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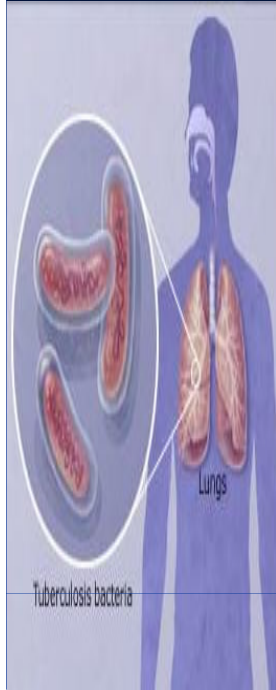


About OMICS Group Conferences

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Diagnostic potential of *M. tuberculosis*-specific proteins and peptides



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Publications >225
Impact Factor >480

Citations = >6400

h-index = 42

i10 index = 125

Theses supervisor
(n=28)

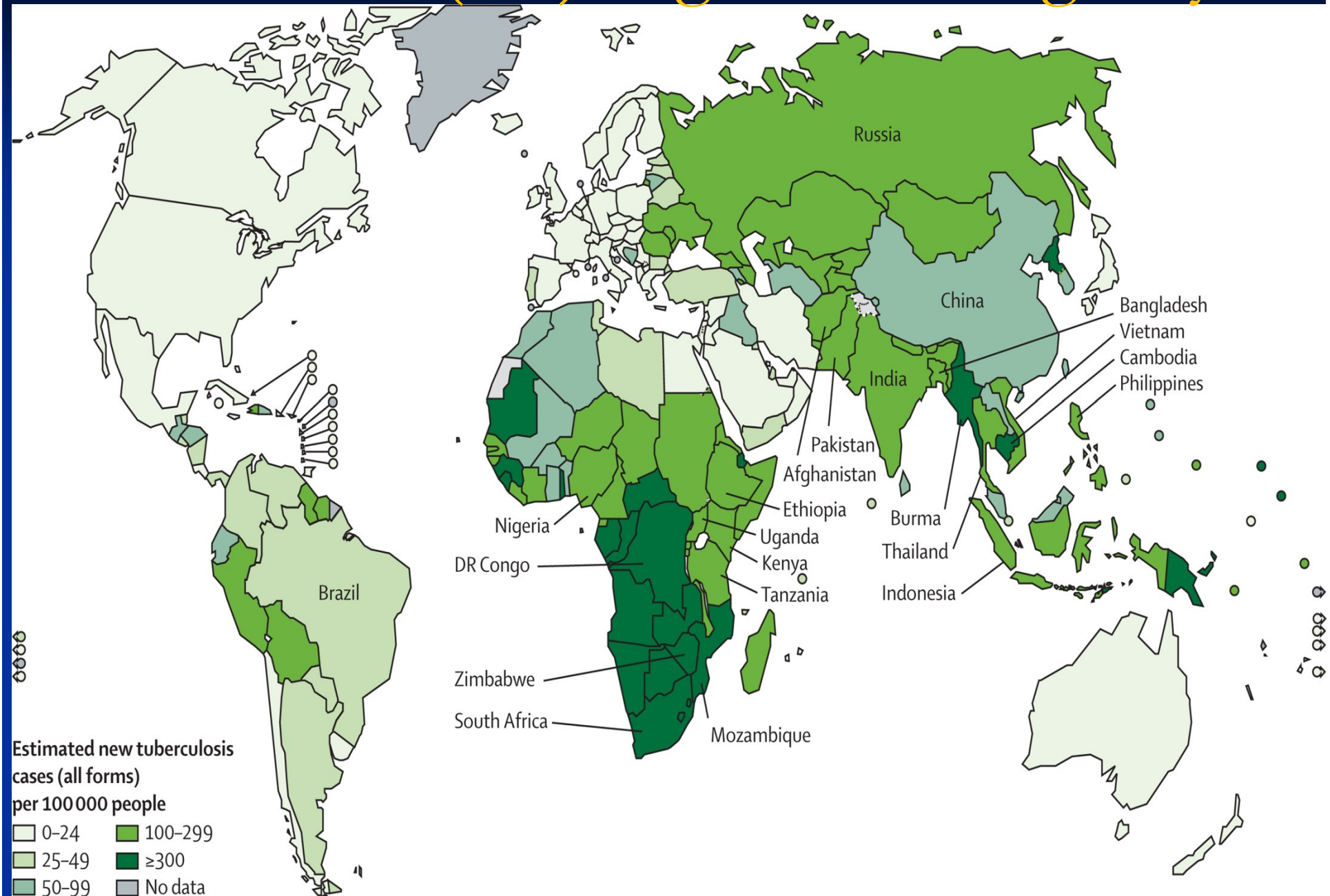
Theses examiner
(n=32)

Invited speaker >³100



Bacteriology 2014

Tuberculosis (TB): a global emergency



Tuberculosis: a global emergency

- TB is the second top most infectious killer of adults, after HIV/AIDS & is among the overall top 10 causes of death in the world
- Annually, 8-9 million people develop active TB disease and 1.3 million people die
- According to the WHO, almost 1/3rd of the world's population is latently infected with *M. tuberculosis*
- HIV and TB co-infection
- MDR and XDR TB
- Failure of BCG vaccine

What is required to control TB?

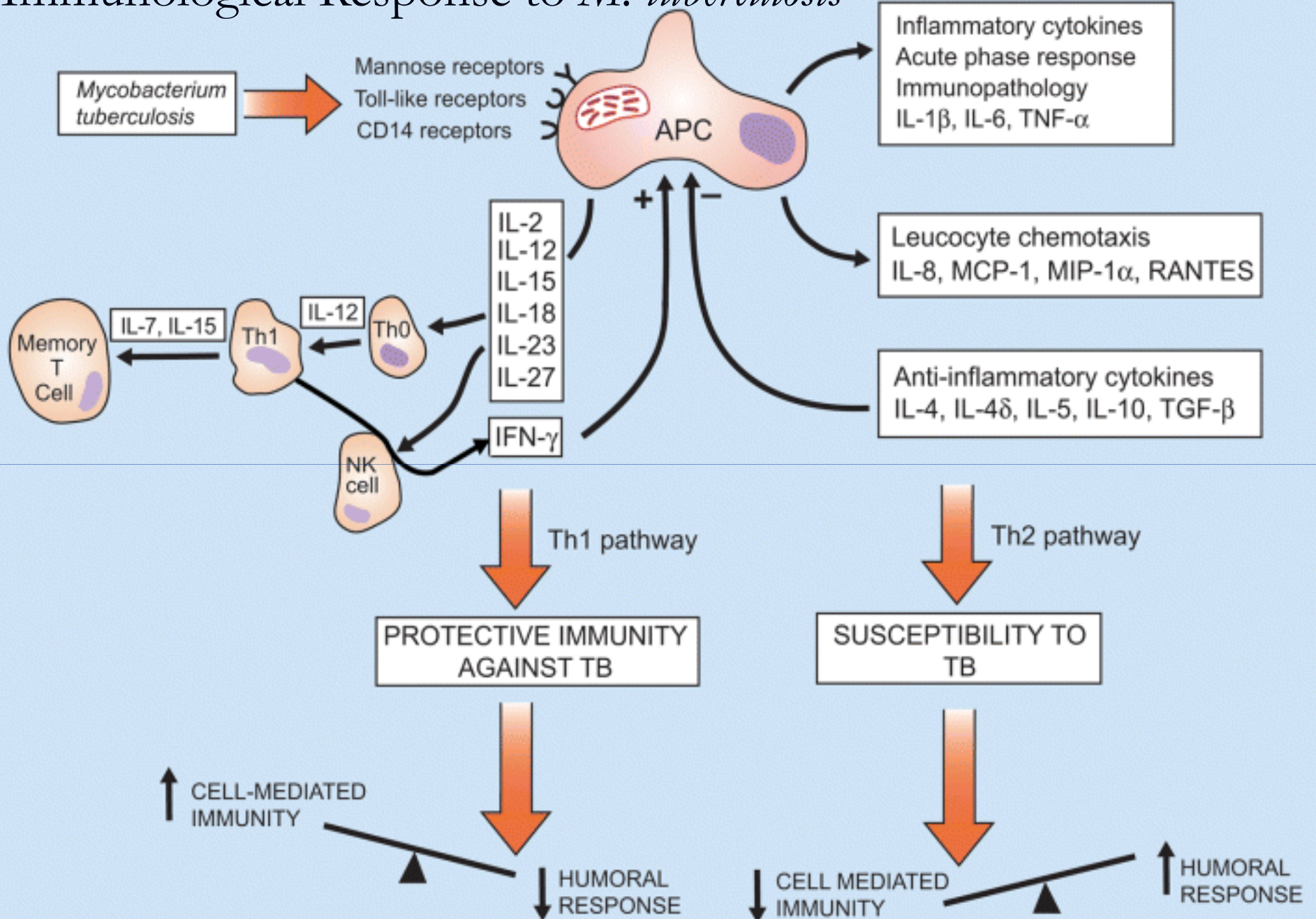
1. Specific diagnosis of both active and latent TB
2. New drugs
3. Effective vaccines

TB Diagnosis

1. Clinical symptoms
2. Chest X-ray
3. Microscopy and culture
4. Molecular methods
5. Immunological methods



Immunological Response to *M. tuberculosis*



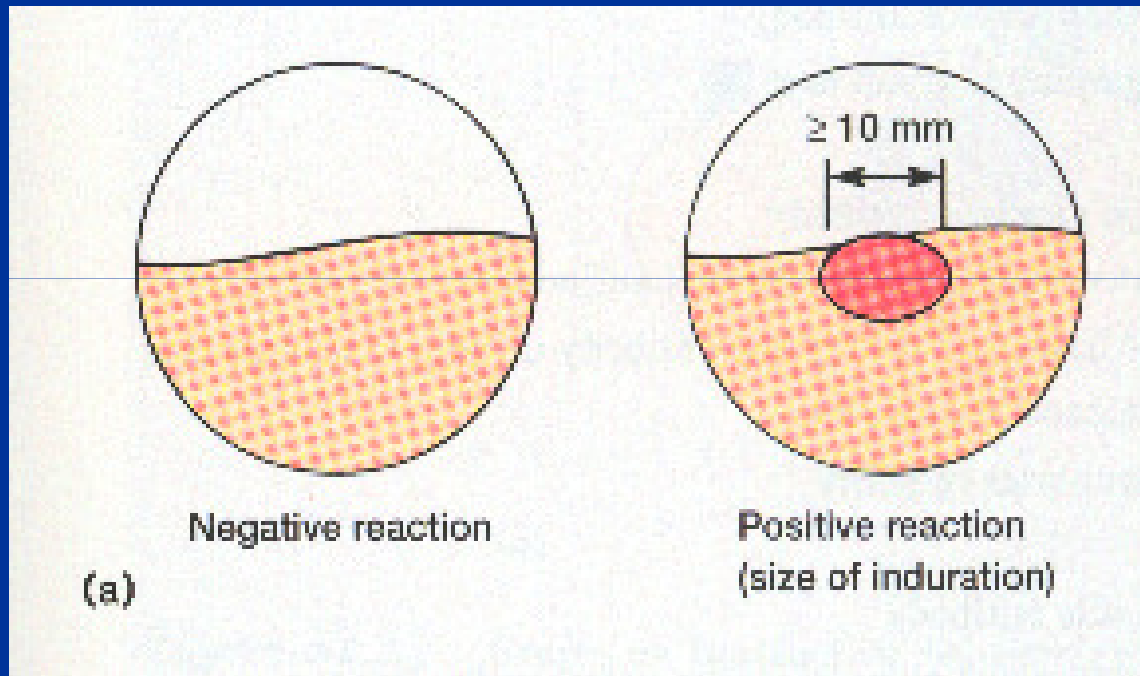
Immunological diagnosis of active and latent TB

▪ Tuberculin Skin test (PPD Test)

- ✓ Crude mixture of culture filtrate of *M. tuberculosis* which contains all types of molecules (species-specific as well as cross-reactive with other mycobacteria)
- ✓ Intra-dermal injection of 0.1 ml of PPD



✓ Immunological response (Erythema and induration) is measured after 48-72 h

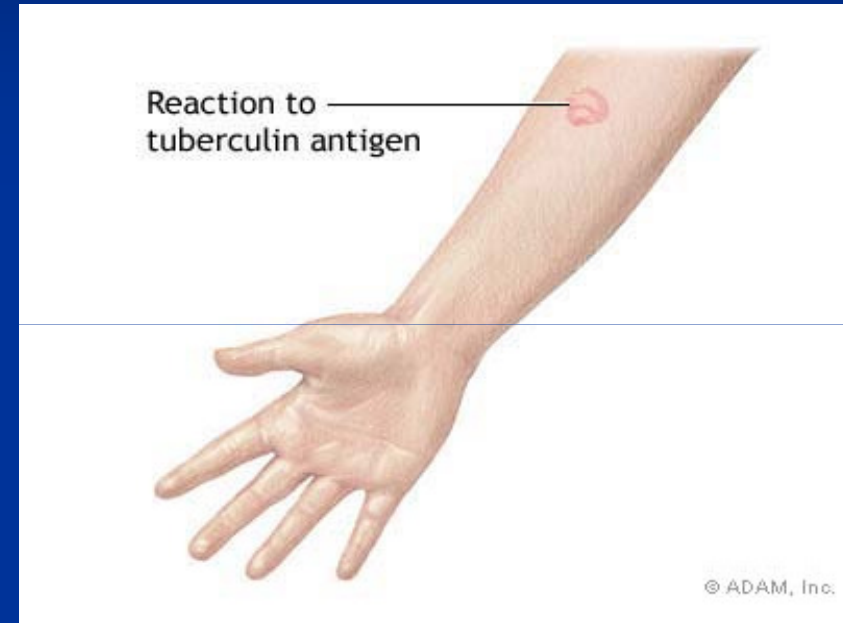


Limitations of the tuberculin (PPD) Test:

Cannot distinguish between:

Active TB disease and

- ♣ Latent TB infection
- ♣ BCG vaccination
- ♣ Exposure to environmental mycobacteria



- ◎ Thus, there is an overwhelming need to identify *M. tuberculosis*-specific antigens as
 - Specific diagnostic reagents

How to obtain *M. tuberculosis*-specific antigens?

- Classical approach:
 - Grow *M. tuberculosis* *in vitro*
 - Purify single proteins using biochemical methods
 - Test the purified proteins for immunological reactivity with patient's samples

Antigens identified using classical approach

- CFP10, ESAT-6 & TB7.7
- Currently used in CMI assays (IFN- γ response assay, IGRA) using peripheral blood cells from patients

These assays are costly, technically demanding & not affordable in TB endemic countries

Simple and cost-effective assays are required

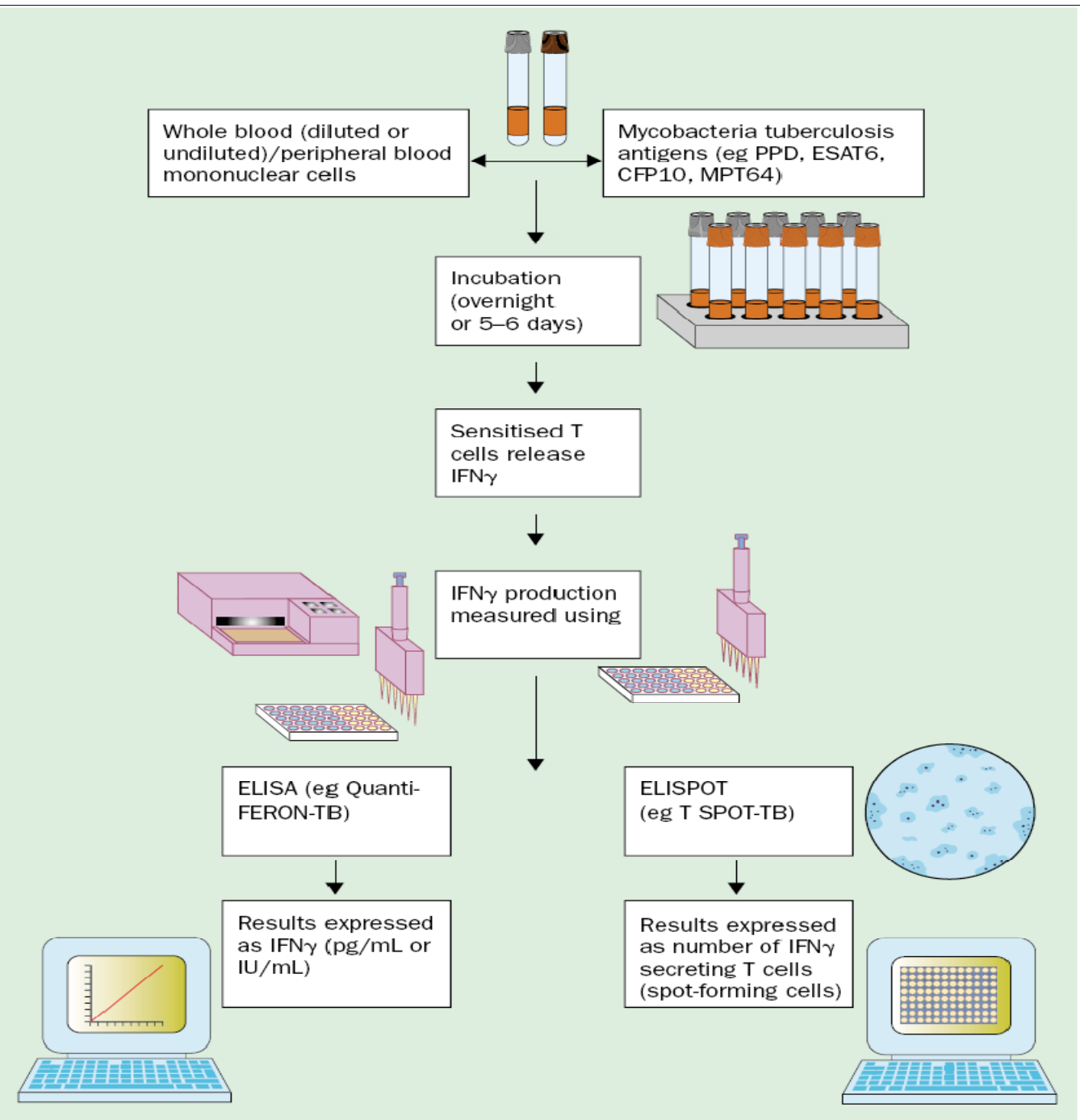
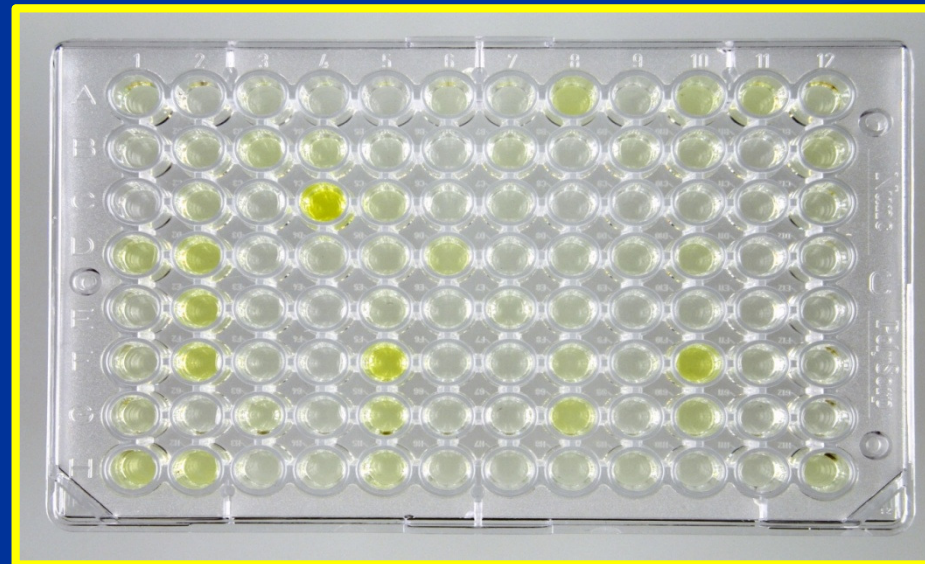


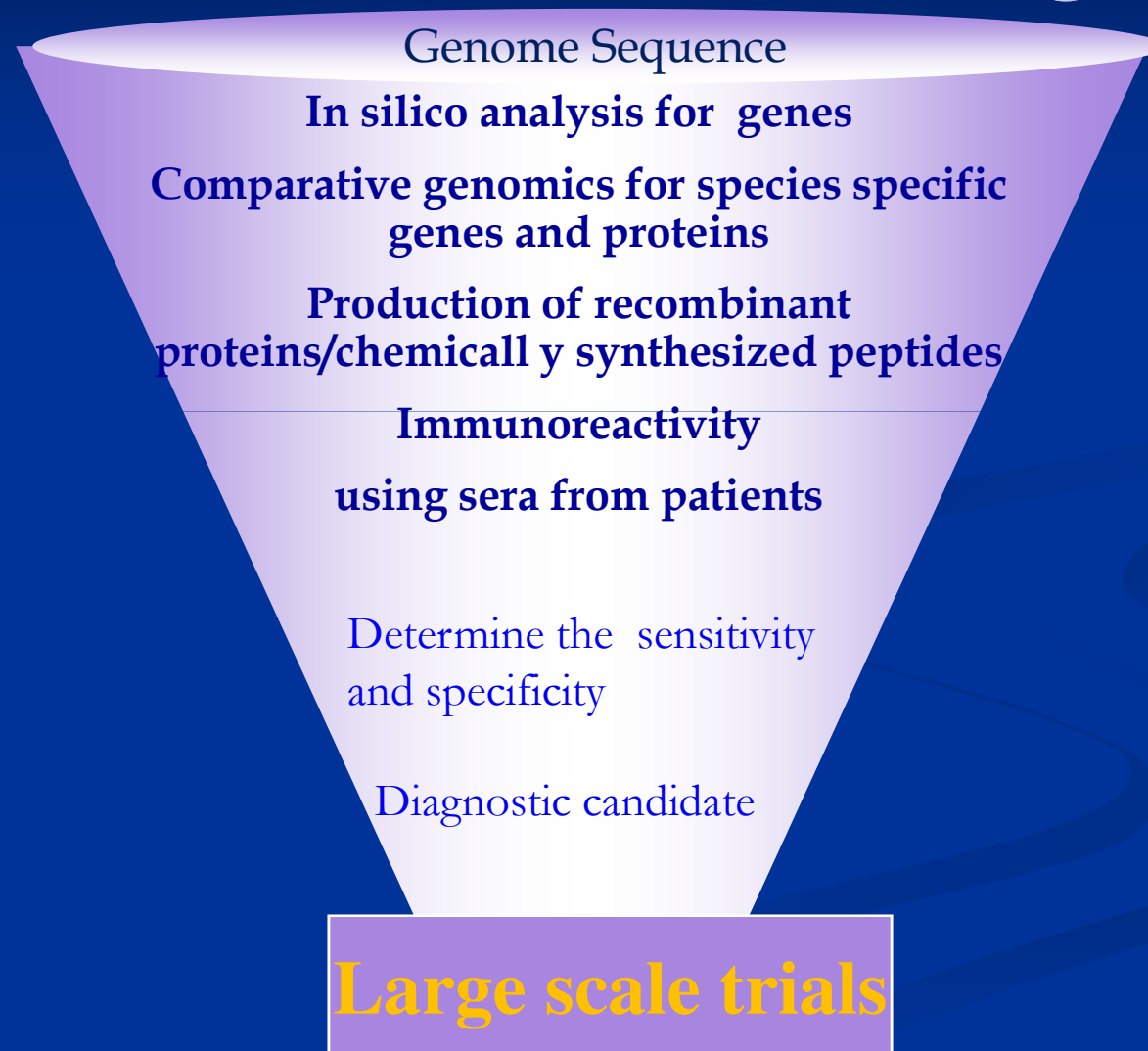
Figure 2. Overview of the interferon- γ (IFN γ) assay technology.

Antibody assays

- Enzyme-Linked-Immunosorbent Assays (ELISA) to detect antibodies to *M. tuberculosis* components in serum samples
- Cost effective, easy to perform



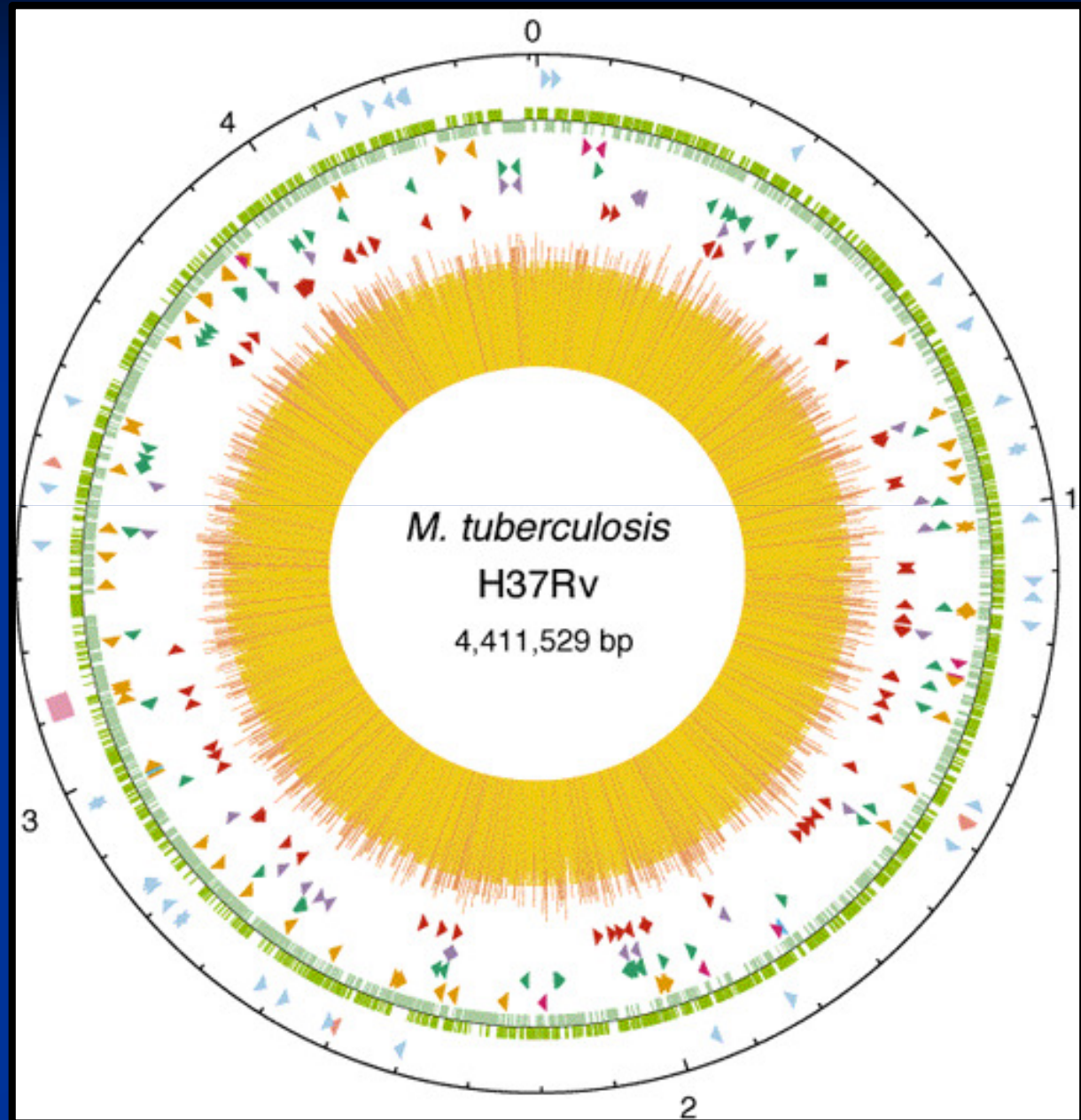
Diagnostic Development in Post Genomic Era: Reverse diagnostic



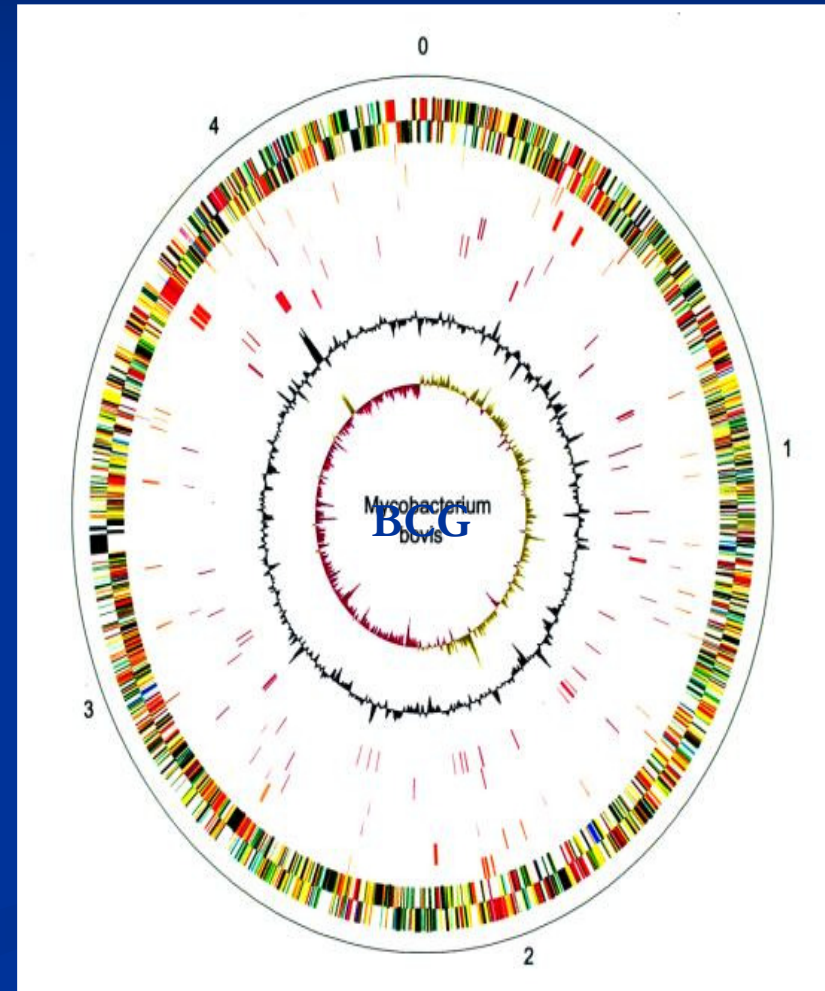
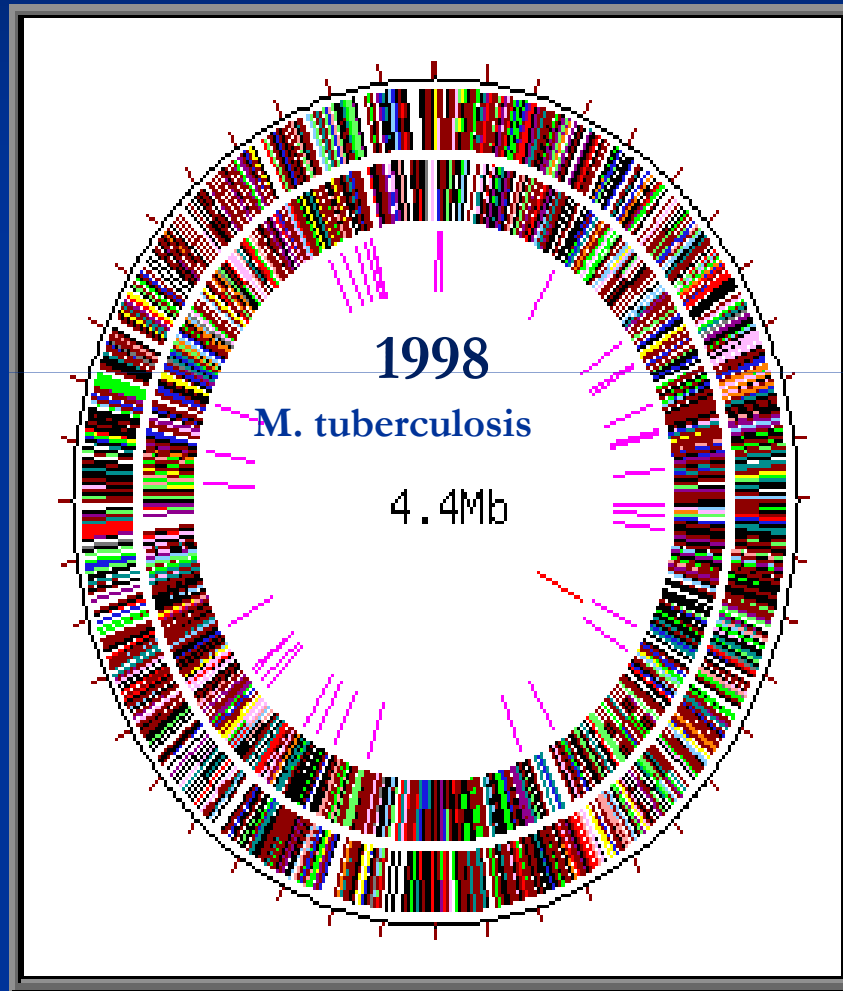
Complete genome sequence of *M. tuberculosis* and gene annotation

Genome: ca 4 MB

Genes: ca 4000

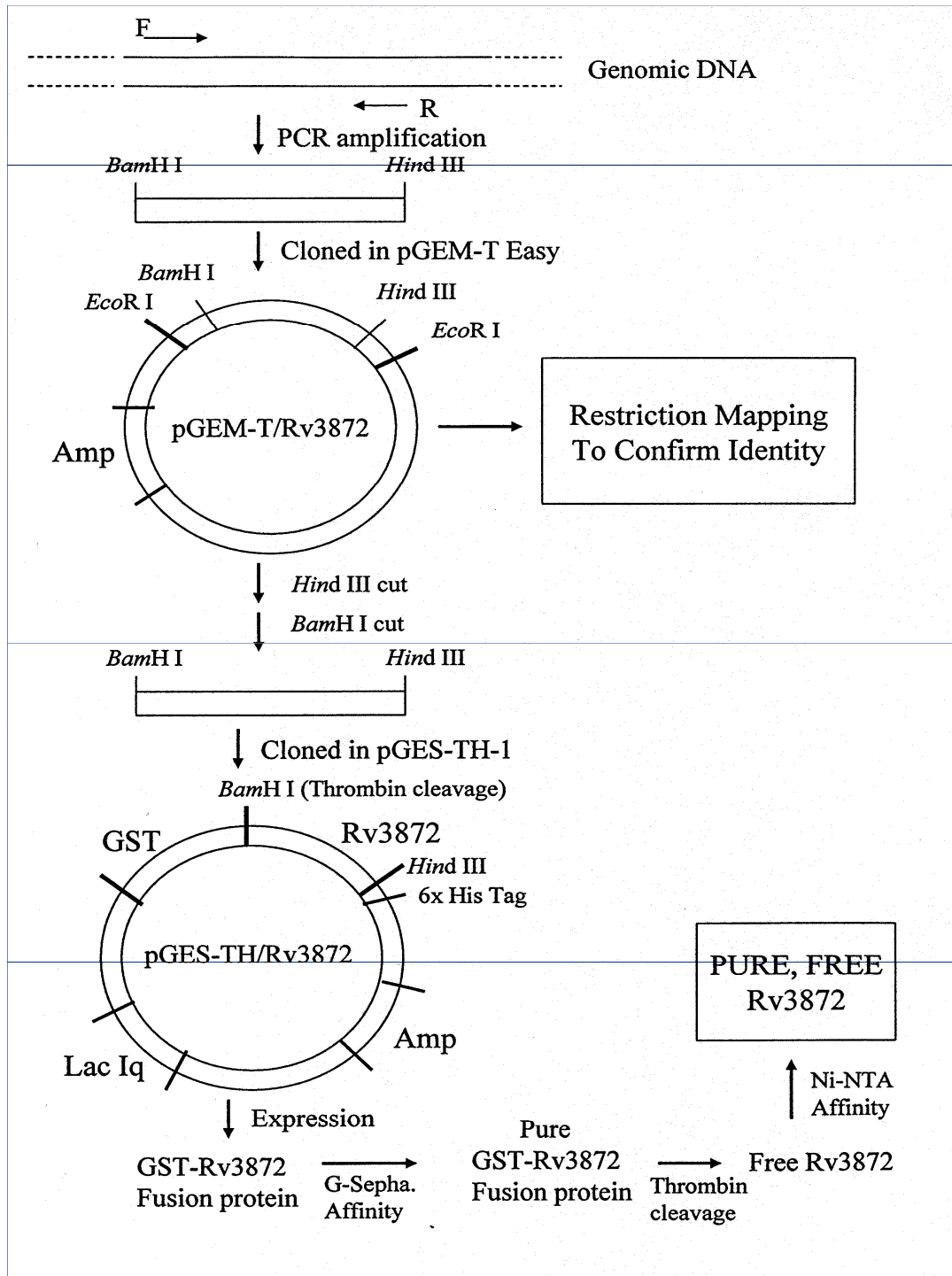


Genomic comparisons of *M. tuberculosis*, *M. bovis* BCG and other mycobacteria



Comparative genomics identified *M. tuberculosis* genomic regions absent in other mycobacteria

Region deleted	<i>M. tuberculosis</i>	<i>M. africanum</i>	<i>M. bovis</i>	BCG
RD1 (12 ORFs)	+	+	+	-
RD4 (3 ORFs)	+	-	-	-
RD5 (5 ORFs)	+	+	-	-
RD6 (11 ORFs)	+	+	-	-
RD7 (8 ORFs)	+	+	-	-
RD9 (7 ORFs)	+	-	-	-
RD10 (3 ORFs)	+	+	-	-
RD11 (5 ORFs)	+	+	-	-
RD12 (4 ORFs)	+	+	-	-
RD13 (16 ORFs)	+	+	+/-	-
RD15 (15 ORFs)	+	+	-	-



Recombinant proteins

- Only six of the 12 targeted RD1 proteins were obtained in purified form.

Inability to amplify full-length gene

Low level of expression

Degradation of the recombinant mycobacterial proteins by *E. coli* proteases

Presence of contaminating *E. coli* components in purified preparations

Overlapping synthetic peptides

P1 MTEQQWNFAGIEAAASAIQGNVTSI

P2 SAIQGNVTSIHSLLEGGKQSLTKLA

P3 EGKQSLTKLAAAWGGSGSEAYQGVQ

P4 SGSEAYQGVQOKWDATATELNNALQ

P5 TATELNNALQNLARTISEAGQAMAS

P6 ISEAGQAMASTEAGNVTGMFA

Synthetic peptides corresponding to proteins predicted in the RDs

RD	Protein	No. of proteins	No. of Peptides
RD1	Rv3871-Rv3878, ORFs 4, 8, 14 and 15	12	220
RD4	Rv0221- Rv0223	3	80
RD5	Rv3117- Rv3121	5	72
RD6	Rv1506c- Rv1516c	11	236
RD7	Rv2346c- Rv2353c	8	167
Total		39	775

Individual peptides were tested for antibody reactivity with pooled TB sera (n=10) in ELISA

Protocol of ELISA using TB sera of pool 1

Rows/ Wells	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	RD1ORF 8 P7	RD1OR9 P6	RD1ORF 9 P14	RD1ORF 9 P22	RD1ORF 9 P30	RD1ORF9 P38	RD1ORF10 P2	RD1ORF10 P10	RD1ORF10 P18	RD1ORF10 P26	<u>RD1ORF1</u> <u>0 P34</u>
B	Sera	RD1ORF 8 P8	RD1OR9 P7	RD1ORF 9 P15	<u>RD1OR9</u> <u>P23</u>	RD1ORF 9 P31	RD1ORF9 P39	RD1ORF10 P3	RD1ORF10 P11	RD1ORF10 P19	RD1ORF10 P27	RD1ORF10 P35
C	CW	<u>RD1ORD</u> <u>8 P9</u>	RD1OR9 P8	RD1ORF 9 P16	<u>RD1OR9</u> <u>P24</u>	RD1ORF 9 P32	RD1ORF9 P40	<u>RD1O3RF</u> <u>10 P4</u>	RD1ORF10 P12	<u>RD1ORF1</u> <u>0 P20</u>	<u>RD1ORF1</u> <u>0 P28</u>	RD1ORF10 P36
D	<u>CW+Sera</u>	RD1ORF 9 P1	RD1OR9 P9	RD1ORF 9 P17	<u>RD1OR9</u> <u>P25</u>	RD1ORF 9 P33	RD1ORF9 P41	RD1ORF10 P5	RD1OR3F1 0 P13	<u>RD1ORF1</u> <u>0 P21</u>	<u>RD1ORF1</u> <u>0 P29</u>	RD1ORF11 P1
E	RD1ORF8 P3	RD1ORF 9 P2	RD1OR9 P10	RD1ORF 9 P18	RD1ORF 9 P26	RD1ORF 9 P34	RD1ORF9 P42	RD1ORF10 P6	RD1OR5F1 0 P14	<u>RD1ORF1</u> <u>0 P22</u>	<u>RD1ORF1</u> <u>0 P30</u>	RD1ORF11 P2
F	RD1ORF8 P4	RD1ORF 9 P3	RD1OR9 P11	RD1ORF 9 P19	RD1ORF 9 P27	RD1ORF 9 P35	RD1ORF9 P43	RD1ORF10 P7	<u>RD1OR7F</u> <u>10 P15</u>	RD1ORF10 P23	<u>RD1ORF1</u> <u>0 P31</u>	RD1ORF11 P3
G	<u>RD1ORF8</u> <u>P5</u>	RD1ORF 9 P4	RD1OR9 P12	<u>RD1OR9</u> <u>P20</u>	RD1ORF 9 P28	RD1ORF 9 P36	RD1ORF9 P44	<u>RD1ORF1</u> <u>0 P8</u>	RD1ORF10 P16	RD1ORF10 P24	<u>RD1ORF1</u> <u>0 P32</u>	RD1ORF11 P4
H	RD1ORF8 P6	<u>RD1ORF</u> <u>9 P5</u>	RD1OR9 P13	RD1ORF 9 P21	<u>RD1OR9</u> <u>P29</u>	<u>RD1OR9</u> <u>P37</u>	RD1ORF10 P1	RD1ORF10 P9	<u>RD1ORF1</u> <u>0 P17</u>	<u>RD1ORF1</u> <u>0 P25</u>	<u>RD1ORF1</u> <u>0 P33</u>	<u>RD1ORF1</u> <u>1</u> <u>P5</u>

Blank ←
 Negative ←
 Ctrl ←
 Positive ←
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All 775 peptides were tested using the above protocol₂₄

Results of ELISA test with pool 1 in terms of OD₄₀₅ values

Rows/ Wells	1	2	3	4	5	6	7	8	9	10	11	12
A	0.094	0.200	0.186	0.179	0.427	0.201	0.181	0.340	0.227	0.157	0.164	<u>0.818</u>
B	0.177	0.197	0.158	0.301	<u>0.546</u>	0.171	0.215	0.136	0.194	0.490	0.436	0.110
C	0.148	<u>1.154</u>	0.185	0.126	<u>0.975</u>	0.148	0.134	<u>0.922</u>	0.129	<u>0.578</u>	<u>0.919</u>	0.137
D	<u>0.676</u>	0.181	0.221	0.167	<u>0.951</u>	0.243	0.146	0.257	0.157	<u>1.147</u>	<u>1.142</u>	0.135
E	0.267	0.168	0.257	0.139	0.210	0.136	0.139	0.182	0.216	<u>0.759</u>	<u>0.710</u>	0.203
F	0.359	0.179	0.187	0.215	0.139	0.136	0.123	0.188	<u>0.515</u>	0.427	<u>0.679</u>	0.231
G	<u>>4</u>	0.165	0.234	<u>0.943</u>	0.360	0.154	0.133	<u>0.522</u>	0.395	0.190	<u>0.674</u>	0.377
H	0.364	<u>0.510</u>	0.186	0.232	<u>0.755</u>	<u>0.712</u>	0.302	0.229	<u>1.125</u>	<u>1.033</u>	<u>0.947</u>	<u>0.520</u>

E/C = OD₄₀₅ value with peptide + sera / OD₄₀₅ value with sera alone

E/C ≥ 2 and OD₄₀₅ values ≥ 0.5 are shown in bold face and underlined

Results of ELISA with peptides (n=47) & sera of TB patients (pool 2)

Protein/Peptide	Mean OD ₄₀₅ values with peptide alone	Mean OD ₄₀₅ values with Sera+Peptide	E/C
RD1ORF2/P24	0.14	0.23	0.23/0.14=1.6
RD1ORF2/P25	>4	>4	>4/>4=1
RD1ORF2/P31	0.21	0.29	0.29/0.21=1.4
RD1ORF6/P2	0.18	0.33	0.33/0.18=1.8
RD1ORF7/P1	0.13	0.22	0.22/0.13=1.6
RD1ORF8/P4	>4	>4	>4/>4=1
RD1ORF8/P5	0.14	0.21	0.21/0.14=1.4
RD1ORF8/P6	0.10	0.17	0.17/0.10=1.6
RD1ORF8/P9	0.58	1.76	1.76/0.58=3
RD1ORF9/P5	0.41	0.45	0.45/0.41=1.1
RD1ORF9/P20	0.11	0.16	0.16/0.11=1.5
RD1ORF9/P23	0.27	0.47	0.47/0.27=1.7
RD1ORF9/P24	0.49	0.99	0.99/0.49=2
RD1ORF9/P25	0.30	0.59	0.59/0.30=1.9
RD1ORF9/P28	0.32	0.42	0.42/0.32=1.2
RD1ORF9/P29	1.5	0.89	0.89/1.5=0.61
RD1ORF9/P37	0.39	0.86	0.86/0.39=2.2
RD1ORF10/P4	1.8	1.5	1.5/1.8=0.83
RD1ORF10/P8	0.13	0.45	0.45/0.13=3.3
RD1ORF10/P15	0.48	0.56	0.56/0.48=1.1
RD1ORF10/P17	0.45	0.53	0.53/0.45=1.2
RD1ORF10/P19	0.35	0.45	0.45/0.35=1.2

Results of ELISA with peptides (n=11) & sera of TB patients (pool 3)

Protein/Peptide	Mean OD ₄₀₅ value with		E/C
	Peptide alone	Peptide+Sera	
RD1ORF8/ P9	0.65	1.5	<u>$1.5/0.65=2.3$</u>
RD1ORF9 /P24	0.41	1.3	<u>$1.3/0.41=3.1$</u>
RD1ORF9 /P37	0.41	0.34	$0.34/0.41=0.85$
RD1ORF10 /P28	1.3	1.5	$1.5/1.3=1.1$
RD1ORF11 /P14	0.28	0.48	$0.48/0.28=1.7$
RD1ORF14 /P10	0.86	0.69	$0.69/0.86=0.8$
RD4ORF2 /P5	1.7	1.8	$1.8/1.7=1.03$
RD6ORF3 /P17	0.45	1.0	<u>$1.0/0.45=2.5$</u>
RD6ORF5 /P10	0.51	1.4	<u>$1.4/0.51=2.7$</u>
RD6ORF10/ P18	0.32	0.45	$0.54/0.32=1.7$
RD6ORF11/ P26	0.45	1.4	<u>$1.4/0.45=3.1$</u>

N=11

Results of ELISA with peptides (n=5) & sera of TB patients (pool 3, and individual serum1-10)

Protein/Peptide	ELISA reactivity (E/C) with											No. of positive sera/total
	Pool 3	S1	S2	S3	S4	S5	S6	S7	S8	S9	s10	
RD1ORF8/P9	<u>2.3</u>	1.6	1.4	1.7	1.7	1.7	<u>3</u>	1.4	1.5	1.2	1.1	1/10
RD1ORF9/P24	<u>3.1</u>	<u>4.5</u>	<u>3.4</u>	1.9	1.7	<u>2.1</u>	<u>2.3</u>	<u>2.8</u>	<u>2.8</u>	<u>2.5</u>	<u>2.7</u>	<u>8/10</u>
RD6ORF3/P17	<u>2.5</u>	<u>2.8</u>	<u>2.7</u>	<u>3.1</u>	<u>2.2</u>	<u>2.7</u>	<u>2.9</u>	<u>3.2</u>	1.5	1.7	1.1	<u>7/10</u>
RD6ORF5/P10	<u>2.7</u>	<u>2.1</u>	1.9	<u>4.4</u>	<u>3.7</u>	<u>3.6</u>	<u>4.2</u>	<u>3.6</u>	<u>5.3</u>	<u>6.9</u>	<u>7.3</u>	<u>9/10</u>
RD6ORF11/P26	<u>3.1</u>	1.9	<u>3.6</u>	<u>5.7</u>	<u>2.8</u>	<u>2.1</u>	<u>3.1</u>	<u>2.2</u>	<u>2.7</u>	<u>2.9</u>	<u>3.2</u>	<u>9/10</u>

Results of ELISA with peptides (n=5) and sera of TB patients (pool 4, and individual serum 1-10)

Protein/Peptide	ELISA reactivity (E/C) with											No. of positive sera/total
	Pool 4	S1	S2	S3	S4	S5	S6	S7	S8	S9	s10	
RD1ORF8 /P9	1	1.2	1.2	1	0.9	1	1	1.1	1.5	1.4	1.6	0/10
RD1ORF9 /P24	<u>3.4</u>	<u>4.5</u>	1.7	<u>2</u>	<u>2.7</u>	<u>3.2</u>	<u>2.2</u>	<u>2.2</u>	<u>2.1</u>	<u>2.9</u>	<u>4.8</u>	<u>9/10</u>
RD6ORF3/ P17	<u>3.5</u>	<u>3</u>	1.9	<u>2</u>	1.4	<u>2.2</u>	<u>11.3</u>	<u>5.9</u>	<u>2.6</u>	<u>2.8</u>	<u>4.6</u>	<u>8/10</u>
RD6ORF5/ P10	<u>3.3</u>	<u>3.4</u>	<u>5.9</u>	<u>7.8</u>	<u>4.9</u>	<u>7.7</u>	<u>3.4</u>	<u>2.6</u>	<u>2.1</u>	<u>3.5</u>	<u>2.2</u>	<u>10/10</u>
RD6ORF11 /P26	<u>2.4</u>	<u>2.2</u>	<u>2.6</u>	<u>3</u>	<u>4.5</u>	<u>3.1</u>	<u>5.6</u>	<u>3</u>	1.9	<u>5.3</u>	<u>2.5</u>	<u>9/10</u>

Results of ELISA with peptides (n=5) and sera of healthy subjects (pool, and individual serum 1-10)

Protein/Peptide	ELISA reactivity (E/C) with											No. of positive sera/total
	Pool	seru m1	seru m2	seru m3	seru m4	seru m5	seru m6	seru m7	seru m8	seru m9	seru m10	
RD1ORF8 /P9	1.2	0.9	1.4	0.1	1.3	1	0.9	1.1	0.8	1.1	1	0/10
RD1ORF9 /P24	1.6	1.3	1.7	1.6	1.5	1.1	1.4	1.3	1.7	1.3	1.7	0/10
RD6ORF3/ P17	1.3	1.3	2.4	1.1	1.4	1.4	1.3	1.2	1.6	1.8	1.6	1/10
RD6ORF5/ P10	<u>5.7</u>	<u>6.6</u>	<u>6.5</u>	<u>4.6</u>	<u>4.9</u>	<u>5.8</u>	<u>6.5</u>	1.3	<u>3.4</u>	1.1	<u>2.1</u>	<u>8/10</u>
RD6ORF11 /P26	1.2	1.3	<u>2.4</u>	1.1	1	1	1.4	1.2	1.5	1.3	1.7	1/10

Consistent peptides' reactivity in terms of percentages

Peptides	TB patients' Sera	Healthy subjects' sera
RD1ORF9 (Rv3876)/P24	70%	28%
RD6ORF3 (Rv1508c)/p17	93%	28%
RD6ORF11 (Rv1516c)/P26	66%	10%
RD6ORF5 (Rv1510c)/P10	46%	50%

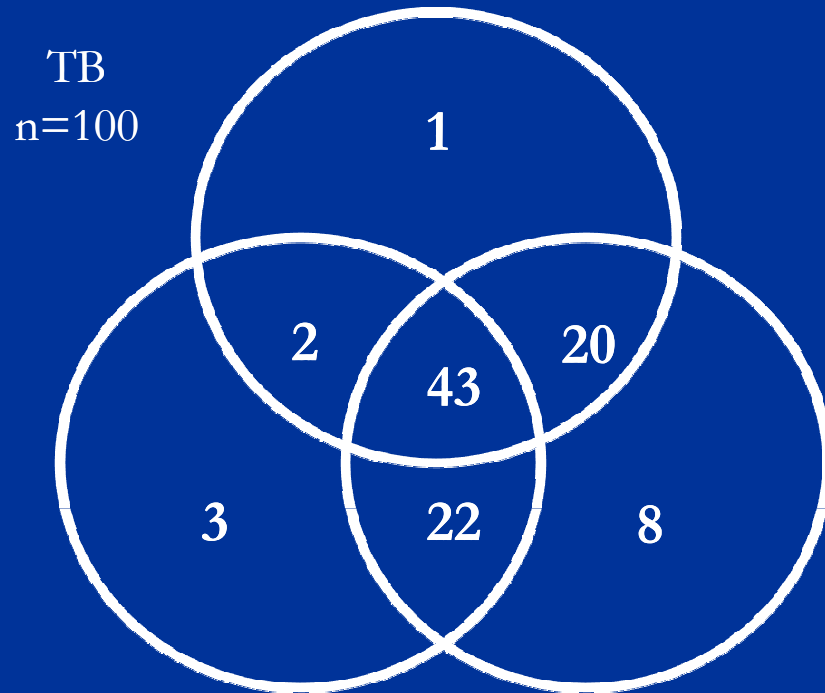
Results of BLAST search for sequence identity of immunodominant peptide aa 136-160 of RD6 protein Rv1510 in various strains of *M. tuberculosis* and other mycobacteria

Mycobacterial species and strains	Sequence	Identity
<i>M. tuberculosis</i> H37Rv, K85, CPHL_A, KZN 605, C, CDC1551, H37Ra, F11, KZN1435, T92, T85, str. Haarlem	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVEGRWLSVGLLSVGVAGFCAQATL Sbjct 127 FVEGRWLSVGLLSVGVAGFCAQATL 151	25/25 100%
<i>M. bovis</i> AF2122/97, BCG, Pasteur 1173P2	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 F E RWLSV LLS+G+AGFC ATL Sbjct 135 FAEARWLSVALLSIGLAGFCLHATL 159	10/25 72%
<i>M. avium</i> 104, paratuberculosis K-10	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE R LSV LLSVG+AGFC ATL Sbjct 117 FVEARPLSVLLLSVGLAGFCVHATL 141	19/25 76%
<i>M. smegmatis</i> str. MC2 155	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE R LSV LLSVG+AGFC ATL Sbjct 129 FVESRALSVALLSVGLAGFCLHATL 153	19/25 76%
M. sp. KMS, M. sp. JLS	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE RWLSV LLS G+AGFC ATL Sbjct 117 FVEARWLSVLLLSAGLAGFCVHATL 141	19/25 76%
<i>M. marinum</i> , <i>M.</i> <i>ulcerans</i> Agy99	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE RWLSVGLLSVG+AGFC ATL Sbjct 103 FVEDRWLSVGLLSVGLAGFCLHATL 127	21/25 84%
<i>M. kansasii</i> ATCC 12478	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE RWLSVGLLSVG+AGFC ATL Sbjct 132 FVEARWLSVGLLSVGLAGFCLHATL 156	21/25 84%

Results of BLAST search for sequence identity of immunodominant peptide aa 361-372 of RD6 protein Rv1516c in various strains of *M. tuberculosis* and other mycobacteria

Mycobacterial species and strains	Sequence				Identity
<i>M. tuberculosis</i> H37Rv, H37Ra, F11, KZN 1435, str. Haarlem, CDC1551, C, 02 1987, 94 M4241A	Query	1	VWPSRLRRGCRA	12	
			VWPSRLRRGCRA		12/12
	Sbjct	362	VWPSRLRRGCRA	373	100%

Rv1516c/P325-336

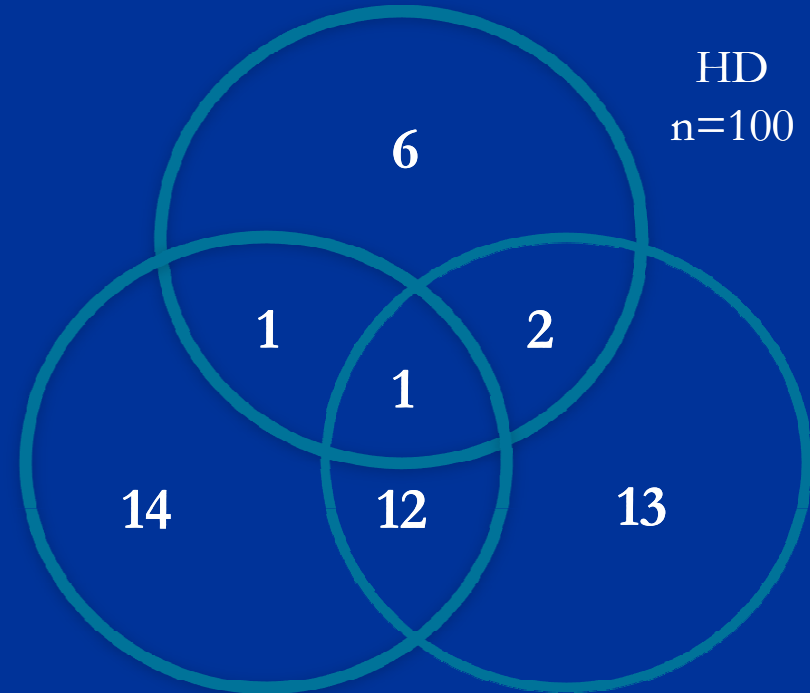


Rv3876/P346-370

Rv1508c/P241-265

- ✓ 87% with 2-3 peptides
- ✓ 12% with 1 peptide
- ✓ 1% didn't show reactivity

Rv1516c/P325-336



HD
n=100

Rv3876/P346-370

Rv1508c/P241-265

- ✓ 16% with 2-3 peptides
- ✓ 33% with 1 peptide
- ✓ 51% didn't show reactivity

Sensitivity, specificity, PPV, NPV and diagnostic accuracy of peptides 346-370 of Rv3876, 241-265 of Rv1508c and 325-336 of Rv1516c individually and in combination

Peptide	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy
346-370 of Rv3876	70%	72%	72%	71%	71%
241-265 of Rv1508c	93%	72%	77%	91%	82%
325-336 of Rv1516c	66%	90%	89%	74%	78%
≥Two peptides	87%	84%	84%	86%	85%

Epitope prediction by using the server ABCpred in the sequence of immunodominant peptide of Rv1510

Peptide	Predicted epitope	Prediction score
136-160 of Rv1510	FVEGRWLSVGLLSVGVAGFCAQATL	
	FVEGRWLSVGLLSVGVAGFC-----	0.65
	FVEGRWLSVGLLSVGVAG-----	0.74
	FVEGRWLSVGLLSVGV-----	0.57
	-----GLLSVGVAGFCAQATL	0.54
	-----SVGLLSVGVAGFCA-----	0.65
	FVEGRWLSVGLLSV-----	0.58
	-----LSVGLLSVGVAG-----	0.55
	-----VGLLSVGVAG-----	0.57

Epitope prediction by using the server ABCpred in the sequence of immunodominant peptide of Rv3876

Peptide	Predicted epitope	Prediction score
346-370 of Rv3876	TQKSLRPAAKGPVKKKVKPQKPKAT	
	TQKSLRPAAKGPVKKKVKPQ-----	0.83
	-----PAAKGPVKKKVKPQKP---	0.78
	----LRPAAKGPVKKKVK-----	0.70
	-----KGPVKKKVKPQKPK--	0.66
	-----GPKVKKKVKPQKP---	0.80
	----LRPAAKGPVKKK-----	0.75
	TQKSLRPAAK-----	0.82
	-----PAAKGPVKKK-----	0.76

Epitope prediction by using the server ABCpred in the sequence of immunodominant peptide of Rv1508c

Peptide	Predicted epitope	Prediction score
241-265 of Rv1508c	ARGLERVVIFVTLGAAAIPAWGVID	
	-----VTLGAAAIPAWGVI-	0.81
	---LERVVIFVTLGAAAIP-----	0.72
	-----FVTLGAAAIPAWGVID	0.67
	-RGLERVVIFVTLGAAAIP-----	0.61
	----ERVVIFVTLGAAAIPAWGVI-	0.57
	-----VVIFVTLGAA-----	0.64
	ARGLERVVIF-----	0.62
	-----VVIFVTLGAAI-----	0.67
	--GLERVVIFVTLG-----	0.59
-----VTLGAAAIPAWG---	0.51	

Epitope prediction by using the server ABCpred in the sequence of immunodominant peptide of Rv1516c

Peptide	Predicted epitope	Prediction score
325-336 of Rv1516c	VWPSRLRRGCRA	
	VWPSRLRRGCRA	0.62
	VWPSRLRRGC--	0.62

**The description of the proteins RD1 Rv3876,
RD6 Rv1508c and RD6 Rv1516c using
TubercuList server**

Protein	Description
RD1 Rv3876 (666 aa)	Conserved hypothetical proline and alanine rich protein of unknown function
RD6 Rv1508c (599 aa)	Probable membrane protein, predicted to be in the GT-C superfamily of glycosyltransferases
RD6 Rv1516c (336 aa)	Probable sugar transferase, involved in cellular metabolism

Raising anti-peptide antibodies in rabbits and ELISA reactivity of sera to immunizing peptides

- Anti-RD1Rv3876, anti-RD6 Rv1508c and anti-RD6 Rv1516c antibodies were raised in 4-8 months rabbits against pools of 11 peptides of each protein

Rv3876	Amino acids sequence
P19	271 PAPLGQPRSYLAPPTRPAPTEPPPS 295
P20	286 RPAPTEPPSPSPQRNSGRRRAERRV 310
P21	301 NSGRRRAERRVHPDLAAQHAAAQPDS 325
P22	316 AQHAAAQPDSITAATTGGRRRKRAA 340
P23	331 TGGRRRKRAAPDLDATQKSLRPAAK 355
P24	346 TQKSLRPAAKGPKVKKVKPQKPKAT 370
P25	361 KVKPQKPKATKPPKVVSQRGWRHWV 385
P26	376 VSQRGWRHWVHALTRINLGLSPDEK 40
P27	391 INLGLSPDEKYELDLHARVRRNPRG 415
P28	406 HARVRRNPRGSYQIAVVGLKGGAGK 430
P29	421 VVGLKGGAGKTTLTAALGSTLAQVR 445

Rv1516c	Amino acids sequence
P12	151 DWGMNIGRHCFSDYAMVAAAGIQPN 175
P13	166 MVAAGIQPNPADYLISLPADYQPT 190
P14	181 ISLPADYQPTAVAAWAPARIPYAIF 205
P15	196 APARIPYAIFGLPSHWLGAPRLGLI 320
P16	211 WLGAPRLGLICYLVALTMAVISPAL 235
P17	226 LTMAVISPAIWAARGARGLERVVIF 250
P18	241 ARGLERVVIFVTLGAAAIPAWGVID 265
P19	256 AAIPAWGVIDRGNSTGFVVPALAY 280
P20	271 GFVVPALAYFVALSRQRWGLATIT 295
P21	286 RQRWGLATITVILAVLVKPFVVLG 310
P22	301 LVKPFVVLGVVLLAARQWRWAGIG 325

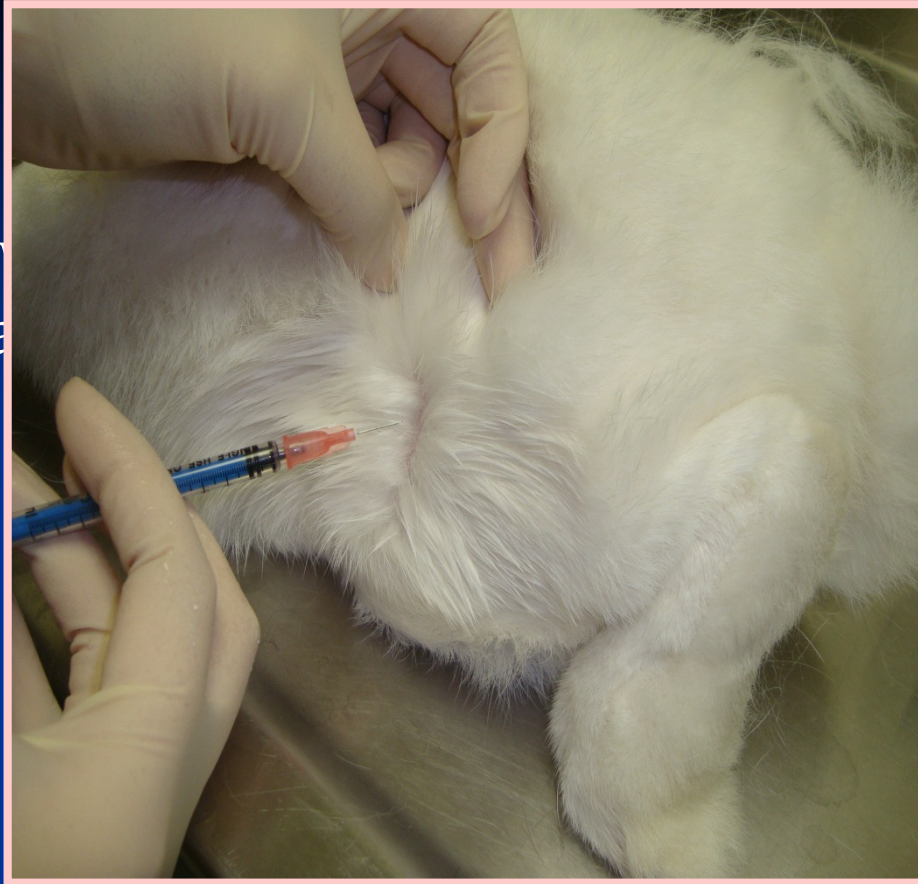
Rv1508c	Amino acids sequence
P16	211 YDDIPADV MPLDWYLHVRHAVHGDI 235
P17	226 HVRHAVHGDI AMLPDTMAVYRRHAQ 250
P18	241 TMAVYRRHAQGMWHNQVVDPPKFWL 265
P19	256 QVVDPPKFWLTQGGPGAATFDAML D 280
P20	271 HAATFDAML DLFPGDPAREELI AVM 295
P21	286 PAREELI AVMADWILRQIANVPGPE 310
P22	301 RQIANVPGPEGRAALQETIARHPRI 325
P23	316 QETIARHPRI AMLALQHRGATPARR 340
P24	331 QHRGATPARRLKTQWRKLAAATPSR 355
P25	346 RKLAAATPSRRGLVDVWPSRLRRGC 370
P26	361 VWPSRLRRGCRA 385

Raising

rabbits

1. Peptides with Freund's adjuvant are

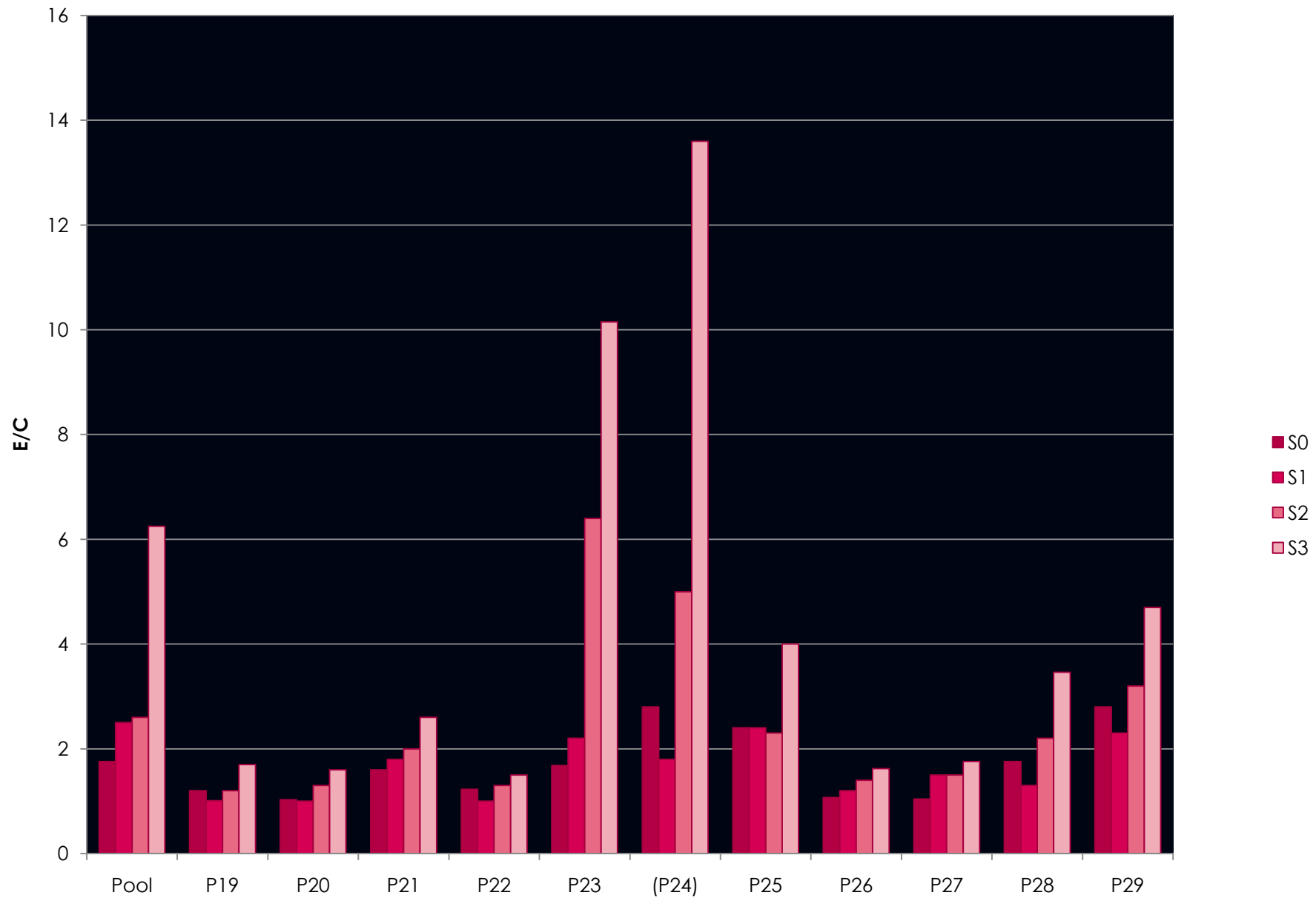
ete Freund's



2. The rabbits were boosted with the same route and amount of emulsified pools of peptide after two weeks of the first immunization and after 1 month of the second immunization

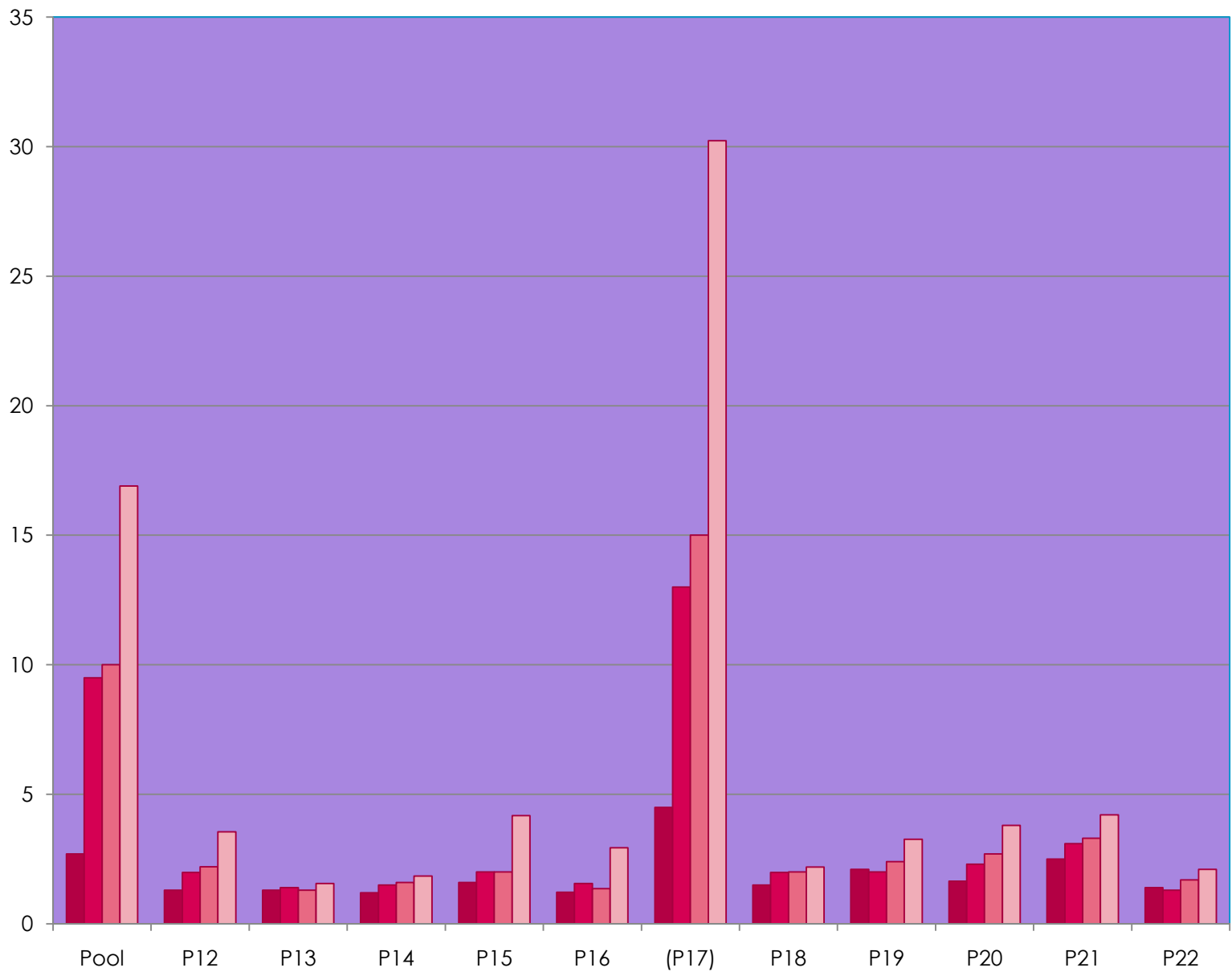
Raising anti-peptide antibodies in rabbits protocol

1. Rabbits were bled from the ear vein before each immunization
2. Sera were used to test for antibody reactivity against the immunizing peptides using ELISA



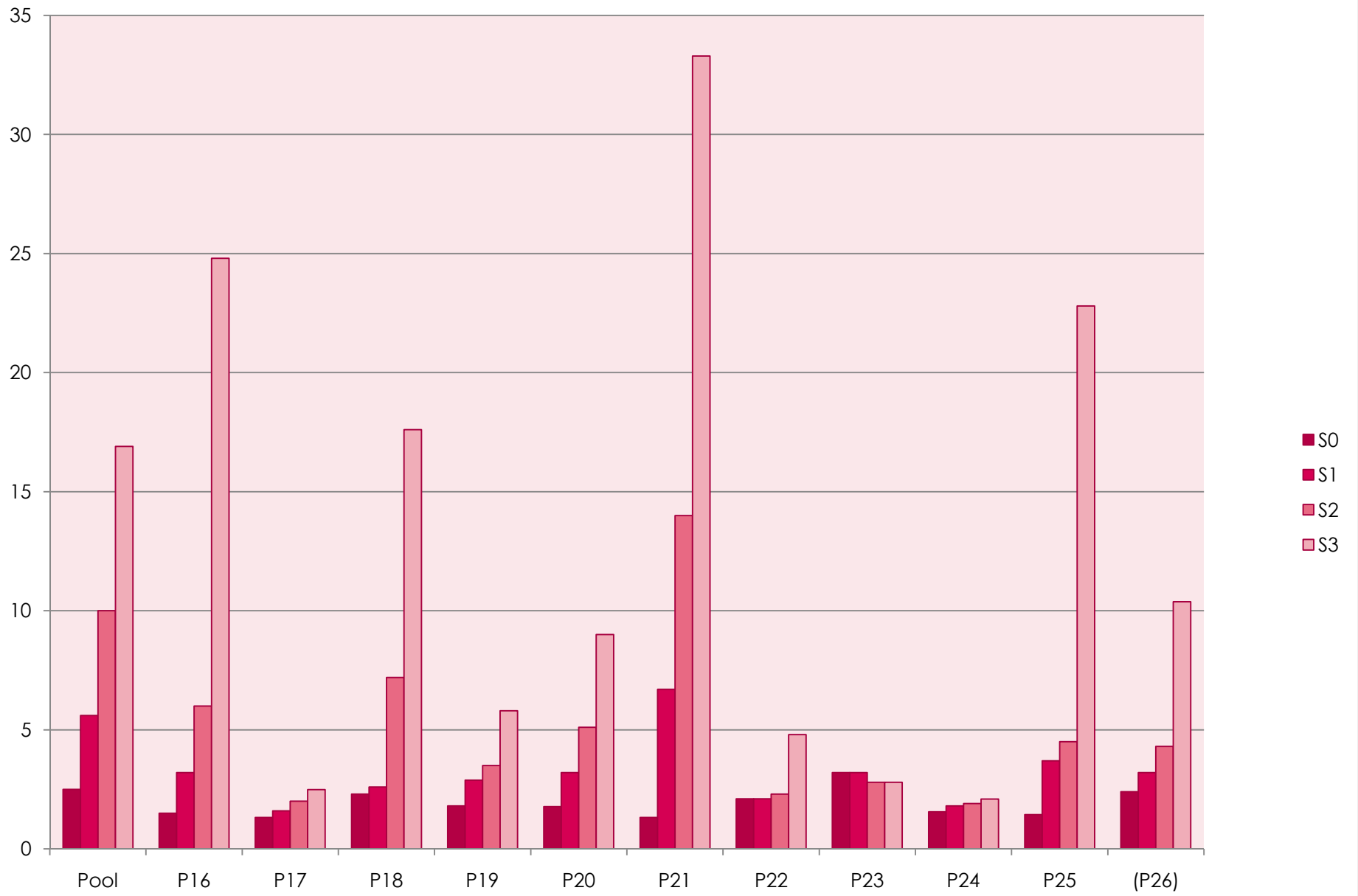
Peptides of Rv3876

E/C



- S0
- S1
- S2
- S3

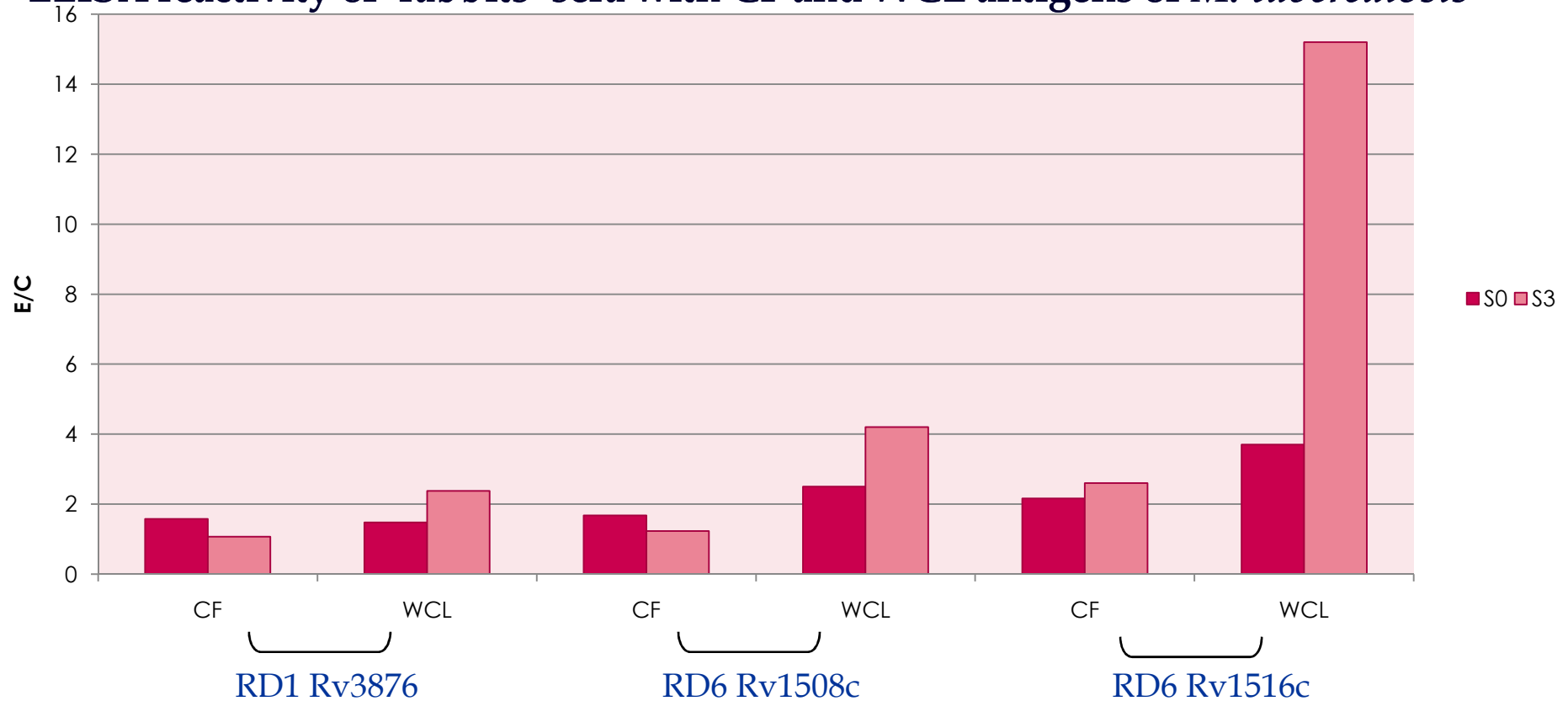
Peptides of Rv1508c



RD6ORF11 (Rv1516c)

Expression of RD1 Rv3876, RD6 Rv1508c and RD6 Rv1516c proteins in *M. tuberculosis*

ELISA reactivity of rabbits' sera with CF and WCL antigens of *M. tuberculosis*



Conclusions

- ✓ Three peptides, i.e. 346-370 of RD1 Rv3876, 226-250 of RD6 Rv1508c and 361-385 of RD6 Rv1516c, were found immunodominant and showed
 - ✓ Strong antibody reactivity with sera of smear and culture positive pulmonary TB patients but weak reactivity with sera of healthy subjects from the community
 - ✓ Thus suggesting their potential in the diagnosis of TB.

Conclusions

- ✓ Anti-peptides antibodies were successfully raised in rabbits by using pools of 11 peptides corresponding to each of the above three proteins
- ✓ All the three peptides found immunodominant with TB sera were also immunodominant with rabbits' sera.
- ✓ The probing of culture filtrates and whole cell lysates of *M. tuberculosis* with the anti-peptide antibodies confirmed their presence in the whole cell lysates, and thus their expression in *M. tuberculosis*.

Acknowledgments

- Collaborator: Prof. Raja'a Al-Attiyah
- Graduate Student: Noura Y Al-Khoudari
- Funding: Kuwait University, Research Sector grants YM08/07 and SRUL02/13

The work was performed at
Thanks for listening



Health Sciences Center - Kuwait



THANKS



IGRA

IFN- γ

RD1

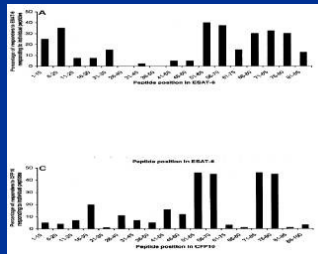


TABLE 1. AMINO ACID SEQUENCES OF OVERLAPPING PEPTIDES FOR ESAT-6 AND CFP-10

Antigen	Amino Acid Sequence
CFP-10	
Peptide 1	MADMKYDAATLQEAQNERISGDL
Peptide 2	GNFERISGDLKTDIQVESTAGLSLQ
Peptide 3	DIQVESTAGLSLQQWRGAGTAQAQAV
Peptide 4	AAGTAQAQAVRFQEAANKIQEELD
Peptide 5	AANKIQEELDESTNIRQAGVQYSR
Peptide 6	IRQAGVQYSRDEEQQALSSQMGE
ESAT-6	
Peptide 1	MTEGQWRFAGIEAASAQIG
Peptide 2	GIEAASAQIGNVTSI
Peptide 3	SAIQGNVTSIHSLLDEGKQSLTLA
Peptide 4	EGKQSLTLAAAWGSGSEAYQGVQ
Peptide 5	SGSEAYQGVQKRWDAATELNHALQ
Peptide 6	TATELNHALQNLARTISEAQAMAS
Peptide 7	NLARTISEAQAMASTEGNVTCMFA



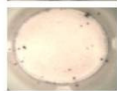
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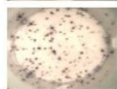
-ve control
Well: A1



+ve control
Well: B1



Panel A
Well: C1



Panel B
Well: D1

PBMC

Whole Blood

T SPOT.TB

QuantiFERON TB Gold In tube

TB Diagnosis

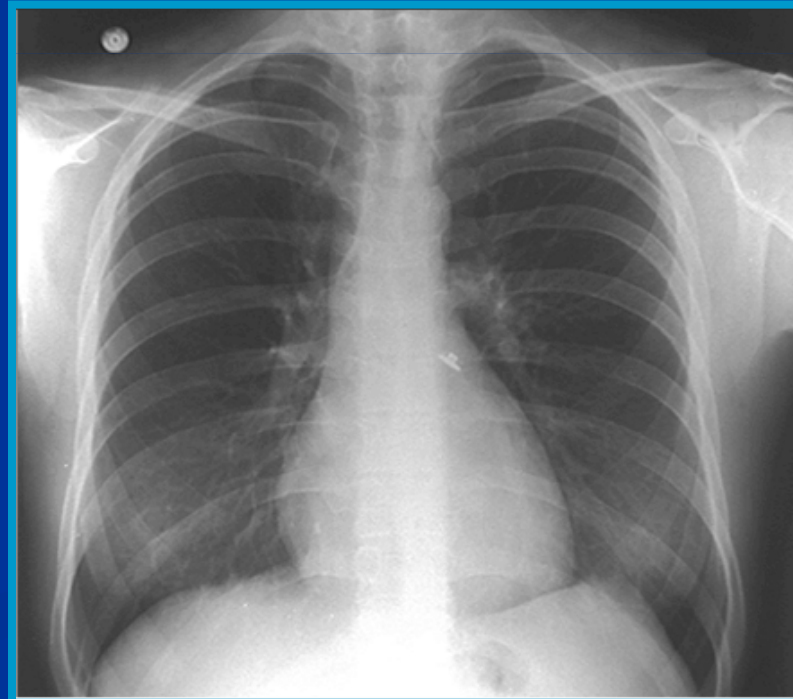
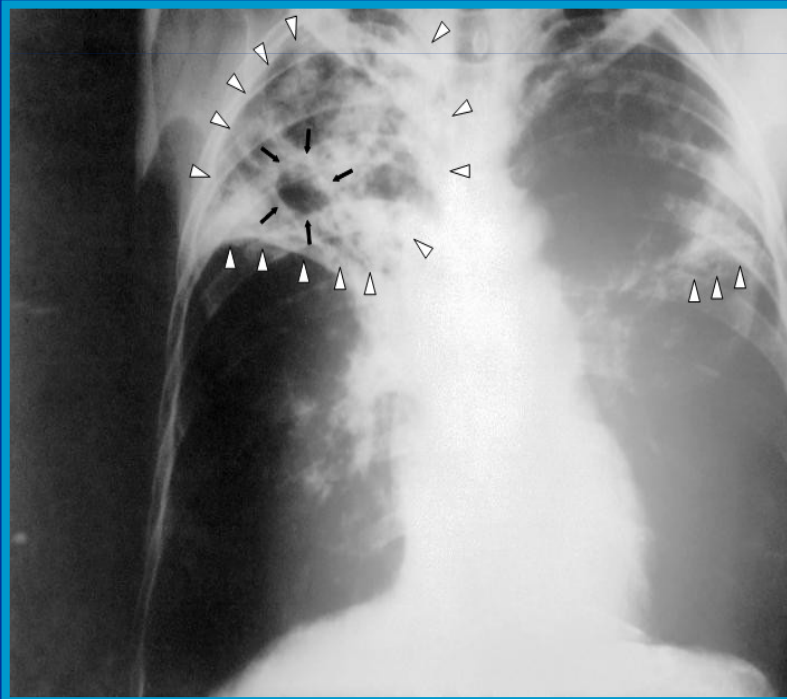
1. Clinical symptoms
2. Chest X-ray
3. Microscopy and culture
4. Molecular and immunological methods



1. Clinical symptoms

2. Chest X-ray

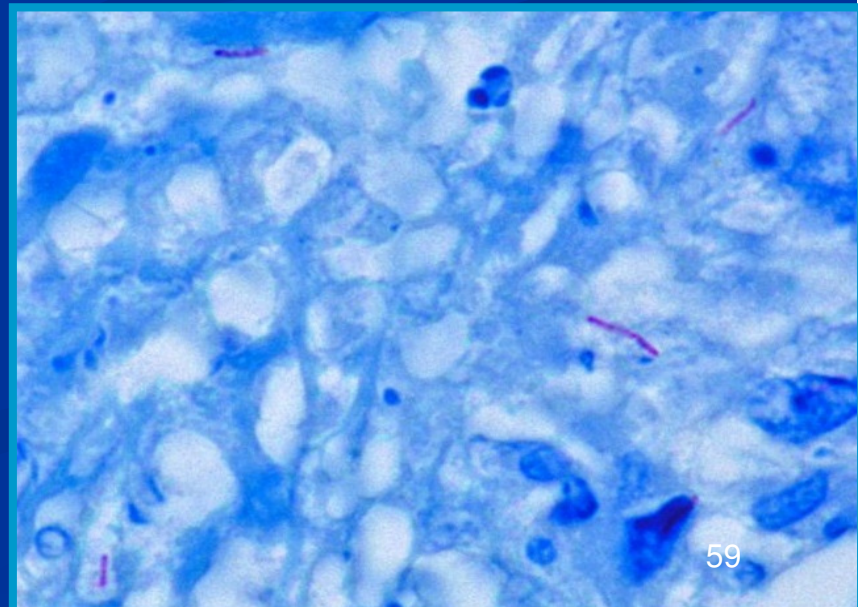
- Rule out diagnosis of active TB



3. Bacteriological examination

- Microscopy

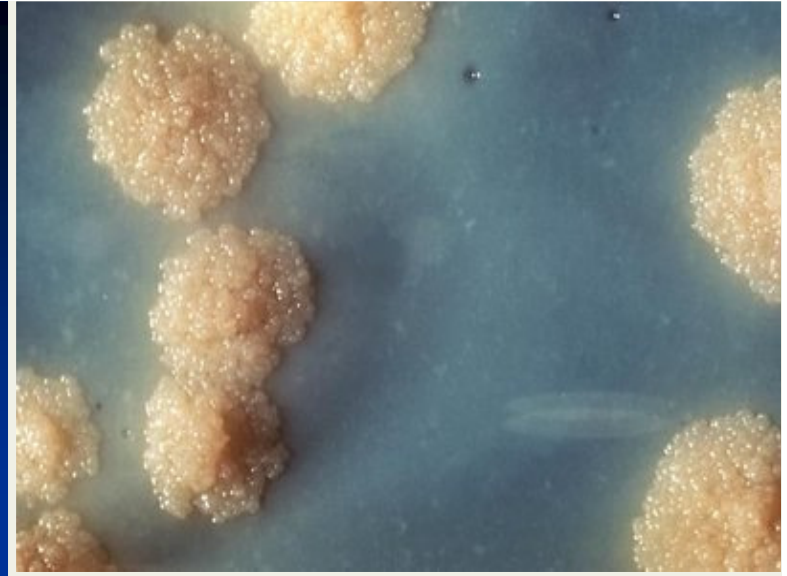
- ✓ Zeihl Neelsen stain, AFB
- ✓ Minimum of 10,000 organisms/ml
- ✓ If bacteria is detected, it doesn't identify the mycobacterial species



3. Bacteriological examination

◎ Culture

- ✓ Middle Brook's medium
- ✓ Lowenstein-Jensen (LJ) medium
- ✓ 10 organisms/ml
- ✓ *M. tuberculosis* takes 4-6 weeks to get visible on media

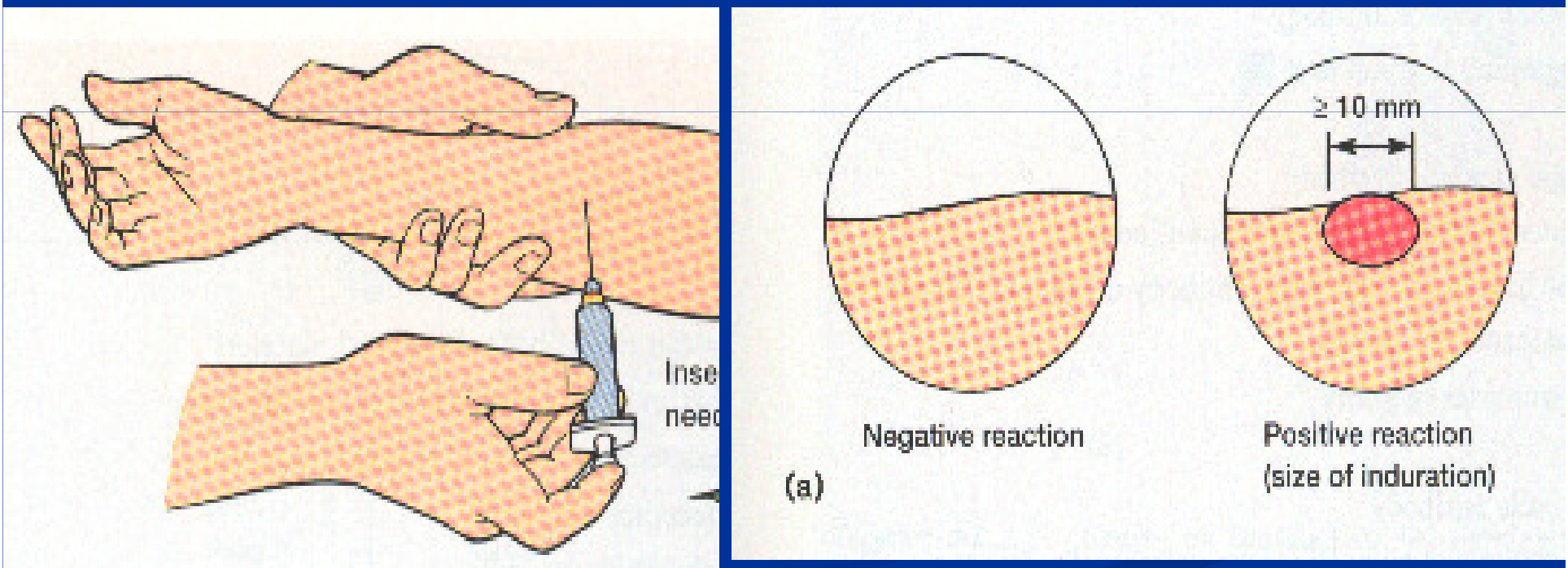


TB Diagnosis (active and latent)?

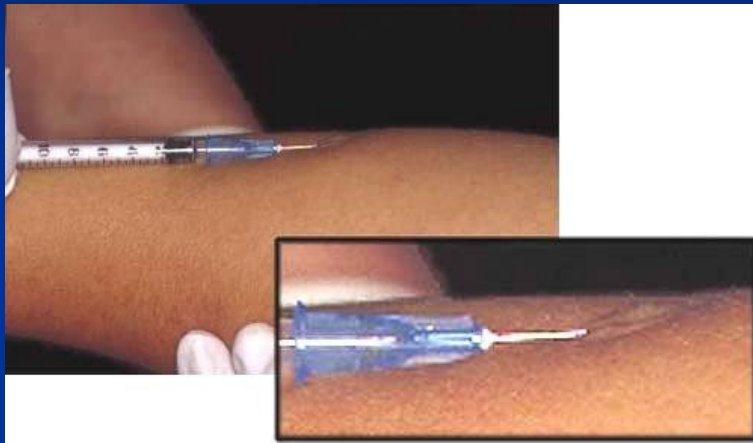
- Clinical symptoms
- Chest X-ray
- Microscopy and culture
- Molecular methods
- Immunological methods



Tuberculin test (TST): Intradermal injection of the antigen in the forearm and reading of delayed type hypersensitivity responses after 48-72 h



Limitations of the TST



- Reagent:
 - Purified protein derivative (PPD) commonly shared among different Mycobacteria (*M. tuberculosis*, BCG and atypical mycobacteria)
- Variability:
 - Reproducibility in giving the test
 - Subjectivity in reading the test
- Logistics
 - Repeat visit needed
 - Wait for 3 days before result



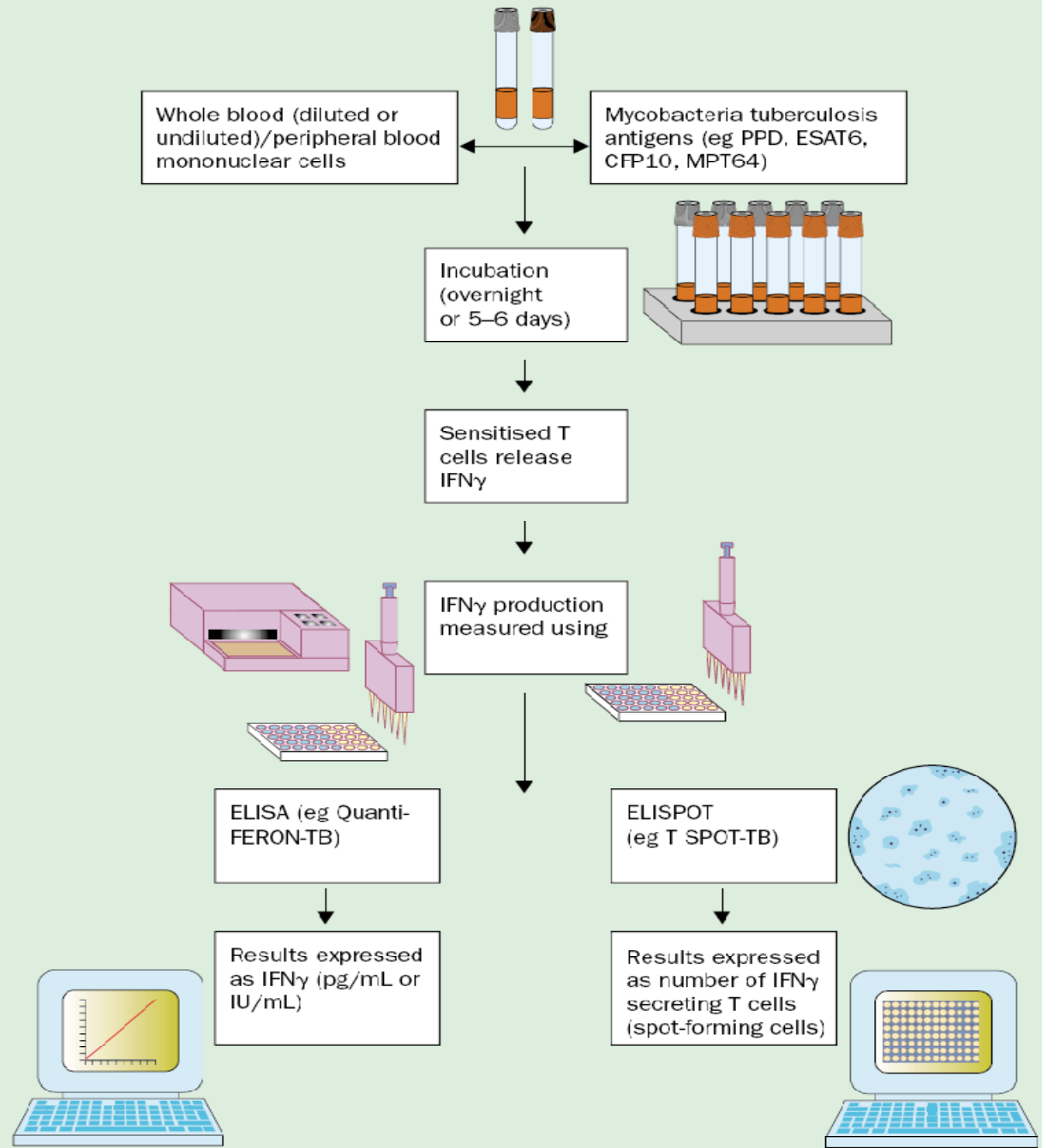
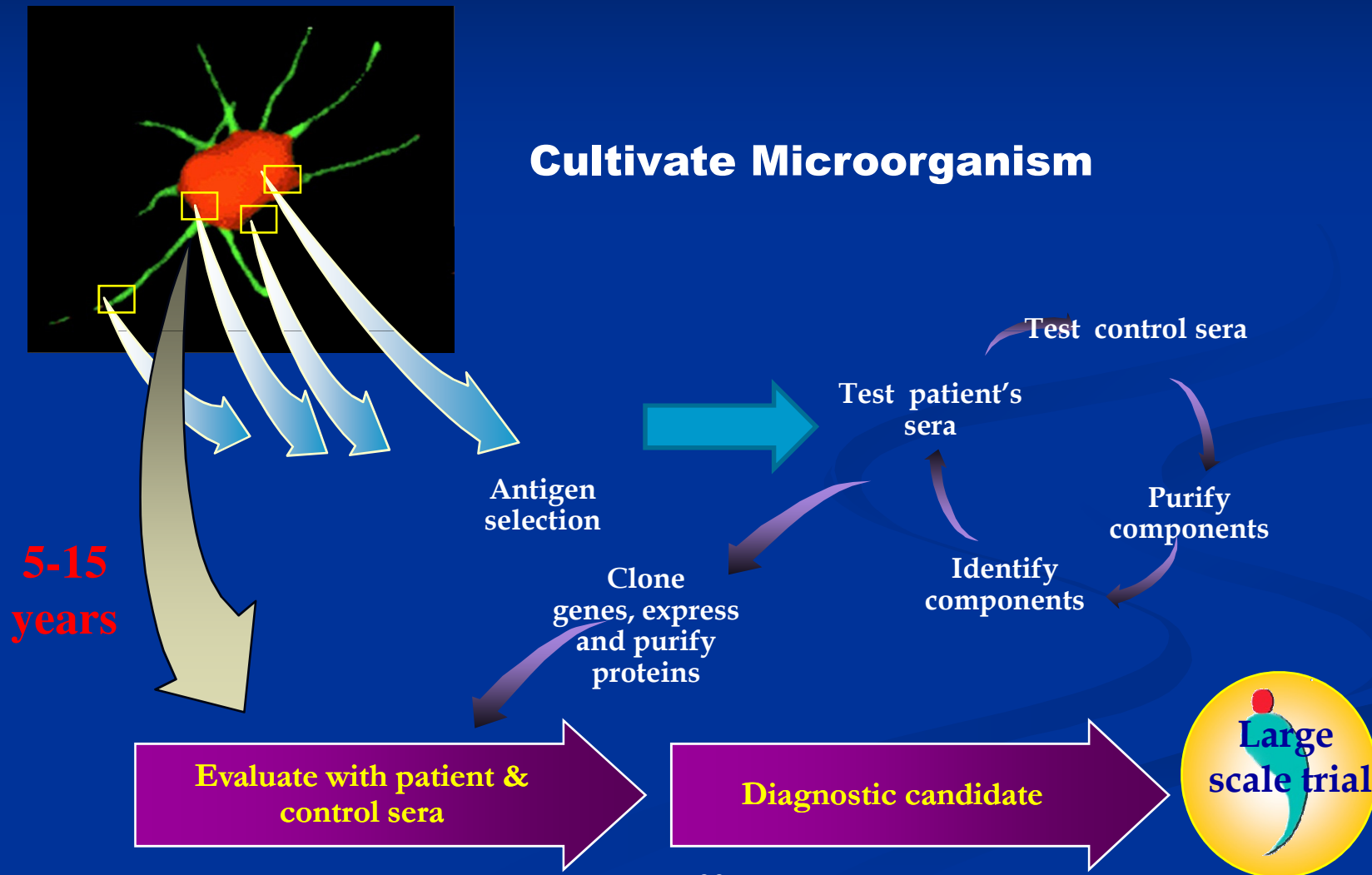


Figure 2. Overview of the interferon- γ (IFN- γ) assay technology.

Animal Work!



Diagnostic development In Pre-genomic era: Conventional approach



Results of BLAST search for sequence identity of immunodominant peptide aa 361-372 of RD6 protein Rv1516c in various strains of *M. tuberculosis* and other mycobacteria

Mycobacterial species and strains	Sequence				Identity
<i>M. tuberculosis</i> H37Rv, H37Ra, F11, KZN 1435, str. Haarlem, CDC1551, C, 02 1987, 94 M4241A	Query	1	VWPSRLRRGCRA	12	
			VWPSRLRRGCRA		12/12
	Sbjct	362	VWPSRLRRGCRA	373	100%

Results of BLAST search for sequence identity of immunodominant peptide aa 241-265 of RD6 protein Rv1508c in various strains of *M. tuberculosis* and other mycobacteria

Mycobacterial species and strains	Sequence	Identity
<p><i>M. tuberculosis</i> H37R_v, CDC1551, H37R_a, F11, KZN 605 K2M 1435, C, str. Haarlem, EAS054, '98-R604 INH-RIF-EM', KZN 4207, T17, GM 1503, EAS054, T85, T92, K85, T46, CPHLA, 021987</p>	<p>Query 1 LTMAVISPAIWAARGARGLERVVIF 25 LTMAVISPAIWAARGARGLERVVIF Sbjct 226LTMAVISPAIWAARGARGLERVVIF250</p>	<p>25/25 100%</p>
<p><i>M. kansasii</i> ATCC 12478</p>	<p>Query 1 LTMAVISPAIWAARGARGLERVVIF 25 LT AV +PA+WAARGARGLERVV+F Sbjct 149 LTGAVFTPAVWAARGARGLERVVVF 173</p>	<p>20/25 80%</p>
<p><i>M. marinum</i></p>	<p>Query 1 LTMAVISPAIWAARGARGLERVVIF 25 L +AV++PA+WA RGARGLER+V F Sbjct 142 LVVAVLTPALWAVRGARGLERLVTF 166</p>	<p>17/25 68% 68</p>



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