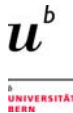


**19<sup>th</sup> SWISS TUBERCULOSIS SYMPOSIUM**  
**Update on culture and identification**

Münchenwiler, 25.03.2010

Dr. Thomas Bodmer, M.D.  
Institute for Infectious Diseases



**Culture of mycobacteria**

---


*“Cultivation of MTB in the laboratory is the gold standard for confirming the diagnosis of tuberculosis.”*

*“The intrinsic growth rate of MTB makes the recovery of the organism in culture a slow process. In traditional egg-based media, growth of colonies of MTB takes between 3 and 6 weeks.”*

*“Obviously, the glacial pace of these traditional culture systems interferes with optimal patient management, and more rapid techniques are required.”*

Oxford Textbook of Medicine, 4<sup>th</sup> ed., 2004; p. 566

---

 Klinische Mikrobiologie

# Sedimentation



Aim:  
„Enrichment und killing“

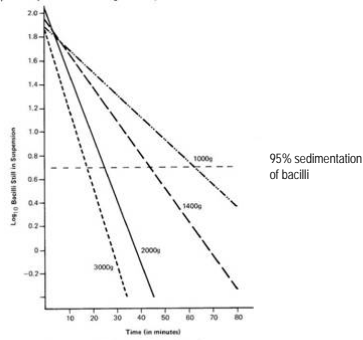


FIGURE 10. Efficiency of centrifugation  
Log10 of percentage of acid-fast bacilli in suspension vs. centrifugation time and g-force. Broken line indicates 95% sedimentation of bacilli for 5% acid in suspension. (x, Lang 87).  
Kent PT & GP Kubica. 1985.

Klinische Mikrobiologie

# Decontamination



Aim:  
„Enrichment and killing“

Method:  
N-Acetyl-L-Cystein-NaOH (NALC)

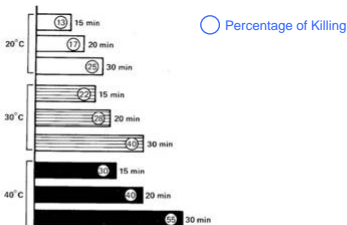



FIGURE 11. Combined lethal effect on mycobacteria of temperature and time of exposure to a potentially lethal digestant

Kent PT & GP Kubica. 1985.

Klinische Mikrobiologie



## „The Killing Fields“

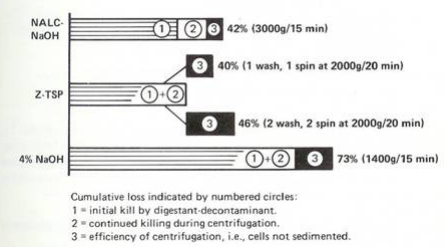
**Aim:**  
*„Enrichment and killing“*

**Method:**  
N-Acetyl-L-Cystein-NaOH (NALC)

**Culture results Berne, 2009:**

Negative	90%
Positive	6%
Contamination*	4%

\*The digestion-decontamination procedure should be as gentle as possible (compatible with an overall contamination rate not in excess of 5%).  
Kent PT & GP Kubica. 1985.




**FIGURE 12. Hypothetical examples of overall kill with different digestants**

\* The tolerance of 50-ml conical bottom centrifuge tubes for g-forces in excess of 3000 RCF varies with the manufacturer (check supplier for this information). The 50-ml conical bottom centrifuge tubes that were first made for use in the tuberculosis laboratory could tolerate only about 3200 g; more recently, tubes that can withstand 5000 g have been manufactured.

\*+ Our recent studies (7) showed that 3000 g for 15 minutes would sediment 95% of the mycobacteria in a digested sputum specimen. From the formula on page 33, it is interesting to speculate that only 5 minutes of centrifugation in new tubes that can tolerate 5000 g would theoretically sediment 95% to 97% of suspended bacilli. Studies to confirm these theoretical figures have not yet been performed.

Kent PT & GP Kubica. 1985.





## Liquid media culture systems




**Bactec® 460-TB**



**Bactec® MGIT 960**



**MB Bact/Alert®**




UNIVERSITÄT  
BERN


## Identification of cultured mycobacteria

---

- > Phenotypic
  - Biochemical tests
  - High-performance liquid chromatography of mycolic acids
  - Immune-chromatography MPT64 (MTB complex)
  - Matrix-assisted Laser Desorption/Ionization – Time-of-flight – Mass-Spectrometry (MALDI-TOF) ?
- > Molecular
  - 65kD hsp gene PCR restriction-enzyme analysis (PRA)
  - Sequencing of house-keeping genes (e.g. the 16S rRNA gene)
  - DNA probe assays (e.g. Accuprobe®, GenoType®, INO LiPA®)
  - DNA chip technology
  - . . .



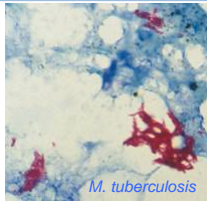
Klinische Mikrobiologie



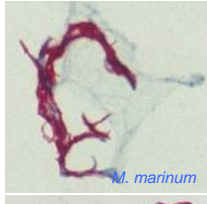
UNIVERSITÄT  
BERN

## High-performance liquid chromatography

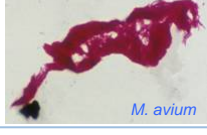
---



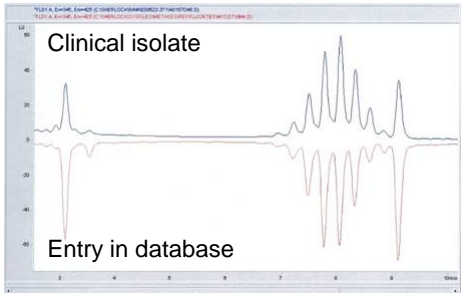
*M. tuberculosis*



*M. marinum*



*M. avium*



Clinical isolate


Entry in database

Journal of Clinical Microbiology, Dec. 2007, p. 3915-3920  
DOI: 10.1128/JCM.01401-07  
Copyright © 2007, American Society for Microbiology. All Rights Reserved. Vol. 45, No. 12


**Rapid, Standardized Method for Determination of *Mycobacterium tuberculosis* Drug Susceptibility by Use of Mycolic Acid Analysis<sup>1</sup>**

Nicole Parrish,<sup>1,2</sup> Gerard Osterhout,<sup>1</sup> Kim Dionne,<sup>1</sup> Amy Sweeney,<sup>1</sup> Nicole Kwiatkowski,<sup>1</sup> Karen Carroll,<sup>1</sup> Kenneth C. Jost, Jr.,<sup>2</sup> and James Dick,<sup>1</sup>  
<sup>1</sup>Department of Pathology, Division of Medical Microbiology, Johns Hopkins University, Baltimore, Maryland,<sup>1</sup> and  
<sup>2</sup>Texas Department of State Health Services, Austin, Texas<sup>2</sup>

Received 18 December 2006/Returned for modification 20 March 2007/Accepted 21 September 2007




Klinische Mikrobiologie




UNIVERSITÄT  
BERN

## MGIT TBc ID Test (BD Diagnostics)


- > immune-chromatographic detection of MPT64 protein in culture media
- > purpose: the rapid identification of *M. tuberculosis* complex strains
- > rapid (15 min.); requires no special equipment
- > applicable for specimens from both liquid and solid media
- > limit of detection:  $10^5$  cfu/ml
- > sensitivity, 99%; specificity, 100%
- > limitations: some *M. bovis* BCG lack MPT64 production; mutations within the *mpt64* gene



Park, MY, *et al.* J. Clin. Microbiol. 2009; **47**: 481-4.



Klinische Mikrobiologie



UNIVERSITÄT  
BERN

## PRA

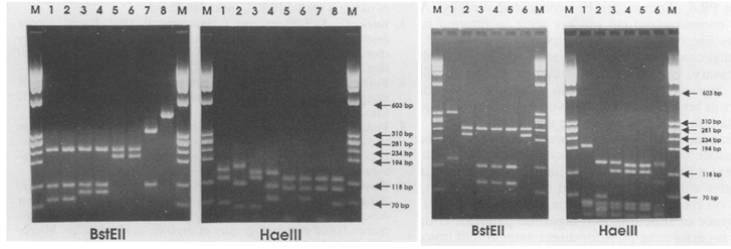
JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1993, p. 175-178  
 0095-1137/93/020175-04\$02.00/0  
 Copyright © 1993, American Society for Microbiology
Vol. 31, No. 2


### Rapid Identification of Mycobacteria to the Species Level by Polymerase Chain Reaction and Restriction Enzyme Analysis

AMALIO TELENTI,<sup>1\*</sup> FRANCINE MARCHESI,<sup>1</sup> MARIANNE BALZ,<sup>1</sup> FRANK BALLY,<sup>1</sup>  
 ERIK C. BÖTTGER,<sup>2</sup> AND THOMAS BODMER<sup>1</sup>


*Institut für Medizinische Mikrobiologie, Universität Bern, Friedbühlstrasse 51, 3010 Bern, Switzerland,<sup>1</sup> and  
 Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, 3000 Hannover, Germany<sup>2</sup>*

Received 29 July 1992/Accepted 26 October 1992





Klinische Mikrobiologie


  
 UNIVERSITÄT  
BERN

## Misidentification of mycobacteria by commercial DNA probe assays

---

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2010, p. 307-310  
 0095-1137/10/\$12.00 doi:10.1128/JCM.01536-09  
 Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Vol. 48, No. 1


### Commercial DNA Probes for Mycobacteria Incorrectly Identify a Number of Less Frequently Encountered Species<sup>∇†</sup>


Enrico Tortoli,<sup>1\*</sup> Monica Pecorari,<sup>2</sup> Giuliana Fabio,<sup>2</sup> Massimino Messinò,<sup>2</sup> and Anna Fabio<sup>2</sup>  
*Regional Reference Center for Mycobacteria, Careggi University Hospital, 50134 Florence, Italy,<sup>1</sup> and Laboratory of Microbiology and Virology, Modena University Hospital, 41124 Modena, Italy<sup>2</sup>*

Received 9 August 2009/Returned for modification 17 October 2009/Accepted 5 November 2009

Although commercially available DNA probes for identification of mycobacteria have been investigated with large numbers of strains, nothing is known about the ability of these probes to identify less frequently encountered species. We analyzed, with INNO LiPA MYCOBACTERIA (Innogenetics) and with GenoType Mycobacterium (Hein), 317 strains, belonging to 136 species, 61 of which had never been assayed before. INNO LiPA misidentified 20 taxa, the majority of which cross-reacted with the probes specific for *Mycobacterium fortuitum* and the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* group. GenoType misidentified 28 taxa, most of which cross-reacted with *M. intracellulare* and *M. fortuitum* probes; furthermore, eight species were not recognized as members of the genus *Mycobacterium*. Among 54 strains investigated with AccuProbe (Gen-Probe), cross-reactions were detected for nine species, with the probes aiming at the *M. avium* complex being most involved in cross-reactions.

---


 Klinische Mikrobiologie


  
 UNIVERSITÄT  
BERN

## Commercial DNA probe systems, part 1


---

- > Comparison of 3 commercial DNA probe systems:
  - AccuProbe (Gen-Probe, San Diego, CA)
  - INO LiPA (Innogenetics, Gent, Belgium)
  - GenoType (HAIN Lifescience GmbH, Nehren, Germany)
- > In the last few years, taxonomic studies have recognized and described many new mycobacterial species, in particular:
  - *Mycobacterium avium* complex (MAC)
  - *Mycobacterium fortuitum* group
- > None of the systems misidentified MTB complex as NTM, but incorrectly assigned to MTB complex:
  - *M. holsaticum* (AccuProbe)
  - *M. riyadhense*, and “*M. simulans*” (GT-CM)

---

Tortoli, E, *et al.* J. Clin. Microbiol. 2010; **48**: 307-10.

---


 Klinische Mikrobiologie

## Commercial DNA probe systems, part 2

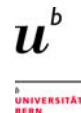


- > GT-CM did not recognize a number of mycobacterial species as members of the genus *Mycobacterium*:
  - *M. elephantis*; *M. frederiksbergense*; *M. hassiacum*; *M. hodleri*; *M. pulveris*; *M. moriokaense*; *M. sphagni* were assigned to the G/C-rich Gram-positive bacilli
  - *M. duvalis* was not recognized by this assay

Tortoli, E, et al. J. Clin. Microbiol. 2010; 48: 307-10.



## MTB complex – from genetic polymorphisms . . .

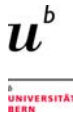


### gyrB Discriminatory Regions

Reference sequences	region 1 (675)	region 2 (756)	region new (1311)	region 3 (1410)	region 4 (1450)
<i>M. tuberculosis</i>	GGTA C GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC G CGAA
<i>M. bovis</i>	GGTA C GAGT	AACGGT A CGG	GGCCGC T GTGA	TGTAA T GAACA	CCGAC T CGAA
<i>M. africanum</i>	GGTA C GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC T CGAA
<i>M. microti</i>	GGTA T GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC T CGAA
<b>Strains tested</b>					
<i>M. tuberculosis</i> (n=5)	GGTA C GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC G CGAA
<i>M. bovis</i>					
subsp. <i>bovis</i> (n=5)	GGTA C GAGT	AACGGT A CGG	GGCCGC T GTGA	TGTAA T GAACA	CCGAC T CGAA
subsp. <i>caprae</i> (n=5)	GGTA C GAGT	AACGGT A CGG	GGCCGC G GTGA	TGTAA C GAACA	CCGAC T CGAA
subsp. C (n=2)	GGTA C GAGT	AACGGT A CGG	GGCCGC G GTGA	TGTAA C GAACA	CCGAC T CGAA
<i>M. africanum</i>					
subtype I (n=5)	GGTA C GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC T CGAA
subtype II (n=5)	GGTA C GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC G CGAA
<i>M. microti</i>					
type llama (n=2)	GGTA T GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC T CGAA
type vole (n=1)	GGTA T GAGT	AACGGT G CGG	GGCCGC T GTGA	TGTAA C GAACA	CCGAC T CGAA

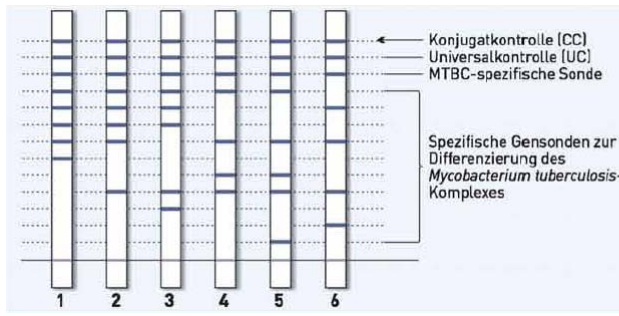
Niemann S., et al. JCM 2000; 38: 3231-3234





## ... to molecular identification

### GenoType® MTBC




Konjugatkontrolle [CC]  
 Universalkontrolle [UC]  
 MTBC-spezifische Sonde

Spezifische Gensonden zur Differenzierung des *Mycobacterium tuberculosis*-Komplexes

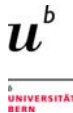
**Spezifische Bandenmuster des GenoType® MTBC**

1. *M. tuberculosis*
2. *M. africanum* I
3. *M. microti*
4. *M. bovis* ssp. *bovis*
5. BCG
6. *M. bovis* ssp. *caprae*

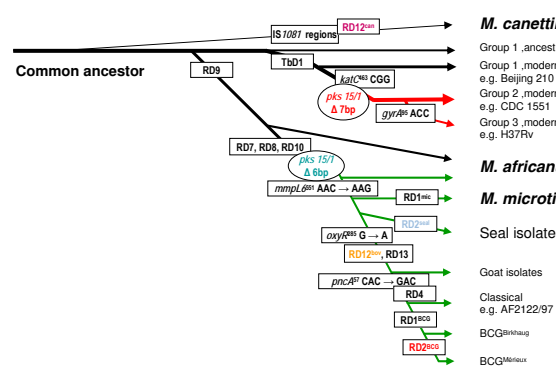
http://www.hain-lifescience.de/pdf/301\_flyer\_dt.pdf



Klinische Mikrobiologie



## MTB – the evolution of a pathogen



***M. canettii***

Group 1 „ancestral“  
Group 1 „modern“  
e.g. Beijing 210

***M. tuberculosis***

Group 2 „modern“  
e.g. CDC 1551  
Group 3 „modern“  
e.g. H37Rv

***M. africanum***

***M. microti***

Seal isolates

Goat isolates

Classical  
e.g. AF2122/97

BCG<sup>Shanghai</sup>

BCG<sup>Molnau</sup>

**Taxonomy**

*M. canettii*

*M. tuberculosis*

*M. africanum*, Type I & II

*M. microti*


*M. pinnipedii*

*M. bovis* subsp. *caprae*

*M. bovis* subsp. *bovis*

*M. bovis* biovar. BCG

Marmiesse M *et al.* 2004. *Microbiol* 150: 483 - 496

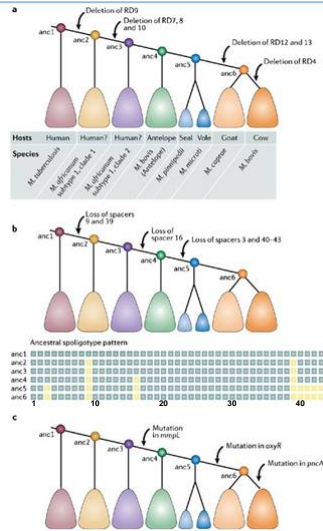


Klinische Mikrobiologie





### Phylogenetic scenario for animal-adapted strains of the *M. tuberculosis* complex



The distribution of phylogenetically informative deletions that are fixed in all descendant clades

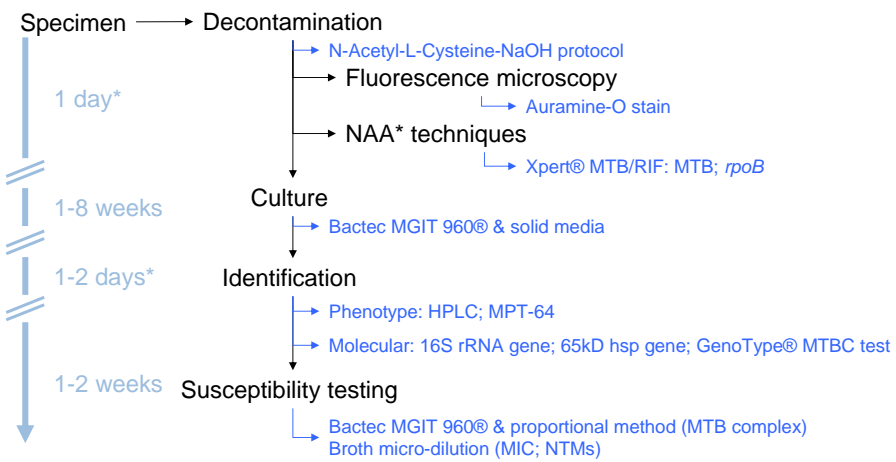
The sequential loss of spoligotype spacer units in the RD9 deleted lineage

The distribution of phylogenetically informative single nucleotide mutations

Smith et al. Nat Rev Microbiol 2006; 4, 670-681



### Laboratory algorithm in 2010



\*working day(s)

\*Nucleic acid amplification



## Culture of mycobacteria



*“Cultivation of MTB in the laboratory is the gold standard for confirming the diagnosis of tuberculosis.”*

*“The intrinsic growth rate of MTB makes the recovery of the organism in culture a slow process. In traditional egg-based media, growth of colonies of MTB takes between 3 and 6 weeks.”*

*“Obviously, the glacial pace of these traditional culture systems interferes with optimal patient management, and more rapid techniques are required.”*

Oxford Textbook of Medicine, 4<sup>th</sup> ed., 2004; p. 566