Potential of Cell-Free DNA in Plasma and Urine for Rapid Detection of *Mycobacterium tuberculosis*

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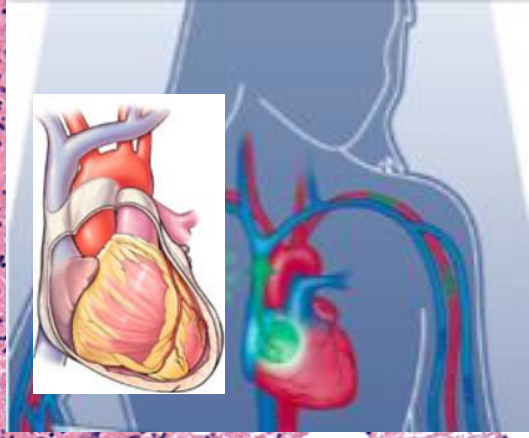
Disclosures

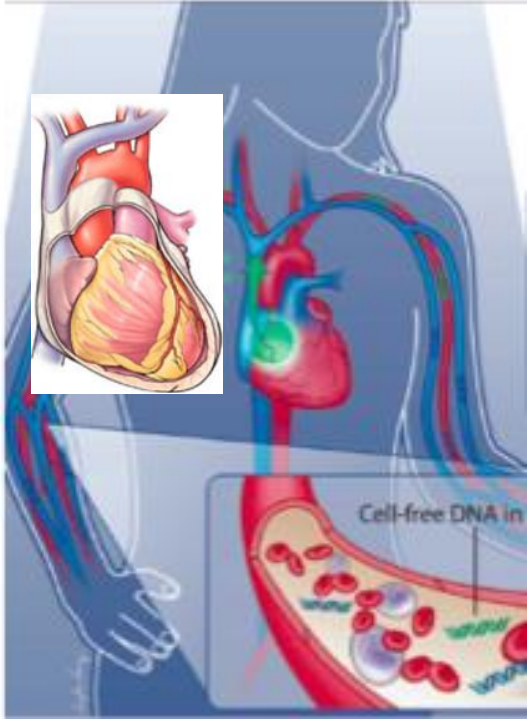
- Banaei:
  - IP interest in GWiS PCR
  - Industry links or funding related to this talk
    - Research support form KariusDx

60 y/o Vietnamese with restrictive pericarditis
Pericardial biopsy
Necrotizing granulomas, no AFB
TB PCR and culture: Positive for MTC
Sputum culture x3: Negative



2/2015

60 y/o Vietnamese with restrictive pericarditis
EDTA Plasma
Deep sequencing of plasma cell-free DNA on Illumina NextSeq500, 75 million reads



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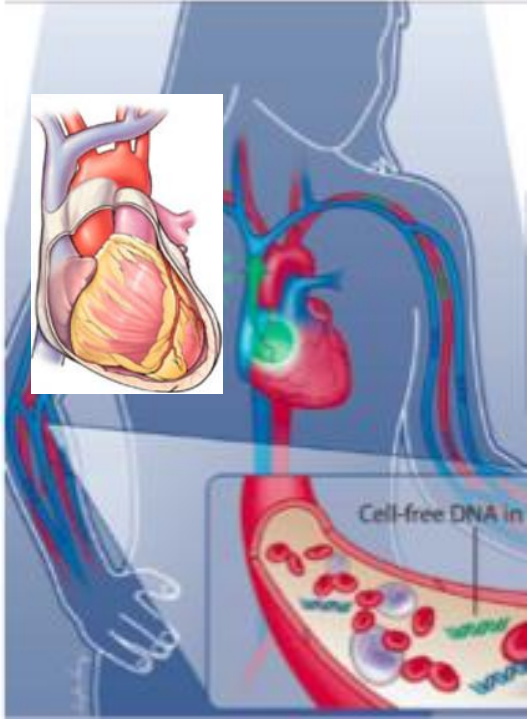

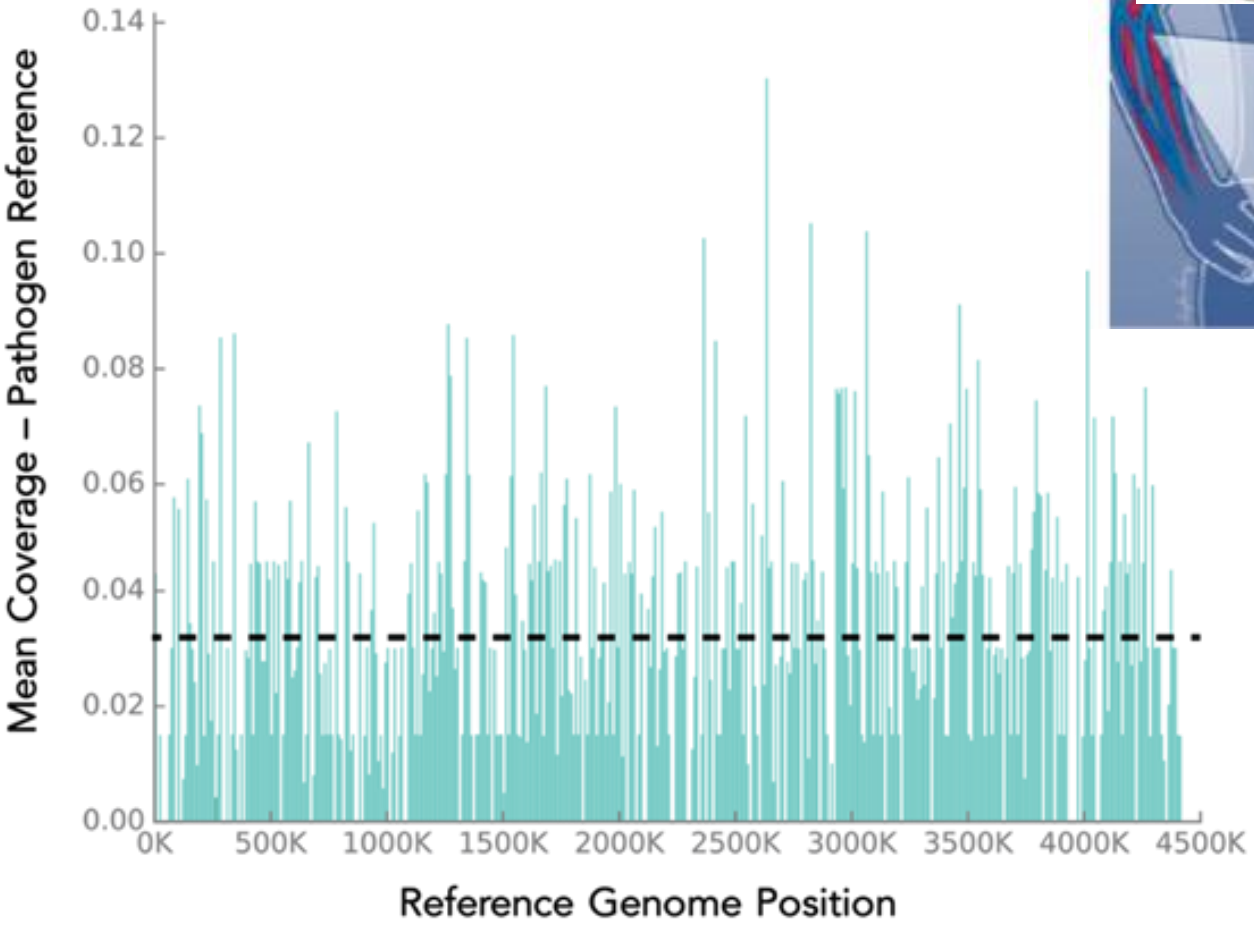

KARIUS

Hong et al ASM Microbe 2016
Hong et al In preparation

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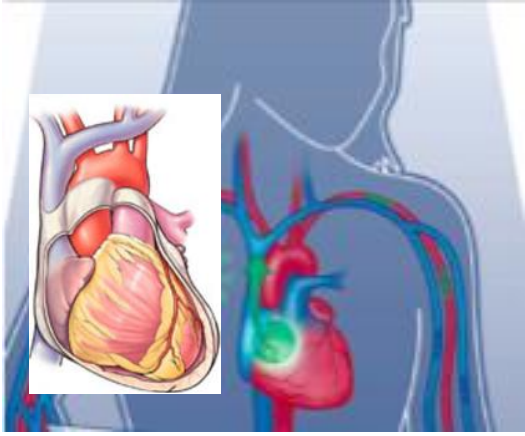
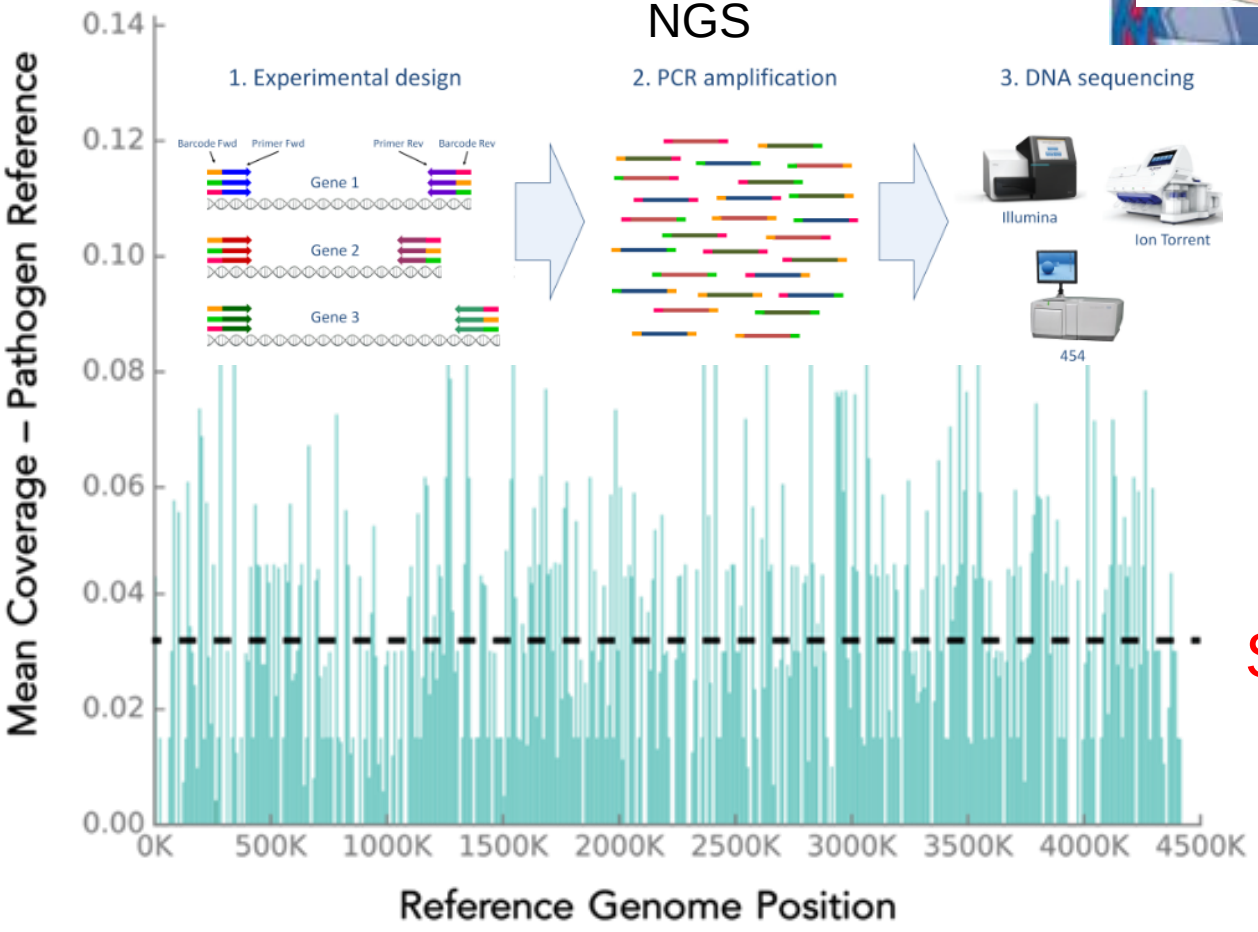




$$$
Complex
Laborious
Technical
Slow
Sensitivity?

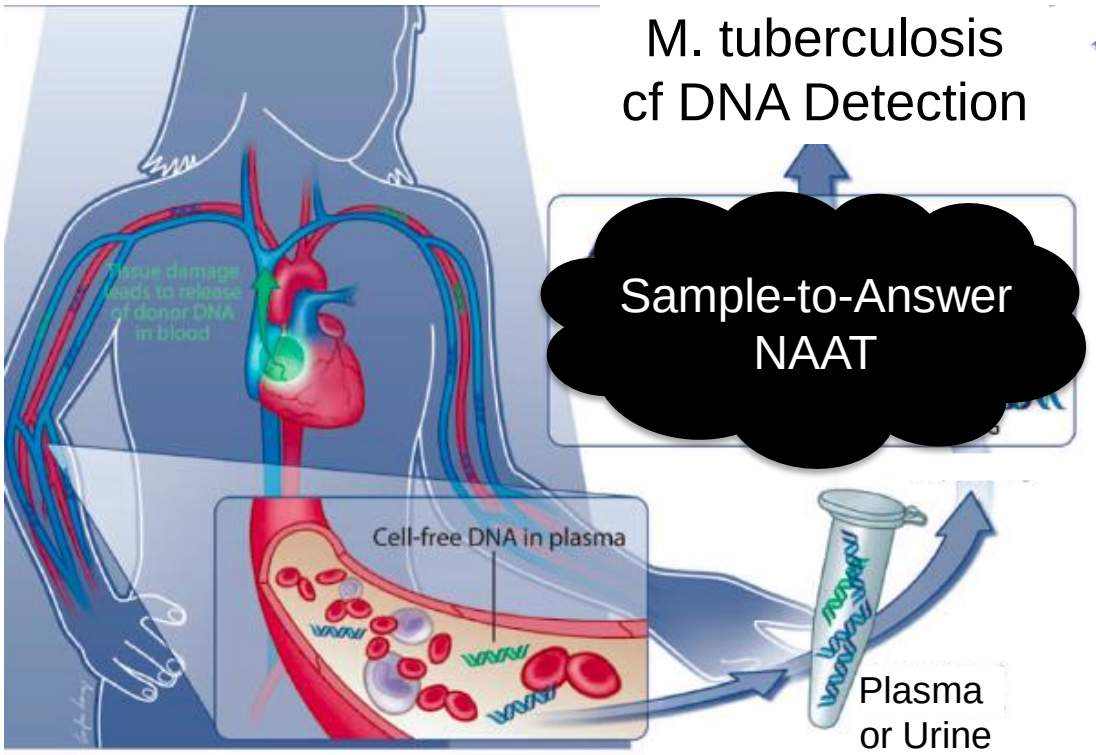
KARIUS

Hong et al ASM Microbe 2016
Hong et al In preparation

Application of cf DNA for Diagnosis of TB

Affordable
Sensitive
Specific
User-friendly
Robust, rapid
Equipment min
Deliverable

Application of cf DNA for Diagnosis of TB



adapted from De Vlaminck Sci Transl Med 2014

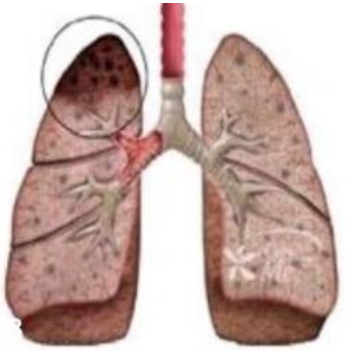
Affordable
Sensitive
Specific
User-friendly
Robust, rapid
Equipment min
Deliverable

Target Population for cf DNA TB Diagnosis

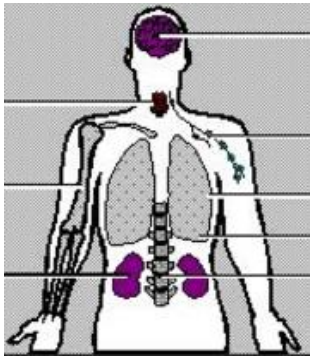
Pediatric

Unproductive

HIV/AIDS



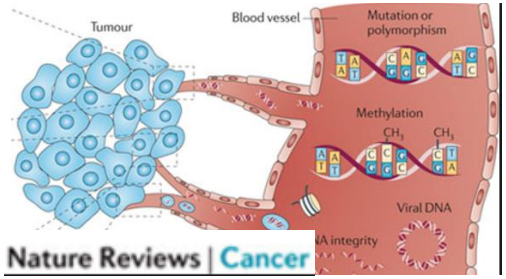
Extrapulmonary



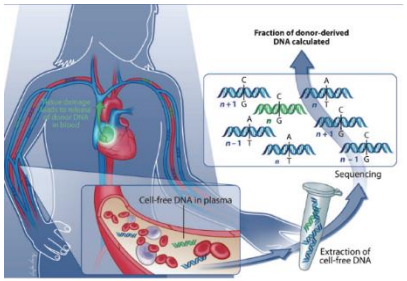
Application of cf DNA in Diagnostics

Fetal aneuploidy

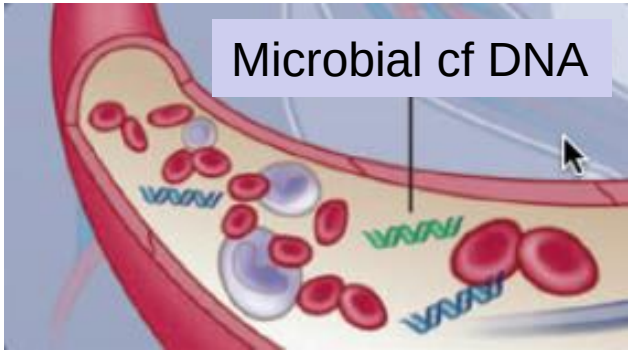
Cancer mutations



Organ rejection



Infectious diseases



- EBV➔nasopharyngeal CA (Cancer Res 1999)
- Invasive fungal infection (CID 2013)

www.thelancet.com/infection Vol 9 August 2009

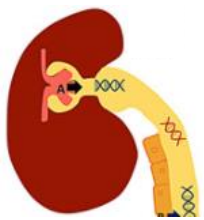
Rapid diagnosis of tuberculosis through the detection of mycobacterial DNA in urine by nucleic acid amplification methods

Clare Green, Jim F Huggett, Elizabeth Talbot, Peter Mwaba, Klaus Reither, Alimuddin I Zumla

	Sensitivity by tuberculosis presentation			Effect of HIV coinfection* on sensitivity		Method of tuberculosis confirmation	Target size bp†
	Pulmonary	Extrapulmonary	Other	HIV positive	HIV negative		
Sechi et al[34]	..	..	13% ND (77/602)	16% (65/412)	6% (12/190)	Suspected—3% (18/602) urine culture positive	182 (566)
Aceti et al[35]	100% (13/13)	..	..	100% (13/13)		Sputum smear or culture	309 (566)
Kafwabulula et al[33]	56% (35/63)	..	..	64% (32/50)	23% (3/13)	Sputum smear or culture	181 (556)
Torrea et al[36]	44% (108/247)	57% (48/84)	..	59% (86/145)	38% (70/186)	Pulmonary tuberculosis diagnosed by sputum smear or culture; extrapulmonary tuberculosis diagnosed by clinical criteria	309 (566)
Rebollo et al[37]‡	7% (2/27)	14% (2/14)	16% D (5/31)	28% (7/25)	6% (2/32)	Culture from any clinical sample and response to treatment	123
Cannas et al[38]§	79% (34/43)	..	..	..	..	Sputum smear or culture	67 (129)
Gopinath and Singh[39]	52% (24/46)	..	..	..	..	Sputum culture	786

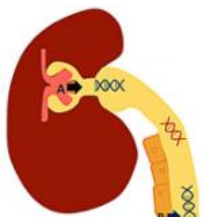
All studies used IS6110 as a target except Gopinath[39] who amplified from cfp32. The tabulated data present the most relevant comparisons between studies relating to transrenal DNA detection. For all studies, except the initial study by Sechi and colleagues[34] who relied on empirical observations, the gold standard for pulmonary diagnosis was sputum smear or culture positives. Data have been divided to distinguish between different presentations where appropriate. D=disseminated. ND=not disclosed. *Studies in which fewer than ten HIV-positive cases included in the study are not detailed. †Size of external product shown in brackets where amplification was nested. ‡0–1 month after presentation and initiation of treatment. §Only data relating to the transrenal DNA are detailed. The authors considered the urine pellet separately, which produced significantly lower detection rates.

Table 1: **Summary of studies on urine-based detection of mycobacterial DNA by PCR amplification**



Accuracy of Urine cf DNA for TB Diagnosis

	Age	Country	TB Type	Cases	Controls	Method	Target	Sensitivity	Specificity
Cannas et al IJTLD 2008	>18 yo	Italy	PTB	43	23	Nested PCR	IS6110	79% (34/43)	100% (23/23)
Fortún et al IJTLD 2014	>18 yo	Spain	EPTB	82	0	TMA	16S rRNA	70% (57/82)	Not Done
			PTB	25				18% (5/25)	
Labugger et al Infection 2017	>18 yo	Germany	PTB	11	8	PCR	IS6110	64%* (7/11)	100% (8/8)



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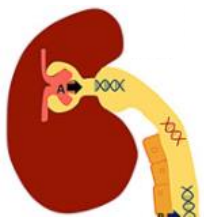
Miliary	Multifocal	LAN	Pleural	Joint
90% (9/10)	67% (16/24)	72% (18/25)	33% (1/3)	45% (5/11)



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	PTB	25				18% (5/25)	
Labugger et al Infection 2017	PTB	11	8	PCR	IS6110	64%* (7/11)	100% (8/8)
						*100% with retesting	

vs Radiology	vs Smear+	vs TTCxP	Wk1	Wk12
↑cfDNA	None	None	↑cfDNA	9/11 Neg

Accuracy of Plasma cf DNA for TB Diagnosis

	Age	Country	TB Type	Cases	Controls	Method	Target	Sensitivity	Specificity
Ushio et al Tuberculosis 2016	>18 yo	Japan	PTB	33	19	Digital PCR	IS6110	65% (21/33)	93% (18/19)
							gyrB	29% (10/33)	100% (19/19)

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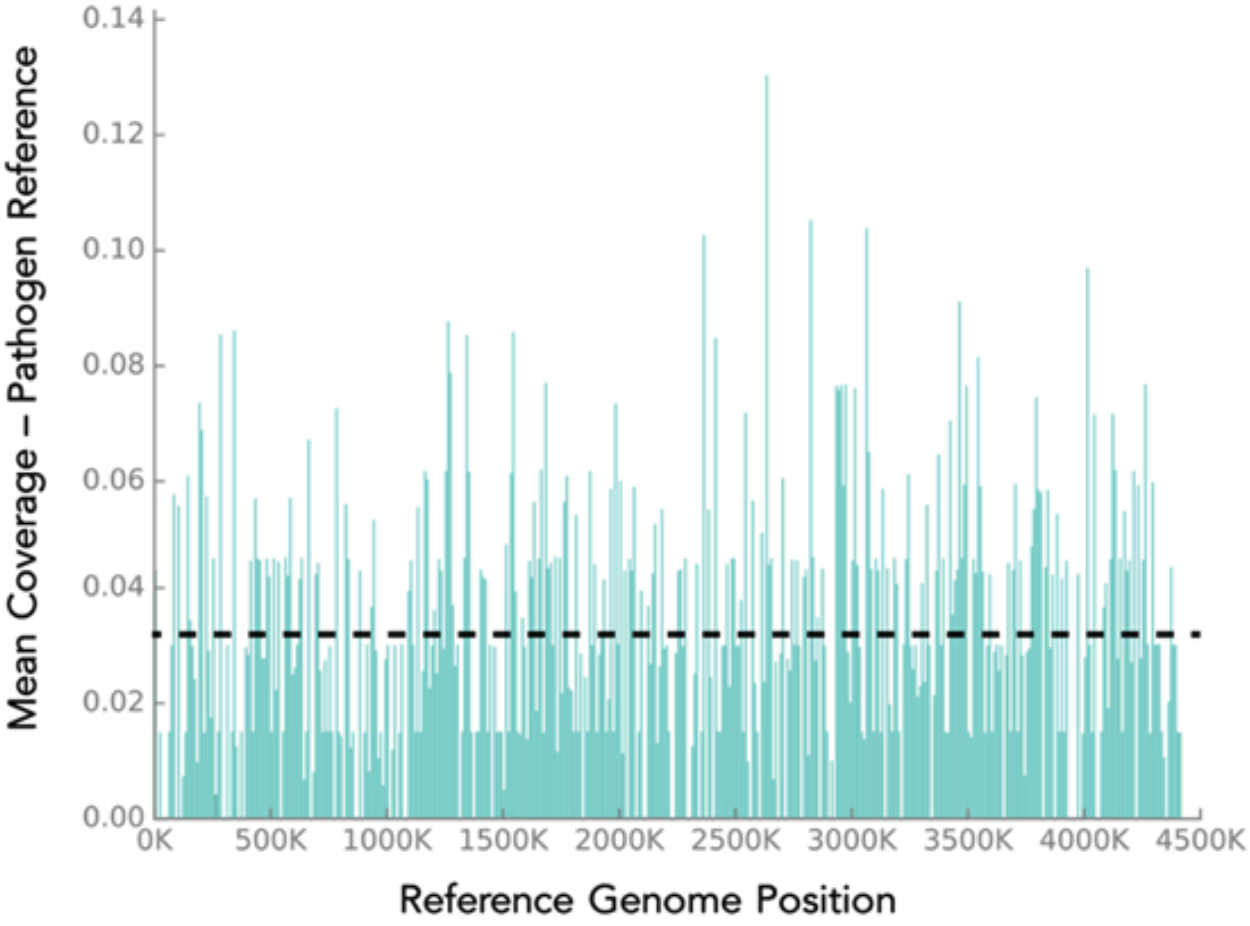
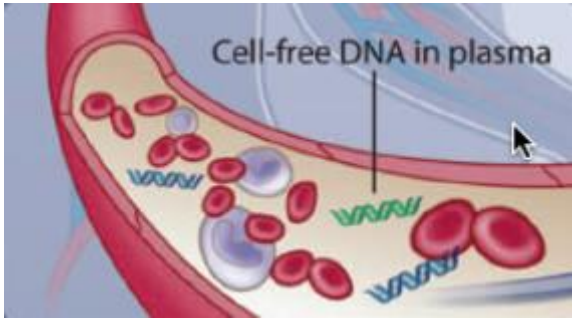
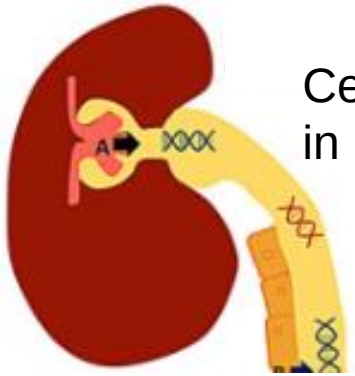
Bilateral vs Unilateral PTB	**PTB+EPTB vs. PTB**
↑cfDNA	↑cfDNA

Genome-Wide Sensitive PCR (GWiS PCR)



Potential of cf DNA in Diagnosis of TB



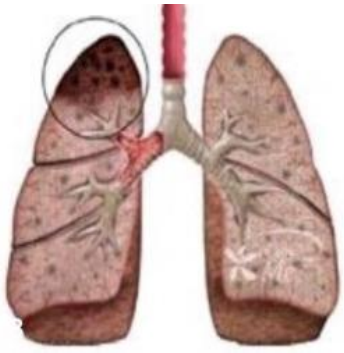


Cell-free DNA in Urine

Accuracy
Sensitivity >70%
Specificity ≈100%

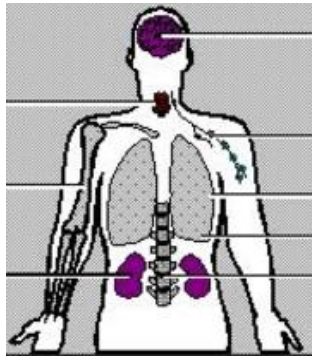
Pediatric

HIV/AIDS



Unproductive

Extrapulmonary



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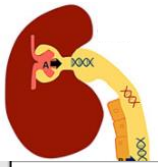
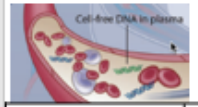


	Age	Country	TB Type	Cases	Controls	HIV+	Smear+	Reference	Sample	Tx Naïve	Preserve	Fresh/Frozen	Vol. (mL)	Extraction	Assay	Method	Target	Sensitivity	Specificity
Cannas et al IJTLD 2008	>18 yo	Italy	PTB	43	23	5%	95%	Culture	Urine	No	EDTA	Frozen	5mL	Mannual/ Resin	LDT	nested PCR	IS6110	79% (34/43)	100% (23/23)
Fortún et al IJTLD 2014	>18 yo	Spain	EPTB	82	0	?	NA	Culture	Urine	Yes	?	?	?	?	MTD (Hologic)	TMA	16S rRNA	70% (57/82)	Not Done
	>18 yo	Spain	PTB	25	0	?	?	Culture	Urine	Yes	?	?	?	?	MTD	TMA	16S rRNA	18% (5/25)	Not Done
Labugger et al Infection 2017	>18 yo	Germany	PTB	11	8	0%	60%	Culture	*Urine	Yes	EDTA	?Fresh	4	Mannual/ Resin	LDT	PCR	IS6110	64%* (7/11)	100% (8/8)



Ushio et al Tuberculosis 2016	>18 yo	Japan	PTB	33	19	0%	100%	Culture	Plasma	?	EDTA	?Fresh	0.2	Qiagen column	LDT	digital PCR	IS6110	65% (21/33)	93% (18/19)
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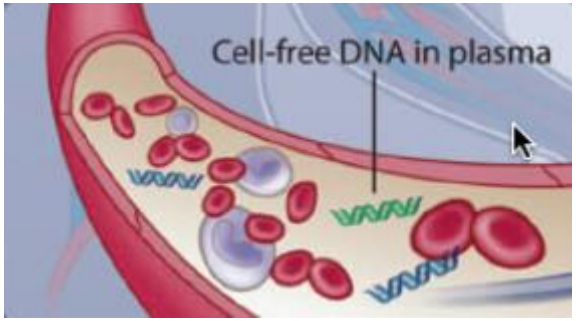
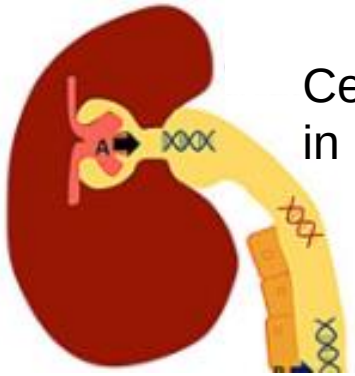
Potential of cf DNA in Diagnosis of TB





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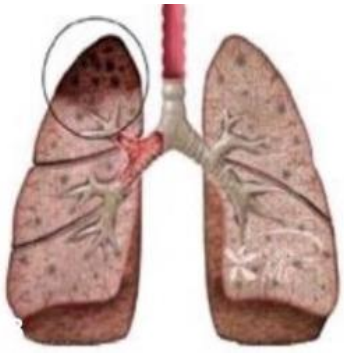
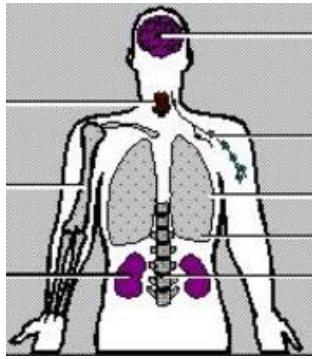


Unproductive

Extrapulmonary



Acknowledgements

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Catherine Hogan
Jason Andrews

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Stanford Pathology