
SEMI
LA VISIÓN GLOBAL DE LA PERSONA ENFERMA
FEMI
XXXI Congreso Nacional de la Sociedad Española de Medicina Interna
OVIEDO
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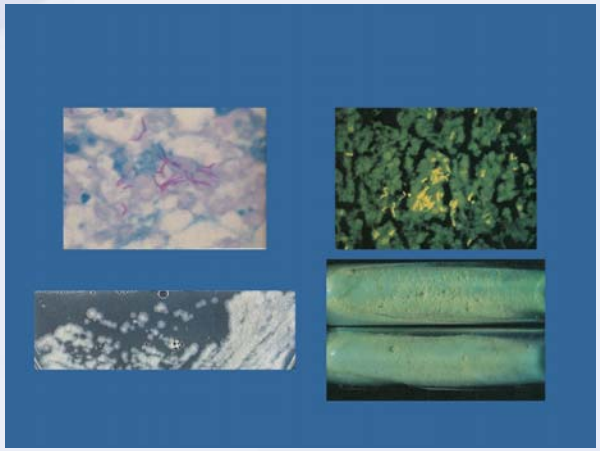
II Congreso Ibérico de Medicina Interna
VII Congreso de la Sociedad Asturiana de Medicina Interna
TUBERCULOSIS 2010: EL DESAFÍO CONTINÚA

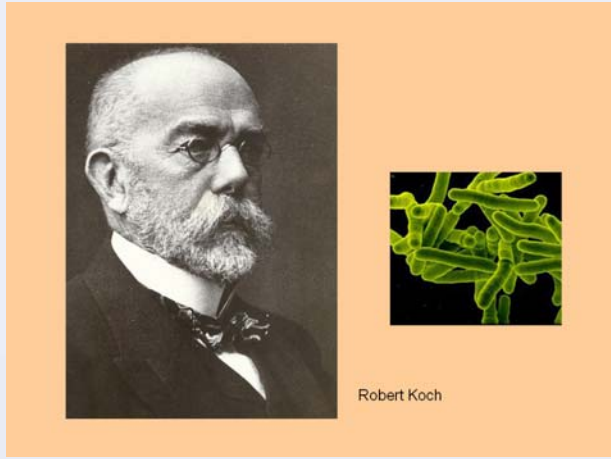
Novedades en el diagnóstico de la Tuberculosis y de la infección tuberculosa latente: ¿siguen vigentes los métodos tradicionales en la era de las técnicas moleculares y de los IGRAs?

Juan José Palacios Gutiérrez

Unidad de Referencia Regional de Micobacterias

Hospital Universitario Central de Asturias

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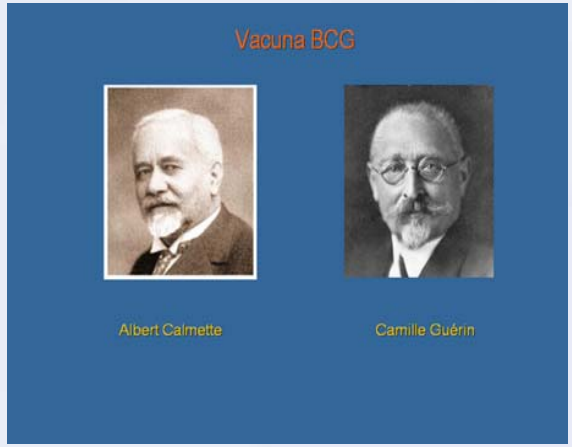
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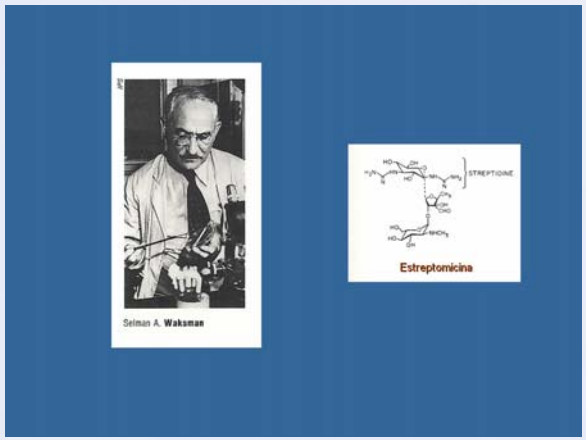
Camille Guérin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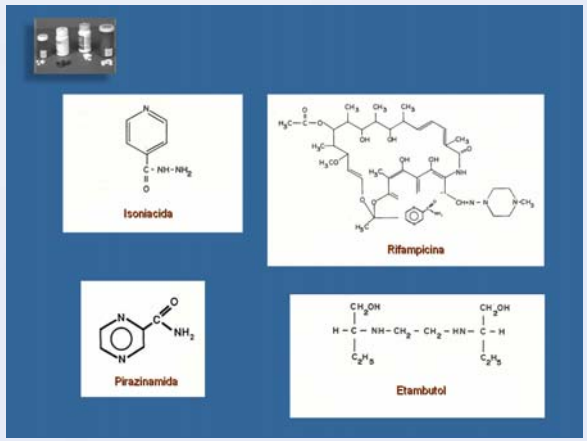
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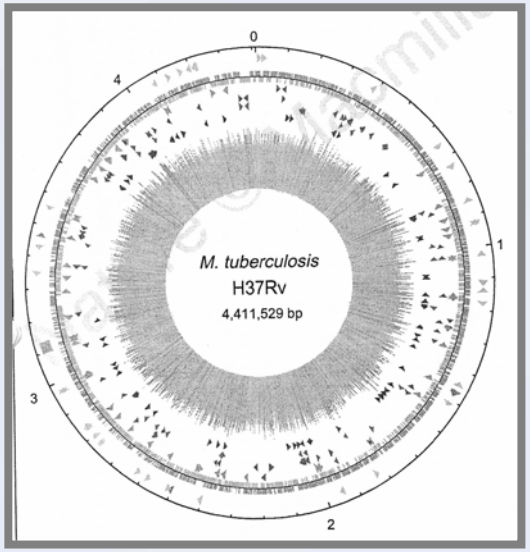
Pirazinamida
Etambutol

Cole S. T. *et al.*
Nature 1998, 393: 537-44



article

Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence

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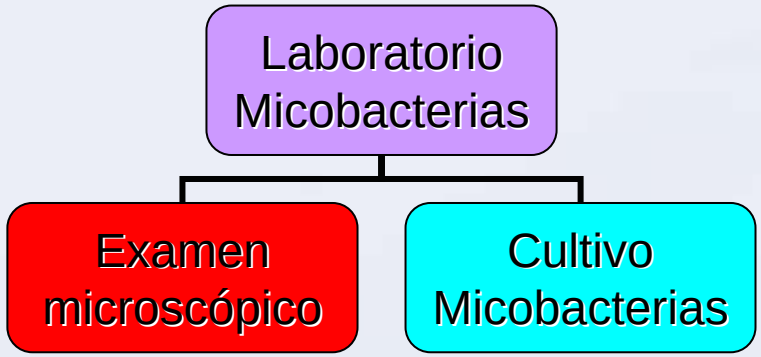
Countless millions of people have died from tuberculosis, a chronic infectious disease caused by the tubercle bacillus. The complete genome sequence of the best-characterized strain of *Mycobacterium tuberculosis*, H37Rv, has been determined and analysed in order to improve our understanding of the biology of this slow-growing pathogen and to help the conception of new prophylactic and therapeutic interventions. The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine + cytosine content that is reflected in the biased amino-acid content of the proteins. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation.



Métodos Fenotípicos

Laboratorio Micobacterias

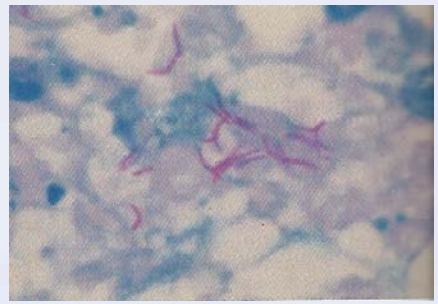
Examen microscópico

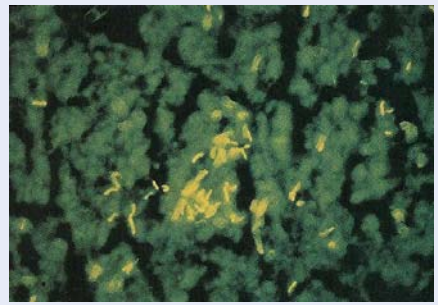
Cultivo Micobacterias

Métodos Fenotípicos

Tinción Carbol Fucsina:
Ziehl-Neelsen, Kinyoun

Tinción fluorescente:
Auramina

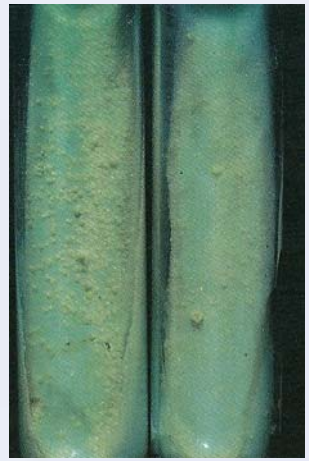




Laboratorio Micobacterias

Examen microscópico

Cultivo Micobacterias



Métodos Fenotípicos

Medios Sólidos:
Löwenstein-Jensen, Coletsos

Medios Líquidos:
Bactec 460
BactAlert3D (MB/BacT)
Bactec MGIT 960

Tenover F. C. *et al.*
J Clin Microbiol 1993; 31:767-70

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1993, p. 767–770
0095-1137/93/040767-04$02.00/0

Vol. 31, No. 4

GUEST COMMENTARY

The Resurgence of Tuberculosis: Is Your Laboratory Ready?

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INTRODUCTION

After years of declining case rates, tuberculosis is again a major public health problem in the United States. To compound the problem, serious outbreaks involving both patients infected with the human immunodeficiency virus (HIV) and HIV-infected and non-HIV-infected health care workers have been noted in several major metropolitan areas. Cases have also increased in other population groups, including the homeless, prisoners, migrant farm workers, and immigrants.

The definitive diagnosis of tuberculosis depends on the isolation and identification of the etiologic agent, *Mycobacterium tuberculosis*, while design of an appropriate therapeutic regimen depends on the results of antituberculosis susceptibility testing. With this information in hand, the necessary infection control procedures and contact tracings can be initiated and informed decisions can be made regarding therapy. If laboratories cannot screen specimens rapidly for acid-fast bacilli, identify isolates in a timely manner, and provide drug susceptibility data in a short period of time, patient care may suffer and infectious patients may continue the chain of transmission. The laboratory has a major role to play in breaking this chain; the importance of completing thorough evaluations of clinical specimens for mycobacteria and, if mycobacteria are present, the importance of obtaining timely species identification and susceptibility testing results cannot be overemphasized. To halt the continuing spread of tuberculosis across the United States and to control transmission within hospitals, laboratories must recognize the urgency and optimize their procedures in reporting results of acid-fast smears, cultures, and drug susceptibility tests to clinicians.

THE TUBERCULOSIS PROBLEM

Tuberculosis is a bacterial disease caused by organisms of the *M. tuberculosis* complex (i.e., *M. tuberculosis*, *M. bovis*, and *M. africanum*). It is transmitted primarily by airborne droplet nuclei produced when individuals with pulmonary or laryngeal tuberculosis sneeze, cough, or speak. Individuals are particularly infectious if they are excreting sufficient bacilli to produce a positive acid-fast-stained preparation of their sputum (1). Infection occurs when susceptible individuals inhale these droplet nuclei. Tuberculosis can occur in any organ of the body (1), although only 5 to 15% of infected individuals will develop active disease within 2 years of primary infection (1, 20). The population groups in the United States that are at increased risk for infection with *M. tuberculosis* include medically underserved, low-income populations, immigrants from countries with a high prevalence of tuberculosis, and residents of long-term-care and correctional facilities. Those at increased risk of developing disease following infection include individuals with HIV infection; close contacts of infectious cases; children less than 5 years old; patients with renal failure, silicosis, and diabetes mellitus; and individuals receiving treatment with immunosuppressive medications.

In the United States, the number of tuberculosis cases reported annually declined steadily between 1953 and 1985; however, in 1986, the rate for newly diagnosed cases increased 1.1% over the preceding year (5). This upward trend has continued; in 1991, a total of 26,283 cases were reported to the Centers for Disease Control (CDC), representing an increase of 18.4% over the number reported in 1985 (8).

Since 1990, nosocomial outbreaks of multidrug-resistant tuberculosis (MDR-TB) involving over 200 patients have been reported to CDC. These outbreaks have included transmission of *M. tuberculosis* to patients, health care workers, and inmates and employees of correctional facilities. Investigation of four such outbreaks that occurred in hospitals in Florida and New York City demonstrated that most cases of MDR-TB occurred in individuals known to be infected with HIV (3, 6, 7, 9, 11, 13, 17). The case fatality rate among patients with active MDR-TB was exceptionally high, 72 to 89%, and the median interval from the time of diagnosis to death was very short, 4 to 16 weeks. Three subsequent outbreaks, involving two additional hospitals and a state correctional facility, are under investigation in New York state (12, 17). At the correctional facility, at least seven inmates and one prison guard have died of MDR-TB.

All of these outbreaks have been characterized by the transmission of strains of *M. tuberculosis* resistant to at least isoniazid and rifampin (MDR), with some strains showing additional resistance to other drugs including ethambutol, streptomycin, ethionamide, kanamycin, and rifabutin. Delays in the laboratory diagnosis and reporting of drug-resistant tuberculosis contributed to the magnitude of these outbreaks since cases were not rapidly identified, the organism was not isolated, or the patients were not put on adequate therapy.

SAFETY IN THE MYCOBACTERIOLOGY LABORATORY

Specimens received in the mycobacteriology laboratory for staining and culture should routinely be considered to contain mycobacteria and, therefore, must be handled in a safe manner. Such specimens may be from patients who are infected with MDR strains of *M. tuberculosis*, which would

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0095-1137/96/$04.00+0

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GUEST COMMENTARY

Diagnostic Mycobacteriology: Where Are We Today?

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INTRODUCTION

Tuberculosis has reemerged as a significant public health problem in certain parts of the United States. In addition, strains of *Mycobacterium tuberculosis* with various patterns of resistance to antituberculosis chemotherapeutic agents have been recovered with increasing frequency (17, 18). The continuation of the human immunodeficiency virus (HIV) epidemic has contributed to both of these problems. In an attempt to address the concurrent problems of the increasing prevalence of tuberculosis and more frequent recognition of multidrug-resistant strains of *M. tuberculosis*, the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., has recently developed and now promulgates recommendations for controlling the transmission of tuberculosis (2, 3).

Obviously, an essential component of any tuberculosis control program is rapid and accurate identification of infected individuals, especially those most likely to transmit viable organisms. In this regard, the CDC has also developed recommendations for standards of laboratory practice regarding detection, identification, and susceptibility testing of *M. tuberculosis* in clinical specimens (17). The three most fundamental aspects of these recommendations are provision of acid-fast bacillus smear results within 24 h of specimen collection, isolation and identification of *M. tuberculosis* within 10 to 14 days, and provision of susceptibility test results within a total of 15 to 30 days. All three of these objectives significantly impact on the function of mycobacteriology sections of clinical microbiology laboratories. Hours of operation, staffing patterns, technology decisions, and the cost of providing service are all profoundly influenced by these proposed standards of laboratory practice.

In particular, the second recommendation of the CDC, isolation and identification of *M. tuberculosis* within 10 to 14 days, would have profound ramifications on the function of clinical microbiology laboratories. A question arises: can this standard be reasonably achieved using current "state-of-the-art" diagnostic methods? An important corollary to this question pertains to the recent licensure of a commercial molecular diagnostic test that will make it possible for even the smallest laboratory to detect and identify *M. tuberculosis* directly in clinical specimens in a matter of hours. What will be the role of direct molecular diagnostic tests in mycobacteriology? What are the performance characteristics of molecular detection assays? Use of such technologies would clearly permit laboratories to meet the standard of 10 to 14 days for the isolation and definitive species identification of *M. tuberculosis*. Will molecular-probe based methods become broadly applied in clinical microbiology laboratories? Should they be used routinely or selectively? What are the fiscal implications of direct molecular detection and identification? These issues serve as the basis for this guest commentary.

Question: Are current CDC recommendations defining 10- to 14-day limits on the recovery and definitive species identification of *M. tuberculosis* achievable by methods other than direct nucleic acid amplification methods?

Answer: Probably not, at least in most laboratories.

Currently, the fastest and most reliable approach to detecting and identifying *M. tuberculosis* in clinical specimens other than use of a direct molecular detection test is use of a broth-based radiometric or continuous monitoring detection system with identification of clinical isolates recovered in culture using a nucleic acid probe-based culture confirmation identification system (8). Seen in Table 1 are the lengths of times to recovery and identification of *M. tuberculosis* obtained in 10 U.S. laboratories during 1993. All 10 laboratories employed a radiometric detection system and nucleic acid probes for identification of isolates. Only 2 of these 10 laboratories, laboratories E and G, met the CDC standard of 10 to 14 days for isolation and identification of *M. tuberculosis*. The wide ranges observed for mean lengths of time to detection and species identification among the 10 laboratories described in Table 1 were explained by differing staffing patterns, variable scheduling of mycobacteria specimen processing and workup of positive cultures, and whether probe-based identification was applied directly to growth in primary cultures or used only to characterize organisms following at least one subculture. Both of the laboratories that met the CDC's recommended standard processed mycobacteriology specimens 7 days per week and aggressively attempted probe-based identification whenever possible directly on growth from primary cultures. In addition, laboratory E worked up positive cultures with probe identification tests 7 days per week.

Question: Would application of nucleic acid amplification assays for detection and identification of *M. tuberculosis* directly in clinical specimens permit laboratories to meet the CDC's recommended standard?

Answer: Possibly, at least for certain specimens.

One nucleic acid amplification assay for the detection and identification of *M. tuberculosis* directly in clinical material, the MTD test (Gen-Probe, Inc., San Diego, Calif.), has recently received Food and Drug Administration (FDA) approval and is now available commercially. The MTD assay is a 4-h procedure based on transcription-mediated amplification of a 16S rRNA target sequence. Results are read with a luminometer. A second amplification method, the Amplicor MTB procedure (Roche Diagnostics Systems, Somerville, N.J.), is currently awaiting FDA approval. The Amplicor MTB assay is predicted on PCR amplification of a 584-bp segment of 16S rRNA; it utilizes a thermocycler and requires 6 h to complete.

In addition to the MTD test and the Amplicor MTB assay, three other direct nucleic acid amplification methods for detecting *M. tuberculosis* are in various stages of development.

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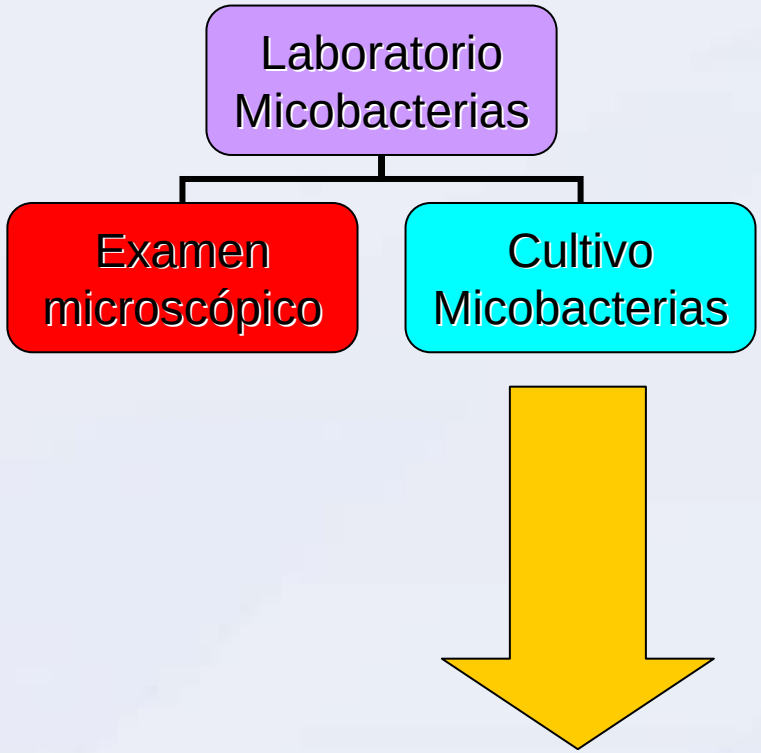
Doern G.V.
J Clin Microbiol 1996; 34:1873-76

Métodos Fenotípicos



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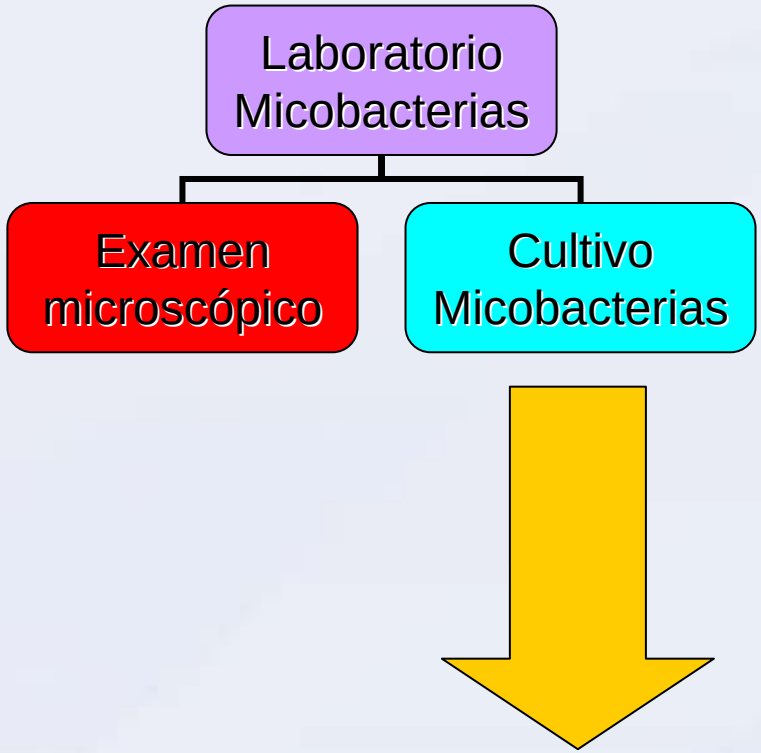
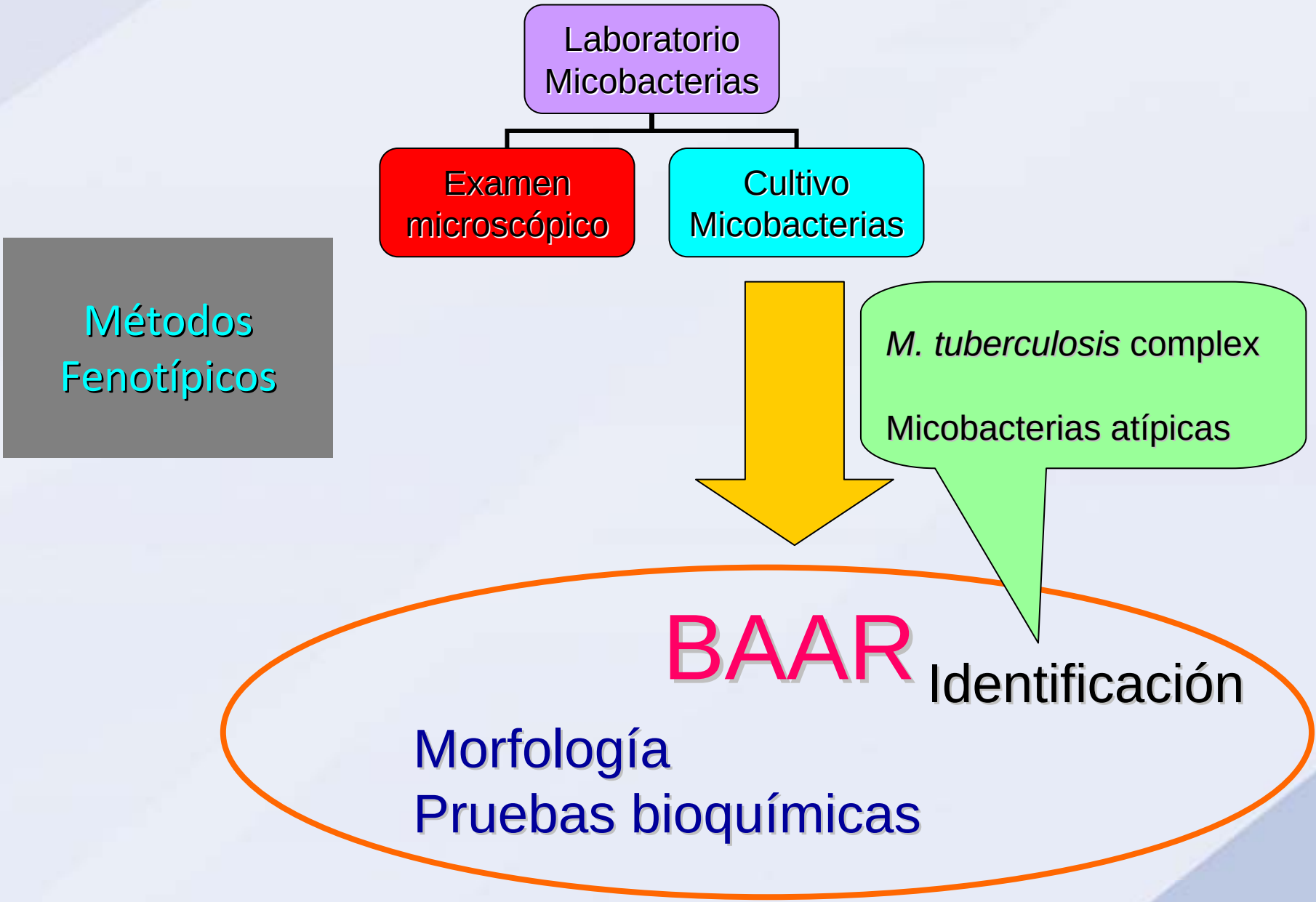
Métodos Fenotípicos



BAAR Identificación

Morfología
Pruebas bioquímicas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Examen microscópico
Cultivo Micobacterias
Métodos Fenotípicos
M. tuberculosis complex
Micobacterias atípicas
BAAR
Identificación
Morfología
Pruebas bioquímicas

Springer B., *et al.*
J Clin Microbiol 1996; 3:296-303

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1996, p. 296–303
0095-1137/96/$04.00+0

Vol. 34, No. 2

Two-Laboratory Collaborative Study on Identification of Mycobacteria: Molecular versus Phenotypic Methods

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Previous studies have indicated hat the conventional tests used for the identification of mycobacteria may (i) frequently result in erroneous identification and (ii) underestimate the diversity within the genus *Mycobacterium*. To address this issue in a more systematic fashion, a study comparing phenotypic and molecular methods for the identification of mycobacteria was initiated. Focus was given to isolates which were difficult to identify to species level and which yielded inconclusive results by conventional tests performed under day-to-day routine laboratory conditions. Traditional methods included growth rate, colonial morphology, pigmentation, biochemical profiles, and gas-liquid chromatography of short-chain fatty acids. Molecular identification was done by PCR-mediated partial sequence analysis of the gene encoding the 16S rRNA. A total of 34 isolates was included in this study; 13 of the isolates corresponded to established species, and 21 isolates corresponded to previously uncharacterized taxa. For five isolates, phenotypic and molecular analyses gave identical results. For five isolates, minor discrepancies were present; four isolates remained unidentified after biochemical testing. For 20 isolates, major discrepancies between traditional and molecular typing methods were observed. Retrospective analysis of the data revealed that the discrepant results were without exception due to erroneous biochemical test results or interpretations. In particular, phenotypic identification schemes were compromised with regard to the recognition of previously undescribed taxa. We conclude that molecular typing by 16S rRNA sequence determination is not only more rapid (12 to 36 h versus 4 to 8 weeks) but also more accurate than traditional typing.

Mycobacteria are aerobic rod-shaped organisms characterized by being acid fast and having a slow growth rate (31). In addition to those of the *Mycobacterium tuberculosis* complex, other species of mycobacteria are opportunistic pathogens and can pose a serious threat to infected individuals (20, 32). It is important to identify mycobacteria to the species level both to address the clinical significance, e.g., isolation of a nonpathogenic versus pathogenic species, as well as to meet the demands of patient management, since treatment regimens for infections caused by one *Mycobacterium* species are often not effective against another.

Identification of mycobacteria to the species level by conventional biochemical tests has been fraught with a long turnaround time, leading to significant delays in diagnosis. Other methods based on lipid analysis, such as high-performance liquid chromatography, thin-layer chromatography, and gas-liquid chromatography, are cumbersome and expensive and are used in only a very few clinical laboratories (3, 11, 13, 16, 20). Identification by use of nucleic acid probes (Gen-Probe, Inc., San Diego, Calif.) is a rapid and widely used procedure, but it covers only a narrow range of mycobacterial species, and problems concerning specificity and sensitivity have been described (2, 4, 6, 12, 15, 17, 25).

To meet the necessity for more rapid species identification and to improve the accuracy of identification of mycobacteria, methods utilizing the amplification of DNA by PCR coupled with restriction enzyme digestion, hybridization, or nucleic acid sequence determination have been developed (5, 7, 19, 21, 23, 26, 27, 29, 30). The use of 16S rRNA gene sequence determination for the routine identification of mycobacteria has led to the suggestion that the standard biochemical reactions used for the identification of mycobacteria may underestimate the complexity of the genus *Mycobacterium*, as genetically distinct species may exhibit similar or identical patterns in these reactions (10). In this study, we sought to systematically compare phenotypic and molecular methods for the identification of mycobacteria by focusing on isolates which yielded inconclusive biochemical test results and thus posed difficulties for identification to the species level.

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MATERIALS AND METHODS

Mycobacterial isolates. Clinical isolates were collected at the Clinical Mycobacteriology Laboratory of the Department of Laboratory Medicine and Pathology, Mayo Clinic and Mayo Foundation, Rochester, Minn. The isolates were grown on Löwenstein-Jensen or 7H10 agar and examined for growth rate, gross and microscopic colony morphology, and pigmentation. Identification to the species level was done by gas-liquid chromatography of short-chain fatty acids (Microbial Identification System; Microbial ID, Newark, Del.) and a battery of biochemical key reactions, including Tween hydrolysis, nitrate reduction, arylsulfatase, urease, tellurite reduction, salt tolerance, and semiquantitative catalase (9, 20). The selection of specific biochemical tests for identification was made on an individual basis after the growth rate, pigment production, and colonial morphologic features were considered. Isolates in this study were identified as "most closely resembling" a particular species.

Isolates were coded (MCRO 1, 2, 5 to 8, 10, 12 to 21, 24 to 30, 32 to 34, 36 to 41, and 45 to 48) and shipped to Hannover for sequence analysis. These isolates could not be definitively identified by the combination of biochemical tests and gas-liquid chromatographic analysis.

Nucleic acid analyses. Identification by 16S rRNA sequence determination was done at the Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover. The methods used have been described previously (10).

Telenti A., *et al.*
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Rapid Identification of Mycobacteria to the Species Level by Polymerase Chain Reaction and Restriction Enzyme Analysis

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A method for the rapid identification of mycobacteria to the species level was developed on the basis of evaluation by the polymerase chain reaction (PCR) of the gene encoding for the 65-kDa protein. The method involves restriction enzyme analysis of PCR products obtained with primers common to all mycobacteria. Using two restriction enzymes, *Bst*EII and *Hae*III, medically relevant and other frequent laboratory isolates were differentiated to the species or subspecies level by PCR-restriction enzyme pattern analysis. PCR-restriction enzyme pattern analysis was performed on isolates (n = 330) from solid and fluid culture media, including BACTEC, or from frozen and lyophilized stocks. The procedure does not involve hybridization steps or the use of radioactivity and can be completed within 1 working day.

Differentiation of mycobacteria to the species level is currently done by time-consuming evaluation of phenotypic and biochemical characteristics. Additional methods such as high-performance liquid chromatography (HPLC) or thin-layer chromatography are limited by the need for standardized growth conditions (4, 13, 18). Gen-Probe (Gen-Probe, Inc., San Diego, Calif.) is a rapid method, but it requires several probes and covers only a limited range of mycobacterial species (14). Sequencing of the 16[illegible] is a powerful technique of differentiati[illegible], it is labor-intensive and difficult t[illegible]use in many clinical laboratories.

We developed a rapi[illegible]ion of the gene encoding fo[illegible]ck protein by the polymerase chain re[illegible] similar approach to the differentiation of m[illegible] has recently been reported by [illegible]aytis et al. ([illegible]65-kDa protein contains epitopes that are unique a[illegible]s as epitopes that are common to various species of [illegible]cobacteria (17). The conserved nature of this gene allows [illegible]differentiation of mycobacteria within 1 day by restriction[illegible]zyme digestion of PCR products obtained by using pr[illegible] common to all mycobacteria.

MA[illegible]D METHO[illegible]

Samples. Forty re[illegible] gated to establish the[illegible] assay was used to det[illegible] isolates.

Sample preparation. A loop [illegible] solid medium (Lowenstein-Jensen or [illegible] agar) was suspended in 1 ml of TE (10 mM [illegible], 1 m[illegible] [pH 8]) and was heat inactivated for 10 m[illegible] at 80[illegible] working with liquid medium (Middlebrook 7H9, [illegible] 12B, or BACTEC 13A) or frozen or rehydrated ly[illegible] stocks, 250 μl was centrifuged and the pellet w[illegible] pended in TE as described above. After inactiva[illegible] bacteria were centrifuged for 15 min, the pellet w[illegible] pended in 100 μl of TE, and 100 μl of glass bead[illegible] was added; the suspended cells were mechanicall[illegible] grated for 2 min in a Mickle apparatus (Mill Works, Gomshall, United Kingdom). After a 10-min centrifugation step, the supernatant was transferred to a new tube. All centrifugation steps were done at 13,000 rpm in an Eppendorf microcentrifuge.

Amplification. Five microliters of lysate was added to each reaction tube. The composition of the PCR mixture (50 μl) was 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 10% glycerol, 200 μM (each) deoxynucleoside triphosphate, 0.5 μM (each) primer, and 1.25 U of *Taq* polymerase (Cetus or Boehringer Mannheim). The reaction was subjected to 45 cycles of amplification (1 min at 94°C, 1 min at 60°C, 1 min at 72°C); this was followed by 10 min of extension at 72°C. Primers Tb11 (5′-ACCAACGATGGTGTGTCCAT) and Tb12 (5′-CTTGTCGAACCGCATACCCT) amplified a 439-bp fragment between positions 398 and 836 of the published gene sequence (17).

Contamination precautions. Amplification tubes for PCR-restriction enzyme pattern analysis (PRA) were prepared in a "PCR-clean" room, while the mycobacterial isolates were inactivated and lysed by staff from the mycobacteriology laboratory. Thereafter, samples were added to the PCR

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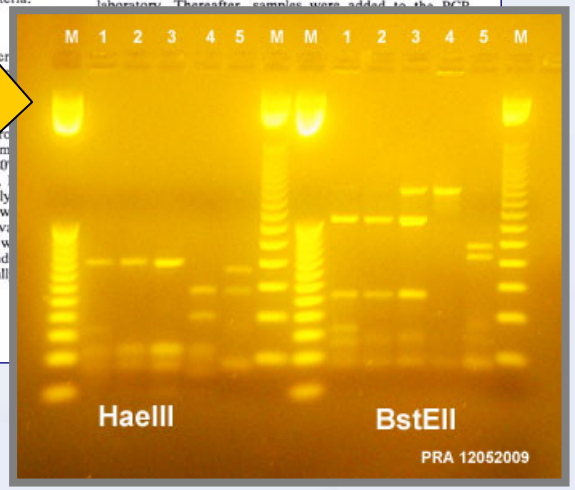

Roth A., *et al.*
J Clin Microbiol 2000; 38:1094-1104

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2000, p. 1094–1104
0095-1137/00/$04.00+0

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Novel Diagnostic Algorithm for Identification of Mycobacteria Using Genus-Specific Amplification of the 16S-23S rRNA Gene Spacer and Restriction Endonucleases

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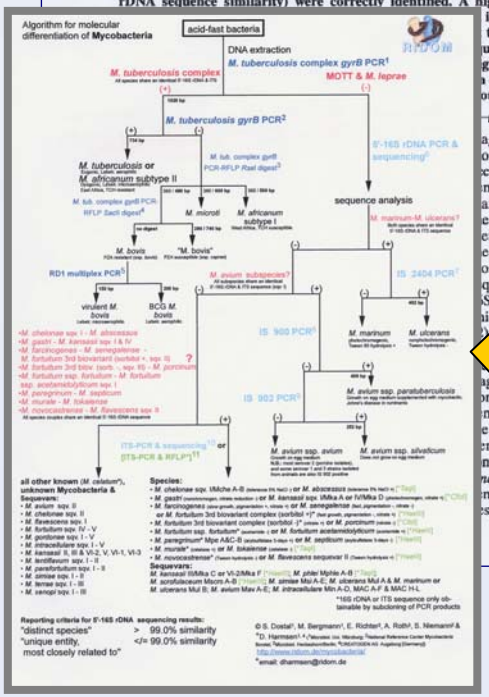
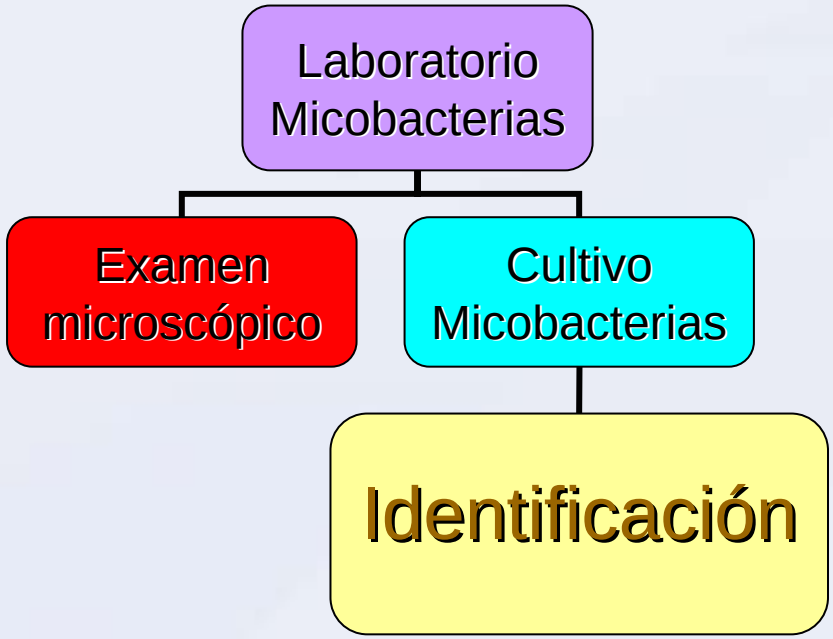
A novel genus-specific PCR for mycobacteria with simple identification to the species level by restriction fragment length polymorphism (RFLP) was established using the 16S-23S ribosomal RNA gene (rDNA) spacer as a target. Panspecificity of primers was demonstrated on the genus level by testing 811 bacterial strains (122 species in 37 genera from 286 reference strains and 525 clinical isolates). All mycobacterial isolates (678 strains among 48 defined species and 5 indeterminate taxons) were amplified by the new primers. Among nonmycobacterial isolates, only *Gordonia terrae* was amplified. The RFLP scheme devised involves estimation of variable PCR product sizes together with *Hae*III and *Cfo*I restriction analysis. It yielded 58 *Hae*III patterns, of which 49 (84%) were unique on the species level. Hence, *Hae*III digestion together with *Cfo*I results was sufficient for correct identification of 39 of 54 mycobacterial taxons and one of three or four of seven RFLP genotypes found in *Mycobacterium intracellulare* and *Mycobacterium kansasii*, respectively. Following a clearly laid out diagnostic algorithm, the remaining unidentified organisms fell into five clusters of closely related species (i.e., the *Mycobacterium avium* complex or *Mycobacterium chelonae-Mycobacterium abscessus*) that were successfully separated using additional enzymes (*Taq*I, *Msp*I, *Dde*I, or *Ava*II). Thus, next to slowly growing mycobacteria, all rapidly growing species studied, including *M. abscessus*, *M. chelonae*, *Mycobacterium farcinogenes*, *Mycobacterium fortuitum*, *Mycobacterium peregrinum*, and *Mycobacterium senegalense* (with a very high 16S rDNA sequence similarity) were correctly identified. A high intraspecies sequence stability and the good



agnosis demand[illegible]ride accurate results in a ore timely fashion (7[illegible]e, efforts for rapid and curate molecular identifi[illegible]been undertaken in re- nt years (13–17, 21–23, 26, [illegible] J. L. Miller, training anual, MIDI Inc., Newark, N.J., [illegible]. Today, sequencing of e 16S RNA gene (rDNA) is rega[illegible]d as the most suitable ethod for identification of mycob[illegible]ria (14, 32). Even so, e high expense, together with a l[illegible]of clinical relevance for ost species identified in routi[illegible]ratory practice, renders quencing acceptable fo[illegible]use. Limitations of the S RN[illegible]he are [illegible]the number of polymor- ic [illegible]*terium* is rather low (13, [illegible]e sequence or a very high [illegible]leads to problems in develop- [illegible]analysis methods, such as restriction [illegible]polymorphism (RFLP) analysis or hybridiza- on w[illegible]robes (3, 6, 15). To meet this need, alternative netic ta[illegible]ets have been studied (13, 16, 22, 27, 28). Of these, e *hsp65* gene has so far been best investigated, and the data re recently improved by inclusion of more species, especially me rapidly growing mycobacteria (e.g., *Mycobacterium che- nae* and *Mycobacterium abscessus*) (5, 21). However, *hsp65* ne-based PCR-RFLP analysis has been impeded by difficul- es, such as minor differences of band sizes between some

Métodos Fenotípicos

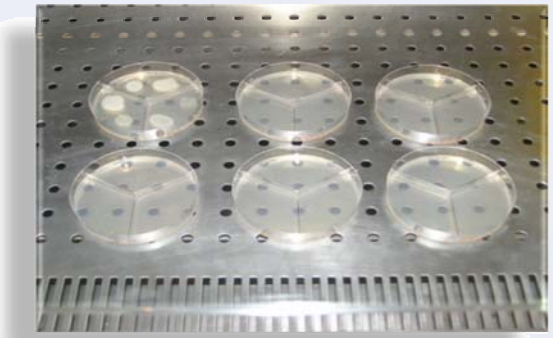


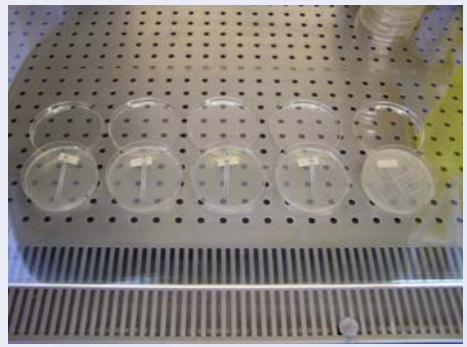
ANTIBIOGRAMA

Laboratorio Micobacterias

Examen microscópico

Cultivo Micobacterias





Métodos Fenotípicos

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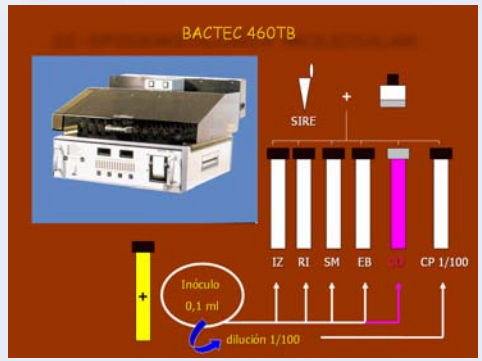


Método de las Proporciones
Bactec radiométrico
MGIT SIRE
Etest

ANTIBIOGRAMA

Situación de las resistencias a fármacos en Asturias

1079 cepas, Unidad de Referencia Regional de Micobacterias período 2004-2009

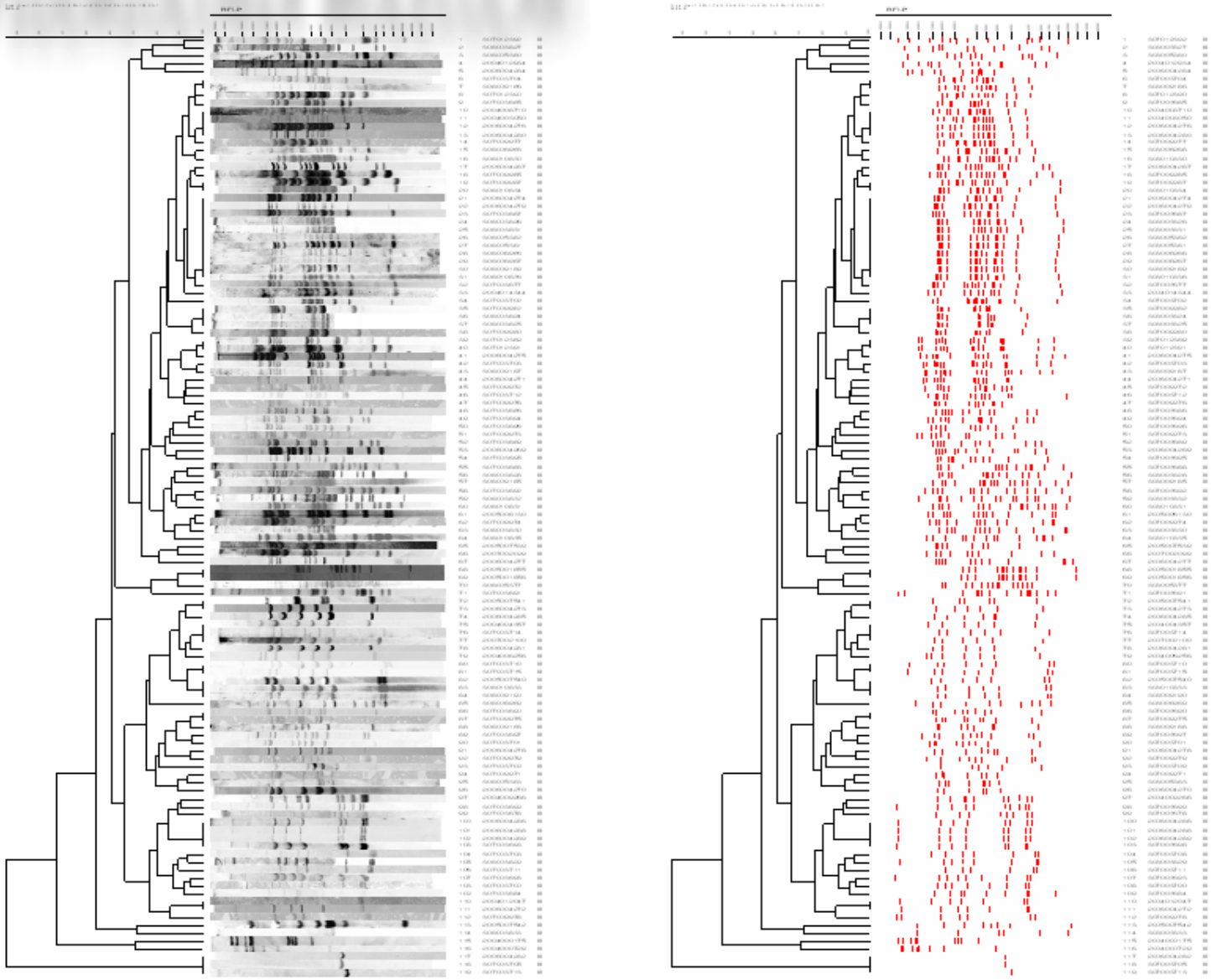
- ✓ 1027 cepas sensibles a todos los fármacos de primera línea — 95,1 %
- ✓ 52 cepas con alguna resistencia (primaria + secundaria) — 4,8 %

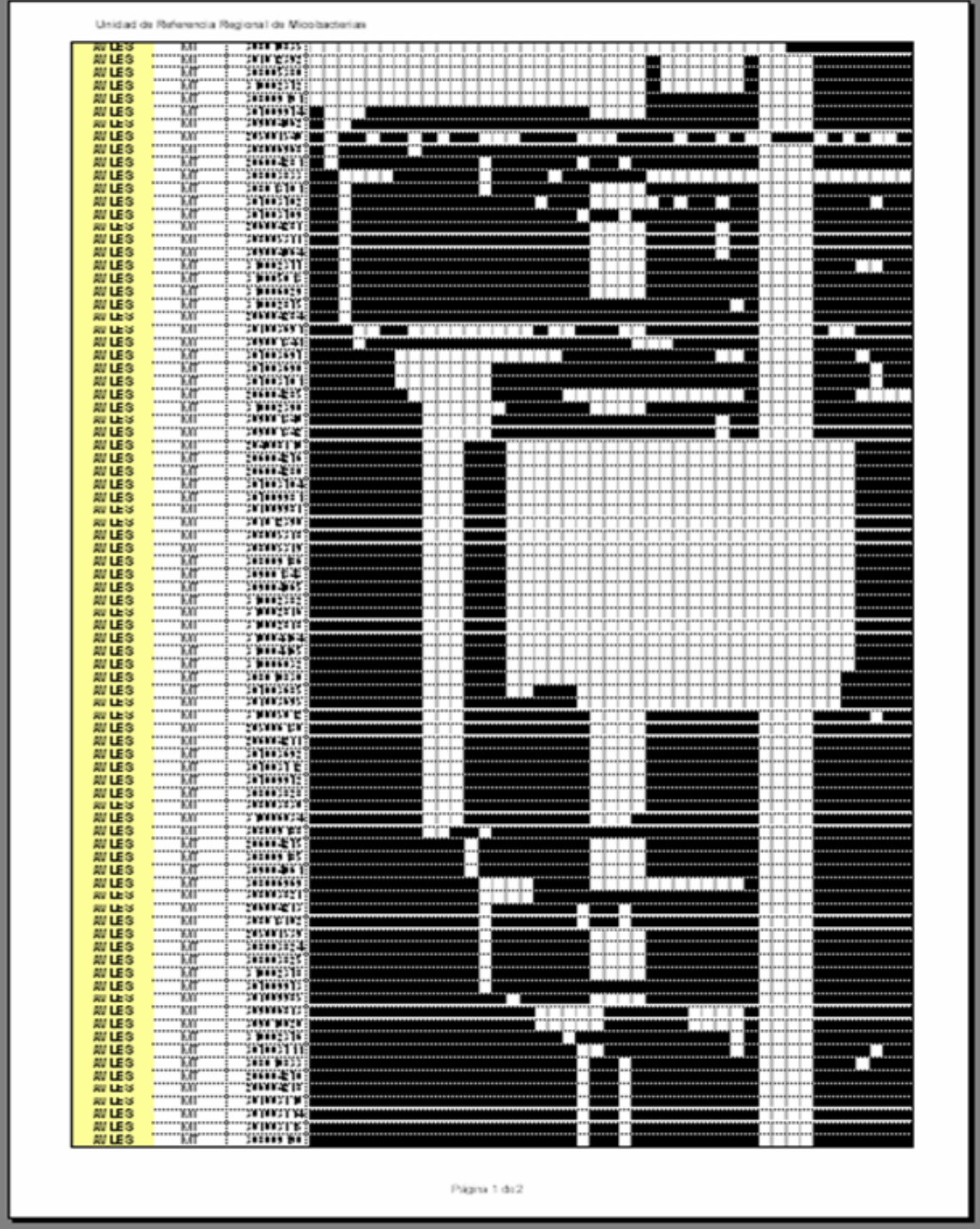
Isoniacida	32	2,9 %
Rifampicina	6	0,5 %
Estreptomicina	13	1,2 %
Etambutol	3	0,2 %
Pirazinamida	16	1,4 %
MDR-TB (resistencia al menos INH+RIF)	6	0,5 %

1 cepa XDR-TB cepas extremadamente resistentes: MDR-TB + resistencia al menos a fluoroquinolonas + cualquier medicamento inyectable de segunda línea: amikacina, kanamicina y capreomicina

RFLP cepas Área III (Avilés) 2004-2008

116 pacientes, Tasa de agrupamiento 37%

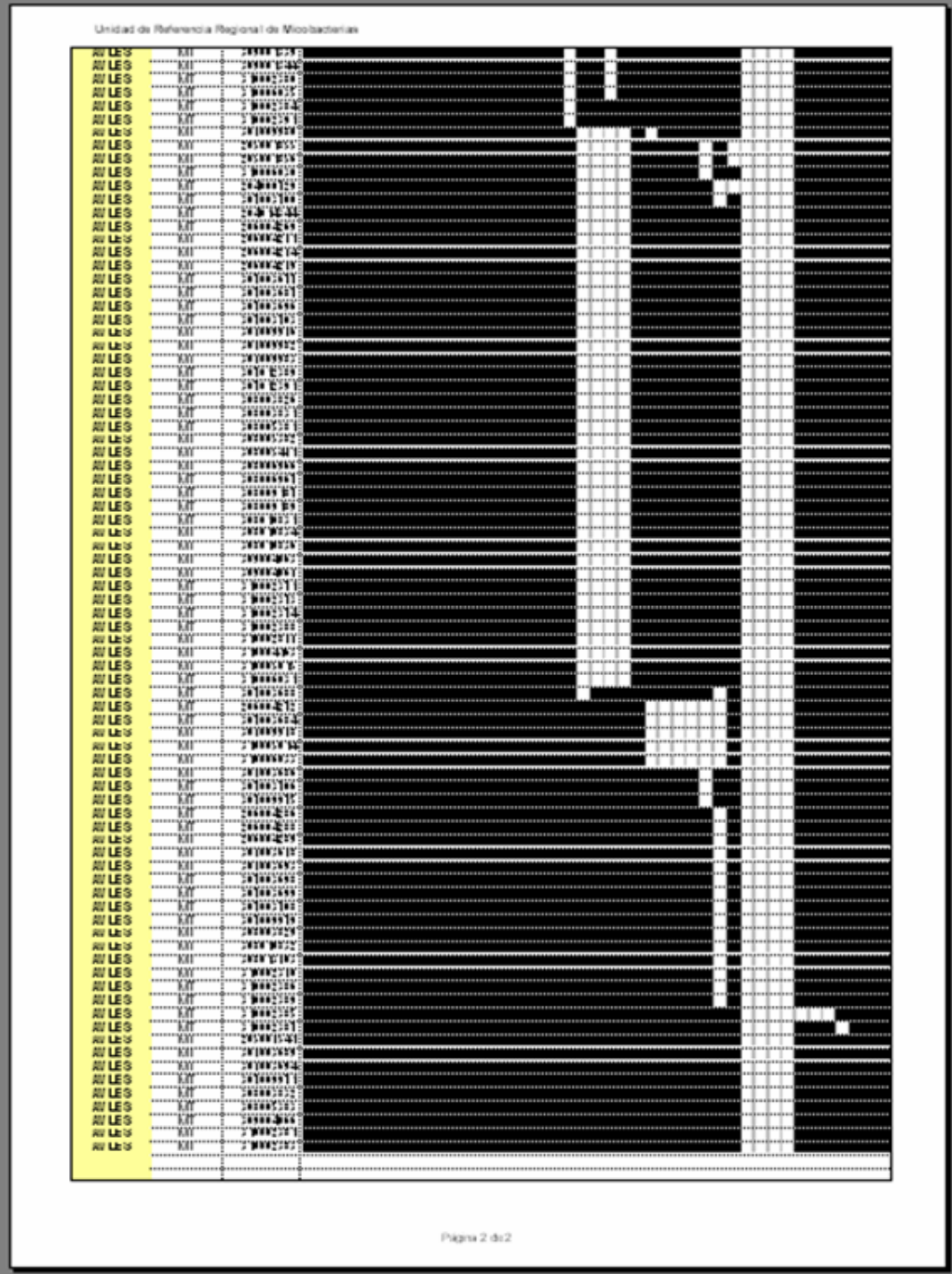




Spoligotyping cepas Área III (Avilés) 2004-2010

Spoligotyping cepas Área III (Avilés) 2004-2010

170 pacientes
Tasa de agrupamiento 77,6%



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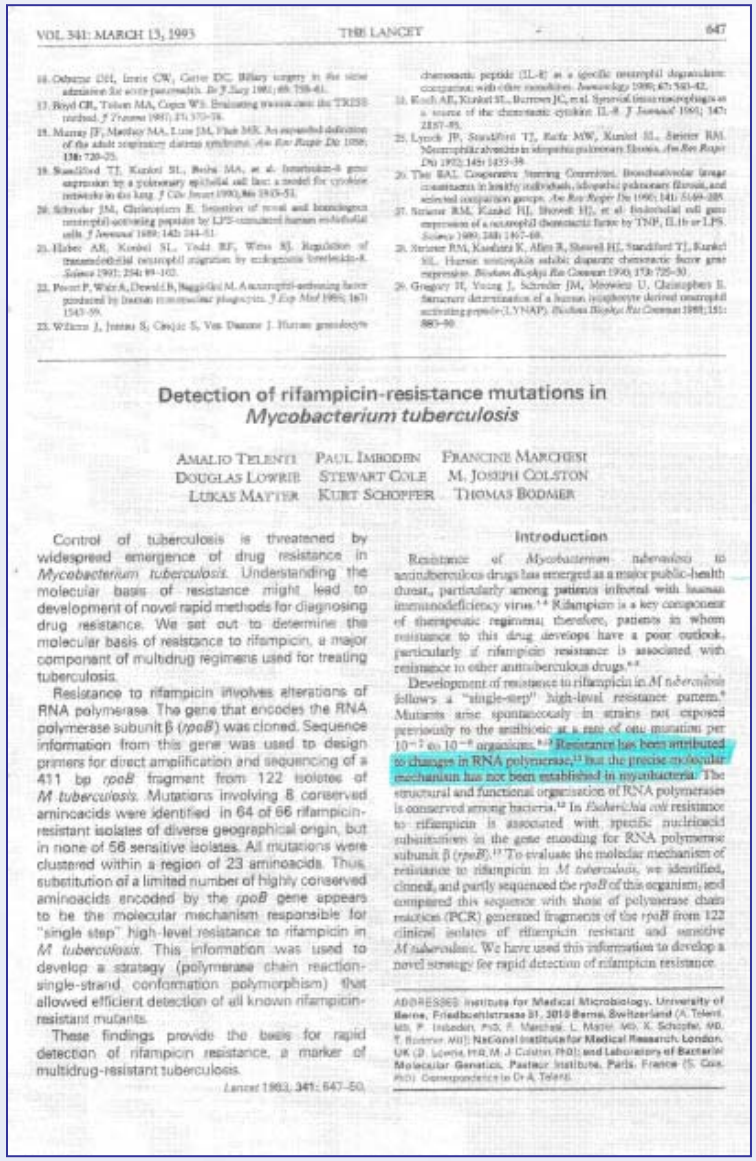

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Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*

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DOUGLAS LOWRIE STEWART COLE M. JOSEPH COLSTON
LUKAS MATTER KURT SCHOPFER THOMAS BODMER

Control of tuberculosis is threatened by widespread emergence of drug resistance in *Mycobacterium tuberculosis*. Understanding the molecular basis of resistance might lead to development of novel rapid methods for diagnosing drug resistance. We set out to determine the molecular basis of resistance to rifampicin, a major component of multidrug regimens used for treating tuberculosis.

Resistance to rifampicin involves alterations of RNA polymerase. The gene that encodes the RNA polymerase subunit β (*rpoB*) was cloned. Sequence information from this gene was used to design primers for direct amplification and sequencing of a 411 bp *rpoB* fragment from 122 isolates of *M tuberculosis*. Mutations involving 8 conserved aminoacids were identified in 64 of 66 rifampicin-resistant isolates of diverse geographical origin, but in none of 56 sensitive isolates. All mutations were clustered within a region of 23 aminoacids. Thus, substitution of a limited number of highly conserved aminoacids encoded by the *rpoB* gene appears to be the molecular mechanism responsible for "single step" high-level resistance to rifampicin in *M tuberculosis*. This information was used to develop a strategy (polymerase chain reaction-single-strand conformation polymorphism) that allowed efficient detection of all known rifampicin-resistant mutants.

These findings provide the basis for rapid detection of rifampicin resistance, a marker of multidrug-resistant tuberculosis.

Lancet 1993; 341: 647–50.

Introduction

Resistance of *Mycobacterium tuberculosis* to antituberculous drugs has emerged as a major public-health threat, particularly among patients infected with human immunodeficiency virus.[1-4] Rifampicin is a key component of therapeutic regimens; therefore, patients in whom resistance to this drug develops have a poor outlook, particularly if rifampicin resistance is associated with resistance to other antituberculous drugs.[5-8]

Development of resistance to rifampicin in *M tuberculosis* follows a "single-step" high-level resistance pattern.[9] Mutants arise spontaneously in strains not exposed previously to the antibiotic at a rate of one mutation per 10^{-7} to 10^{-8} organisms.[9,10] Resistance has been attributed to changes in RNA polymerase,[11] but the precise molecular mechanism has not been established in mycobacteria. The structural and functional organisation of RNA polymerases is conserved among bacteria.[12] In *Escherichia coli* resistance to rifampicin is associated with specific nucleoacid substitutions in the gene encoding for RNA polymerase subunit β (*rpoB*).[13] To evaluate the molecular mechanism of resistance to rifampicin in *M tuberculosis*, we identified, cloned, and partly sequenced the *rpoB* of this organism, and compared this sequence with those of polymerase chain reaction (PCR) generated fragments of the *rpoB* from 122 clinical isolates of rifampicin resistant and sensitive *M tuberculosis*. We have used this information to develop a novel strategy for rapid detection of rifampicin resistance.

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Telenti A. *Thorax* 1998; 53:793-97

Thorax 1998;53:793–797

Genetics and pulmonary medicine • 5

Series editors: J Britton, J Hopkin

Genetics of drug resistant tuberculosis

A Telenti

The emergence of multidrug-resistant tuberculosis (MDR-TB), generally defined as resistance to at least isoniazid and rifampicin, has generated concern for the future of tuberculosis control.[1] The global magnitude of the problem is not well known. Most of the available studies are non-representative surveys of a population or a country, frequently failing to discriminate between primary and acquired resistance. However, emerging data (fig 1) suggest that, while multidrug resistance may not be a widespread problem, it remains a public health threat in areas with a high prevalence of tuberculosis and suboptimal tuberculosis control programmes.[2] Progress in understanding of the basis of drug action and resistance is the key to development of diagnostic strategies, novel drugs and treatment programmes, and to gaining insight into the pathogenicity of drug resistant strains.

Mechanisms of resistance and drug targets in tuberculosis

Bacteria use a number of strategies to achieve drug resistance. These can be roughly summarised into three categories: (1) barrier mechanisms (decreased permeability and efflux pumps); (2) degrading or inactivating enzymes—for example, β-lactamases; and (3) drug target modifications—for example, single mutation in a key gene. The genetic information for such properties may be acquired via exogenous mobile genetic elements such as plasmids or transposons, or it may reside in the chromosome.

Mycobacteria are not basically different from many other bacteria in that they use several of these strategies. Firstly, mycobacteria are characterised by a specialised cell wall which displays significantly reduced permeability to many compounds.[3] Secondly, mycobacteria produce degrading enzymes such as β-lactamases[4] and other drug-modifying enzymes. These are among the factors cited to explain the natural resistance of many mycobacterial species to frequently used antibacterial agents.

Resistance to agents used for the treatment of tuberculosis generally depends on the third general mechanism of resistance described—that is, modification by mutation of key target genes. Thus, acquisition of resistance in *Mycobacterium tuberculosis* derives from chromosomal mutational events. MDR-TB reflects the stepwise accumulation of individual mutations in several independent genes[5] and not the "block" acquisition of multidrug resistance.

A considerable amount of work has been devoted in the last few years to understanding mechanisms of resistance and to identifying the genes involved. The use of molecular data is already helping the development of novel ways of detecting MDR-TB earlier.[6 7] A summary of our current knowledge is presented in table 1.

RESISTANCE TO ISONIAZID

There is now a large body of information, both genetic and biochemical, on the multistep process involved in the activation of isoniazid prodrug into a potent derivative, and its final action on the mycolic acid biosynthesis.[8] Isoniazid is actively taken up by *M tuberculosis* and is oxidised by the mycobacterial catalase-peroxidase. Absence of catalase activity has long been recognised as a marker for isoniazid resistance and it has now been shown to result from mutation of this enzyme.[9 10] This phenomenon is observed in approximately 50% of clinical strains.

In the presence of an intact catalase-peroxidase an active intermediate is generated which will inhibit the activity of an enzyme involved in the synthesis of mycolic acids: the enoyl-ACP reductase, encoded by *inhA*.[8 11] Mutations in the *inhA* region appear to be responsible for resistance in approximately 25% of clinical isolates and are generally associated with low level isoniazid resistance (MIC ≤1 mg/ml) (table 1).[8 11 12] Most mutations result in upregulation of the *inhA* gene expression and thus in increased amounts of the corresponding enzyme which overwhelms the inhibitory action of the drug. Rarely, mutations have occurred at the site of interaction with the activated form of isoniazid.[8 11 13] Availability of the three-dimensional structure of the enoyl-ACP reductase has allowed a detailed analysis of the interaction of the enzyme with isoniazid, thus setting the basis for future rational drug design strategies.[14 15]

After the identification of the *katG* and *inhA* genes it was apparent that 10–20% of isoniazid resistant isolates lacked mutations in either gene. Search for additional genes led to the identification of the *ahpC* gene which encodes the alkyl hydroperoxide reductase.[12 15 16] Mutations in *ahpC*, identified in approximately 10–15% of clinical isolates,[12 17] may not have a causal role in resistance, and rather serve to identify major lesions in *katG*.[12 15] Unknown

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Telenti A. *Thorax* 1998; 53:793-97

Table 1 Mechanisms of drug resistance in Mycobacterium tuberculosis

Antimycobacterial agent	*Mechanism of action*	*Genes involved in resistance*	*Frequency of mutations associated with resistance*	*Mechanism of resistance*
Isoniazid	Inhibition of mycolic acid biosynthesis	(i) *katG* (catalase-peroxidase)	(i) 42–58%	(i) Mutations in *katG* result in failure to generate an active intermediate of isoniazid
		(ii) *inhA* (enoyl-acyl carrier protein reductase)	(ii) 21–34%	(ii) Over expression of *inhA* allows continuation of mycolic acid synthesis
		(iii) *ahpC* (alkyl hydroperoxide reductase)	(iii) 10–15%	(iii) *ahpC* mutations may just serve as a marker for lesions in *katG*
Rifampicin	Inhibition of transcription	*rpoB* (β subunit of RNA polymerase)	96–98%	Mutations in *rpoB* prevent interaction with rifampicin
Streptomycin	Inhibition of protein synthesis	(i) *rpsL* (ribosomal protein S12)	(i) 52–59%	Mutations prevent interaction with streptomycin. Resistance not associated with mutation in *rpsL* or *rrs* is usually low level
		(ii) *rrs* (16S rRNA)	(ii) 8–21%	
Ethambutol	Inhibition of arabinogalactan and lipoarabinomannan biosynthesis	*embcAB* (arabinosyl transferase)	47–65%	Over expression or mutation of EmbB allow continuation of arabinan biosynthesis. Resistance not associated with EmbB mutation is usually low level
Pyrazinamide	Unknown	*pncA* (pyrazinamidase-nicotinamidase)	72–97%	Loss of pyrazinamidase activity results in decreased conversion of pyrazinamide to pyrazinoic acid, the putative active moiety
Fluoroquinolones	Inhibition of the DNA gyrase	*gyrA* (DNA gyrase subunit A)	75–94%	Mutations in *gyrA* prevent interaction with fluoroquinolones Mutations in *gyrB* and efflux may contribute to resistance

Fluit AD C. *et al.*

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Vol. 14, No. 4

Molecular Detection of Antimicrobial Resistance

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Vol. 46, No. 2

MINIREVIEW

Evolution of Drug Resistance in *Mycobacterium tuberculosis*: Clinical and Molecular Perspective

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The story of antituberculosis chemotherapy is a miniature of the history of anti-infective chemotherapy. In the first half of the 20th century the problem of tuberculosis appeared insoluble: the lipid-rich cell wall was believed to make chemotherapy impossible (21). This gloomy view seemed to be confirmed when the first antibiotics developed, sulfonamides and penicillin, had no useful activity against *Mycobacterium tuberculosis*. With this in mind it is easy to understand the early euphoria surrounding Albert Schatz and Selman Waksman's discovery of streptomycin while working at Rutgers University in New Jersey (70) and Harold Lehmann's discovery of *para*-aminosalicylic acid (PAS) shortly afterwards (47).

The clinical trials that followed the description of streptomycin rapidly dispersed the first hopes of a conquest of tuberculosis. Although patients improved compared with those patients not on therapy (the British Medical Research Council [BMRC] trial is widely considered to have been the first randomized controlled clinical trial), relapse occurred in many patients and the organisms were found to be resistant to streptomycin. Combined streptomycin and PAS trials proved that combination therapy prevented the emergence of resistance (14). The subsequent descriptions of isoniazid (19), pyrazinamide (52), rifampin (34), ethambutol (30), and other drugs gave the medical community the basic tools for tuberculosis control. The subsequent series of trials conducted under the auspices of the U.S. Public Health Service, the BMRC, and others produced data indicating that cure rates of over 95% with minimal relapse rates were possible in as little as 6 months, a reduction from the first regimens, which required treatment for 2 years (13, 22, 32, 33). Using these tools many countries have seen the virtual eradication of tuberculosis (82) and others, including some of the poorest, have seen a steady decline in the disease until the human immunodeficiency virus (HIV) epidemic caused the number of cases to spiral out of control (74). The tragedy of tuberculosis treatment is that, 50 years after the introduction of effective specific chemotherapy, the number of cases is higher worldwide and, more threateningly, there is an increasing number of cases of infections with organisms resistant to the major antituberculosis agents (25, 26, 62).

The circumstances in which drug resistance emerges are well known and have been so since shortly after the first clinical trials became available and their lessons were digested (51). In recent years the molecular basis for the mechanism of action of antituberculosis agents and the way in which the organisms become resistant have begun to be unraveled. In this review the clinical circumstances of resistance are described. The molecular mechanisms whereby resistance emerges are also outlined together with the insights that this brings to controlling the threat of an epidemic of multiple-drug resistance.

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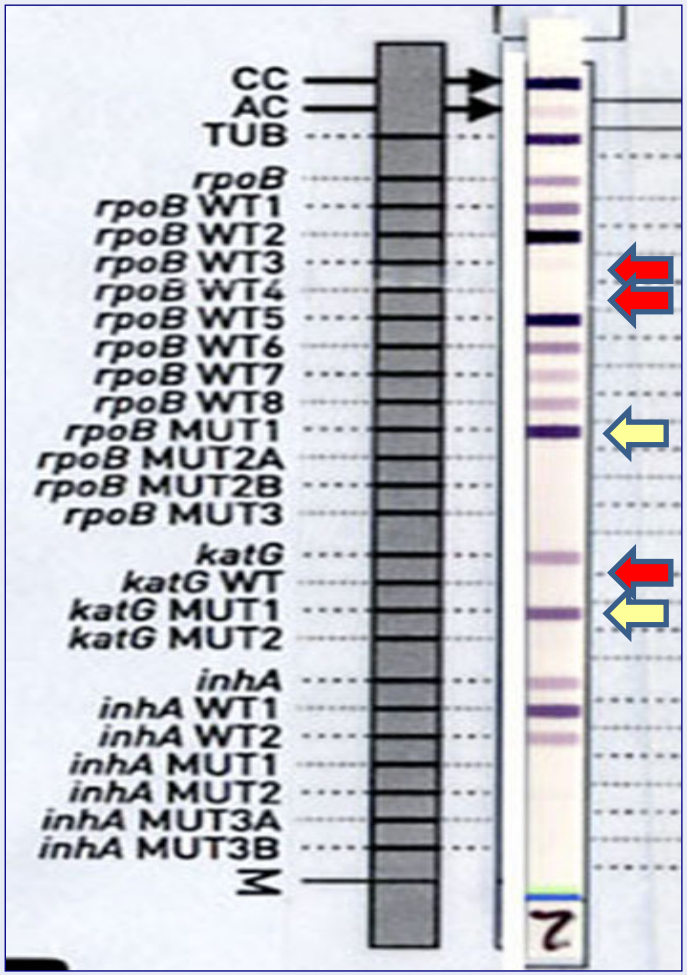
CLINICAL CIRCUMSTANCE FOR RESISTANCE DEVELOPMENT

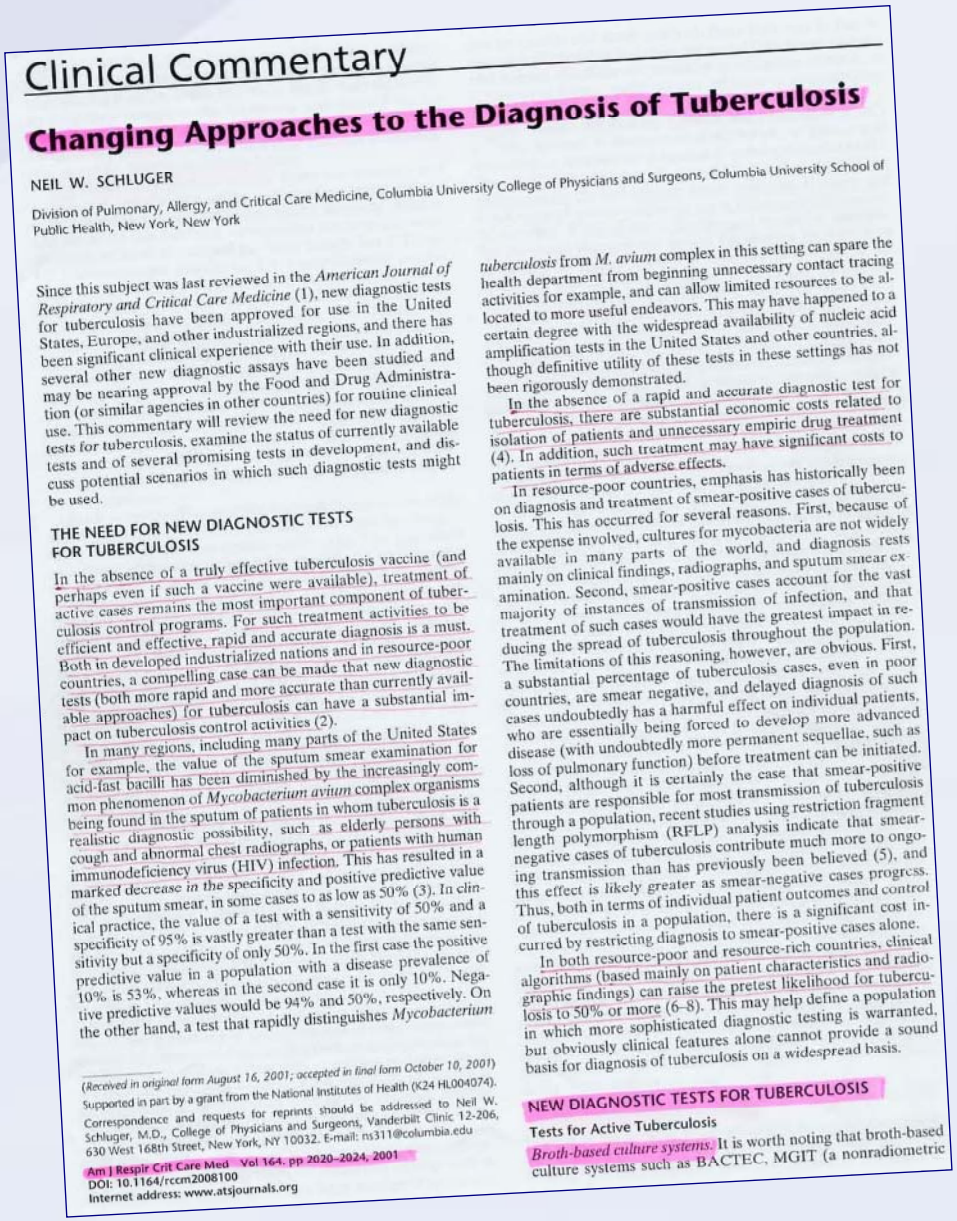
The approach to chemotherapy for tuberculosis is very different from that for other bacterial infections. The organism has a long generation time and a capacity for dormancy, when its low metabolic activity makes it a difficult therapeutic target (53, 61, 83). In addition, *M. tuberculosis* may be located in pulmonary cavities, empyema pus, or solid caseous material, where penetration of antibiotics is difficult or the pH is sufficiently low to inhibit the activity of most antibiotics (29, 43). A series of animal and human clinical trials has led to the concept that there are different populations of bacteria present within the host. (8–10, 44, 57). Organisms in pulmonary cavities are thought to be multiplying in an aerobic environment and consequently behave in a way that can be mimicked by in vitro tests. Organisms located within caseous foci are in a milieu where the low pH is likely to inhibit the activity of agents such as aminoglycosides but to provide the conditions necessary for pyrazinamide activity. Bacteria found within macrophages probably only exhibit occasional spurts of metabolism and may be in relatively microaerophilic conditions, where mycobacterial dormancy can be induced (83).

Each of the antituberculosis drugs has a major role in dealing with one of these populations. For example, isoniazid is critical early in therapy; its bactericidal activity rapidly reduces the sputum viable count because it is active mainly against the organisms growing aerobically in pulmonary cavities (23, 40). Pyrazinamide is only active at low pH, making it ideally suitable for killing the organisms inside caseous necrotic foci. This explains the finding that pyrazinamide appears to have no benefit after the second month of therapy (27). Rifampin is important in killing organisms that are metabolizing slowly, killing the persisters, and sterilizing the patient's sputum, as demonstrated by animal studies (35) and clinical trials (27). Mathematical models suggest that increases in the size of the starting bacterial population are associated with the emer-

Diagnóstico rápido de MDR-TB



- ✓ Usado para la detección rápida de resistencias a INH y RIF
- ✓ Basado en hibridación de DNA amplificado procedente de cultivos de *Mycobacterium tuberculosis*
- ✓ En la tira : sondas que representan regiones de los genes *rpoB*, *kat G* e *inhA* y sus principales mutaciones
- ✓ La ausencia de hibridación a alguna de estas sondas específicas indica la existencia de ése tipo de mutación con o sin hibridación la zona de mutaciones más frecuentes.
- ✓ En nuestro caso: D516V para *rpoB*
  S315T1 para *katG*



Clinical Commentary

Changing Approaches to the Diagnosis of Tuberculosis

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Since this subject was last reviewed in the *American Journal of Respiratory and Critical Care Medicine* (1), new diagnostic tests for tuberculosis have been approved for use in the United States, Europe, and other industrialized regions, and there has been significant clinical experience with their use. In addition, several other new diagnostic assays have been studied and may be nearing approval by the Food and Drug Administration (or similar agencies in other countries) for routine clinical use. This commentary will review the need for new diagnostic tests for tuberculosis, examine the status of currently available tests and of several promising tests in development, and discuss potential scenarios in which such diagnostic tests might be used.

THE NEED FOR NEW DIAGNOSTIC TESTS FOR TUBERCULOSIS

In the absence of a truly effective tuberculosis vaccine (and perhaps even if such a vaccine were available), treatment of active cases remains the most important component of tuberculosis control programs. For such treatment activities to be efficient and effective, rapid and accurate diagnosis is a must. Both in developed industrialized nations and in resource-poor countries, a compelling case can be made that new diagnostic tests (both more rapid and more accurate than currently available approaches) for tuberculosis can have a substantial impact on tuberculosis control activities (2).

In many regions, including many parts of the United States for example, the value of the sputum smear examination for acid-fast bacilli has been diminished by the increasingly common phenomenon of *Mycobacterium avium* complex organisms being found in the sputum of patients in whom tuberculosis is a realistic diagnostic possibility, such as elderly persons with cough and abnormal chest radiographs, or patients with human immunodeficiency virus (HIV) infection. This has resulted in a marked decrease in the specificity and positive predictive value of the sputum smear, in some cases to as low as 50% (3). In clinical practice, the value of a test with a sensitivity of 50% and a specificity of 95% is vastly greater than a test with the same sensitivity but a specificity of only 50%. In the first case the positive predictive value in a population with a disease prevalence of 10% is 53%, whereas in the second case it is only 10%. Negative predictive values would be 94% and 50%, respectively. On the other hand, a test that rapidly distinguishes *Mycobacterium tuberculosis* from *M. avium* complex in this setting can spare the health department from beginning unnecessary contact tracing activities for example, and can allow limited resources to be allocated to more useful endeavors. This may have happened to a certain degree with the widespread availability of nucleic acid amplification tests in the United States and other countries, although definitive utility of these tests in these settings has not been rigorously demonstrated.

In the absence of a rapid and accurate diagnostic test for tuberculosis, there are substantial economic costs related to isolation of patients and unnecessary empiric drug treatment (4). In addition, such treatment may have significant costs to patients in terms of adverse effects.

In resource-poor countries, emphasis has historically been on diagnosis and treatment of smear-positive cases of tuberculosis. This has occurred for several reasons. First, because of the expense involved, cultures for mycobacteria are not widely available in many parts of the world, and diagnosis rests mainly on clinical findings, radiographs, and sputum smear examination. Second, smear-positive cases account for the vast majority of instances of transmission of infection, and that treatment of such cases would have the greatest impact in reducing the spread of tuberculosis throughout the population. The limitations of this reasoning, however, are obvious. First, a substantial percentage of tuberculosis cases, even in poor countries, are smear negative, and delayed diagnosis of such cases undoubtedly has a harmful effect on individual patients, who are essentially being forced to develop more advanced disease (with undoubtedly more permanent sequellae, such as loss of pulmonary function) before treatment can be initiated. Second, although it is certainly the case that smear-positive patients are responsible for most transmission of tuberculosis through a population, recent studies using restriction fragment length polymorphism (RFLP) analysis indicate that smear-negative cases of tuberculosis contribute much more to ongoing transmission than has previously been believed (5), and this effect is likely greater as smear-negative cases progress. Thus, both in terms of individual patient outcomes and control of tuberculosis in a population, there is a significant cost incurred by restricting diagnosis to smear-positive cases alone.

In both resource-poor and resource-rich countries, clinical algorithms (based mainly on patient characteristics and radiographic findings) can raise the pretest likelihood for tuberculosis to 50% or more (6–8). This may help define a population in which more sophisticated diagnostic testing is warranted, but obviously clinical features alone cannot provide a sound basis for diagnosis of tuberculosis on a widespread basis.

NEW DIAGNOSTIC TESTS FOR TUBERCULOSIS

Tests for Active Tuberculosis

Broth-based culture systems. It is worth noting that broth-based culture systems such as BACTEC, MGIT (a nonradiometric

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Am J Respir Crit Care Med Vol 164. pp 2020–2024, 2001
DOI: 10.1164/rccm2008100
Internet address: www.atsjournals.org

Schluger N.W.
Am J Respir Crit Care Med 2001; 164:2020-24

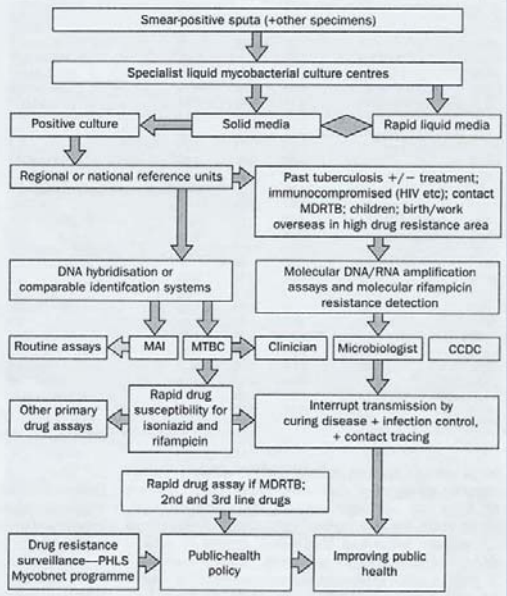
Drobniewski F.A., *et al.*
Lancet Infect Dis 2003; 3:141-7

Modern laboratory diagnosis of tuberculosis

F A Drobniewski, M Caws, A Gibson, and D Young

One-third of the global population is believed to be infected with bacteria of the *Mycobacterium tuberculosis* complex, the causative agent of tuberculosis. More than 8 million new cases of tuberculosis occur annually leading to 2 million deaths. Mortality is particularly high in those coinfected with HIV and where the bacteria are multiple-drug-resistant strains—ie, strains resistant to at least isoniazid and rifampicin. Early diagnosis of tuberculosis and drug resistance improves survival and by identifying infectious cases promotes contact tracing, implementation of institutional cross-infection procedures, and other public-health actions. This review addresses significant advances made in the diagnosis of infection, clinical disease, and drug resistance over the past decade. It proposes operational criteria for a modern diagnostic service in the UK (as a model of a low-incidence country) and explores some of the economic issues surrounding the use of these techniques.

Lancet Infect Dis 2003; 3: 141–47



Model cost-effective national tuberculosis diagnostic and reference laboratory scheme. MAI=Mycobacterium avium-intracellulare; MTBC=Mycobacterium tuberculosis complex; CCDC=consultant in communicable disease control; MDRTB=multidrug-resistant tuberculosis; PHLS=public health laboratory service.

The 20th century saw many advances in the battle against tuberculosis but still this disease kills between 2 and 3 million people worldwide annually, and is resurgent in regions of the world where it was once thought to be conquered. It has been estimated that one-third of the world's population—almost 2 billion people—is infected with the causative bacterium, *Mycobacterium tuberculosis*. The emergence of drug resistance, especially multiple drug resistance, and increasing coinfection with HIV has fuelled the current pandemic.

The incidence of *M tuberculosis* began to decline rapidly from the start of the 20th century in developed countries with improvements in sanitation and housing. These trends were accelerated by the introduction of BCG vaccination and the discovery of antimicrobials such as streptomycin, which were used in effective combinations established in a series of landmark trials by the British Medical Research Council, the USA Public Health Service, and their partners.[1] The development of these standardised short-course drug regimens of 6–8 months supported by accurate quality-assured laboratory services is at the core of modern tuberculosis-control programmes.

Here we review recent advances in the diagnosis of tuberculosis, clinical infection, and drug resistance, and propose a scheme for a modern diagnostic service in a low-incidence country (figure).

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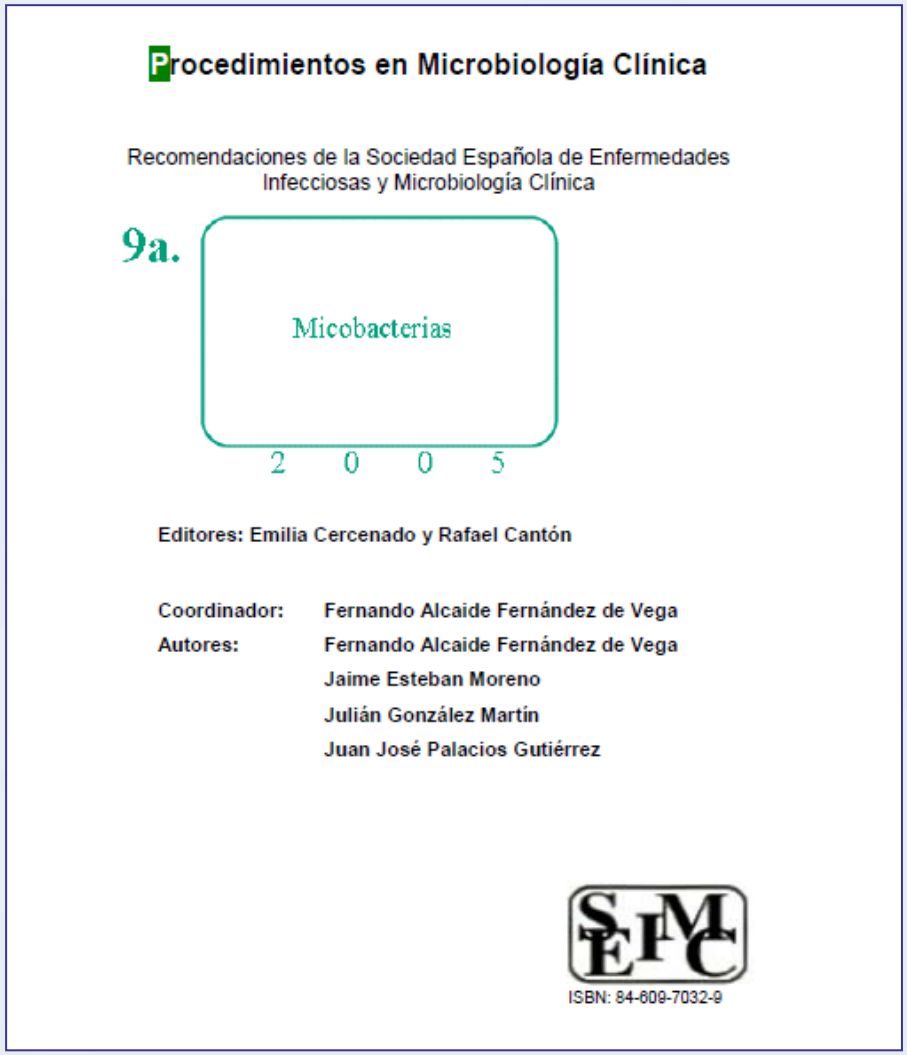
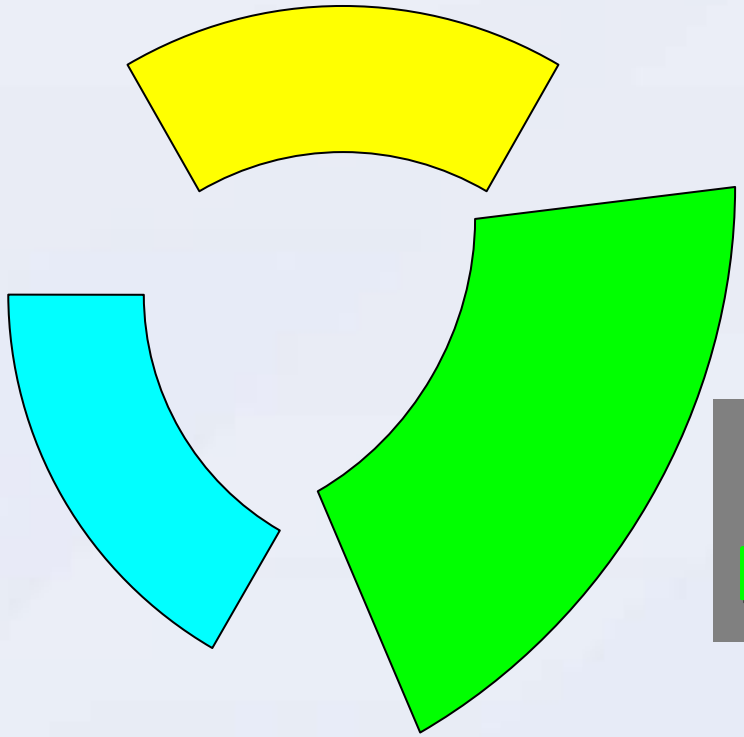
Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica

Procedimientos en Microbiología Clínica, 2005

Micobacterias 9a



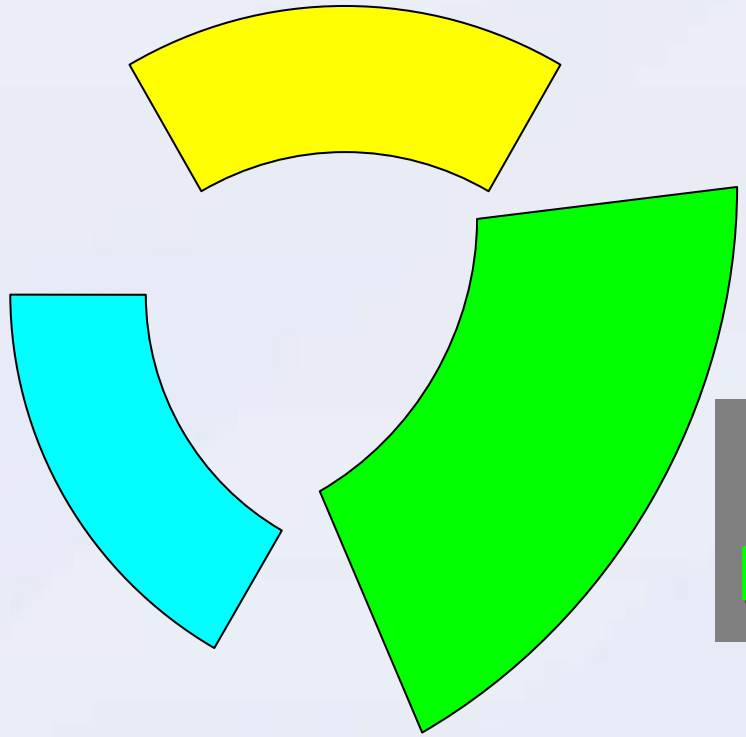
Métodos Moleculares



Métodos Fenotípicos

Laboratorio Micobacterias

Métodos Moleculares



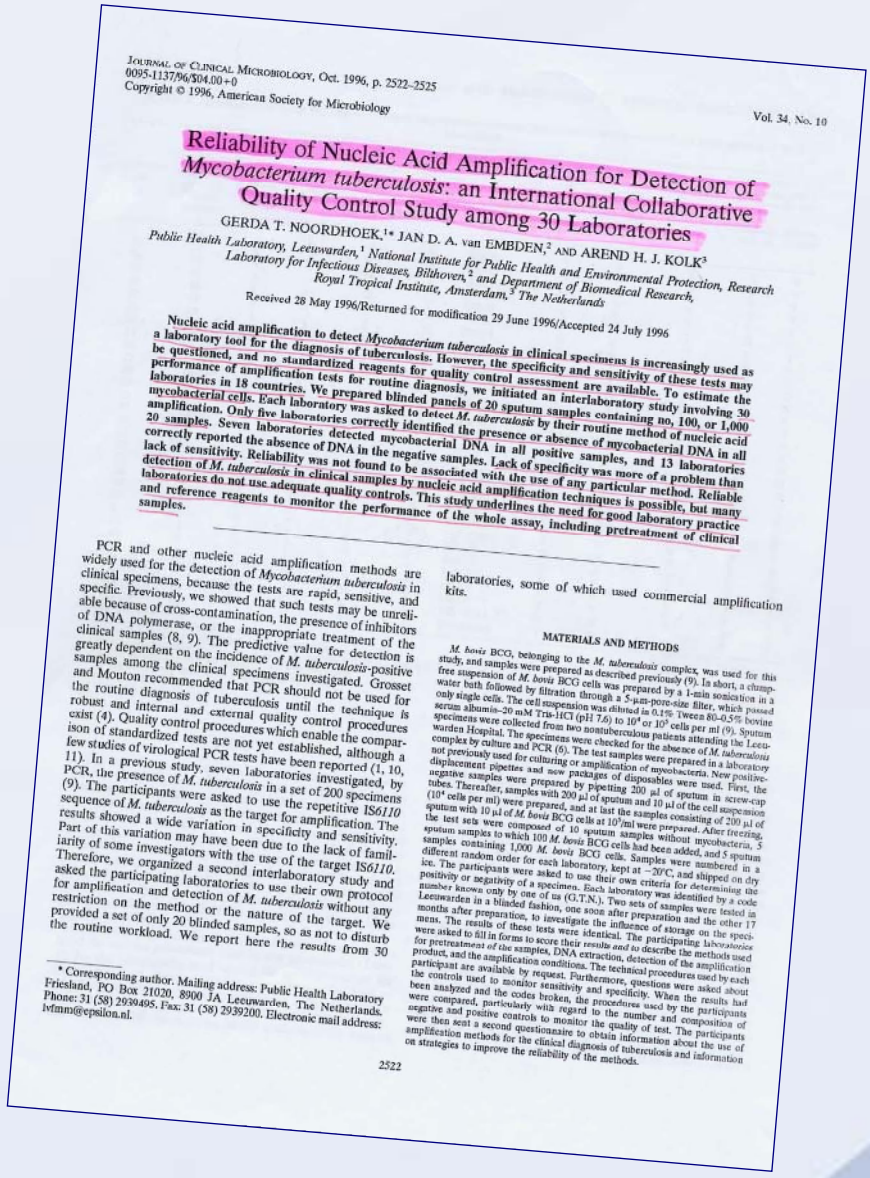
Métodos Fenotípicos

Laboratorio Micobacterias

Las mejores herramientas que permitan suministrar información útil al clínico

Técnicas dirigidas al diagnóstico directamente en la muestra clínica

Noordhoek GT. *et al.*
J Clin Microbiol 1996, 34: 2522-25



JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1996, p. 2522–2525
0095-1137/96/$04.00+0

Vol. 34, No. 10

Reliability of Nucleic Acid Amplification for Detection of *Mycobacterium tuberculosis*: an International Collaborative Quality Control Study among 30 Laboratories

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Nucleic acid amplification to detect *Mycobacterium tuberculosis* in clinical specimens is increasingly used as a laboratory tool for the diagnosis of tuberculosis. However, the specificity and sensitivity of these tests may be questioned, and no standardized reagents for quality control assessment are available. To estimate the performance of amplification tests for routine diagnosis, we initiated an interlaboratory study involving 30 laboratories in 18 countries. We prepared blinded panels of 20 sputum samples containing no, 100, or 1,000 mycobacterial cells. Each laboratory was asked to detect *M. tuberculosis* by their routine method of nucleic acid amplification. Only five laboratories correctly identified the presence or absence of mycobacterial DNA in all 20 samples. Seven laboratories detected mycobacterial DNA in all positive samples, and 13 laboratories correctly reported the absence of DNA in the negative samples. Lack of specificity was more of a problem than lack of sensitivity. Reliability was not found to be associated with the use of any particular method. Reliable detection of *M. tuberculosis* in clinical samples by nucleic acid amplification techniques is possible, but many laboratories do not use adequate quality controls. This study underlines the need for good laboratory practice and reference reagents to monitor the performance of the whole assay, including pretreatment of clinical samples.

PCR and other nucleic acid amplification methods are widely used for the detection of *Mycobacterium tuberculosis* in clinical specimens, because the tests are rapid, sensitive, and specific. Previously, we showed that such tests may be unreliable because of cross-contamination, the presence of inhibitors of DNA polymerase, or the inappropriate treatment of the clinical samples (8, 9). The predictive value for detection is greatly dependent on the incidence of *M. tuberculosis*-positive samples among the clinical specimens investigated. Grosset and Mouton recommended that PCR should not be used for the routine diagnosis of tuberculosis until the technique is robust and internal and external quality control procedures exist (4). Quality control procedures which enable the comparison of standardized tests are not yet established, although a few studies of virological PCR tests have been reported (1, 10, 11). In a previous study, seven laboratories investigated, by PCR, the presence of *M. tuberculosis* in a set of 200 specimens (9). The participants were asked to use the repetitive IS*6110* sequence of *M. tuberculosis* as the target for amplification. The results showed a wide variation in specificity and sensitivity. Part of this variation may have been due to the lack of familiarity of some investigators with the use of the target IS*6110*. Therefore, we organized a second interlaboratory study and asked the participating laboratories to use their own protocol for amplification and detection of *M. tuberculosis* without any restriction on the method or the nature of the target. We provided a set of only 20 blinded samples, so as not to disturb the routine workload. We report here the results from 30 laboratories, some of which used commercial amplification kits.

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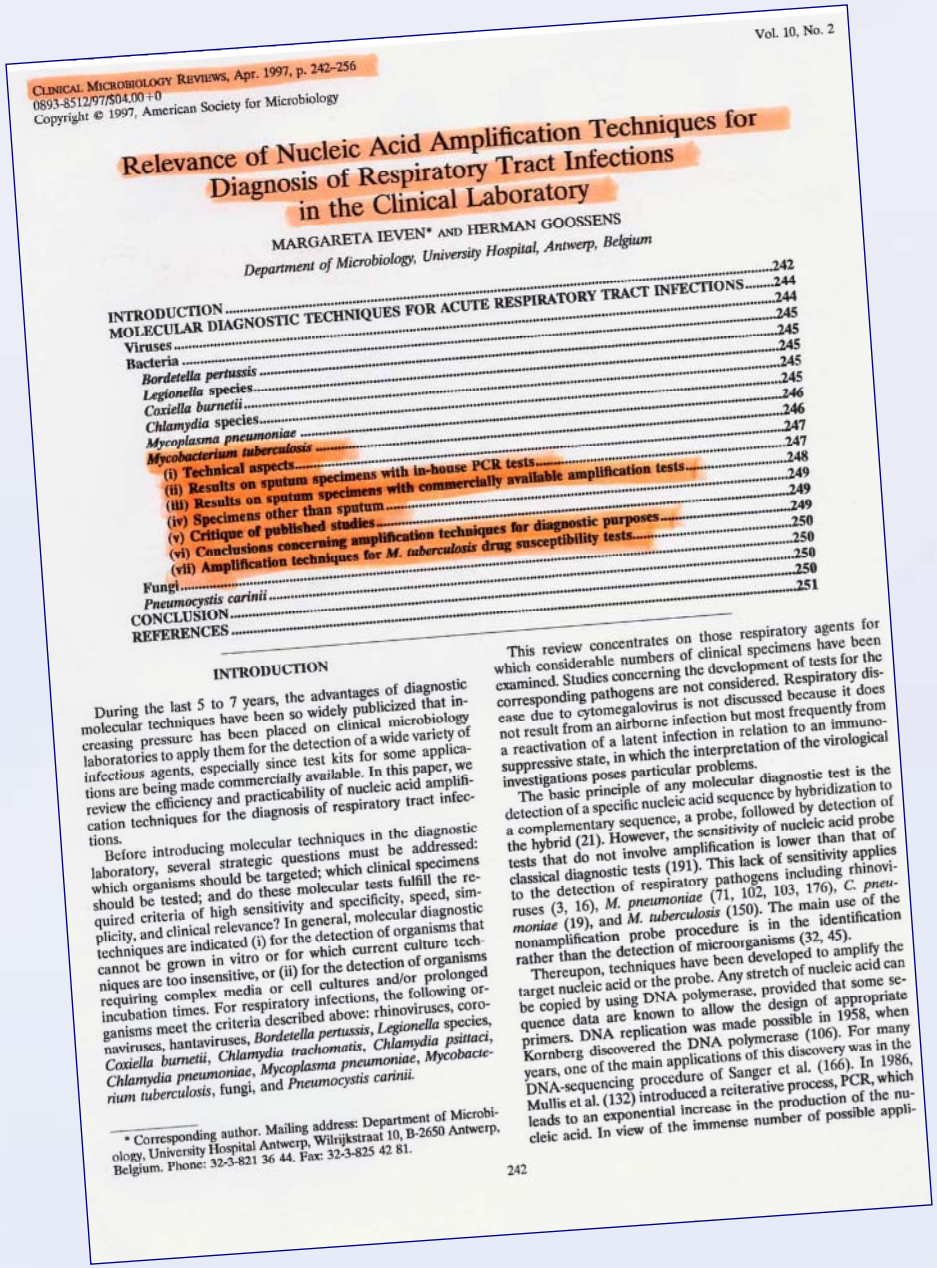
MATERIALS AND METHODS

M. bovis BCG, belonging to the *M. tuberculosis* complex, was used for this study, and samples were prepared as described previously (9). In short, a clump-free suspension of *M. bovis* BCG cells was prepared by a 1-min sonication in a water bath followed by filtration through a 5-μm-pore-size filter, which passed only single cells. The cell suspension was diluted in 0.1% Tween 80–0.5% bovine serum albumin–20 mM Tris-HCl (pH 7.6) to 10^4 or 10^5 cells per ml (9). Sputum specimens were collected from two nontuberculous patients attending the Leeuwarden Hospital. The specimens were checked for the absence of *M. tuberculosis* complex by culture and PCR (6). The test samples were prepared in a laboratory not previously used for culturing or amplification of mycobacteria. New positive-displacement pipettes and new packages of disposables were used. First, the negative samples were prepared by pipetting 200 μl of sputum in screw-cap tubes. Thereafter, samples with 200 μl of sputum and 10 μl of the cell suspension (10^4 cells per ml) were prepared, and at last the samples consisting of 200 μl of sputum with 10 μl of *M. bovis* BCG cells at 10^7/ml were prepared. After freezing, the test sets were composed of 10 sputum samples without mycobacteria, 5 sputum samples containing 1,000 *M. bovis* BCG cells had been added, and 5 sputum samples containing 100 *M. bovis* BCG cells. Samples were numbered in a different random order for each laboratory, kept at −20°C, and shipped on dry ice. The participants were asked to use their own criteria for determining the positivity or negativity of a specimen. Each laboratory was identified by a code number known only by one of us (G.T.N.). Two sets of samples were tested in Leeuwarden in a blinded fashion, one soon after preparation and the other 17 months after preparation, to investigate the influence of storage on the specimens. The results of these tests were identical. The participating laboratories were asked to fill in forms to score their *results and to* describe the methods used for pretreatment of the samples, DNA extraction, detection of the amplification product, and the amplification conditions. The technical procedures used by each participant are available by request. Furthermore, questions were asked about the controls used to monitor sensitivity and specificity. When the results had been analyzed and the codes broken, the procedures used by the participants were compared, particularly with regard to the number and composition of negative and positive controls to monitor the quality of test. The participants were then sent a second questionnaire to obtain information about the use of amplification methods for the clinical diagnosis of tuberculosis and information on strategies to improve the reliability of the methods.

Ieven M. *et al.*
Clin Microbiol Rev 1997, 10: 242-56



CLINICAL MICROBIOLOGY REVIEWS, Apr. 1997, p. 242–256
0893-8512/97/$04.00+0

Vol. 10, No. 2

Relevance of Nucleic Acid Amplification Techniques for Diagnosis of Respiratory Tract Infections in the Clinical Laboratory

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INTRODUCTION

During the last 5 to 7 years, the advantages of diagnostic molecular techniques have been so widely publicized that increasing pressure has been placed on clinical microbiology laboratories to apply them for the detection of a wide variety of infectious agents, especially since test kits for some applications are being made commercially available. In this paper, we review the efficiency and practicability of nucleic acid amplification techniques for the diagnosis of respiratory tract infections.

Before introducing molecular techniques in the diagnostic laboratory, several strategic questions must be addressed: which organisms should be targeted; which clinical specimens should be tested; and do these molecular tests fulfill the required criteria of high sensitivity and specificity, speed, simplicity, and clinical relevance? In general, molecular diagnostic techniques are indicated (i) for the detection of organisms that cannot be grown in vitro or for which current culture techniques are too insensitive, or (ii) for the detection of organisms requiring complex media or cell cultures and/or prolonged incubation times. For respiratory infections, the following organisms meet the criteria described above: rhinoviruses, coronaviruses, hantaviruses, *Bordetella pertussis*, *Legionella* species, *Coxiella burnetii*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Mycobacterium tuberculosis*, fungi, and *Pneumocystis carinii*.

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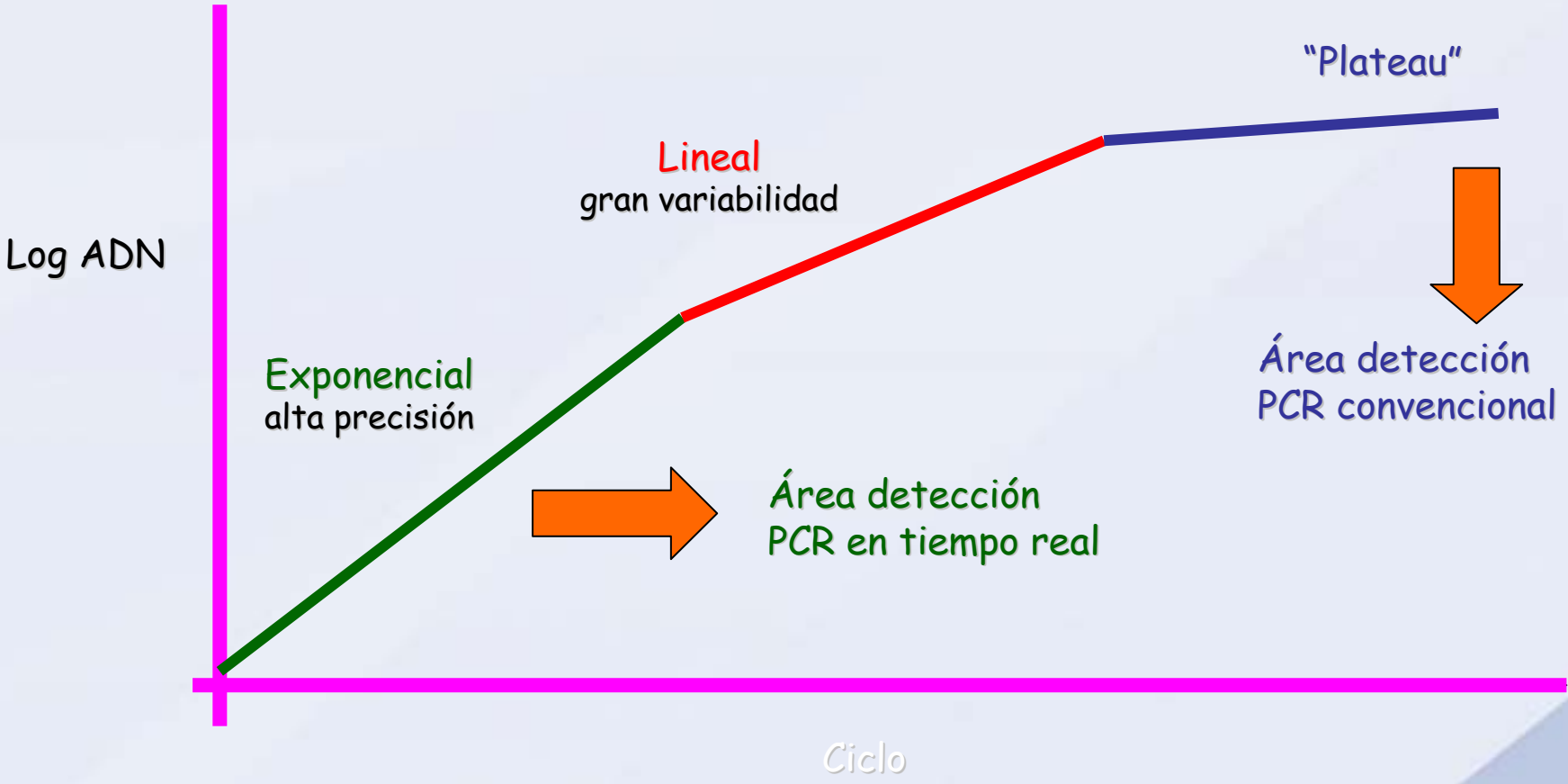
This review concentrates on those respiratory agents for which considerable numbers of clinical specimens have been examined. Studies concerning the development of tests for the corresponding pathogens are not considered. Respiratory disease due to cytomegalovirus is not discussed because it does not result from an airborne infection but most frequently from a reactivation of a latent infection in relation to an immunosuppressive state, in which the interpretation of the virological investigations poses particular problems.

The basic principle of any molecular diagnostic test is the detection of a specific nucleic acid sequence by hybridization to a complementary sequence, a probe, followed by detection of the hybrid (21). However, the sensitivity of nucleic acid probe tests that do not involve amplification is lower than that of classical diagnostic tests (191). This lack of sensitivity applies to the detection of respiratory pathogens including rhinoviruses (3, 16), *M. pneumoniae* (71, 102, 103, 176), *C. pneumoniae* (19), and *M. tuberculosis* (150). The main use of the nonamplification probe procedure is in the identification rather than the detection of microorganisms (32, 45).

Thereupon, techniques have been developed to amplify the target nucleic acid or the probe. Any stretch of nucleic acid can be copied by using DNA polymerase, provided that some sequence data are known to allow the design of appropriate primers. DNA replication was made possible in 1958, when Kornberg discovered the DNA polymerase (106). For many years, one of the main applications of this discovery was in the DNA-sequencing procedure of Sanger et al. (166). In 1986, Mullis et al. (132) introduced a reiterative process, PCR, which leads to an exponential increase in the production of the nucleic acid. In view of the immense number of possible appli-

Fases de la PCR



Limitaciones de la PCR convencional

- Se analiza exclusivamente el producto final (fase de "plateau")
- Requiere procesamiento post-PCR
- Los resultados no pueden expresarse como números
- Mayor duración del ensayo

Brocolo F. *et al.*
J Clin Microbiol 2003, 41: 4565-72

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2003, p. 4565–4572
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.10.4565–4572.2003

Vol. 41, No. 10

Rapid Diagnosis of Mycobacterial Infections and Quantitation of *Mycobacterium tuberculosis* Load by Two Real-Time Calibrated PCR Assays

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Sensitive and specific techniques to detect and identify *Mycobacterium tuberculosis* directly in clinical specimens are important for the diagnosis and management of patients with tuberculosis (TB). We developed two real-time PCR assays, based on the IS*6110* multicopy element and on the *senX3-regX3* intergenic region, which provide a rapid method for the diagnosis of mycobacterial infections. The sensitivity and specificity of both assays were established by using purified DNA from 71 clinical isolates and 121 clinical samples collected from 83 patients, 20 of whom were affected by TB. Both assays are accurate, sensitive, and specific, showing a complementary pattern of *Mycobacterium* recognition: broader for the IS*6110*-based assay and restricted to the *M. tuberculosis* complex for the *senX3-regX3*-based assay. Moreover, the addition of a synthetic DNA calibrator prior to DNA extraction allowed us to measure the efficiency of DNA recovery and to control for the presence of PCR inhibitors. The mycobacterial burden of the clinical samples, as assessed by direct microscopy, correlates with the *M. tuberculosis* DNA load measured by the *senX3-regX3*-based assay. In addition, reduced levels of *M. tuberculosis* DNA load are present in those patients subjected to successful therapy, suggesting a potential use of this assay for monitoring treatment efficacy. Therefore, these assays represent a fully controlled high-throughput system for the evaluation of mycobacterial burden in clinical specimens.

Tuberculosis (TB) remains one of the major public health problems worldwide (5, 25), particularly due to the appearance of drug-resistant *Mycobacterium tuberculosis* strains that render TB control programs more cumbersome (16, 24). Diagnostic tests devoted to the rapid, sensitive, and specific identification of the causative agent are key elements for successful health programs aimed at disease control. Moreover, the accurate determination of mycobacterial burden might be beneficial for fast assessment of patient response to standard therapy, especially in those patients suspected of harboring resistant *M. tuberculosis* strains (24). Traditional laboratory techniques (22, 34), such as direct microscopy observation and *Mycobacterium* culture on semisolid or liquid medium, are far from being sensitive and specific or adequate for a fast *M. tuberculosis* identification. Moreover, the harsh decontaminating procedures combined with the lack of homogeneity of the sputum and the tendency of *Mycobacterium* to clump render even quantitative culture systems unreliable.

Detection of *M. tuberculosis*-specific DNA sequences might represent a more sensitive and fast diagnostic target (9, 27, 29, 36); however, the successful use of DNA amplification techniques is strongly dependent on the choice of the target sequence (12, 28). Moreover, since respiratory tract specimens are naturally contaminated by many different species of commensal and pathogenic microorganisms, a high degree of specificity for *M. tuberculosis* recognition is mandatory.

PCR-based systems require, in addition, an efficient extraction and purification procedure for the DNA, which is further complicated by the physical peculiarity of the sputum and by the high lipid content of the mycobacterial cell wall. Thus, all the available techniques for mycobacterial DNA extraction require manipulation steps, which result in an unpredictable loss of starting material. At present, there are few methods available for real-time quantification of *M. tuberculosis* DNA (3, 7, 15, 17), but none allow for the control of both the efficiency of the extraction procedure and the presence of PCR inhibitors.

Here, we describe the development of two real-time calibrated PCR assays for the rapid, sensitive, and accurate determination of *M. tuberculosis* DNA burden directly from clinical samples. In our assays, the problems of DNA extraction efficiency and PCR inhibitors have been solved by using a synthetic DNA molecule, termed calibrator, specifically detected by an ad hoc-designed probe which does not cross-react with *Mycobacterium* sequences. The calibrator permits us to control each sample for the presence of PCR inhibitors, to determine a cutoff value of sensitivity for negative samples, and to normalize positive samples for the efficiency of DNA recovery (1). These assays, which amplify two distinct regions of the *M. tuberculosis* genome, one fragment of the IS*6110* multicopy element (32) and one of the *senX3-regX3* intergenic region

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† Present address: Pharmacia, Pharmacology Dept., Gene Expression Unit, 20014 Nerviano, Milan, Italy.

Ventajas de la PCR cuantitativa en tiempo real

- Ensayo dinámico, se analiza la fase exponencial
- No requiere procesamiento post-PCR ("sistema cerrado")
- Los resultados son cuantitativos
- Menor duración del ensayo

Tabla 7. Sistemas comerciales de amplificación genética para la detección directa del complejo *M. tuberculosis* en la muestra clínica.

Nombre comercial	Método de amplificación	Diana	Volumen de muestra (µl)	Técnica de detección	Automatización	Control interno	FDA
AMTD2	TMA	16S rRNA	450	Quimioluminiscencia	NO	NO	SI
AMPLICOR	PCR	16S rRNA	100	Colorimétrica	SI	SI	SI
DTB	SDA	IS*6110* / 16S rRNA	500	Fluorométrica (ET)	SI	SI	NO
LiPA	Nested PCR	*rpoB*	500	Colorimétrica	SI	NO	NO
RealArt MTB	Real Time PCR	16S rRNA	10	Fluorométrica	SI	SI	NO

FDA: Aprobada por la FDA.

Helb D. *et al.* *J Clin Microbiol* 2010; 48: 229-37

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0095-1137/10/$12.00 doi:10.1128/JCM.01463-09

Rapid Detection of *Mycobacterium tuberculosis* and Rifampin Resistance by Use of On-Demand, Near-Patient Technology▽†‡

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107 sputum samples 29/29 **100% smear-positive culture-positive** cases and 33/39 **84.6% smear-negative culture-positive** cases, ***M. tuberculosis*** was not detected in 25/25 (100%) of the culture negative samples.

64 smear-positive culture-positive sputa Xpert MT/RIF detected 63/64 **98.4% culture-positive cases** and 9/9 **100% cases of rifampin resistance**. Rifampin resistance was excluded in 54/55 (98.2%) susceptible cases.

Blakemore R. *et al.* *J Clin Microbiol* 2010; 48: 2495-2501

JOURNAL OF CLINICAL MICROBIOLOGY, July 2010, p. 2495–2501 Vol. 48, No. 7
0095-1137/10/$12.00 doi:10.1128/JCM.00128-10

Evaluation of the Analytical Performance of the Xpert MTB/RIF Assay▽†

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The Xpert MTB/RIF assay correctly identified all 79 *M. tuberculosis* isolates and correctly excluded all 89 nontuberculosis isolates

RIF resistance was correctly identified in all 37 resistant isolates and in none of the 42 susceptible isolates

Banada PP. *et al.* *J Clin Microbiol* 2010; 48: 3551-57

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2010, p. 3551–3557 Vol. 48, No. 10
0095-1137/10/$12.00 doi:10.1128/JCM.01053-10

Containment of Bioaerosol Infection Risk by the Xpert MTB/RIF Assay and Its Applicability to Point-of-Care Settings▽

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These results suggest that benchtop use of the Xpert MTB/RIF assay limits infection risk to the user.

Boehme CC. *et al.* *N Engl J Med* 2010; 363: 1005-1015

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Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis 98.2% and 124 of 171 with smear-negative tuberculosis 72.5%. The test was specific in 604 of 609 patients without tuberculosis 99.2%

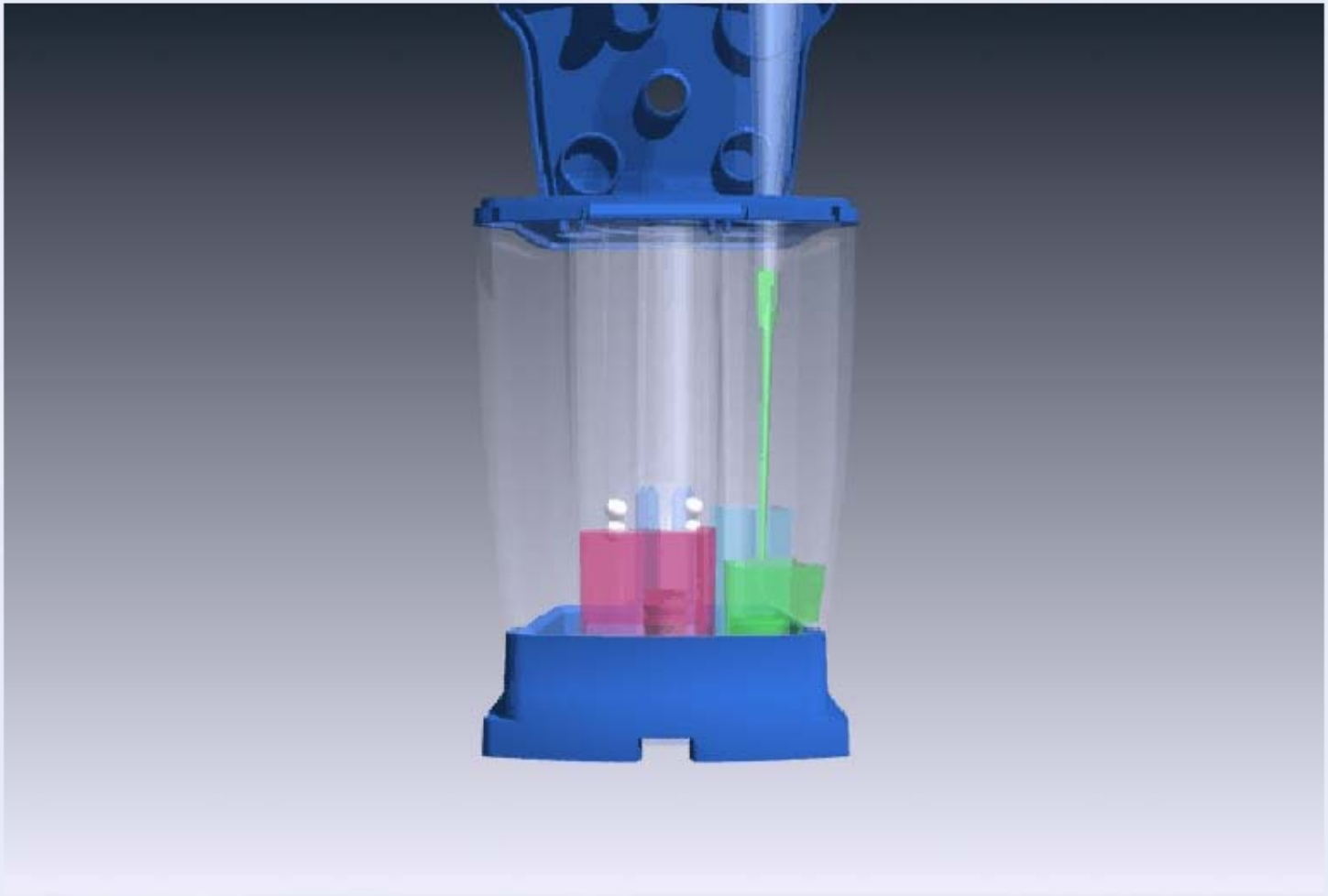
MTB/RIF test identified 200 of 205 patients 97.6% with rifampin-resistant bacteria and 504 of 514 98.1% with rifampin-sensitive bacteria

Xpert MTB/RIF

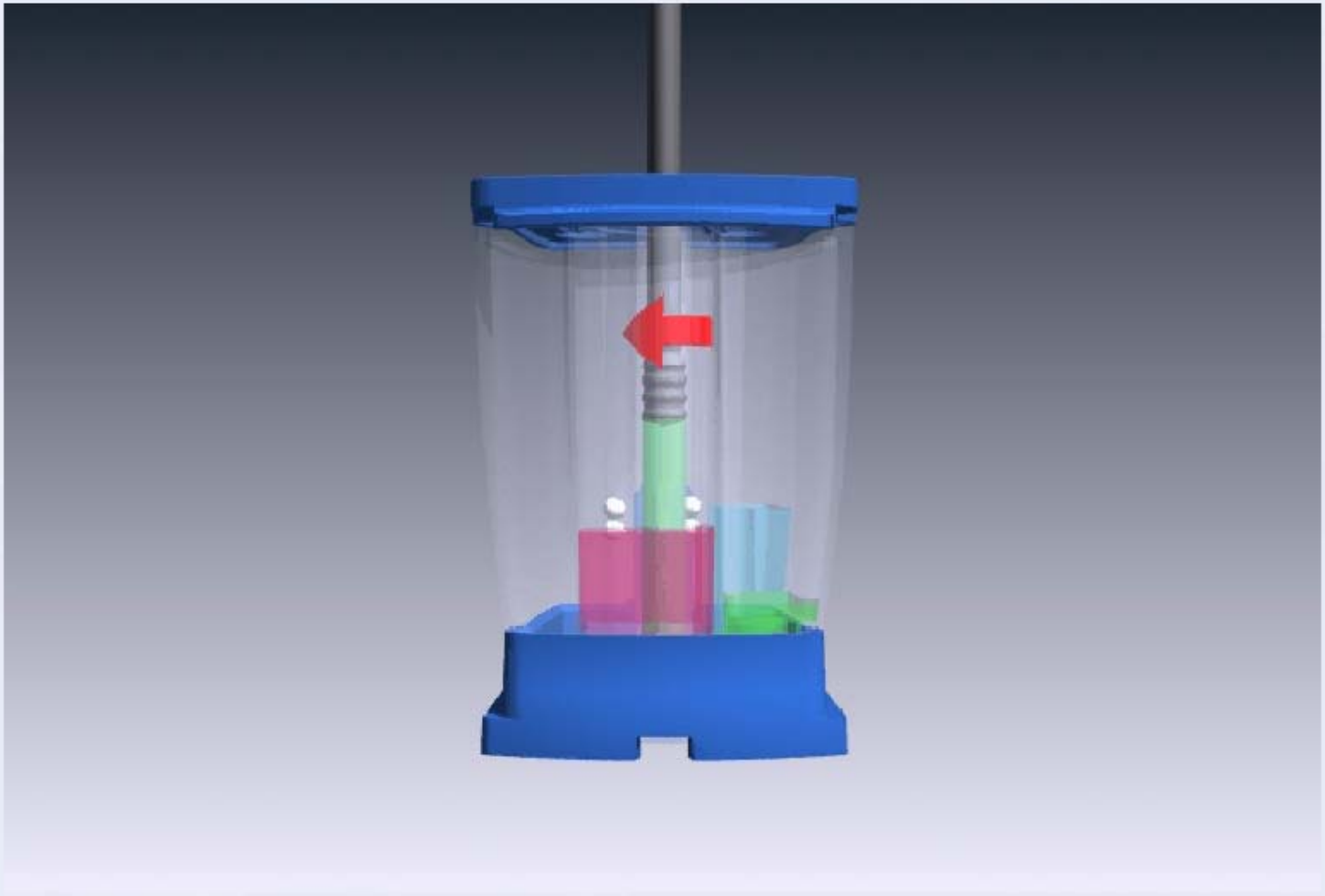
15 minutos

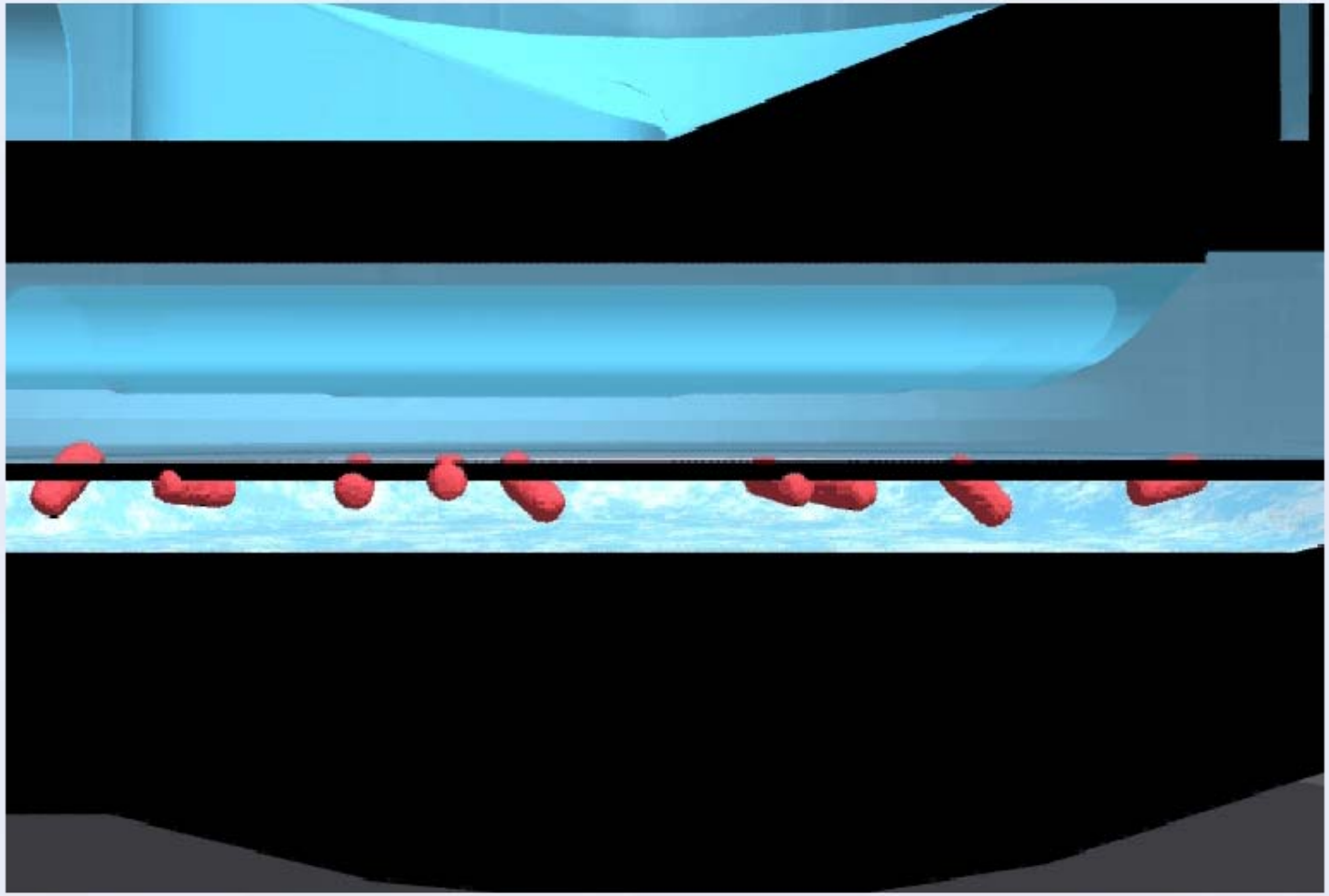
1 minuto

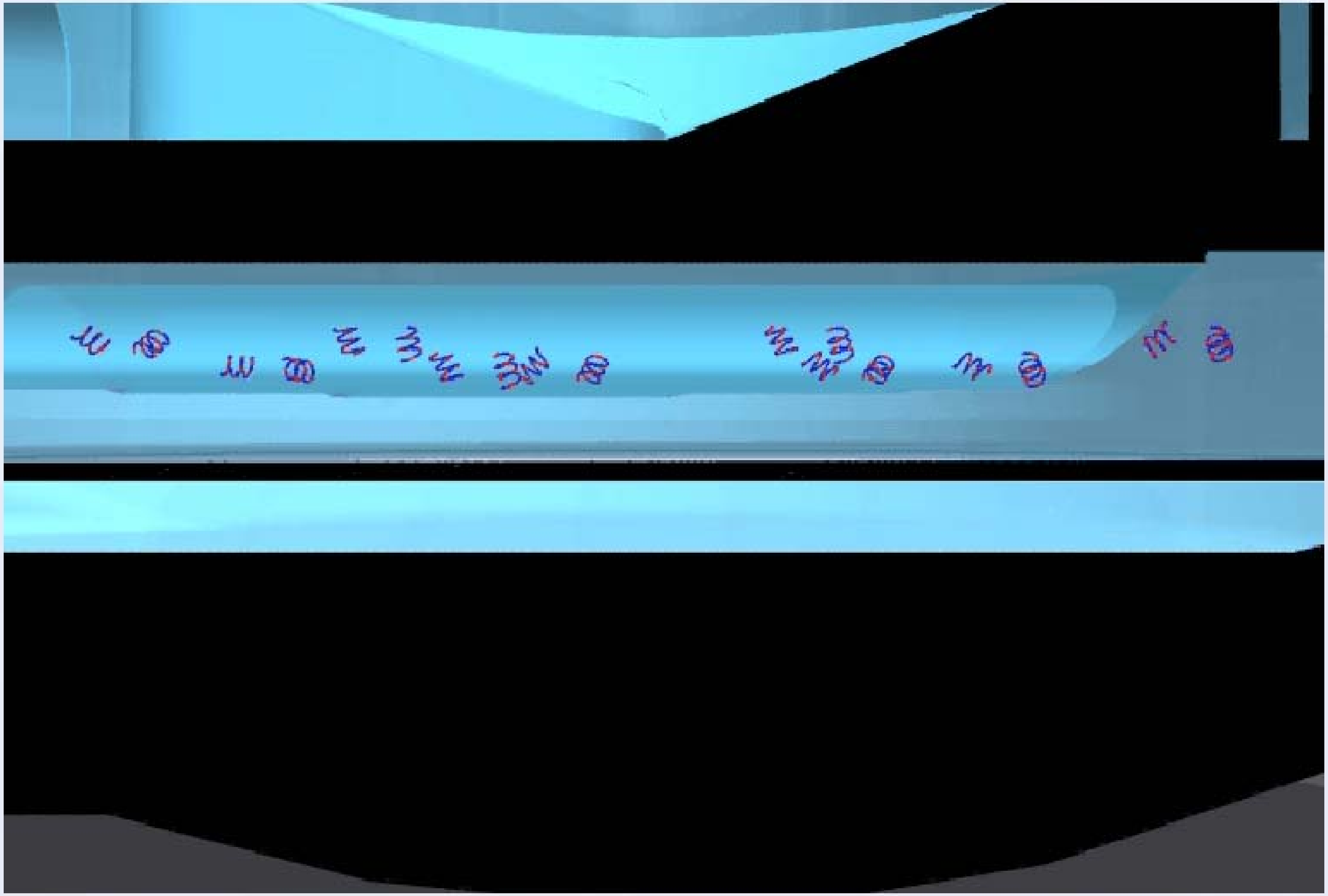
1h 40 minutos

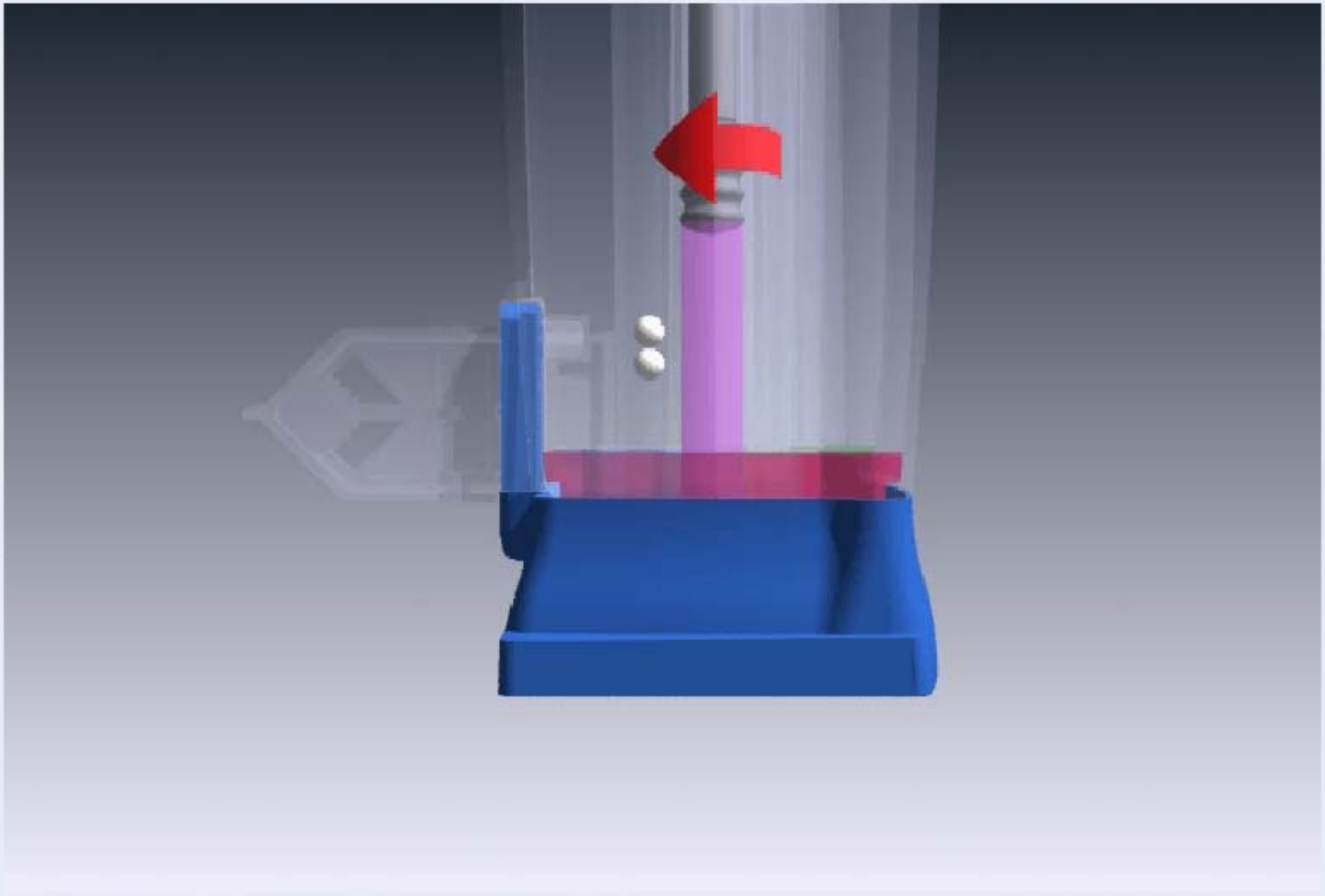


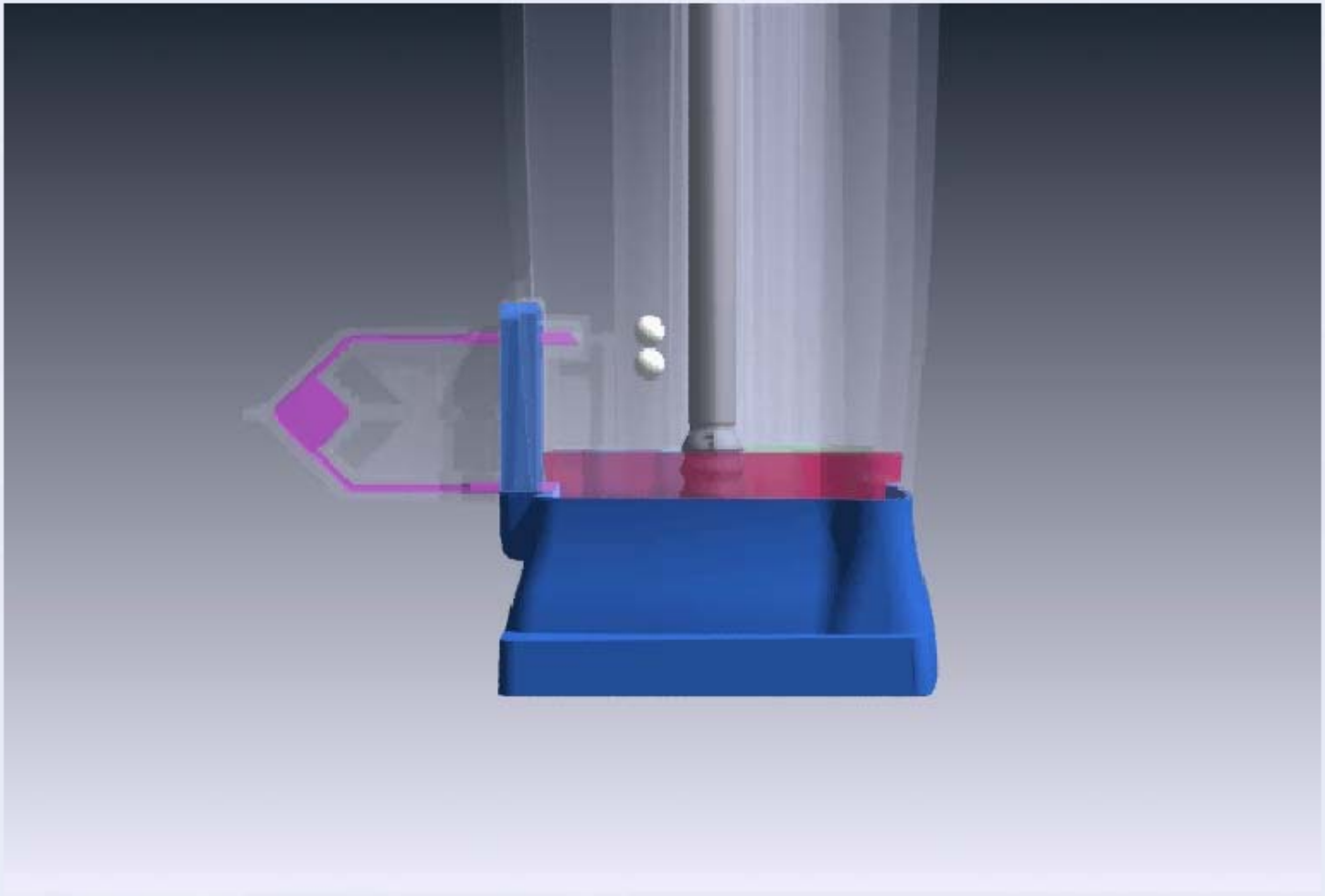

GeneXpert.
GeneXpert
Xpert MRSA

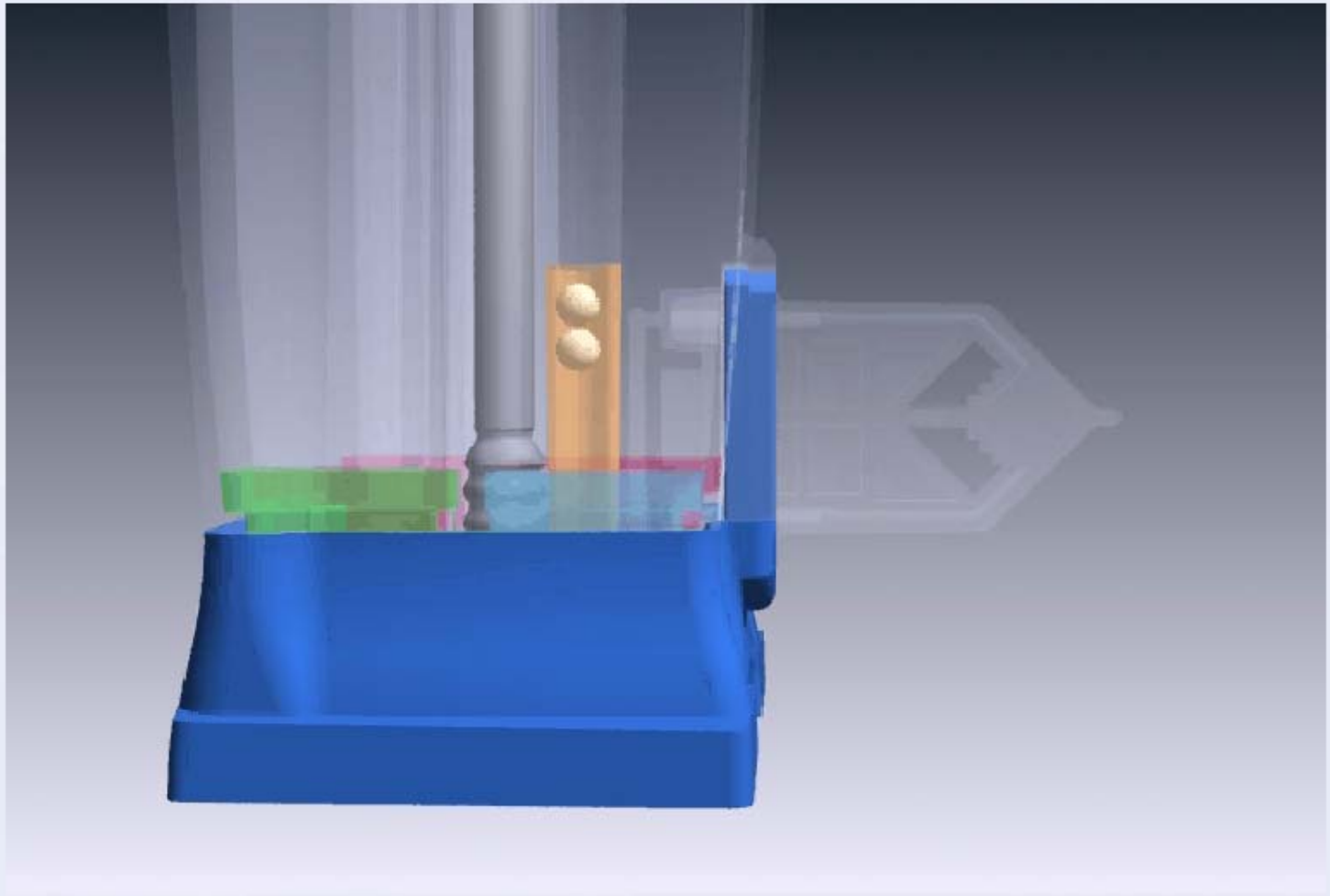


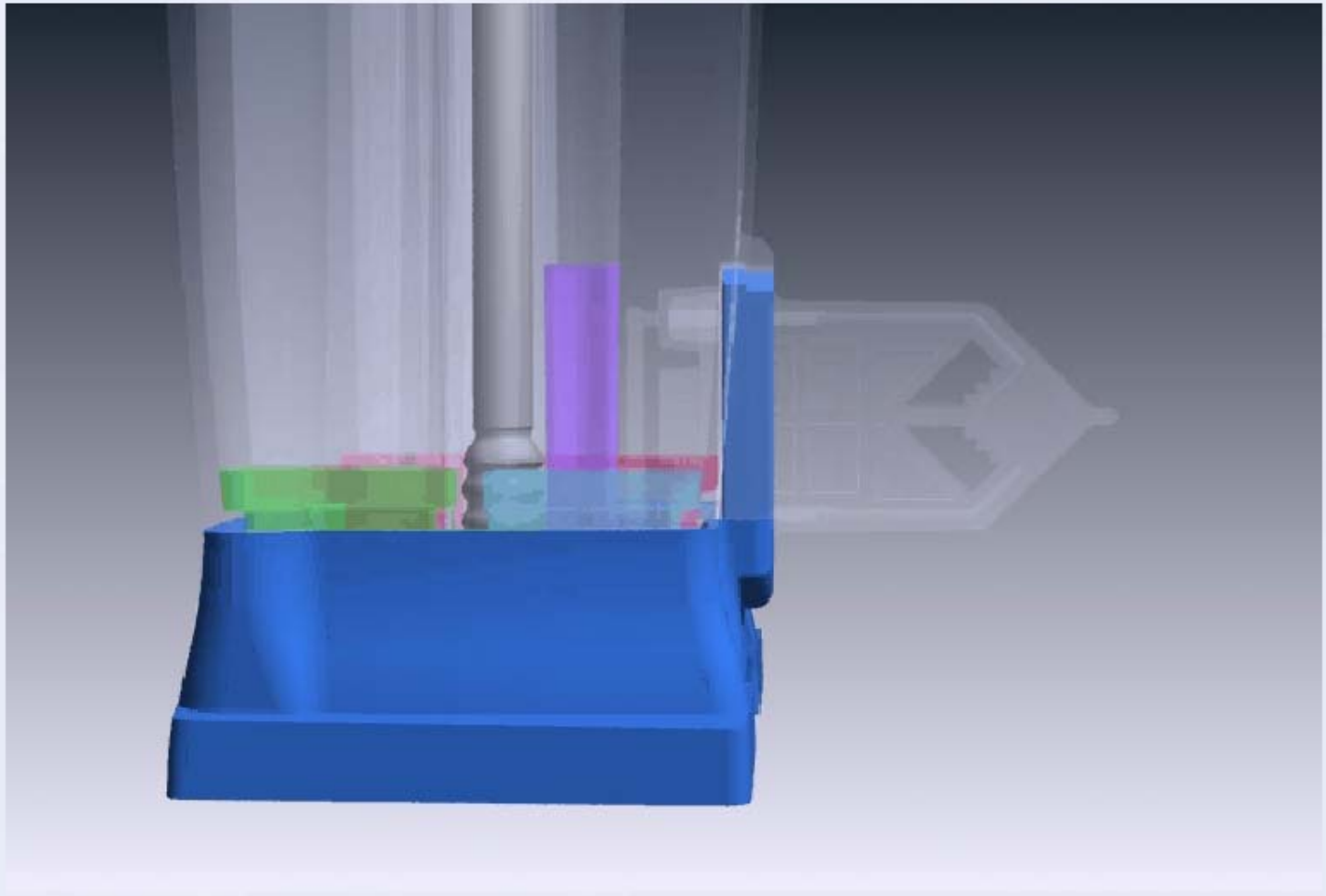


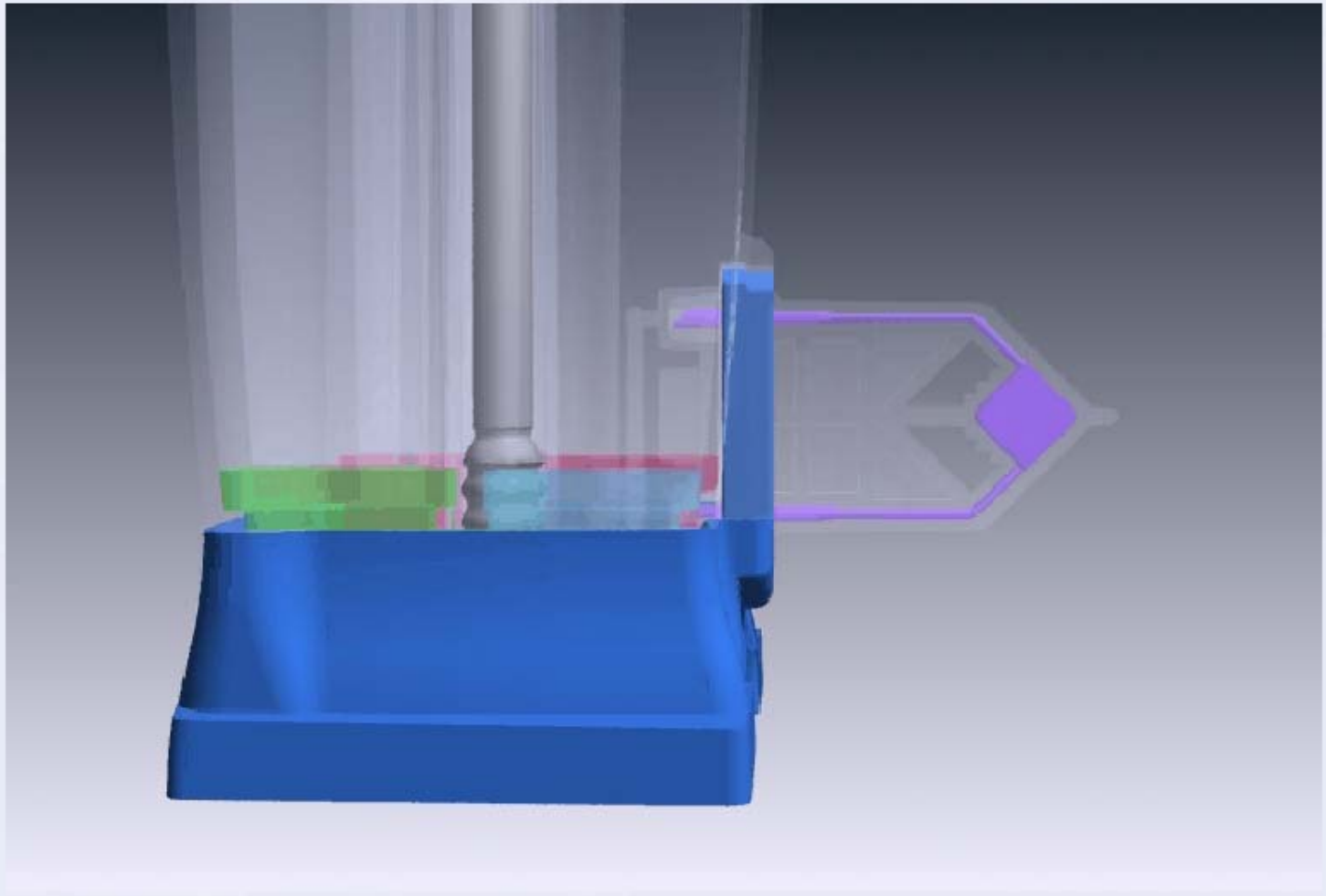


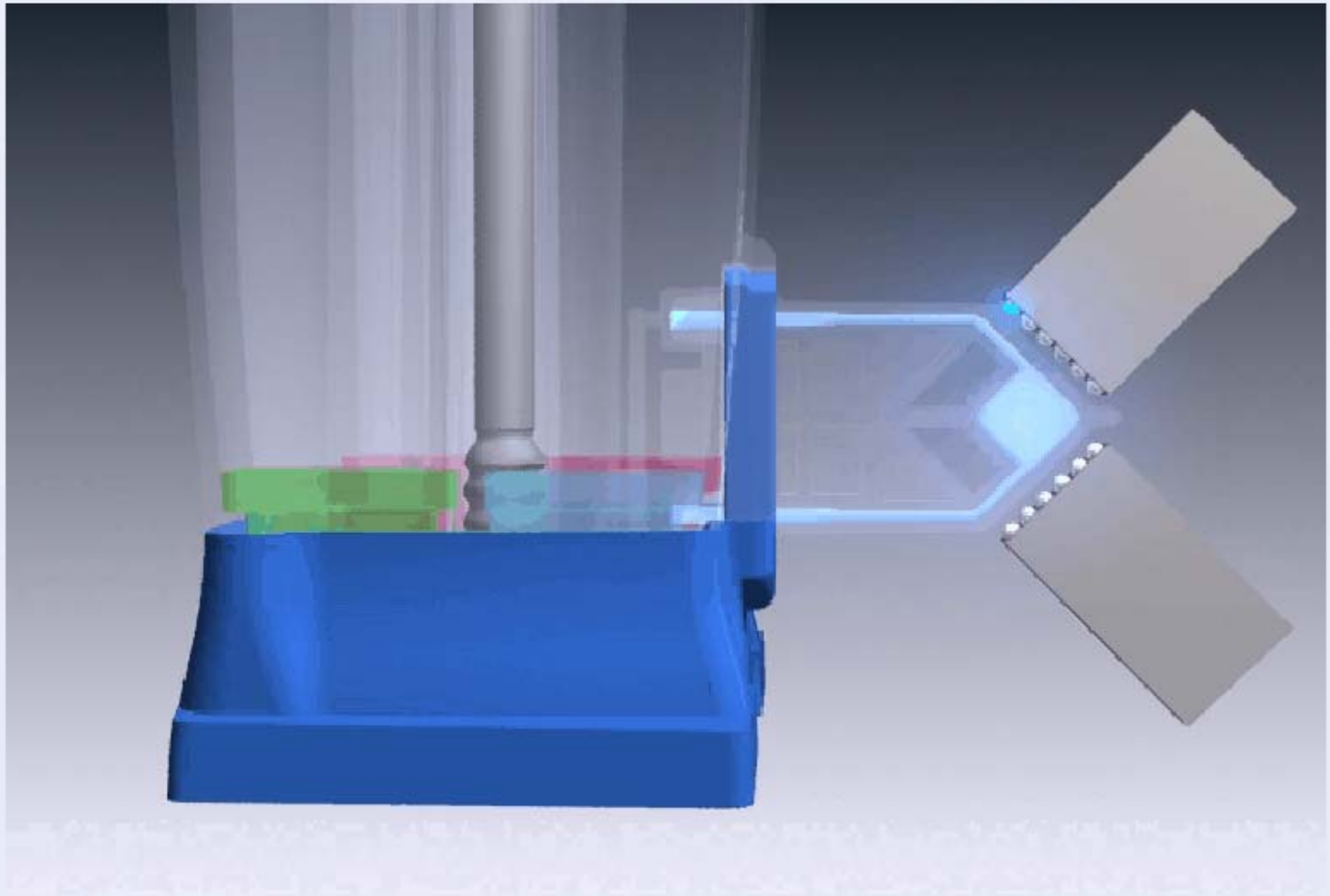


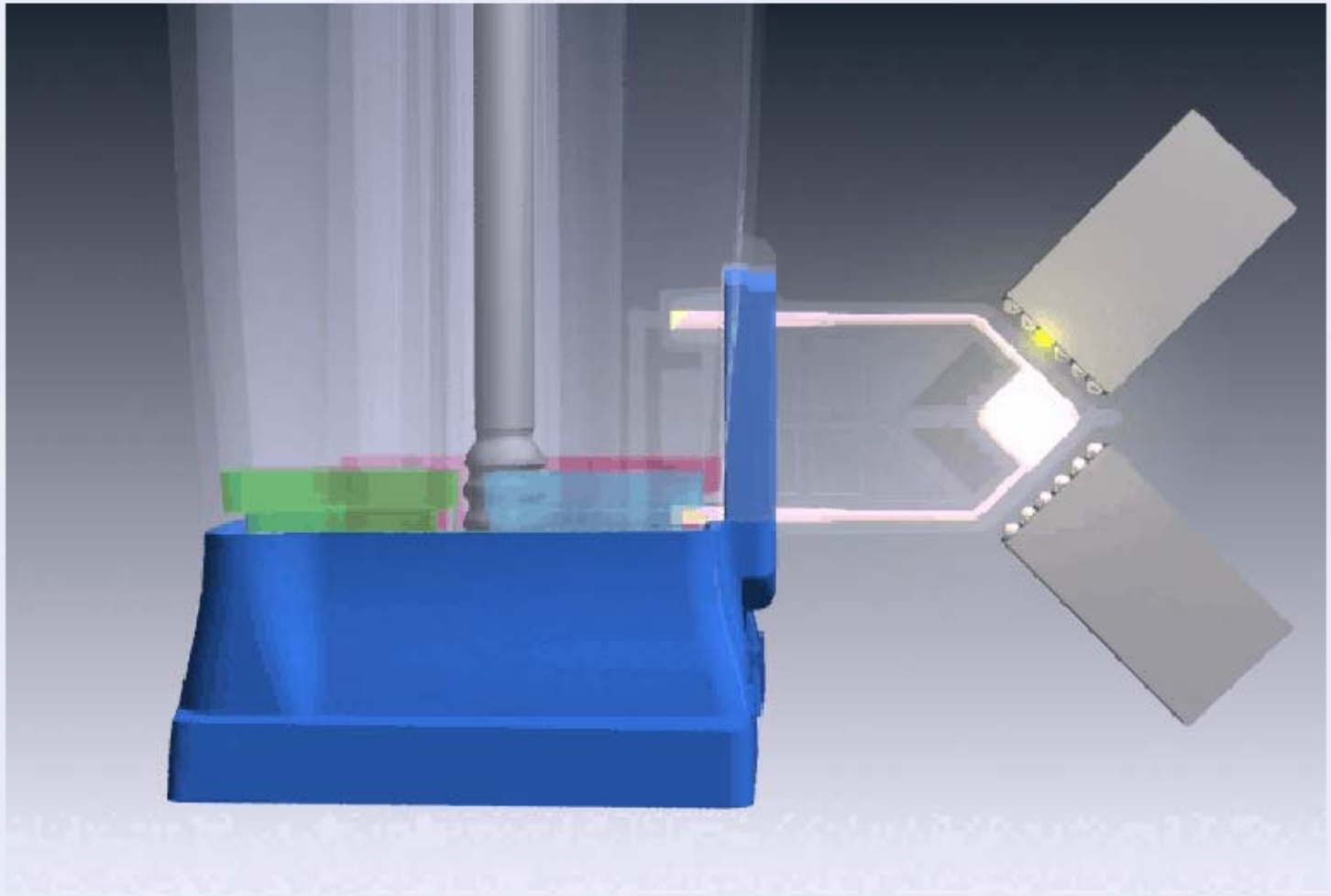


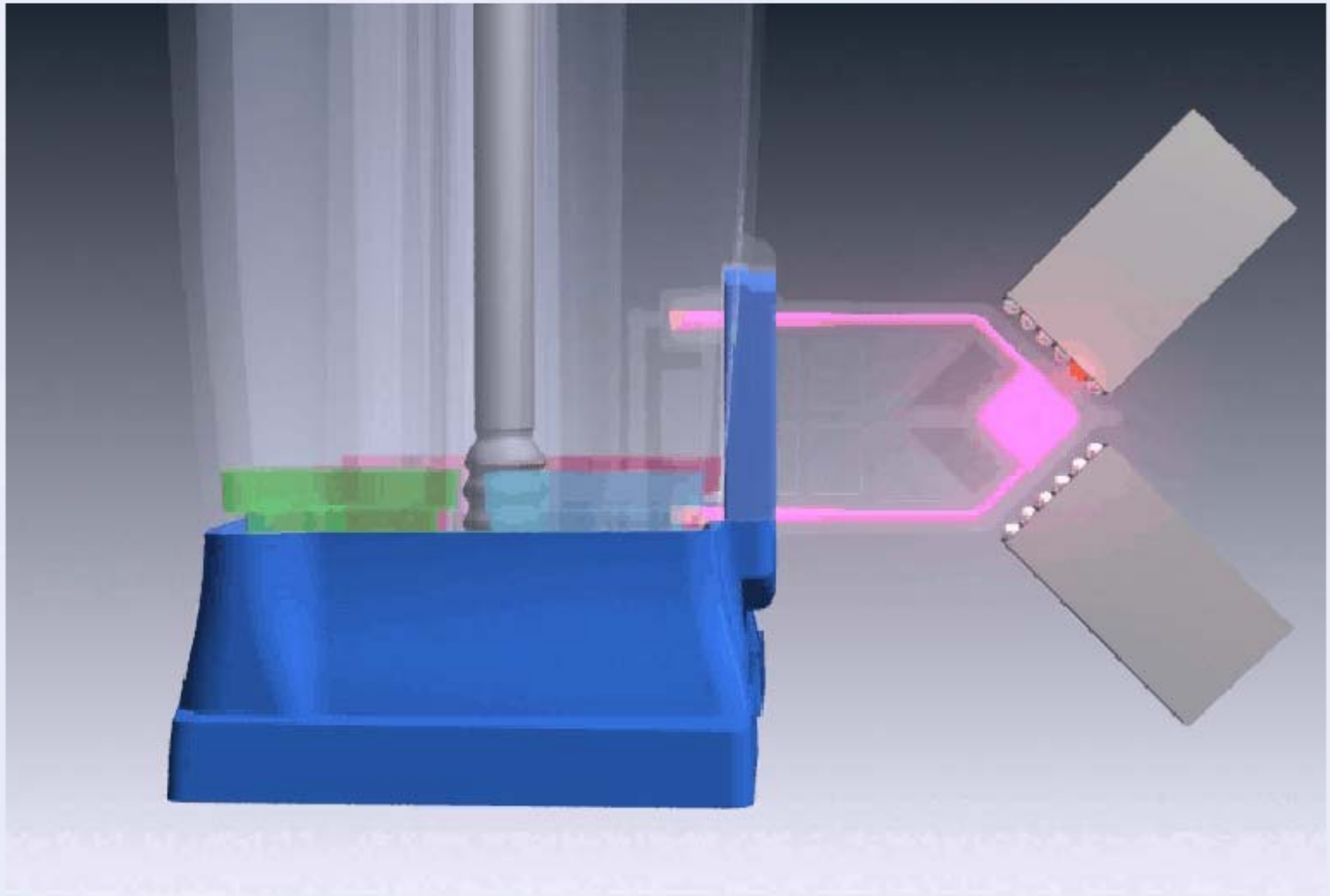


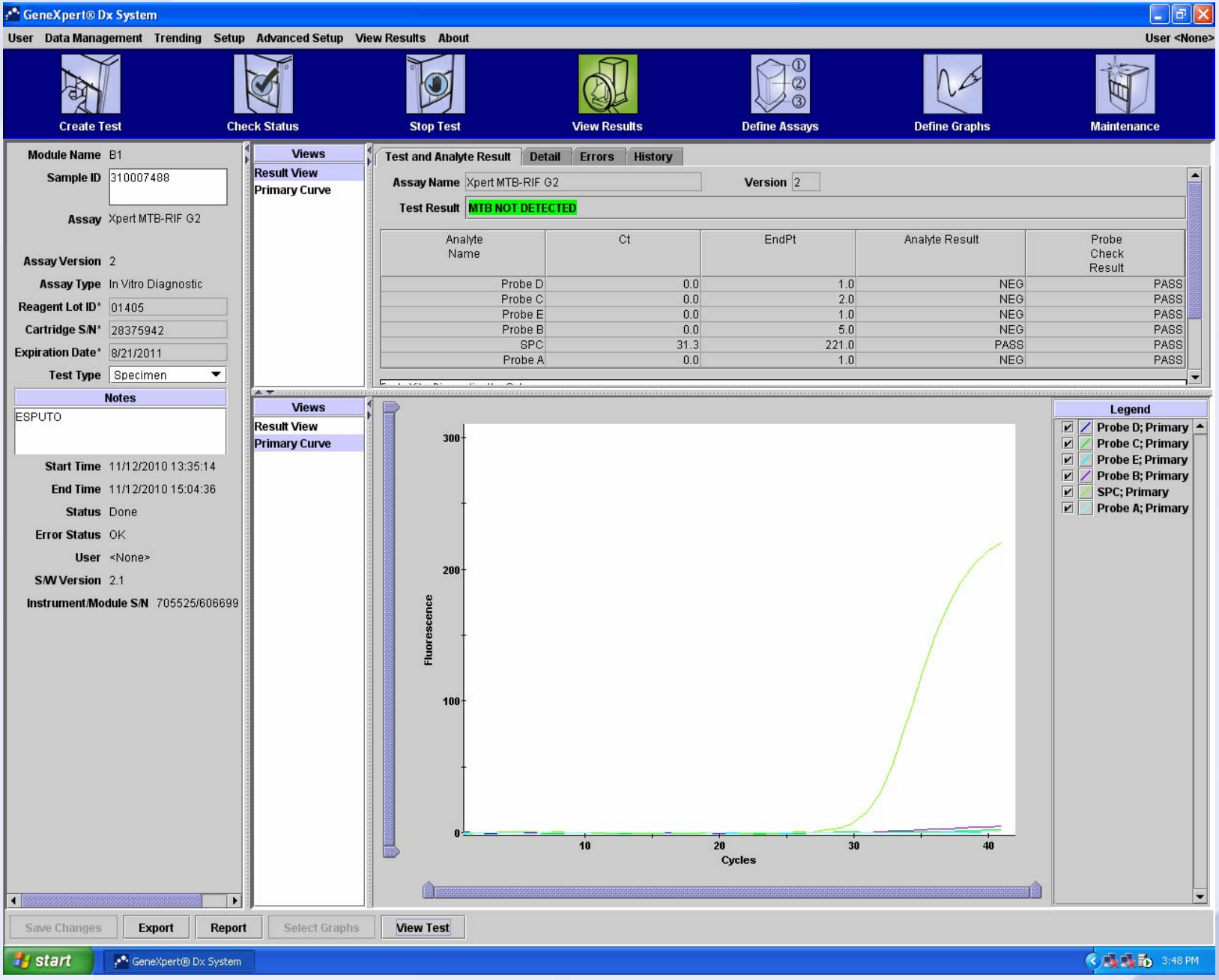








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User <None>
Create Test
Check Status
Stop Test
View Results
Define Assays
Define Graphs
Maintenance
Module Name B1
Sample ID 310007488
Assay Xpert MTB-RIF G2
Assay Version 2
Assay Type In Vitro Diagnostic
Reagent Lot ID* 01405
Cartridge S/N* 28375942
Expiration Date* 8/21/2011
Test Type Specimen
Notes
ESPUTO
Start Time 11/12/2010 13:35:14
End Time 11/12/2010 15:04:36
Status Done
Error Status OK
User <None>
S/W Version 2.1
Instrument/Module S/N 705525/606699
Views
Result View
Primary Curve
Test and Analyte Result
Detail
Errors
History
Assay Name Xpert MTB-RIF G2
Version 2
Test Result MTB NOT DETECTED
Analyte Name | Ct | EndPt | Analyte Result | Probe Check Result
Probe D | 0.0 | 1.0 | NEG | PASS
Probe C | 0.0 | 2.0 | NEG | PASS
Probe E | 0.0 | 1.0 | NEG | PASS
Probe B | 0.0 | 5.0 | NEG | PASS
SPC | 31.3 | 221.0 | PASS | PASS
Probe A | 0.0 | 1.0 | NEG | PASS
Views
Result View
Primary Curve
Fluorescence
300
200
100
0
10
20
30
40
Cycles
Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
SPC; Primary
Probe A; Primary
Save Changes
Export
Report
Select Graphs
View Test
start
GeneXpert® Dx System
3:48 PM

GeneXpert PC 10/16/2010 10:17:54

Test Report

Sample ID: 310006921
Test Type: Specimen

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF G2	2	In Vitro Diagnostic

Test Result: **MTB NOT DETECTED**

Test and Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	0	1	NEG	PASS
Probe C	0	1	NEG	PASS
Probe E	0	-2	NEG	PASS
Probe B	0	2	NEG	PASS
SPC	28.9	208	PASS	PASS
Probe A	0	-1	NEG	PASS

User:	<None>		
Status:	Done	Start Time:	10/15/2010 12:08:20
Reagent Lot ID*:	01403	End Time:	10/15/2010 13:37:09
Expiration Date*:	7/31/2011	Module Name:	A4
Cartridge S/N*:	28277455	Module S/N:	606181
S/W Version:	2.1	Instrument S/N:	705524
Notes:	AS. TRAQUEAL		
Error Status:	OK		

Errors

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* indicates that a particular field is entered using a barcode scanner

For In Vitro Diagnostics Use Only.

GeneXpert® Dx System

User | Data Management | Trending | Setup | Advanced Setup | View Results | About | User <None>

Create Test | Check Status | Stop Test | View Results | Define Assays | Define Graphs | Maintenance

Module Name B3
Sample ID 310007477
Assay Xpert MTB-RIF G2

Assay Version 2
Assay Type In Vitro Diagnostic
Reagent Lot ID* 01405
Cartridge S/N* 28375908
Expiration Date* 8/21/2011
Test Type Specimen

Notes
AS. BRONQUIAL

Start Time 11/12/2010 11:55:08
End Time 11/12/2010 13:24:22
Status Done
Error Status OK
User <None>
S/W Version 2.1
Instrument/Module S/N 705525/606703

Views
Result View
Primary Curve

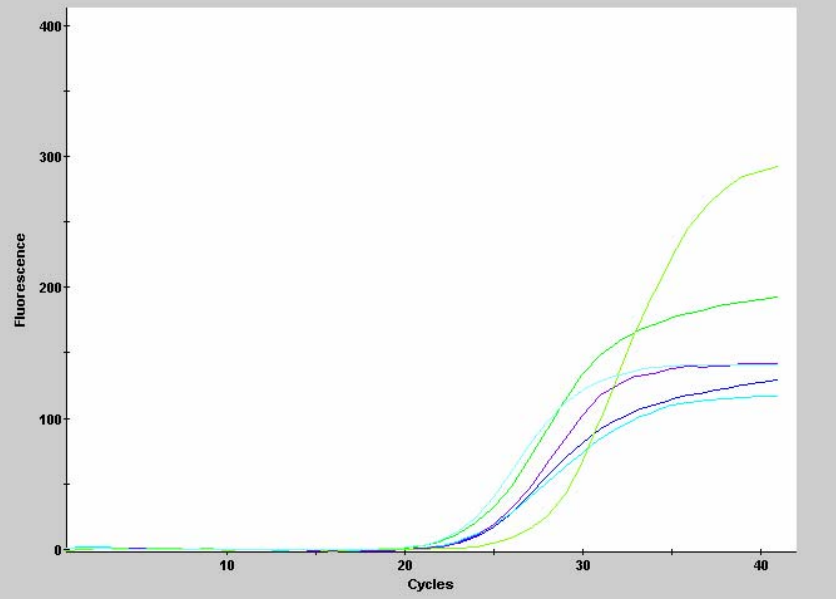
Test and Analyte Result | Detail | Errors | History

Assay Name Xpert MTB-RIF G2 Version 2

Test Result MTB DETECTED LOW;
Rif Resistance NOT DETECTED

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	25.3	130.0	POS	PASS
Probe C	24.0	193.0	POS	PASS
Probe E	25.2	117.0	POS	PASS
Probe B	25.1	142.0	POS	PASS
SPC	27.5	292.0	NA	PASS
Probe A	23.6	141.0	POS	PASS

Views
Result View
Primary Curve



Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
SPC; Primary
Probe A; Primary

Save Changes | Export | Report | Select Graphs | View Test

start | GeneXpert® Dx System | Document1 - Microsof... | 3:50 PM

GeneXpert PC 11/16/2010 15:22:41

Test Report

Sample ID: 310007523
Test Type: Specimen

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF G2	3	In Vitro Diagnostic

Test Result: MTB DETECTED VERY LOW;
Rif Resistance NOT DETECTED

Test and Analyte Result

Analyte Name	Ct	End Pt	Analyte Result	Probe Check Result
Probe D	32.0	84	POS	PASS
Probe C	30.2	166	POS	PASS
Probe E	31.2	93	POS	PASS
Probe B	31.7	113	POS	PASS
SPC	34.1	185	NA	PASS
Probe A	29.7	146	POS	PASS

User:	<None>		
Status:	Done	Start Time:	11/16/2010 09:46:35
Reagent Lot ID*:	02003	End Time:	11/16/2010 11:14:29
Expiration Date*:	10/2/2011	Module Name:	B1
Cartridge S/N*:	28873252	Module S/N:	606699
S/W Version:	2.1	Instrument S/N:	705525
Notes:	esputo		
Error Status:	OK		

Errors
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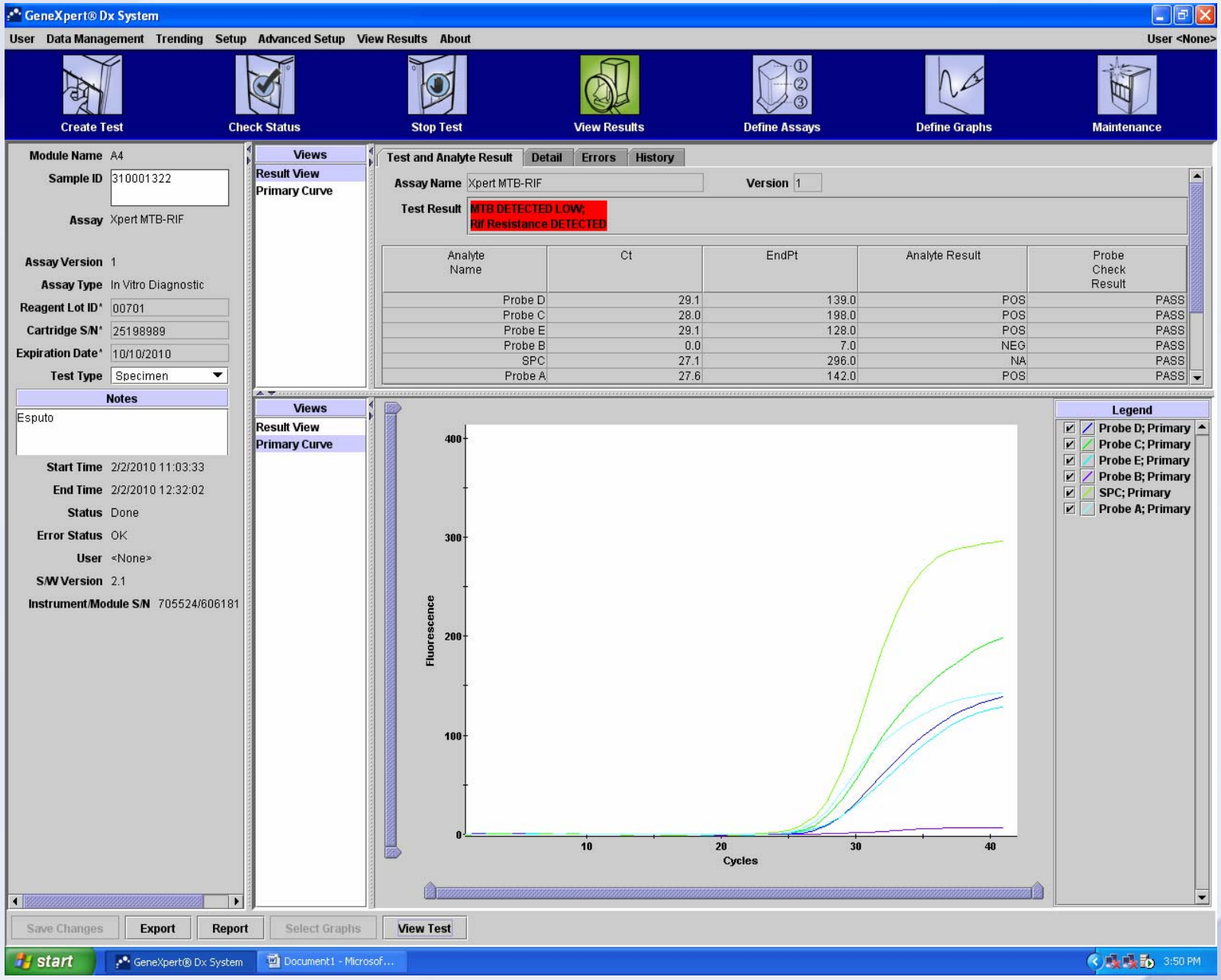
* indicates that a particular field is entered using a barcode scanner

For In Vitro Diagnostics Use Only.


GeneXpert® Dx System
User Data Management Trending Setup Advanced Setup View Results About
User <None>
Create Test
Check Status
Stop Test
View Results
Define Assays
Define Graphs
Maintenance
Module Name A4
Sample ID 310001322
Assay Xpert MTB-RIF
Assay Version 1
Assay Type In Vitro Diagnostic
Reagent Lot ID* 00701
Cartridge S/N* 25198989
Expiration Date* 10/10/2010
Test Type Specimen
Notes
Esputo
Start Time 2/2/2010 11:03:33
End Time 2/2/2010 12:32:02
Status Done
Error Status OK
User <None>
S/W Version 2.1
Instrument/Module S/N 705524/606181
Views
Result View
Primary Curve
Test and Analyte Result
Detail
Errors
History
Assay Name Xpert MTB-RIF
Version 1
Test Result MTB DETECTED LOW;
Rif Resistance DETECTED
Analyte Name
Ct
EndPt
Analyte Result
Probe Check Result
Probe D 29.1 139.0 POS PASS
Probe C 28.0 198.0 POS PASS
Probe E 29.1 128.0 POS PASS
Probe B 0.0 7.0 NEG PASS
SPC 27.1 296.0 NA PASS
Probe A 27.6 142.0 POS PASS
Views
Result View
Primary Curve
Fluorescence
400
300
200
100
0
10
20
30
40
Cycles
Legend
Probe D; Primary
Probe C; Primary
Probe E; Primary
Probe B; Primary
SPC; Primary
Probe A; Primary
Save Changes
Export
Report
Select Graphs
View Test
start
GeneXpert® Dx System
Document1 - Microsof...
3:50 PM

Sample ID: 310001322
Test Type: Specimen

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF	1	In Vitro Diagnostic

Test Result: MTB DETECTED LOW;
Rif Resistance DETECTED

Test and Analyte Result

Analyte Name	Ct	End Pt	Analyte Result	Probe Check Result
Probe D	29.1	139	POS	PASS
Probe C	28.0	198	POS	PASS
Probe E	29.1	128	POS	PASS
Probe B	0	7	NEG	PASS
SPC	27.1	296	NA	PASS
Probe A	27.6	142	POS	PASS

User:	<None>		
Status:	Done	Start Time:	2/2/2010 11:03:33
Reagent Lot ID*:	00701	End Time:	2/2/2010 12:32:02
Expiration Date*:	10/10/2010	Module Name:	A4
Cartridge S/N*:	25198989	Module S/N:	606181
S/W Version:	2.1	Instrument S/N:	705524
Notes:	Esputo		
Error Status:	OK		

Errors
<None>

* indicates that a particular field is entered using a barcode scanner

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Xpert MTB/RIF

Nuestra experiencia

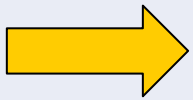
Resultados globales

Resultados globales

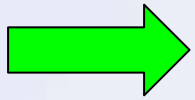
	n
Esputo	368
Aspirado bronquial	197
BAL	49
Orina	29
LCR	81
Líquido pleural	159
Líquido ascítico	24
Líquido articular	10
Líquido pericárdico	4
Sangre	16
Biopsia	69
Exudado purulento	55
PAAF	22
Médula ósea	12
Jugo gástrico	10
Heces	3
Total	1.108

Resultados globales

	n
Esputo	368
Aspirado bronquial	197
BAL	49
Orina	29
LCR	81
Líquido pleural	159
Líquido ascítico	24
Líquido articular	10
Líquido pericárdico	4
Sangre	16
Biopsia	69
Exudado purulento	55
PAAF	22
Médula ósea	12
Jugo gástrico	10
Heces	3
Total	1.108



614 muestras respiratorias



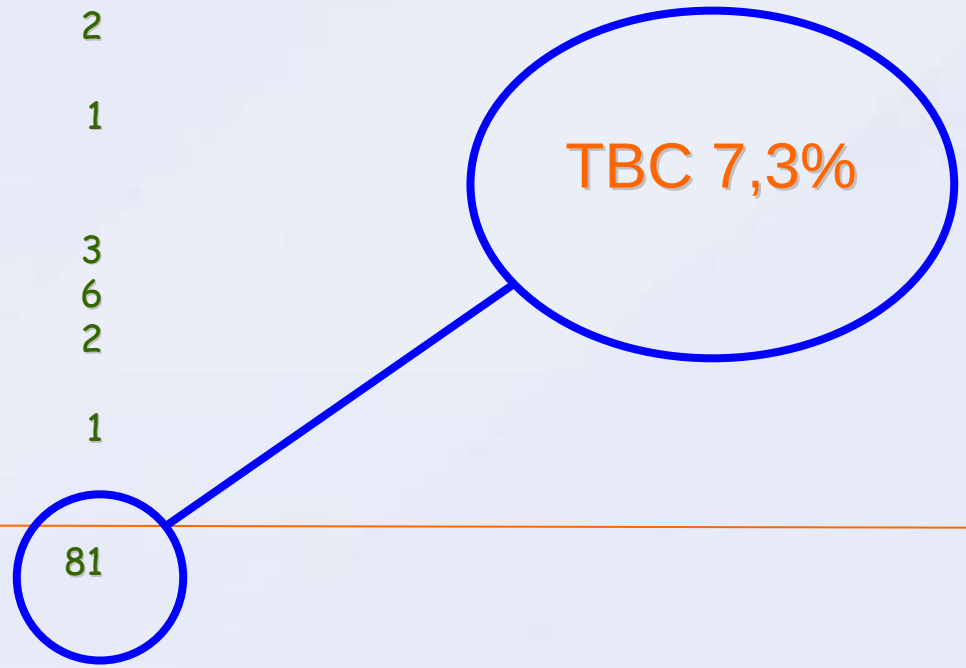
494 muestras extra-respiratorias

Resultados globales

	n	TBC
Esputo	368	43
Aspirado bronquial	197	14
BAL	49	3
Orina	29	4
LCR	81	2
Líquido pleural	159	2
Líquido ascítico	24	
Líquido articular	10	1
Líquido pericárdico	4	
Sangre	16	
Biopsia	69	3
Exudado purulento	55	6
PAAF	22	2
Médula ósea	12	
Jugo gástrico	10	1
Heces	3	
Total	1.108	81

Resultados globales

	n	TBC
Esputo	368	43
Aspirado bronquial	197	14
BAL	49	3
Orina	29	4
LCR	81	2
Líquido pleural	159	2
Líquido ascítico	24	
Líquido articular	10	1
Líquido pericárdico	4	
Sangre	16	
Biopsia	69	3
Exudado purulento	55	6
PAAF	22	2
Médula ósea	12	
Jugo gástrico	10	1
Heces	3	
Total	1.108	81



Resultados globales

	n	TBC	BK (+)
Esputo	368	43	32
Aspirado bronquial	197	14	2
BAL	49	3	
Orina	29	4	1
LCR	81	2	
Líquido pleural	159	2	
Líquido ascítico	24		
Líquido articular	10	1	
Líquido pericárdico	4		
Sangre	16		
Biopsia	69	3	1
Exudado purulento	55	6	
PAAF	22	2	
Médula ósea	12		
Jugo gástrico	10	1	1
Heces	3		
Total	1.108	81	37

Resultados globales

	n	TBC	BK (+)
Esputo	368	43	32
Aspirado bronquial	197	14	2
BAL	49	3	
Orina	29	4	1
LCR	81	2	
Líquido pleural	159	2	
Líquido ascítico	24		
Líquido articular	10	1	
Líquido pericárdico	4		
Sangre	16		
Biopsia	69	3	1
Exudado purulento	55	6	
PAAF	22	2	
Médula ósea	12		
Jugo gástrico	10	1	1
Heces	3		
Total	1.108	81	37

56,6% Baciloscopia POSITIVA

34/60

14,2% Baciloscopia POSITIVA

3/21

45,6% Baciloscopia POSITIVA

Resultados globales

	n	TBC	BK	MGIT 960	
			(+)	(+)	NEG
Esputo	368	43	32	42	1
Aspirado bronquial	197	14	2	14	
BAL	49	3		2	1
Orina	29	4	1	4	
LCR	81	2		2	
Líquido pleural	159	2		2	
Líquido ascítico	24				
Líquido articular	10	1		1	
Líquido pericárdico	4				
Sangre	16				
Biopsia	69	3	1	3	
Exudado purulento	55	6		6	
PAAF	22	2		2	
Médula ósea	12				
Jugo gástrico	10	1	1	1	
Heces	3				
Total	1.108	81	37	79	2

Resultados globales

	n	TBC	BK	MGIT 960		BacTAlert 3D	
			(+)	(+)	NEG	(+)	NEG
Esputo	368	43	32	42	1	43	
Aspirado bronquial	197	14	2	14		14	
BAL	49	3		2	1	3	
Orina	29	4	1	4		4	
LCR	81	2		2		2	
Líquido pleural	159	2		2		1	1
Líquido ascítico	24						
Líquido articular	10	1		1		1	
Líquido pericárdico	4						
Sangre	16						
Biopsia	69	3	1	3		3	
Exudado purulento	55	6		6		5	1
PAAF	22	2		2		2	
Médula ósea	12						
Jugo gástrico	10	1	1	1		1	
Heces	3						
Total	1.108	81	37	79	2	79	2

Resultados globales

	n	TBC	BK	MGIT 960		BacTAlert 3D		Löw-Jensen	
			(+)	(+)	NEG	(+)	NEG	(+)	NEG
Esputo	368	43	32	42	1	43		38	5
Aspirado bronquial	197	14	2	14		14		14	
BAL	49	3		2	1	3		3	1
Orina	29	4	1	4		4		3	1
LCR	81	2		2		2		2	
Líquido pleural	159	2		2		1	1	2	
Líquido ascítico	24								
Líquido articular	10	1		1		1		1	
Líquido pericárdico	4								
Sangre	16								
Biopsia	69	3	1	3		3		3	
Exudado purulento	55	6		6		5	1	5	1
PAAF	22	2		2		2		1	1
Médula ósea	12								
Jugo gástrico	10	1	1	1		1		1	
Heces	3								
Total	1.108	81	37	79	2	79	2	72	9

Resultados globales

	n	TBC	BK	MGIT 960		BacTAlert 3D		Löw-Jensen		Xpert MTB/RIF	
			(+)	(+)	NEG	(+)	NEG	(+)	NEG	(+)	NEG
Esputo	368	43	32	42	1	43		38	5	42	1
Aspirado bronquial	197	14	2	14		14		14		14	
BAL	49	3		2	1	3		3	1	2	1
Orina	29	4	1	4		4		3	1	4	
LCR	81	2		2		2		2		2	
Líquido pleural	159	2		2		1	1	2		1	1
Líquido ascítico	24										
Líquido articular	10	1		1		1		1			1
Líquido pericárdico	4										
Sangre	16										
Biopsia	69	3	1	3		3		3		3	
Exudado purulento	55	6		6		5	1	5	1	5	1
PAAF	22	2		2		2		1	1	2	
Médula ósea	12										
Jugo gástrico	10	1	1	1		1		1		1	
Heces	3										
Total	1.108	81	37	79	2	79	2	72	9	76	5

Resultados COMPARATIVOS

	Examen microscópico		
	POS (n=37)	NEG (n=44)	Total (n=81)
Bactec MGIT 960	37	42	79
BacTAlert 3D	37	42	79
Löw-Jensen	37	35	72
Xpert MTB/RIF	37	39	76

Resultados COMPARATIVOS

	Examen microscópico		
	POS (n=37)	NEG (n=44)	Total (n=81)
Bactec MGIT 960	37	42	79
BacTAlert 3D	37	42	79
Löw-Jensen	37	35	72
Xpert MTB/RIF	37	39	76

Resultados GLOBALES

		TBC	No TBC	Total
Xpert MTB/RIF	POS	76	0	76
	NEG	5	1027	1032
	Total	81	1027	1108

Sensibilidad 93,8% Especificidad 100%

VPP 100% VPN 99,5% Concordancia 99,5%

Resultados MUESTRAS RESPIRATORAS

		TBC	No TBC	Total
Xpert MTB/RIF	POS	58	0	58
	NEG	2	554	556
	Total	60	554	614

Sensibilidad 96,6% Especificidad 100%

VPP 100% VPN 99,6% Concordancia 99,6%

Resultados
MUESTRAS EXTRA-RESPIRATORAS

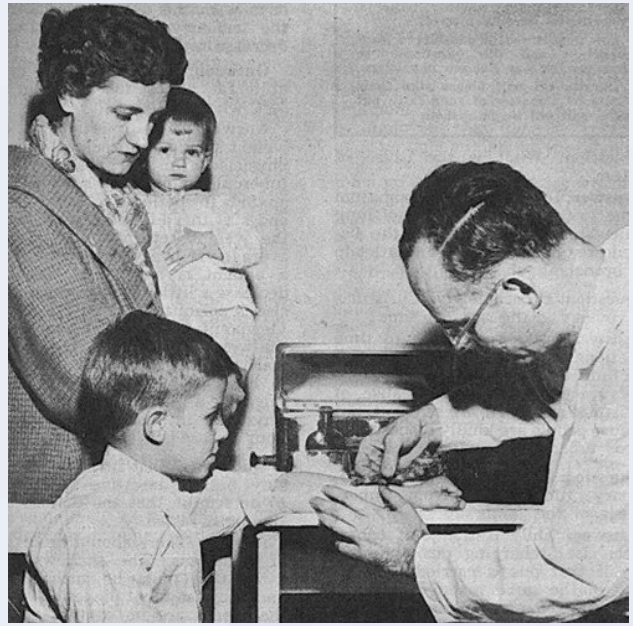
		TBC	No TBC	Total
Xpert MTB/RIF	POS	18	0	18
	NEG	3	473	476
	Total	21	473	494

Sensibilidad 85,7% Especificidad 100%

VPP 100% VPN 99,3% Concordancia 99,2%

Avances en el diagnóstico de la Infección tuberculosa

Prueba de la Tuberculina

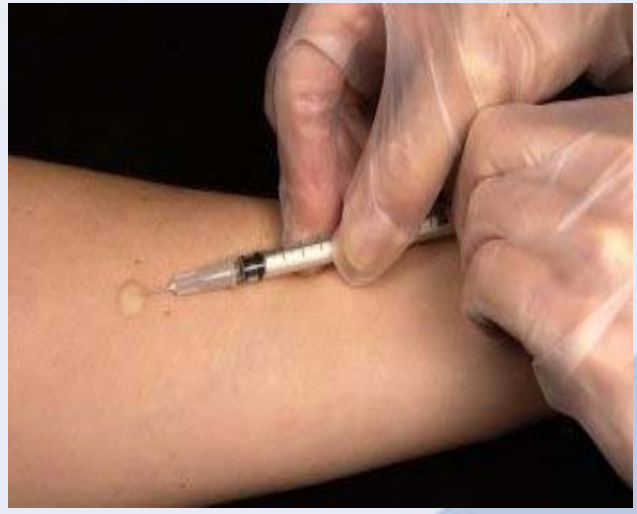


El diagnóstico de la Infección tuberculosa latente se sustenta en la prueba cutánea de la tuberculina (Mantoux).

Es una prueba diagnóstica con más de 100 años de antigüedad.

La prueba de la tuberculina pone de manifiesto un estado de hipersensibilidad del organismo frente a las proteínas del bacilo tuberculoso, que se adquiere después de una infección producida por *M. tuberculosis*

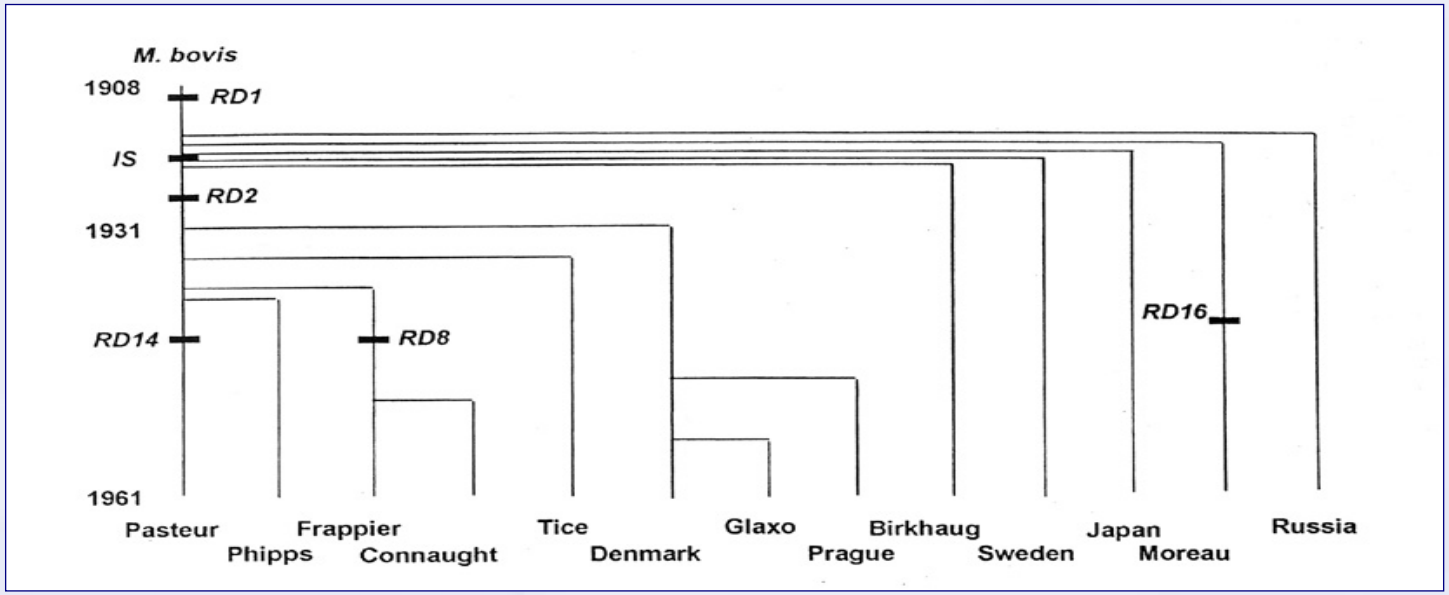
- Proteínas filtradas de cultivos (Derivado Proteico Purificado, PPD)
- Inyección intradérmica
- La induración se mide 48-72h después



IGRAs *Interferon-gamma-release assays*

"Pruebas ***in vitro*** que detectan la liberación de **Interferón-gamma** en respuesta a una estimulación con antígenos tuberculosos específicos **ESAT-6** (*early-secreted antigenic target* 6), **CFP-10** (*culture filtrate protein* 10), **TB7.7(P4)**

IGRAs fundamentos

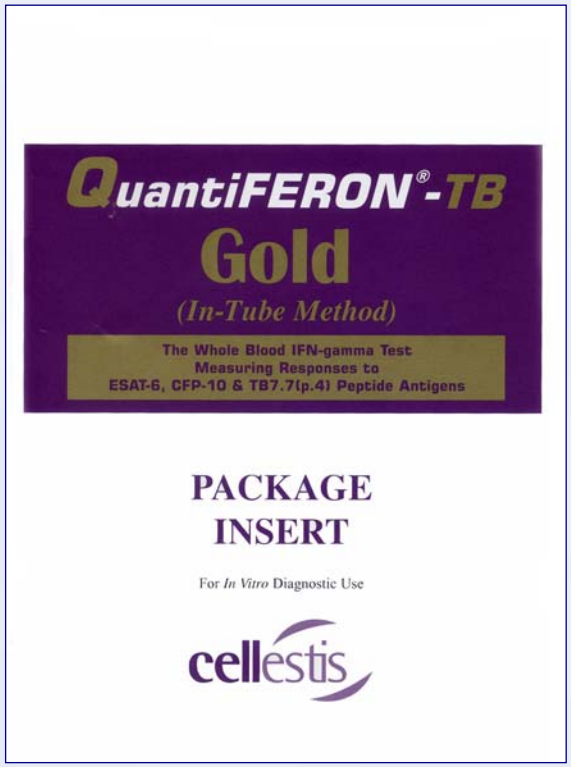


- Los antígenos utilizados ESAT-6 y CFP 10 derivan de la región RD1 de MTB
- La región RD1 no está en las cepas de BCG (se pierde durante la derivación de *M. bovis BCG* de *M. bovis* entre 1908 y 1921 en el Instituto Pasteur)
- No se producen falsos positivos en individuos vacunados con BCG
- La región RD1 tampoco está presente en la mayoría de las micobacterias ambientales, excepto *M. kansasii, M. marinum, M. szulgai*

Formato de IGRAs disponibles:

- QuantiFERON-TB Gold In-Tube (Cellestis, Australia)
- T-SPOT.TB (Oxford Immunotec, U.K.)

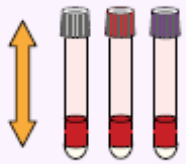
QuantiFERON-TB Gold In-Tube



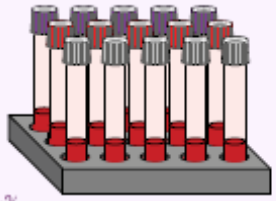
QuantiFERON®-TB Gold In-Tube

Assay Quick Reference Guide

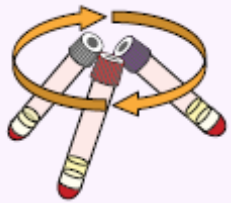
Part 1. Blood Incubation and Harvesting



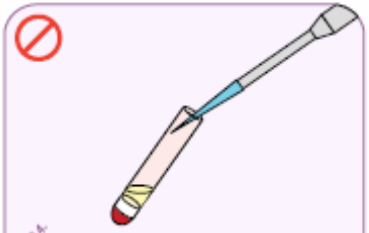
Step 1. After blood collection, mix QuantiFERON®-TB Gold tubes thoroughly, by shaking vigorously for 5 seconds.



Step 2. As soon as possible, and within 16 hours of collection, incubate tubes upright at 37°C for 16-24 hours.

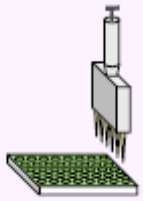


Step 3. Centrifuge tubes at 2000-3000 g (RCF) for 15 minutes.

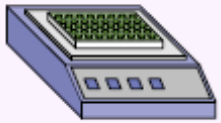


Step 4. Harvest at least 200 µL plasma from each tube. Store in racked microtubes or uncoated microplates.

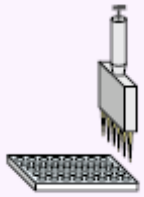
Part 2. Human IFN-γ ELISA



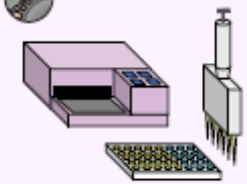
Step 5. Add 50 µL of working conjugate to each well. Add 50 µL of plasma or standard.



Step 6. Shake covered plate for 1 min. Incubate for 120 minutes at room temperature.



Step 7. Wash plate ≥ 6 times. Add 100 µL of substrate. Incubate 30 min. at room temperature.



Step 8. Add 50 µL of stop solution. Read absorbance within 5 min. at 450 nm (620-650 nm ref).

Step 9. Calculate results using QuantiFERON®-TB Gold In-Tube Analysis Software.

QuantiFERON-TB Gold In-Tube

1 of 2

cellestis

Version 2.50

QuantiFERON®-TB Gold In-Tube Results

Test Date: jueves 11 octubre 2007
Operator: delfi
Run Number: 1
Kit Batch Number: 50131

Valid ELISA test run.

Results

Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
11275	0.10	0.11	20.33	0.01	20.23	NEGATIVE
11345	0.06	1.34	12.04	1.28	11.98	POSITIVE
11346	0.08	1.43	20.87	1.37	20.81	POSITIVE
11463	0.07	0.07	4.16	0.00	4.09	NEGATIVE
11464	0.10	0.12	9.04	0.02	8.94	NEGATIVE
11465	1.23	1.75	20.41	0.52	19.18	POSITIVE
11600	0.12	0.48	8.34	0.36	8.22	POSITIVE

Signature _____ Date _____

cellestis

Version 2.50

QuantiFERON®-TB Gold In-Tube Results

Test Date: martes 4 mayo 2010
Operator: Admin
Run Number: 1
Kit Batch Number: 1

Valid ELISA test run.

Results

Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
310003649	0.08	0.34	13.57	0.26	13.49	NEGATIVE
310003670	0.09	0.09	13.57	0.00	13.48	NEGATIVE
310003681	0.22	0.21	13.57	-0.01	13.35	NEGATIVE
310003684	0.08	0.10	13.57	0.02	13.49	NEGATIVE
310003686	0.09	2.31	3.01	2.22	2.92	POSITIVE
310003687	0.07	0.07	13.57	0.00	13.50	NEGATIVE
310003706	0.06	0.06	13.57	0.00	13.51	NEGATIVE
310003727	0.28	2.31	6.76	2.03	6.48	POSITIVE
310003741	0.05	0.06	3.30	0.01	3.25	NEGATIVE
310003742	0.06	0.25	1.73	0.19	1.67	NEGATIVE
310003744	0.08	1.40	13.57	1.32	13.49	POSITIVE
310003746	0.05	0.05	13.57	0.00	13.52	NEGATIVE

Signature _____ Date _____

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB [IU/mL]	Report/Interpretation
≤ 8.0	< 0.35	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35	< 0.5	**Indeterminate**[3]	Results are indeterminate for TB-Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0[4]	Any	Any		

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB [IU/mL]	Report/Interpretation
≤ 8.0	< 0.35	≥ 0.5	**Negative**	*M. tuberculosis* infection NOT likely
	≥ 0.35 and < 25% of Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35	< 0.5	**Indeterminate**[3]	Results are indeterminate for TB-Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0[4]	Any	Any		

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL][1]	QuantiFERON®-TB [IU/mL]	Report/Interpretation
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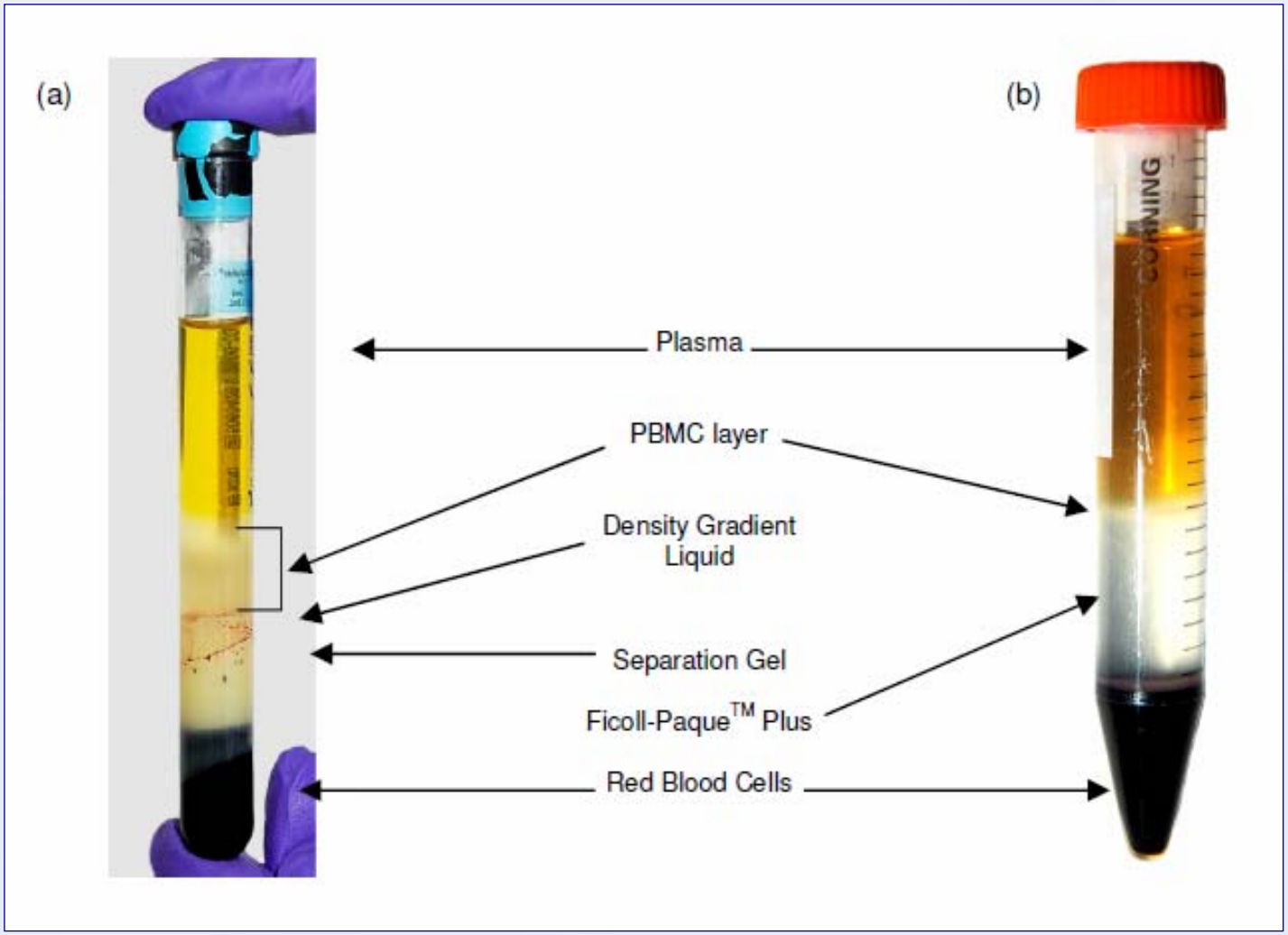
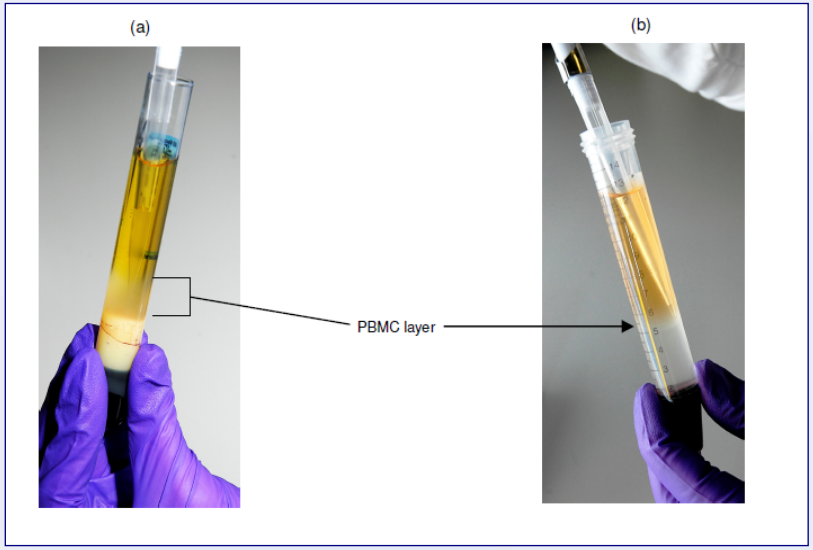
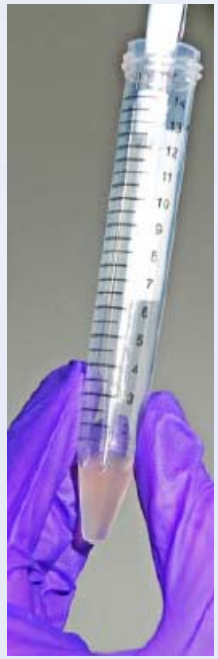
	≥ 0.35 and ≥ 25% of Nil value	Any	**Positive**[2]	*M. tuberculosis* infection likely
	< 0.35	< 0.5	**Indeterminate**[3]	Results are indeterminate for TB-Antigen responsiveness
	≥ 0.35 and < 25% of Nil value	< 0.5		
> 8.0	Any	Any		

T-SPOT.TB

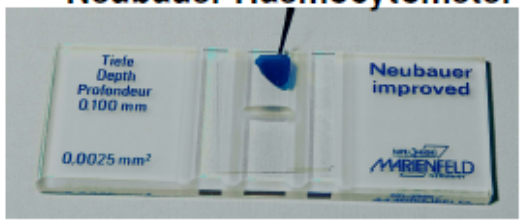

T-SPOT.TB
8
An in vitro diagnostic test for the detection of effector T cells that respond to stimulation by Mycobacterium tuberculosis antigens and is intended for use as an aid in the diagnosis of tuberculosis infection
12 x 8 well strips (24 tests)
PANEL A
PANEL B
Oxford Immunotec
Substrate solution
LOT 886
2011-05-04
25 mL


(a)
(b)
Plasma
PBMC layer
Density Gradient Liquid
Separation Gel
Ficoll-Paque™ Plus
Red Blood Cells
CORNING

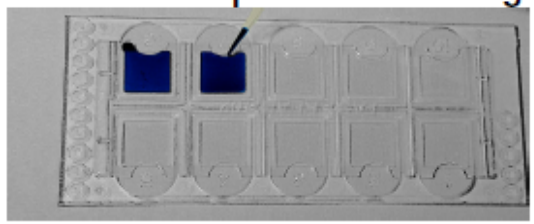

(a)
(b)
PBMC layer

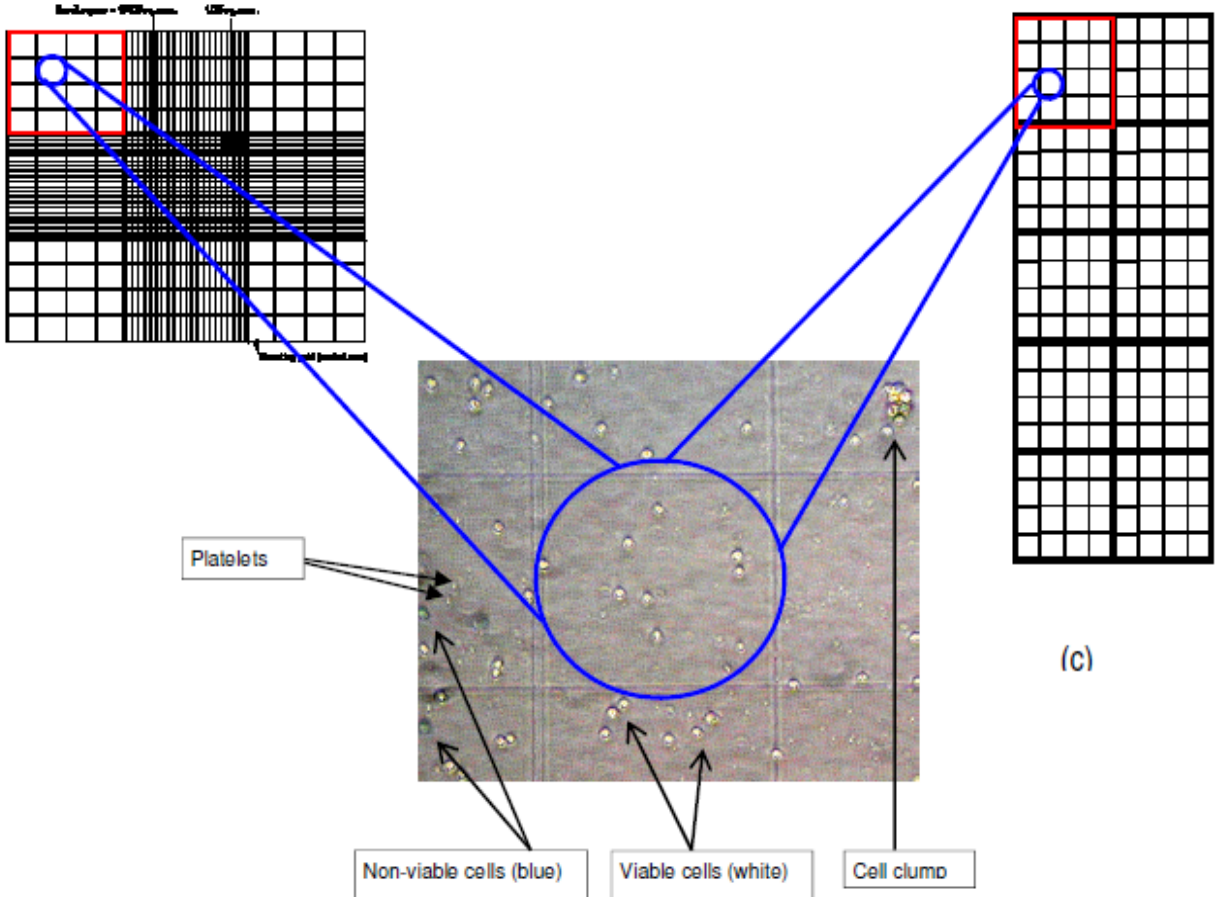


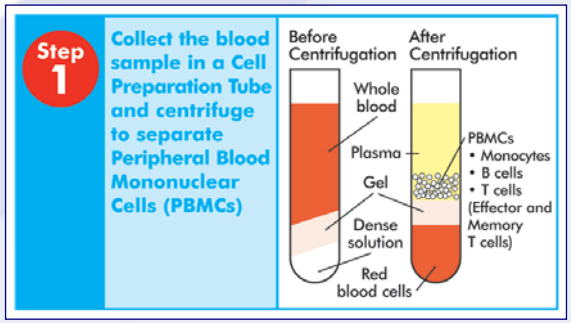
Neubauer Haemocytometer



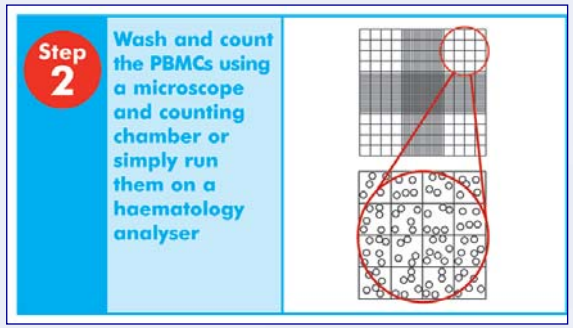
Fast-Read Disposable Counting



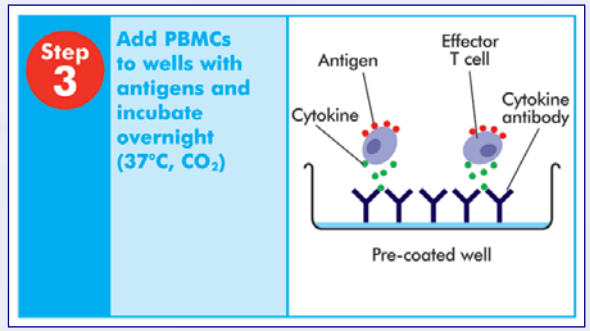




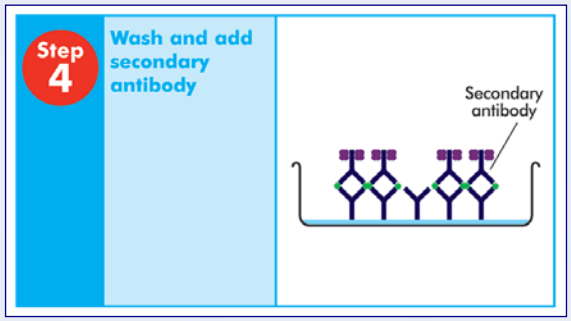
Extracción de sangre usando un tubo Vacutainer CPT® o tubo con gradiente de ficoll. Extracción capa linfocitaria



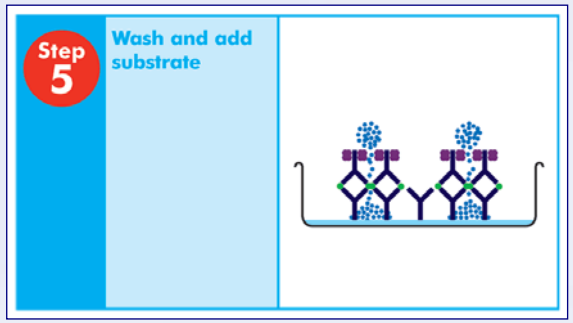
Lavado y recuento celular.



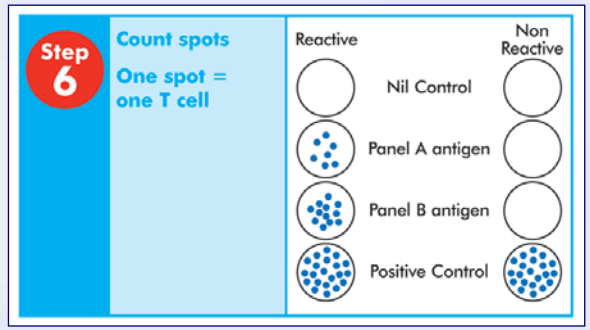
Se añaden las células y los antígenos TBC al pocillo correspondiente, se incuba una noche en estufa CO_2 Los anticuerpos capturan el IFN-gamma



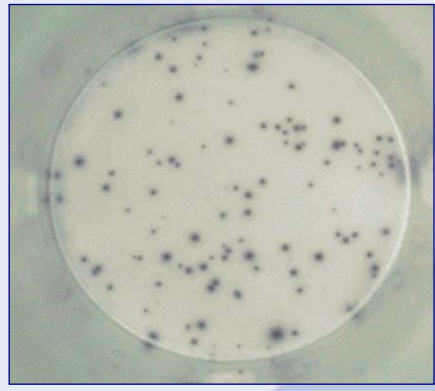
Se lava el pocillo y se añade el 2º anticuerpo conjugado, se incuba 1 hora

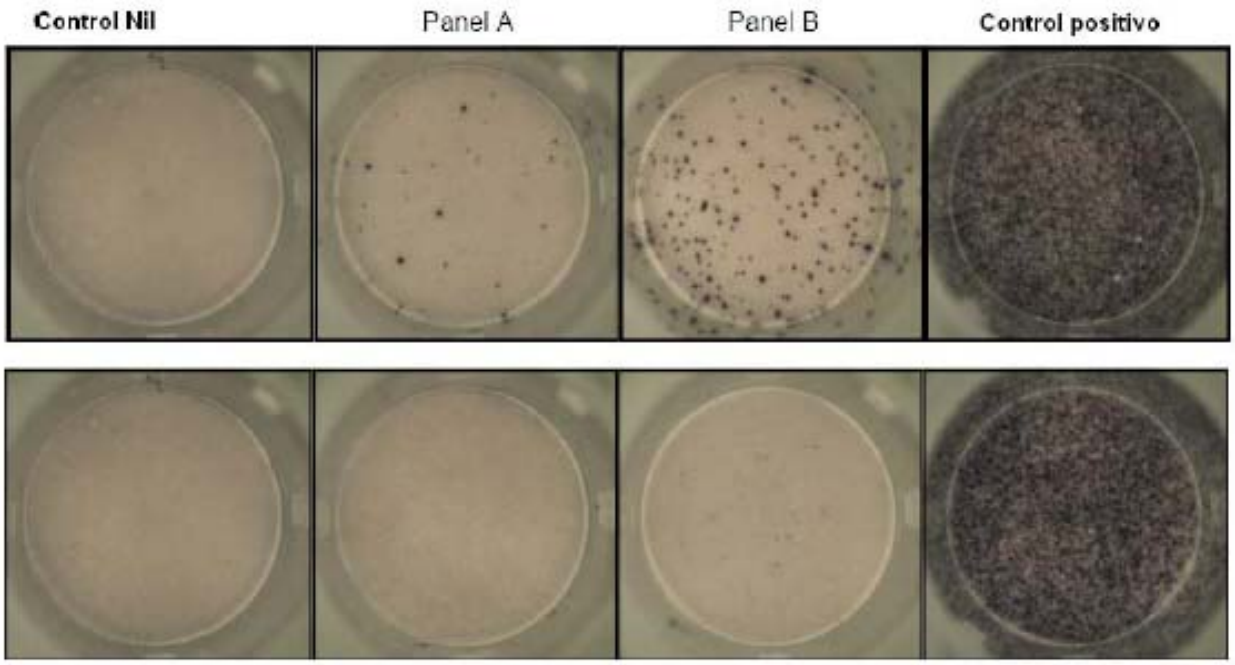


Se lava el pocillo y se añade el sustrato, se incuba 7 minutos. Se cuentan, de manera manual o automatizada, las manchas generadas



Resultado




Control Nil
Panel A
Panel B
Control positivo
Muestra reactiva
¡Muestra no reactiva

Resultado positivo:

- Control Negativo = **0 – 5** manchas

+

Manchas Panel A / B **–** Manchas Control Negativo **≥ 6**

- Control Negativo = **6 – 10** manchas

+

Manchas Panel A / B **≥ 2x** Manchas Control Negativo

Resultado indeterminado:

- Control Positivo = **<20** manchas

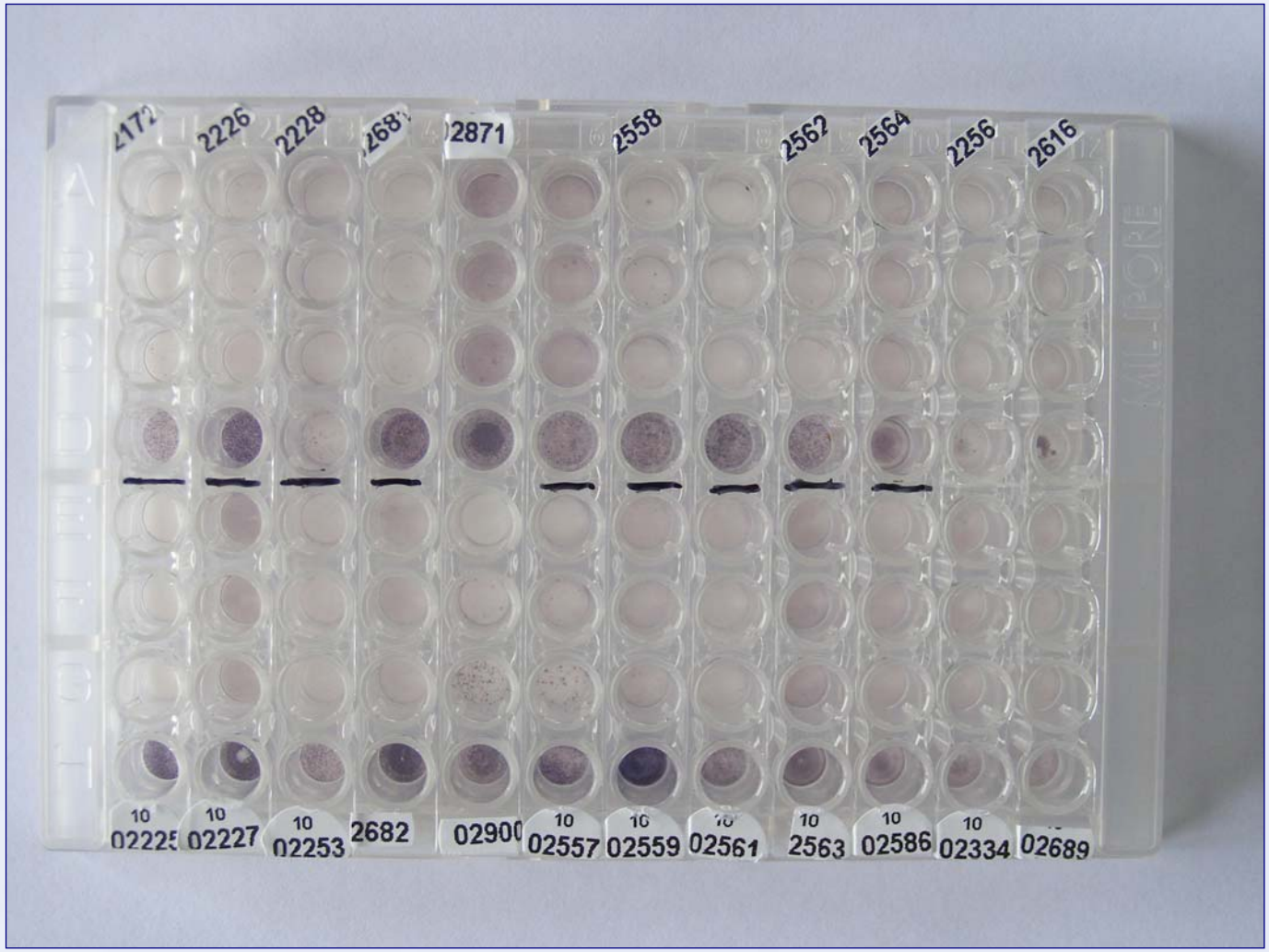
+

Panel A / Panel B **No reactivos**

- Control Negativo = **>10** manchas

¿contaminación? ¿secreción espontánea de IFN-gamma?

- Alto *background* en todos los pocillos


2226
2228
2871
2558
2562
2564
2256
2616
10
02227
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02586
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02334
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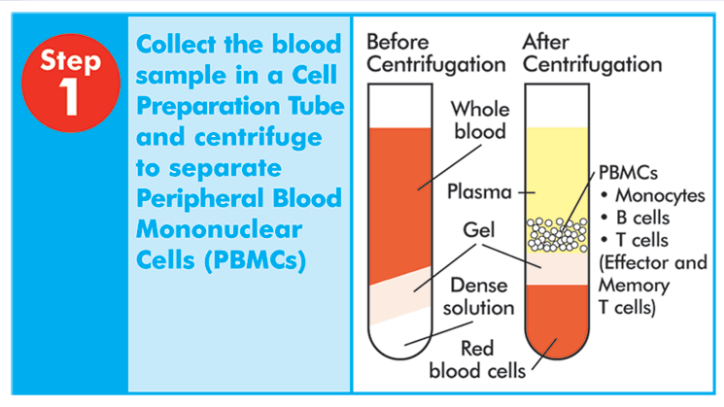
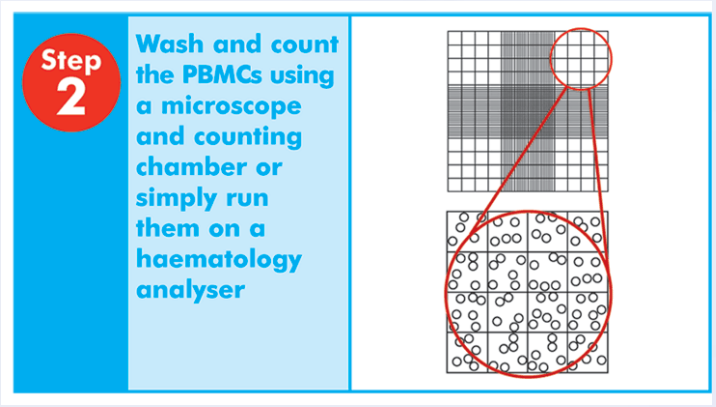


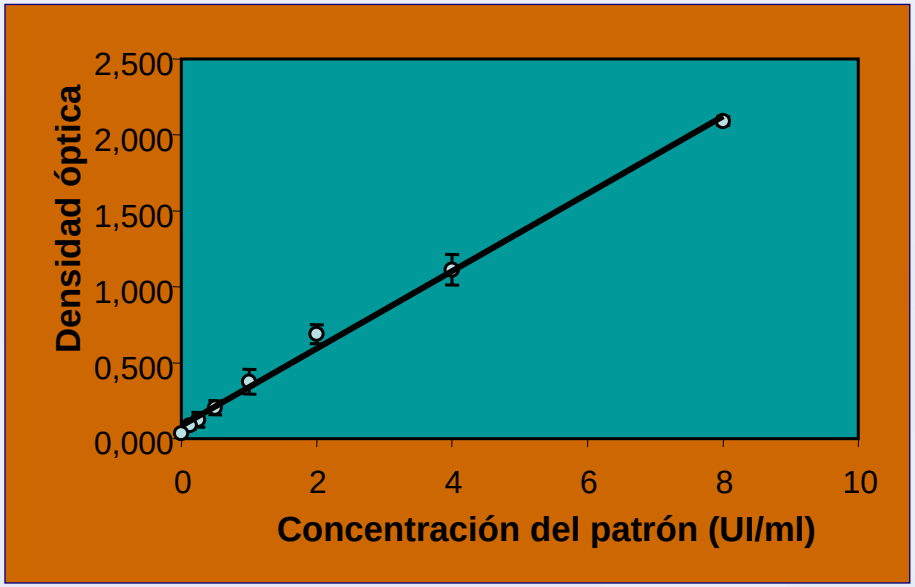


Diferencias: lavado y recuento de linfocitos T

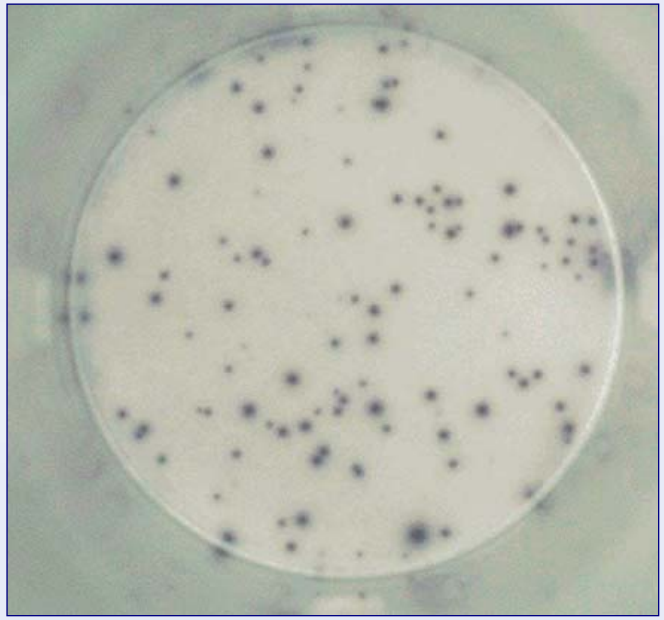
- El lavado y el recuento de los linfocitos T mejoran la sensibilidad
- El lavado elimina las citoquinas de fondo que podrían ocasionar resultados inespecíficos
- El recuento permite corregir las muestras de los pacientes con ↓ células T

Todas las muestras se ajustan aprox. 250.000 células lo que permite establecer un punto de corte más preciso

Diferencias: Resultados



Curva estándar



Lectura absoluta

SENSIBILIDAD-ESPECIFICIDAD IGRAs

Meta-análisis

Diel R *et al*.

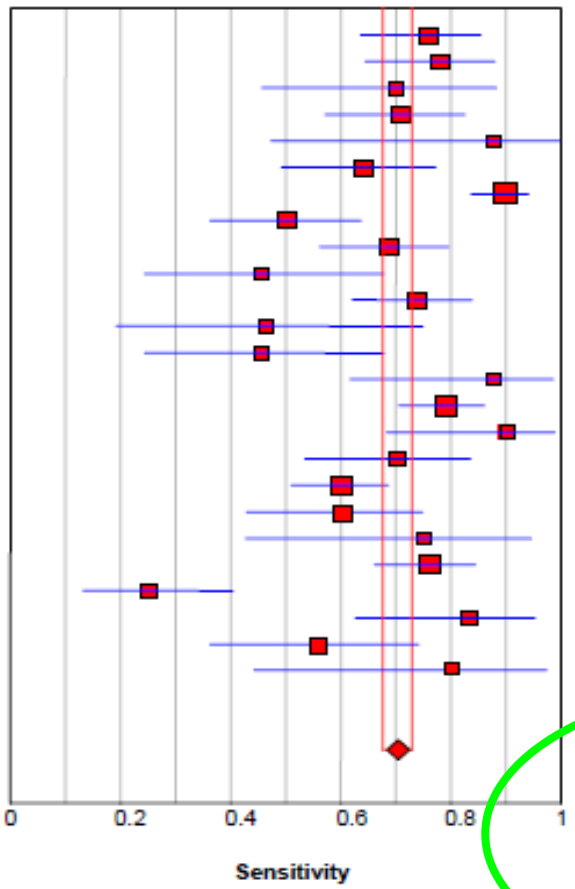
Chest 2010, 137:952-968

Tuberculina

Sensibilidad

70%

Figure 2: Forest plot of TST sensitivity



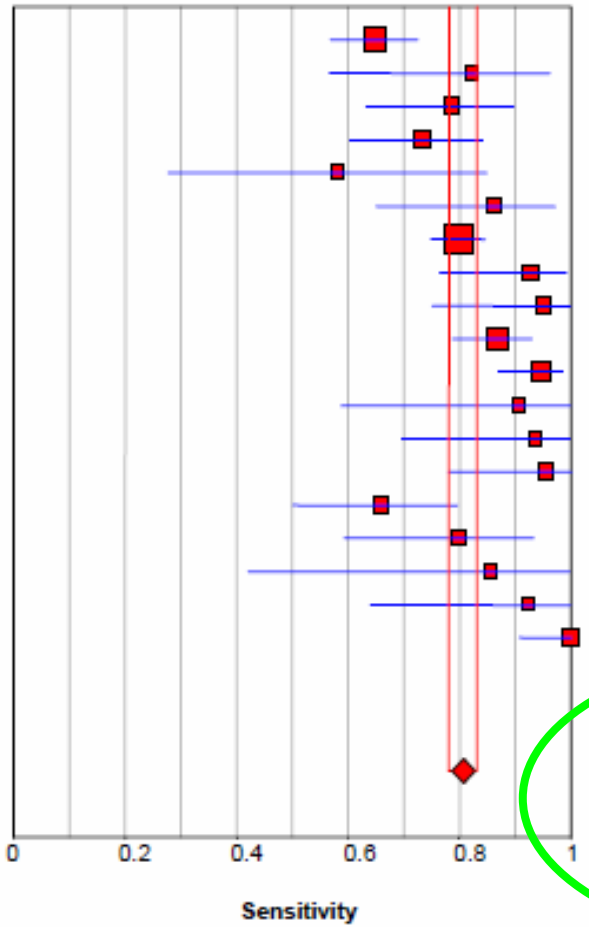
Study, Year [Reference]	Sensitivity (95% CI)		Patients (n/n)
Mori et al. 2004[3]	0.76	(0.64 - 0.85)	50/76
Kang et al. 2005[4]	0.78	(0.64 - 0.88)	42/54
Ferrara et al. 2006[5]	0.70	(0.46 - 0.88)	14/20
Lee et al. 2006[6]	0.71	(0.57 - 0.82)	39/55
Jafari et al. 2006[7]	0.88	(0.47 - 1.00)	7/8
Kobashi et al. 2006[8]	0.64	(0.49 - 0.77)	32/50
Tsiouris et al. 2006[9]	0.90	(0.84 - 0.94)	131/146
Detjen et al. 2007[10]	0.50	(0.36 - 0.64)	28/28
Kang et al. 2007[11]	0.69	(0.56 - 0.79)	46/67
Kim et al. 2007[12]	0.45	(0.24 - 0.68)	10/22
Mazurek et al. 2007[13]	0.74	(0.62 - 0.84)	51/69
Vincenti et al. 2007[14]	0.46	(0.19 - 0.75)	6/13
Bartu et al. 2008[15]	0.45	(0.24 - 0.68)	10/22
Bianchi et al. 2009[16]	0.88	(0.62 - 0.98)	14/16
Dosanjh et al. 2008[17]	0.79	(0.71 - 0.86)	98/119
Gerogianni et al. 2008[18]	0.90	(0.68 - 0.99)	18/20
Jafari et al. 2009[19]	0.70	(0.53 - 0.83)	28/40
Kobashi et al. 2008[20]	0.60	(0.51 - 0.68)	78/130
Kobashi et al. 2008[21]	0.60	(0.45 - 0.74)	29/48
Palazzo et al. 2008[22]	0.75	(0.43 - 0.95)	9/12
Soysal et al. 2008[23]	0.76	(0.66 - 0.84)	70/92
Kabeer et al. 2009[24]	0.25	(0.13 - 0.40)	11/44
Kampmann et al. 2009[25]	0.83	(0.63 - 0.95)	20/24
Kobashi et al. 2009[26]	0.57	(0.39 - 0.74)	20/35
Nicol et al. 2009[27]	0.80	(0.44 - 0.97)	8/10

Pooled Sensitivity = 0.70 (0.67 to 0.72)
Chi-square = 128.00; d.f. = 24 (p = 0.0000)
Inconsistency (I-square) = 81.3 %

QuantiFERON TB Gold-In Tube

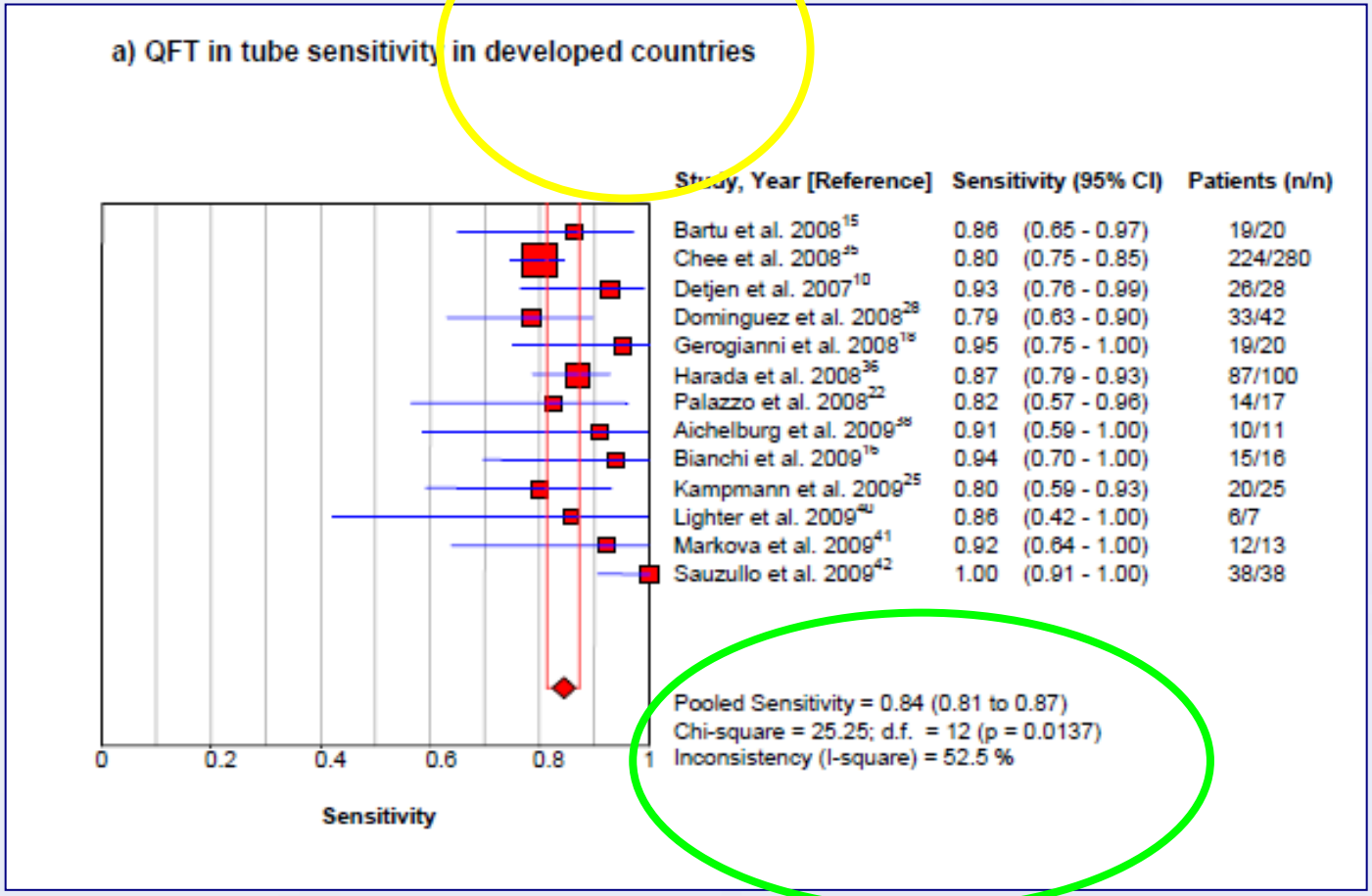
Sensibilidad 81%

Figure 3: Forest plot of QFT in tube sensitivity



Study, Year [Reference]	Sensitivity (95% CI)		Patients (n/n)
Tsiouris et al. 2006[9]	0.65	(0.57 - 0.72)	100/154
Palazzo et al. 2008[22]	0.82	(0.57 - 0.96)	14/17
Dominguez et al. 2008[28]	0.79	(0.63 - 0.90)	33/42
Pai et al. 2007[33]	0.73	(0.60 - 0.84)	44/60
Baba et al. 2008[34]	0.58	(0.28 - 0.85)	7/12
Bartu et al. 2008[15]	0.86	(0.65 - 0.97)	19/22
Chee et al. 2008[35]	0.80	(0.75 - 0.85)	224/280
Detjen et al. 2007[10]	0.93	(0.76 - 0.99)	26/28
Gerogianni et. al. 2008[18]	0.95	(0.75 - 1.00)	19/20
Harada et al. 2008[36]	0.87	(0.79 - 0.93)	87/100
Katiyar et al. 2008[37]	0.95	(0.87 - 0.99)	72/76
Aichelburg et al. 2009[38]	0.91	(0.59 - 1.00)	10/11
Bianchi et al. 2009[16]	0.94	(0.70 - 1.00)	15/16
Chegou et al. 2009[39]	0.96	(0.78 - 1.00)	22/23
Kabeer et al. 2009[44]	0.66	(0.50 - 0.80)	29/44
Kampmann et al. 2009[45]	0.80	(0.59 - 0.93)	20/25
Lighter et al. 2009[40]	0.86	(0.42 - 1.00)	6/7
Markova et al. 2009[41]	0.92	(0.64 - 1.00)	12/13
Sauzullo et al. 2009[42]	1.00	(0.91 - 1.00)	38/38

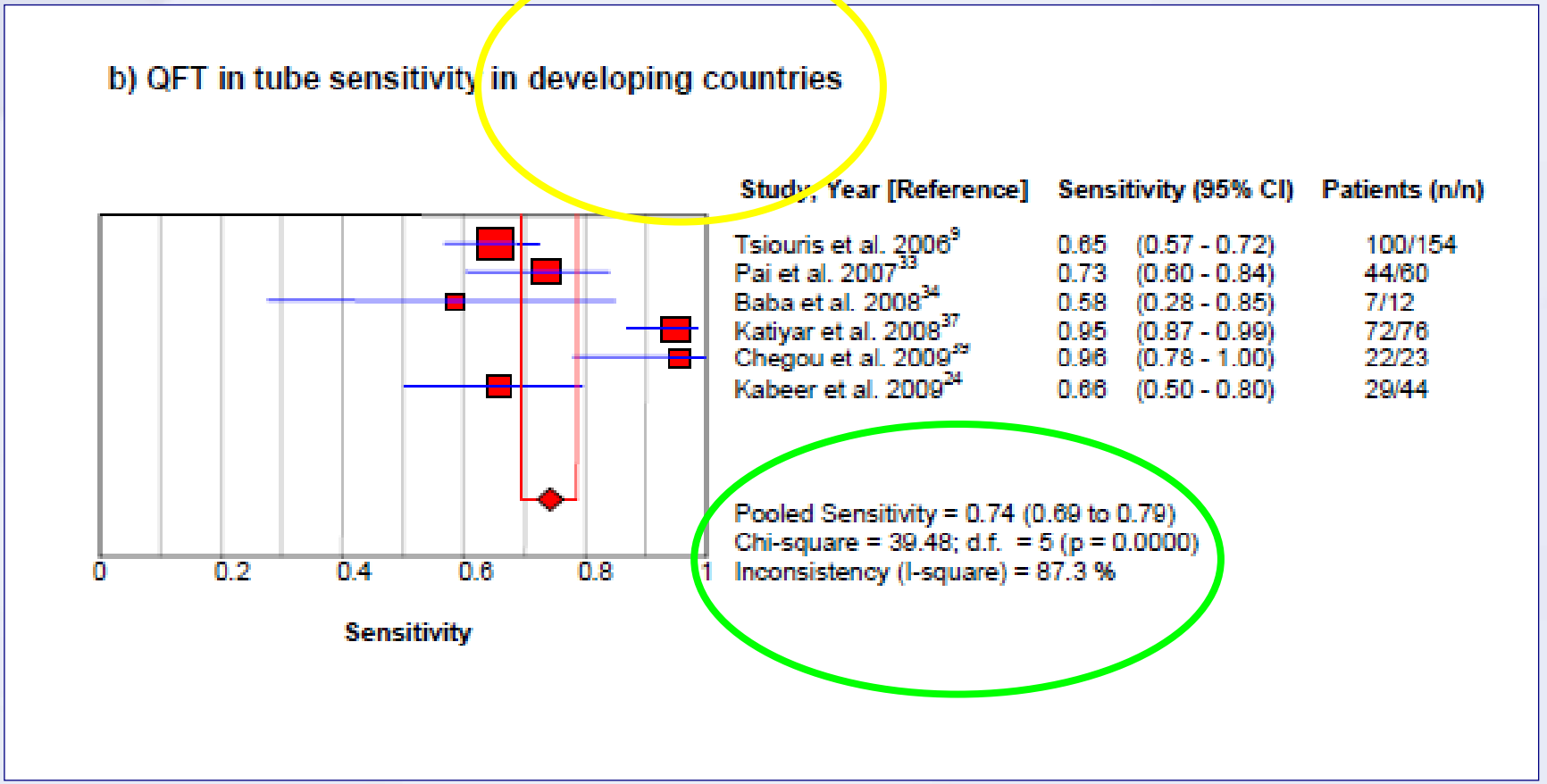
Pooled Sensitivity = 0.81 (0.78 to 0.83)
Chi-square = 79.90; d.f. = 18 (p = 0.0000)
Inconsistency (I-square) = 77.5 %



QuantiFERON TB Gold-In Tube

Sensibilidad

84%



QuantiFERON TB Gold-In Tube

Sensibilidad

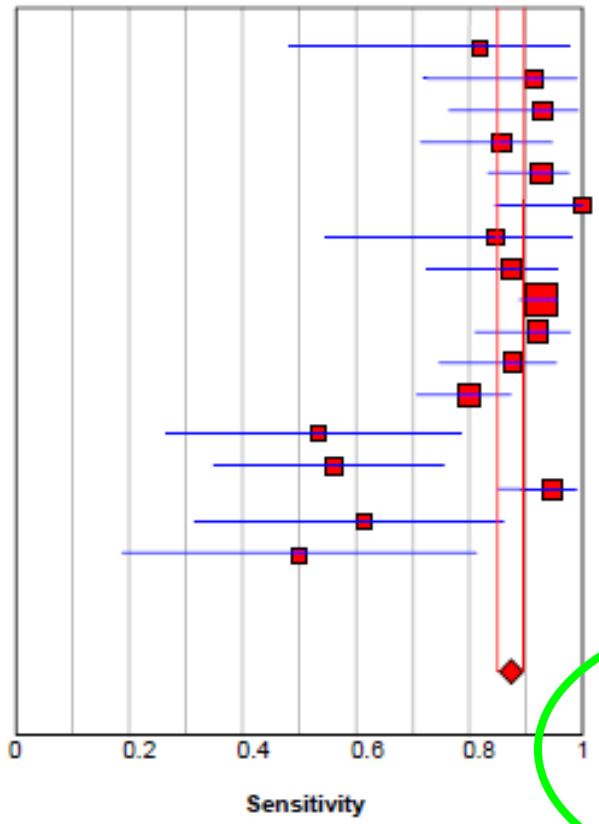
74%

T-SPOT.TB

Sensibilidad

87,5%

Figure 4: Forest plot of T-SPOT.*TB* sensitivity



Study, Year (Reference)	Sensitivity (95% CI)		Patients (n/n)
Ferrara et al. 2006[5]	0.82	(0.48 - 0.98)	9/11
Goletti et al. 2006[43]	0.91	(0.72 - 0.99)	21/23
Detjen et al. 2007[10]	0.93	(0.76 - 0.99)	26/28
Dominguez et al. 2008[28]	0.86	(0.71 - 0.95)	36/42
Kang et al. 2007[11]	0.93	(0.83 - 0.98)	62/67
Kim et al. 2007[12]	1.00	(0.85 - 1.00)	22/22
Vincenti et al. 2007[14]	0.84	(0.55 - 0.98)	11/13
Wang et al. 2007[44]	0.87	(0.73 - 0.96)	34/39
Chee et al. 2008[35]	0.93	(0.89 - 0.96)	254/274
Higuchi et al. 2009[45]	0.92	(0.81 - 0.98)	47/51
Kobashi et al. 2008[47]	0.88	(0.75 - 0.95)	42/48
Soysal et al. 2008[23]	0.80	(0.71 - 0.87)	80/100
Warier et al. 2009[46]	0.53	(0.27 - 0.79)	8/15
Kampmann et al. 2009[25]	0.56	(0.35 - 0.76)	14/25
Kim et al. 2009[47]	0.95	(0.85 - 0.99)	53/56
Markova et al. 2009[41]	0.62	(0.32 - 0.86)	8/13
Nicol et al. 2009[27]	0.50	(0.19 - 0.81)	5/10

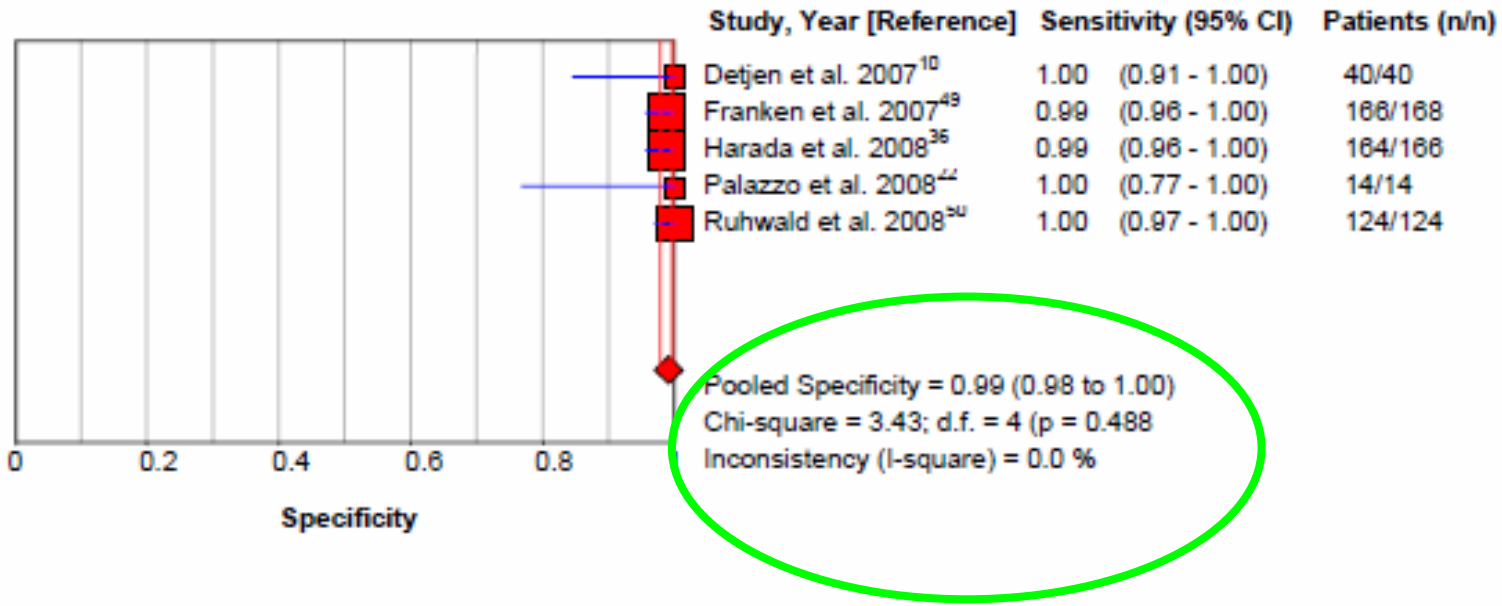
Pooled Sensitivity = 0.875 (0.85 to 0.90)
Chi-square = 65.59; d.f. = 16 (p = 0.0000)
Inconsistency (I-square) = 75.6 %

ESPECIFICIDAD
IGRAs

QuantiFERON TB Gold-In Tube

Especificidad 99%

Figure 5: Forest plot of QFT in tube specificity

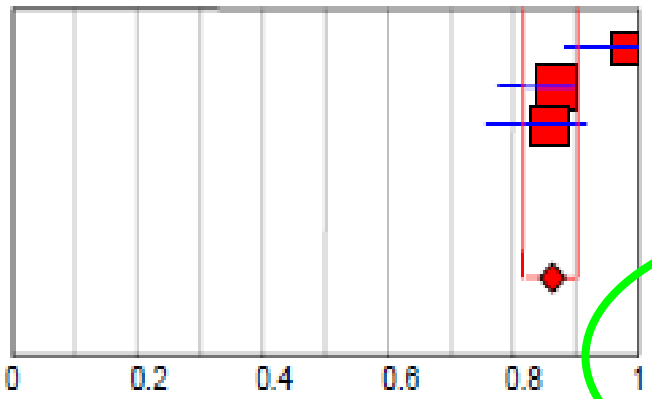


T-SPOT.TB

Especificidad

86%

Figure 6: Forest plot of T-Spot.*TB* specificity



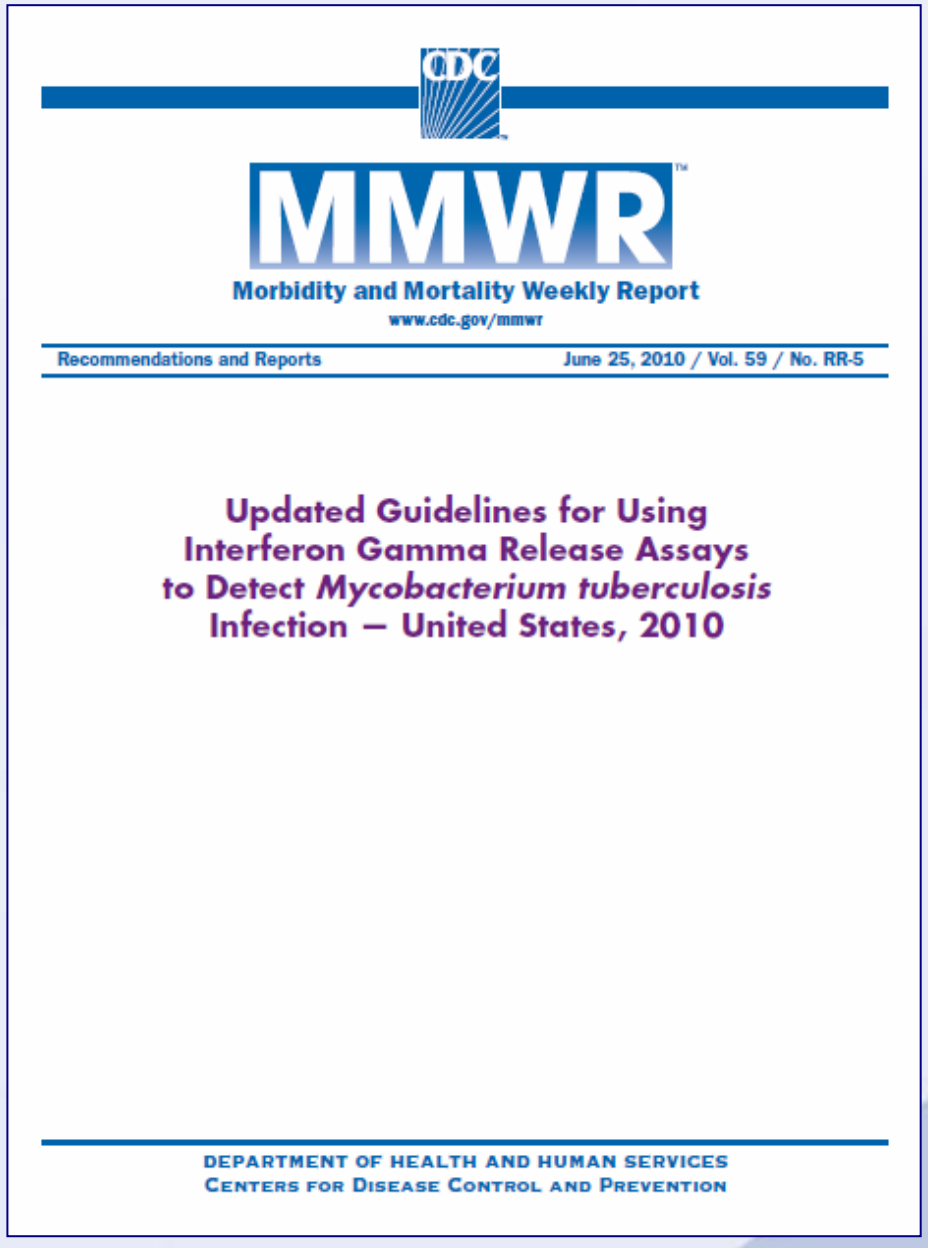
Study, Year [Reference]	Sensitivity (95% CI)		Patients (n/n)
Detjen et al. 2007[10]	0.98	(0.87 - 1.00)	39/40
Lee et al. 2006[6]	0.85	(0.77 - 0.90)	111/131
Higuchi et al. 2009[45]	0.83	(0.74 - 0.91)	70/84

Pooled Specificity = 0.86 (0.81 to 0.90)
Chi-square = 6.97; d.f. = 2 (p = 0.031)
Inconsistency (I-square) = 71.3%

Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection

Morbidity and Mortality Weekly Report MMWR

June 25, 2010 / Vol.59 / No. RR-5



Respaldo al uso de IGRAs

- QuantiFERON Gold **se puede emplear en todos los casos** en los que la prueba de la tuberculina está actualmente recomendada

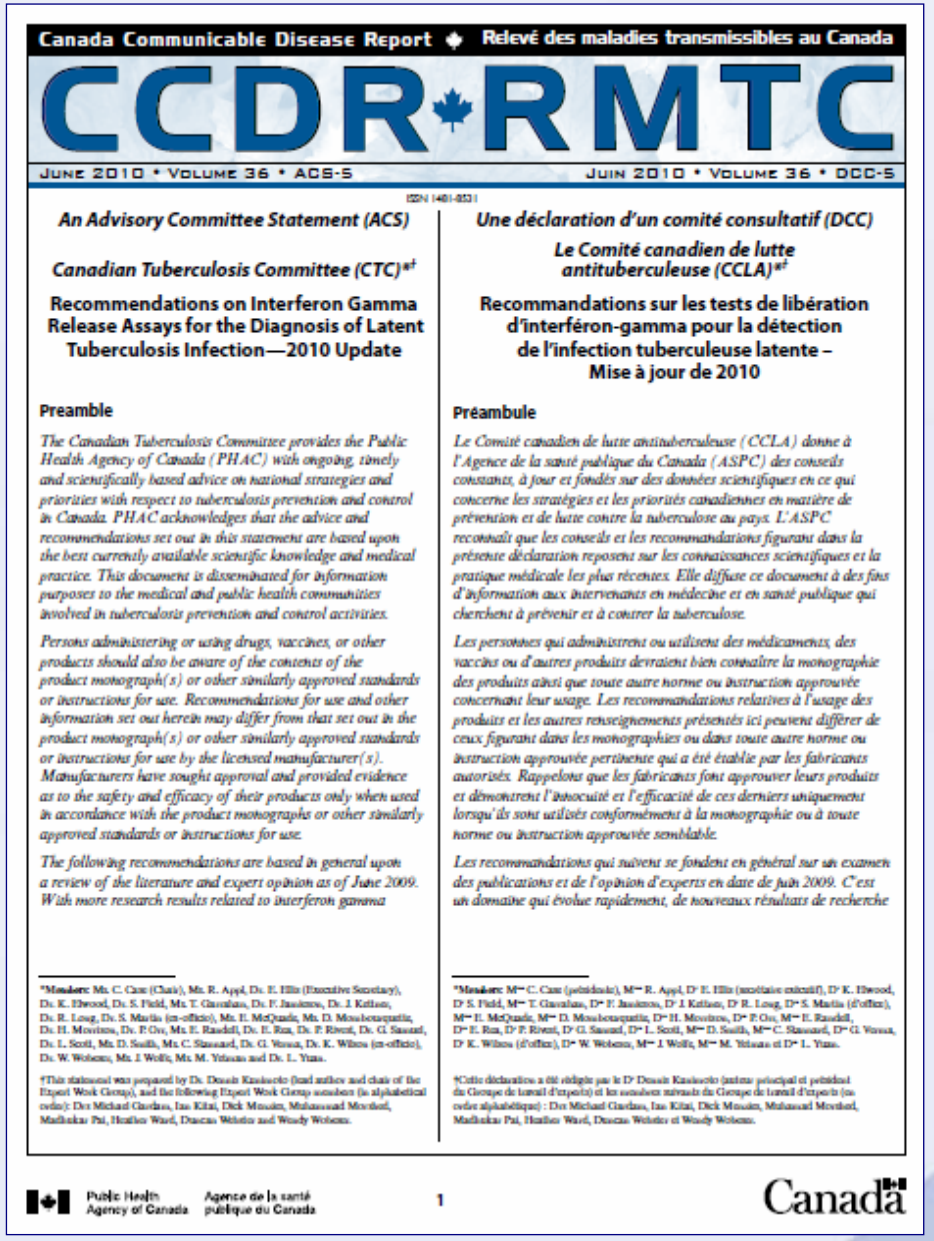
- QuantiFERON Gold **deberá utilizarse en lugar** de la prueba de la tuberculina y **no además** de ella

- **No se recomienda usar solamente** QuantiFERON Gold para excluir LTBI **en**:
  - ✓ Inmunodeprimidos
  - ✓ niños menores de 5 años de edad
  - ✓ Pacientes que van a ser tratados con antagonistas TNF-α

Recommendations on Interferon Gamma Release Assays for the Diagnosis of Latent Tuberculosis Infection-2010 Update

Canada Communicable Disease Report CCDR

June 2010, volume 36, ACS-5



Canada Communicable Disease Report ◆ Relevé des maladies transmissibles au Canada

CCDR•RMTC

June 2010 • Volume 36 • ACS-5 Juin 2010 • Volume 36 • DCC-5

An Advisory Committee Statement (ACS)

Canadian Tuberculosis Committee (CTC)[*†]

Recommendations on Interferon Gamma Release Assays for the Diagnosis of Latent Tuberculosis Infection—2010 Update

Preamble

The Canadian Tuberculosis Committee provides the Public Health Agency of Canada (PHAC) with ongoing, timely and scientifically based advice on national strategies and priorities with respect to tuberculosis prevention and control in Canada. PHAC acknowledges that the advice and recommendations set out in this statement are based upon the best currently available scientific knowledge and medical practice. This document is disseminated for information purposes to the medical and public health communities involved in tuberculosis prevention and control activities.

Persons administering or using drugs, vaccines, or other products should also be aware of the contents of the product monograph(s) or other similarly approved standards or instructions for use. Recommendations for use and other information set out herein may differ from that set out in the product monograph(s) or other similarly approved standards or instructions for use by the licensed manufacturer(s). Manufacturers have sought approval and provided evidence as to the safety and efficacy of their products only when used in accordance with the product monographs or other similarly approved standards or instructions for use.

The following recommendations are based in general upon a review of the literature and expert opinion as of June 2009. With more research results related to interferon gamma

Une déclaration d'un comité consultatif (DCC)

Le Comité canadien de lutte antituberculeuse (CCLA)[*‡]

Recommandations sur les tests de libération d'interféron-gamma pour la détection de l'infection tuberculeuse latente – Mise à jour de 2010

Préambule

Le Comité canadien de lutte antituberculeuse (CCLA) donne à l'Agence de la santé publique du Canada (ASPC) des conseils constants, à jour et fondés sur des données scientifiques en ce qui concerne les stratégies et les priorités canadiennes en matière de prévention et de lutte contre la tuberculose au pays. L'ASPC reconnaît que les conseils et les recommandations figurant dans la présente déclaration reposent sur les connaissances scientifiques et la pratique médicale les plus récentes. Elle diffuse ce document à des fins d'information aux intervenants en médecine et en santé publique qui cherchent à prévenir et à contrer la tuberculose.

Les personnes qui administrent ou utilisent des médicaments, des vaccins ou d'autres produits devraient bien connaître la monographie des produits ainsi que toute autre norme ou instruction approuvée concernant leur usage. Les recommandations relatives à l'usage des produits et les autres renseignements présentés ici peuvent différer de ceux figurant dans les monographies ou dans toute autre norme ou instruction approuvée pertinente qui a été établie par les fabricants autorisés. Rappelons que les fabricants font approuver leurs produits et démontrent l'innocuité et l'efficacité de ces derniers uniquement lorsqu'ils sont utilisés conformément à la monographie ou à toute norme ou instruction approuvée semblable.

Les recommandations qui suivent se fondent en général sur un examen des publications et de l'opinion d'experts en date de juin 2009. C'est un domaine qui évolue rapidement, de nouveaux résultats de recherche

*Members: Ms. C. Carr (Chair), Ms. R. Appl, Dr. E. Ellis (Executive Secretary), Dr. K. Elwood, Dr. S. Field, Ms. T. Gavaghan, Dr. F. Jamieson, Dr. J. Kellner, Dr. R. Long, Dr. S. Martin (ex-officio), Ms. E. McQuaid, Ms. D. Mousbourquette, Dr. H. Moustou, Dr. P. Orr, Ms. E. Randell, Dr. E. Rea, Dr. P. Rivest, Dr. G. Sauvé, Dr. L. Scott, Ms. D. Smith, Ms. C. Sinnaeve, Dr. G. Verma, Dr. K. Wilson (ex-officio), Dr. W. Webster, Ms. J. Wolfe, Ms. M. Yetman and Dr. L. Yuan.

†This statement was prepared by Dr. Dennis Kunimoto (lead author and chair of the Expert Work Group), and the following Expert Work Group members (in alphabetical order): Drs Michael Gardam, Ian Kitai, Dick Menzies, Muhammad Morshed, Madhukar Pai, Heather Ward, Duncan Webster and Wendy Webster.

*Membres: Mme C. Carr (présidente), Mme R. Appl, Dr E. Ellis (secrétaire exécutif), Dr K. Elwood, Dr S. Field, Mme T. Gavaghan, Dr F. Jamieson, Dr J. Kellner, Dr R. Long, Dr S. Martin (d'office), Mme E. McQuaid, Mme D. Mousbourquette, Dr H. Moustou, Dr P. Orr, Mme E. Randell, Dr E. Rea, Dr P. Rivest, Dr G. Sauvé, Dr L. Scott, Mme D. Smith, Mme C. Sinnaeve, Dr G. Verma, Dr K. Wilson (d'office), Dr W. Webster, Mme J. Wolfe, Mme M. Yetman et Dr L. Yuan.

‡Cette déclaration a été rédigée par le Dr Dennis Kunimoto (auteur principal et président du Groupe de travail d'experts) et les membres suivants du Groupe de travail d'experts (en ordre alphabétique) : Drs Michael Gardam, Ian Kitai, Dick Menzies, Muhammad Morshed, Madhukar Pai, Heather Ward, Duncan Webster et Wendy Webster.

Respaldo al uso de IGRAs

- **IGRAs y tuberculina no permiten distinguir** entre **LTBI y enfermedad activa**

- **IGRAs** presentan una **elevada especificidad en vacunados** 93-99%, son más consistentes los estudios con QFT que con T-SPOT.TB (derivan de la experiencia con la versión ELISPOT)
- **Tuberculina especificidad alta** (aprox. 97%) en **no vacunados** y es **variable o baja** (aprox.60%) **en vacunados**

- **IGRAs sensibilidad** en **TBC activa 75-90%** (QFT aprox. 75-80%, T-SPOT.TB aprox. 90%), tuberculina similar a QFT inferior a T-SPOT.TB
- Existen **pocos estudios**, y son **muy heterogéneos**, sobre **sensibilidad IGRAs en inmunodeprimidos**, T-SPOT.TB parece superar a la tuberculina

- **IGRAs en estudio de contactos** se correlacionan bien con otros marcadores pero **no necesariamente mejor que la tuberculina en todas las poblaciones**

- **IGRAs** se correlacionan **mejor que la tuberculina** en poblaciones con **incidencia baja + BCG**

. . .

- Siempre se encontrarán **casos con discordancias entre IGRAs y tuberculina** pero **no siempre podrán ser explicadas.** Parecen reducirse cuando se modifican los puntos de corte
- **Discordancias** significativas **en personal sanitario tuberculina+,IGRAs-**

- **IGRAs en <18 años sensibilidad** en **TBC activa variable** T-SPOT.TB parece superior a QTF
- **Discordancias** frecuentes en niños **tuberculina+,IGRAs-**

- **IGRAs están descritas conversiones y reversiones, su valor pronóstico se desconoce**

- En la actualidad **se desconoce el valor predictivo de IGRAs,** los **resultados** de los 3 estudios publicados **no son consistentes.**

Algunas consideraciones…

QuantiFERON-TB Gold In-tube

Parece más recomendable para el ámbito extra-hospitalario y para estudios de contactos más o menos numerosos

T-SPOT.TB

Parece más recomendable para el ámbito hospitalario, especialmente en pacientes con inmunodepresión, o en valoraciones previas a terapias con inmunosupresores en general y anti-TNF en particular

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Parece más recomendable para el ámbito hospitalario, especialmente en pacientes con inmunodepresión, o en valoraciones previas a terapias con inmunosupresores en general y anti-TNF en particular

…¿Deberían coexistir?

¿también con la tuberculina?

QuantiFERON TB GOLD IN-TUBE vs T-SPOT.TB

Resultados contexto Enfermedad Inflamatoria Intestinal

- 92 pacientes, resultados coindidentes ambos IGRAs 87%

77	Resultado NEGATIVO coincidente	83,7%
3	Resultado POSITIVO coincidente	3,2%
6	T-SPOT.TB POSITIVO/ QuantiFERON NEGATIVO	6,5%
3	QuantiFERON POSITIVO/T-SPOT.TB NEGATIVO	3,2%
3	Resultado INDETERMINADO T-SPOT.TB/ QuantiFERON NEGATIVO	3,2%

Total positivos 12 (13%)

9 T-SPOT.TB

6 QuantiFERON TB GOLD In-Tube

Conclusiones

Resultados contexto Enfermedad Inflamatoria Intestinal

Un 13% de los pacientes fue diagnosticado de Infección tuberculosa

Al considerar cualquier resultado IGRA positivo como criterio de infección tuberculosa, la realización de ambas pruebas EN ESTE TIPO DE PACIENTES aumenta la sensibilidad diagnóstica entre 25-50%

En pacientes inmunodeprimidos debería tenerse en cuenta ADEMÁS el impacto sobre los resultados de “algunos eventos” evidenciados con ambos IGRAs

cellestis

Version 2.50

QuantiFERON®-TB Gold In-Tube Results

Test Date: martes 4 mayo 2010
Operator: Admin
Run Number: 1
Kit Batch Number: 1

Mínimo 0,15

Valid ELISA test run.

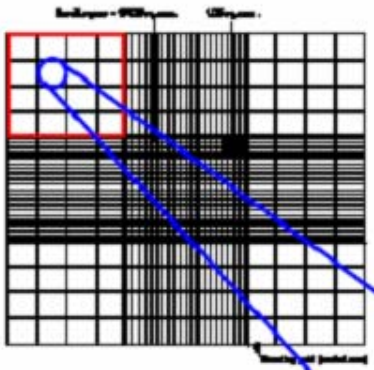
Results

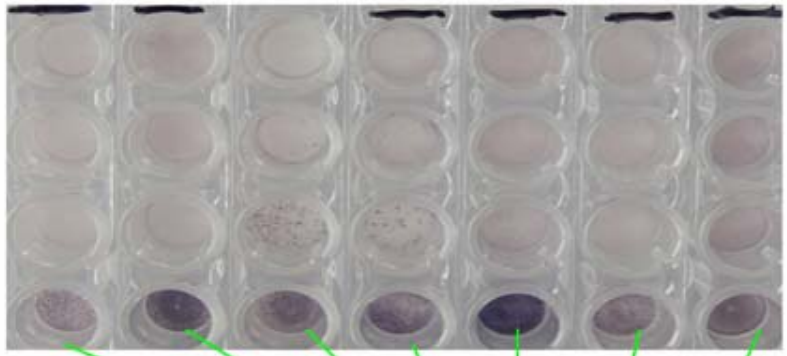
Subject ID	Nil	TB Ag	Mitogen	TB Ag-Nil	Mitogen-Nil	Result
310003649	0.08	0.34	13.57	0.26	13.49	NEGATIVE
310003670	0.09	0.09	13.57	0.00	13.48	NEGATIVE
310003681	0.22	0.21	13.57	-0.01	13.35	NEGATIVE
310003684	0.08	0.10	13.57	0.02	13.49	NEGATIVE
310003686	0.09	2.31	3.01	2.22	2.92	POSITIVE
310003687	0.07	0.07	13.57	0.00	13.50	NEGATIVE
310003706	0.06	0.06	13.57	0.00	13.51	NEGATIVE
310003727	0.28	2.31	6.76	2.03	6.48	POSITIVE
310003741	0.05	0.06	3.30	0.01	3.25	NEGATIVE
310003742	0.06	0.25	1.73	0.19	1.67	NEGATIVE
310003744	0.08	1.40	13.57	1.32	13.49	POSITIVE
310003746	0.05	0.05	13.57	0.00	13.52	NEGATIVE

Signature _____ Date _____

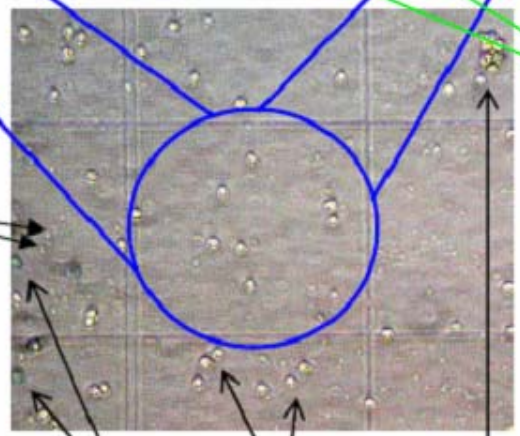
Neubauer Haemocytometer

<250.000 células





Control positivo
Ok



Platelets

Non-viable cells (blue)

Viable cells (white)

Cell clump

PROBLEMAS DE INTERPRETACIÓN EN LOS ENSAYOS BASADOS EN LA LIBERACIÓN DE INTERFERÓN GAMMA (IGRAs).

Argüelles Menéndez P, Gutiérrez Fernández C, Penedo Pallares A[1], Chamorro López L, Palacios Gasos M, Palacios Gutiérrez JJ[1].
Servicio de Bioquímica Clínica, Hospital Universitario Ramón y Cajal. Madrid.
[1]Unidad de Referencia Regional de Micobacterias, Hospital Universitario Central de Asturias. Oviedo.

INTRODUCCIÓN

Los Interferon Gamma Release Assays (IGRAs) se basan en la detección del IFN-gamma liberado como respuesta a la estimulación *in vitro* de las células T sensibilizadas presentes en sangre periférica con antígenos específicos de *M. tuberculosis*. Existen dos formatos disponibles comercialmente: QuantiFERON-TB Gold In-Tube® (Cellestis) -QFT- y T-SPOT.TB ® (Oxford Immunotec) –TSTB-. Esta tecnología esta diseñada para el diagnóstico de la infección tuberculosa latente (ITL); se evita la subjetividad en la interpretación de los resultados, incluye controles positivos para identificar a los pacientes anérgicos y no se ve afectada por la vacunación con BCG. Desde el año 2008, en nuestra Unidad se emplea QFT de manera rutinaria en pacientes con sospecha de tuberculosis y en el contexto del estudio de contactos. Realizando además TSTB cuando se pretende descartar ITL en pacientes inmunodeprimidos, en edades pediátricas, o previo a la instauración de anti-TNFα. Hemos analizado 1425 muestras y constatado la existencia de un 20 a 30% de resultados en el tubo/pocillo control positivo en los cuales a pesar de que se cumplen los criterios mínimos exigidos por cada fabricante, podrían ser considerados "no totalmente óptimos" (QFT: valor en el tubo mitógeno ≤5 UI/ml; TSTB: pocillo control positivo >20 *spots* pero muestra de <250.000 células/ml). Creemos que esto influenciará la sensibilidad y valor predictivo negativo del IGRA.

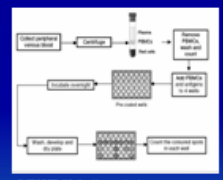
OBJETIVO

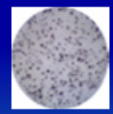
En el contexto del diagnóstico de la ITL, comparar los resultados obtenidos por QFT y TSTB, en el grupo de pacientes inmunodeprimidos, pediátricos, y previo a terapia anti-TNFα, prestando especial atención a aquellos pacientes en los cuales los valores obtenidos en los controles positivos de sus muestras de sangre "no eran totalmente óptimos".

MATERIAL Y MÉTODOS

En QFT se procede mediante ELISA al análisis y cuantificación de la respuesta. TSTB es un tipo de ensayo ELISPOT. Se estudiaron 75 pacientes. A todos se les realizó de manera simultánea QFT y TSTB.

QuantiFERON-TB Gold In-Tube® (Cellestis)





T-SPOT.TB ® (Oxford Immunotec)

RESULTADOS

En 62/75 muestras (82,6%) se cumplían "de manera óptima" los requisitos al menos para uno de los IGRAs empleados, mientras que en 13/75 muestras (17,3%) se evidenciaban las variables objeto de este estudio. En el caso del TSTB encontramos 31/75 muestras (41,3%) en las cuales no fue posible inocular 250.000 células si bien el control positivo presentaba >20 spots (resultado interpretable); de ellas 25 eran negativas, 5 positivas y 1 indeterminada. En el caso del QFT encontramos 24/75 muestras (32%) en las cuales el control positivo (mitógeno) era >0,5, pero <5 UI/mL (resultado interpretable); de ellas 22 fueron negativas y 2 positivas. En 2/25 muestras negativas por TSTB la prueba QFT realizada simultáneamente fue positiva (8%), a su vez en 2/22 muestras negativas por QFT la prueba TSTB fue positiva (9,1%).

CONCLUSIONES

En pacientes inmunodeprimidos, edad pediátrica y especialmente en chequeo previo a la instauración de terapia anti-TNF α creemos justificada la estrategia de utilización de dos IGRAs simultáneamente. Nuestros resultados evidencian casi un 10% de falsos negativos de cada prueba considerada individualmente. En un contexto clínico como el descrito, el impacto económico nos parece justificado frente a las repercusiones clínicas de un falso negativo. Lo comentado no es un inconveniente para que cualquiera de los IGRAs comercializados pueda ser considerado adecuado para población general y ser utilizado de manera individualizada.

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75 pacientes

Resultados contexto Inmunodeprimidos, terapia anti-TNF, edad pediátrica

62 al menos uno de los IGRAS "Ok" 82,6%

13 los dos IGRAs "no Ok" 17,3%

T-SPOT.TB 31 (41,3%) <250.000 células

- 25 NEGATIVOS (2 fueron QTF POSITIVO)
- 5 POSITIVOS
- 1 INDETERMINADO

QuantiFERON 24 (32%) Mitógeno >0,5 PERO inferior al 40% de la media

- 22 NEGATIVOS (2 fueron T-SPOT.TB POSITIVO)
- 2 POSITIVOS

75 pacientes

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- 2 POSITIVOS

En estas situaciones se evidencia de un incremento de falsos negativos de un IGRA respecto al otro

Conclusiones

Inmunodeprimidos, terapia anti-TNF, edad pediátrica

Al considerar cualquier resultado IGRA positivo como criterio de infección tuberculosa, la realización de ambas pruebas EN ESTE TIPO DE PACIENTES aumenta la sensibilidad diagnóstica entre 28-71%

En un contexto clínico como el descrito el impacto económico nos parece justificado

PAPEL DE LAS NUEVAS TÉCNICAS DE PCR A TIEMPO REAL A PROPÓSITO DE UN CASO DE TUBERCULOSIS.

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Servicio de Bioquímica Clínica, Hospital Universitario Ramón y Cajal. Madrid.
[1]Unidad de Referencia Regional de Micobacterias, Hospital Universitario Central de Asturias. Oviedo.

HISTORIA CLÍNICA

Motivo de consulta: Mujer de 14 años que acude por fiebre, tos y expectoración. Historia actual: Diagnosticada de neumonía en LMD el 11 de enero de 2010, a tratamiento con Augmentine 10 días, con persistencia de clínica y Rx, acude nuevamente a Urgencias donde se trata con Octegra y tras 4 días de tratamiento, al persistir tos, fiebre y expectoración, se decide ingreso. Antecedentes personales: NAMC. No DM ni HTA ni DL. No hábitos tóxicos. No antecedentes médicos de interés.

EXPLORACIÓN

T^a 38.8ºC. Sat.O2.: 98%. TA: 110/80. Eupneica en reposo y normocoloreada. AP: crepitantes en costado dcho. Abdomen anodino. EEII: sin edemas.

PRUEBAS COMPLEMENTARIAS

RX.Tórax: Condensación en LMD con infiltrado nodular en LID. En Rx posterior se objetivó derrame pleural derecho.
ECG: R. sinusal a 100 lpm.
Ecografía pleural D: Mínimo derrame pleural con algún septo interior. Condensación LID.
ANALÍTICA: Ure: 20 mg/dL, Cre: 0.62 mg/dL, Na: 138 mM/L, K: 4.25 mM/L, PCT: 0.42 ng/mL, PCR: 15.57mg/L, GOT: 18 U/L, GPT: 17 U/L, FA: 78 U/L, GGT: 25 U/L, BT: 0.5 g/dL, LDH: 303 U/L. BK esputo: Negativa. QUANTIFERON: Positivo. Hemocultivos: Negativos. Urinocultivo: Negativo. Cultivo esputo: Negativo.
PCR esputo (Xpert MTB/RIF): Positivo para MTB complex, detectada resistencia a rifampicina. Cultivo micobacterias: Positivo para *M. tuberculosis* complex multisensible.

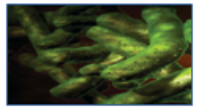
Test Result:	MTB DETECTED LOW; Rif Resistance DETECTED			
Test and Analyte Result				
Analyte Name	**Ct**	**EndPt**	**Analyte Result**	**Probe Check Result**
Probe D	29.1	139	POS	PASS
Probe C	28.0	198	POS	PASS
Probe E	29.1	128	POS	PASS
Probe B	0	7	NEG	PASS
SPC	27.1	296	NA	PASS
Probe A	27.6	142	POS	PASS

EVOLUCIÓN

Tras tratamiento inicial con R+I+P+E, la paciente sufre intolerancia digestiva y empeoramiento radiológico. La situación clínica junto con la información disponible en ese momento por PCR en esputo (mutación en el gen *rpoB*) motivó que se cambiara el tratamiento inicial para cubrir la posibilidad de multirresistencia (R+ E+ P+Mx+ Lnz). En los cultivos se aisló e identificó *Mycobacterium tuberculosis*, el antibiograma demostró que la cepa era sensible a todas las drogas de primera línea incluida rifampicina; asimismo se confirmó por 2 métodos complementarios (INNOLiPA RIF TB, Genotype MDRplus) que la cepa presentaba la mutación en el gen *rpoB* detectada en la muestra clínica. Se instauró nuevamente tratamiento con Rifater, añadiéndose esteroides orales por síndrome febril persistente. En el momento del alta el derrame pleural había desaparecido y la fiebre remitido con los esteroides. La evolución clínica ha sido favorable, actualmente se encuentra pendiente de completar el tratamiento específico.

DIAGNÓSTICO DEFINITIVO

Tuberculosis pulmonar, cepa de *M. tuberculosis* sensible a drogas de primera línea, incluida rifampicina, en presencia de mutación en el gen *rpoB* que no se expresa.



DISCUSIÓN

La tecnología Xpert MTB/RIF (Genexpert, Cepheid) permite el diagnóstico de la tuberculosis directamente en la muestra clínica y a la vez la detección de mutaciones en el gen *rpoB* (lo que suele ir asociado a resistencia a la rifampicina). El formato es compacto y de fácil realización. Se amplifica una zona diana específica del genoma de *M. tuberculosis* que se evidencia por hibridación con sondas unidas a marcadores fluorogénicos. Las mayores ventajas residen en su rapidez (1h, 40 min) y en el formato compacto, lo que permite su realización de manera individualizada "a demanda" y dar soporte al clínico para la toma de decisiones anticipándose a la positividad de los cultivos. Sin embargo, en cuanto a la detección de resistencia a la rifampicina, conviene recordar que, en la tuberculosis, el criterio de sensibilidad o resistencia a un fármaco se sigue sustentado en el antibiograma (>1% de población micobacteriana resistente), la presencia de mutaciones en genes que codifican resistencias a fármacos no conlleva "resistencia segura", simplemente alerta y anticipa de esa posibilidad, que debería ser confirmada siempre para lo cual sigue resultando imprescindible el cultivo. Sirva de ejemplo nuestro caso. Creemos que los métodos moleculares y tradicionales deben coexistir en el laboratorio de micobacterias.

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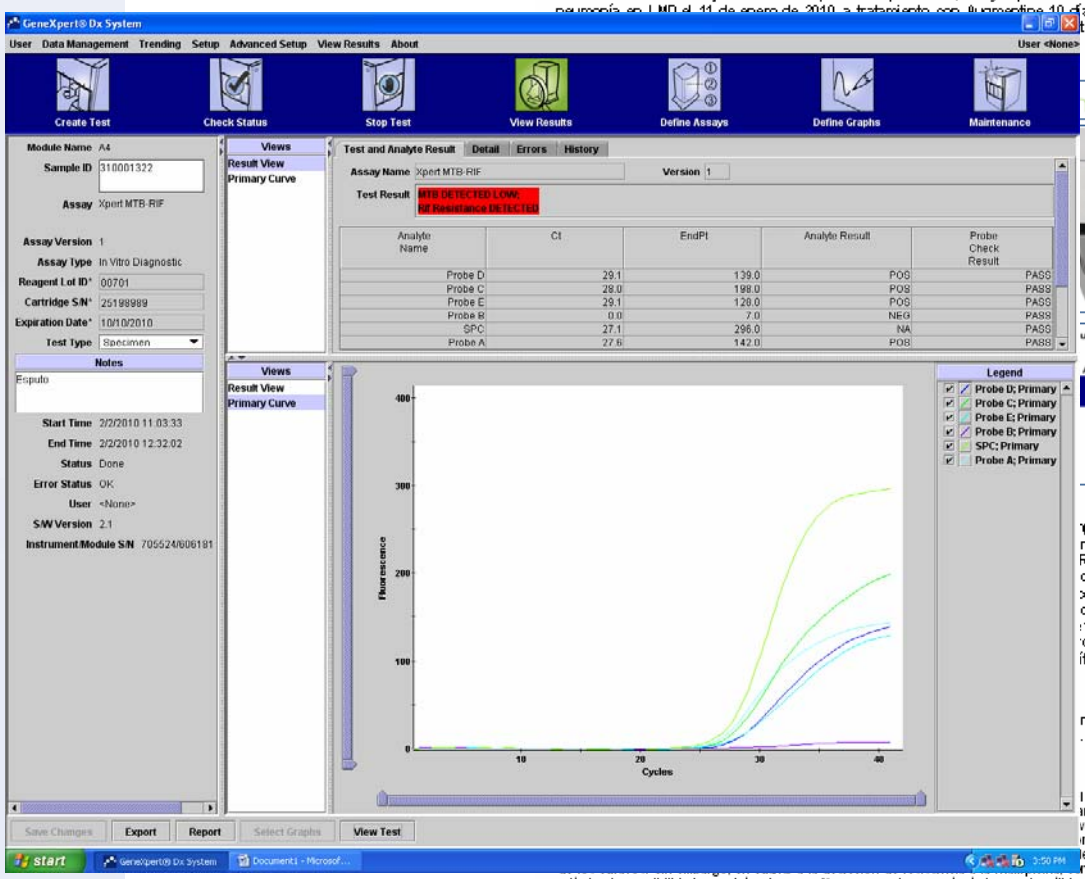

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Test Type: Specimen

Assay Information

Assay	Assay Version	Assay Type
Xpert MTB-RIF	1	In Vitro Diagnostic

Test Result: MTB DETECTED LOW; Rif Resistance DETECTED

Test and Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	29.1	139	POS	PASS
Probe C	28.0	198	POS	PASS
Probe E	29.1	128	POS	PASS
Probe B	0	7	NEG	PASS
SPC	27.1	296	NA	PASS
Probe A	27.6	142	POS	PASS

User:	<None>		
Status:	Done	Start Time:	2/2/2010 11:03:33
Reagent Lot ID*:	00701	End Time:	2/2/2010 12:32:02
Expiration Date*:	10/10/2010	Module Name:	A4
Cartridge S/N*:	25198989	Module S/N:	606181
S/W Version:	2.1	Instrument S/N:	705524
Notes:	Esputo		
Error Status:	OK		

Errors
<None>

* indicates that a particular field is entered using a barcode scanner

For In Vitro Diagnostics Use Only.

GeneXpert® Dx System Software Version 2.1 Page 2 of 2

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Assay	Assay Version	Assay Type
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Test Result: MTB DETECTED LOW; Rif Resistance DETECTED

and Analyte Result

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Probe C	28.0	198	POS	PASS
Probe E	29.1	128	POS	PASS
Probe B	0	7	NEG	PASS
SPC	27.1	296	NA	PASS
Probe A	27.6	142	POS	PASS

User: <None>
Status: Done
Reagent Lot ID*: 00701
Start Time 2/2/2010 11:03:33
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…odule S… 606181
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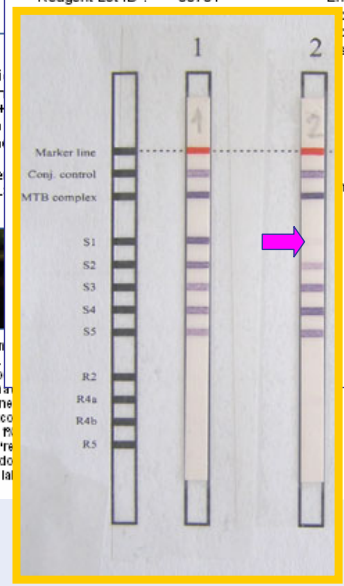
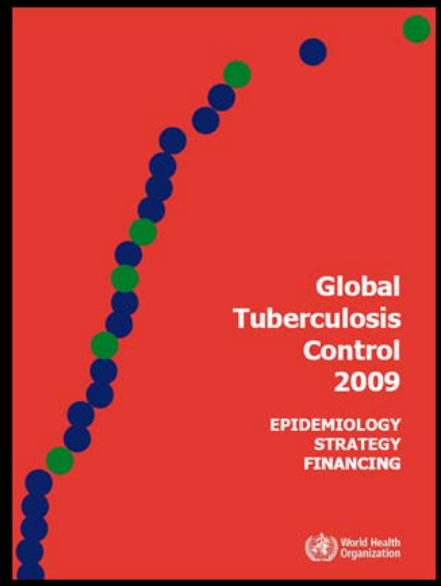
Page 2 of 2



- **Objetivos de la OMS en 1991:**
  - ✓ Detección del 70% de casos infecciosos
  - ✓ Curación de >85% de ellos
  - ✓ DOTS



Organización Mundial de la Salud

ESTRATEGIA ALTO A LA TUBERCULOSIS Stop TB Partnership

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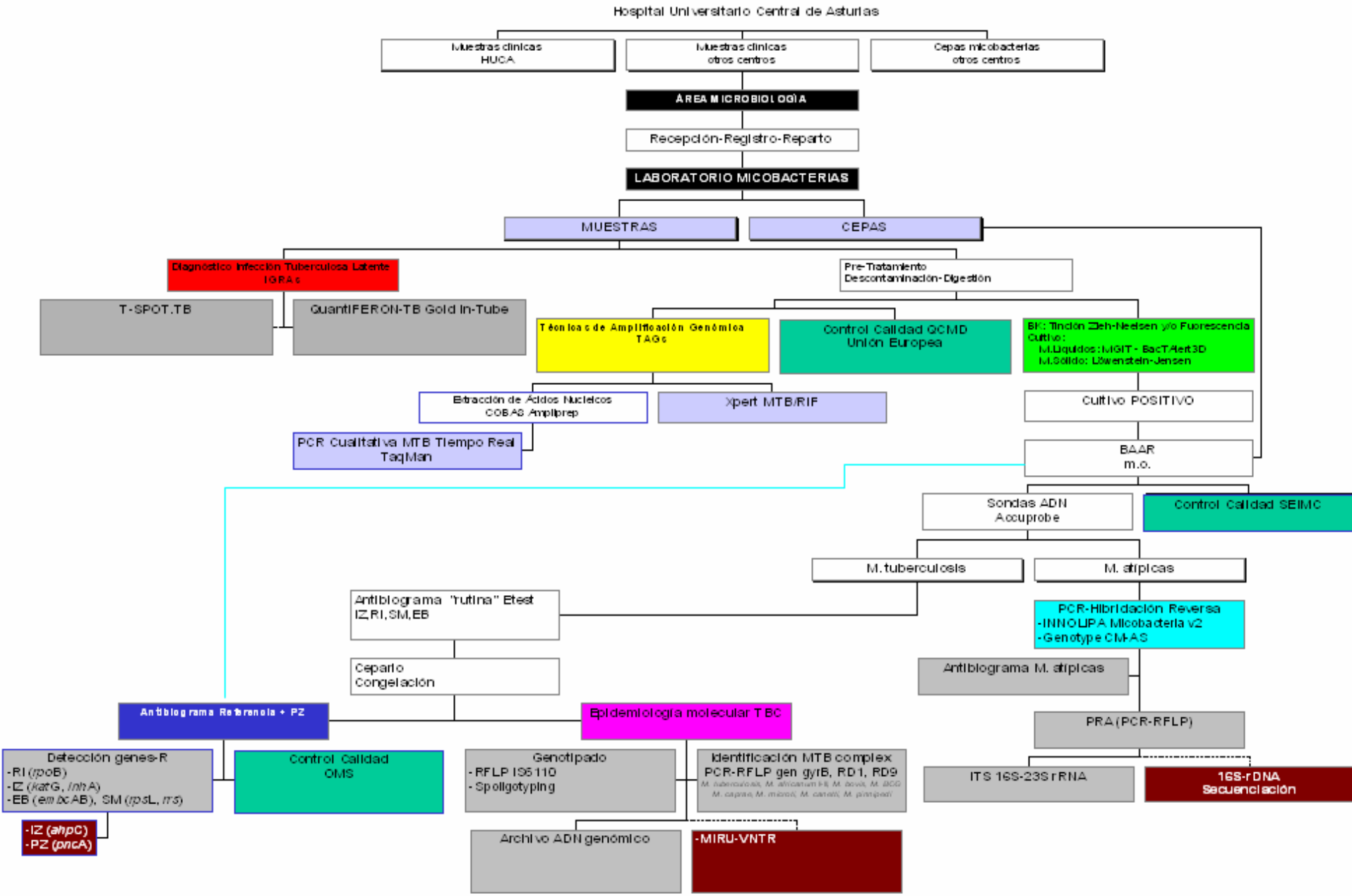
VISIÓN	**UN MUNDO LIBRE DE TUBERCULOSIS**
FINALIDAD	Reducir marcadamente la carga mundial de tuberculosis para 2015, en consonancia con los Objetivos de Desarrollo del Milenio y las metas de la alianza Alto a la Tuberculosis
OBJETIVOS	• Conseguir el acceso universal a un diagnóstico de calidad y a un tratamiento centrado en el paciente • Reducir el sufrimiento humano y la carga socioeconómica asociados a la tuberculosis • Proteger a las poblaciones vulnerables contra la tuberculosis, la tuberculosis/VIH y la tuberculosis multirresistente • Apoyar el desarrollo de nuevos medios y hacer posible que se usen pronto y eficazmente
METAS	• ODM 6, Meta 8: Detener y comenzar a reducir la incidencia de tuberculosis para 2015 • Metas relacionadas con los ODM y apoyadas por la alianza Alto a la Tuberculosis: ▫ 2005: detectar al menos el 70% de los casos de tuberculosis infecciosa y curar al menos el 85% de ellos ▫ 2015: reducir la prevalencia de tuberculosis y la mortalidad por esa causa en un 50% respecto a 1990 ▫ 2050: [illegible] problema de salud pública (1 caso por millón de habitantes)

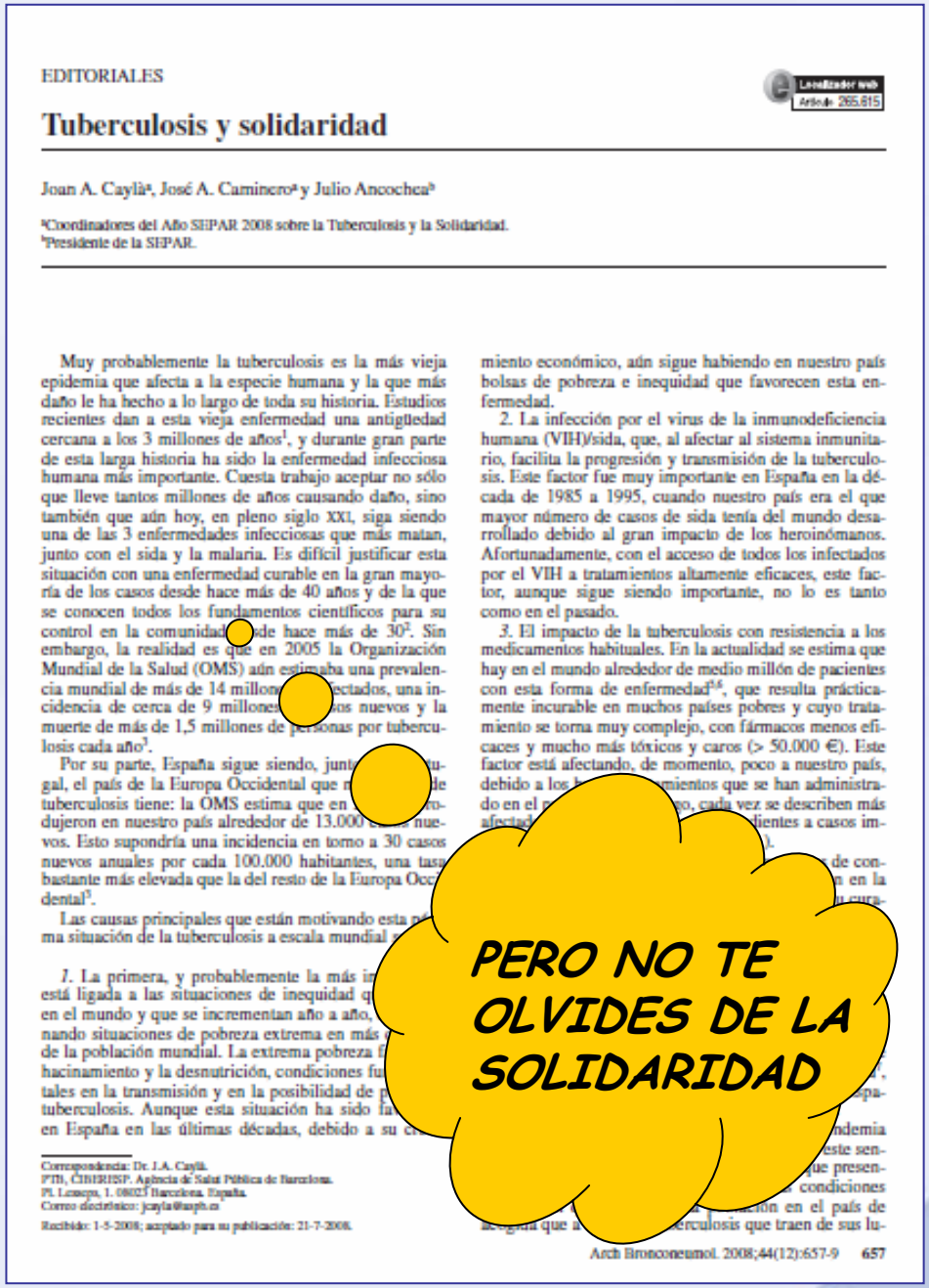
- **Objetivos de desarrollo del milenio:**
  - ✓ Para 2015: Reducción a la mitad de la prevalencia y las muertes de los niveles de 1990
  - ✓ Para 2050: incidencia global <1 por 1.000.000 (indice actual 125 por 100.000)

Stop TB Partnership IN THE FIGHT AGAINST TB THE HERO COULD BE YOU

www.stoptb.org/globalplan/assets/documents/globalplanfinal.pdf


Hospital Universitario Central de Asturias
Muestras clínicas HUCA
Muestras clínicas otros centros
Cepas micobacterias otros centros
ÁREA MICROBIOLOGÍA
Recepción-Registro-Reparto
LABORATORIO MICOBACTERIAS
MUESTRAS
CEPAS
Diagnóstico Infección Tuberculosa Latente IGRAs
T-SPOT.TB
QuantiFERON-TB Gold In-Tube
Pre-Tratamiento Descontaminación-Digestión
Técnicas de Amplificación Genómica TAGs
Control Calidad QCMD Unión Europea
BK: Tinción Ziehl-Neelsen y/o Fluorescencia
Cultivo:
M.Líquidos: MGIT - BacT/Alert 3D
M.Sólido: Löwenstein-Jensen
Extracción de Ácidos Nucleicos COBAS Ampliprep
Xpert MTB/RIF
Cultivo POSITIVO
PCR Cualitativa MTB Tiempo Real TaqMan
BAAR m.o.
Sondas ADN Accuprobe
Control Calidad SEIMC
M.tuberculosis
M. atípicas
Antibiograma "rutina" Etest IZ,RI,SM,EB
PCR-Hibridación Reversa
-INNO LIPA Micobacteria v2
-Genotype CM-AS
Cepario Congelación
Antibiograma M. atípicas
Antibiograma Referencia + PZ
Epidemiología molecular TBC
PRA (PCR-RFLP)
Detección genes-R
-RI (rpoB)
-IZ (katG, inhA)
-EB (embCAB), SM (rpsL, rrs)
Control Calidad OMS
Genotipado
- RFLP IS6110
- Spoligotyping
Identificación MTB complex
PCR-RFLP gen gyrB, RD1, RD9
M. tuberculosis, M. africanum I/II, M. bovis, M. BCG
M. caprae, M. microti, M. canetti, M. pinnipedii
ITS 16S-23S rRNA
16S-rDNA Secuenciación
-IZ (ahpC)
-PZ (pncA)
Archivo ADN genómico
-MIRU-VNTR

Caylà JA *et al*
Arch Bronconeumol 2008, 44: 657-59



EDITORIALES

Tuberculosis y solidaridad

Joan A. Caylà[a], José A. Caminero[a] y Julio Ancochea[b]

[a]Coordinadores del Año SEPAR 2008 sobre la Tuberculosis y la Solidaridad.
[b]Presidente de la SEPAR.

Muy probablemente la tuberculosis es la más vieja epidemia que afecta a la especie humana y la que más daño le ha hecho a lo largo de toda su historia. Estudios recientes dan a esta vieja enfermedad una antigüedad cercana a los 3 millones de años[1], y durante gran parte de esta larga historia ha sido la enfermedad infecciosa humana más importante. Cuesta trabajo aceptar no sólo que lleve tantos millones de años causando daño, sino también que aún hoy, en pleno siglo XXI, siga siendo una de las 3 enfermedades infecciosas que más matan, junto con el sida y la malaria. Es difícil justificar esta situación con una enfermedad curable en la gran mayoría de los casos desde hace más de 40 años y de la que se conocen todos los fundamentos científicos para su control en la comunidad desde hace más de 30[2]. Sin embargo, la realidad es que en 2005 la Organización Mundial de la Salud (OMS) aún estimaba una prevalencia mundial de más de 14 millones de afectados, una incidencia de cerca de 9 millones de casos nuevos y la muerte de más de 1,5 millones de personas por tuberculosis cada año[3].

Por su parte, España sigue siendo, junto con Portugal, el país de la Europa Occidental que mayor carga de tuberculosis tiene: la OMS estima que en 2005 se produjeron en nuestro país alrededor de 13.000 casos nuevos. Esto supondría una incidencia en torno a 30 casos nuevos anuales por cada 100.000 habitantes, una tasa bastante más elevada que la del resto de la Europa Occidental[3].

Las causas principales que están motivando esta preocupante situación de la tuberculosis a escala mundial son:

1. La primera, y probablemente la más importante, está ligada a las situaciones de inequidad que existen en el mundo y que se incrementan año a año, generando situaciones de pobreza extrema en más del 20% de la población mundial. La extrema pobreza favorece el hacinamiento y la desnutrición, condiciones fundamentales en la transmisión y en la posibilidad de padecer tuberculosis. Aunque esta situación ha sido favorable en España en las últimas décadas, debido a su crecimiento económico, aún sigue habiendo en nuestro país bolsas de pobreza e inequidad que favorecen esta enfermedad.

2. La infección por el virus de la inmunodeficiencia humana (VIH)/sida, que, al afectar al sistema inmunitario, facilita la progresión y transmisión de la tuberculosis. Este factor fue muy importante en España en la década de 1985 a 1995, cuando nuestro país era el que mayor número de casos de sida tenía del mundo desarrollado debido al gran impacto de los heroinómanos. Afortunadamente, con el acceso de todos los infectados por el VIH a tratamientos altamente eficaces, este factor, aunque sigue siendo importante, no lo es tanto como en el pasado.

3. El impacto de la tuberculosis con resistencia a los medicamentos habituales. En la actualidad se estima que hay en el mundo alrededor de medio millón de pacientes con esta forma de enfermedad[3,5], que resulta prácticamente incurable en muchos países pobres y cuyo tratamiento se torna muy complejo, con fármacos menos eficaces y mucho más tóxicos y caros (> 50.000 €). Este factor está afectando, de momento, poco a nuestro país, debido a los [illegible] que se han administrado en el [illegible], cada vez se describen más afectados [illegible] pacientes a casos im-

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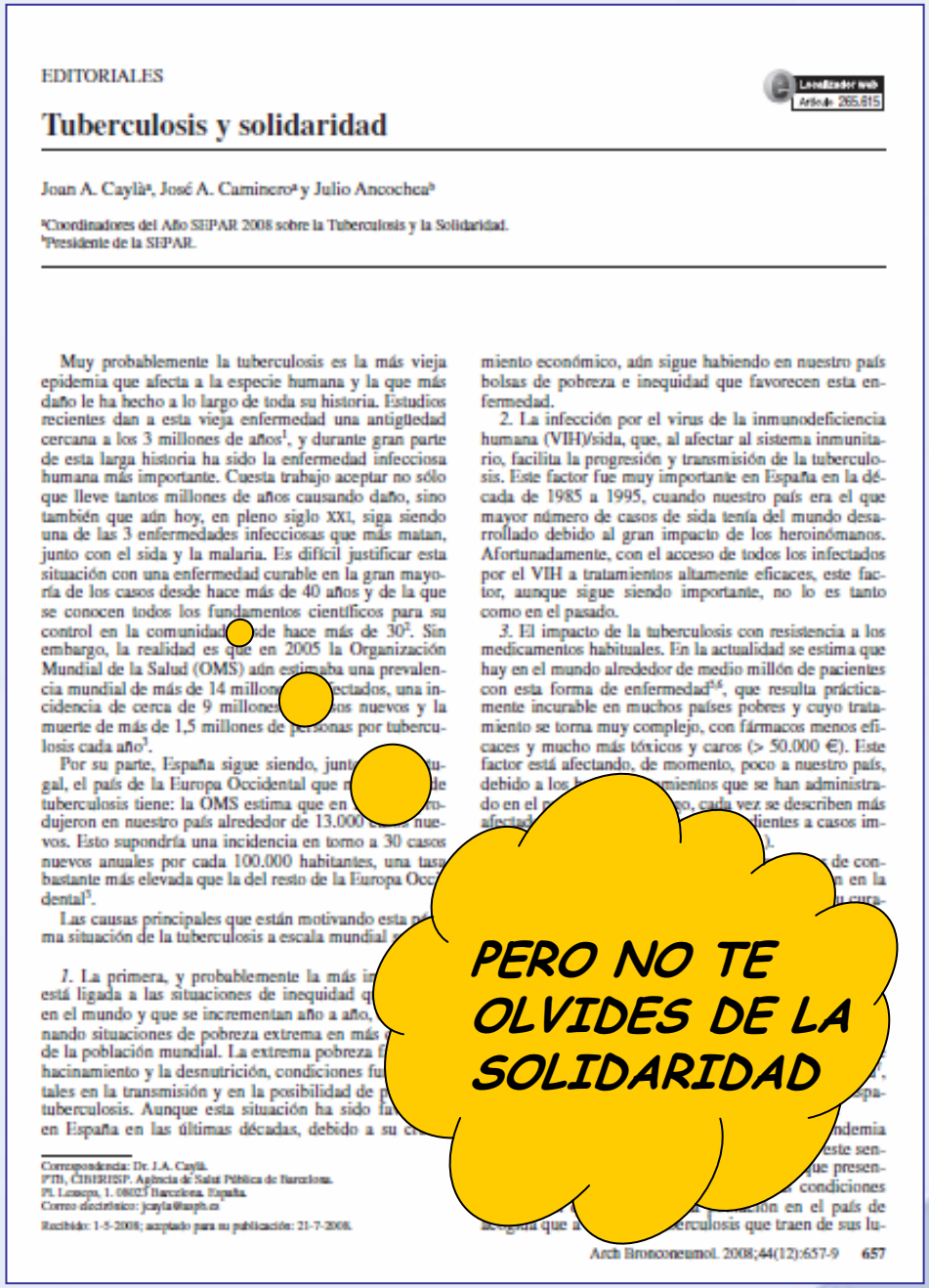
PTI, CIBERESP. Agència de Salut Pública de Barcelona.
Pl. Lesseps, 1. 08023 Barcelona. España.
Correo electrónico: jcayla@aspb.es

Recibido: 1-5-2008; aceptado para su publicación: 21-7-2008.

PERO NO TE OLVIDES DE LA SOLIDARIDAD

Caylà JA *et al*
Arch Bronconeumol 2008, 44: 657-59

Gracias



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Las causas principales que están motivando esta preocupante situación de la tuberculosis a escala mundial son:

1. La primera, y probablemente la más importante, está ligada a las situaciones de inequidad que existen en el mundo y que se incrementan año a año, generando situaciones de pobreza extrema en más del 50% de la población mundial. La extrema pobreza favorece el hacinamiento y la desnutrición, condiciones fundamentales en la transmisión y en la posibilidad de padecer tuberculosis. Aunque esta situación ha sido favorable en España en las últimas décadas, debido a su crecimiento económico, aún sigue habiendo en nuestro país bolsas de pobreza e inequidad que favorecen esta enfermedad.

2. La infección por el virus de la inmunodeficiencia humana (VIH)/sida, que, al afectar al sistema inmunitario, facilita la progresión y transmisión de la tuberculosis. Este factor fue muy importante en España en la década de 1985 a 1995, cuando nuestro país era el que mayor número de casos de sida tenía del mundo desarrollado debido al gran impacto de los heroinómanos. Afortunadamente, con el acceso de todos los infectados por el VIH a tratamientos altamente eficaces, este factor, aunque sigue siendo importante, no lo es tanto como en el pasado.

3. El impacto de la tuberculosis con resistencia a los medicamentos habituales. En la actualidad se estima que hay en el mundo alrededor de medio millón de pacientes con esta forma de enfermedad[4,5], que resulta prácticamente incurable en muchos países pobres y cuyo tratamiento se torna muy complejo, con fármacos menos eficaces y mucho más tóxicos y caros (> 50.000 €). Este factor está afectando, de momento, poco a nuestro país, debido a los tratamientos que se han administrado en el pasado, cada vez se describen más afectados, pacientes a casos im-

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