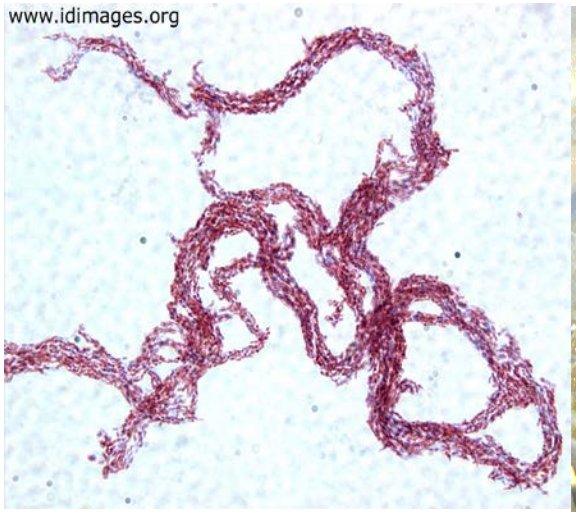
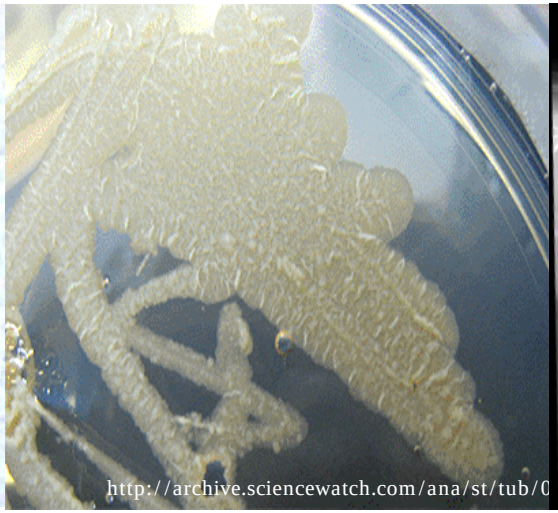
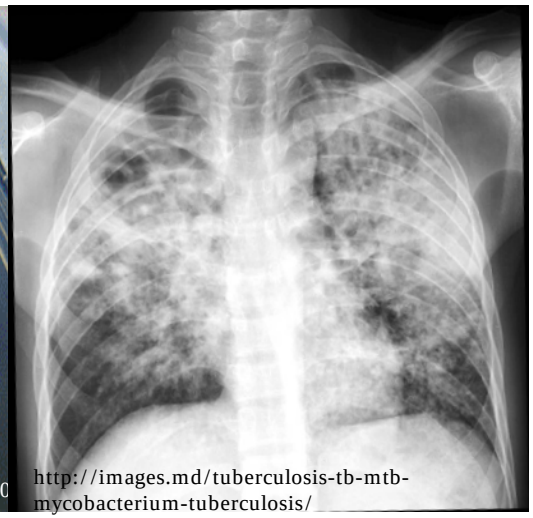
Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Mycobacterium tuberculosis Case Study







Aliberkys Benso (Lopez) M.S
Microbiologist
WSLH

Coming up next…

1. Background on the patient
2. Patient's *M. tuberculosis* infection chronology
3. Treatment and follow up testing
4. Ana Guaracao Ph.D summer study
5. Significance of evaluating *M. tuberculosis* cell viability
6. Review of new assays on development to assess *M. tuberculosis* cell viability

Background on the patient

Demographics:

Age: 58 yrs
Father : Native American-Alaskan
Mother : Caucasian

Life style:

- Vietnam veteran
- Heavy smoker
- Recovering Alcoholic
- Previous use of Cocaine
- Hobbies: Native American rituals
  Motorcycle riding
- Lives with mother and brother

Medical history

- Post Traumatic stress disorder
- Hepatitis C
- Sinusitis
- Ankylosing spondylitis

From Motorcycle Accident to TB infection

April 16, 2004: → Motorcycle accident → Milwaukee Hospital

5 months ↓

- Concussion
- Broken nose
- Memory loss
- Pain in left anterior chest
- Hemoptysis
- Subjective fevers
- Sputum blood tinged
- **SPOT in LUNG**

September 09, 2004: Pulmonologist appointment(-Lung lesion increased)

September 22, 2004: - BAL collected, Smear Positive (2+)

September 23, 2004: - Admitted to the hospital as presumptive TB pt

Treatment and Follow up Testing

1st line drugs

-IIRE and PZA

September 23 - 1st week of April

6 sputum collected
5 smear negative
1 smear positive
1 culture positive

2010
Diagnosed *M. avium complex*

Follow up Testing

Specimens	DOC	SMR	TB/MAC PCR	MGIT +	TB/MAC PCR (from MGIT)	CULTURE
1	4/4/2015	+ Few AFB	**TB CT=34.30** MAC CT=31.45	_	TB CT=0	TB NEGT
2	4/9/2015	1-9/10 F	-	4/17/15	TB CT=0 MAC CT=0	MAC POST
3	4/13/2015	>9/ F	-	4/15/15	TB CT=0 MAC CT=24.01	MAC POST
4	4/14/2015	>9/ F	-	4/21/15	TB CT=0 MAC CT= 24.88	MAC POST
5	5/2/2015	1-9/100 F	-	5/14/15	TB CT= 0 MAC CT= 27.98	MAC POST
6	5/3/2015	>9/F	-	5/11/15	TB CT =0 MAC CT= 26.90	MAC POST
7	5/4/2015	>9/F	-	5/10/15	**TB CT= 38.14** MAC CT=25.68	MAC POST
8	7/28/2015	>9/ F	-	8/6/15	**TB CT=40.33** MAC CT=29.16	MAC POST
9	7/29/2015	>9/F	-	8/6/15	**TB CT= 40.66** MAC CT=29.07	MAC POST

Follow up Testing

Specimens	DOC	SMR	TB/MAC PCR	MGIT +	TB/MAC PCR (from MGIT)	CULTURE
10	6/24/2016	>9/F	**TB CT= 29.39** MAC CT =31.00	7/2/16	TB CT= 0 MAC CT=29.22	MAC POST
11	6/25/2016	>9/F	**TB CT= 34.82** MAC CT= 33.11	7/3/16	TB CT=0 MAC CT= 29.46	MAC POST
12	6/26/2016	1-9/F	-	7/3/16	TB CT=0 MAC CT=29.60	MAC POST
13	7/28/2016	1-9/F	-	8/7/16	**TB CT= 39.28** MAC CT=26.69	MAC POST
14	7/29/2016	1-9/F	-	8/7/16	TB CT=0 MAC CT=26.75	MAC POST
15	7/30/2016	1-9/F	-	8/5/16	TB CT=0 MAC CT =0	MAC POST

Using PCR CT values to predict viability

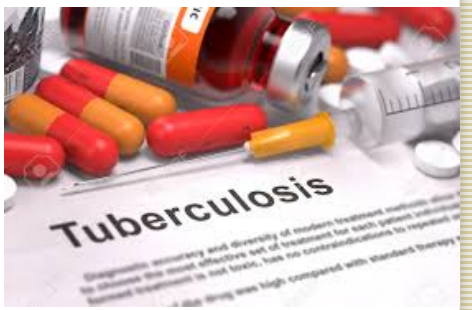
Ana Guaracao Ph.D

Senior microbiologist at WSLH

	1st TB PCR	Days between PCRs	2nd TB PCR
Average for **31 Positive** TB cultures	Ct=25.18	19	Ct= 18.6
Average for **5 Negative** TB cultures	Ct=37.2	25	Ct= 28.38
Average for **36 total** cultures	Ct=26.85	20	Ct= 19.95

- ✓ Decrease in Ct values between 1st and 2nd PCR are not indicative of viable *M. tuberculosis* .
- ✓ Include more specimens for more statistically significant data.
- ✓ Continue this study including Follow- Up specimens to compare CT values from before and after commencing treatment .

Significance of Evaluating *M. tuberculosis* Viability



1. Assessing treatment response

2. Minimize toxicity

3. Prevent development of drug resistanc

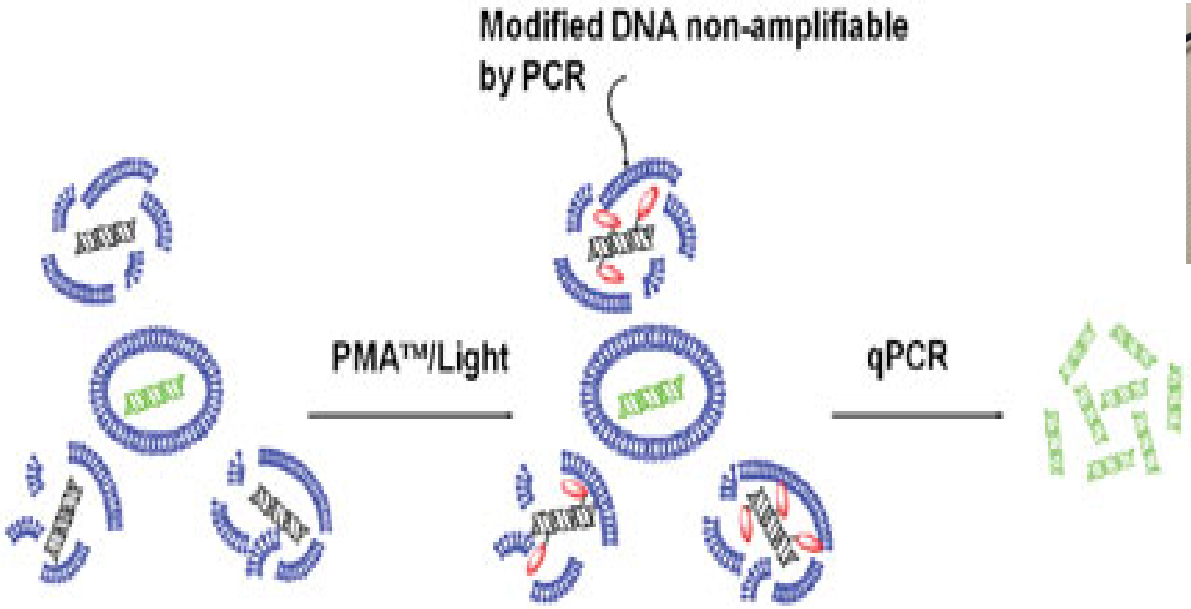
Ongoing Research on Cell Viability Assay Development

1. *M. tuberculosis* Viability by PMA Real-Time PCR

2. *M. tuberculosis* Viability by Microscopy (Fluorescein Diacetate (FDA)
3. Assessing *M. tuberculosis* viability by Flow Cytometry

4. Nile-Red labeled Auramine-O staining smear

M. tuberculosis Viability by PMA Real-Time PCR



Advantages

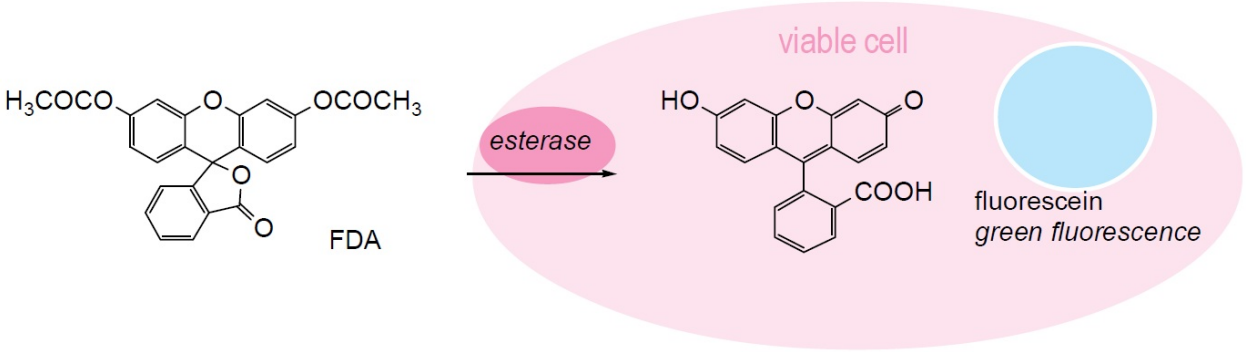
- Comparable with NALC-NaOH
- Used in primary specimens

Disadvantage

- Cells with compromised membrane integrity

M. tuberculosis Viability by Microscopy

Fluorescein Diacetate (FDA)



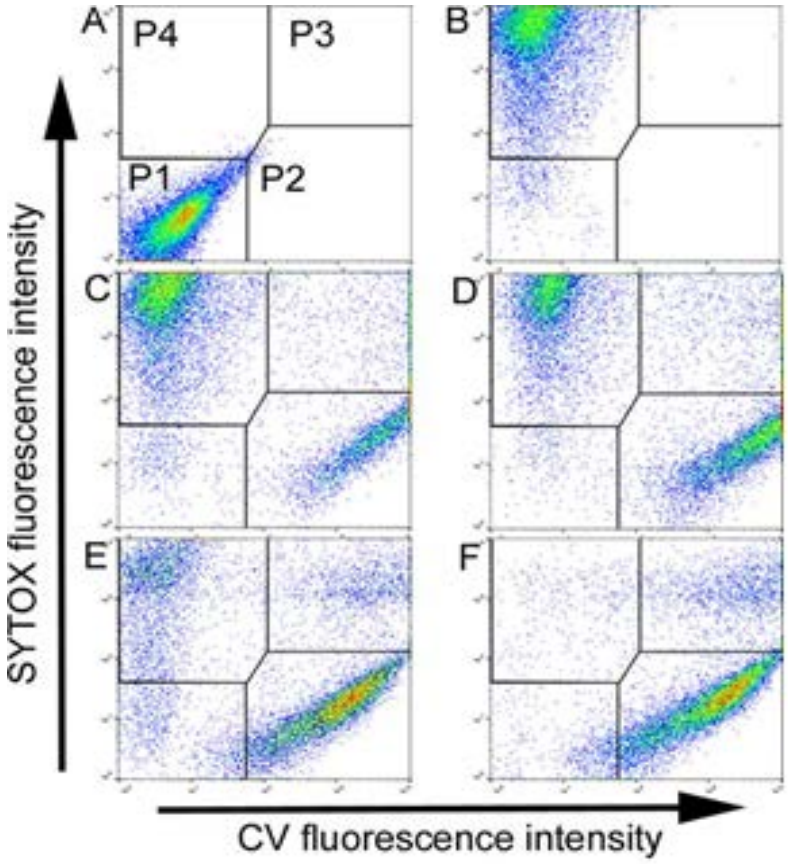
Advantages

- Does not require sophisticated laboratory infrastructure
- Used as early indicator of poor treatment response
- Permits early identification of drug resistance

Disadvantage

- Only useful to assess sputum from smear positive pulmonary TB pt
- Requires trained lab personal
- Does not identifies the cause of treatment failure

Assessing *M. tuberculosis* viability by Flow Cytometry



Live cells → **Calcein-violet with Acetoxy-Methyl Ester**

-permeates cell membranes

-intake or efflux depends on growth rate

Dead cells → **Sytox Green**

-penetrates through damaged cell membranes

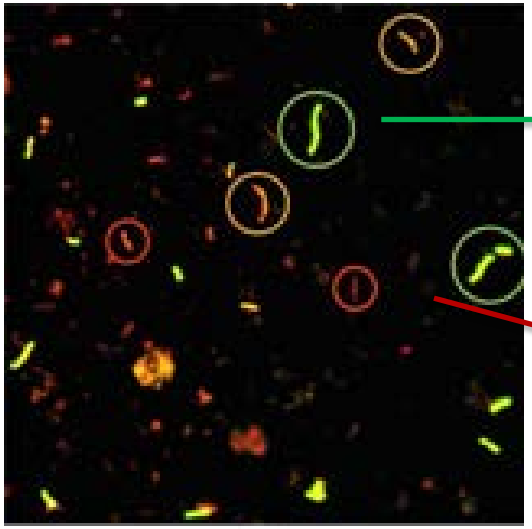
-binds to DNA

Charlotte Louise Hendon-Dunn et al. Antimicrob. Agents Chemother. 2016;60:3869-3883

Auramine labeled Nile-Red staining smear

M. tuberculosis In vitro studies show :

- MTB becomes persistent
- Metabolic downregulation
- Stops replication
- Decrease ATP production
- Shifts energy from carbohydrates to fatty acids



Tuberculosis 2015 95, 770-779DOI: (10.1016/j.tube.2015.08.001)

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