Serological LF POC Test for Active TB

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WHO Policy: Commercial Serological Tests for TB (2011)

GRADE evidence profiles and final policy recommendations

It is therefore recommended that these tests should not be used in individuals suspected of active pulmonary or extra-pulmonary TB, irrespective of their HIV status. (India Banned current Serogical tests).

Implications for further research

Targeted further research to identify new/alternative point-of-care tests for TB diagnosis and/or serological tests with improved accuracy is strongly encouraged.

Why Did The Serological Tests Fail?

- No rational selection of antigens
  - Lysates/Culture filtrates of *M. tb*
  - Cell-wall proteins difficult to obtained
  - Proteins highly expressed in bacteriological media
  - Proteins eliciting antibodies in immunized animals
  - Proteins not specific for *M. tb*
  - No understanding of antibody responses in human TB
    - chronic disease
    - spectrum of clinical, bacteriological, radiological immunological manifestations
    - Bacterial adaptation results in transcriptome alterations
- No systematic evaluation of serological tests

Rational Antigen Selection

Focus on antibody responses in humans…..

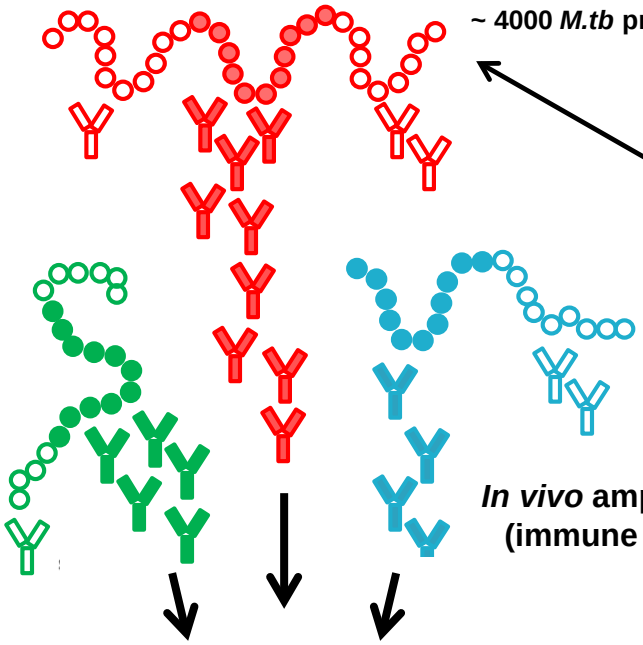
- *M. tb* specific antigens
- Highly immunogenic cell-wall proteins
- Immunodominant regions of 3 cell-wall proteins
- Rational, careful selection of peptides
  - Antibodies in HIV- and HIV+ TB patients
  - Not recognized by Abs in PPD+, NTBLD, HHC, HIV+TB-
  - TB-endemic countries (except HIV+TB-)

Serological Test

Molecular Test

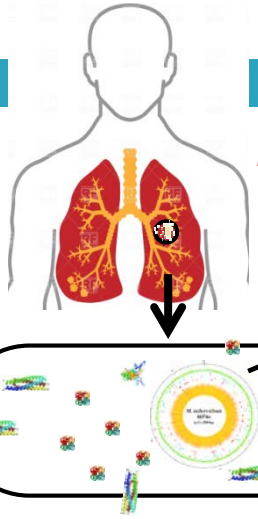
M. tb specific CW proteins

~ 4000 *M.tb* proteins



In vivo amplification (immune system)

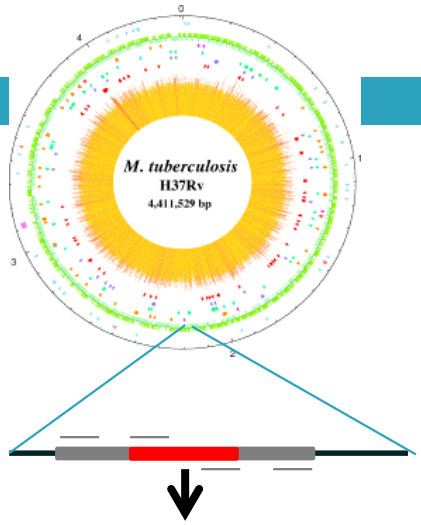
Detect Abs to *M. tb* specific target epitopes



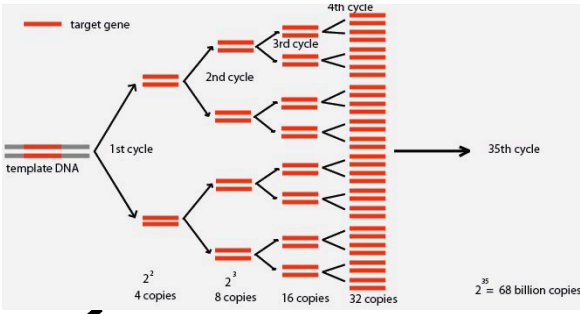
M.tb

M. tb specific gene

~ 4000 *M.tb* genes

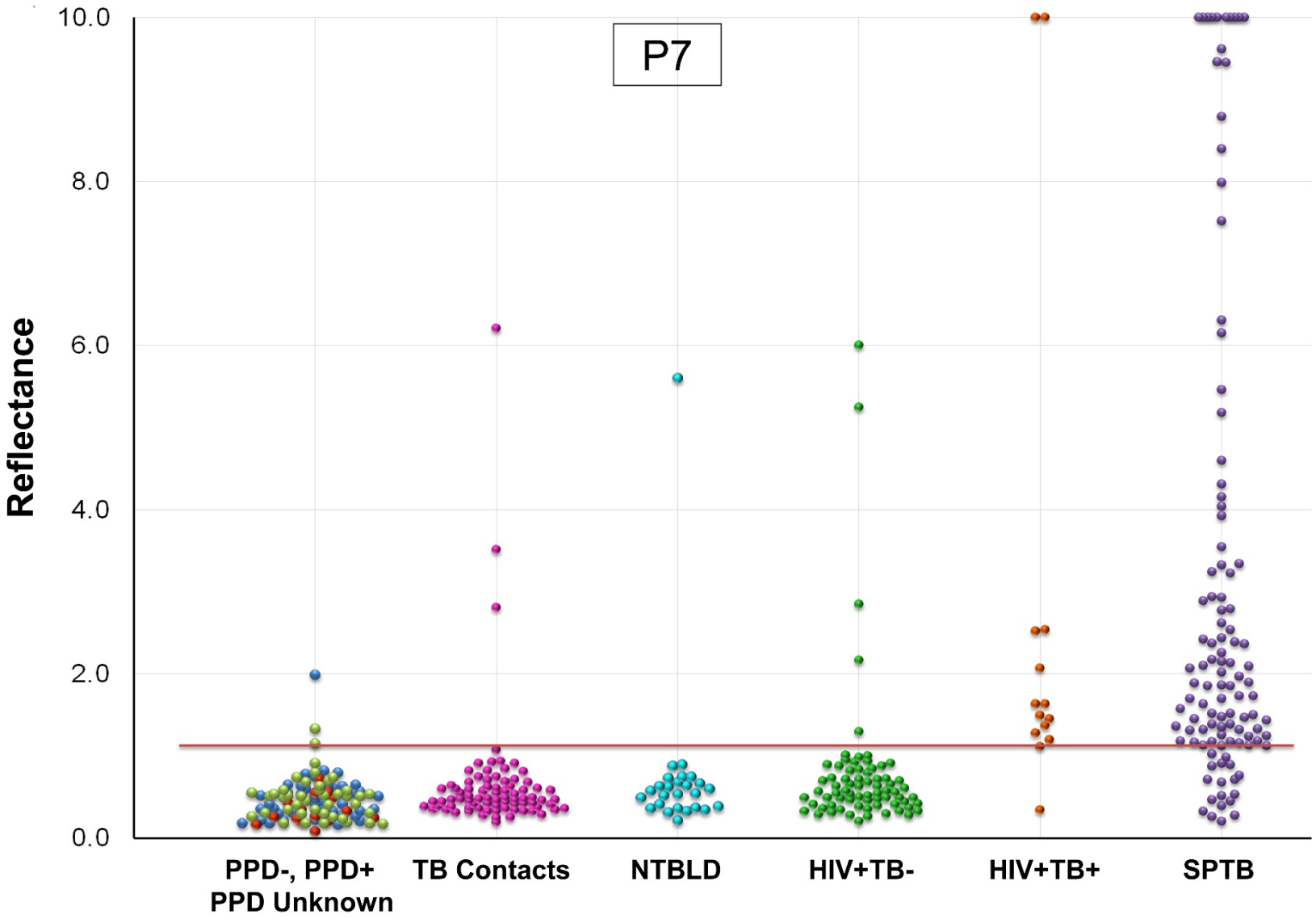


In vitro amplification of gene (PCR)

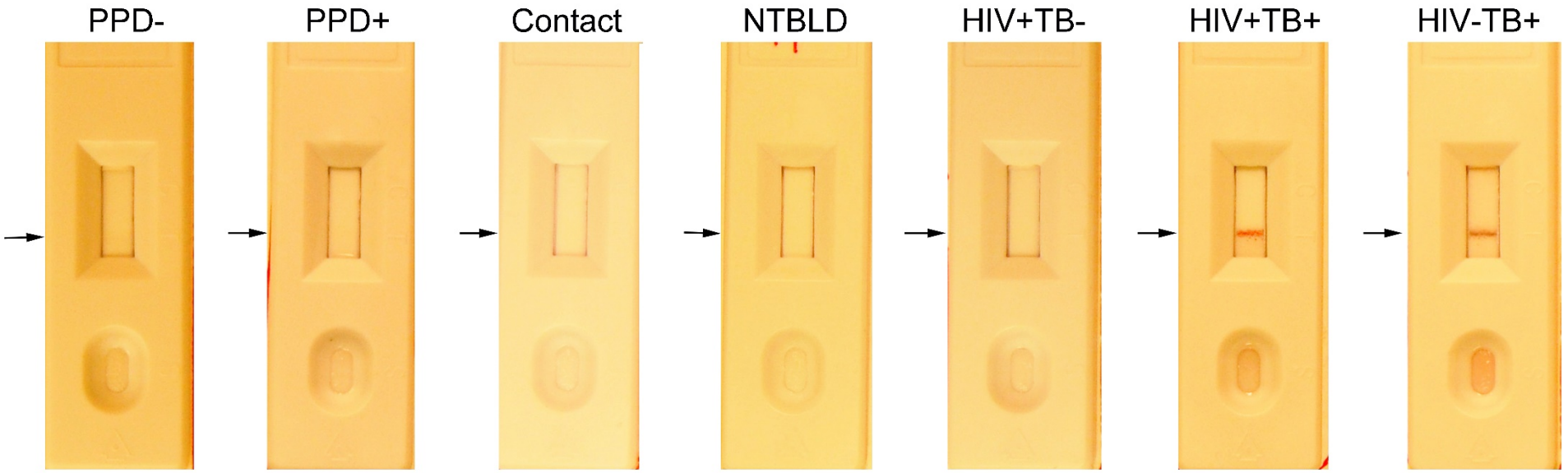


Detect amplified *M. tb*-specific target gene

Reactivity of Sera (n = 400) with P7 on LF



Reactivity of Peptide P7 with Sera on LF flow

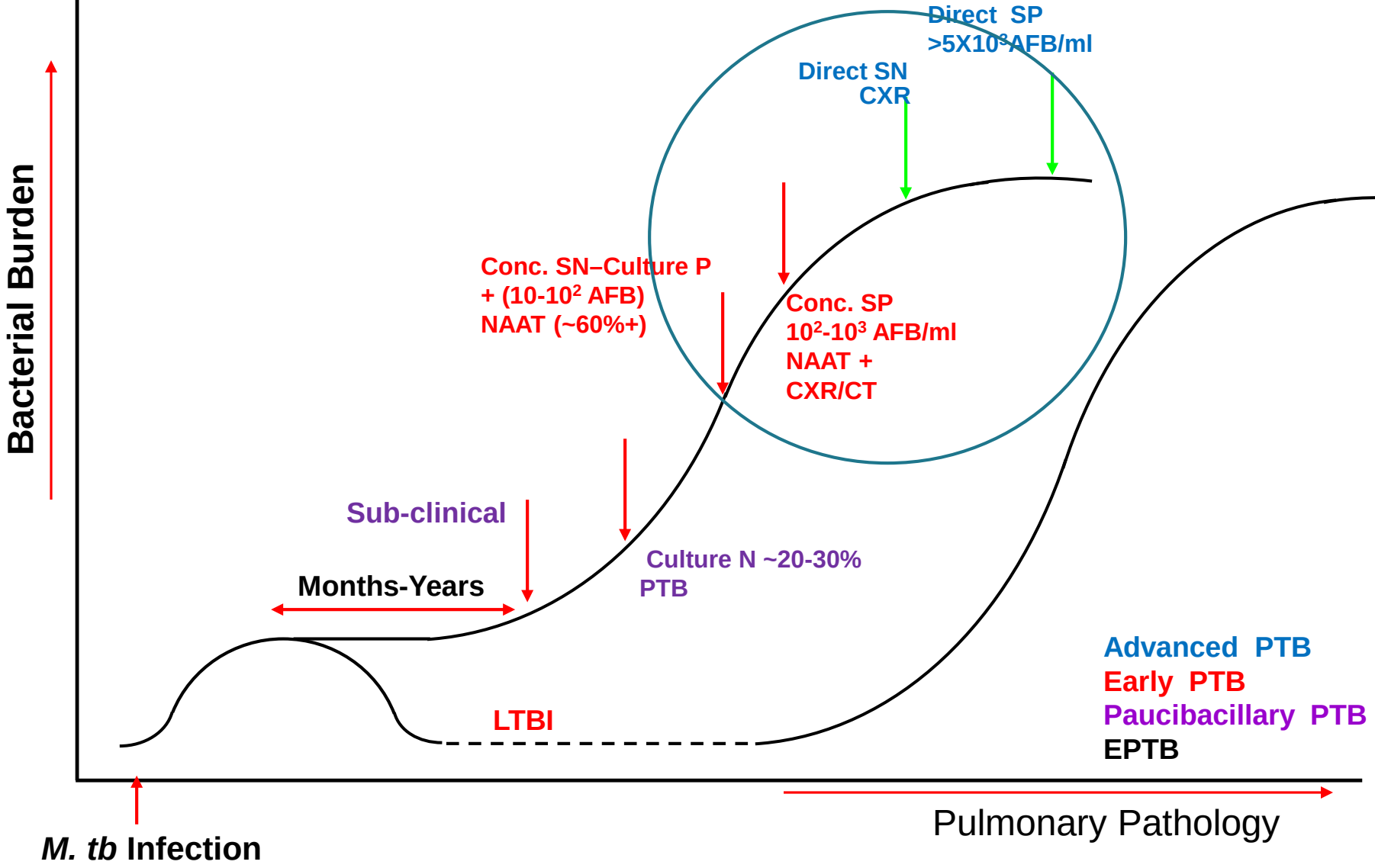


Peptides and Peptide Combinations on LF

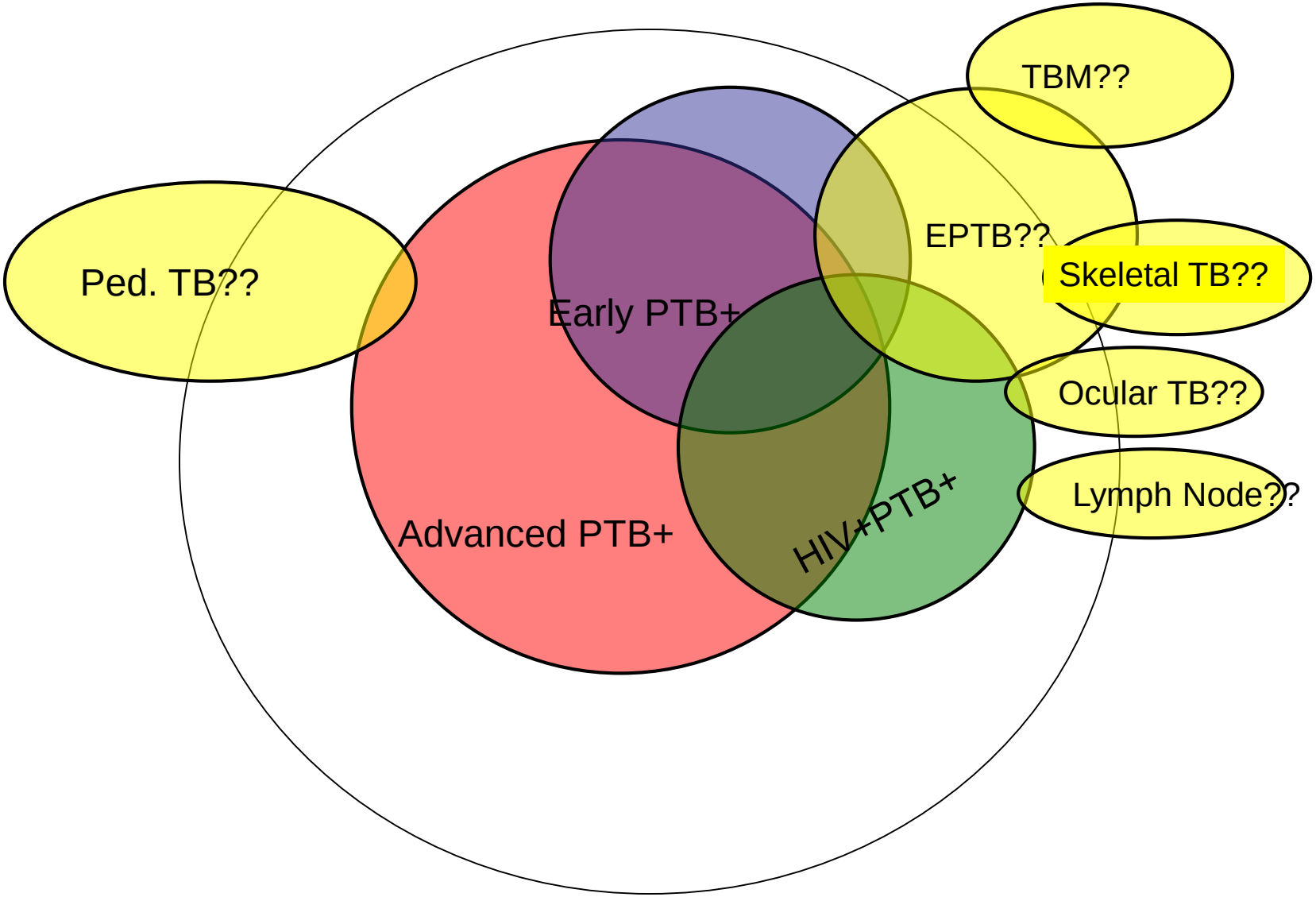
Peptide	Sensitivity (95% CI)	Specificity (95% CI)
P1	58.1% (48.9, 66.9)	99.4% (96.9, 100.0)
P6	71.0% (62.1, 78.8)	92.1% (87.2, 95.6)
P7	82.3% (74.4, 88.5)	93.8% (89.2, 96.9)
P1 + P6	86.3% (79.0, 91.8)	92.1% (87.2, 95.6)
P1 + P7	91.9% (85.7, 96.1)	93.8% (89.2, 96.9)
P6 + P7	87.1% (79.9, 92.4)	90.5% (85.2, 94.3)
P1 + P6 + P7	94.4% (88.7, 97.7)	90.5% (85.2, 94.3)

Gonzalez JM, Francis B, Burda S, Hess K, Behera D et al (2014): Development of a POC test for TB based on multiple immunodominant epitopes of *M. tb* specific cell-wall proteins. Plos One 9(9), e106279

Need for Immunological Detection



Challenges of Immunological Detection



Epitope Mapping of *M. tb* Specific CW Proteins on MA

Protein	# of Peptide	HIV- ENC PTB (13)	HIV- EC PTB (7)	HIV- ANC PTB (10)	HIV- AC PTB (10)	HIV-EPTB (10)	HIV+ PTB (10)
Protein Z	394	13	50	34	5	10	91
Protein-J	100	2	3	11	34	2	1
Protein-W	66	2	2	9	25	1	1
Protein-C	163	1	3	35	52	1	4
Protein-M	77	1	1	16	25	1	4
Protein-N	90	1	1	12	31	1	2
Protein-L	73	2		20	23	1	2
Protein-O	186	3	5	33	66	2	
Protein-R	54	2	4	11	32	2	
Protein-G	90		2	15	28	1	
Protein-F	51		1	20	15	1	5
Protein-Q	119		3	12	34	2	5
Protein-K	104	1		17	37		2
Protein-U	62	1		3	18		1
Protein-D	73		1	7	18		11
Protein-H	46		1	2	12		5
Protein-P	110		1	12	36		1
Protein-S	62			11	24	1	
Protein-V	41			5	10	1	1
Protein-E	75			12	24		3
Protein-X	65			11	18		2
Protein-Y	45			2	13		
TBB-P2	68	13	11	9	16	3	4
TBB-P1	73		5	11	27		1
TBB-P3	50		9	7	12		5
Immunodominant (40%)							

Current Status

Optimization of peptide-based LF test for replacing microscopy

Identification of peptides for detection of paucibacillary TB ongoing

Simple, affordable, sensitive, specific, implementable, POC serological test will be possible to devise.

The Team

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