Working together to fight poverty-related diseases

2004 Annual Report

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Message from CEO

When the Tuberculosis Diagnostics Initiative (TBDI) was first established at the World Health Organization in 1997, the main driver was the notion of making DOTS (Directly Observed Treatment Short-course) easier to implement, in the belief that expansion of DOTS services would improve case detection as well as rates of cure.

By 2000, when TBDI received support from the Gates Foundation, a survey of industry activities had identified more than 50 companies with TB diagnostic tests in their research and development portfolio. Progress in test development was critically slowed by lack of funding in many cases, but more importantly by lack of information about the types of tests needed, limited access to reference clinical materials, unavailability of experienced clinical trial sites, and limited familiarity with developing world markets.

In response, TBDI developed a series of activities, including establishing a Specimen Bank, developing trial sites, collecting market data, and supporting test development research, intended to accelerate commercial development of improved diagnostics. Unfortunately, this work alone could not ensure that new tools would be developed, or that, once developed, they would be available at an affordable price to meet the needs of disease-endemic countries.

To help meet this challenge FIND was established in 2003 to work in contractual partnerships with industry and academia to develop new diagnostic technologies for uptake in disease-endemic regions worldwide. In this short period FIND has taken important strides towards establishing itself as a lead agency in the field of diagnostics, but already it has become clear that its work must be based on two main operating principles.

The first is that FIND must continually refine and update its vision, its strategic direction, and its project portfolio. Ideas and technologies are not fixed, and the flexibility to adapt to new concepts and innovations is fundamentally important.

The second principle, related to the first, is that FIND must base its field work on a strong, precise, and dynamic project management system. Given the range of products with which FIND will ultimately be involved, and the geographic and intellectual breadth of activities, a clear and well-organized project management system, in which all staff members are engaged, is critical.

The next two or three years will be a critical period in our work but I am confident that FIND will deliver on its mission - a credit to its staff and its partners.

Giorgio Roscigno, Chief Executive Officer

Major activities and milestones

This is FIND's first report on a full year of activities. In July 2003 FIND was established through a grant from the Bill and Melinda Gates Foundation to promote and facilitate the development, evaluation, and use of improved diagnostics for tuberculosis in order to upgrade patient care and disease control in endemic settings. Major activities toward this objective were:

Strategy and partnership building – Creating links with industry, academic centers, international health agencies, donors, and national governments in order for FIND to fulfill its mission of delivering affordable new diagnostic technologies to the populations in need.

Development – Partnering with industry and academic researchers in order to advance promising reagents and platforms, for which there exists proof of principle, into optimized diagnostic products for the detection of TB cases, mycobacterial drug susceptibility testing, and latent infection.

Evaluation – Funding contract and public health laboratories to evaluate the performance characteristics of market-ready tests in regulatory-quality laboratory and field trials.

Demonstration – Collaborating with public health authorities to demonstrate the feasibility and programmatic impact on patients and TB control programs and thus generate objective evidence for the broader uptake of new diagnostic tests.

The activities, outcomes, and plans are reported on these four objectives.

Evolution of FIND's strategy

FIND's mission to *accelerate the development, evaluation and appropriate use of improved TB diagnostics in developing countries* has not changed. The strategy for fulfilling that mission, however, has shifted significantly, following experiences and lessons learned since 2003, especially as informed by its patient-centered strategic plan.

FIND's strategic approach at the beginning was to prioritize the diagnostic needs, and to partner with companies holding in their portfolios promising tests for those indications. Prioritization of diagnostic needs (see table below, with diagnostics listed more or less in rank order of their importance to global TB control) was based on:

1) the number of individuals that would directly benefit by an improved tool;

2) the importance of specific populations (such as smear-positive patients) to disease control efforts; and

3) the degree of medical benefit that new technologies could offer over existing tests.

Table 1: Priorities for TB diagnostics development

Indication	**Disease to be detected**	**Size of test population**
Case Detection	Pulmonary TB with high bacterial load Pulmonary TB with low bacterial load Extra-pulmonary and pediatric TB	100-200 million 100-200 million 5-50 million
Drug susceptibility testing	MDR-TB for treatment MDR-TB for surveillance	10 million 100,000
Latent TB Infection	Latent TB for treatment Latent TB for surveillance	unknown test-dependent

During the last two years, it became clear that this strategic approach could be improved in two ways. First, by considering more closely diagnostic needs as dictated by the patient, rather than as dictated by disease epidemiology. This was based on a greater understanding of the diagnostic process as gained through:

1) an analysis of the global market for TB diagnostics that estimated global TB diagnostic expenditures, determines current and potential market size respectively, for existing and new tools, outline the costs of tool development; and estimate financial and social returns on investment in diagnostics R&D;

2) a cross-sectional study quantifying TB diagnostic delay and associated costs and morbidities in 1600 newly diagnosed TB patients and 1000 patients symptomatic with respiratory disease in four disease endemic countries (India, Peru and Zambia, Thailand);

3) two mathematical modeling exercises to estimate the epidemiologic impact of specific types of new diagnostics.

The results of these studies, carried out as part of the joint work plan with the Special Programme for Research and Training in Tropical Diseases, (TDR) will be reported in full by TDR. Core information gained in these studies that informed the FIND strategy is described below.

Market Analysis

As shown in Table 2, the direct global spending (excluding indirect patient costs) on TB diagnostics is over a billion dollars a year, with more than half of that money spent on microscopy and culture alone.

Table 2: Current global direct expenditures on TB diagnostic tests

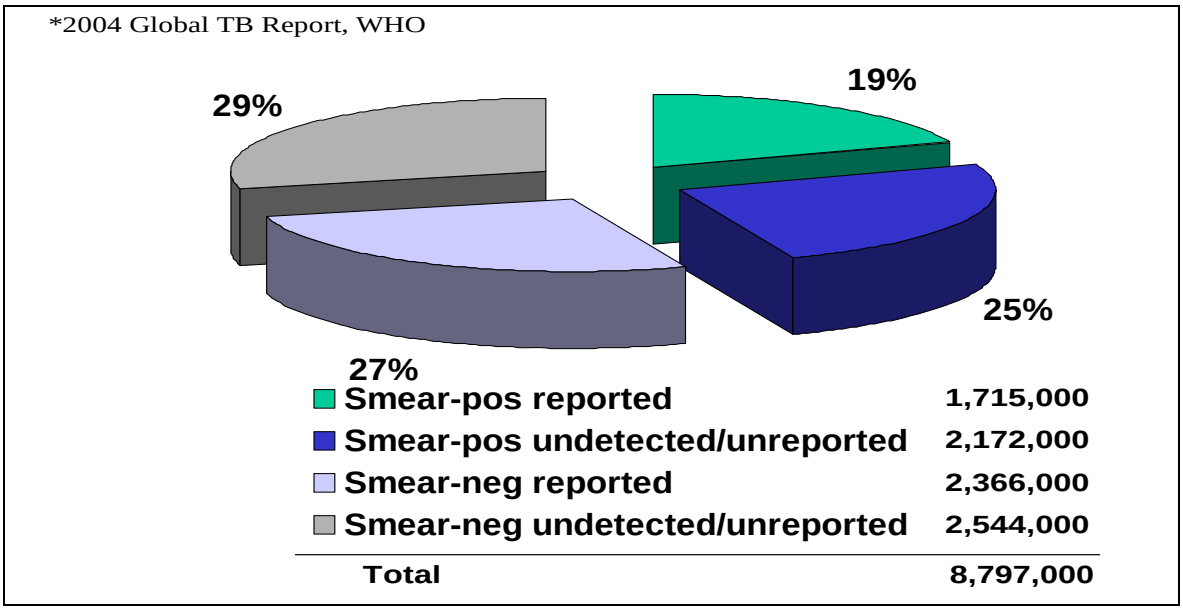
Microscopy	**$324,906,257**
Culture	**$376,258,898***
Xray	**$509,406,090**
Mantoux	**$580,955,889**
NAAT	**$35,119,542***
Total:	**>1.2 billion**

*Manufacturers' costs applied. Reimbursement costs may be higher.

The majority of all diagnostic testing, 70% of which is done in the 22 high-burden countries (HBCs), is done using non-commercial bacteriology (microscopy and locally-prepared egg-based media) and radiography, with over 150 million such tests done annually. There is a much smaller volume of commercial testing done which results in US$ 75 million in sales split roughly evenly between automated culture systems and nucleic acid amplification test (NAAT) kits.

With this level of investment however, the return is poor. As the figure below shows the diagnostic yield of this expenditure is limited, with only 19% of all incident TB cases detected and reported as smear-positive.*

Figure 1: Proportion of new TB cases diagnosed annually



As shown in Table 3, the availability of laboratory facilities where testing can take place (gathered from a country-by-country survey performed as part of the market analysis) is one, but not the only, reason for the distribution of types of testing. Culture facilities for TB are widely available in the US and Europe, with a culture-capable laboratory for every 1000-4000 TB suspects.

Among the 22 countries accounting for 85% of the global TB burden, however, only Brazil and Russia have more than one culture laboratory per 10,000 TB suspects. Among the high-burden countries in Africa, culture-capable laboratories play a negligible role in TB diagnosis, with an average of only one such facility per 500,000 TB suspects. Indeed, 90% of all testing using culture performed in HBCs takes place in just two countries, South Africa and the Russian Federation.

Despite an average of 1.7 microscopy centers per 100,000 population among the 22 high-burden countries, logistical problems (missing or broken materials, strikes, lack of trained personnel) make access to microscopic diagnosis difficult, and nearly 5 million incident TB cases, including 2,172,000 smear-positive cases and 2,544,000 smear negative cases, are either undetected or unreported.

Table 3: Availability of diagnostic services

	Population (millions)	Gross National Index	**DST** labs/100k population	**DST** labs/100k TB suspects	**Culture** labs/100k population	**Culture** labs/100k TB suspects	**Microscopy** labs/100k population	**Microscopy** labs/100k TB suspects	**Health posts**/100k population	**Health posts**/100k TB suspects
North America	328	37,610	0.10	64.2	0.35	226.4	0.88	570	1.46	951
Europe	459	22,850	0.16	34.0	0.44	95.3	0.49	106	3.89	851
Japan	127	34,510								
Other High Income	30	18,000	0.11	15.7	0.35	49.4	0.96	135	4.33	608
Total from 22 HBC	3,892	869	0.02	1.0	0.06	3.6	1.16	67	8.06	466
Rest of World	1,383		0.06	1.8	0.08	2.5	1.37	41	8.87	263
Total	6,219	5,500	0.04	2.2	0.11	5.8	1.12	59	7.40	388

Diagnostic delay study

Data from this study are still being analyzed but demonstrate, not surprisingly, that local factors are important to determining whether or not patients move quickly through the diagnostic process. General to all sites was the finding that patients select their initial health care providers based on convenience and trust, and may not reach established microscopy centers until having already sought care at two to three other locations.

In Lima, 22% of 259 TB patients first sought health care from pharmacists. Once they consulted a physician, only 56% of TB patients were requested to submit sputum specimens and did so. In Chennai, 13% of 1000 patients being evaluated for symptomatic respiratory disease did not complete the diagnostic process, and 11% of patients in whom TB was detected were not notified of the diagnosis. In Lusaka, on the other hand, due primarily to the necessity for patients to purchase the sputum collection container, only 0.5% of patients completed the diagnostic process and only 6 of 600 patients even submitted a single sample.

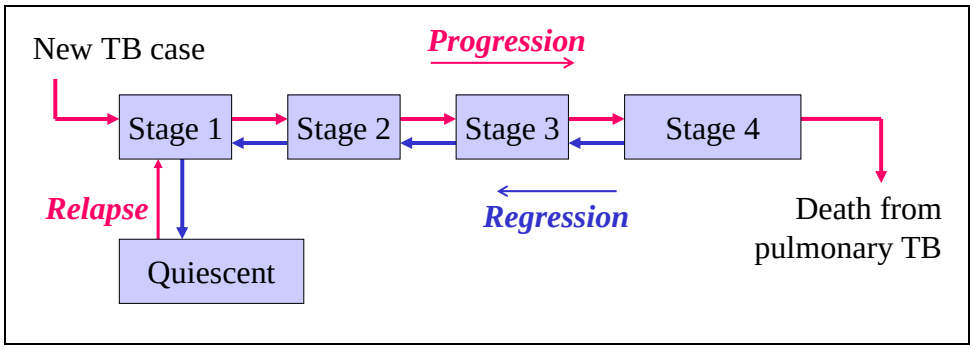
Delays to diagnosis within the health system varied widely, but were in many cases substantial, and could be limited by introducing technologies that could be used more peripherally, where patients first seek care.

Mathematical models

Two mathematical models were constructed for different purposes. First, in order to assist in the planning process for improvements in diagnostics services as called for in the *Global Plan to Stop TB II*, a conventional mathematical model assuming linear transmission from undetected cases was developed (Dye, *et al*. *work in progress*). That model examined the impact of 5 diagnostic technologies with different dates and speeds of implementation and penetration. This model predicted that point-of-care tests that could be used to detect early disease if launched by 2010 would have a large impact on incidence and mortality within 5 years and would affect country capacity to reach Millennium Development Goals. Culture and other high performance tests implemented at referral sites had little impact on disease control.

Second, a patient-centered mathematical model that did not include a transmission component (de Vlas, *et al*., *manuscript in preparation*) was constructed to replicate the progression of tuberculous disease, the movement of patients through the health system, and the relationship between the two (see Figure 2).

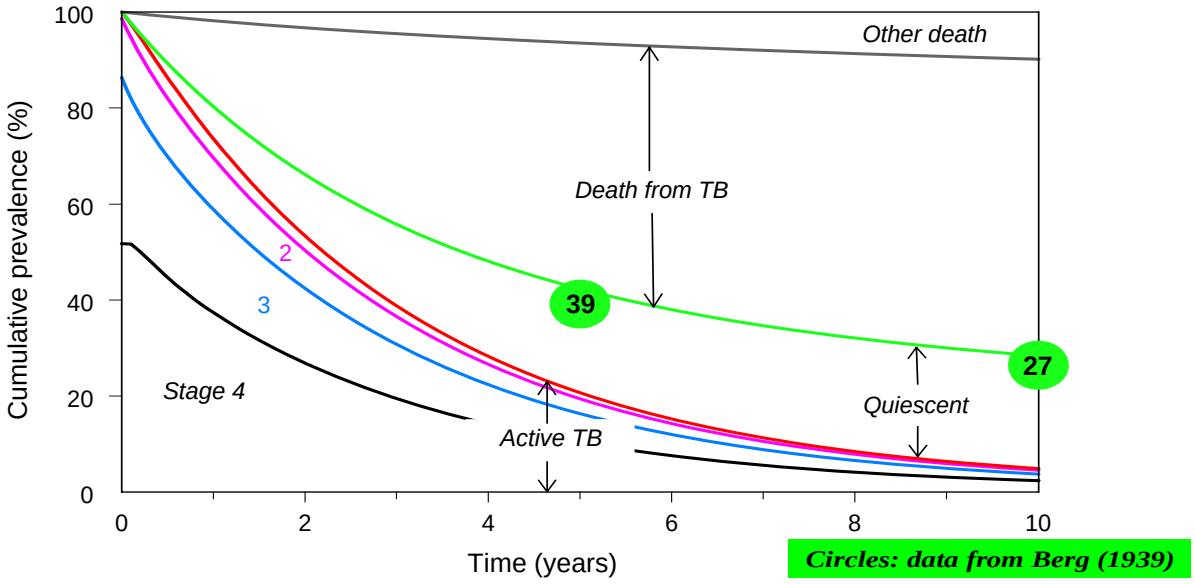
Figure 2: Progression of TB diseases



As shown above, a stylized biological model of the natural history of TB disease progression was constructed, and parameter assumptions were made for HIV-positive and HIV-negative cohorts about the rate of progression and regression through disease stages (to death), the sensitivity of microscopy, culture, and x-ray at each stage, and the rates of care-seeking and work interruption at each stage.

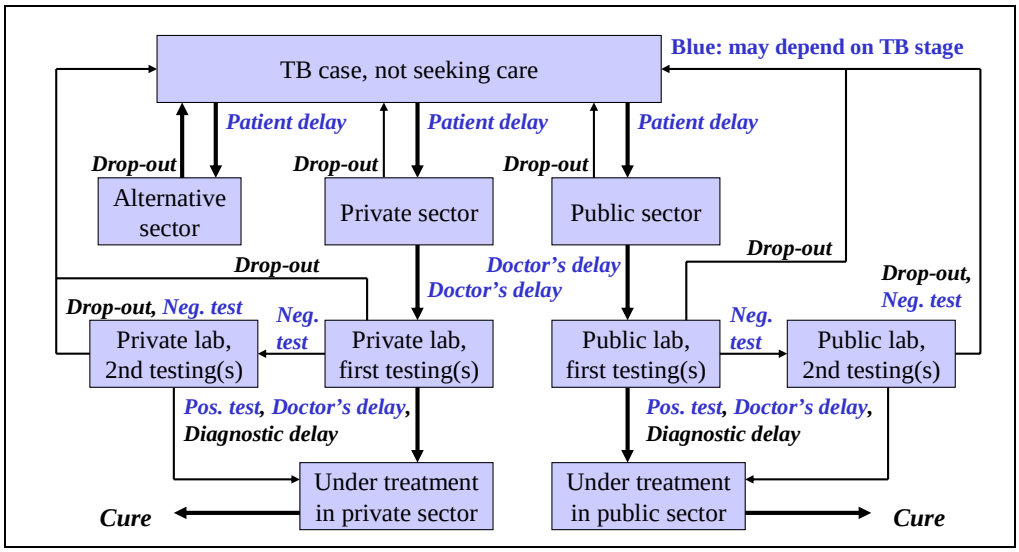
The parameter assumptions for the HIV-negative cohort were tested against natural history data on death rates from the pre-antibiotic era, as depicted in Figure 3. The green line on the graph below shows the predicted death rates in a hypothetical HIV-cohort using the model, with the circles indicating two data points measured in sanatoria populations in the 1930s (Berg, G. *ACTA Tuberculosea Scandinavica* 1939 Suppl IV:1-207).

Figure 3: Natural history of disease progression in a hypothetical cohort of HIV-uninfected TB patients



Similarly, a model of a theoretical health system was constructed, with parameter assumptions made for each cohort and disease stage about the rate of movement through, and rate of dropout from, the health system. This is shown in Figure 4 below.

Figure 4: Patient flow in a hypothetical health system

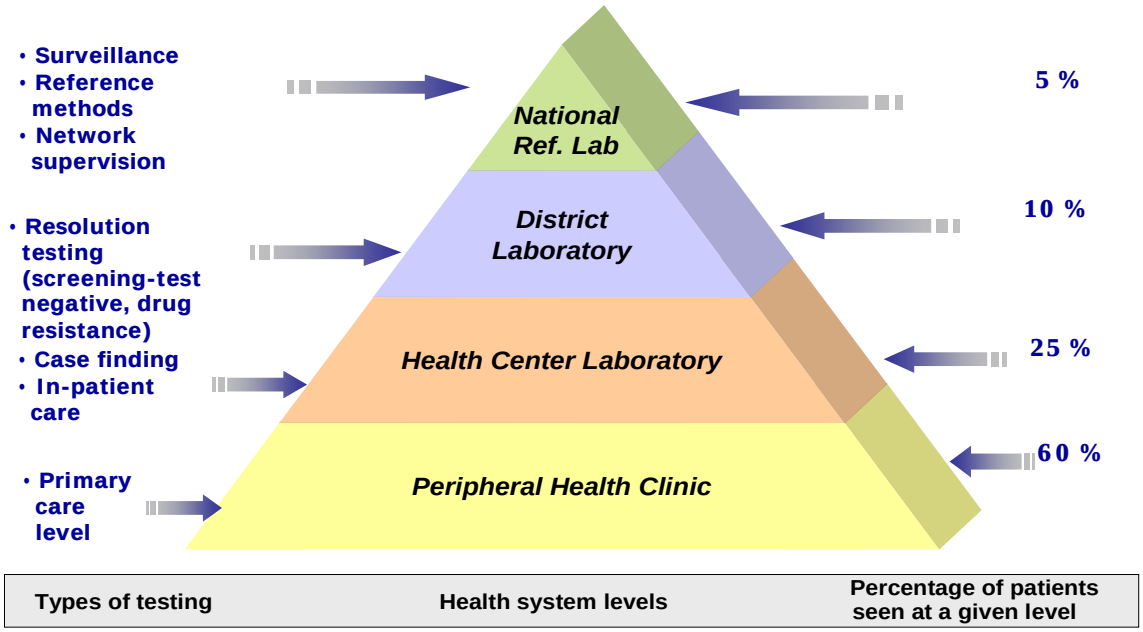


The primary outcome measures in this model were degree of delay and dropout, number of patients correctly placed on treatment, disease prevalence, and mortality. This model found that even without including a transmission component, the number of cases put on treatment and the total diagnostic delay were most dramatically reduced by point-of-care tests, even if test performance was suboptimal.

Taken together, these three quantitative analyses of the diagnostic process led to a more sophisticated understanding of how and where diagnostic technologies could be used to have the greatest impact. A revised strategy was designed which focused on the individual to be tested and 1) considered levels of the health system where technologies would be used, and 2) prioritized testing at point-of-care.

The strategy for targeting technologies to specific levels of the health system can be described briefly. As shown in the schematic below (Figure 5) for a developing country health system, a single national reference laboratory oversees the work of a limited number of district or regional referral laboratories, which in turn provide supervision for a network of microscopy centers currently responsible for reporting smear-positive cases. Below this level, however, there exist many more numerous peripheral clinics where microscopy services are generally not available, but where the majority of primary care takes place.

Figure 5:Priority setting by needs of different levels of the health system



The numbers on the right side of the diagram are meant to illustrate that the majority of patients first seek care at the periphery of the health system. The capacity to detect and confirm TB at the microscopy center and, better yet, at the peripheral clinic, would not only increase the fraction of cases detected and treated but, more importantly, would abbreviate the delay associated with referral through the health system to a specialized laboratory.

Technologies are still needed at higher levels of the health system, where more complex medical decisions need be made, e.g., changing treatment regimens, diagnosis and management of patients with extrapulmonary and AFB smear-negative pulmonary TB, as well as the diagnosis and treatment of multidrug-resistant tuberculosis. In this setting, human resources and infrastructure are more widely available, and more sophisticated technologies could be implemented.

Although patients may be fewer in number than those seen at peripheral health centers and the overall disease burden and impact on transmission less significant, the investment required to bring entirely new technologies to these laboratories will be higher due to the performance characteristics and level of sophistication required to meet the medical criteria. FIND's strategy is to introduce incremental improvements to current technologies while designing and working on simple broad technological platforms to integrate the diagnosis of multiple diseases at this level of the health system.

Customer requirement documents have now been drafted that specify the characteristics of the tests needed at each level of the health system. The prioritization of test development for point-of-care testing is reflected in the budget for 2005-2008, which allocates 60% of all spending toward these technologies.

The second improvement in FIND's test development strategy was to broaden our consideration of technology solutions. We moved since 2003 from a list of available TB applications to a more structured approach for selecting technology platforms, matching both the complexity of testing and the sophistication of information (result) with the level of the health system at which it would be used. This approach also allowed us to consider how additional disease parameters might be tested on the same platform in the future.

Partnership Building

FIND has a broad array of communication designed to create and maintain effective relationships with a range of individuals and institutions. Beyond this, FIND has recognized three partnership needs that are critical to its success:

- Industry
- The World Health Organization
- Clinical diagnostic research facilities in high-burden settings

Industry

To date, legal agreements have been signed with four diagnostic companies. In all of FIND's contractual agreements with industry partners, methods are clearly defined to ensure that each dollar invested results in a return for the public sector in the form of affordability and access. This may be achieved in a variety of ways, depending on the nature of the project, the maturity of the technology, the size of the company, and the size of FIND's total investment. When there is significant intellectual property (IP), FIND typically seeks an irrevocable, royalty free license to the IP for the public sector in developing countries. If necessary to ensure access, FIND may purchase the IP outright. In other cases, when IP is either irrelevant or not negotiable, negotiated product pricing may be the primary mechanism to ensure affordability and access. Each of the first three agreements entered into in 2004 has distinctive features:

Biotec Laboratories, UK: Projects for detection of MTB and for detection of rifampicin-resistance in two days directly from sputum based on phage replication in MTB. Financial weakness in the company motivated FIND to protect its investment in this technology by purchasing the core intellectual property and holding it through the period of joint development.

Salubris Inc, USA*:* Development of new culture system (also functional for speciation and susceptibility testing) based on colorimetric solid media. The right to have this media manufactured by an outside facility if the company was unable to supply the product in appropriate volume and at suitable cost or quality, or to convert the media to a dry form for reconstitution if stability of prepared media was a limiting feature, were included in the contract.

Becton, Dickinson & Company, USA: Projects for TB culture and drug susceptibility testing using liquid media with fluorescent indicator of growth (Mycobacterium Growth Indicator Tube). This agreement to demonstrate the feasibility of scaling up use of this culture system in the public sector of developing countries centered on an existing product already in the market. FIND was able to negotiate a large cost reduction in the media and detection equipment.

The World Health Organization (WHO)

Close collaboration with WHO is of fundamental importance to FIND as products move through demonstration projects toward implementation. By geography and history, FIND has a close relationship with the Stop TB Department (STB) at WHO and has extended that relationship over the past two years. Examples of this collaboration include:

- FIND co-sponsorship with WHO and TDR of an "Expert Consultation on optimizing diagnosis of tuberculosis through improved smear microscopy and diagnostic algorithms."

- FIND leadership of the Stop-TB Working Group on Diagnostics.
- FIND chairmanship of the Stop-TB Subgroup on Laboratory Strengthening meetings.
- FIND chairmanship of WHO Mega Meeting sessions on TB control.
- Invitation for FIND to join the core group of the TB/HIV Working Group of Stop-TB.
- Advanced discussions with WHO concerning a shared staff member to focus on laboratory issues, including the use of new technologies.

Clinical diagnostic research facilities in high-burden settings

One of the primary roles of FIND in its relationships with industry is to understand the needs of, and have access to, patients and health systems in developing countries. Access to trial sites, to reference clinical materials, and to national control programs is essential. Below are three of the most important partnerships FIND has developed with groups operating research programs in high-burden settings.

With the Consortium to Respond Effectively to the AIDS/TB Epidemic (CREATE), FIND has partnered to demonstrate the feasibility of MGIT testing in developing world settings to improve the detection of HIV patients coinfected with TB. Through CREATE, which includes partners Johns Hopkins University (South Africa and Brazil), London School of Hygiene and Tropical Medicine (South Africa, Zambia), and Aurun Health Research (South Africa), FIND is able to demonstrate the MGIT technology in ~60,000 patients over a 2-year period. An activity of this scale is only possible through partnership.

With the University of Munich, which operates a research unit in a high-burden setting in Mbeya, Tanzania, and with the University of Cayetano Heredia in Lima, Peru, FIND has signed agreements that will ensure clinical evaluation capacity for multiple products. With core support coming from FIND to maintain laboratory and clinical personnel throughout the period of investment, FIND has access to high quality laboratory facilities, experienced investigators, strong data management, and high patient volumes. These sites will be used for the collection of additional reference materials (an estimated 5000 samples will be needed to support R&D activities over the coming three years) as well as to execute laboratory and clinical evaluations of products in the FIND portfolio.

With MSF Holland, PATH, GENETUP, a German NGO, the US Centers for Disease Control and Prevention (CDC), and the UK Health Protection Agency, FIND is able to mount demonstration projects in sites that have external technical support and external funding in Uzbekistan, Tanzania, Nepal, Kenya, and the Russian Federation.

Information gathering and sharing

Information gathering*:* With the understanding that the success of FIND hinges on its ability to identify and capture promising diagnostic technologies, FIND invested significant effort during its first two years of operation on information gathering. This was done through literature review; conference attendance; meetings of the FIND Scientific Advisory Committee; discussions with leaders in the diagnostics industry; meetings with scientific staff of the National Institutes of Health, the Wellcome Trust and other donors; review of biodefense projects funded by the US military (review panel); referral from BMGF and other donors receiving submissions on diagnostics; and personal contact with technology developers. FIND visited or received over 40 companies to review technologies in detail and consider their relevance to the FIND portfolio.

Information sharing*:* Details of FIND activities are communicated through various channels, and with different goals depending on the target audience. Though there is considerable overlap, four target audiences have been defined: the general public, the scientific community, the business community, and the TB public health community.

General public: The goal of informing the general public of our activities is to highlight the importance of diagnostics for public health, to provide a reliable source of information about specific technologies, and to attract new people and ideas. The FIND website and FIND newsletter are designed to be accessible to the general public.

Website: The FIND website contains basic information on how and why FIND was established, and provides details of directors and staff, objectives, activities, press releases and copies of presentations. Revised three times since being established in late 2003, hits to the website have steadily increased.

Newsletter: FIND's newsletters provide activity updates, a calendar of events, and technology partnership announcements. They are posted on the website and published in 2,000 copies for distribution to the global TB community.

Scientific Community*:* The goal of sharing information with the scientific community is to garner their interest and involvement in FIND activities, to maintain professional credibility, and to influence thinking about diagnostic R&D that is underway in various institutes. Articles in the scientific literature, presentations at technical conferences, publication of Requests for Applications (RFAs) and personal contact are the most important forms of communication for this audience. Indicators of impact include good convening power, strong relationships with leading research institutions, agreements from research institutions to give FIND special access to intellectual property, and responses to RFAs.

***Publications**:* During 2004, FIND scientific staff published a number of articles and one book chapter in the peer-reviewed scientific press:

- Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. Nature Reviews Microbiology 2004;2:231-240.
- Kocagoz T, O'Brien R, Perkins M. A new colorimetric culture system for the diagnosis of tuberculosis. (letter) Int J Tuberc Lung Dis 2004;8:1512
- Perkins MD, Small P. Admitting Defeat. Int J Tuberc Lung Dis ; (in press)
- Small P, Perkins MD. Developing New TB Diagnostics: A Global Call to Action. Lancet Infect Dis. (in press)
- Perkins MD, Roscigno G, Zumla A. Progress towards improved tuberculosis diagnostics in developing countries. Lancet (in press)
- Perkins MD, O'Brien RJ. New diagnostics for tuberculosis: an essential element for global control and elimination. Marcel Dekker (in press)

***Presentations**:* Since the launch of FIND, staff members have given numerous presentations at national and international meetings (see Table 4 below).

***Commercial / Industrial Partners**:* Information sharing with commercial groups is critical to the success of existing partnerships and to the creation of new ones. Most direct communication related to projects takes place within the project management framework, is covered by confidentiality agreements and is not shared with the public. New agreements are usually announced with a press release. Indicators of success include milestone progress toward development objectives, the number of companies having heard about FIND who seek to partner with FIND, and corporate information such as website hits and share prices following press events.

FIND has issued press releases to announce the signing of partnership agreements with several technology companies: Biotec Laboratories Ltd. of the UK, Salubris Inc. and Becton Dickinson of the USA.

***Tuberculosis Public Health Community**:* The Tuberculosis Public Health Community is critical to the uptake and appropriate use of any of the technologies arising from FIND activities. Information is shared with this community through a number of venues, with the principle goals of developing a shared vision related to new diagnostics for TB, assisting planning towards testing and implementation of new tests, dispelling distrust and/or anxiety about research activities, and giving perspective to potential donor agencies about how FIND's activities fit into overall TB control efforts. Indicators of success include requests for country visits by National Tuberculosis Program (NTP) managers and Ministries of Health, requests by technical agencies for intellectual input to symposia and other fora, inclusion in WHO and IUATLD closed consultations, and donor support for FIND activities.

***Sponsored symposia**:* FIND sponsored or shared sponsorship of major symposia on diagnostics on five occasions since 2003:

- Paris October 2003 – IUATLD 34th World Conference on Lung Health
- Moscow June 2004 – IUATLD 3rd European Regional Congress on Lung Health
- Paris October 2004 – IUATLD 35th World Conference on Lung Health

Workshops: In its capacity as the lead agency of the Stop-TB Working Group on Diagnostics, FIND hosted Working Group meetings in October 2003 and October 2004. In addition, FIND has hosted technical meetings in collaboration with WHO and TDR.

***Commercial facilitation**:* FIND has continued to work in partnership with TDR to carry out core activities toward facilitating commercial tool development. These include maintenance of the WHO/TDR TB Specimen Bank, development of a *M. tuberculosis* strain bank, provision of GLP/GCP training for diagnostic trial sites, and development of a *Global Analysis of the Market for Tuberculosis Diagnostics*.

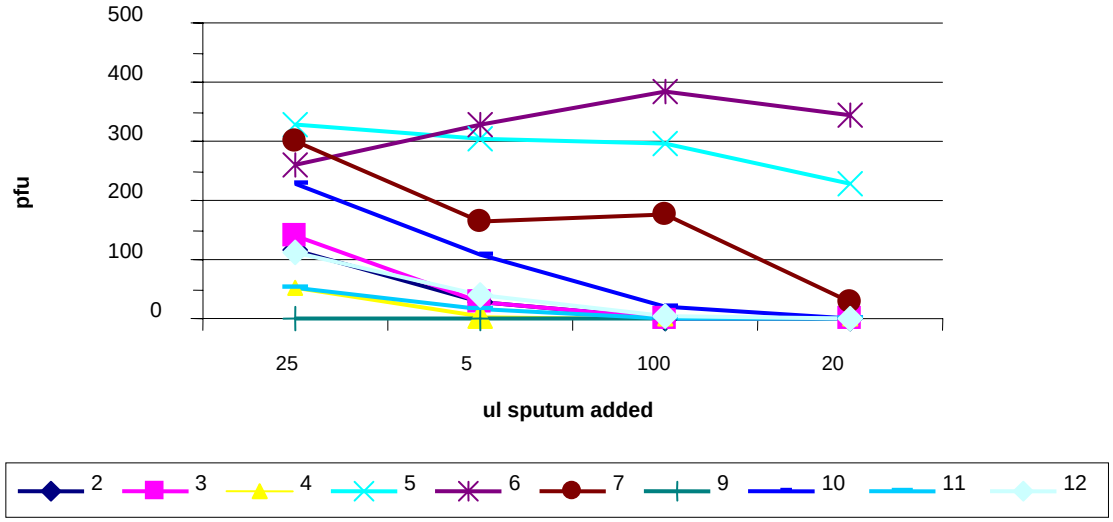
Table 4: Scientific presentations by FIND staff in 2004

Date	Venue	Topic	Speaker	Venue
Sep-03	New Delhi	Addressing obstacles to wider appropriate use of rapid tests	Perkins	PATH India Workshop
Oct-03	Paris	The role of FIND in development, evaluation and demonstration of new TB diagnostics	Roscigno	FIND symposium, 34th IUATLD World Congress of Lung Health
Nov-03	Cape Town	Health and poverty: Skipping generations in technology is possible solution	Roscigno	Meeting at MRC
Feb-04	Algiers	Local manufacturing of essential medicines	Roscigno	15th Conference of the Africa Region IUATLD
Apr-04	Washington	"FIND strategy, objectives, and key factors of success	Roscigno	USAID
Apr-04	Washington	New TB diagnostics in context	Perkins	World Bank
May-04	The Hague	What are the new diagnostic tools and where will they come from?	Perkins	KNCV: Wolfheze Workshops on Tuberculosis Control
Jun-04	Torino	Pharmaceutical technology transfer to developing countries	Roscigno	-
Jun-04	Moscow	New TB Diagnostics: FIND Priorities and Plans	Perkins	3rd Congress European Region IUATLD Symposium
Jul-04	Brasilia	Perspectives for the Development and Evaluation of New TB Diagnostic Methods	Perkins	National TB Meeting: Brazil
Aug-04	Dar Es Salaam	The Initiative on Pharmaceutical Technology Transfer	Roscigno	WIP-ARIPO Round Table
Aug-04	Manila	New diagnostics modalities in TB	Roscigno	11th Annual Philippine Coalition Against Tuberculosis
Sep-04	Rome	Partnerships for Health: Evolution and challenges	Roscigno	EDCTP forum
Oct-04	Cape Town	The role of diagnostics in resource poor setting: FIND	Roscigno	ROCHE-sponsored symposium
Oct-04	Seoul	"Creating capacities and improving access for essential medicines	Roscigno	Korea Ministerial Conference
Oct-04	Paris	New diagnostics modalities in TB	Roscigno	FIND symposium, 35th IUATLD World Congress
Oct-04	Perugia	Biologic Repositories to Support Diagnostics Research and Development	Perkins	International Society for Biological and Environmental Repositories
Oct-04	Paris	History and objectives of the Diagnostics Working Group of Stop-TB	Perkins	35th IUATLD World Congress of Lung Health
Oct-04	Paris	A Public Sector Engine for Diagnostics Development	Perkins	35th IUATLD World Congress of Lung Health
Nov-04	Paris	The FIND/TDR workplan: A vehicle for laboratory strengthening	Perkins	Stop-TB Working Group on DOTS-expansion, Subgroup on Lab Strengthening

Development of phage assays

Removal of phage inhibitor*:* As a first step to identifying possible inhibitors to the *FASTPlaque* TB reaction, a model system was established to study inhibition. Media inoculated with known concentrations of *M. tuberculosis* was used to produce a predicted number of viral plaques. Small aliquots from different sputum samples (25, 50, 100 or 200 μl) were added to the reaction at the time of incubation and the effect on pfu measured. An impressive dose-effect was noted, with 2-log reductions in plaque number after the addition of just 100 μl of most sputum samples.

Figure 6: Dose related inhibition of plaque assay



The degree of inhibition varied between specimens (83% ±26 (mean± 1sd)). Almost all sputum tested (>99%) showed inhibition of at least >50%. Less than 1% of sputa tested contained no plaque assay inhibitory activity. It was found that sputum specimens could be stored at -70^{o}C without loss in the activity of the inhibitory activity. This has facilitated storage of specimens for further testing.

Work over the past 4 months has been ongoing to test a number of methods to remove or overcome the inhibition. In summary, three methods reduced inhibitory activity in unprocessed sputum. Washing the sputum reduced inhibition by ~25%. Heating the unprocessed sputum or treating with trypsin reduced inhibition by 60 to 70%. DNase, lipase, chondroitinase, 1-antitrypsin and activated charcoal had no effect on the assay. In contrast, amylase, synthetic low molecular weight protease inhibitors (Sigma cocktail) and DTT inhibited the assay.

Passing unprocessed sputum through different sized molecular weight cut-off (MWCO) membranes indicates that ~90% of the inhibition is due to a factor(s) with a MWCO >100,000 and ~10% is due to a factor(s) with a MWCO <5,000.

***Phage concentration**:* A number of other attempts were made to optimize the assay including simply increasing or decreasing the phage concentration from the standard 10^9 pfu/ml. The number of plaques formed per reaction with input concentrations of phage from 10^7 to 10^{10} pfu/ml was studied. Going beyond this concentration would present important manufacturing difficulties. In general, the number of plaque forming units (pfu) per colony forming unit (cfu) increased with phage titer up to a concentration of 10^9. There was little additional yield from 10^{10} pfu/ml and greater risk of breakthrough phage not killed by virucide. Lengthening the period of phage infection did not result in an important increase in infection efficiency.

***Altered timing of assay steps**:* To date, a review of the data suggests that it may not be possible to meet the original product specification targets while keeping the two day turnaround time for results. It was hypothesized that increasing the resuscitation period from one hour to 1-3 days might increase the number of infection-susceptible *M. tuberculosis* organisms in the sample. Alternatively, allowing a 1-3 day period for post-virucide phage amplification (PVA) (cell lysis and release of 100-200 phage particles per cfu) could improve the sensitivity of the readout.

The effect of several combinations of resuscitation (0, 1, 2 & 3 days) and PVA (0, 1 & 2 days) has been investigated with decontaminated smear positive samples. This work indicates that a 3 day resuscitation step, followed by a 1 day PVA remains the best option for improving the phage signal. Preliminary studies using a 3 day resuscitation step, 1 day PVA step, and 7H9/OADC virucide neutralization were performed using smear-positive sputum samples diluted until AFB concentrations were $<10^4$. The new method resulted in an increased number of plaques in 42 of the 47 plates with from which quantitative comparisons between the pfu yield of the conventional and new method were possible. An increase in average plaque number of 100-fold (1.8 $\log_{10}$) was found.

Though the value of the phage-based assay decreases the longer the total time required for testing takes, it is also true that laboratory work flow has never been adequately considered in test design, and it is likely that flexibility in the length of certain steps could be a significant advantage to the test. The current target version of the assay would allow Monday to Wednesday processing only, without requiring weekend reading. Unfortunately, the PVA step is ineffective unless the virucide is completely neutralized. A number of chelating agents, including desferoxamine and EDTA have been studied, but for the time being, virucide is being neutralized with large volumes (>15ml) of 7H9 media plus OADC. This presents logistical problems and cannot be considered a final solution.

FIND is to review the 4th quarterly report from Biotech in 2005, to evaluate the *FASTPlaque* case detection test and related costs.

Phage assay for rifampin resistance

This test had completed development at the time of the Biotec contract, and was included in a large comparative evaluation of DST methods in Peru funded by TDR and monitored by FIND (described below in the evaluation section). At the time of FIND's 11/04 visit to the Peru site, accompanied by a review of the preliminary data, it was clear that excess contamination of the *FASTPlaque Response* plates was likely to be a problem.

This problem had not been seen in the only other study completed before that time, a small study in Cape Town, but the observation reinforced similar findings in Port Elizabeth. There was some relationship with the bacterial burden, with 2 to 3+ AFB positive samples being much more likely to be contaminated but less likely to be indeterminate (due to inadequate plaque number in the control plate).

As a result of the high contamination rate this product went back into development. An antibiotic cocktail that was non-confounding to the rifampin resistance determination was identified and proven to reduce contamination rates without altering performance. This product then returned to the evaluation phase with only a four month delay in study conclusion.

Sequella: FIND convened a panel of scientists in February 2004 to review the information available about the use of transdermally delivered TB antigens, specifically MPT64, in a patch test to detect tuberculosis. The conclusion of that meeting was that the evidence was strong enough to merit a more carefully controlled study of the method. FIND entered into lengthy negotiations with Sequella for access to the technology in exchange for phased support of 1) completion of development of a generation 1 product, 2) clinical evaluation, demonstration and launch of generation 1 product, and 3) development and evaluation of a generation 2 product.

FIND terminated the development project on MPT64 in November of 2004 due to poor product performance.

Evaluation studies: TK Medium

TK Medium (Salubris, Inc, Cambridge, MA) is a novel, solid mycobacterial growth medium that supports rapid growth of *M. tuberculosis*. This medium has multiple color dye indicators that enable early visual detection of mycobacterial growth, as well as the visual differentiation of mycobacterial growth from contamination. The original reddish color of the medium turns yellow with mycobacterial growth, and green with growth of other bacteria or fungi. The change in color depends on metabolites and enzymes produced by different microorganisms and occurs before colonies become apparent. TK Medium does not contain any radioactive material or fluorescent dye and does not require a scintillation counter, UV light, or other detection system for evaluation of culture tubes. The color change can easily be evaluated by the naked eye. Standard TK Medium contains no added antibiotics, and TK Selective Medium contains antimicrobials to inhibit growth of contaminating organisms such as those

frequently found in respiratory specimens. TK Medium is used for the speciation of mycobacterial isolates at the level of *M. tuberculosis* complex and MOTT bacilli.

Initial evaluations of the TK Medium in Turkey indicate that the culture system is as sensitive as Löwenstein-Jensen (LJ) medium, the most widely used solid culture system throughout the world, and has a much more rapid time to detection (14 vs. 28 days), approaching that of liquid culture systems that are the standard for TB diagnosis in industrialized countries (10 days).

Following laboratory validation studies that are being conducted by the CDC in Atlanta, the TK Medium system will be evaluated in three TB laboratories in North America and in laboratories in South Africa and Brazil. The studies in the latter two sites are being overseen by investigators from Johns Hopkins University with financial support from the U.S. Agency for International Development.

Demonstration studies

It is generally accepted that more rapid, effective, and easily implemented TB diagnostic tests can improve both patient care and disease by decreasing detection delay in patients seeking care and increasing access to detect otherwise missed cases. This would result in decreased TB morbidity, mortality, and disease transmission.

However, the ability of a new diagnostic test to deliver these benefits may not be evident solely on the basis of analytic data or controlled trials of test performance. For this reason, FIND believes that large-scale demonstration projects are required to provide the evidence that new tests that perform well in controlled settings can have an important medical and public health impact when implemented in programmatic settings. These projects are intended to demonstrate the feasibility, scaled-up performance, patient and/or public health impact, and, where possible, the cost-effectiveness of using a new technology. Data from these studies will enable technical bodies such as the WHO to recommend these tests for routine use and provide Ministries of Health and other purchasers of TB diagnostics justification to implement these tests in national tuberculosis control programs.

One of FIND's initial demonstration projects is assessing the Mycobacterial Growth Indicator Tube (MGIT) culture system from Becton Dickinson for TB diagnosis in developing countries. The purpose of this project is to demonstrate the feasibility and impact of MGIT technology in two specific areas where new diagnostic TB tests are badly needed: improved case finding among HIV-infected persons with TB and expanded drug susceptibility testing (DST) for persons at risk of multidrug-resistant (MDR) TB. MGIT is more sensitive than traditional solid culture on LJ media and provides results much more quickly. Thus, access to and sustainable implementation of this technology in high-burden countries would represent significant progress towards improved TB management.

In areas where TB rates have increased greatly in association with the HIV epidemic, the most widely used TB diagnostic test, AFB smear-microscopy, is inadequate. This is because the large majority of HIV-associated TB cases are AFB smear-negative. In the absence of mycobacterial culture, the complicated and lengthy diagnostic algorithms used to diagnose AFB smear-negative cases are insufficiently precise, and many patients die before the diagnosis of TB is made. Rapid culture methods, such as MGIT, provide the opportunity to quickly and accurately diagnose these cases and implement TB treatment, thus both decreasing HIV-associated TB morbidity and mortality and interrupting TB transmission.

In the initial MGIT demonstration projects for case-finding in TB/HIV, FIND is partnering with CREATE, whose purpose is to organize, implement and evaluate novel strategies to reduce morbidity and mortality from TB in populations with high rates of HIV and TB co-infection. MGIT technology is being used for improved TB case detection in CREATE projects in Zambia, South Africa, and Brazil. FIND is also planning similar projects in Tanzania and Kenya, partnering with CDC, PATH, and several academic institutions working in HIV/AIDS.

Alarming increases in MDR TB, which is not treatable with the currently available first-line TB drugs, have been found in a number of countries throughout the world. In response to this problem, the WHO Stop TB Department has established the DOTS-Plus strategy and the Green Light Committee, a mechanism to provide second-line TB drugs to programs that qualify to undertake MDR TB treatment. However, a major impediment to expansion of MDR TB treatment is lack of DST capability in many programs. To address this problem, FIND is working with WHO and other technical partners to implement MGIT DST demonstration projects in DOTS-Plus sites. Projects in Uzbekistan, Nepal, and the Russian Federation are being implemented with assistance from MSF-Holland and GENETUP, a German NGO working in Nepal, and the UK Health Protection Agency.

Accomplishments

FIND's top four accomplishments in 2004:

a) Attracting and maintaining a group of motivated top professionals (full-time and consultants) and positioning FIND at the forefront of international efforts to develop TB diagnostics.

b) Establishing and validating the FIND business and intellectual property model of collaboration with industry, both major multinationals (e.g. BD and Eiken) and smaller biotechnology companies.

c) Identifying key existing technologies and including them in a professionally managed portfolio of products, bringing first world technologies to the public health sector of developing countries and displaying a promising portfolio of TB diagnostics adapted to all levels of the public health system.

d) Demonstrating the capacity to end projects that do not meet performance milestones.

Challenges

The simple and efficient organization structure of FIND and its capacity to develop and execute contracts has greatly facilitated the administrative work needed to reach its goals. There remain several important challenges, including the technical complexity of TB test development, the weaknesses of some of FIND's corporate partners, and the poor human resource and technical infrastructure at sites where new technologies will be used. Mechanisms to address each of these challenges are in development:

- Technical challenges: *M. tuberculosis* is a slow-growing organism, difficult to lyse, present in low copy numbers, provoking a heterogeneous immune response, and commonly mixed in a complex and variable sample matrix – sputum. FIND is using a multi-pronged approach to development work, using 7 different technologies (antibody detection, antigen detection, bacterial cultivation, phage replication, optical detection, electronic sensing, and molecular amplification) to develop case detection tools. Supporting multiple approaches dilutes the risk associated with dependence on a single technology.

- Weakness of corporate partners: Many of the smaller biotechnology companies partnering with FIND have reduced technical teams and very limited financial strength. FIND has implemented a process for intellectual property management and contract negotiation that preserves its right to find additional or alternative partners should the principal partner be unable to meet its obligations. For technical support, FIND has been able to convene the leading scientists in relevant areas to join product development groups.

- Poor site infrastructure: Most of the countries with a high burden of TB have notoriously weak laboratory infrastructure. FIND has a two-pronged approach. One, developing technologies that depend as little as possible on laboratory infrastructure and two, working closely with the Laboratory Strengthening Subgroup of the Stop-TB partnership to develop a strategic plan and implementation calendar for improving laboratory capacity in disease-endemic settings.

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