Developing essential scientific capability in countries with limited resources

On-site training in modern molecular technology and capacity-building in less developed countries are essential for the effective prevention and control of emerging, reemerging and endemic infectious diseases.

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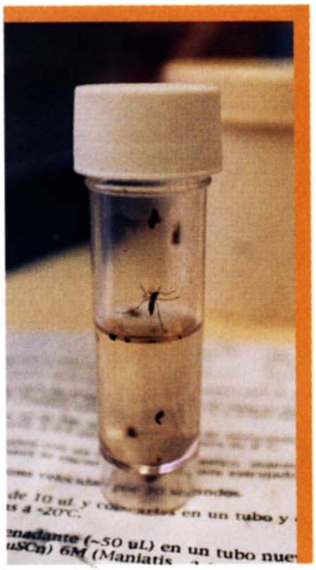
In October 1995, an outbreak of an acute febrile illness with hemorrhagic manifestations was reported in the Northern Nicaraguan community of Achuapa[1], which was initially presumed to be dengue fever. Three months earlier, reverse transcriptase-polymerase chain reaction (RT-PCR) detection and typing of dengue virus directly from serum samples (ref. 2 and manuscript in preparation) had been introduced in the Department of Virology at the Centro Nacional de Diagnóstico y Referencia (CNDR), where a number of ongoing PCR projects have been developed over eight years of collaborative efforts. In response to the outbreak in Achuapa, Nicaraguan scientists at the CNDR performed RT-PCR for dengue detection; however, the samples came up negative, even though all the positive and inhibition controls behaved as expected. After working through the weekend and processing numerous samples in duplicate to confirm the results, they realized that the etiologic agent was not dengue virus, and that other pathogens had to be considered. This outbreak sparked a great deal of international interest, and much effort was invested on the part of several international scientific institutions (most notably, the US Centers for Disease Control and the Cuban Instituto de Medicina Tropical "Pedro Kouri"). Eventually the agent was identified as the bacteria *Leptospira*[1], and the appropriate antibiotics were administered. So as to be better prepared for possible future outbreaks, Nicaraguan scientists are establishing classical isolation methods of *Leptospira*, as well as PCR detection of *Leptospira* at minimal additional cost.

As the timely diagnosis and control of infectious diseases becomes an ever more pressing need, developing countries must obtain access to the most appropriate modern technologies and must maintain high-level diagnostic and epidemiologic capabilities. This is especially true now that emerging and reemerging diseases, such as AIDS, tuberculosis, cholera and dengue, are no longer confined to a particular corner of the planet and are rapidly becoming worldwide health concerns. National health objectives in less developed countries, as well as global efforts to establish and maintain effective disease surveillance networks, will benefit from on-site training in modern molecular technology, strengthening local networks of laboratories, and developing reliable communication links.



Insect vectors can be used as samples for PCR — for example, to detect dengue virus in infected *Aedes aegypti* mosquitoes.

The AMB/ATT Program

The adaptation of modern biomedical technologies to on-site conditions and training local scientists in their use for appropriate application to relevant local problems are the objectives of a program that has been developed over the past eight years[3]. This model for the appropriate transfer of molecular technology for use in public health and biomedical sciences has been successfully applied in several Latin American countries. The Applied Molecular Biology/Appropriate Technology Transfer (AMB/ATT) Program is a three-phase program consisting of a series of hands-on workshops conducted in countries with limited resources. Phase I introduces molecular techniques for diagnosis and environmental surveillance of locally prevalent infectious diseases, Phase II oversees the application of these techniques in molecular diagnostic and epidemiological studies, and Phase III fosters the use of molecular biology to address relevant biomedical research and public health objectives. Although there are advanced scientific institutions in less developed countries, the AMB/ATT Program focuses on countries and regions where there is limited working knowledge of molecular technology.

The basic principle of the AMB/ATT Program is to take science to the problem. Workshops are conducted entirely in the language of the host country, and instructors include local scientists and participants in previous courses. An inexpensive, do-it-yourself approach to implementing molecular techniques is stressed, with an emphasis on having a solid understanding of the procedures and reagents. Simple but effective methods are taught to avoid sample cross-contamination problems, and innovative solutions are sought to overcome the material constraints. Participants are provided with training in molecular technology, good laboratory practice and the scientific method. Additionally, project development and grant-writing skills are taught, because another objective is for the participants to obtain direct funding for their projects and to administer them independently.

The initial workshops were given in Nicaragua in 1991 and 1992 (ref. 4). Since then, Phase I and II workshops have been given in three other Latin American countries, involving participants from an additional 12 countries. Phase II and Phase III projects are ongoing in Nicaragua, Ecuador, Guatemala and Mexico; they include the molecular characterization of *Leishmania*, the epidemiological surveillance of dengue directly from patient sera by RT-PCR, RT-PCR detection and typing of dengue virus in its mosquito vector, multiplex PCR detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and the molecular diagnosis of tuberculosis.

To give a more detailed example, dengue fever and the more severe form of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) — one of the most medically important arthropod-borne diseases — has spread alarmingly throughout Latin America during the last 15 years[5]. Since previous infection with one of the four dengue serotypes is an important risk factor for developing DHF/DSS upon infection with a heterotypic serotype[6,7], it is crucial to determine which serotypes of dengue virus are circulating where and when. In July 1995, we introduced RT-PCR detection and typing of dengue virus directly from patient serum samples (ref. 2 and manuscript in preparation) in the Department of Virology at the CNDR in Nicaragua. We immediately examined samples from the current dengue epidemic and were able to obtain preliminary data regarding circulating serotypes. This was very exciting because it was the first time that the Nicaraguans had been able to type their own dengue strains, since they have not had access to cell culture facilities for viral isolation and have had to resort to the costly and lengthy process of sending a limited number of samples for typing in laboratories in other countries. As a complement to the implementation of RT-PCR for dengue, a small cell culture facility has also been established for isolation of dengue virus to create an autochthonous strain bank for further genetic investigations.

Molecular techniques

Molecular techniques, such as the polymerase chain reaction and nonradioactive DNA probes, can offer specific advantages when adapted to local human and material resources. When appropriately applied, these molecular methods can be more specific, sensitive, versatile, rapid, safer and less costly than alternative methods. For example, during the Phase II AMB/ATT course in Quito, Ecuador, a patient with presumed leishmaniasis was admitted to the hospital at the workshop site. Since the leishmaniasis team was at that moment testing a number of PCR assays for detection of *Leishmania* parasites in clinical samples, a biopsy from this patient's lesions was included in the current experiment. Results later that day confirmed the presence of leishmanial DNA in the patient's lesions and further identified the parasite as belonging to the *Leishmania braziliensis* complex. Interestingly, the classical methods used for immediate analysis of the same samples yielded negative results because of insufficient sensitivity of these techniques, even though the case was clinically and epidemiologically compatible with a diagnosis of leishmaniasis. In addition to clinical samples, specimens from vectors and environmental sources can be examined by molecular means.

Since molecular techniques are based on analysis of the organism's DNA, important genetic information can be obtained, as in the simultaneous detection and strain characterization (typing) of dengue virus or *Leishmania* parasites. An unexpected demonstration of the genetic specificity of these techniques occurred recently during an international course using the AMB/ATT format to introduce molecular techniques as applied to infectious disease diagnosis. Latin American course organizers had chosen PCR detection of *Leishmania* as one example, and workshop instructors were testing this assay on-site the week before the course to anticipate any possible technical and logistical problems. We were all perplexed, however, when the cultured reference strains of *Leishmania* supplied by a local laboratory gave negative results in the PCR assay even though the positive controls (purified leishmanial DNA) resulted in amplified fragments of the expected size. When a series of additional *Leishmania* cultures were tested, it was determined that we had inadvertently detected a laboratory contamination with a morphologically similar but genetically distinct parasite in the original cultures.

On-site laboratories and personnel trained in molecular diagnosis and epidemiology provide readiness for an immediate response to health care crises. DNA-based techniques can be rapidly adapted to the detection of any organism, including recently identified emerging pathogens. Personnel and laboratories can be used immediately for detection of a new pathogen simply by providing new primers or probes and reaction conditions at little extra cost. These resources can also serve to rule out possible pathogens, as in the Nicaraguan outbreak in Achuapa.

Workshop participants learn to apply samples to an agarose gel for electrophoretic analysis of PCR products.

On-site capacity-building

Adapting technologies to existing situations is important because the material conditions on-site (availability of water, electricity, materials, reagents) are very different from laboratories in the developed world where scientists are often trained. For example, during the pre-course preparation for the first of a series of workshops in Quito, Ecuador, course instructors were testing the manual amplification of the *Vibrio cholerae* toxin-encoding operon and were increasingly frustrated when all the water baths appeared to be broken and could not reach the temperature needed for the denaturation step. That is, until we realized that at 9000 feet above sea level, water boils at 89 °C, and therefore, no water bath was ever going to reach our target temperature of 94 °C. After some brainstorming, a layer of oil was put on the "faulty" water bath to approximate a closed system, and thereby, a compromise temperature of 92 °C was achieved. However, the oil then efficiently erased the tube labels written with permanent marker; thankfully, the reaction tubes had been placed in a predetermined order in the floating racks so the experiment was decipherable — and successful. Conducting training courses under conditions that most closely approximate the true working environment of the participating scientists teaches problem-solving and demonstrates that it is possible to modify the technology in question appropriately, building a sustainable base for long-term implementation.

Low-cost alternatives — including in-house preparation of reagents from crude ingredients, simplification of protocols, and recycling — make molecular technology feasible in a low-budget situation. Even the risk of sample cross-contamination, the major drawback of PCR, can be minimized using low-cost techniques. We have avoided major contamination problems by strict adherence to a number of simple but effective procedures such as the physical separation of reaction preparation, sample preparation and product analysis; the use of bleach, which destroys unwanted DNA, to clean pipettors and work surfaces; the storage of reagents in small aliquots; and the inclusion of multiple controls in every experiment. By using this low-budget approach, it is possible to carry out techniques like PCR for approximately one-hundredth the cost of commercially available assays.

Recycling materials can reduce laboratory costs considerably. Here, gloves are labeled for reuse in the sample preparation room.

Overcoming the technical hurdles is part of the challenge; other impediments to developing local scientific capability include the paucity of scientific careers, the intellectual isolation of scientific centers, poor infrastructure in many areas (communications, information, transportation, utilities), and low levels of governmental investment in education, health and science. The AMB/ATT approach to on-site training attempts to address a number of these issues. Establishing resident expertise and routine use of molecular techniques supports scientific careers on-site by helping to minimize the emigration of scientists and developing a format for their engagement in country-specific activities. Communication networks among course participants and instructors work to reduce scientific isolation. Training in grant-writing skills helps scientists take advantage of international sources of funding and such national sources as are available.

In terms of improving communication, in-country workshops serve to overcome resistance and to build confidence in handling computers and modern information systems. Use of electronic mail between collaborators reinforces its role as a vital link, especially given the difficulty and cost of regular phone or facsimile communications in many regions. The courses contribute to the formation of national, regional and international collaborations and information-sharing networks. These in turn strengthen active disease surveillance and awareness on the part of trained local personnel, resulting in more timely reporting of critical information about potential emerging diseases into international surveillance networks. In-country capacity building is not a replacement for disease surveillance by international organizations but rather a complementary component.

Appropriate technology transfer

One of the most important elements of "appropriate technology transfer" is the transfer process itself. Successful transfer requires a knowledge-based, participatory, responsible approach that responds to local initiative and has local relevance. The tools required to understand the principles underlying the technology must be transferred along with the technology itself. Although the initial transfer of knowledge and skills is rapid and can often be accomplished in an intensive training workshop, the implementation process is gradual and requires sustained follow-up. For this reason, the AMB/ATT Program incorporates the follow-up process into its structure by its organization into successive phases. Intraregional cooperation and the use of regional expertise is a priority, since the most relevant information and incentive for one country considering a new technology is the experience of a neighbor in a similar situation.

One critical point is the necessity for a rigorous case-by-case analysis to determine whether application of the new technology is appropriate; implementation should only be considered if the new techniques confer an advantage over existing methods. For instance, the idea is not that PCR is a panacea to be applied to every infectious disease, but rather that it represents another tool, which can in some cases provide critical genetic information or needed results more rapidly or inexpensively than alternative methods.

Although the AMB/ATT program focuses on molecular techniques, the same principles of simplification and knowledge-based transfer of advanced technologies to strengthen local laboratories can be applied to many disciplines in different parts of the world.

Acknowledgments
This work has been supported in part for the John D. and Catherine T. MacArthur Foundation, the New England Biolabs Foundation, and the American Society for Biochemistry and Molecular Biology.

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