

# TBM Diagnostics: Xpert Ultra

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# Minnesota-Makerere-Mbarara Meningitis Collaboration



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# Diagnostics History

- Ziehl–Neelsen stain, 1882-1883
- Culture
  - 1882 Koch => Solid Media
  - 1980 BD Bactec instrument
  - 1998 MGIT 960 instrument
- PCR
  - 1990's Gen-Probe & Roche
  - Dec 2010 Xpert MTB/RIF
  - **2017 Xpert MTB/RIF Ultra**

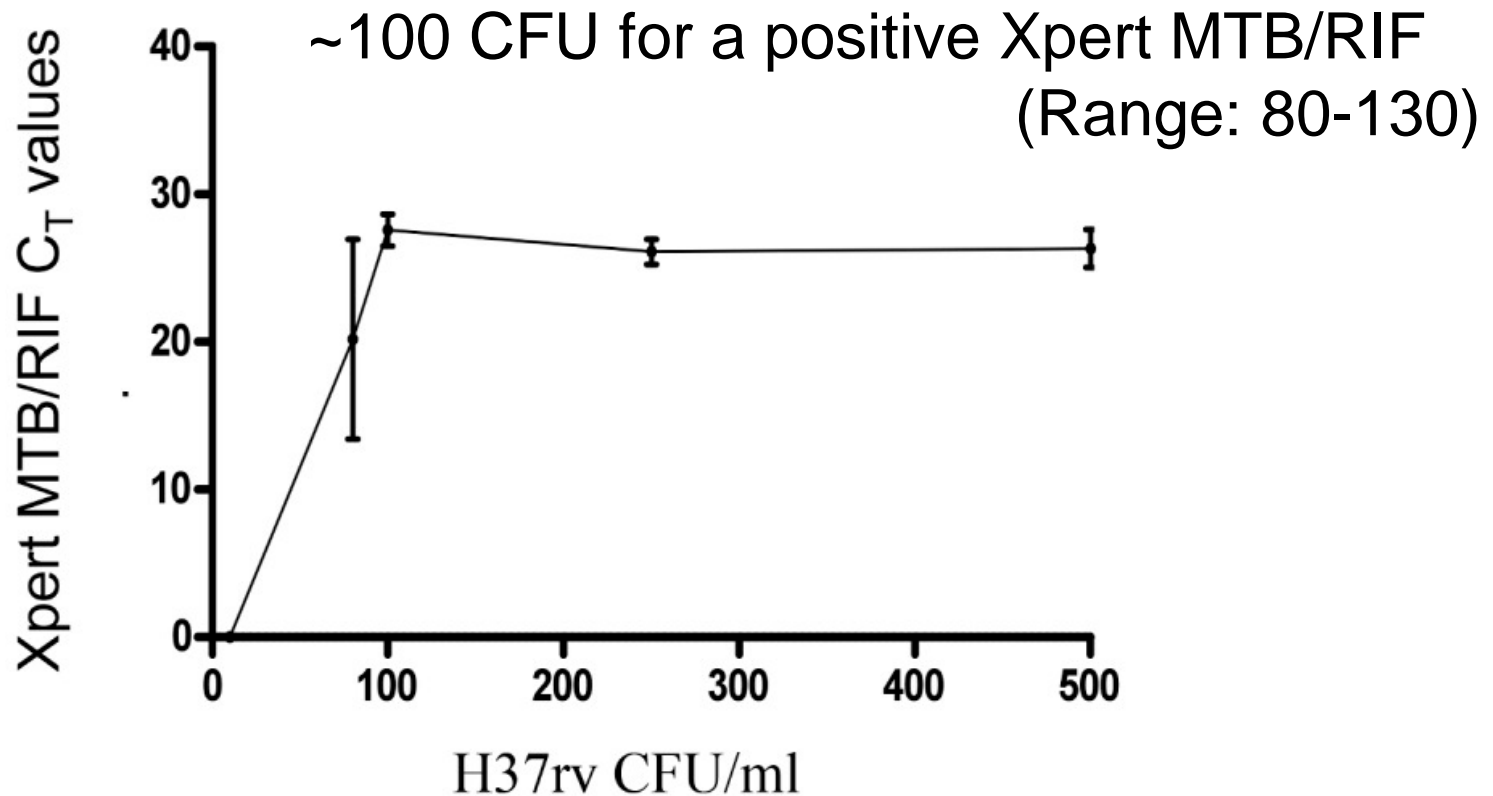


# Analytical Sensitivity

- Ziehl–Neelsen stain, ~5000 CFUs
- Culture ~ 10 CFUs
  
- Xpert MTB/RIF ~100 CFUs



# Xpert: Analytical Sensitivity



# Analytical Sensitivity

- Ziehl–Neelsen stain ~ 5000 CFUs
- Culture ~ 10 CFUs
  
- Xpert MTB/RIF ~ 100 CFUs
- Xpert MTB/RIF Ultra ~ 10 CFUs



# Xpert MTB/RIF

- More volume = More sensitivity
- Patel *et al.*
  - 1mL raw = 47% sensitivity vs. Culture
  - 3mL centrifuged = 82% sensitivity vs. Culture
- Nhu *et al.*
  - centrifuged all CSF, Sensitivity 59% vs. Case Definition
  - Xpert CSF tested: 17% <.8mL; 61% .8-2mL; 22% >2mL
- Bahr *et al.*
  - 27% sensitivity with 2mL CSF; 72% sensitivity centrifuged
  - Culture 71% sensitivity



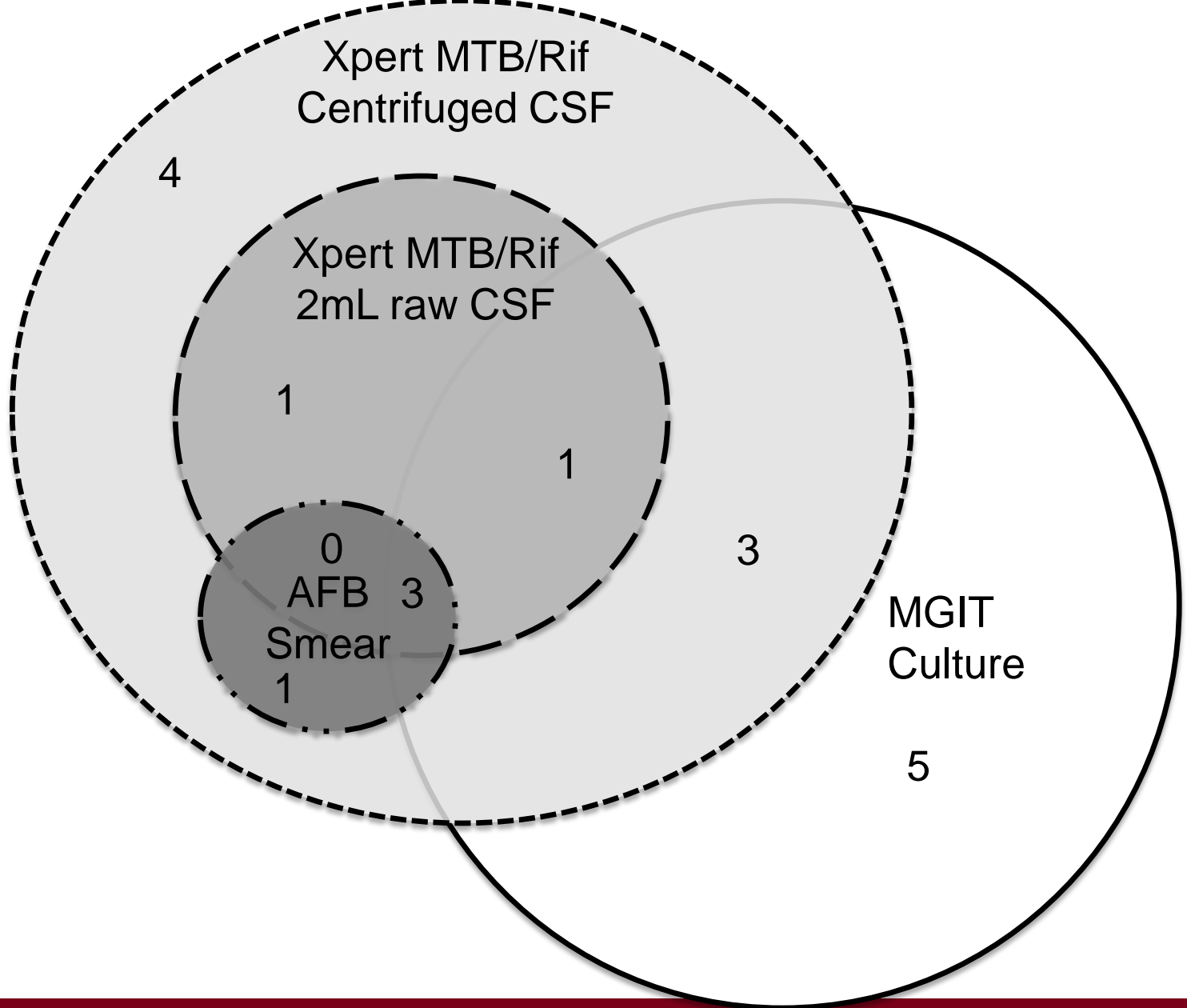
# Uganda TBM Results 2015

CSF Test	N	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Xpert 2mL un-centrifuged CSF	107	28% (5/18)	100% (89/89)	100% (5/5)	87% (89/102)
Xpert centrifuged CSF	95	72% (13/18)	100% (77/77)	100% (13/13)	94% (77/82)
Culture	80	71% (12/17)	100% (63/63)	100% (12/12)	93% (63/68)
AFB Smear by Microscopy	107	22% (4/18)	100% (89/89)	100% (4/4)	86% (89/103)

Xpert Protocols:  
P=0.008 by McNemar's test







# Xpert MTB/RIF Ultra



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# What's new?

- Xpert<sup>®</sup> MTB/RIF Ultra
  - Larger PCR reaction chamber
  - Improved PCR Probes
    - *M tb* Targets: IS6110, IS1081, rpoB
  - Sensitivity of ~10 CFU/mL
  - Pulmonary clinical trial (FIND & TB-CDRC)



# Xpert Ultra in TBM

- Prospectively 129 recruited subjects in Mbarara, Uganda.
- April 2015- Dec 2016
- Received Xpert Ultra in Nov 2016
- Tested in Dec 2016
- Manuscript written Jan 2016



# Xpert Ultra Methods

Reference standard:

- 1) Any positive TB test
- 2) Probable / Definite TBM per Uniform Case Definition – excluding Xpert Ultra results

Median of 8mL CSF collected, centrifuged  
2mL pellet divided for testing.



# Participant Characteristics

Characteristics	TB Meningitis N=22	Other Meningitis N=107	P value
Age, years	32 (30-34)	34 (29-43)	0.24
Men, percent	59% (13/22)	54% (58/107)	0.82
Headache duration, days	7 (6.5-14)	4.5 (7-14)	0.83
CD4 T cell count, cells/ $\mu$ L	72 (43-124)	88 (15-226)	0.83
Serum C-reactive protein, mg/L	49 (16-74)	52 (11-108)	0.48
CSF white cells/ $\mu$ L	12 ( $\leq$ 4-130)	5 ( $\leq$ 4-38)	0.24
CSF lymphocytes, %	70% (65-83%)	75% (59-80%)	0.89
CSF total protein, mg/dL	255 (90-510)	170 (65-365)	<b>0.023</b>
CSF glucose, mg/dL	49 (32-71)	70 (49-95)	<b>0.002</b>
Antecedent/Prior TB diagnosis	18% (4/22)	3.3% (3/91)	<b>0.026</b>
Physician TB meningitis diagnosis*	59% (13/22)	43% (44/102)	0.24
Alive at discharge / last contact	50% (11/22)	70% (64/91)	0.082



# CSF Diagnostic Performance

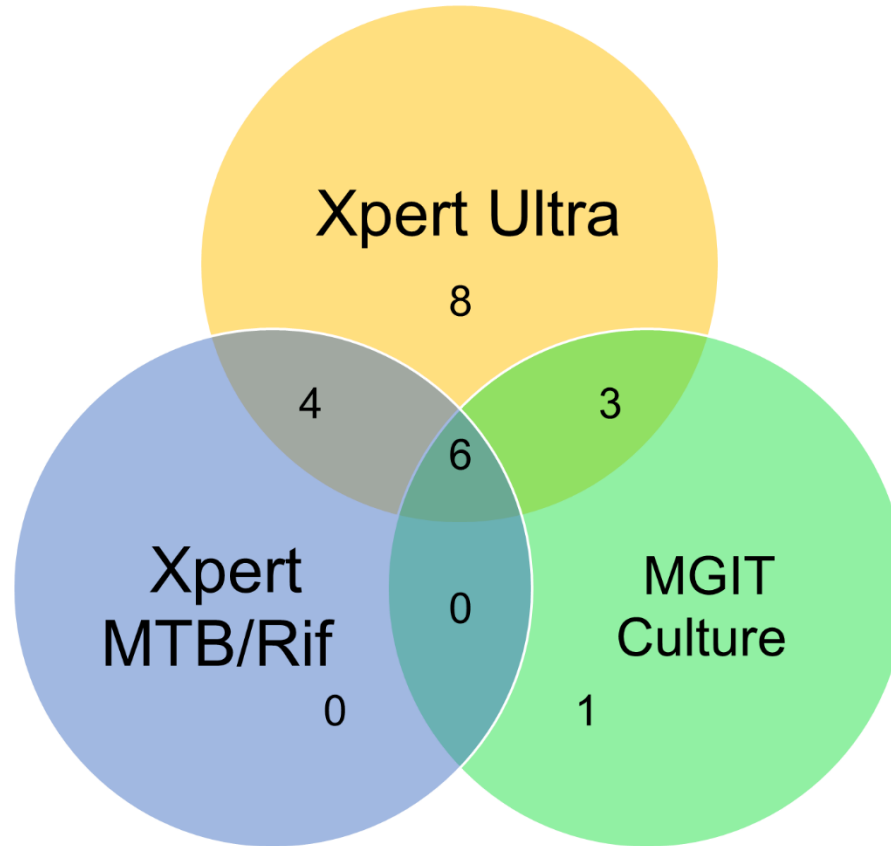
CSF Test	Volume Tested	N	Sensitivity vs. composite end point	Sensitivity vs. case definition	Assay Error Rate
<b>Xpert Ultra</b>	0.5mL	129	95% (21/22)	70% (16/23)	2.3% (3/129)
<b>Xpert MTB/Rif</b>	1.0mL	129	45% (10/22)	43% (10/23)	4.7% (6/129)
<b>MGIT Culture</b>	0.5mL	129	45% (10/22)	43% (10/23)	1.6% (2/129)

Composite end-point included any positive CSF Xpert Ultra, Xpert, or Bactec Mycobacterial growth indicator tube (MGIT) culture. Sensitivity vs. uniform clinical case definition for definite (n=14) or probable (n=9) TB meningitis excluded Xpert Ultra results in defining case status.

Error in culture reflects contamination with non-tuberculous mycobacterium growth



# Venn Diagram



TB Negative n=107

Of 8 participants positive by Xpert Ultra only, 6 had recently initiated HIV therapy, and all 8 were negative by testing with:

- Cryptococcal Antigen
- Gram's stain,
- culture, and
- multiplex PCR for 14 common meningitis pathogens (Biofire).



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# Next Gen Sequencing for Confirmation

Sequencing from the residual volume left over in the Xpert Ultra cartridges

ID	Xpert Ultra Category	MGIT Culture	Xpert MTB/RIF	Next Generation Sequencing of <i>M. tuberculosis</i> Genes
5001	Trace	Negative	Negative	IS6110 detected
5004	Very Low	Positive	Positive	IS6110, IS1081, rpoB (WT) detected
5005	Trace	Positive	Negative	Negative*
5103	Trace	Positive	Negative	IS6110 detected
5194	Very Low	Positive	Positive	IS6110, IS1081, rpoB (WT) detected
5116	Trace	Negative	Negative	IS6110 detected
5285	Very Low	Negative	Positive	IS6110, IS1081, rpoB (WT) detected



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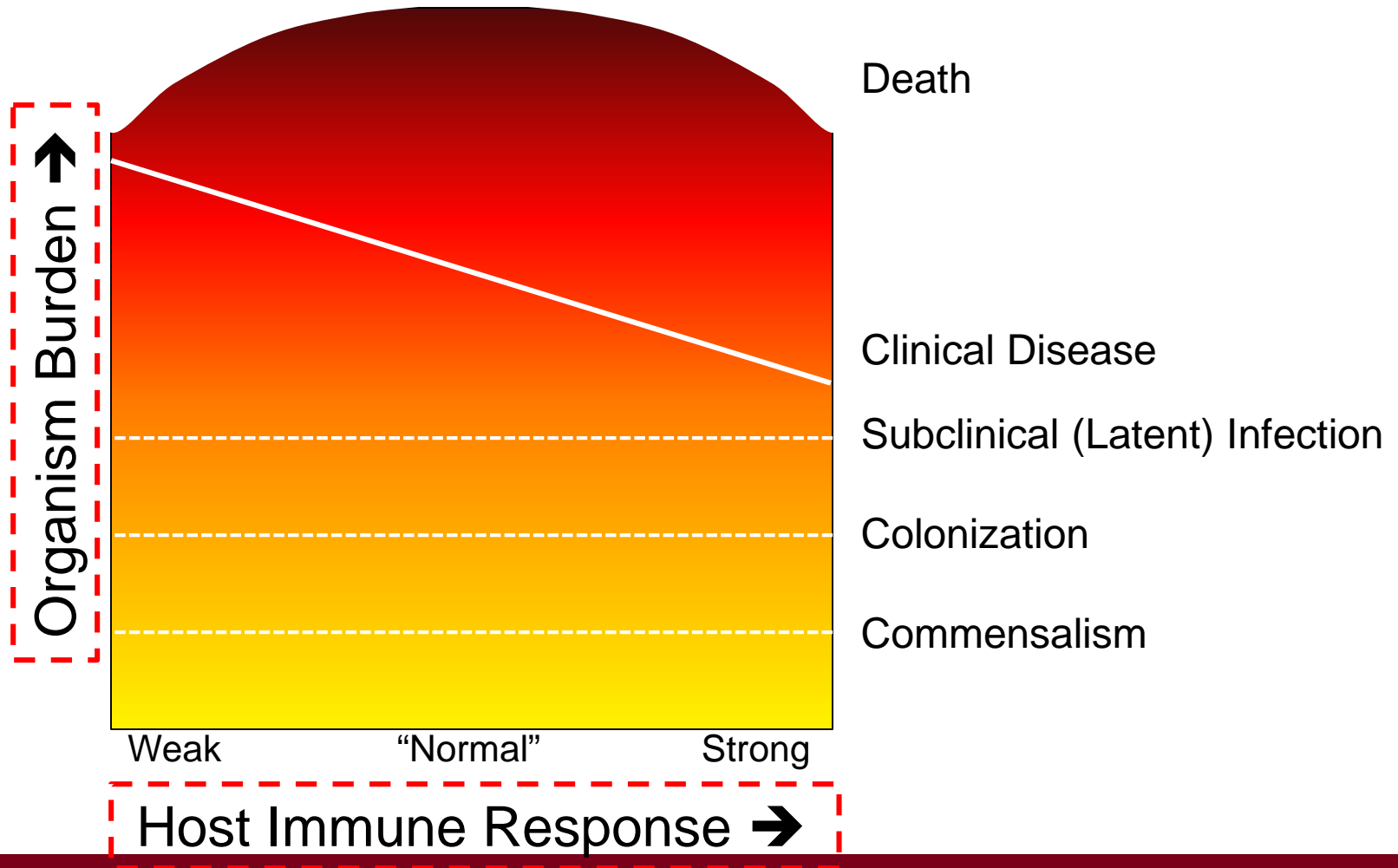
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# Pathogen-Response Framework

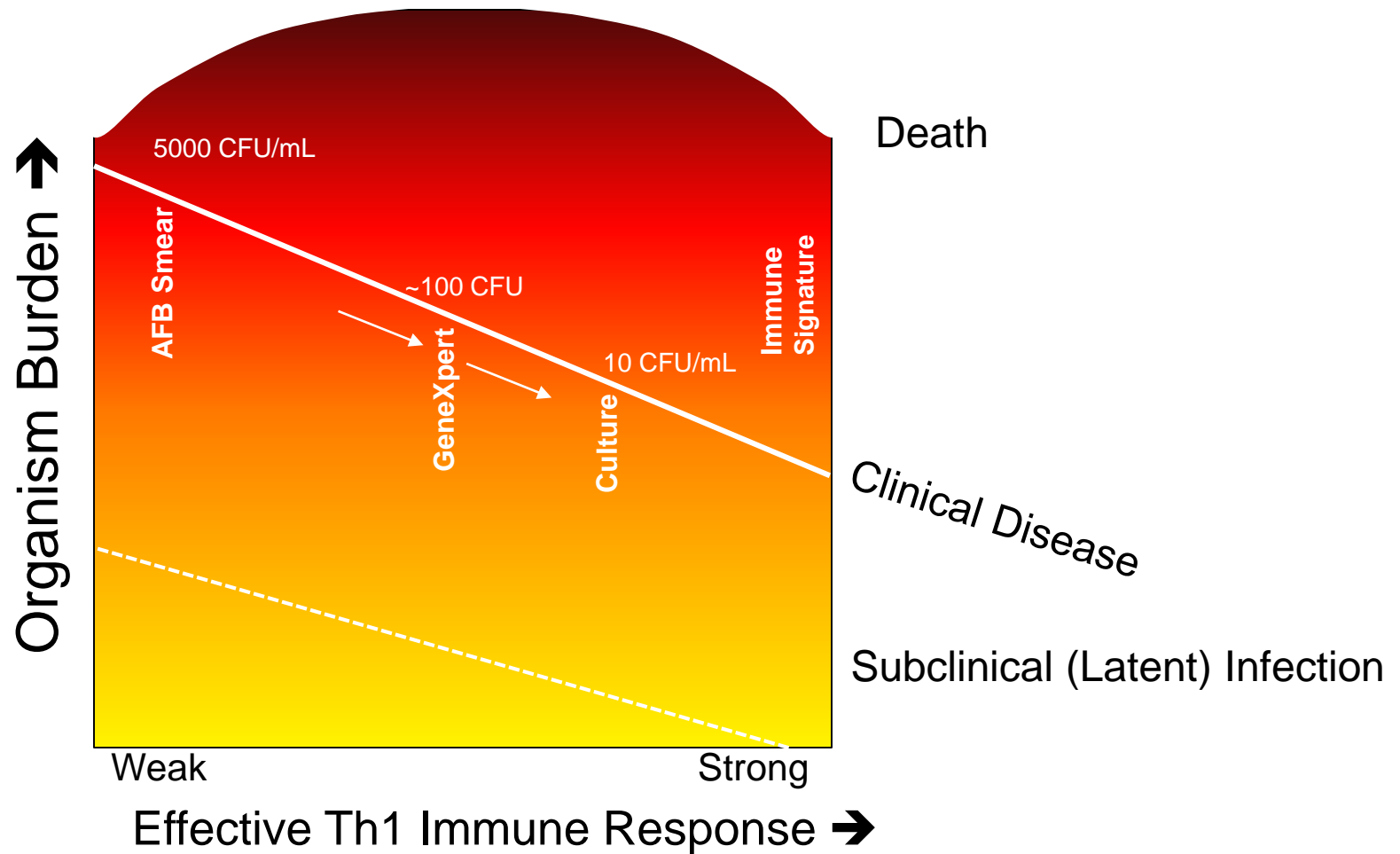


Modified from:  
Casadevall A, Pirofski L. The damage-response framework of  
microbial pathogenesis. *Nature Rev Microbiol* 2003; 1:17-24



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# Diagnostic Framework: TB Meningitis



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