up, and introduction (see Figure C2). The strategy is relevant for any organization(s) or partnership(s) pursuing thermostable vaccines of interest to GAVI. Public- and private-sector stakeholders should be consulted and

engaged from initial assessment to final introduction to ensure that decisions are driven by data collected from both sectors. Consensus building will be critical to success.

Figure C2. Proposed strategic approach to vaccine stabilization



C.2.5.1.Situation analysis

Background research on the technical and commercial aspects of vaccine stabilization should be completed by implementing groups. Members of the New Technologies Working Group have already initiated research on many of these topics. This work can serve as a starting point for future efforts by others.

Technical: Collect and analyze available data on current status of research

Available information on stabilization materials and methods, application to vaccines, production and processing equipment, and delivery formats should be reviewed and analyzed. Preliminary information is included in this report and a summary of available data on sugar-glass stabilization of vaccines is included as Appendix A.

Supply: Conduct background research on stability technology companies and vaccine producers pursuing stable formats

Attempts should be made to identify key companies willing to participate in private/public sector research to improve stability of vaccines of interest to GAVI. Vaccine producers should be limited to those that are United Nations (UN) prequalified suppliers[UNICEF2003a] or deemed capable of prequalification.

Intellectual property (IP): Assess the intellectual property position of intended products

Private- and public-sector partners pursuing research, and hopefully thermostable vaccine products of interest to GAVI, should review, synthesize, and document

patents, proprietary know-how, and trade secrets pertaining to their materials and methods in order to determine their likely impacts on the development and commercialization of proposed stabilized vaccines.

Regulatory: Investigate potential regulatory pathways for stabilized vaccines

For planning and budgeting purposes, it is important to understand the potential regulatory requirements for vaccine stabilization projects from the perspective of the national regulatory authorities as well as the World Health Organization. A paper entitled “Regulatory Considerations for Thermostable Vaccine Projects” is available as an initial attempt to analyze this subject. [Chaloner-Larsson2002]

Demand: Assess the demand of the global public-sector market for current and new vaccines and project (based on current trends) the vaccine market over the next ten to fifteen years

It is likely to take seven to twelve years for reformulated vaccines or newly developed thermostable vaccines to reach the marketplace. Therefore, it is essential that targeted vaccines are ones that will be relevant over the next few decades. A strategic market forecast for the period 2003 to 2013 has been drafted and can be made available to interested parties.[O’Connell2003] The spreadsheet can be used to develop consensus among public-sector partners about the market as well as to narrow the list of priority vaccines for stabilization. In addition, the forecast can serve to inform private sector partners about potential markets for particular products.

Market drivers: Identify factors affecting the market (such as alternative or emerging new technologies in the field of immunization)

The market for thermostable vaccines will be dependent upon the evolution of stabilization technology and vaccine markets. Stabilization technologies have application far beyond childhood vaccines, for example, with foods, diagnostic reagents, and bio-terrorism vaccines. The progress made with these other products will greatly affect the viability of stabilization technology companies and the vigor with which intellectual property is protected. The vaccine market is also experiencing flux as developed-and developing-country markets diverge, companies consolidate, and vaccine producers from emerging countries come on line. Keeping abreast of the changing nature of both these markets and the characteristics of their respective market drivers will be critical to understanding how to further develop the emerging thermostable vaccine market.

C.2.5.1. Prioritization

The demand assessment should serve to narrow or validate the list from which potential vaccine candidates for stabilization can be identified. We propose that a process occur by which candidate stabilization projects are prioritized based on potential public health impact as well as technical and commercial viability.

C.2.5.2.1.Public health considerations

Three major topics should be considered during the initial prioritization process and are addressed in the sections that follow.

- Should existing traditional vaccines be rendered thermostable? If so, what are the key public health impact factors that can be used to prioritize among this group?
- How can sugar-glass stabilization be applied to vaccines in development?
- Because sugar-glass stabilization transforms vaccines into dried products, what formats are appropriate for use in developing-country immunization programs?

Existing vaccines

At minimum, criteria for selection of vaccines for stabilization should include potential impact on program operations, program outcomes, and health outcomes. As an initial attempt at prioritization of existing vaccines, we recommend that priority be given to stabilization of vaccines with the attributes described below. The attributes are not listed in order of importance. Further refinement of the prioritization process should be conducted by implementing organizations and must be followed by economic analysis to ensure feasibility.

Vaccines damaged by freezing

Heat damage to vaccines can often be prevented through the early warning given by vaccine vial monitors (VVMs). Because VVMs change color gradually with exposure to heat over time, it is possible to use a VVM to identify a vial of vaccine that has received some heat exposure, but is not yet at the discard point, so that it can be used immediately or so that the cold chain conditions under which it is stored can be improved.[WHO2002a] Freezing, however, is a threshold phenomenon and once it occurs to freeze-sensitive vaccines, the vaccines must be discarded.[WHO2002b] Therefore, vaccines damaged by freezing are especially good candidates for stabilization that will render them impervious to both extreme hot and cold temperatures.

Vaccines used in mass campaigns or supplementary immunization activities

Vaccines that are delivered in campaign settings or supplementary immunization settings are ideal candidates for stabilization as these activities could be conducted entirely free of cold chain constraints. Examples include outreach with tetanus toxoid to women of childbearing age in areas at high risk for tetanus or mass campaigns with yellow fever vaccine to prevent outbreaks.[WHO2002c]

Vaccines, that if thermostable, would greatly reduce cold chain needs for routine immunization programs

Stabilizing one vaccine type that is routinely delivered with other vaccines may not provide added benefit to immunization programs unless it greatly frees up cold chain capacity or unless the other vaccines are also stabilized. For example, the cold chain impact of converting only BCG vaccine to a thermostable format would be less than the impact of stabilizing only pentavalent (DTP-hepatitis B-Hib) vaccine as the former is routinely provided in 20-dose vials that take up little cold chain space per dose, while the latter is usually provided in 2-dose vials.[UNICEF2003b]

Freeze-dried vaccines

Many problems exist with use of current freeze-dried vaccines in developing country settings.[WHO2000] Such vaccines must be transported with a diluent vial whose contents are added to the vaccine at the point of use with a special reconstitution syringe. Because freeze-dried vaccines do not contain preservatives, microorganisms that enter the vial can grow quickly. Therefore the reconstituted vaccine must be kept cold at the point of use and the contents must be discarded within a few hours. Safety is potentially compromised if the incorrect diluent is used or if safe injection practices are not followed during the reconstitution step or during withdrawal of doses. Deaths have resulted when children received reconstituted vaccine kept for more than six hours.[WklyEpiRec1996] Freeze-dried vaccines are therefore ideal candidates for stabilization if the proposed thermostable format eliminates the reconstitution step.

High-priced vaccines

The impact of losing an expensive vaccine to heat or freeze damage or post-reconstitution discard helps to justify the added cost to obtain a thermostable product. Full economic analyses must be carried out, but on a cursory level, a high-priced vaccine should be prioritized over a low-price vaccine if all other factors are equal.

An initial attempt at prioritizing key existing vaccines used in immunization programs as candidates for stabilization based on the above criteria is included as Table C2. It is the opinion of this working group that stabilization of multivalent vaccines offers the greatest public health impact among vaccines used in immunization programs today.

C.2.5.2.1.2.New vaccines

Vaccines in development that are likely to be integrated into developing-country immunization programs, such as new formulations of meningococcal and multivalent vaccines, should all be considered prime candidates for stabilization. For many products, integration of stabilization materials and methods can still occur prior to the clinical trial phases and may yield superior products in terms of safety, efficiency, and effectiveness. Ideally, all new vaccines will be thermostable in the future. For this to occur, the vaccines must all be developed with appropriate thermostability specifications.

C.2.5.2.1.2.Guidelines for selection of vaccine format

Once candidates are selected, important guidelines must be followed to ensure that an appropriate vaccine format and mode of delivery is used for the thermostable vaccine. At a minimum the thermostable vaccine should not increase logistical complexity for national immunization programs

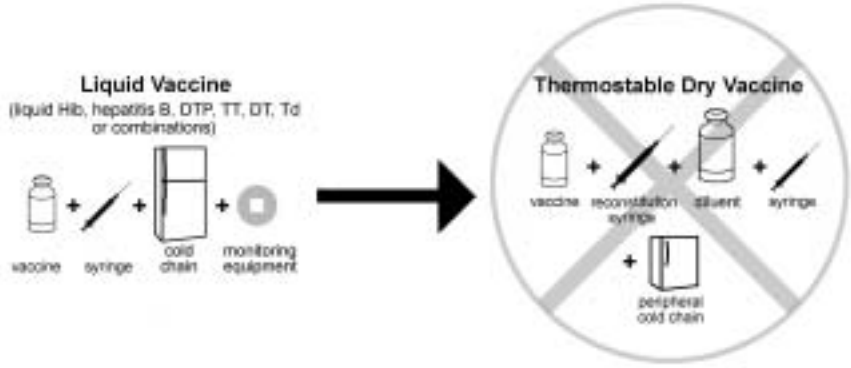
For example, it is not acceptable to convert an existing injectable liquid vaccine (TT, DTP, hepatitis B, liquid Hib—or combinations of these vaccines) into a stable-dry vaccine that must be manually reconstituted and delivered by syringe and needle. Such a conversion would increase logistical complexity and decrease safety. Figure C3 outlines the required equipment for both the liquid and stable-dry vaccines in vials. Unlike the liquid vaccine, the stable-dry vaccine would require a diluent vial and reconstitution syringe. The stable-dry vaccine would also lose its stability once reconstituted, meaning that some means of keeping the product cold would be necessary at the point of use and like existing reconstituted vaccines, the remaining contents would have to be discarded within a few hours.

Table C2: Initial Prioritization of Candidate Vaccines[1] for Stabilization Based on Potential Public Health Impact

Current Attribute/ Benefit of stabilization	**DTP-HB-Hib[2] or DTP-HB**	**Yellow Fever (YF)**	**Measles**	**Hepatitis B vaccine**	**Bacille Calmette Guérin (BCG)**	**Tetanus Toxoid (TT)**	**Oral Polio Vaccine (OPV)**
Freeze sensitive/ Would render vaccine freeze-stable	Yes	No	No	Yes	No	Yes	No
Used in campaigns or supplementary immunization/ Activities could be conducted without cold chain	No	Yes	Yes	Rarely[3]	No	Yes	Yes
Removal from the cold chain during routine immunization would have significant impact/Frees up significant cold chain capacity or eliminates need for ice packs	Yes	No	Yes,[4] If BCG also removed	No	Yes,[4] If measles vaccine also removed	No	No
Freeze-dried vaccine/Improves safety by eliminating manual reconstitution	Sometimes[5]	Yes	Yes	No	Yes	No	No
High price vaccine[6]/Likely to be more cost-effective	Yes	Yes	No	Yes	No	No	No
Priority	Higher ➡	➡	➡	➡	➡	➡	Lower

[1] Key vaccines currently used in developing country immunization programs were considered for this exercise.
[2] Diptheria-Tetanus-Pertussis-Hepatitis B-*Haemophilus influenzae* type B vaccine
[3] Used for birth dose outreach in some countries, but often in combination with other vaccines (e.g., TT and OPV).
[4] In many countries, if both BCG and measles vaccine were thermostable, ice or ice packs would not be required at the point of immunization for keeping reconstituted vaccines cold.
[5] Some brands of DTP-HB-Hib contain freeze-dried Hib.
[6] Weighted average price over $0.30 per dose. [UNICEF2003]

Figure C3: Example of an unacceptable thermostable vaccine product. Conversion of an existing liquid vaccine (vial format) to a thermostable-dry vaccine (vial format) results in increased equipment needs and decreased safety via the manual reconstitution step.



The thermostable vaccine should not decrease safety for vaccinators or recipients. In the example given in Figure C3, the added manual reconstitution step would increase risks via potential vial contamination, additional opportunity for needle-stick injury, and/or potential use of incorrect amount or type of diluent. *Negative effects on safety are unacceptable trade-offs for thermostability.*

C.2.5.2.2.Private sector considerations

C.2.5.2.21.Selection of private sector partners

It is imperative that vaccine producers engaged in public/private-sector research to advance thermostable vaccines be either United Nations prequalified [UNICEF2003a] or deemed capable of such qualification. Some vaccine producers have stabilization technology expertise in-house, others have established relationships with stabilization technology companies, and a third category may benefit from introduction to sources of stabilization expertise. This latter category is likely to include emerging vaccine producers as well as vaccine development groups such as those supported through The Vaccine Fund. Ideally, projects will be undertaken with multiple stabilization technologies and multiple vaccine producers to spread risks and encourage competition.

C.2.5.2.2.2.Technical and commercial viability

Identification of the costs, risks, and benefits of pursuing a specific product via public-private partnership will be essential in order to predict and ensure success. This process will be similar to that described for accelerating the availability of vaccines for the developing world.[McKinsey2001] [André2002]

Industry partners will need to formulate a business plan that takes into account risks and costs related to:

- Technical feasibility
- Clinical trials
- Production equipment purchase, installation, and validation
- Regulatory review and approval
- Opportunity costs
- Intellectual property and know-how
- Market demand and expected return on investment
- Availability of staff and financial resources
- Potential benefits will also be assessed as part of the business plan and include:
- Competitive advantage/increased price
- Reduced costs through production efficiencies—such as
  - Improved yield,
  - improvements in combination vaccine formulation,
  - larger batch sizes,
  - longer-term storage of bulk and final product
- Reduced shipping and storage costs:
  - decreased internal wastage,
  - ability to ship and store vaccines without refrigeration
- Enhanced revenue—through the ability to utilize stabilization methods and equipment for more lucrative products

This analysis will determine the industry partner's level of interest and the type and magnitude of public-sector assistance needed to drive the project forward.

C.2.5.2.3.Economic Analyses

Information gathered on the costs of achieving a thermostable vaccine product can be used to further quantify the value proposition. Economic analyses will be critical in order to understand the potential impact of developing, producing and using thermostable vaccines on key stakeholders including industry, immunization programs, health providers, beneficiaries and society. Such analysis will help to further clarify prioritization of resources and should be continually updated as data become available throughout the life of a project.

For example, cost-effectiveness analysis could be used to evaluate the overall impact of introducing a thermostable vaccine into an immunization program. Costs might include:

- Higher prices
- Increased logistics of having multiple formats (thermostable and standard) of vaccines
- Program costs, including communication and training requirements for introducing and handling new thermostable vaccines
- Benefits such as savings or improved health outcomes might include:
- Improvements in immunization effectiveness. With existing vaccines, when temperature damage goes unnoticed children may receive heat- or freeze-damaged vaccine. Thermostable vaccines should prevent such situations
- Elimination of wastage that now occurs when temperature-damaged vaccines are identified and discarded
- Reduction in expiry date discards due to longer product shelf lives.
- Lower cold chain costs through storage of thermostable vaccines at ambient temperatures
- Elimination of some cold chain equipment or decrease in required capacity
- Reduced requirements for temperature monitoring devices
- Improved immunization coverage

  Outreach is more difficult when vaccines must be kept at 2°-8°C, particularly with freeze-dried vaccines that must be kept cold after reconstitution. Thermostable vaccines can greatly reduce logistical and equipment needs during outreach if all vaccines being transported are thermostable.
- Improved safety by reducing risks due to contamination and/or improper reconstitution

  Some formats of thermostable vaccines will improve upon current freeze-dried vaccines by eliminating the need for reconstitution or the need to keep reconstituted vaccines cold.

C.2.5.3.Structuring projects

Roles for the public sector to serve as a risk-sharing partner should be identified by the implementing organizations and are likely to vary depending on the project(s) being pursued. Potential roles for the public sector are similar to those used to accelerate availability of vaccines for the developing world and could include:

Development partner and facilitator

The public sector can serve to “push” the technology forward. For example, public sector organizations with research facilities could conduct focused research and development (e.g., toxicology studies on a new excipient or scale-up of a stabilized “orphan vaccine”), with the added benefit that resulting data could be made publicly available. Other “push” mechanisms

include providing financial support through grants or loans for specific activities such as clinical trials or production scale-up, developing specifications, assisting with regulatory issues, or securing access to key component technologies.

Purchaser or influencer of demand

By engaging potential purchasers of thermostable vaccines early on and assisting with characterization of the market for such products, the public sector can help to "*pull*" these technologies forward and prepare for their introduction. Minimization of demand uncertainty through advance purchase agreements, price guarantees, advocacy, and supportive policy can leverage the efforts of the private sector to make these products available.

C.2.5.4.Product development and scale-up

The product development and scale-up process will vary according to vaccine type and format, stabilization materials and methods, and required production equipment. Development of a new or reformulated thermostable vaccine will be a lengthy and expensive process and may require between seven and twelve years to reach the point of availability for use in developing country immunization programs.

C.2.5.5.Introduction

Close coordination and planning will need to occur between implementing groups, the World Health Organization, and vaccine purchasers including the Supply Division of the United Nations Children's Fund and the Pan American Health Organization. Prequalification, pricing, and other procurement issues will need to be addressed. In addition, mechanisms for targeted distribution of thermostable vaccines to countries that can utilize them to their full potential should be established, in anticipation of the availability of thermostable products.

It is likely that thermostable vaccines can be handled according to the existing "good storage practices for pharmaceuticals."[WHO storage] Such products do not require refrigeration, but simply storage in dry, well-ventilated premises at temperatures up to 30°C. A higher upper-temperature limit should be possible with thermostable vaccines. As product development progresses, policy development, advocacy, training materials development, and plans for pilot and final introduction should also progress. Management of a mix of vaccines that are freeze sensitive, heat sensitive, and thermostable will be required until such time that thermostable vaccines are the norm in developing country immunization programs.

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C.3. Appendix A . Sugar-Glass Stabilization of Vaccines: an overview of existing data

Introduction

While a number of companies have conducted research applying sugar-glass materials and techniques to stabilize vaccines, few study results have been released to the public domain. Available data are sparse and often include only summaries of results without details regarding methodology. This paper attempts to consolidate and briefly summarize data that have been made publicly available since 1990. Baseline information is provided on the stability of current vaccine formulations, where available, and followed by data on the sugar-glass formulations.

Stabilization successes

Diphtheria and tetanus toxoids, pertussis and combined vaccines (DT and DTP)

Stability of current formulations
Heat stability of the diphtheria and tetanus toxoid components of DTP has been shown to vary according to producer. In some DTP vaccines, these components can withstand exposure to temperatures above 37ºC lasting several weeks. Storage at 45º for 8 weeks produced a loss in potency for both tetanus and diphtheria toxoids of about 40%. Both components are destroyed in 3 to 5 hours when stored at 60ºC. In addition, both may change their appearance and lose potency when frozen as the gel structure of the aluminum-based adjuvant used is destroyed by freezing.[1]
Pertussis potency is more difficult and expensive to measure than the diphtheria and tetanus components of DTP, but several valuable studies have shown that it is also more sensitive to degradation at elevated temperatures than the other two components. At 37°C, potency declines rapidly at a degradation rate between 1 and 6% (per day?). Storage at 50 to 56°C brings about rapid and complete loss of potency of the pertussis component.

Stability of sugar-glass formulations
Sugar-glass drying has significantly increased the temperature stability of DTP, DT, and separate pertussis and tetanus toxoid components as shown in several studies. (See Table C3 for results.)

Table C3: Stability of sugar-glass formulations of diphtheria and tetanus toxoids, pertussis and combined vaccines

Vaccine	Results
DTaP	"Trehalose-dried DTaP antigens and adjuvant were shown to be biologically and chemically unaltered after storage at 60°C for 12 weeks. Preclinical investigations demonstrated immunogenicity and potency." [2]
DT	The percent of active antigen, as measured by ELISA, for both diphtheria and tetanus toxoid in the combined vaccine dropped by less than 10% after drying with trehalose and storage at 45°C for 35 weeks.[3,4] See Figure C.3.1.
DT	*In vitro* and *in vivo* testing showed no significant loss of activity for trehalose-dried DT after 1 year at 37°C or 60°C.[4]
Pertussis (acellular)	*In vitro* and *in vivo* testing showed no significant loss of activity for trehalose-dried pertussis antigens after 1 month storage at 60°C and 1-3 months at 37°C.[4]
TT	One study showed less than 10% drop in activity after 35 weeks at 45°C as compared to a loss of 50% antigenicity and virtually all activity for the control within 2.5 weeks of storage at the same temperature. [4]
TT	Another study showed no significant loss of activity for trehalose-dried TT after 1 year at 37°C or 60°C.[4]
TT	"TT can be kept totally stable at temperatures ranging from -70°C to 37°C for over 9 months." [4]
TT	TT was stabilized by drying as complex glass microspheres and suspended in oil or a perfluorocarbon. These samples were stored along with a glassified dry vaccine at 37°C for 3 months. At the end of this time, the dry vaccine was rehydrated. All formulations gave high levels of protective antibody in preclinical testing, but a rise in titre over time was noted for the vaccine in oil and perfluorocarbon.[5]

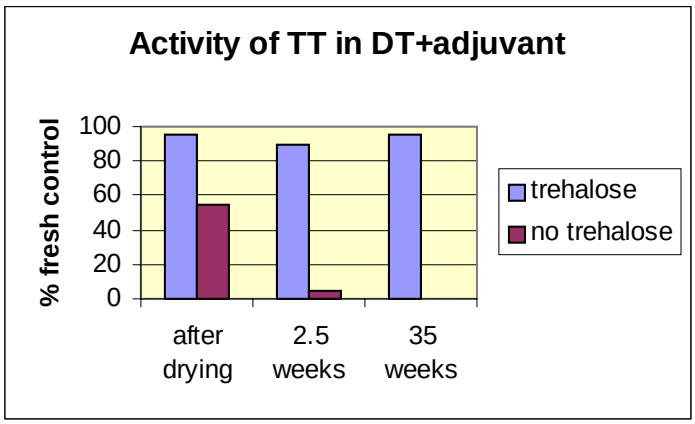

Figure C.3.1 . Activity of tetanus toxoid in reconstituted diphtheria tetanus and adjuvant vaccine. Samples were dried in the presence or absence of trehalose and stored at45°C for up to 35 weeks.[3]

Hepatitis B vaccine

Stability of current formulations
Hepatitis B is supplied in a liquid form, consisting of the surface antigen (HBsAg) adsorbed on aluminum salt. It is stable for several years when stored at 2°-8°C, and can maintain potency for short periods at elevated temperatures. However, studies have shown that significant degradation in potency can occur after as few as 4 months at 20°-26°C, 7 days at 36°-40°C, and 3 days at 45°C for plasma-derived vaccine. [1]

Stability of sugar-glass formulations
Plasma-derived hepatitis B surface antigen was 100% active after sugar-glass drying and storage for 4 weeks at 45°C.[4]

Measles Vaccine

Stability of current formulations
Live measles vaccine is notoriously unstable at elevated temperatures and historically difficult to stabilize. The development of an effective stabilizer and WHO establishment of a requirement for heat stability of freeze-dried measles resulted in an improved quality of measles vaccines on the market.[1] However, the vaccine remains one of the most heat labile used in immunization programs. Currently available freeze-dried measles vaccine is very stable at temperatures below 0°C zero, but degrades at elevated temperatures, especially those exceeding 40°C.[1] It has been shown to maintain required infectivity titre for only about 7 days at 37°C. Reconstituted measles vaccine must be discarded within hours.

Stability of sugar-glass formulations
Two studies have demonstrated the increased stability of measles vaccine dried with sugars. In the first, measles vaccine stabilized with sugar-glass drying maintained potency after 4 months storage at 37°C (see Figure C.3.2).[4] In the second, sugar-glass dried measles vaccine lost no activity after 2 months at room temperature compared to commercial freeze-dried vaccines which lost 90% of original titre in the same time.[4]

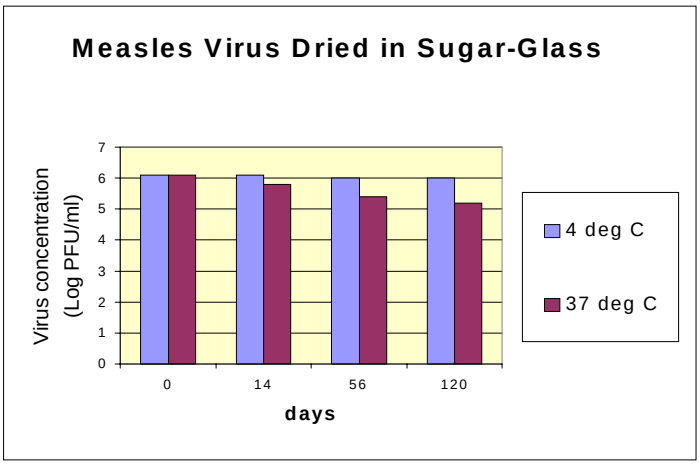

Figure C.3.2. Assayed titres of measles vaccine after drying in sugar-glass and storage up to 120 days at various temperatures.[4] The WHO allowable drop in titre for measles vaccine is 1 log over 7 days at 37°C.[4]

Influenza (PR8) Vaccine

No loss of "antigenicity" and similar or improved immunogenicity were shown for spray-dried influenza vaccine with adjuvants after 9 months of storage at temperatures ranging from -70°C to 37°C (see Figure C.3.3).[4]

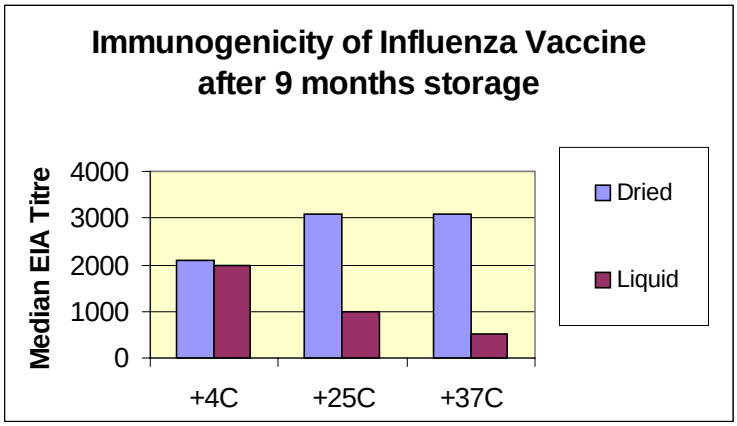

Figure C.3.3. Immunogenicity measured in mice 4 weeks after injection with vaccine that had been stored for nine months.[4]

Note on aluminum adjuvant

One barrier to vaccine stabilization through freeze-drying has been the instability of the important adjuvant aluminum hydroxide when frozen. Freezing causes extensive morphological changes to the aluminum gel structure.[1] Aluminium hydroxide dried in the presence of trehalose exhibited no significant loss of gel structure after 4 weeks at 45°C.[3]

Stabilization challenges

It was found that in order to stabilize oral polio (Sabin 1) vaccine, trehalose must be present inside and outside the capsid, and most methods of introducing trehalose inside the capsid were unsuccessful [2,4]. Also, stabilizing live bacteria vaccines often requires manipulation of growth conditions, making them more difficult to work with than inactivated vaccines or viral vaccines.[6,7]

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C.4. Reducing Dependency on the Cold Chain: additional Suggestions for Future Research

Background

Improperly maintained or outdated refrigeration equipment, poor compliance with cold chain procedures, inadequate monitoring, and poor understanding of the dangers of vaccine freezing contribute to the weakness of the existing cold chain. Emphasis has long been placed on keeping vaccines cold, with less attention devoted to prevention of vaccine damage from freezing. Published reports and field evidence demonstrate that freezing of freeze-sensitive vaccines in the cold chain is commonplace, potentially resulting in widespread delivery of vaccines whose potency has been compromised. [Hanjeet1996, Guthridge1997, Wawryk1997, Bass1996, Lugosi1990, Bell2001]

While sugar-glass stabilization was identified by the Global Alliance for Vaccines and Immunization (GAVI) Research and Development Task Force as a research priority, other areas of research to reduce dependency on the cold chain were identified by the Task Force that merit further consideration by GAVI partners. These areas of research are likely to have shorter time lines and lower investment requirements. All center on facilitating the elimination of freeze damage to vaccines, investigating the feasibility and impact of altering vaccine transport and storage practices, and developing or advancing superior equipment.

One area of research originally identified by the Task Force—assessment of the impact of freeze damage on vaccine immunogenicity—is not included in this research portfolio. The topic was discussed at the “Overcoming Freezing in the Cold Chain” meeting held by the Technology and Operations Panel at the World Health Organization (WHO) headquarters in Geneva in February 2003.[WHO2003a] This panel determined that adequate evidence exists that freezing damages some vaccines. Rather than fund additional laboratory research, WHO is working with vaccine manufacturers to obtain and analyze existing data on the effects of freezing on vaccines.

Suggested Areas of Research

Validate New Vaccine Transport and Storage Procedures
Enable individual countries to assess and characterize cold chain freezing

Provision of assessment tools to individual countries that enable the identification and characterization of vaccine freezing in the cold chain will increase awareness of freezing problems and focus limited resources on effective interventions to reduce them. A generic protocol is being developed by WHO and PATH that can be adapted by countries and used to design cold chain freezing assessments.[Nelson2003] Regional meetings to disseminate study outcomes, followed by technical assistance, could also help countries develop locally appropriate plans for reducing freeze damage to vaccines.

Assess impact of a Vaccine Vial Monitor (VVM)-managed cold chain to reduce freezing

Expansion of VVMs to all vaccine vials could enable a reliable two-temperature cold chain. One temperature system could maintain current icepack transport methods and requisite freezers and equipment for non-freeze-sensitive vaccines. A second temperature system could include mechanisms to protect freeze-sensitive vaccines during transport and storage (see Section 2.1.3.). VVMs on all vaccine vials would help ensure that heat exposure limits are not exceeded. Monitoring and evaluating VVM-identified vaccine wastage would identify flaws in the cold chain and facilitate strategic investments to strengthen the cold chain. Additionally, VVMs are a critical monitoring tool during outreach to extend vaccination coverage.

Initiatives such as this are currently limited by the fact that countries receive inconsistent mixtures of vaccines with and without VVMs and can not depend upon access to VVMs for vaccine management. Since 2000, United Nation's Children's Fund (UNICEF) has specified that all vaccines be supplied with VVMs. At present, approximately half of vaccine suppliers to UNICEF include VVMs on all their vaccines.[WHO2003b] The New Technologies Working Group of GAVI's Research and Development Task Force is supportive of the recent GAVI Board recommendations to accelerate implementation of VVMs on all vaccines. [GAVI2002]

Model flexible cold chain policies in several countries

Application of information on the extent and location of vaccine freezing in the cold chain from above mentioned cold chain freezing assessments would motivate countries to introduce new procedures to reduce freezing. Options to reduce vaccine freezing with more flexible cold chain policies include the use of chilled water packs during transport and storage in air-conditioned rooms. These strategies should also be evaluated for cost and impact on cold chain capacity. WHO is currently working on a "flexible cold chain" policy document to assist countries with modeling and implementation of these policies to reduce freeze damage to vaccines.
Develop "freeze-proof" refrigeration and outreach technologies such as:

New solar or solar-hybrid refrigerators

Health clinics serving areas with intermittent electricity are often forced to use inefficient and freeze-prone kerosene refrigerators. Lower-cost solar systems are increasingly available and include solar-hybrid refrigerators that can be powered using solar, electricity, or batteries. In addition to reducing freezing, these improved solar systems could simplify operations, and be more reliable and cost effective. These technologies could be validated and refined for developing country use.

Phase change materials (PCMs) for ice-free refrigeration and outreach

The cold temperatures required to freeze ice packs in refrigerator/freezers and the transport of vaccines with unconditioned ice packs are sources of freeze damage to vaccines. [Lloyd2001] Elimination of the need for ice, while maintaining specified cold chain temperatures, would be of great value. Replacement of ice packs with new PCMs, such as eutectic salt solutions or paraffin-based products, would eliminate the need to achieve temperatures below 0°C in order to produce ice packs. The trade-off,

however, is that the cooling capacity of PCMs is less than that of ice for the same weight. Efficient insulation and improved refrigeration technology could be used to "freeze" the PCM materials, but not freeze vaccines. An added advantage is that such an ice-free cold chain would reduce energy requirements for vaccine refrigeration.

New technologies to eliminate freezing in vaccine carriers

Although above mentioned PCMs will prevent freezing problems during outreach and can be introduced into new or expanded outreach programs, ice packs will remain ubiquitous in cold chain transport as most central facilities have ice pack freezers. As a short-term solution, new cold box liners or other materials could be developed for use inside existing vaccine carriers to protect vaccines from freezing.

New technologies to eliminate vaccine freezing in ice-lined refrigerators (ILRs)

New ice-free, cold chain technologies will provide safe and fuel-efficient solutions for cold chain systems in the future; however, there are a large number of ILRs in district-level cold chains around the world. ILR technology is inexpensive,high-volume, and holds cold temperatures after long power outages, but is prone to sub-zero temperatures and has been identified as one of the major causes of vaccine freezing in district level cold chains. Retrofitting these ILRs with specially designed heating elements could prevent the problem of vaccine freezing in existing ILRs. Other solutions may include refilling the ice lining with materials such as paraffin-based PCMs that freeze at temperatures higher than that of vaccines.

Identify and promote low cost and accurate freeze indicator technologies

Detection of freezing of sensitive vaccines is critical both in order to discard those vaccines exposed to freezing temperatures as well as to provide a means to assess weak points in the cold chain. The primary freeze indicator technology used today, FreezeWatch™[6], has some limitations in terms of accuracy and shelf life. Alternatives, like simple thermometers, while inexpensive and relatively easy to read, do not provide temperature history and their accuracy varies. Electronic thermometers vary from complex to small digital readout devices. While accurate, re-usable and useful these devices are expensive. Low-cost and accurate, freeze indicator technologies are needed to document, characterize and help correct freezing problems during vaccine shipping and storage.

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[6] FreezeWatch is a trademark of the 3M Corporation.

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D. Improving tools to measure performance of immunization services by developing a non-invasive (oral fluid) field assay to measure protective levels of tetanus antitoxin

D.1. Executive Summary

Programmes to immunize individuals, particularly those to immunize children in developing countries are essential for global health against infectious diseases. Methods to actively monitor the effectiveness of vaccination efforts are few, and the extent to which programmes meet their expectations is unknown. The Working Group was tasked with identifying technology that would offer a non-invasive means to assess the effectiveness of immunization programmes, while being appropriate for field use as well as in laboratories that possess only basic capabilities.

Antibodies in blood to tetanus toxoid almost never occur in persons who have not been immunized. Therefore, their presence usually verifies adequate immunization efforts and can be used as a marker to monitor the effectiveness of vaccine efforts. Currently, measurement of tetanus antibodies is routinely assessed in blood to determine protection following immunization. However, the obtainment of blood requires an invasive method of collection, is sometimes difficult to implement in remote locations, requires sharp objects (needles) that are dangerous for health care workers, and results in sharp waste materials that can jeopardize the safety of others. These issues can be addressed by the collection and use of alternative body fluids for testing such as oral fluid samples. A test designed to assess sufficient levels of antibodies to tetanus toxoid in oral fluids is proposed as a non-invasive and safe means to monitor whether immunization programs have been effective in providing protection.

Oral fluids, collected from the oral cavity, are composed of saliva and a fluid derived from blood. This fluid, crevicular fluid, is a filtrate of plasma that can be preferentially and easily collected from the area at the tooth-gum margin. Although at concentrations lower than in blood, antibodies in crevicular fluid can be detected with the use of highly sensitive tests. A number of oral fluid collection devices are commercially available, and oral fluids collected by these devices have proven effective for the identification of a number of infections agents if appropriately sensitive tests are employed.

The Working Group reviewed current technologies and determined that the development of an oral fluid test to effectively measure protection to tetanus toxoid is feasible, and would be valuable to assess immunization programmes. Such a test could be developed and validated in a reasonable period of time (1-2 years), and could be configured in a rapid test format (completion in less than 30 minutes) for ease of use by a variety of health care personnel. Several collection and testing devices, presently available and proven effective for other infectious agents, could be exploited and appropriately modified for detecting tetanus antibodies. The Group recommended that the test be made in a format that allows both point-of-care testing (on site), and the collected samples suitable for easy transport at ambient temperatures to a laboratory where testing can be batch-tested by individuals other than those making the collections.

The project is envisioned to require:

(1) Collection of appropriate oral fluid/serum sample pairs to be used as calibrators for test development,
(2) assessment of different oral fluid collection devices for their ability to effectively collect samples rich in crevicular fluid with adequate antibody levels,
(3) modification of existing tetanus antibody tests to perform with oral fluids e.g., increased sensitivity) and assessment,
(4) replacement of this method with a rapid test format and re-assessment,
(5) extensive validation of the rapid oral fluid test using large numbers of sample pairs, and
(6) standardization of the product for manufacturing.

Groups to be considered to undertake test development include industry and academia. Industrial partners offer the advantages of experience in development and bringing products to market, but may be limited by the expected low return of profits because of relatively low numbers of products required. Academic support is generally faster, less expensive, and intellectual property issues may be less complicated. Ideally, a collaboration between industry and academia may result in speed of test development, rapid standardization, and at more reasonable costs.

D.2. Research Plan and recommendations

D.2.1.Task of the Working Group

The Working Group was tasked with determining the feasibility of having a non-invasive test made available for monitoring the effectiveness of immunization programmes in developing counties. The objective is to develop a simple test that can identify antibodies to tetanus toxoid in easily-collected oral fluid (saliva) samples. The test should be of sufficient sensitivity and specificity to make it useful to determine, under field-use conditions, whether an individual has received a tetanus vaccine(in the case of toddlers it can be presumed the vaccine received would have been the triple diphtheria-tetanus-pertussis vaccine)that has elicited a protective level of tetanus antitoxins. The test would thus be a surrogate marker of the effectiveness of the immunization programme in the area being surveyed - at least for the triple vaccine – regarding the storage,transport and administration of vaccine.

D.2.2.Issues to be Addressed

The following issues to be addressed were identified:

Is technology available that can be used to meet the task, and can such a test be developed in a reasonable time frame (1-2 years)?

Should the test be devised for point-of-care testing at field sites, or should specimens be collected and transported to a central location for batch-testing under optimal laboratory capabilities while, at the same time, addressing transport issues?

Can test sensitivity and specificity requirements be met?

What factors need to be addressed related to production and subsequent manufacturing?

D.2.3.Background

D.2.3.1.Introduction

Rapid and simple-to-perform test technologies allow for the detection of antibodies to a wide variety of infectious agents. In addition, tests have been successfully developed that can detect antibodies in several body fluids, including serum, whole blood from fingerstick, oral fluids, and urine (Constantine et al., 1992).Tests that use sample media that are collected non-invasively (i.e., oral fluids, urine, fingerstick whole blood) are in routine use in laboratories throughout the world, and have been shown to possess sensitivities and specificities equivalent to more conventional tests that use serum or plasma samples. Such simple tests that use alternative body fluids that are non-invasively collected can be exploited can be devised to identify individuals who have been effectively immunized, thereby verifying the successful efforts of immunization programmes.

D.2.3.2.Monitoring Immunization Programmes

Programmes to immunize individuals, particularly those to immunize children in developing countries are many, but their ability to reach large numbers of persons, and the effectiveness of the efforts are questionable. Methods to actively monitor protection from infection after vaccination and to determine adequate antibody responses in vaccinees currently include self-reporting or documentation at households, but these methods may not be accurate, and are labor intensive and costly (M. Levine, personal communication).Better methods to verify the effectiveness of programmes are needed.

The presence of antibody as a result of vaccination is an undisputed measure that vaccination has been accomplished. However, antibody as a result of vaccination must be differentiated from the presence of antibody acquired from natural infection. As a surrogate to verify immunizations, the determination of antibody status to tetanus toxoid has been proposed to be effective. Because antibodies to tetanus almost never occur in persons who have not been immunized, their presence verifies vaccination and suggests adequate immunization efforts. Furthermore, antibody levels, measured in international units, can be assessed to determine if immunization has been effective in producing protective levels (>0.01 IU/ml) (WHO, 1993).Several reports have shown that populations can been monitored for responses to tetanus toxoid through serologic testing using serum samples (Deming et al., 2002; WHO, 2000).

D.2.4.Rationale

The availability of a simple test using non-invasively collected samples such as oral fluids would be an effective and cost-savings tool for GAVI to ensure that immunization programmes were accomplishing their intended goals. This would be used as a supplement for verifying that, in particular, children were receiving effective immunizations to protect them against a variety of infections. The proposed test would be appropriate for point-of-care testing (in the field), as well as at reference centres.

D.2.5.Oral Fluids as a medium for Testing

D.2.5.1.Origin and Definitions

It has been known for almost 20 years that antibodies to infectious agents can be detected in oral fluids (Johnson et al., 1988). The original testing was for the detection of anti- HIV IgA immunoglobulin that was derived from the secretory mucosa or salivary glands. However, it was later found that IgG antibodies are present in oral fluid and can be detected with an equivalent accuracy as the detection of antibodies in blood. Oral fluid testing for the detection of antibodies to some infectious agents, including HIV, is now performed routinely, and the isotype of antibody that is detected is IgG (Klokke et al., 1991). When first attempted, the detection of IgG in oral fluids was difficult (test sensitivity was poor). However, this less than optimal sensitivity was not caused by the absence of antibodies in the fluid but rather to a relative insensitivity of the assays for detection of low quantities of immunoglobulin. This was verified later with the development of exquisitely sensitive assays, specifically designed for the testing of oral fluids (Holm-Hansen et al., 1993). Furthermore, the availability of specifically constructed oral fluid collection devices offered preferential collection of appropriate fluids (crevicular fluid) in a standardized manner. As the composition of oral fluids became better understood, the manufacturers of tests appropriately modified their tests to obtain the necessary increase in sensitivity

Oral fluid is different than saliva; it contains not only pure saliva from the salivary glands, but other secretions from the mucosa of the oral cavity, and crevicular fluid that is derived from capillaries beneath the tooth-gum margin (Tamashiro and Constantine, 1994).For this reason, the term oral fluid should be used rather than saliva.Because crevicular fluid is a transudate derived from blood (plasma), oral fluid samples contain plasma from the blood (the same fluid used for testing serum with serum-based tests).Therefore, the testing of oral fluid is really the testing of plasma in the oral cavity. However, the concentration of antibodies in this oral fluid is much less that in plasma because of the dilutional effect of fluids from the salivary glands (true saliva), necessitating the requirement for extremely sensitive tests that are able to detect small quantities of antibody.

The term whole saliva or mixed saliva has been proposed for the mixture of the salivary gland secretions, crevicular fluid, and other products of the oral mucosa, collectively. Whole saliva contains parotid, submandibular, and minor salivary gland secretions as well as mucin, bacteria, leukocytes, sloughed epithelial cells, and mucosal transudate. Whole saliva can be

collected directly from the oral cavity either by dribbling or spitting. The two main types of fluids found in whole saliva from the oral cavity are pure saliva and crevicular fluid.Pure saliva originates in the salivary glands and can be collected only by special procedures.

In contrast, crevicular fluid (CF), also commonly referred to as gingival CF, gingival crevicular transudate, oral mucosal transudate (OMT), is derived as an interstitial transudate passively transported from the capillary bed beneath the buccal and gingival mucosa to the oral cavity (Lehner, 1992).From the blood capillaries, the transudate diffuses through the subepithelial tissue and then the junctional epithelium and collects in the gingival crevice as CF before flowing into the oral cavity to mix with pure saliva.The origin and flow of CF into the oral cavity is depicted in Figure D1.CF is composed of humoral and cellular components from blood, but at different concentrations due to the transudation process. Concentrations of the components are much lower in the oral fluid because of the dilutional effect from pure saliva and other secretions. CF can be easily collected from the gingival crevices at the tooth-gum margin and the buccal mucosa with special collection devices.

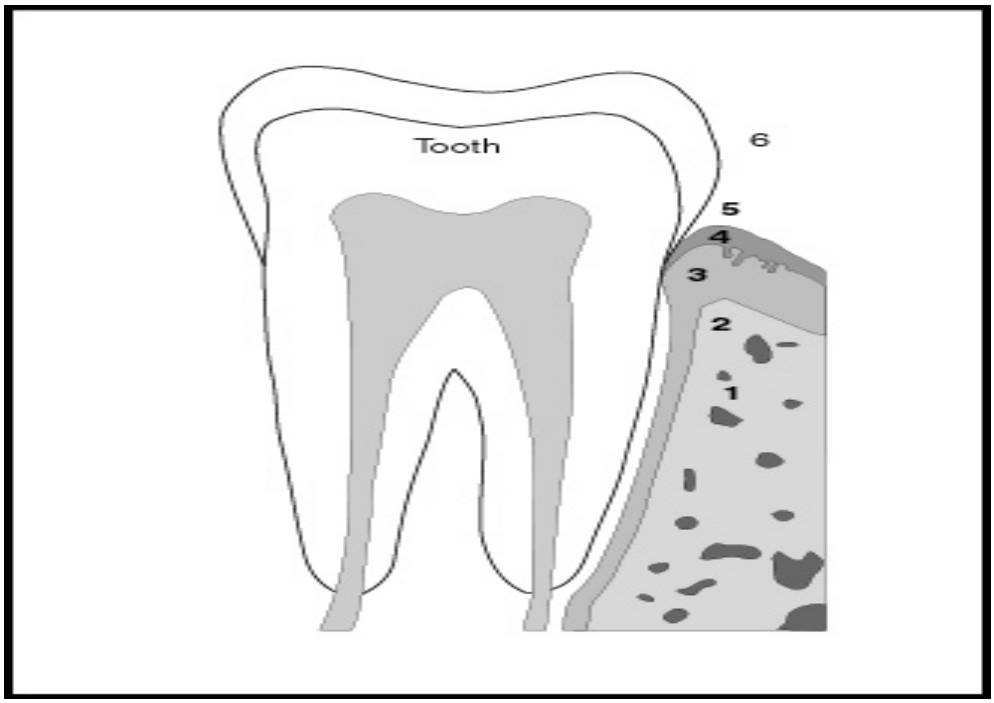


Figure D1. Flow of mucosal transudate from capillaries to oral cavity: 1) blood capillary; 2) subepithelium tissue;3) junctional epithelium;4) sucular epithelium; 5) gingival fluid; and 6) saliva.

The predominant immunoglobulin in CF is IgG; therefore, when whole saliva is collected for analysis, there are significant levels of IgG that can be detected, although at a lower level due to a dilutional effect from pure saliva.Because these levels of IgG are low, many concerns have been raised over the ability to detect the IgG.However, it has been conclusively shown that ELISA, Western blot, and rapid assays that use oral fluids can perform as well as serum-based tests (Saville et al., 1997; Constantine et al., 1992).The key is to have specifically designed tests that are much more sensitive than

serum-based tests; thus the sensitivity of the tests compensate for the lower levels of antibodies in oral fluids.

Table 1 shows the composition of different isotypes and concentrations of antibodies in oral fluids.Importantly, it should be noted that the concentration of IgG in whole saliva is 1000 times less than in plasma, and that of CF is about 4 times less.If specific devices are used to preferentially collect CF, the concentration from the collection devices is about 1/400 of that in plasma (Tamashiro and Constantine, 1994). Consequently, if oral fluid tests are to accurately detect specific antibodies in oral fluids collected with these devices, the tests must be made to be about 400 times more sensitive.

Component	IgG	IgM	IgA
Plasma	14,730	1,280	2,860
Parotid saliva	0.36	0.43	39.5
Crevicular fluid	3,500	250	1,110
Whole saliva	14.4	2.1	194

Table 1.Mean Immunoglobulin Concentrations (mg/l) in Plasma and Selected Oral Fluid Components.

Table 2 shows the concentrations of IgG in the different oral fluids when collected by specific devices (Parry, 1989).It should be noted that the concentration of IgG in oral fluids may not be as important as the sensitivity of the test used to test that fluid.In other words, specific tests are designed to work effectively with

specific collection devices.

Fluid	Mean Concentration (mg/l)
Dribbled saliva	12.1-14.4
Crevicular fluid-rich saliva	
Foam swab	14.4
Sampler/Omni-Sal	23.5
OraSure	40.5
Salivette	9.6
Parotid saliva	0.2
Crevicular fluid	3,500
Plasma	12,500

Table 2.Comparison of the Concentrations of IgG in Oral Fluids Using Different Collection Methods.

D.2.5.2.Advantages of Oral Fluids as a Testing Medium

Oral fluid samples can be collected easily, with little instruction, and can be administered by any health care workers without the need for special training (e.g., phlebotomy).In addition, some populations oppose giving blood specimens, and therefore the ability to collect and test oral fluid (or other type) samples allows the determination of the prevalence of HIV in populations where little information is available because of objection to blood collections. Oral fluids can be collected in a variety of field settings, are safer, involve non-invasive collection methods, and are particularly valuable in developing countries where there are cost and personnel limitations. Oral fluid collection methods allow storage in their transport media for up to 21 days at room temperature before testing, and the collection devices are easily stored and stable at room temperature for years. As a noninvasive and painless collection method, the use of oral fluids is much more acceptable compared with phlebotomy by many individuals and offers a potential for a higher degree of collection compliance among subjects being tested for surveillance purposes; this may also reduce sampling bias. Individuals, such as children and others whose blood may be difficult to obtain can be sampled easily. In general, most volunteers do not have complaints about oral fluid collection devices, and some studies have shown that approximately 69% of the volunteers preferred oral fluid collection to having their blood drawn (Major et al., 1991Peralta et al., 2001).Oral fluid testing has also been applied in the evaluation of antibody responses in volunteers receiving a recombinant HIV vaccine, and this method may be an easier alternative to the collection of blood from groups of vaccinees that must be monitored for response to the vaccine. Finally, the use of stabilizers in oral fluid collection systems reduces the effects of unfavorable transport/storage conditions, such as climate, high temperatures, and lack of refrigeration facilities in developing countries. However, stabilizers may not be necessary if testing is performed quickly.

Concern has been raised about the levels of specific antibody in oral fluids as compared to blood during early infection or after vaccination. Studies performed to assess antibodies in oral fluids to measles, mumps, and rubella from persons who had been immunized and were seroconverting, showed equivalent intervals of time for the detection of antibodies in serum and oral fluid pairs (Thieme, 1994).The sensitivity and specificity for detection of measles, mumps, and rubella using the OraSure collection device and an ELISA method were 97% and 100%, 94% and 94%, and 98% and 98%, respectively. Thus, there is no reason to believe that antibodies to any infectious agent are present in blood before in oral fluids; after all, they are the same antibodies, just present in different compartments. Another concern with the testing of fluid from the oral cavity has been the potential for interference. However, in a large study of different populations, the accuracy of testing oral fluids has not been found to be compromised by the presence of oral lesions or a history of smoking (Tamashiro and Constantine, 1994). Also, there does not appear to be any correlation between antibody levels in oral fluid and dental conditions such as the number of teeth, the visible plaque index, or the percentage of periodontal pockets. Antiviral antibodies in oral fluids

have even been demonstrated in edentulous and partially dentate individuals (Bragg et al., 1991).

D.2.5.3. Collection Methods for Oral Fluids (Crevicular Fluid)

For most effective and accurate collection of oral fluids, specific oral fluid collection systems must be used because these provide a more standardized means with highly reproducible results, and collect oral fluids that are rich in crevicular fluid that contains IgG antibodies. The development of specific oral fluid collection devices has made collection of specimens easier as well as allowing one fluid to be collected preferentially over another, although all samples will be a mixture of oral fluids. The target for collection is CF, which is high in IgG content and that is derived from the blood. The ratio of types of fluid that is collected will depend on the device, where the device is placed in the oral cavity, and the amount of salivation occurring during sampling. Most devices that have been developed consist of an absorbent device, which can be a foam swab, rayon ball or roll, or a pad on a plastic stem. The device is usually removed from an individual package and placed in the oral cavity or chewed for several minutes. After saturation of the device, it may be removed and placed in a transport buffer tube containing antimicrobial agents (and sometimes, antiproteolytic stabilizers), or processed immediately. To obtain the oral fluid, some devices are subsequently centrifuged or filtered with a serum separator, while others (e.g., rayon ball) are simply compressed to expel the fluid. Once collected, oral fluid specimens are stable for up to three weeks at room temperature if placed in a preservative, or even longer at refrigerated or frozen temperatures (package insert, OraSure).The four most recognized commercial oral fluid devices are the Sampler (previously called the Omni-Sal) from Saliva Diagnostics Systems; the OraSure (OraSure Technologies, previously Epitope); the Salivette (Sarstedt); and the Orapette (Trinity Biotech). Each is described below.

The Sampler system consists of an absorbent pad that is placed under the tongue for two minutes and includes an indicator in the plastic stem that identifies when the amount of oral fluid collected is sufficient.Following collection, the saturated pad is placed in a buffer solution for transport to the laboratory. The stem can easily be removed and the pad is either centrifuged or compressed with a serum separator to obtain the oral fluid. The Sampler, placed under the tongue, collects oral fluids rich in CF. The antiproteolytic agents and stabilizers present in the transport buffer allow the oral fluid samples to be stable at room temperatures for periods up to 3 weeks according to the manufacturer.

The OraSure system, licensed by the FDA in 1994, is very similar to the Sampler in appearance, but the absorbent pad is placed and held stationary along the tooth-gum margin between the lower gum and the inside of the cheek for two minutes; it collects a whole saliva sample that is rich in CF. The pad is also chemically treated (contains a hypertonic salt solution and

gelatin) to enhance the collection of CF; thus, the oral fluid sample is concentrated preferentially with CF. After collection, the pad is placed in 800 µl of a preservative solution, and the oral fluid is then eluted by centrifugation. This can be stored at 4-35°C (39-98°F) for 21 days before testing, and samples can be shipped through the mail at room temperature without refrigeration. This OraSure collection system has been shown to be capable of successfully detecting antibodies to HIV, measles, mumps, HCV, and H. pylori in oral fluids. Figure D2 shows the OraSure oral fluid collection device

Figure D2.The OraSure collection device.

The Salivette collection device consists of a cotton-wool roll that is placed in the oral cavity for 1 minute. As the roll is masticated, a mixture of fluids is absorbed which are then eluted by centrifugation. Conflicting results have been reported on the reliability of the Salivette to collect oral fluid with detectable levels of immunoglobulin.

The Orapette is a treated rayon ball (similar to a cotton ball) that is placed in the oral cavity and moved around the inside of the mouth closely near the gums to stimulate and collect CF at the tooth-gum margin. The rayon ball has been chemically treated to absorb protein, and individuals are instructed to move the ball around the mouth with their tongue, paying particular attention to the gingiva around the teeth in order to preferentially collect CF. As the ball becomes saturated with oral fluid (preferentially CF), it is removed by the subject and placed in a disposable syringe-like plastic device that compresses the rayon and expels the eluate while trapping particulate matter in the rayon. About 1 ml of oral fluid is collected in this system. One disadvantage of the Orapette is that the sample must be tested immediately, refrigerated for short periods, or frozen for later testing; there is no preservative material to prevent bacterial growth. Figure D3 shows the

Orapette and its associated syringe-like device for collection and expelling of oral fluid, respectively.

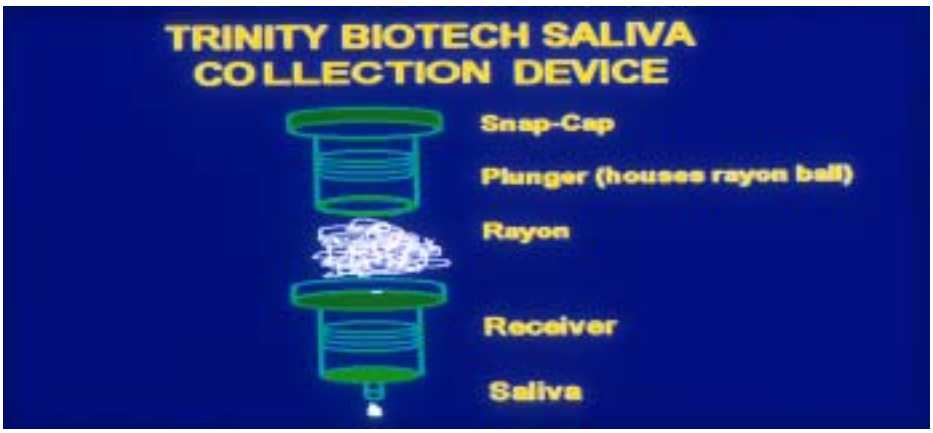


Figure D3.The Orapette and syringe-like device.

D.2.5.4.Oral Fluid Testing Systems

D.2.5.4.1. Enzyme ImmunoAssays (EIA):

EIA methods such as ELISA tests for the detection of antibodies to infectious agents are well known and respected. Their main advantages are relative simplicity and the ability to test large numbers of samples simultaneously (up to 90 in a three hour run).Their main disadvantages include the requirement for instrumentation (that requires stable electricity and is of high cost), calibrated pipettes for delivery of reagents (which are not always available in developing countries), and relatively long turn around time for results (as compared to rapid tests).ELISA methods are avilable for testing fingerstick blood, oral fluids, and urine for evidence of antibodies to a variety of infectious agents. Several EIA methods are available that can be used with oral fluids for testing; again, these are specially designed (or modified) tests and not the ones used for testing serum. The development of an oral fluid test for tetanus toxoid using oral fluids collected by this system would require calibration, optimization, and validation. The OraSure collection device is designed for use with ELISA type assays, and performs well for HIV. There is no reason to suspect that this collection device would not be suitable for collection of samples for the subsequent detection of tetanus toxoid antibodies.

D.2.5.4.2.Rapid Tests:

Rapid HIV tests have been developed that can accurately detect antibodies in oral fluids; e.g., antibodies to HIV (Saville et al., 1997).These are configured as dot-blot types, lateral flow types, or dipsticks.As examples of the most popular oral fluid rapid tests are the flow-through and a collection/testing One-Step assay (OraQuick).

The OraQuick is a combination collection and lateral flow testing device to detect antibodies (to HIV). Although the FDA has licensed it for detection of HIV antibodies only for fingerstick samples, it is available internationally for use with oral fluids. Consisting of an absorbent (porous) pad on a stick coupled to a lateral flow testing device, it is used to swab once around the gums, and placed in a vial of buffer solution. Following a 20-minute incubation, the results are read like other lateral flow rapid tests and a control line is included. Figure D4 shows the latest technology in rapid tests, by combining a rapid collection/testing/lateral flow technology that allows any one of a variety of testing media to be used by the same device; i.e., serum, plasma, venipuncture whole blood, fingerstick blood, or oral fluid. This is an example of a truly One-Step rapid collection and testing device. An example of a combination collection/rapid testing system is the OraQuick from OraSure Technologies (for HIV).

Figure D4: A rapid, one-step, combination collection/testing rapid assay that uses oral fluids (OraQuick).

D.2.5.4.3.Simple Tests:

Simple tests are defined as tests that are easy to perform, but require more than 30 minutes for completion (Constantine et al., 1992).However, although relatively long turn around time as compared to rapid tests, there is usually little hands-on time.An example of a simple test that is designed to test oral fluid samples is a dipstick method that requires about two hours for completion.A major advantage of this test over rapid tests is its ability to test large numbers of samples.The test is a dipstick technique that uses a plastic comb with “teeth” to which HIV peptides are contained (on the flat surface of each tooth of the comb).The solid phase comb has separate “spots” with different

antigens to detect several viruses (e.g., HIV-1 and HIV-2), and has twelve teeth (for 12 separate tests).All reagents are contained in a cassette in rows (wells) in a developing plate.Oral fluid is placed in the first row of the cassette and the teeth of the comb are inserted into the wells containing different samples.The antigens on the teeth react with the antibodies in the samples, and after an incubation, the comb is removed and inserted into the next row, which contains a wash solution.Subsequently, the comb is moved to adjacent rows of the cassette that contain conjugate, buffers, and substrate.Only a pipette is needed to accurately measure and dispense the samples into each row.These tests provide documentation of the results as well as contain an internal control that indicates the presence of total IgG in the saliva sample.The test can test ten samples at a time plus controls, and it is easy to begin another one or two sets of ten specimens during the incubation periods of the first set.Thus, about 30 specimens could be tested easily in about 1 hour with minimal hands-on time.An example of a simple test designed to test oral fluids is the ImmunoComb II (Orgenics) for HIV detection.

D.2.5.4.4. Advantages of Rapid Tests over EIA Methods

Simplicity

All rapid tests are simple to perform, but are differentiated from “simple tests” based on their ability to offer results in less than 30 minutes. However, there are different degrees of simplicity, ranging from the simplest where just the sample is added to the device, to other rapid tests that require multiple procedural steps including a pre-dilution of the sample, timed incubations, comparison of results to some standard to gauge the intensity of the reaction, and some that produce results that are less than easy to read and interpret. Nevertheless, all are simple to perform in comparison to methods such as EIAs and Western blots. Most do not require pipettes that must be calibrated, but use disposable droppers that deliver predetermined volumes of sample or reagents, making delivery quite simple.

Another advantage that rapid tests offer due to their simplicity is point of care testing where collection and testing can be performed simultaneously (rapid oral fluid test), an attribute that could never be accomplished before because of the required sample processing. The combination of collection and testing eliminates the need for phlebotomy, eliminates the requirement for laboratories and centrifuges to process samples before testing, and it decreases turn around time for results substantially. Rapid assays that allow the use of alternative body fluids such as oral fluids and fingerstick, are ideally applicable for hard-to reach populations and where rapid turn around times for results are needed. Finally, rapid tests can be considered as simpler than EIA tests because they do not require instrumentation, which necessitates maintenance and routine quality control checks.

Robustness

A number of rapid tests are considered to be robust, particularly as compared to EIA screening tests. For example, some rapid assays can be stored at ambient temperatures (room temperature) or have a wide range of storage temperatures (18-33 C) that allows them to be transported easily, even carried within a pocket. This eliminates the need for a cold-chain (refrigerated transport boxes) and allows the use of smaller transport containers. The allowance for higher temperature storage (33C) is particularly valuable in laboratories where there is poor temperature control and outside temperatures are high. Also, many rapid tests allow an extended time to read results (e.g., 15-60 minutes) after the last reagent is added. This not only gives flexibility in their use, but the stability of results for extended times allows verification of results in real-time by other personnel. Because many rapid tests are designed to be stable at ambient temperatures, their reagents are usually in a dried form. This allows a longer shelf-life, as compared to some EIA methods where liquid conjugates, for example, have a short duration for use. Finally, many rapid tests are packaged in sealed packages, allowing ease of transportation and resistance to spillage of liquid reagents.

Speedy Results

Rapid assays can produce results in as little as 1 minute, and all produce

Results within 30 minutes. Most require about 15 minutes from addition of the sample. Although speedy results are not always needed, depending on the testing purpose, there is no debate that this is a clear benefit in many testing situations. As applied to immunization studies, it could be envisioned that immediate results while the individual was at the clinic would allow for immunizations to be conducted if it were found that the desired level of protective antibodies was not present. However, if large numbers of sera are to be tested, the advantage of rapid tests to provide speedy results for a large number of individuals is lost.

Internal Test Attributes

Most rapid assays incorporate an internal procedural control to verify that all

reagents are performing adequately and that the sample has been added (an important attribute that EIAs lack). This internal control, usually an antibody to human IgG, detects any immunoglobulin in a sample, and is primarily incorporated to eliminate false negative results that could occur due to failure to add a sample (Ketema et al., 2001). However, it also acts to verify that the conjugate system is working and that proper wicking and movement of reactants are occurring as expected. If the control does not perform as expected, the results are considered invalid.

Increased Compliance for Testing

Rapid tests that use oral fluids or fingerstick samples have been shown to be valuable for certain populations that are reluctant to give blood (for religious reasons) or for populations where blood is difficult to obtain. Although these media can be used with EIA tests, it has been shown that compliance for testing increases when rapid tests that use these media are offered. Several studies have verified that rapid tests in conjunction with non-invasive collection methods are preferred, and result in the increased testing of individuals (Peralta et al., 2001). Furthermore, these tests can be used for group collections (a number of persons can collect oral fluids simultaneously), thereby increasing compliance from peer pressure, and resulting in rapid results for a large number of persons within minutes without the requirement for blood drawing.

Use in Developing Countries

Perhaps most importantly on a global basis, is the application of rapid tests in developing countries (Constantine et al., 1992). The use of rapid tests is already widespread and addresses the financial restraints and infrastructure issues that are omnipresent in developing countries. A lack of adequate resources limits the laboratory capabilities because of infrastructure limitations (e.g., stable electricity and equipment), and the training of personnel. Rapid assays address these issues quite effectively. It can be reflected in early studies that were performed in Tanzania where rapid tests were used in a hotel room and at night when the temperature was cooler and within the acceptable range for testing (Constantine, unpublished). Also, because of a lack of electricity, testing was performed by candlelight. This exemplifies the potential for rapid tests to “get the job done”. With the advent of newer rapid tests that do not require refrigeration, their use becomes even more attractive for developing countries. In addition, a number of developing country issues can be addressed by rapid tests that use fingerstick or oral fluid specimens. Rapid tests are also being used effectively in mobile vans and recreational centers for outreach programs to get more individuals tested.

D.2.5.4.5. Principles of Rapid Tests

D.2.5.4.5.1.Dot-Blot Rapid Assays

Rapid HIV assays that incorporate “spotted” antigens on solid support paper and that are usually configured so that the sample and reagents pass through the membrane to an absorbent pad while reactions take place on the membrane are referred to as “dot-blot” or “flow-through” assays (Constantine et al., 1992). Many yield results within five or ten minutes some in as little as three minutes. They are easy to perform, but do require multiple steps in comparison to the newer lateral flow and one-step rapid assays. In typical dot-blot assays, the antigens are passively blotted (adsorbed) onto the support membranes usually by hydrophobic interactions, and are most often placed as a small circle (dot) or line.

These antigens are usually recombinant, synthetic peptides, or a combination of the two.A plastic device holds the solid support and contains absorbent pads under the membrane to collect the serum and reagents after the reaction with the antigens has occurred on the membrane. The procedure is usually initiated by the addition of a buffer first which acts to wet the solid support membrane (usually nitrocellulose), followed by 1 or 2 drops of sample. Following this, more buffer is added, and subsequently a conjugate (and substrate if the conjugate is an enzyme).In some rapid tests, anti-human immunoglobulin attached to enzymes are the conjugates that bind to the antibody in the sample. The addition of a suitable substrate produces a color on the paper at the site (dot or line) where antigen was attached. More commonly, conjugates consist of a Protein A colloidal gold reagent. Protein A, a substance derived from the cell wall of the bacterium *Staphylococcus aureus*, has a strong propensity to bind to most human immunoglobulin of the IgG class, thereby reacting with an antibody to HIV that has been captured by coated antigen. The colloidal gold portion of the conjugate is responsible for visualization of antibody reaction at the antigen site on the membrane. This conjugate system has the advantage of increasing the speed of the test because no substrate step is needed. In addition, the Protein A colloidal gold reagent can be freeze-dried (lyopholized) to enhance stability, thereby allowing storage at room temperature; i.e., does not require refrigeration. The dot blot assays require successive steps of adding several reagents, either with timed periods (1-2 minutes) between additions, or simply just waiting until each reagent becomes absorbed through the membrane. This requirement is considered as a disadvantage to some users because the waiting for absorption makes testing many samples simultaneously difficult. Since reactions or background color may proceed with time, it is important to read the results within the specified time interval, unless a stop solution is included. Most all of these dot blot assays contain an internal control dot indicating that the test is working properly, that all reagents are good, and all have been added. The main purpose is to show that the sample was added because all samples have human immunoglobulin. If this control does not show the expected result, the test is not performing properly or the sample was not added, and the test result is labeled as invalid. Figure D5 shows an example of a dot-blot test with positive results and the internal control.

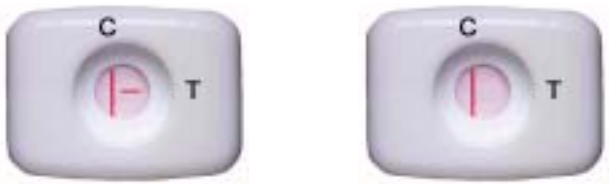


Figure D5: Typical dot-blot rapid assay showing a control line (vertical line at C) and a positive test result (horizontal line at T).

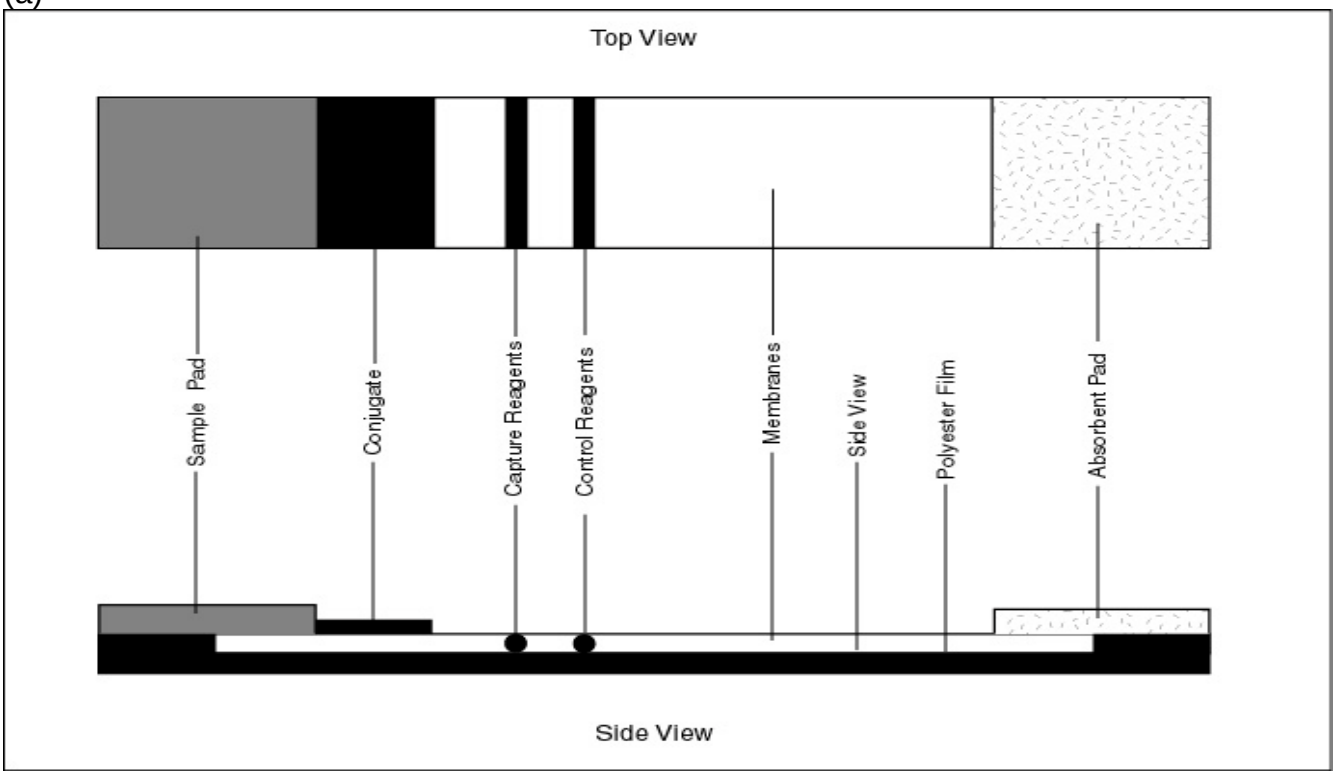
D.2.5.4.5.2.Chromatographic (Lateral Flow) and One-Step Assays

Newer rapid technologies have been introduced which offer added advantages over the dot-blot and other rapid tests (Ketema et al., 2001).

These advantages include more simple procedures, “one-step” procedures (One-Step assays), room temperature storage, some incorporating an antigen sandwich configuration (3rd generation) to increase sensitivity, and the practical advantage that these assays are not fraught with the problem of poor flow-through that is noted with some flow-through rapid devices because the reagents wick horizontally or vertically by capillary action. These rapid assays, also called *immunochromatographic or lateral flow* assays, have all reagents contained in the device, or are a test strip in a tube-like device. The reagents are contained in a flat cartridge device, usually made of plastic or paper, and includes a strip that contains the antigens that will capture antibody in the sample. Whole blood, oral fluid, or serum (depending on the test) is placed at one end of the device (or the strip is placed in a tube containing the sample) and the sample is allowed to diffuse along the strip by the process of chromatography (diffusion or wicking). Impregnated reagents in the strip, often a protein A colloidal gold reagent, allow the antibody/antigen/conjugate reactions to occur without further additions of reagents, while other assays require just one other addition of buffer after sample addition. The simplified procedure decreases the chances of technical error, making these assays more foolproof. The results are usually a “test line” which indicates reactivity of antibody with the immobilized antigens, and a “control line” that acts as a procedural control. These tests can be completed usually in less than 15 minutes, are easily read, and contain a built-in quality control mechanism (procedural control).Therefore, these types of rapid assays offer attractive features, they are accurate, and may be the types of tests that will be widely selected in the future.

Figure D6a depicts the design of immunochromatograhic assays; Figure D6b shows a typical test device and Figure D6c shows representative results by these assays.

(a)


Top View
Sample Pad
Conjugate
Capture Reagents
Control Reagents
Membranes
Side View
Polyester Film
Absorbent Pad
Side View

(b)

(c)

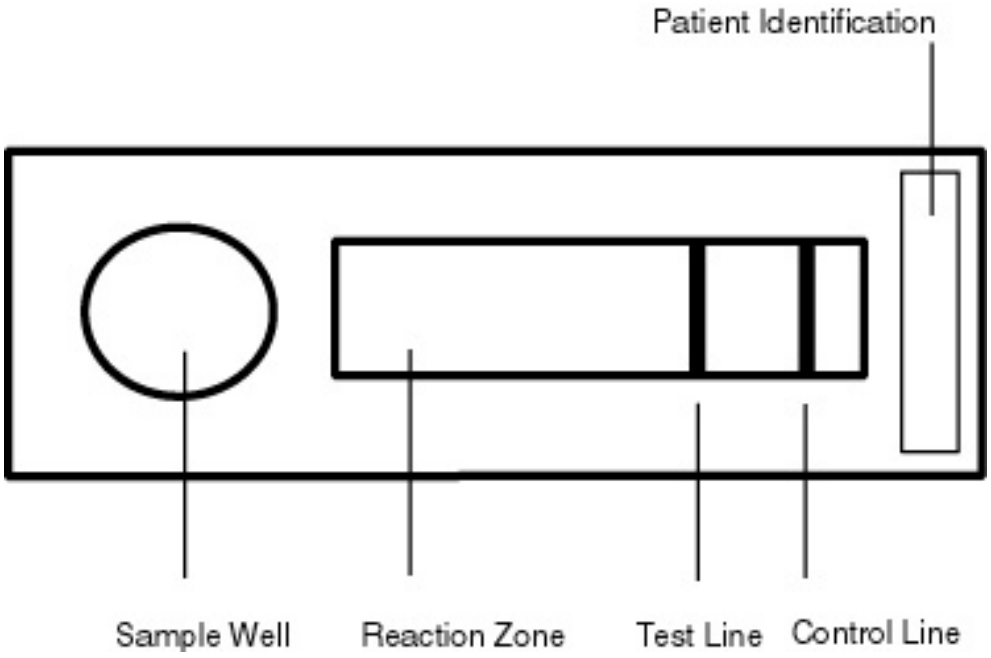


Figure D6: Typical immunochromatographic, lateral flow, devices showing (a) the configuration, (b) a typical device (Uni-Gold Recombigen HIV), and (c) results (test and control lines).

Another lateral flow test, the Determine (Abbott) shown in Figure D7, is a unique modification that lacks a plastic cartridge (the solid support is just the membrane on a thin flat plastic sheet) and comes in “cards” of 10 tests (Abbott, Determine package insert).These cards are easy to transport and store, and are thin enough to place 100 tests in a shirt pocket. The test requires no reagents, just the addition of serum or plasma, can be stored at a wide range of temperatures (2-30 C), and is therefore a truly “One-Step” lateral flow test. This test was designed specifically to be portable and inexpensive; it is extremely simple, requiring only the addition of serum or plasma (i.e., no reagents). However, if whole blood (venipuncture or fingerstick) is used, there is a requirement that one drop of a “chase” buffer must be added after the sample. The Determine incorporates the same 3rd generation format (antigen sandwich) in which the detecting reagent is an antigen labeled with a selenium-colloid (rather than Protein A) to give a red color reaction. The test requires 50ul of serum, plasma, venipuncture whole blood, or blood collected via fingerstick; blood from the finger is collected using a calibrated capillary tube. The test is read after 15 minutes, but not longer than 60 minutes after addition of the sample.

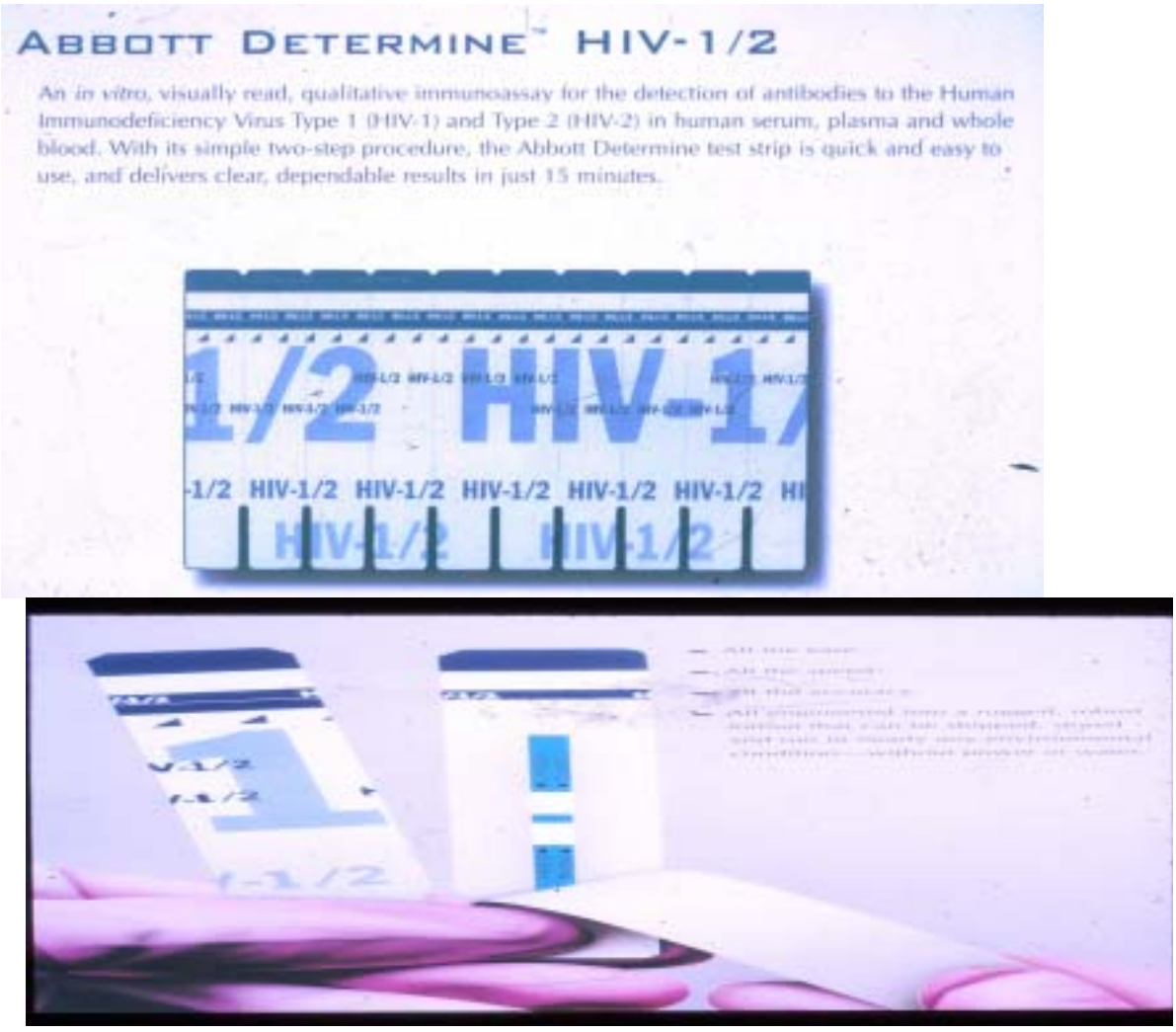


Figure D7: A One-Step lateral flow rapid test: the Determine.

D.2.5.4.5.3.Instruments for Reading Rapid Assays

Several instruments are available for reading results from rapid assays. These instruments offer simple use, rapid reading, permanent documentation of results, and decrease the subjective interpretation which is always associated with rapid tests. Some instruments are battery operated, and some connect with a computer to provide additional information about the sample (e.g., intensity of reaction).One such instrument that is capable of reading rapid test results is shown below in Figure D9.

Figure D9: A rapid test reader (EY Laboratories).

D.2.6. Research Plan

D.2.6.1. Identifying the Research and Production Group

Groups that can be considered to undertake test development include industry and academia. Industrial partners offer the advantages of experience in development and bringing products to market. However, they require high financial support, particularly if the resultant product will not yield high returns, and the company must introduce a new effort into their current plans. The number of tests required may be a major issue in soliciting industrial support if the number of tests required for the overall project is relatively small. Academia support is generally faster, less expensive, and Intellectual Property issues may be less complicated. The disadvantages include finding the right partner that has some experience in translating research methods into standardized products, a lack of manufacturing experience, and production of the final product.

Ideally, a collaboration between industry and academia may result in speed of test development at more reasonable costs (by academia), and rapid standardization and manufacturing (by industry).

D.2.6.2. Format of the Test

Rapid tests allow for point of care testing, immediate results (which may assist with re-immunization if necessary), eliminate loss-to-follow-up for needed activities, and can be performed in remote areas without the need for transport of specimens. Because they can also be performed in reference

laboratories, they offer flexibility and are the choice for this project. The test development effort will be based on modifications of existing rapid tests that are in routine use and have been adapted for use with oral fluid specimens. The format must also support ambient temperature transport (and high temperatures), and no need for refrigeration at storage. Current technologies allow these requirements to be met.

D.2.6.3. Selecting Oral Fluid Collection Device

Time and cost-savings could be realized by selecting an already existing oral fluid collection device. Several are on the market and have been documented to be effective in collecting appropriate samples for antibody testing. The two major candidates are the OraSure collecting device (OraSure Technologies) which has been described and would be useful if specimens are to be transported long distances over several weeks. It has been validated with ELISA type tests, but could conceivably be validates with a rapid test. The second candidate would be the combined collection/testing device (OraQuick), appropriate for POC testing. If selected, the “test” part of the device would need to be modified for the detection of Tetanus antibodies by using different antigens.

D.2.6.4. Test Development

Rapid or Simple method. Although none currently exist for tetanus antibody detection, translation of known rapid technology should be relatively easy. If sensitivity for detection of 0.01 IU/ml proves to be problematic, amplification methods can be explored. Although rapid and simple assays have been shown to have equivalent analytical sensitivity to ELISA methods, several means exist to increase sensitivity, if required. Colormetric amplification methods include tyramide signal amplification (TSA) where an enzyme reaction produces intermediates that deposit on membranes or solid supports to act as additional substrates for more enzyme/colorimetric reactions. This increases sensitivity by amplifying the normal signal. Other research methods exist to accomplish an increase in sensitivity by similar mechanisms. These have been applied to two rapid methods and have shown proof of principle. All components necessary to develop a rapid or simple assay system to detect tetanus toxoid antibodies are available, and have been optimized for rapid tests. The only component in such systems that is needed is the antigen. The C fragment of tetanus toxoid has been shown to work well in ELISA systems. Antibody reactivity to this antigen has been correlated with protective responses at 0.01 IU/ml. This antigen is readily available, but its concentration and orientation on membranes or other solid supports will need to be optimized empirically.

D.2.6.5.Test Calibration and Validation

The rapid method would require calibration against samples having varying concentrations of antibodies to tetanus, and would subsequently need to be

validated against blind panels of well-characterized samples. Such samples would need to be procured during test development. Acquisition of such samples is not anticipated to be problematic (are readily available). The existing ELISA method using serum for the detection of tetanus antibodies would act as the reference test. The targeted population for evaluation and validation would be those with ages between 12-18 years. During the development process, it would need to be determined if total IgG immunoglobulin levels would need to be measured in oral fluid to verify that the sample is adequate. Finally, because breast milk may contain IgA as well as IgG antibodies from the mother, the degree that these antibodies may interfere with the assessment of IgG in the oral cavity of infants who may be breast-feeding would need to be determined.

D.2.6.6.Test Cost

Based on the currently available rapid tests, and oral fluid technology, it is anticipated that the final product for tetanus toxoid detection would be sold for between $5-15 per test.

D.2.7. Intellectual Property (IP) and Royalties issues

Depending on who develops the test, IP and royalty issues must be addressed.This is usually accomplished at the time of contract negotiations.

D.2.8. Schedule and Time Frame

The Research Agenda, showing tasks and time-lines, is shown in the chart on the next page .

D.2.9.Recommendations of the Working Group

(1) Solicit, via RFA, a collaborative initiative between industry and academia

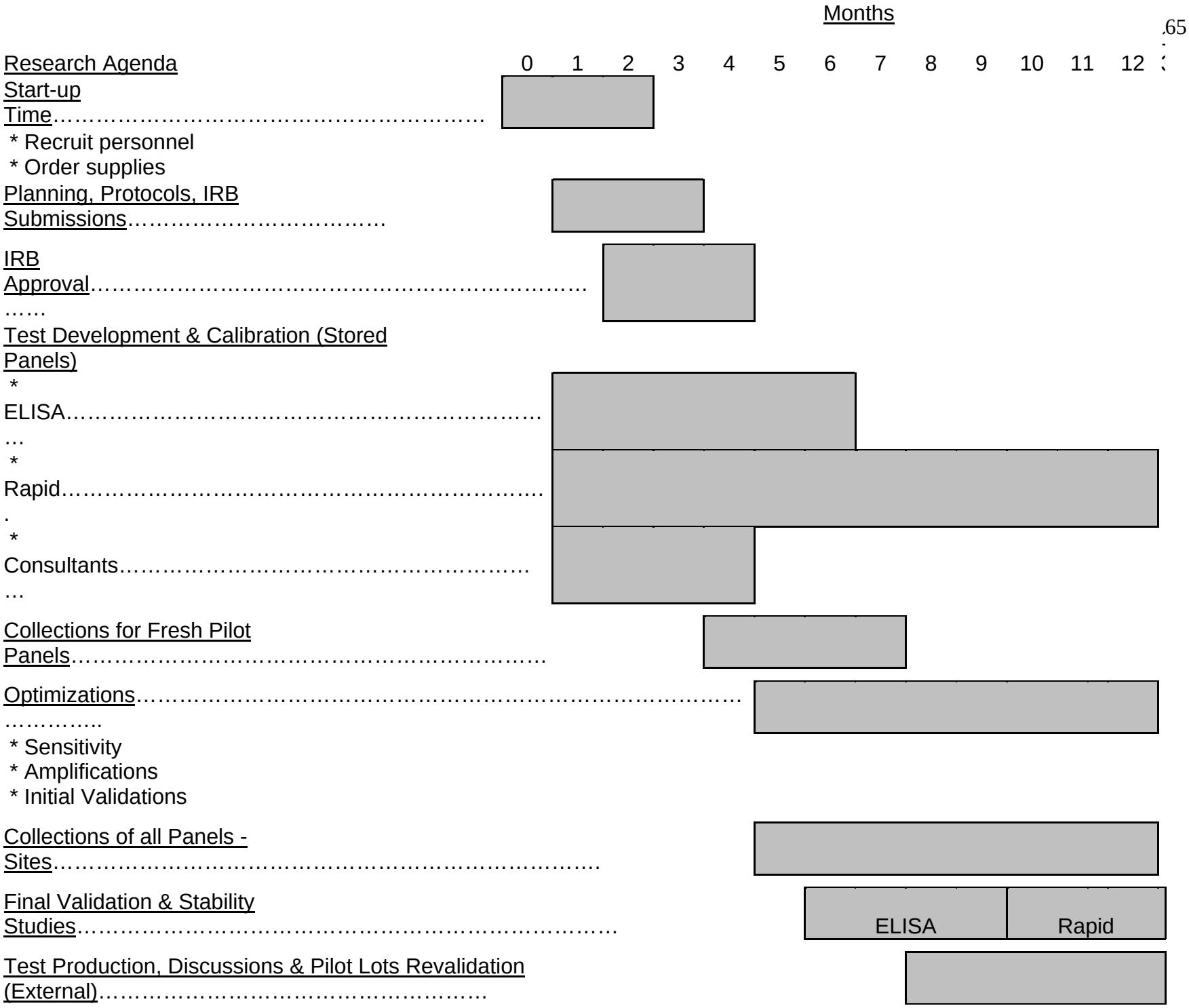
(2) Select a rapid, point-of-care test that allows simultaneously both collection and testing

(3) Strongly consider selecting a collection device or test system that is already established and has been validated to detect antibodies to infectious agents

(4) Make available resources that allow for maximum provisions to complete the project within one to two years from the time of contact. This would include appropriate consultants

(5) Establish field sites for collection of samples and validation of the test.

TT Oral Fluid Test Development



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D.2.11.References

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D. E. The priority to reduce infectious wastes and ultimately eliminate the use of sharps: Devices to defang needle-syringes after use

E.1 Executive Summary - Defangers

E.1.1. Definition and description

Defangers are a group of commercially-marketed and investigational medical devices which remove or destroy the needle of needle-syringes used for injections. Electric models destroy the needle by passing a large current through it. Manual defangers cut the needle (and in some cases, the syringe tip), or sometimes pull it out from permanent attachment with its hub or with the syringe. Removed needles drop into a separate container.

E.1.2. Unsafe injections; potential advantages and disadvantages of defangers

Improper reuse of unsterile injection equipment is a serious cause of iatrogenic disease in curative medicine in the developing world, and a concern for immunization programs, which are estimated to account for ten percent of all injections worldwide. Needlestick injuries threaten health care workers, ancillary hospital personnel, and the general community exposed to “sharps” waste improperly disposed in the environment.

Defangers which destroy or segregate the needle from other components of the medical waste stream offer the potential, when used as intended, to reduce an increasing volume of sharps as a result of the growing uptake of auto-disable (A-D) needle-syringes. If defangers are actuated by the health worker immediately after administering each injection, they may also decrease the likelihood of accidental needlestick injuries if sharps boxes are unavailable or overfilled. Defangers capable of destroying both the needle and the syringe of conventional (non-A-D) needle-syringes might also reduce the scandal of their improper pilferage, “recycling”, resale, and unsterile reuse.

The advantages just described for defangers, however, remain theoretical. To date, there have been no independent, well-designed, controlled, scientific studies to support such claims. It is entirely possible that the additional defanging step might lead to an increased frequency of needlestick injuries from the extra manipulation involved. This is particularly possible if defangers are not available within one or two paces of every locus where patients are injected, requiring health workers to walk elsewhere with an exposed needle, or to “batch” the used needle-syringes for later defanging. The latter would require someone to reach dangerously into a container of used needle-syringes in order to insert each into a defanging device.

E.1.3. Limitations of existing sharps waste management

The safe and proper disposal of sharps waste is a growing challenge. Many developing country health authorities assign it little priority. There are drawbacks to each of the options for sharps waste: **(1)** burnable sharps disposal boxes, **(2)** incinerators, and **(3)** disposal pits in the ground. Promising alternative waste technologies have yet to become economically practical for developing countries: **(1)** recycling syringe plastic, and **(2)** melters to create "bricks" of encapsulated needles in syringe plastic.

E.1.4. Market considerations and potential demand for defanger devices

Existing electric defangers range in cost from approximately US$100 to $800.Existing manual defangers range in cost from approximately US$50 to $300, depending on features. Investigational defangers are targeted to sell for less than $20 each. Assuming proper defanger use requires one to be located within reach of every health care worker administering a vaccination, the theoretical global demand for defangers is estimated at 0.5 to 1.0 million defangers needed for the 74 Vaccine Fund-eligible countries, or 1.0 to 1.25 million for all 165 developing countries.

E.1.5. Specifications for defangers supported by GAVI

The working group proposed a number of performance and design specifications for defangers whose research, development, promotion, and distribution would be funded or facilitated by the Global Alliance for Vaccines and Immunization (GAVI) for use in developing countries. The major specifications, *inter alia*, were that the defanger should: **(1)** not require electricity or batteries; **(2)** defang all currently available A-D syringes as well as all non-A-D plastic syringes of nominal capacity sizes from 1 mL up to 25 mL; **(3)** defang syringes with either Luer cone (slip), Luer lock, or snap-on type interfaces with their needle hubs; **(4)** render unreusable non-A-D syringes defanged by the device; **(5)** leave no needle stub on the defanged syringes that could produce a percutaneous injury; **(6)** provide a clearance (or a safety shield) for the operating hand of at least 15 cm; **(7)** demonstrate a lifetime of at least 25,000 defangs; and **(8)** use disposable, fill-level-marked, transparent or translucent needle containers with permanently locking closures.

E.1.6. Research, development, and implementation agenda

Given the unproven claims for defangers, it is recommended that defanger developers and manufacturers be given approximately four months to modify their products or prototypes in order to satisfy the proposed specifications. These should then be tested at the bench by independent testing

laboratory(ies). Defangers found to meet the specifications should then be evaluated in field trials in a variety of settings in representative developing countries of Africa, Asia, and the Americas.

The field evaluations should be conducted by competent institutions or agencies which do not have any compromising relationships to defanger developers, intellectual property holders, or manufacturers. The evaluations should include passive (uncontrolled) studies, as well as controlled trials (and head-to-head, if multiple devices are submitted) in both routine immunization settings and special (mass) vaccination campaigns. They should use objective, quantitative research endpoints such as rates of needlestick injury, proportions of needle-syringes defanged, and frequency of defanger breakdown and other causes of non-use, such as loss, theft, disappearance, or deliberate intention to "recycle" non-A-D needle-syringes. Subjective health care worker experience and preferences towards the devices, as well as economic analyses should also be studied. The suggested research agenda might require from 12 to 24 months to conclude, and cost from US$0.5 to $1.5 million.

Upon review of the results of such trials, GAVI would decide whether and to what extent defangers should be purchased, promoted, distributed, and/or bundled with donated vaccine in eligible Vaccine Fund countries. It would then select an implementing organization or entity to carry out the program, including design and management of training syllabi and other rollout materials and ancillary needs. Based on estimates mentioned above, full rollout of defanger technology in all Vaccine Fund eligible countries might cost US$20 million over several years.

E.2. Preface - Defangers

(This preface [starting p. 70], and its disclaiming footnote, might properly belong merged somehow into the "plenary" introduction for all three technologies, back in section B, with any redundant points removed. The focus below on defangers would also need to be modified to apply equally to all three technologies)

The Global Alliance for Vaccines and Immunization (GAVI) is opening a "window 3" to make monies from the Vaccine Fund available for research and development (R&D) efforts that would facilitate reaching GAVI goals. At its Board meeting of 11 March 2002, it requested further development of priorities and plans for R&D spending in its delineated technology project areas (www.vaccinealliance.org/reference/seventh_board/priority.html):

- "Decreased dependence upon and streamlining of the cold chain"
- "Improved tools to measure immunization services performance."
- "Reducing infectious wastes and ultimately eliminating the use of sharps (needles and syringes)."

In regard to the 3rd bullet priority (reducing and eliminating sharps), several preliminary research topics were floated, including the following (www.vaccinealliance.org/reference/seventh_board/word/techprojects.doc):

• "Reducing infectious wastes and ultimately eliminating the use of sharps (needles and syringes)."
• "Devices to "defang" syringes– Short term"
• "Aerosol administration of measles vaccine during masscampaigns – Short & medium term"
• "Urban disposal of needles & syringes (incinerators and grinders) – Medium term"
• "Multi-dose jet guns – Medium term"

E.2.1. Terms of reference - Defangers

The GAVI R&D New Technologies Task Force, led by Drs. Marie-Paule Kieny, Myron Levine, and Rino Rappuoli, formed a working group under the chair of Dr. Francis Andre, and tasked it to address the second bullet above, on needle defangers, as one of its first agenda items. (Two other items relate to the cold chain and diagnostic tools to assess immunization program progress.) The assignment was to review the field of needle defangers and make recommendations on an agenda for further research and implementation efforts. This report is the result of that effort.[7] ~~(NOTE: This footnote properly belongs moved into the plenary section B.)~~

[7] Mention throughout this document of trade names and commercial products is for information and identification only, and does not imply endorsement nor recommendation for use by the Global Alliance for Vaccines and Immunization, the World Health Organization, nor the agencies and institutions of the working group members.

E.3. Introduction - Defangers

E.3.1. Definitions - Defangers

The following terms used in this document are defined as follows, in order to avoid possible ambiguity of meaning from their non-specific usage in other publications and presentations.

E.3.1.1 "*Auto-disable*" (A-D). An auto-disable needle-syringe is one designed to prevent inadvertent or intentional reuse by limiting the number of plunger movements and incorporating either a fixed, non-removable needle, or a detachable needle that becomes fixed upon insertion onto the syringe.[WHO1998b] (Formerly referred to as "auto-destruct".)

E.3.1.2 *needle-syringe* = a syringe and its attached needle, either removable or permanently affixed, either auto-disabling or of conventional disposable or resterilizable type (plural: needle-syringes)

E.3.1.3 needle *defanger* = a device which removes or destroys the needle of a needle-syringe unit, for which its mechanism of action is unspecified (see sublevel below for more specific devices within this category)

E.3.1.3.1. needle *remover* = a manual defanger device which physically separates the needle from the syringe by applying a mechanical pulling force or by cutting the syringe barrel or tip (interface with the needle hub), and the needle hub, if attached).

E.3.1.3.2. needle *destroyer* = a defanger device which obliterates the needle attached to a syringe, usually by means of combustion and vaporization from the heat generated by passing an electric current through the needle

E.3.1.4. needle *container*=the receptacle within or attached to the defanger device which holds the needles removed from syringes (or its swarf), until they can be disposed of. The container may be intended to be disposed along with the needles, or to be reused after its contents are disposed.

E.3.1.5 needle *swarf* = the residue and ash that remains from the combustion and disintegration of needles destroyed by the heat of electrical current in electric needle destroyer devices

E.3.1.6 *sharp* (noun) = medical instrument or device which has a sharp edge or point capable of puncturing or lacerating the skin, including, *inter alia,* needles, scalpels, lancets, lances, tines, breakable glass, hematocrit tubes or drug ampoules (plural: sharps)

E.3.1.7 *stub* (noun) = The portion of a needle, often blunted or deformed (but potentially sharp), remaining attached to a syringe after the action of a needle destroyer or needle cutter

E.4. Background - Defangers

E.4.1. Unsafe injections in the world

The safety of the estimated 12 to 16 billion injections which are administered each year worldwide have come under increasing scrutiny and concern. From 50 percent to 90 percent of these injections are estimated to be carried out with unsterile needle-syringes that may contain traces of blood or tissue fluid from previous patients, creating great potential for iatrogenic transmission of infections such as human immunodeficiency virus (HIV), hepatitis B (HBV), and hepatitis C (HCV), among others.[Simonsen1999]

The World Health Organization (WHO) estimates unsafe injections cause 21.7 million new HBV infections in developing and transitional countries, constituting 33 percent of the burden of this disease in such regions.[WHO2001] In the same group of countries, the resulting annual HCV incidence from injections was estimated at 2 million cases, comprising 42 percent of the total. Globally, HIV transmitted iatrogenically by needle is estimated to cause 96,000 infections per year, or two percent of the total. These iatrogenic infections are estimated to lead to the worldwide loss of 9.18 million disability adjusted life years (DALYs) from the premature disability and death of the victims between the years 2000 and 2030.[Dziekan2003]

Some have postulated recently that the great pandemic of AIDS in Africa is primarily of such nosocomial cause of transmission, rather than by sexual transmission.[Brewer2003, Gisselquist2003] But this hypothesis is disputed by other authorities whose research continues to support the primacy of sexual transmission for AIDS cases in Africa, but who nonetheless agree that unsafe injections account for substantial numbers, although a minority of cases.[WHO2003b, Walker2003]

Entire “recycling” industries have developed in some developing countries to collect or scavenge used injection equipment, superficially clean it, and repackage it to be sold, sometimes masquerading it as new.[Kunskulniti1991, Gunn1992, Muller2001a, Muller2001b, Mujeeb2003, Talaat2003]

E.4.2. Needlestick injuries

Injuries from pricks with needle-syringes and other sharps occur not only among health care workers such as nurses and doctors and their auxiliary support staff such as janitors and laundry workers. Needlestick injuries also occur to collectors and scavengers of medical equipment, as well among the general population from exposure to needle-syringes improperly disposed in the community environment.

The risk of infection from the stick of a needle which had been used in an infected patient is estimated from empirical observations to be about 30% for hepatitis B, 2% for hepatitis C, and 0.3% for human immunodeficiency virus.[CDC2001] Research on needlestick injuries in the United States revealed that most of them occurred during and after injection while the needle was still in control of the person administering the injection.[EPINET]

In developing countries, the frequency of needlesticks among health care workers can be substantial.An occupational health survey in India, for example, revealed an astounding rate of 600-700 needlesticks per 1000 health care workers per week,[Muller2001a] equivalent to 36 sticks per health care worker per year.

To reduce the risk of needlesticks, the WHO and the United Nations Children's Fund (UNICEF) advise that used needle-syringes be placed immediately into nearby special sharps disposal boxes without any further manipulation, such as recapping.[WHO2002d]

E.4.3. Auto-disable syringes

To overcome the dangerous practice of intentional or inadvertent reuse of unsterile needle-syringes, current WHO and UNICEF policy recommends use only of auto-disable (A-D) needle-syringes which are incapable of re-use.[WHO1999b] A consensus has developed that donors of vaccine must "bundle" with them a corresponding number of A-D syringes. This has been successful in increasing their use in many developing countries, and thus reducing the frequency of improper reuse. Given current trends, it is estimated that in the year 2005 alone, 700 million A-D syringes will be purchased.[PATH2001a] Of course, bundling policies that apply primarily to immunization,[WHO1999b] will have little impact on the ten-fold-larger number of injections per year in curative medicine.

Ironically, the success of increasing utilization of A-D syringes is resulting in greatly increased volumes of potentially infectious "sharps". This increased volume is welcome, as it reflects safer injections, which previously might otherwise have resulted in the improper recycling and reuse of needle-syringes. However, the increase complicates the problem of safe disposal.

E.4.4. Proper sharps handling

Policies for the proper containment and disposal of sharps medical waste have the goals to reduce needlestick injuries, prevent improper reuse of needle-syringes, and reduce volume of medical waste and its risk to the community and environment.[Prüss1999]

WHO currently recommends that sharps be immediately discarded after use into a dedicated disposal box,[WHO2002d] which costs approximately US$0.70 for one box holding about 100 needle-syringes (~US$0.007 per disposed needle-syringe). When full, the sharps disposal box should be incinerated, or burned in the open if incinerators are not available. In the best of practices, the Program for Appropriate Technology in Health (PATH, Seattle, USA) estimates that 100 needle-syringes disposed in this way will require 5 liters of volume in sharps boxes.[PATH2001a] In the worst of practices which have been observed in many places, needle-syringes are disposed in the regular trash, where open piles are susceptible to scavenging.

E.4.5. Waste management limitations

The reality is that national and district health authorities in many developing countries demonstrate little interest, involvement, and commitment for the safe disposal of needles and syringes, as illustrated by experience in Cambodia[Laurent1998] and the Eastern Mediterranean.[Zghondi2002] For these reasons, among others, existing practices and technology for disposal of medical waste are still inadequate and limited.[PATH2001b]

Burnable sharps disposal boxes (1) may not be supplied or available in sufficient numbers, (2) may not be removed promptly and become dangerously overfilled, (3) may be pilfered to improperly “recycle” non-A-D needle-syringes, and (4) when burned in the open release toxic pollutants from the syringe plastic.

E.4.5.2. Incineration

Incinerators which reach at least 800º C, in theory, can serve to nearly completely eliminate sharps waste, except for a small residue of sterile ash and metallic residue. But they have their limitations. They are costly, engender community resistance to potential pollution, and often lack an infrastructure and the fuel resources to deliver waste to centrally located units. Better performing models need refined fuel (petrol liquid gas [PLG] or kerosene) in order to achieve the higher temperatures needed for combustion with a minimum of pollution. Poorly functioning models can leave “hedgehogs” of unburned plastic lumps with protruding needles. Open burning at lower temperatures of certain plastics can release toxic pollutants.[MRC1999]

Some jurisdictions have instituted limitations on incineration on grounds of environmental protection. India, for example, restricts such treatment of syringes because of concerns for toxics produced by burning PVC (polyvinyl chloride) plastic. It requires non-plastic waste be burned in advanced two-chambered incinerators operating at 800º C and 1050º C, respectively, per chamber.[Muller2001a] To further minimize pollution from improper burning, some Indian states forbid waste generators to operate their own incinerators; rather, they must bring waste to centralized approved ones.[Dalal2001]

E.4.5.2.1 Incinerators:

The Montfort incinerator is designed to be built on-site with locally-available materials. It uses a minimum of fuel,[PATH2001b] estimated from experience in Senegal at 2 liters of kerosene per burn.[Faye2003] Manufactured incinerators include models made by SICIM (Italy), Vulcain (France), and Medecin 400 (South Africa).[PATHundeated, WHO2002d]

The SICIM Pioneer “auto-combustion” incinerator, which costs approximately US$2,500,[WHO2002d] is fueled by the syringe plastic itself. A study in Cambodia found its use to cost overall about $4,500 per year.[Lydon2002] This estimate included the associated costs of safety boxes (29%), training (16%), the incinerator itself (14%), and its housing (15%). This was equivalent to US$0.08 per incinerated syringe for routine immunization and US$0.02 for routine and supplemental campaigns. This is almost as much as the cost of an A-D needle-syringe itself, which currently costs approximately US$0.06 to $0.07 through UNICEF procurement. However, the SICIM incinerator failed efficiently to burn large quantities of a load of syringes and needles in a field trial in Cambodia.[Laurent1998] Poor combustion led to smothering of the fire and thick smoke. Molten plastic also leaked from the unit.

E.4.5.3. Disposal pits

Digging pits in the ground is an option for local disposal of the residue of sharps waste from either burned disposal boxes, from the incomplete combustion of incinerators, or the direct deposits of unprocessed needle-syringes. Ideally, such excavations should have protective covers and access points to avoid access. Such disposal pits have the disadvantages of **(1)** becoming accessible through erosion or excavation, **(2)** not practical in areas of high water table, which may lead to contamination of drinking water.

E.4.5.4. Alternative processes for medical waste

Various alternatives for processing medical waste include thermal treatment at different temperatures, chemical inactivation such as with chlorine, irradiation, and biological processes using enzymes.[HCWH2001] Each have their own advantages and disadvantages in developing country settings.

E.4.5.4.1. Shredding and disinfection

Shredding and disinfection through wet chemicals or pressurized steam is a possibility for urban settings, but is not yet practical for primary care facilities.[WHO2002d] However, development of the right kind of pressure cooker might be a solution.[PATH2001b]

E.4.5.4.2 Needle-syringe melters

One attractive approach for medical waste is to use relatively low controlled temperatures over several hours to melt needle-syringes into harmless plastic blocks or other shapes of potential utility (see **Error! Reference source not found.**). This method requires only a controlled temperature of 175º C,[PATH2001b] and has already been commercialized as the Demolizer™ (see **Error! Reference source not found.**),[UNIVEC2003] among other devices of U.S. and European manufacture. Melting renders the needles harmless by embedding their sharp points, and sterilizes pathogens at the same time.

Figure E1. "Bricks" of approximately 100 melted needle-syringes produced by the Demolizer™ melter device.Needles are embedded safely within the brick (photograph courtesy: Anthony Battersby).

Figure E2. The table top Demolizer™ device which heats needle-syringes until they melt and then cool into a solid piece. (UNIVEC, Farmingdale, NY, www.univec.com)

These tabletop devices cost about $4,000 and require electricity, which are two obstacles to widespread developing country use.[WHO2002d] Development of low-cost non-electrical melters which could maintain proper time-temperature profiles (to avoid unwanted combustion of the plastic), while using readily available fuels, would be of potentially great utility.[Lloyd2000,PATH2001b]

E.4.5.4.3. Needle-shielding syringes

Various needle-shielding syringes are marketed as SESIPs ("Sharps with Engineered Sharps Injury Protection") in developed countries to satisfy occupational safety and health regulations.[OSHA2001] (www.med.virginia.edu/medcntr/centers/epinet/safetydevice.html#2). They remain, however, too costly for developing country use.

A most elegant example is a needle-syringe product in which upon completion of injection a spring propels the needle back into the barrel of the syringe, where it remains inaccessible (VanishPoint® syringe, Retractable Technologies, Little Elm, Texas). By avoiding the final push on the plunger at the end of injection, health workers can avoid activation of the spring and thus continue to use the needle-syringe again and again. This technology has not yet been adapted into an A-D needle-syringe.

E.4.6. Hierarchy of controls

The overarching GAVI priority under which defangers are being considered is to reduce the frequency and ultimately eliminate the use of sharps in immunization programs because of the hazards they pose. It is thus useful to keep in mind a well-known concept in occupational health: the "Hierarchy of Controls", which lists solutions in order of their effectiveness and preference:

(1) Eliminate the hazard or use a safer alternative device (e.g. use needle-free vaccination, such as jet injectors, oral, intranasal, or aerosol vaccine delivery).

(2) Isolate the hazard from people by redesigning equipment (engineering controls) (e.g., automatic needle-shielding devices to prevent needlesticks, A-D syringes to prevent reuse, ventilation system design to minimize need for putting on masks).

(3) Administrative controls to change the way the job is done (e.g., accounting for used needles to avoid diversion to the recycling market).

(4) Work practice controls to change the way the job is done (e.g., "don't recap needles", "defang immediately after injection", "deposit in disposal pits", and the training needed to effect these guidelines).

(5) Personal protective equipment (e.g., use of gloves, and the training needed to effect their proper use)

Defangers would thus fall into levels (2) and (4) above. Of course, it is unreasonable to expect them to be the ultimate or complete solution to the problem of sharps. Diverse solutions at different points of use might best address the sharps problem:during injection, after use but before disposal, during disposal, after disposal, and during or after final destruction.In any case,

defangers may have a useful role to play in some situations, and the research agenda is designed to determine those circumstances.

E.5. Existing needle defangers

E.5.1. Marketed defanger products

E.5.1.1. Electrical needle destroyers

The most widely used types of defangers are electrical models, sometimes referred to as *needle destroyers*, which have existed since the 1970s. PATH invited multiple manufacturers of needle destroyers worldwide to provide samples of their product, and arranged for telephone interviews with nine respondents during the period of October 1999 through February 2000.[PATH2000]

The PATH survey determined that most destroyers operate on mains (wall) electric current and apply a direct current (DC) electrical voltage across the needle in order to destroy it. Others use mains current to recharge 5-to-10 ampere-hour sealed lead acid batteries, which make them quite heavy. They usually have inline fuses, protective covers, and a removable swarf container. More expensive and sophisticated needle destroyers regulate the voltage and current, detect the presence of an object at orifice, and have adjustable settings. Many have features such as an indicator of battery charge, a counter for the number of needles incinerated, a fan to gather fumes into an internal filter, shields to contain any blood aerosol, a fragrance to counter the odor of burning blood and needles, and a tray for disposing of defanged syringes.

E.5.1.1.1. Examples of electrical needle destroyers

(1) Needle-Ease® (~$150 for personal model 2501, Sharps Elimination Technologies, Inc., Denver, Colorado, USA; URL: http://needle-ease.com/needleeasesystem.htm);

(2) ELSAfe-99™ (~$95, PT Elektindodaya Pakarnusa, Indonesia; URL: www.nusaweb.com/nusashop/elsa/elsafe-99a.html);

(3) NicSafe 1800™ (~$266, NIC Americas, Inc, Duluth, Georgia, USA, subsidiary of MediSys PLC, UK; URL: http://207.111.197.53, see **Error! Reference source not found.**);

(4) Needlyzer™ (~$550-$800, MedPro, Inc., Lexington, Kentucky, USA; URL: www.needlyzer.com, see **Error! Reference source not found.**);and the

(5) Sharp$_X$™ (~$995, Biomedical Disposal, Inc., Norcross, Georgia, USA; URL: www.biodisposal.com/products.htm, see **Error! Reference source not found.**).

E.5.1.1.2. Features of electrical needle destroyers

Prices of basic models ranged from “under $100” to $250.More complex ones cost from $295 to $799. Their weights ranged from 1 to 4 kg. Battery-operated devices destroyed from 50 to 300 needles per charge. Battery recharge times varied from 1 to 8 hours. Swarf container capacities ranges from about 500 to 5000 needles. Incineration temperatures were reported between about 800 to 1600º C. Incineration times varied from 0.5 to 2 seconds. The stub lengths ranged from 1 to 3 mm. Testing found no pathogens in the swarf.

As of 2000, a total of four needle destroyers have received premarket approval from the U.S. FDA for sale in the United States: the NicSafe 1800™ in 1997, the Needle-Ease® and the Needlyzer™ in 1998, and the Sharp$_X$™ in 1999.[PATH2000]Outside the U.S.A., few countries regulate this medical technology; Hungary, Argentina, and France are reported to have devices approved for marketing.

Figure E3. NicSafe 1800™ electric needle destroyer

Figure E4. Needlyzer™ electric needle destroyer

Figure E5. Sharpx™ electric needle destroyer

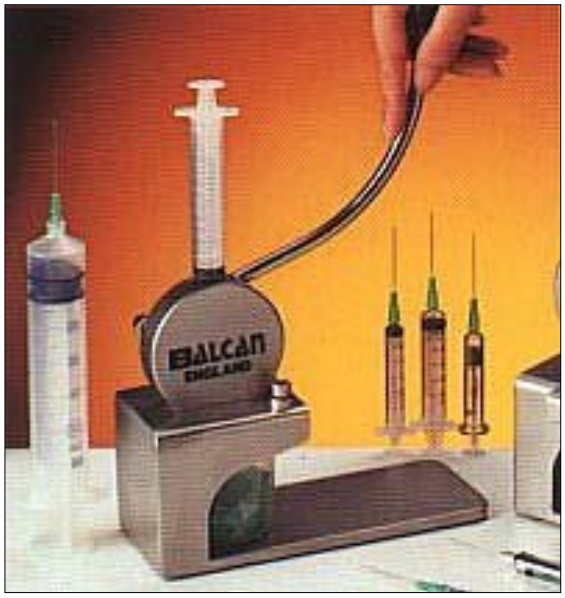
E.5.1.2. Commercial manual needle defangers

The Destructor™ (Balcan Engineering Ltd., Woodhall Spa, Lincolnshire, UK) is among the most widely distributed manually-operated needle defangers (see Figures E6a and b).[Balcan2001, Balcan2003, Rinfret2003] It is the only manual defanger listed in the WHO-UNICEF Product Information Sheets.[WHO2000b] The Balcan Destructor™ features two simultaneous cutting operations, one for the needle and the other for the syringe tip, rendering both unusable. The claimed blade life is 200,000 cuts, however no data to support this is provided. WHO and UNICEF recommend it be disassembled and cleaned at the end of every immunization session.[WHO2000b]

The lid for the container is conveniently stored within the device underneath the open container. The container plastic may contain chlorine, making it less safe to burn than other plastics. Observers on the GAVI working group believe it may need a longer lever arm to keep the hand operating the cutting lever further away from the inlet opening.

For the standard model Destructor™, which can only accommodate normal Luer slip syringes and their needles, the retail (internet) is GBP £174.50 (US $273). A “multi purpose” model capable of accepting screw-hubbed needles and Luer lock fittings costs £197.50 ($309). Disposable needle containers, holding about 250 needles each, cost £0.50 ($0.78), which averages $0.003 per needle collected.

PATH surveyed the large and growing market for needle-destruction devices in India as a result of national legislation.[Dalal2001] In 2001, the needle and syringe disposal products industry addressing this mandate for proper handling of sharps was characterized by small, unorganized manufacturers using simple technology, such as cutting pliers, needle cutters, electrical needle destroyers, microwaves, and shredders. Costs of these devices ranged from a low of around U.S.$1.10 to over $1,000 for microwaves and shredders.



Figures E6a & b. Balcan Destructor™ manual needle defangers. Notice: only syringe tip (and needle and hub, if attached) enters device for cutting.

E.5.2. Investigational manual needle defangers

PATH has filed a patent application for one manual defanger devices, developed, in part, with support from the U.S. Agency for International Development's HealthTech program and the Bill & Melinda Gates Foundation's Appropriate Technologies in Health program.[PATH2001b] One prototype of an inexpensive and portable needle puller has been disclosed (see **Error! Reference source not found.**).

Figure E7. Prototype PATH needle defanger with rectangular clear plastic needle container and table base

PATH obtained feedback on its prototype(s) from Indian health care workers:[Muller2001a, Muller2001b]

The findings were:

(1) a preference for the table-top model over the hand-held one for its solid feel and better functioning.

(2) re: the table-top model, they liked its stability, ease of use, effectiveness, intuitive design, simplicity, safety, ability to see what happens to the needle and when it is contained, its durability, and single-action pulling.

(3) re: the hand-held model, they liked its lightness and portability.

(4) The majority of Indian testers put their thumbs on the movable lever and their other fingers on the stationary handle, which was the opposite of PATH's design intent.

(5) The testers expressed the opinion that the tip of each needle still needs disinfection or destruction (as with an electric destroyer), despite its complete encasement within the defanger's needle container (possible fear of its getting opened and causing needlestick?).

Observers on the GAVI working group believe the PATH devices may need a wider shield to protect the hand which squeezes the pulling lever from a misdirected needle.

E.5.3. Experience with existing products

E.5.3.1. Developed countries

Most defanger devices in current use in developed countries are electrical needle destroyers.

Their costs and maintenance requirements make them unsuitable for use in many developing country settings, particularly in peripheral health centers.

Besides relying on electricity, which may not always be available, their electrodes wear out, particularly if excessively large needles are inserted.

Some will not work with gauges larger than 19.

E.5.3.2. Developing countries

E.5.3.2.1 India

PATH has collected much opinion and input regarding needle defangers from Indian government officials, non-governmental organizations, health care workers, and private manufacturers as part of its ongoing defanger project in India (see section E5.2).[Muller2001a, Muller2001b, PATH2003] The defanger market in India is driven by its national 1998 Biomedical Waste Handling and Management law, which, as of December 2002 applies to all health facilities.[Dalal2001] All such institutions generating biomedical waste are required to comply with rules for safe disposal promulgated by the Indian Ministry of Environment and Forests.

Under these regulations, best practices observed in a few hospitals included: **(1)** burning off the needles with electric devices, **(2)** cutting off the tip of separable syringes to prevent their reuse, **(3)** decontaminating separated needles and syringes in chlorine solution (or autoclaving them), **(4)** shredding syringes before collection and sale to plastic recyclers, **(5)** incinerating and depositing segregated needles in landfills, **(6)** soaking needle stubs and hubs after destruction in chlorine solution before disposal, and **(7)** encasing sharps in cement under a new foundation slab.[Muller2001a, Muller2001b]

Worst practices observed among auxiliary nurse midwives working in peri-urban immunization outreach included: **(1)** recapping disposable needles, often with an unsafe, vertical, two-handed method, **(2)** manual removal of needles from glass syringes and placing into a plastic bag or cardboard box for storage, **(3)** returning needles to the health sub-center, **(4)** digging a [not-so-deep] hole to bury needles but leaving much sharps waste on the adjacent ground, and **(5)** resterilizing disposable needles for reuse.[Muller2001a] Also: **(6)** batching used syringes for later tip destruction with an electric defanger, and **(7)** carrying needles removed from glass syringes on a tray down the hallway, grabbing them with forceps, and inserting them into a remotely-located needle destroyer.[Muller2001b]

PATH-sponsored field assessments of defangers are underway in India, intended primarily for feedback into the design process from subjective user input.[PATH2003] As of May 2003, results are not yet available.

E.5.3.2.2. Africa

South Africa's immunization program, a past user of needle defangers, has reportedly abandoned them, as they did not appear to reduce the frequency of needlesticks.[Catlin2003] In Egypt, despite the use of defangers, 40 percent of janitors were reported to receive needlesticks over the prior three months.[Bodenschatz2001] Consequently, infection control policies were revised to withdraw them from use.

In Senegal and Côte d'Ivoire, PATH is planning field assessments of one thousand Balcan Destructor™ devices as part of a demonstration project scheduled from March to September 2003.

E.5.3.3. Drawbacks to electric defangers

Drawbacks to existing electric needle-destroyer technology include: **(1)** downtime due to frequent power shortages, **(2)** high price, **(3)** immobility of heavy tabletop models, **(4)** frequent replacement of worn electrodes, **(5)** inconsistent or incomplete destruction of the needles, leaving sharp stubs, **(6)** splattering or misting of blood (see **Error! Reference source not found.**), **(7)** generation of obnoxious fumes, noise, and sparks, and **(8)** the resulting prohibition of their use in explosive atmospheres where oxygen and anesthesia are in use. [Muller2001a, PATH2000, PATH2003]

Figure E8. Splatter around inlet hole and adjacent countertop after clinical use of electrical needle destroyer device of Brazilian manufacture (photograph courtesy: Anthony Battersby).

E.6. Policy issues for defangers

E.6.1. Correct use and other principles

A consensus has developed on certain principles and goals regarding defanger devices and the handling of sharps waste in general:[Pruss1999, WHO2000a, WHO2002a, Bouvet2002, Catlin2003, Battersby2003, WHOundated-a, WHOundated-b, WHOundated-c]

(1) A device must be located in the immediate vicinity of all locations where injections are given with needle-syringes to be defanged. Thus, one must be at every patient bedside in clinical facilities, and within one step of all loci where vaccinations are administered.

(2) Defanging must occur immediately after the injection has been completed, before the needle-syringe leaves the hand of the person who performed the injection.

(3) All waste handling technologies must fit into an overall framework of administration and management, involving policy guidelines, supply chain logistics, training for behavioural change, and supporting legislation. “The system is the problem – not technology. “The management of operational constraints is the key to success vs. failure.” The weakest link defines the strength of the system.”

(4) Ideal technologies would avoid additional steps in handling the sharps waste stream. The KISS principle is paramount (“Keep it simple, stupid”). If things can go wrong, they will.

(5) Final destruction or disposal of waste should occur as near as possible to the point where the waste is generated.

(6) The person or entity that creates the waste should have the obligation to destroy it. Responsibility should not be divided.

(7) Waste is ideally recycled and converted to a safe, usable form.

(8) Human nature dictates that people will “improve” on approved procedures. Supplies of consumables which must never run out will nonetheless do so.

E.6.2. Information gaps and unresolved questions

E.6.2.1. Scientific evidence

There is as yet no published, peer-reviewed, scientific evidence supporting contradictory claims that defangers will either decrease or increase the frequency of needlestick injuries to health care workers, or have other positive or negative impacts on the safe and proper handling of sharps waste. User satisfaction is not suitable evidence of safety or efficacy. Documentation of safety and efficacy and other claimed benefits is required before widespread distribution and promotion of defanger devices is warranted.

E.6.2.2. Prior claims and research

In an unpublished internal document, PATH reported:

"Extensive product testing of needle removers has been conducted for the past three years with thousands of needles. Health workers and immunization experts in India, Senegal, China, and Cambodia have performed several thousand mock uses of needle removers with sterile needles with no needlestick injuries. Needle removers were used for immunization needle disposal in four Indian static health facilities and on two outreach visits during a controlled field trial in 2001. No needlesticks occurred with more than 700 injections in busy clinics.". PATH2003

PATH has released a commissioned study by a private testing laboratory on the splatter resulting from the use of five defanger devices, a pair of pliers, and a WHO safety box:NAMSA2002 Five defangers were reported as "negative for splatter", including PATH's identified needle puller, Balcan's identifiable Destructor™, and the three other non-identified devices. Also negative around its entry hole was the WHO safety box. Only the common workshop pliers were found positive.

E.6.2.3. Reusable versus disposable needle containers

There is no consensus for whether or not the needle container of defangers should be entirely disposable, or reusable (after emptying its contents into a disposal pit or other depository.

E.6.3. Pros and cons of needle defangers

As mentioned in section E.6.2, thorough, unbiased, and controlled field evaluations of the efficacy and safety of needle defanger technology have yet to be performed. Thus, there is yet no reliable data demonstrating that they represent an improvement over current recommended methods of handling sharps.Catlin2003 Their potential advantages and disadvantages remain hypothetical, but can be described, as follows:

E.6.3.1 Anticipated benefits and advantages

Destroying or damaging the needle of a needle-syringe, and separating it from the syringe immediately after injection, may have several benefits:

E.6.3.1.1. Reduce overall volume of sharps waste

The volume of overall "sharps waste" requiring puncture-proof containers and special handling[Pruss1999] is greatly reduced.[PATH2001a, PATH2002b] PATH demonstrated that separation of needles from syringes

reduces sharps volume by 90 percent or more (see **Error! Reference source not found.**).[PATH2002c] Moreover, syringes pack more closely together without needles attached (see **Error! Reference source not found.**). Compared to a volume when disposed together of 100 percent, needles alone occupy about 1 percent and syringes alone about 55 percent,[PATH2002c] representing an overall reduction in waste volume of 44 percent.

E.6.3.1.2. Reduce needlestick injuries

Defanging could reduce the incidence of needlestick injuries to health care workers and others exposed to sharps in the downstream handling and processing of medical waste.

E.6.3.1.3. Prevent reuse of non-A-D syringes

Destruction of the needle of conventional needle-syringes prevents improper reuse of the needle. Some defangers may also disable non-A-D syringes, also rendering impossible the intentional or inadvertent reuse of the syringe component.

E.6.3.1.4. Facilitation of medical waste collection

Facilitation of the recommended segregation of medical waste,[Pruss1999] allowing defanged syringes to be collected and handled along with other “infectious waste” in less expensive yellow plastic bags until disposal.[PATH2001b, Zghondi2002]

E.6.3.1.5. Facilitate recycling of plastic

If recycling of the plastic content of syringes is intended, early removal of the needles may render these syringes safer and easier to ship and process.[PATH2002b]

E.6.3.1.6. Cost savings

Assuming segregated needles can be disposed economically in local, deep, protected pits, reducing the volume of waste can result in economies as a result of the need for fewer sharps boxes, and the decreased costs of transporting them to incinerators or other sites of ultimate disposal.

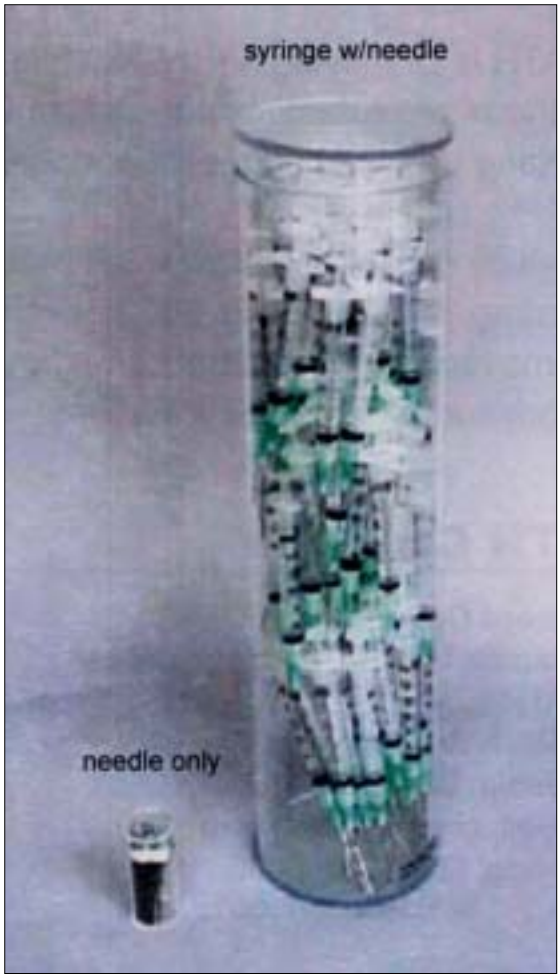


Figure E9. Segregation of needles from needle-syringes reduces sharps volume by 90 to 99 percent.[PATH2002c] **(photograph courtesy: PATH)**

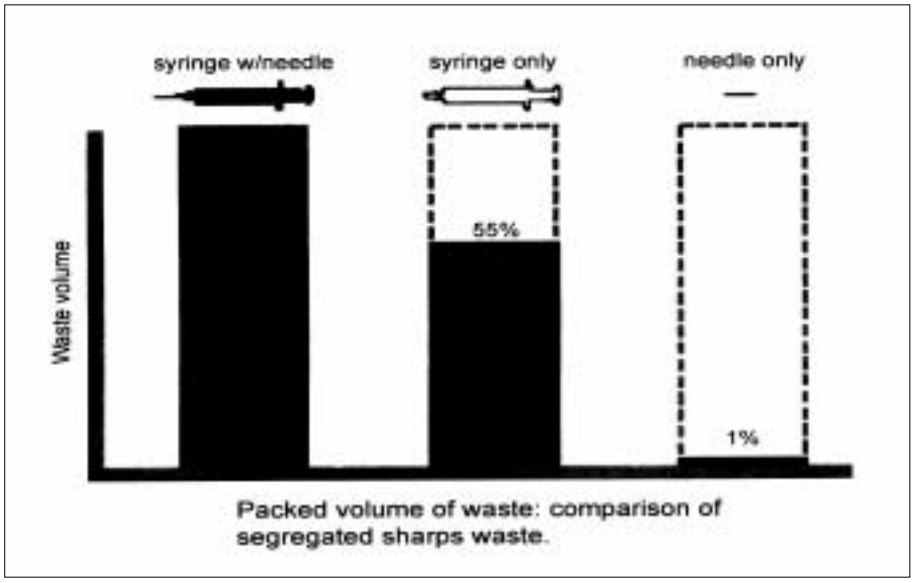


Figure E10. Separating needles and syringes reduces overall volume of waste stream.[PATH2002c] **(graph courtesy: PATH)**

E.6.3.2. Potential risks and disadvantages

On the other hand, the extra equipment and steps involved in defanging needle-syringes may result in the following risks and costs:

E.6.3.2.1. Increased risk of needlestick injuries

Paradoxically, needle defangers may actually result in an increase in the number of needlestick injuries to health care workers because of increased handling of exposed sharps and delays in disposal. If economic constraints result in insufficient numbers of defangers to permit them to be located within one step of every locus of an injection, uncapped needles may be transported to other parts of a room, or even into different rooms, in order to reach the defanger.[Lydon2002, Catlin2003]

Despite the recommendation to immediately defang after injection, harried nurses may elect to stockpile used needle-syringes in order to defang them all in batches after a busy immunization session. This might especially apply in special mass vaccination campaigns where the attraction of division of labor might result in a different person performing the defanging operation. An increased risk of needlesticks may result from the vaccinator handing the used needle-syringe to another person, or dropping it into a container from which another person must retrieve it in order to defang it.

Needlesticks may also occur upon the periodic emptying of the needle container and cleaning of the defanger. Other unforeseen aspects of the design and performance of the defanger may results in needlesticks during their use. Devices requiring one hand to hold the defanger and the other hand to guide the needle-syringe into the inlet opening have the potential for the needle to hit the holding hand. There are anecdotal reports that the forces generated by some defangers can propel a syringe by mechanical rebound across the room.[Catlin2003]

For the above reasons, regulators in the United States promulgate strict rules for defanger devices, on the grounds that they increase the handling of sharps.[PATH2000] The relevant U.S. Occupational Safety and Health Administration (OSHA) rule is:

"… *contaminated needles and other contaminated sharps shall not be bent, recapped, or removed …unless the employer can demonstrate that no alternative is feasible or that such action is required by a specific medical or dental procedure.*"[OSHA2001]

It also states:

"*Shearing or bending of contaminated needles is prohibited.*"

The U.S. Food and Drug Administration (FDA) classifies needle destroyers ("sharps needle destruction devices") into class III, deeming them to carry the greatest risk of harm to the patient and/or the user (compared to classes I and II).[FDA2001] As a result, needle destroyers require a thorough review of safety and efficacy under its "premarket approval" (PMA) route. They cannot use the simpler "510(k)" mechanism for marketing clearance of lower-risk class I and II devices by showing "substantial equivalence" to an existing "predicate" device.

In its guidance document for industry,[FDA2001] FDA warns that "*each additional step (manipulation) in the needle disposal process has the potential for increasing the risk to the user.*" In addition to requiring the submission of standard physical descriptions and bench testing results on applicant devices, FDA requires clinical studies at a minimum of three locations for each [type of] health care setting where the device may be used. These studies must provide the rationale for testing all safety and effectiveness claims for the device, and provide measurable endpoints related to the claims. The studies must detail all outcomes and problems, including operational failures, or other ergonomic outcomes and misuse considerations.

E.6.3.2.2. Non-use for behavioral, logistical, and economic reasons

Even if provided in sufficient numbers for every locus of vaccination, defangers still may not be used. First, it requires a change in established practice, which requires additional training and expense to overcome. "Any product that increases the workload and requires additional effort on the part of the healthcare worker is likely to face some resistance," stated a PATH-commissioned report.[Dalal2001]

Second, workers in outreach programs, particularly those integrated with other primary health care interventions (such as maternal and child health etc.) may already have too much other equipment to carry to include the additional weight of a defanger. Or they may simply forget to bring it along.

Third, there may be insufficient tabletop space near the patient's bed or other immunization site, or on a mobile injection cart, to set the device and operate it in a stable fashion.

Fourth, if the manual forces required to operate the device are sufficiently high, fatigue may lead to non-use.

Fifth, defanger devices may not be able to overcome various economic incentives for health care workers or clinics to generate extra income by selling used injection equipment to scavengers and recyclers.[Kungskulniti1991, Gunn1992, Huysman1994, Hunt2001, Lydon2002, Dawn2003, ID21-2003]

As one report from India about defangers stated, “Dysfunctional behavior such as pilferage, especially in the government hospitals, and the economics of making a living out of waste can be barriers in the Indian context.”[Dalal2001]

To control this practice, PATH once recommended careful accounting of syringes supplied and/or injections given, compared to the number of syringes or syringe boxes accumulated or destroyed, to detect diversions at the clinic or en route to final disposal.[PATHundated] But it is likely such accounting can be “fiddled” to hide reuse and diversion of used needle-syringes.

E.6.3.2.3. Administrative and financial obstacles to proper use

As with most sharps waste technologies, defangers require good management of logistics, supervision of staff, and financial resources for maintenance. Many defangers have consumable components that need to be perpetually resupplied, such as needle or swarf containers, or electrodes for electric devices. Might defangers suffer the same fate as other technologies that turned out to be inappropriate? The developing world is littered with solar refrigerators and incinerators in disrepair or not used for lack of fuel, motivation, spare parts, etc.As mentioned earlier, healthcare waste disposal is perceived as a low priority in many countries.

E.6.3.2.4. Maintenance and cleaning

Most defangers will require periodic emptying of needle or swarf containers after approximately every 100 uses. Periodic cleaning will be needed to remove deposits left by the blood and/or medication content of the needles and syringes.

E.6.3.2.5. Non-defangable sharps

Some sharps cannot be defanged by some devices -- such as very large needle-syringes, introducers, IV needles, arterial blood gas sets, lancets, scalpels, and certain glass items. Health facilities may assume defangers will obviate the need for continued purchase of sharps boxes, and stop providing them. This occurred in Egypt[Bodenschatz2001] In such cases, large sharps may end up in a regular unprotected container.

E.6.3.2.6. Illusory savings in waste stream processing

Defanged syringes potentially containing blood or tissue fluid would be classified as infectious waste. There is some uncertainty whether the

proper disinfection and processing of such waste really saves labor or money. If they end up deposited in the same burial pit as defanged needles, has there really been sufficient benefit to justify the investment in defangers?

Recycling of plastic from defanged syringes may not make economic sense. In Moldova, recycling efforts were reported to have failed because of **(1)** improper waste segregation at the source, **(2)** non-collection of the materials by the designated recycler due to shortages of vehicle fuel, **(3)** transport costs exceeding the scrap value paid for the plastic, and **(4)** low demand for the finished product made from the recycled plastic.[Battersby2003]

E.7. Market considerations and size estimates

E.7.1. Developed countries

The U.S.A. and other developed countries are primarily focused on needlestick injuries at the point of care, given their general conformance with recommended disposal practices such as sharps disposal boxes at each point of use and their routine collection, removal, and proper disposal.In the richer countries, once the needle-syringe combination is placed in a sharps container, relatively very few injuries occur “downstream” in the chain of removal by housekeeping staff, processing by medical waste service companies, and final destruction and disposal by incineration or other means.

Thus, in developed countries, the benefits of destroying a needle and/or its accompanying syringe may be outweighed by the risks of additional steps in handling the sharp before depositing in the safety container. As mentioned above, U.S. regulations forbid the use of needle destroyers in most clinical situations. However, in some unusual situations, there may be clear advantages to destroying the needle before deposit into a sharps box, such as environments where such boxes are susceptible to break-in and theft by drug addicts seeking injection equipment.

E.7.2. Developing countries

E.7.2.1 India

There is little available data on the market for needle defangers in the developing world except for India. Its market is probably more advanced than in any other country as a result of national and local regulations mentioned in sections E.4.5.2 and E.5.3.2 above. A survey commissioned by PATH found 11 different needle/syringe cutters and 17 different needle burners on the Indian market in 2001.[Dalal2001] Based on increasing conformance with the new Indian rules, this report estimated the need for

58,000 needle pullers in 2000-01, 86,900 in 2001-02, 159,600 in 2002-03, 42,600 in 2003-04, and 44,000 in 2004-05.[Dalal2001]

The first market niche to be targeted for PATH's investigational needle puller was immunization outreach programs, in which 400,000 Auxiliary Nurse Midwives work, each of whom would need a defanger.[Muller2001b]

In the curative medicine sector, it is possible to estimate the number of defangers that might be needed by using the reported number of hospital beds in India (896,767). Assuming one should be at or near each bedside at a ratio of one defanger per four patients would require 225,000 defangers.

Dalal Consultants also summarized the overall medical and sharps waste burden in the 32 States and Union Territories of India.[Dalal2001] They assumed 1-to-2 kg of medical waste generated daily per bed in a typical hospital, and 0.6 kg in a general practice medical clinic. Using a total of 896,767 hospital beds from Government health statistics, they estimated 1,345,151 kg per day of overall waste, of which ten percent was infectious (134,515 kg), and one-quarter of the latter was "sharps" (33,629 kg).

Dalal also estimated the total number of used disposable syringes that would be generated nationwide in India during a special mass immunization campaign to reach all persons throughout India.[Dalal2001] A UNICEF official contacted in India by Dalal estimated an average of 10,000 syringes would be needed for a typical Primary Health Center (PHC), and 7,000 for a Primary Health Sub-Center (PHSC). Dalal then multiplied these numbers by the number of PHCs (22,010) and PHSCs (136,339) reported in Rural Health Statistics in India (published by the Directorate General of Health Services, Ministry of Health and Family Welfare). The total number of syringes needed for a single-dose campaign was thus 1,174,473,000 (1.17 billion).

E.7.2.2. Modeling developing market demand

To understand the financial burden for GAVI and other donors which might bundle defangers with provision of vaccines, the working group informally commissioned Anthony Burton and Philippe DuClos of WHO to model the theoretical maximum demand for defangers to be used in immunization programs in the 74 Vaccine Fund-eligible countries and the total of 165 developing countries.

E.7.2.2.1.Assumptions, scenarios, caveats

The underlying assumption was that a defanger would be needed at each locus where vaccinations are administered. Two parallel approaches were used: The "population scenario" model was based on the assumption that an immunization site serves, on average, 5,000 persons in the population, and each site would need one defanger. The

second model, a “health care worker numbers scenario”, assumed that eighty percent of all health care workers give vaccinations and each would need one defanger. Neither model directly estimates the number of treatment rooms and bedside locations in the curative sector, except to multiply immunization sector estimates by a factor of ten based on their relatives sizes.

E.7.2.2.2. Findings

In the *population scenario*, data from the Exanded Programme on Immunization (EPI) of WHO/UNICEF and from the United Nations Development Programme was used to estimate that all 165 developing countries would have 1.11 million vaccination sites, while the subset of Vaccine Fund-eligible countries would have 815,000 sites (See Table E1).

Table E1. Estimates of vaccination and curative medicine sites where needle defangers might be used (after Burton and DuClos).

Context Data				Modeled Immunization Injection Sites			
				Assumption I – One site per 5,000 population	Assumption II – One site per 80% of all health professionals	Summary from Immunization Site Models	All Injections Estimate (10% vaccines, 90% curative)
Markets	Countries	Popul.	Annual Births				
Global	191	6.44 bn	133.1 m				
Developed	26	0.87 bn	9.2 m				
Developing – all	165	5.57 bn	123.9 m	1,114,000	1,154,000	1.0 – 1.25 m	10.0 - 12.5 m
Developing - *VF-eligible	74	4.08 bn	95.7 m	815,000	600,000	0.5 – 1.0 m	5.0 – 10.0 m

* VF = GAVI Vaccine Fund

In the *health care worker numbers scenario*, data from WHO Estimates of Health Personnel[WHO1998a] were used to estimate the need for 1.154 million immunization defangers in developing countries overall, and 600,000 among the subset of Vaccine Fund-eligible countries.

Amalgamating the two models produced a broadened conservative estimate of 1.0 to 1.25 million defangers that could be needed for immunization in all developing countries, and 0.5 to 1.0 million defangers for the Vaccine Fund-elegible countries alone.

E.8. Design and performance specifications

Various specifications for design and performance features of needle defangers have been suggested in draft documents by staff of WHO WHO2002c and internal reports of PATH.PATH2001a The working group has amalgamated and adopted many, but not all, of these proposals into the design and performance specifications specified in the Executive Summary above and detailed in the section E14.2 Annex below. The working group recommends these specifications as the basis for further research, development, promotion, and distribution of defanger devices to be funded or facilitated by GAVI for use in developing countries. Of course, they should be subject to modification, addition, and deletion, as indicated upon further reflection and experience from the field.

E.9. Policies and principles for defanger research

WHO recommends that medical devices should “undergo testing or clinical trials to substantiate intended benefit.”WHO2002b This is wise advice for any technology requiring considerable investment and carrying the potential for harm. GAVI should not accept claims unsupported by convincing, scientific data collected under conditions of intended use in the field (in contrast to the ideal conditions of the laboratory bench). Given the purported advantages and contradictory potential disadvantages described in section E6.4 above for defangers, it is essential to ascertain carefully any unforeseen and unintended consequences of technology (détournement de technique).

E.9.1. Independent research entities

For such research to be credible, it should be conducted by one or more independent entities without conflicts of interest in defanger technology. Such conflicts might apply to defanger designers with or without intellectual property (IP) rights, those involved with ongoing defanger R&D projects, defanger manufacturers, and field collaborators with employees compensated by such organizations. Of course, once the necessary research stage is completed and a decision is made to implement and promote defanger technology (see below), there ought be no bar to engaging the most experienced organization(s) in this field, regardless of their IP interests or R&D involvement.

The research entity(ies) chosen to conduct the research described below would ideally be selected by competitive review of request for proposals (RFP) from among universities, non-governmental organization, government or international agencies with appropriate developing country field research experience and methodological expertise conducting controlled research trials.

E.9.2. Principles of evaluation

In the evaluation of defangers by the proposed research agenda, it is necessary to apply some key principles:

First, the benefit or outcome the devices are expected to achieve should be defined beforehand, then measured quantitatively.

Second, units of analysis and units of measurement for the benefit must be established. The unit of analysis could be each device in the study or could be each facility. Units of measurement might be, for example, needlestick injury rates per 10,000 needle-syringes or per 10,000 person-hours of employee use.

Third, rate comparisons must be between comparable situations; not mass campaign versus routine clinical use.

Fourth, time-density must be adjusted for accuracy of exposure: “Person-years” of use may not be comparable between developed and developing-country settings, because of vacation, sick leave, and rotation to other clinical jobs. In such case, person-hours may be a better denominator than person-days or person-years.

Fifth, outcomes should be measured as population attributable risk, not as a proportion of all injuries associated with various devices.

Sixth, baseline rates for endpoints or outcomes need to be established in the study itself, either by controlled sites or crossover design. One should not extrapolate rates and assumptions from other facilities or other countries to the study sites.

Seventh, although employee preferences and subjective feedback are important input, they are not a surrogate for the proper evaluation of safety and efficacy. Moreover, employees asked questions by their employers or by funders of the study are likely to yield biased responses; these limitations should be declared and minimized as much as possible.

Eighth, when more than one defanger is being evaluated, head-to-head comparisons are desirable.

E.10. Recommended research agenda

E.10.1. Screening at the bench for inclusion into field trials

The first step should be to screen eligible devices for field evaluations to select those which satisfy the desired design and performance specifications discussed elsewhere in this document, as subsequently modified. Defanger developers and manufacturers should be invited to submit devices by a reasonable 4-month deadline to permit modifications to existing devices for conformance with the specifications. One or more independent bench laboratory testing facilities should be contracted to test and review the submitted devices for conformance with the specifications.

E.10.2. Passive field observation studies

Large-scale evaluation of defangers might be conducted in both “passive” as well as “active” study designs. Active trials (see below) would involve a more rigorous controlled comparison and a higher degree of involvement and ongoing

monitoring and surveillance by the research entity(ies) than would passive studies.

Observation under such intensive circumstances is well known to be capable of influencing participants towards normative behavior which can lead to misleading conclusions. Passive studies, on the other hand, might have an advantage of providing a clearer indication of whether defangers would be used in the manner and to the degree intended when health care workers are no longer subject to such careful observation and measurement.

In a passive field observation study design, a large number of defanger devices (1,000?, 10,000?, 100,000?) might be furnished to targeted health care workers in selected representative districts or provinces in a country. A routine amount of training and promotion for their use also would be provided at the beginning, including construction or identification of intended disposal sites for defanged needles, such as local needle pits. Continuing support for shipping consumable supplies, effecting repairs, and training new health care workers would be provided by the national immunization program to an extent reasonable to expect they routinely could achieve.

The research entity would not return until surprise, unannounced visits 6, 12, or 18 months later to a random sample of study sites, and to health care workers on outreach, to measure simple endpoints, such as:

(1) Number and proportion of distributed devices still in current use

(2) Number and proportion of devices broken or malfunctioning

(3) Number and proportion of devices missing, stolen, or unaccounted for

(4) Number of defangs performed as recorded by internal mechanical counters

(if available in defangers studied; see section E14.2.2.(2))

(5) Number of defanged needles properly disposed in the needle pit compared to records of total needle-syringes furnished and vaccine doses administered during the intervening period

E.10.3. Field evaluation trials for safety and efficacy

E.10.3.1. World regions and representative countries for study

Defanger evaluations should be conducted in each of the three major regions of the developing world:Africa, Asia, and Latin America/Caribbean.In each region, at least two representative countries should be selected for field evaluation trials. These may or may not be the same countries in which passive field observation studies will also be performed (see section E10.2).

E.10.3.2. Randomization and controls

Controlled field trials of sufficient size and duration for statistical validity should be conducted in randomly selected districts or provinces or other suitable administrative units. Control may be effected by using either separate non-intervention sites or by crossover design comparing endpoint measures in the same site before and after intervention.

E.10.3.3. Comparative study arms

When more than one device is found to satisfy the specification criteria for proceeding to field trials (see section E.11.3), trials should be designed as much as practical to make head-to-head comparisons between different devices.

E.10.3.4. Immunization settings to assess

When practicable, field studies should involve separate, parallel evaluations of defangers in different immunization situations where defangers might be useful:

(1) Routine immunization in fixed centers such as clinics or community hospitals

(2) Routine immunization in “outreach” by mobile health care workers who travel to villages and patient homes to administer immunizations.

(3) Special immunization activities such as mass vaccination campaigns and national immunization days.

E.10.3.5. Endpoints

Among possible quantitative endpoints related to injection practices which have been suggested are:[WHO2003a, Catlin2003, Battersby2003]

(1) Needlestick incidence among nurses, doctors, persons operating defangers and other health personnel, including summary data on injury circumstances

(2) Numbers over time of distributed devices in use, lost or unaccounted for (as with the endpoints suggested for “passive studies” (section **Error! Reference source not found.**))

(3) Magnitude of use recorded by internal counters (section **Error! Reference source not found.** (a)) as a proportion of all needle-syringes that should have been defanged according to records of needle-syringe procurement and vaccination doses administered

(4) Quantitative measures over time of the actual needle-disposal practices and locations of disposal at the start, during, and conclusion of the intervention

(5) Proportion of needle-syringes targeted for defanging actually defanged

(6) Proportion of syringes not rendered completely unusable by the defanger

(7) Frequency of observed episodes of accumulation of two or more needle-syringes not defanged immediately after injection (“batching”, as might occur during a busy vaccination session)

(8) Frequency of observed or recorded visible splashes, needles falling out, or other potential sources of contamination while handling, closing, and disposing of needle containers

(9) Proportion and frequency over time of breakage of defanger devices

Among possible quantitative endpoints related to waste handling practices are:

(1) Needlestick injury rates among waste handlers

(2) Measured volumes of sharps and non-sharps (defanged syringes) medical waste before, during, and at the conclusion of the study

(3) Proportion of health care facilities using various methods of disposal, including recycling, for [segregated] needle waste and non-sharps waste

(4) Diversion of non-A-D needles and syringes to the recycled market for potential reuse

E.10.4. Economic studies

In conjunction with and using some preliminary results from field trials, economic analyses should be commissioned to more accurately estimate the overall costs of needle defangers. These should be amortized at the observed usage rates and over their expected lifetimes, and converted to a cost per individual needle-syringe defanged. Additionally, the observed breakage, loss, and theft rates should be used to estimate the actual lifetimes and longevity of individual devices to be expected in field situations (in contrast to bench measurements of defanging capability).

E.10.5. Behavioral studies, subjective data, focus groups

In parallel with the above field studies, health care worker experience, satisfaction or dissatisfaction, preferences, and other feedback (ergonomic and otherwise) should be collected on their use of defangers. These might be conducted by guided questionnaires, in-depth interviews, focus groups, direct observation, or other methods.[PATH2001a]

Such behavioral research should also explore the economic incentives and disincentives to using defanger devices (including income derived from illicit diversion of non-A-D syringes to the recycling market. This will be essential to answer the critical question, “If defangers are provided by GAVI or other donors, will they be used?”

E.10.6. Demonstration projects

The agenda for research described above should be distinguished from field demonstration projects by defanger developers and manufacturers. The latter field activities have a vital role to play in improving the design of new devices and understanding the market and its needs for this technology. They help understand various design tradeoffs, including cost versus durability, portability versus static use, reusable versus disposable needle containers, and alternative syringe disabling options."[Vail2002]

Such demonstration projects are to be encouraged, and their findings taken into account by GAVI upon its review of the results of the research agenda described above. However, demonstration projects should not be considered a substitute for independent, scientific evaluation of defanger safety and efficacy.

In Senegal in 2002 and 2003, PATH is sponsoring pilot demonstration projects with defangers in the St. Louis and Matam areas. The purpose is to address waste management problems revealed from a national injection safety assessment and an EPI review in 2002.[Vail2002] Among the prior practices identified were **(1)** reuse of syringes in the absence of sterilization (25% of facilities), **(2)** needlestick injuries (52% of health care workers in prior year, with two-handed recapping common), **(3)** use of sterilizable equipment (67% of facilities) with fewer having steam sterilizers (58%), **(4)** burning waste (68%), and (5) the absence of a waste management policy.

In India, the introduction of A-D syringes prompted proposals from WHO for pilot testing of needle defangers which would assess the performance and acceptability of defangers, observe their impact on safety and needlestick injuries, on waste collectors and the community. It would also look at improperly discarded sharps found in the vicinity of health facilities.[WHO2002a] Two sites are being considered: Kerala state or in the New Delhi area, and both rural and urban settings would be studied. PATH is undertaking a field demonstration project whose results should be known in 2004.[PATH2003]

E.11. Implementation plan

E.11.1. Research oversight and contracting

Upon GAVI's initial decision to pursue the above agenda to investigate needle defangers, it would be necessary to identify an appropriate institution to manage the administrative and financial responsibilities to announce, identify, contract with, and provide oversight of the actual organization(s) to conduct the research.

E.11.2. Research timeline and budget

Given the suggested approximate 4-month interval before submission of devices for bench testing of specifications performance in accordance with section E14, it is reasonable to expect six months before the results of such screening are available to select device(s) for field evaluation. These bench laboratory assessments, including empirical measurement of claimed lifetimes, are likely to cost approximately US$30,000 to $50,000 per device.

Concurrent with the above screening process, it is likely to require four months to announce competitive RFPs and review proposals for the selection of competent research organizations to conduct the field evaluation research agenda outlined above in sections **Error! Reference source not found.** and **Error! Reference source not found.**

After the research entity(ies) is (are) selected, the entire research agenda, including passive and active studies in two countries on each of the three continents, and including various immunization settings, are likely to require an additional 12 to 24 months for completion. Although proposed budgets are likely to vary widely and ultimately be determinative, it is expected that the entire field evaluation component might cost anywhere from US$0.5 to $1.5 million, including the cost to procure sufficient devices and supplies to study one device. If multiple devices are to be compared head-to-head, these overall cost estimates would be expected to increase perhaps 30% for two devices, and 60% for three devices.

E.11.3. Implementation decision point

Upon the conclusion of the research studies, approximately 1-½ to 2 years after its initial decision to pursue research on defangers, it would be incumbent for the GAVI Board, its secretariat, or other delegée to make the “GO / NO GO” decision for further development and implementation of needle defangers as an integral part of GAVI-supported immunization programs.

If the decision is “GO”, then the next steps would be to identify and finance a suitable organization to lead that effort, which would including development of business cases and plans, and further details and implementation for rollout. Although impossible to predict what the ultimate cost of each defanger would be, if one assumes US$20.00 each, and accepts the estimates that up to one million defangers are needed for Vaccine Fund-eligible countries, the total hypothetical cost of complete implementation would be $20 million, spread out over the years required for rollout.

E.11.4. Defanger quantities, costs, and rollout

- Quantities needed. Based on the estimates of "market" need summarized above, it is assumed 1 million defangers hypothetically would be needed to completely serve every Vaccine-fund eligible country. However, a realistic assumption would be that research would find defangers of practical use in only half (500,000) of all immunization loci.
- Replacement quantities. In addition, to replace devices as they reach their service life of 25,000 defangs, new devices would be needed in perpetuity at a ratio of 1 for every 25,000 doses of injectable vaccine supplied.
- Purchase costs. Costs to manufacture each device meeting the specifications above are estimated to range from a low of US$10.00 to a high of $20.00.
- Ancillary costs. Costs of shipping, training, administration, etc., might constitute 25 percent of purchase cost, or an additional $2.50 to $5.00 per device.
- Recurring/disposable costs. Disposable needle containers which hold 100 needles and satisfy other specifications might cost US$0.25 each, which converts to $0.0025 per injection.
- Overall costs. Thus, the hypothetical cost of purchasing devices to reach half (500,000) every immunization locus in Vaccine Fund countries would total as follows:

  Initial device purchase - $5 million to $10 million (

  Initial ancillary costs - $1.25 m to 2.5 m

  Replacement devices (incl. ancill.) - $0.0009 to $0.0018 per injection

  Disposables costs - $0.0025 per injection

- Rollout estimates. A realistic expectation would be that such costs would be spread out over several years. An implementation program is likely to distribute only 100,000 devices in year 1 of a rollout ($1.25 to $2.5 million), another 100,000 in year 2 and succeeding years (same annual costs). Thus, complete rollout would require 5 years.

E.12. Acknowledgements - Defangers

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E.14. Annex - Defanger Specifications

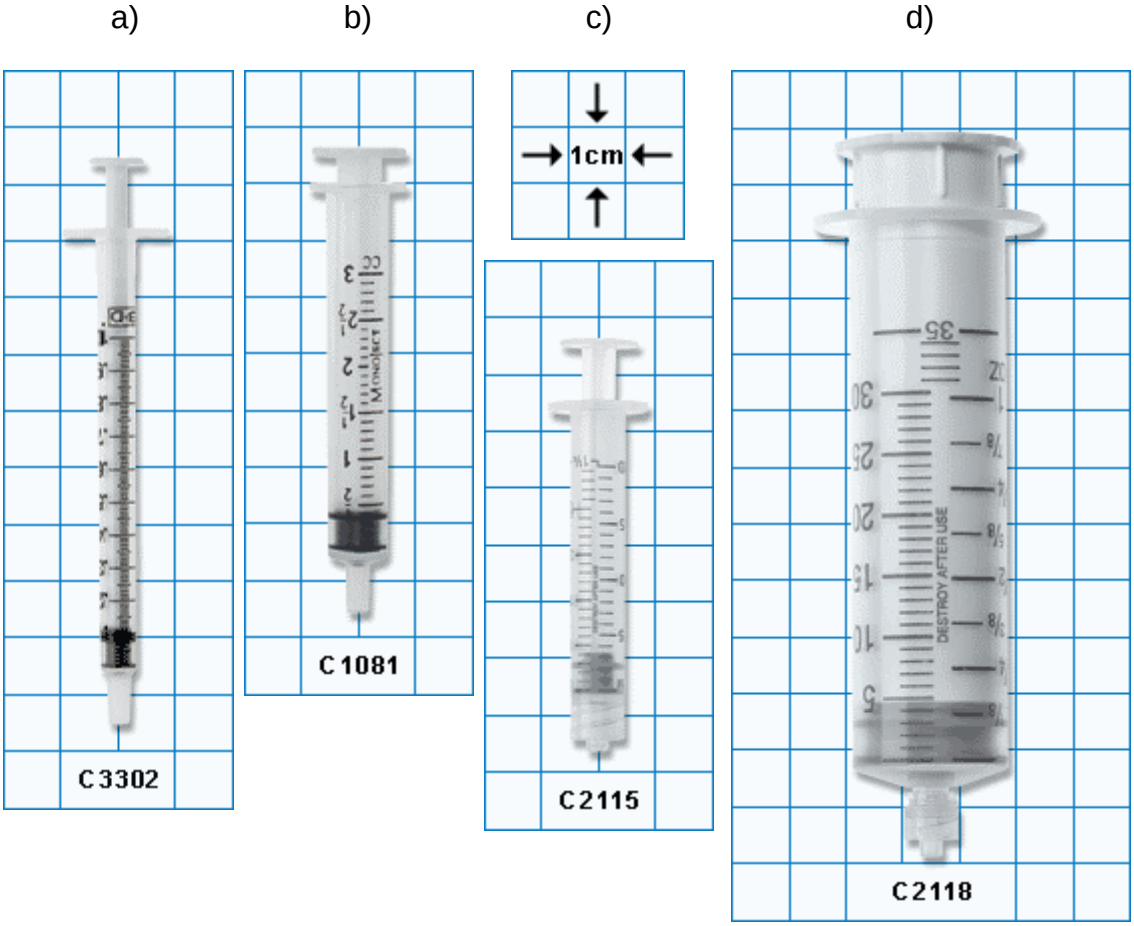
E.14.1. Primer on Needle-syringe Interface Types

In order to visualize and understand the function of needle defangers and the performance specifications to be discussed below, it may be useful to present the physicial characteristics of needle-syringes in common use in developing countries. **Error! Reference source not found.** illustrates three difference brands of A-D needle-syringe or syringe.

Figure E11. Examples of auto-disable (AD) syringes with either fixed needles (top and middle) or standard ANSI Luer taper (cone) interface for attachment of separate needle (bottom; plunger tip locks inside luer cone upon completion of injection).

Error! Reference source not found.a, b, c, and d illustrate various diameters and needle-syringe interfaces used commonly in all countries. The two syringes on the left have Luer taper)or “cone”) interfaces for a “slip” fitting with a needle hub. The two syringes on the right have Luer “lock” interfaces (with threads) for the needle attachment. Luer standards are described in American National Standards Institute document ANSI/HIMA MD70, 1-1983and in International Organization for Standardisation document ISO 594/1,2 1986-1991.

Obviously, defangers intending to disable syringes with Luer-lock interfaces will need entry holes wide enough to accommodate them. Additional, cones which assist the use in guiding the needle-syringe tip into the entry hole will need to be of sufficient angle to permit the entry of large-volume syringes with wide diameters, as in the “d” photograph.



Figures E11a, b, c, d. Examples of conventional plastic syringes with Luer standard tapered interfaces for removable needles: “Luer slip” (1st and 2nd from left: 1mL and 3 mL) and Luer "lock" (3rd and 4th from left: 1 mL and 30 mL).

A manufacturer-prefilled glass syringe with fixed needle is illustrated in **Error! Reference source not found.**. Although such packaging is not currently in common use in developing country public immunization programs, someday it might be for expensive new vaccines whose manufacturers do not want to create separate filling lines for multi-dose packaging.

Figure E12. Example of prefilled glass syringe with fixed needle.

E.14.2. Recommended Defanger Design and Performance Specifications

E.14.2.1. Major Specifications

Defangers to be provided and promoted under GAVI auspices for use in developing country immunization programs should:

(1) Not require electricity or batteries, but operate entirely by mechanical (muscle) power, manual or pedal

Although electricity is available in many urban and district health facilities, mains outlets are unlikely to be close and convenient to every locus of vaccination. Health workers may frequently forget to charge defangers operating on rechargeable batteries, and replacing battery packs once they cannot hold their charge may be problematic. Also, the increasing use of alcohol hand rubs would create a fire hazard in the presence of electrically-operated defangers.

(2) Defang all currently available A-D syringes, as well as all non-A-D syringes (plastic and glass) of nominal capacity sizes from 1 mL up to 25 mL;

Large-volume syringes of 25 mL capacity are used to reconstitute 50-dose vials of vaccines used in mass campaigns, such as to prevent measles, yellow fever, and meningococcal disease. Prefilled glass syringes may someday appear in developing country immunization programs for newer, more expensive vaccines in which manufacturers may for cost reasons not wish to alter monodose/prefilled-syringe filling lines used for developed world products.

(3) Defang non-A-D plastic syringes with interfaces with their needle hubs of either Luer cone (slip) type, Luer lock type, or snap-on type;

It is reasonable and desirable for defangers to be capable of working with all common needle interfaces found on syringes that might be used in immunization settings.

(4) Render unreusable non-A-D syringes defanged by the device;

In conventional needle-syringes in which the needle and syringe are separable, cutting or otherwise destroying only the needle does not solve the problem that syringes are often rinsed, repackaged, and recycled for (non-sterile) reuse. The defanger should disable both the needle and the syringe, preferably in one step.

(5) Leave no needle stub on the defanged syringes that could produce a percutaneous injury; The remaining syringe after the needle has been disable, removed, or destroyed should no longer carry a risk of sharps injury.

(6) Provide a clearance (or a safety shield) of at least 15 cm (6 inches) between the hand holding or operating the defanger and the nearest point along the imaginary central axis extending above the entry inlet hole where the needle is inserted into the device;

Observations by working group members of how both marketed and investigational needle defangers functioned generated concern about the possibility of needlesticks occurring to the hand that held the device and/or applied the necessary force to defang the needle. There appeared to be insufficient clearance or safety shielding between the position of this hand and the route that the needle-syringe – guided by the other hand – would take towards the entry hole of the defanger. See Figures E6a and b, and **Error! Reference source not found.**. Inadvertent bumping or inattention of the health worker might knock or lead the needle into the operator's other hand. A clearance of 15 cm (~6 inches), or a shield of similar radius around the entry hole axis, seemed a reasonable margin of safety.

(7) Demonstrate a lifetime of at least 25,000 defangs;

This arbitrary number was based on an average of 100 defanging operations per day at each vaccination "station" in a routine immunization clinic, for a typical 250 work days per year (5 days times 50 weeks).

The lifetime shall be determined by the manufacturer based on empirical testing. The claimed lifetime may be expressed either in terms of its total number of defangs, or the period of time it may be used at specified expectations for the frequency of usage (i.e., defangs per week, month, or year). If the defanger is designed to stop working after

a limited time or number of operations, the total number of operations shall be adopted as the claimed lifetime.

(8) Use disposable needle containers with permanently locking closures. The level of maximum recommended filling shall be marked on each needle container, which shall have transparent or translucent walls to permit visual indication of the degree of filling.

There would be a temptation to empty the needles from filled containers by pouring them into a disposal pit or other waste site, in order to recycle and re-use the container in the defanger itself, or for some other purpose. Sturdy containers with good closures have many practical uses. Emptying of the needles from the containers appears to increase the risk of dropping and scattering them with consequent possibility of needlestick. Requiring the closure (e.g., a screw cap top) to lock permanently into place once the filled needle container is covered may reduce the utility and attractiveness of such containers.

(9) Minimize or avoid splatter of the liquid content of needle-syringes within the vicinity of the defanger.

(10) Be amenable to easy periodic cleaning of external and internal parts and their disinfection with chlorine or other solution or sterilization with steam;

The internal components of defangers, particularly moving parts such as blades, are likely to become “gummed” up with the dried residue of vaccine or tissue fluid contained within the defanged needle-syringes. This will likely require cleaning to maintain functionality. The ability to be disinfected or sterilized periodically is essential.

E.14.2.2. Minor Specifications and Additional Desirable Features

Although not absolutely essential, defangers for use in developing country immunization programs ideally would:

(1) Not cause undue fatigue for health workers using them for up to 100 consecutive defanging operations;

The working group is unaware of any available testing methodology or ergonomic specification to quantify this requirement, which can be modified and adapted accordingly when such a method is identified.

(2) Have non-resettable internal counters to determine the number of needle-syringes defanged

This feature would be of particular importance, and perhaps a mandatory requirement, for field evaluation studies to determine and compare with greater precision the actual usage of devices placed into

use. Counters would also be of use outside of research settings for better management and accounting of sharps handling practices.

(3) Have a cycling time of 10 seconds or shorter between defanging operations on consecutive needle-syringes;

(4) Have a design which prevents needles falling out of the unit when inverted or dropped;

(5) Be capable of defanging needles from 18 to 32 gauge, and up to 5 cm (2 inches) in length, including butterfly sets;

(6) Be capable of withstanding vibration, shock, and free fall 1 meter onto a standard hard surface (steel plate), and still retain its performance capabilities, in accordance with international standards for such testing.

Defangers should be sturdy enough to withstand the rigors of field use in developing countries. The vibration and shock test should be performed in accordance with IEC document 60068-2-64 "Environmental testing – Part 2: Test methods - Test Fh: Vibration, broad band random (digital control) and guidance".

The free fall test shall be performed as specified in IEC 60068-2-32. "Test Ed: Free fall". During these testing procedures, if a needle container breaks such that it is obvious to the user, it should be replaced and the test continued until all specified steps have been performed. Such breakage of the needle container should not, by itself, disqualify the device.

(7) Have needle containers and their closures of widest diameter no greater than 9 cm in order to pass freely through inlet pipes of nominal 10 cm (3.9 inches) inner diameter, as specified for protected needle pits.

One choice for local disposal of sharps waste, developed by Médecins Sans Frontières, is the use of protected disposal pits dug into the ground with access only through a pipe extending above ground.[PATHundated] Compatibility of needle containers with this system would be desirable.

(8) Have a capacity of the needle container of at least 100 average size needles;

(9) Have a means to store the cover or closure of the needle container in or attached to the defanger, so that it is not misplaced before needed for closing the filled container;

(10) Have the needle container built of materials which do not produce toxic or environmentally hazardous emissions when burned at temperatures of unassisted open combustion (~250º -to- ~750º C).

This requirement generally would discourage the use of halogenated polymers, such a polyvinyl chloride (PVC).[Pruss1999]

(11) Have needle container and their closures with warning labels and color-coding and symbols to identify them as sharps containers;

(12) Have a puncture-resistant needle container which resists tearing, abrasion, chemical damage from chlorine disinfectant, and liquid leakage.

This would requires a minimal puncture force of 12.5 Newtons, according to relevant WHO specifications.[WHO1999a]

(13) Have a weight of 750 grams or less (including the weight of a full needle container) for devices intended to be held in the hand to operate them;

(14) Have features or accessories to avoid sliding or tipping on surfaces of potential use, including the ground, for devices which operate by placement on a firm surface;

PATH recommends a topple angle of >20º, which precludes tipping over when placed on an incline of lesser slope.[PATH2001a]

(15) Have a conical or concave surface which guides the needle into the entry hole of the defanger, permitting an initial entry angle of up to 45º from the central axis of the entry hole.

(16) Be of such simplicity of operation and maintenance in the field to require no more than a primary education to use it properly;

(17) Be accompanied with necessary maintenance tools, if any;

(18) Be labeled on the device itself with permanently imprinted or attached non-verbal symbols for critical guidance for safe and proper use;

(19) Be accompanied by instructions for proper use, cleaning, and maintenance in both text in the languages of the destination country of use and in non-verbal pictures or drawings.

(20) Produces minimal or no spraying or splashing onto accessable nearby surfaces of the liquid contents of the needle-syringe being defanged;

(21) Be portable, light, easy, reusable, reliable, intuitive, durable (1-3 years), withstand hot/moist/dusty/tropical environments, and cost from US$2-to-$7;

Such miscellaneous criteria were suggested by PATH in connection with its planned demonstration project in India.[Muller2001a, Muller2001b]

F. Additional sharps-reduction technologies for future consideration

A number of other promising technologies fall within the priority of the GAVI Board to "reduce infectious wastes and ultimately eliminate the use of sharps (needles and syringes)". The working group did not have time to address these in the first round, but considers them worthy of future consideration for support via the research and development window. They include:

F.1. “Low-hanging fruit” - short term

F.1.1. Needle-free Jet Injection

Administration of conventional vaccines by needle-free jet injectors designed for either routine clinic use or high-workload mass vaccination campaigns have the advantage of 50 years of proven efficacy in delivering dozens of current off-the-shelf products. Existing multi-use-nozzle jet injectors (MUNJIs) like the Ped-O-Jet®, Med-E-Jet®, Imo-Jet®, etc. are in limbo because of concerns for bloodborne pathogen transmission between consecutive vaccinees. R&D support is needed for a generation of safe, affordable disposable cartridge-jet-injectors (DCJIs), as well as to overcome safety concerns with bench and clinical evaluations of re- engineered MUNJIs and newly-developed disposable-nozzle jet injectors (DNJIs). c

F.1.2. Needle-syringe melters

This existing expensive technology described briefly in section E.4.6.4.2 above would benefit from applied research to lower its cost and adapt it to work without electricity using available fuels in developing countries. This might involve simple technology for automatic temperature regulation, which is the key technical challenge.

F.2. "Medium-hanging fruit" - mid-term:

F.2.1 Plastic needles.

Existing waste disposal technologies like incineration, melting, and recycling would work better if the needles were made of plastic readily blunted by heat Imagine simply passing the used needle for a half-second into a candle flame to melt its tip into a harmless lump.

F.3. "High-hanging fruit" - long-term:

F.3.1 Cutaneous Vaccination.

Intradermal vaccination using reduced quantities of existing vaccines have been a well-documented but little-used niche in immunization for several decades. Recently, the field of cutaneous delivery of vaccine antigens has burgeoned as the role of the skin's dendritic ("Langerhans") cells is recognized. These cells

take up antigen, transport them to deeper lymphoid tissues, and present them to begin the immune response. Dozens of biotech companies are working on all manner of ways to deliver antigen (and drugs) into the epidermis without using needles, but many lack the resources to conduct clinical trials to prove the principle of their method.

Various such technologies to pass antigen through the barrier of the stratum corneum - the upper layer of dead cells -are now being studied and might accelerate with R&D funding support. These include blowing properly-milled powders into the skin with supersonic gas. Others are developing various techniques to create pathways through the stratum corneum, such as microscopic tines or needles which mechanically poke or abrade the skin. One strategy is to burn tiny holes in the stratum corneum using painless brief electric currents. Another is to abrade the stratum corneum with a laser beam, after which antigen can be applied directly to the exposed epidermis until the stratum corneum regenerates within a few hours. A third is to attach and pull off sticky tape to remove the stratum corneum before applying the antigen. Others have found that simple passive diffusion of antigen across the stratum corneum is facilitated by a novel enteric toxin adjuvant.

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