

**Evaluation of a Multiplexed PCR and Melt
Curve Analysis Method for Rapid
Identification of *Mycobacterium
tuberculosis* complex (MTBC) from
Positive MGIT Broths**

April 18, 2016

Stacy White, PhD



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

Background

- AZ State Laboratory introduced MALDI-TOF for mycobacterium identification
 - great for solid growth
 - problems with MGIT broth
- Primary ID method from broth HPLC
 - technically demanding
 - extensive experience
 - technical support diminished
- Requirement for a alternative method to identify from broth
 - probes?
 - PCR?





Multiplex Real-Time PCR Assay and Melting Curve Analysis for Identifying *Mycobacterium tuberculosis* Complex and Nontuberculous Mycobacteria

Jeong-Uk Kim, Choong-Hwan Cha, and Hae-Kyong An

Department of Laboratory Medicine, Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung, South Korea

A multiplex real-time PCR assay and melting curve analysis for identifying 23 mycobacterial species was developed and evaluated using 77 reference strains and 369 clinical isolates. Concordant results were obtained for all 189 (100%) isolates of the *Mycobacterium tuberculosis* complex and 169 (93.9%) isolates of nontuberculous mycobacteria. Our results showed that this multiplex real-time PCR assay is an effective tool for the mycobacterial identification from cultures.



J. Clin. Microbiol. February 2012 vol. 50 no. 2 483-487



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

PCR/Meltcurve Analysis

- products of amplification reaction subjected to gradual temperature increase
- presence of dsDNA intercalating molecule (EvaGreen)
 - fluoresces when dsDNA present
- loss of fluorescence as DNA strands dissociates
- melting temp (T_m) defined as the temperature 50% dissociation



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

PCR/Meltcurve Analysis

- Targets:
 - MTBC (IS6110)
 - *M.avium* (16SrRNA)
 - *M. intracellulare* (16SrRNA)
- PCR and meltcurve analysis in the the same tube
- relatively cheap-
 - no need for primer/probes combos
 - single reaction
- easy setup/fast results



PCR/Meltcurve Timeline



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

Validation Study

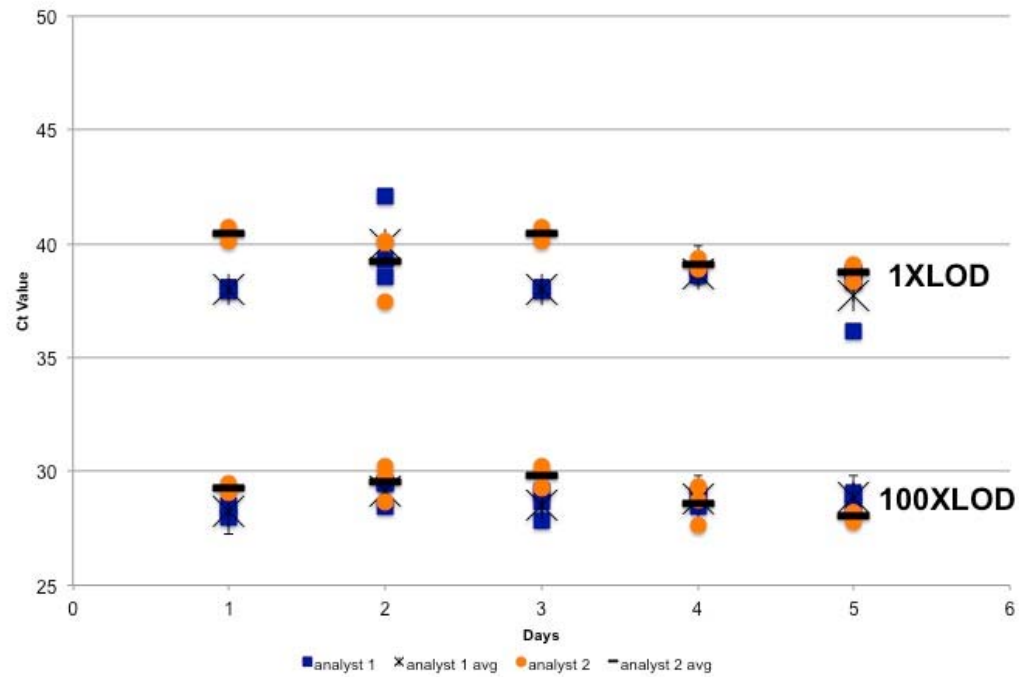
- sensitivity
- reproducibility
- specificity
- method to method



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

Reproducibility



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

Inclusivity

Spoligotype	C _t	T _m (°C)
776377704020771	26.19	85.36
775777606060731	27.31	85.34
777740607760771	28.43	85.42
000000000003771	27.99	85.35
777776777760601	32.38	85.49
776177607760771	26.55	85.44
477776770000000	29.26	85.37
477760017413071	29.89	85.43
777776777760771	29.98	85.63
577776770000000	29.51	85.34
676773777677600	33.26	85.56
770003400003071	27.42	85.57
676773777776000	32.28	85.56
000000007700171	27.99	85.50
676773777776000	32.31	85.3
67777477413771	24.57	85.39
640013377776000	30.98	85.49
67777477413771	26.21	85.39

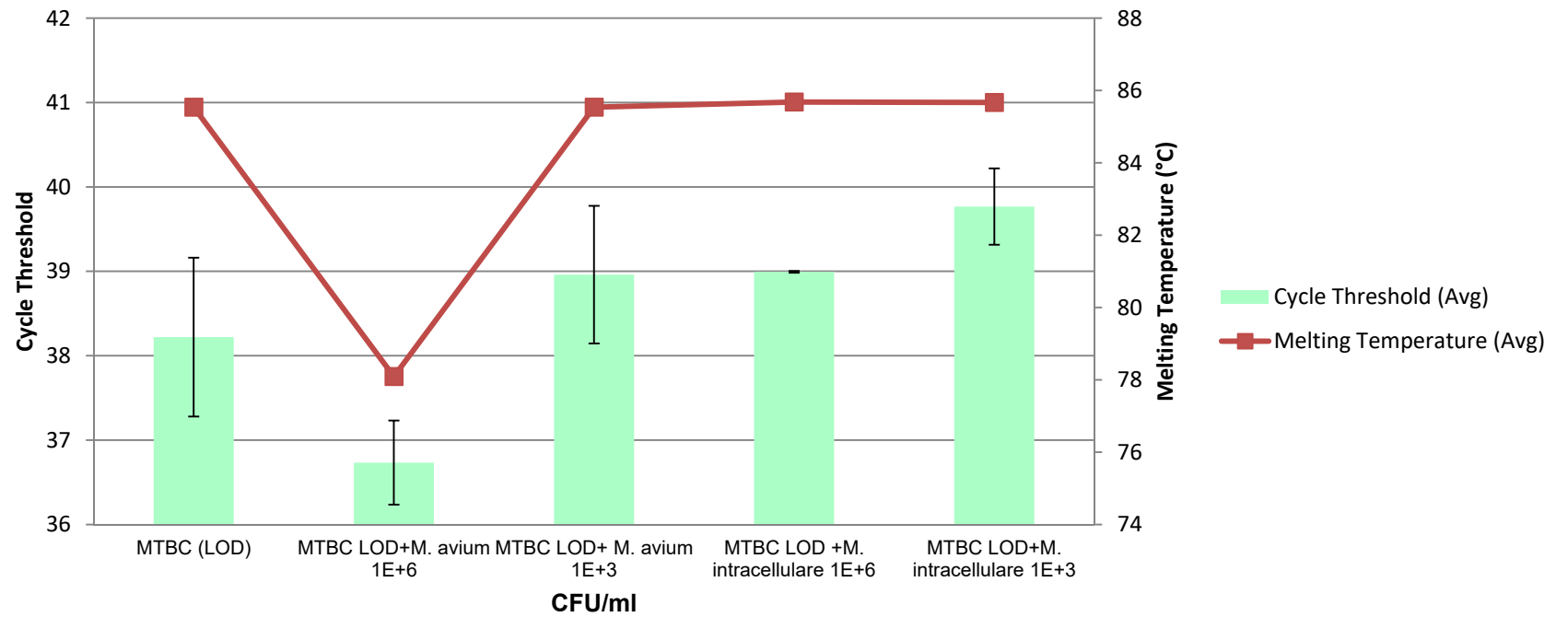
Cross reactivity and Interference

Organism (ATCC#)	w/o MTBC		w/MTBC	
	C _t	T _m (°C)	C _t	T _m (°C)
<i>Pseudomonas aeruginosa</i> (10145)	0	0	33.10	85.38
<i>Acinetobacter baumannii</i> (19606)	0	0	35.65	85.59
<i>Bordetella bronchiseptica</i> (10580)	0	73.96	34.28	85.54
<i>Haemophilus influenzae</i> (10211)	0	73.26	34.86	85.65
<i>Staphylococcus epidermidis</i> (12228)	0	0	34.13	85.50
<i>Bordetella parapertussis</i> (15989)	0	0	34.88	85.48
<i>Neisseria meningitidis</i> (13077)	0	0	34.85	85.35
<i>Streptococcus salivarius</i> (13419)	0	0	34.22	85.48
<i>Staphylococcus aureus</i> (25923)	0	0	33.31	85.32
<i>Candida albicans</i> (60193)	0	0	35.24	85.42
<i>Eikenella corrodens</i> (BAA 1152)	0	0	34.29	85.46
<i>Bordetella pertussis</i> (9340)	0	0	34.25	85.49
<i>Streptococcus pneumoniae</i> (6305)	49.6	73.54	35.21	85.45
<i>Corynebacterium diphtheriae</i> (13812)	0	74.05	34.52	85.53
<i>Klebsiella pneumoniae</i> (33495)	0	0	35.83	85.59
<i>Moraxella catarrhalis</i> (10973)	49.53	77.05	37.54	85.49
<i>Streptococcus pyogenes</i> (19615)	42.39	76.85	32.13	85.38
<i>Mycobacterium kansasii</i> (12478)	0	0	31.23	85.56
<i>Mycobacterium goodii</i> (14470)	0	0	31.91	85.67
<i>Mycobacterium scrofulaceum</i> (19981)	44.99	77.11	33.29	85.63
<i>Mycobacterium mucogenicum</i> (49650)	44.74	80.1	32.40	85.80
<i>Mycobacterium celatum</i> (51131)	0	0	30.64	85.68
<i>Mycobacterium chelonae</i> (35752)	0	0	33.38	85.73



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans



ARIZONA DEPARTMENT OF HEALTH SERVICES

Health and Wellness for all Arizonans

Method Comparison

MTBC Positive (Recovered) = 105

	MTBC Detected	MTBC Not Detected
HPLC	43	62
PCR/Meltcurve	103	2

MTBC Negative (Not Recovered)=164

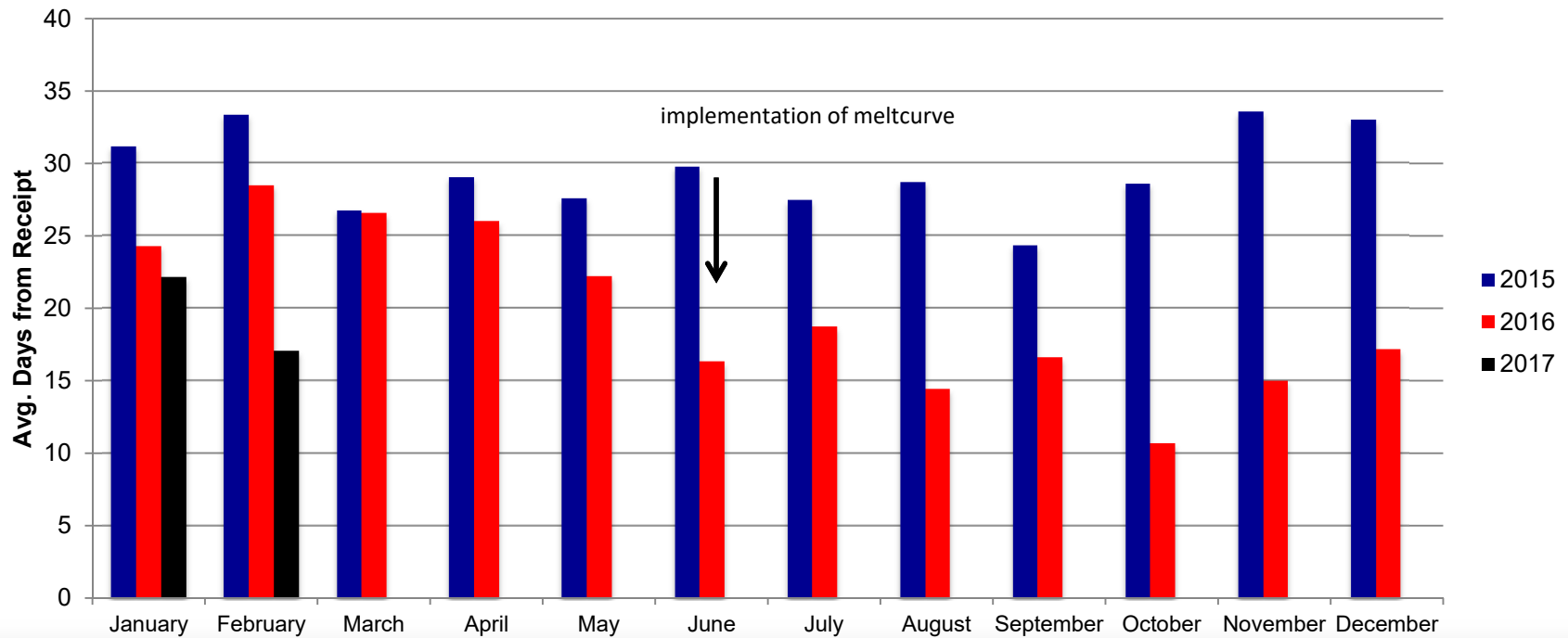
	MTBC Detected	MTBC Not Detected
HPLC	0	164
PCR/Meltcurve	0	164



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

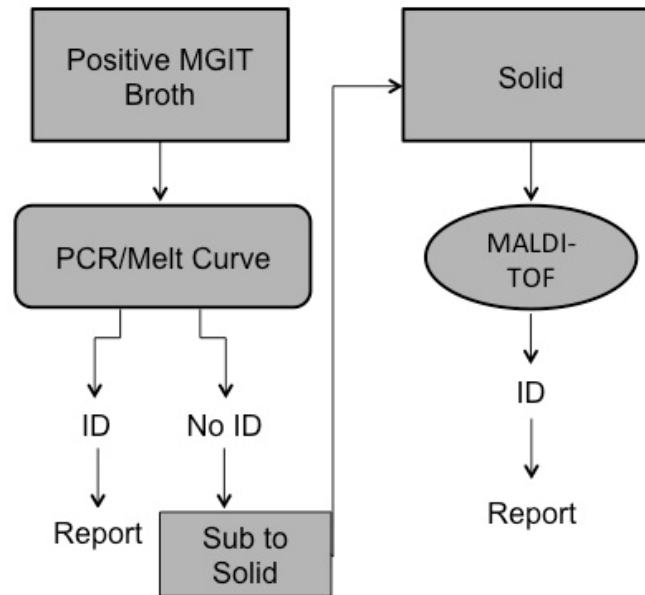
TAT Improvement



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans

IDENTIFICATION WORKFLOW:



Conclusion

- The PCR/meltcurve assay demonstrated specificity and reliably detected MTBC in MTBC positive MGIT broths
- Due to ease and simplicity, the PCR/meltcurve assay has replaced the HPLC for the identification of MTBC in positive MGIT broths at ASPHL, which has impacted identification turnaround times
- The multiplexed PCR assay is designed to detect MAC as well as MTBC, further investigation is needed to determine if MAC can be reliably identified from MGIT broths.
 - high levels of *M.avium* affects low level of MTBC detection



THANK YOU

Stacy White, PhD

Stacy.White@azdhs.gov | 602-542-1025

azhealth.gov

 @azdhs

 facebook.com/azdhs



ARIZONA DEPARTMENT
OF HEALTH SERVICES

Health and Wellness for all Arizonans