TB MOLECULAR DIAGNOSTIC TESTING AT WADSWORTH CENTER: PERFORMANCE EVALUATION AND POTENTIAL IMPACT ON PUBLIC HEALTH

Vincent Escuyer, Ph.D.
Wadsworth Center, New York State Department of Health

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PERFORMANCE EVALUATION OF MOLECULAR DIAGNOSTIC TESTS FOR TUBERCULOSIS

The Association of Public Health Laboratories (APHL), in cooperation with the U.S. Centers for Disease Control and Prevention (CDC) Division of Tuberculosis Elimination (DTBE), is seeking to award one-time funding to APHL member state and local public health laboratories for the purpose of evaluating the performance of molecular diagnostic tests for tuberculosis and increasing evidence-based knowledge regarding the most appropriate use of these assays in settings with both high and low burdens of tuberculosis. Funds may be used to support operational initiatives which aim to improve current molecular diagnostic services or seek to provide evidence to develop efficient and effective laboratory algorithms incorporating these tests into the overall laboratory system.

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TB MOLECULAR ASSAYS AT THE WADSWORTH CENTER

Real time PCR for MTBC and MAC DNA detection

Target: IS6110 (MTBC) and 16S-23S rRNA Internal Transcribed Spacer (ITS)

Primary specimens and isolates

Real Time PCR for species identification within MTB complex

Targets: RD1, RD4, RD9, RD12, ext-RD9

Primary specimens and isolates

PCR/Pyrosequencing for detection of mutations associated with drug resistance

Targets: *rpoB* (Rifampin), *katG* and *inhA* (Isoniazid)

WORKFLOW
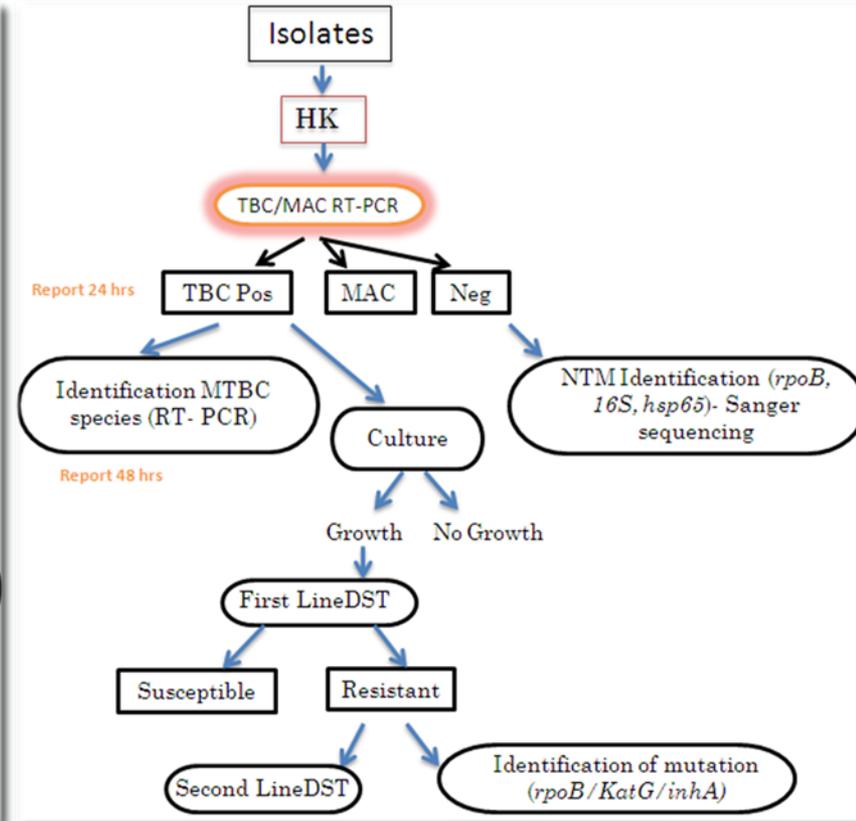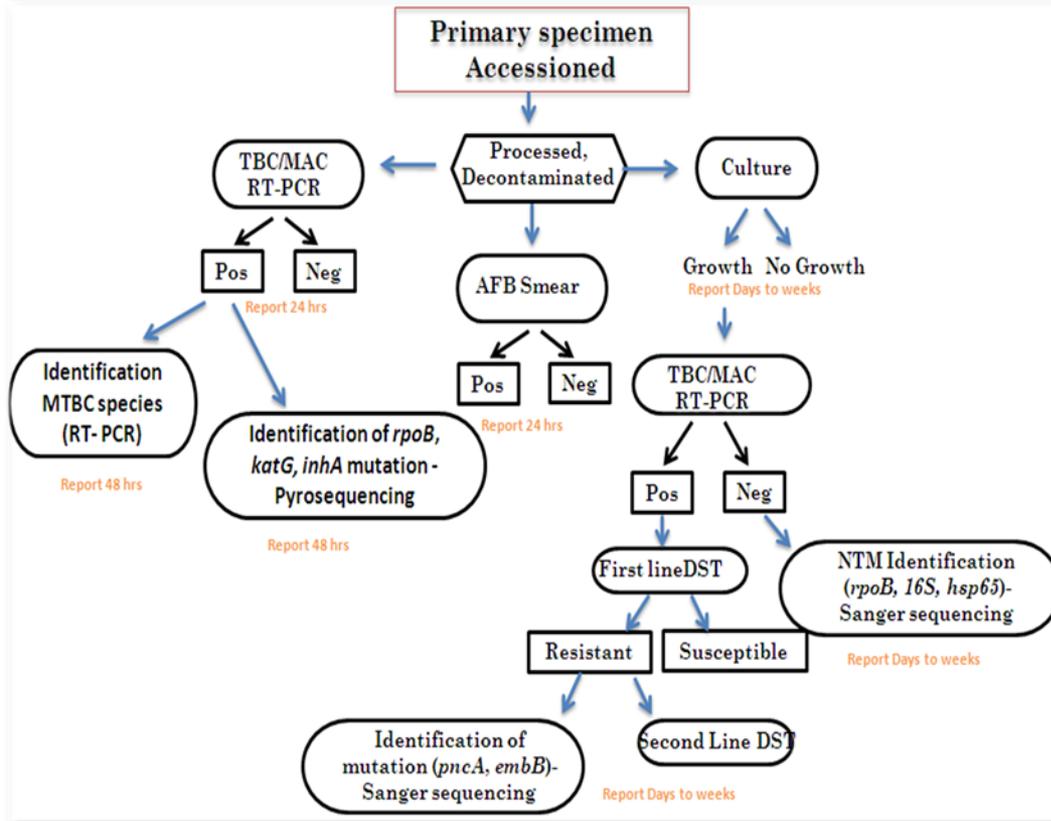
AIMS

1. Impact of NAAT use in smear negative patients.

2. Impact of early detection of *M. avium* complex DNA concurrently with *M. tuberculosis* complex

3. Correlation between MTBC real-time PCR results and smear readings

4. Impact of early *rpoB, katG* and *inhA* testing

1. IMPACT OF NAAT USE IN SMEAR NEGATIVE PATIENTS.

Culture	Real-time PCR		
	Pos	Neg	Inconclusive
Pos	27	8	0
Neg	16	452	4
Total (507)	43	460	4

Sensitivity	81.4%
Specificity	96.7%
PPV	68.6%
NPV	98.3%

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TREATMENT INFORMATION FOR 18 / 51 PATIENTS.

- ➢ **13 were empirically started on treatment before any specimen was sent to the laboratory.**

- ➢ **Remaining 5 patients were started on treatment solely based on the NAAT results, within an average of 24 hours upon receipt of the results.**

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CONCLUSIONS-AIM 1

➢ **51 TB cases (10%) detected out of the 507 smear negative patients tested**

➢ **Specificity of our TBC Real-time PCR on AFB negative specimens was very high, signifying that this assay can be used to "rule in" TB on this type of specimens.**

➢ **Sensitivity, although higher than the values previously published for other NAAT's is still too low to use our assay alone to "rule out" TB despite an excellent Negative Predictive Value**

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2. IMPACT OF EARLY DETECTION OF *M. AVIUM* COMPLEX

2.a. Number or specimens PCR-positive for MAC

Smear		Culture	
	Number of MAC Positive Specimens by real-time PCR	Number of PCR Positive Specimens in MGIT	Number of PCR Positive Specimens on 7H10 medium
None	5	4	4
Rare (1+)	19	19	19
Few (2+)	22	22	19
Moderate (3+)	15	15	14
Numerous (4+)	16	16	13
Total	77	76 (98.7%)	69 (89.6%)

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2.b. Two examples of early treatment removal

Specimen #	Date Received	Date PCR Positive Result	Date Positive Culture (MGIT)	Date positive Culture (7H10)	Treatment Start Date	Treatment removal Date
12-28954	9/10/12	9/11/12	9/14/12	9/19/12	9/7/12	9/14/12
12-29373	9/13/12	9/14/12	9/15/12	9/19/12	9/12/12	9/18/12
12-29960	9/18/12	9/19/12	9/20/12	10/1/12	8/31/12	10/1/12
12-30348	9/20/12	9/21/12	9/25/12	10/3/12	9/12/12	11/20/12
12-31712	10/1/12	10/2/12	10/5/12	10/19/12	9/26/12	11/2/12
12-40237	12/18/12	12/19/12	12/25/12	Negative	12/18/12	**12/21/12**
13-06640	3/11/13	3/12/13	3/14/13	3/19/13	3/8/13	**3/13/13**
13-09113	4/4/13	4/5/13	4/5/13	4/23/13	3/31/13	4/19/13

2c. Economical impact assessment for the laboratory

	MAC PCR testing only	MAC PCR and Culture	MTBC PCR, Culture and DST complete testing
Cost/ specimen	$4.08	$12.27	$110.46
Estimated total cost for average of 200 MAC positive specimens	$12,816.00	$18,454.00	For an estimated 10/ year- $2304.60
Savings with MTBC/MAC real-time PCR testing only	NA	$5638.00	$2304.60
Total anticipated savings		$7942.60	

CONCLUSIONS-AIM 2

➤ **A total of 77 specimens were evaluated for this study. All AFB smear positive/ MAC- PCR positive specimens were also culture positive. Additionally 4 out of 5 AFB smear-negative/MAC-PCR positive specimens were also culture positive.**

> ➤ **Elimination of any type of culture and additional confirmation work for all specimens initially found positive for MAC by real-time PCR?**

➤ **Need to reach out to the health care community to educate and raise awareness**

➤ **The utilization of the MTBC/MAC real-time PCR with no additional testing on MAC positive specimens and additional efforts to prevent MTBC testing on MTBC/MAC mixed specimens would potentially save an average of ~$8000.00.**

➤ **5 out of 367 isolates received during the contract period, were a mixed MAC/MTBC culture, as determined by Real Time PCR. Prevented the initiation of MTBC testing until a pure culture was obtained**

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Microscopy value	Quantity	IS6110				extRD9				extRD9 (1:5 dilution)			
		CT value range	Average CT value	Count/# of specimens	Median	CT value range	CT value	Count/# of specimens	Median	CT value range	CT value	Count/# of specimens	Median
Numerous	(4+) >9 AFB/field	19.79-30.07	24.83	52/26	25.22	22.56-33.72	26.79	26/26	27.19	24.16-33.75	28.76	26/26	29
Moderate	(3+) 1-9 AFB/field	24.89-31.44	26.91	28/14	26.65	25.65-33.59	29.9	14/14	29.9	27.4-34.7	31.18	14/14	31.17
Few	(2+) 1-9 AFB/10 fields	24.65-34.50	30.91	22/11	31.4	29.46-36.16	32.4	11/11	32.16	28.22-38.33	33.69	11/11	33.39
Rare	(1+) 1-9 AFB/100 fields	28.05-41.16	32.36	20/10	31.47	31.86-41.16	35.06	10/10	34.7	33.46-41.07	36.34	9/10	36.35
None	No AFB seen	33.31-38.35	36.14	8/4	36.46	37.69-38.53	38.11	2/4	38.11	38.5	38.5	1/4	38.5

Can we predict the quantity?

Sample #	IS6110 average value	extRD9 value	extRD9 1:5 value	predicted quantity	actual quantity	
13-11849	26.95	28.3	29.13	Numerous	Numerous	
13-12725	37.46	undet	undet	None	None	14 right
13-13376	33.89	36.16	38.33	Rare	Few	13 wrong
13-12048	31.22	32.3	34.59	Few	Few	
13-11252	20.83	22.68	23.04	Numerous	Numerous	
13-10998	23.17	24.86	25.98	Numerous	Numerous	
13-10283	28.36	31.65	33.46	Few	Moderate	
13-09545	29.44	31.63	33.34	Few	Moderate	
13-09231	34.6	38.87	39.99	None	None	
13-09059	39.82	41.16	undet	None	Rare	
13-13474	20.26	23.19	24.16	Numerous	Numerous	
13-13476	33.15	32.34	33.9	Few	Few	
13-13649	33.06	35.17	38.08	Rare	Few	
13-13949	38.83	undet	undet	None	None	
13-14547	21.47	25.24	27.06	Numerous	Numerous	
13-14548	33.99	40.65	39.18	Rare	Few	
13-14782	31.29	33.89	35.93	Rare	None	
13-14861	36.64	undet	undet	None	Rare	
13-14863	31.05	34.83	36.97	Rare	Rare	
13-15085	33.64	39.85	39.12	Rare	Rare	
13-15086	33.62	38.81	40.26	Rare	None	
13-15250	29.03	32.45	34.45	Few	Rare	
13-15253	37.37	39.79	undet	None	Rare	
13-15254	35.16	undet	38.91	None	Rare	
13-15307	38.28	undet	undet	None	None	
13-15499	35.12	33.79	35.38	Rare	None	
13-15502	31.04	31.93	33.92	Few	Few	

CONCLUSIONS-AIM 3

➢ **IS6110 real time PCR: some correlation between Ct values and smear status**

➢ **Clear improvement with the use of a mono-copy target (ext-RD9).**

➢ **Correlation was 52%. Predictive value is still currently too low to consider replacing acid fast staining with real-time PCR.**

➢ **Human factor for smear reading: most of the discrepancies were found with specimens with low concentrations of organisms (Smear – or 1+).**

➢ **Further testing needs to be performed: Increased number of specimens and titration curve with control specimens carrying a defined concentration of bacilli. compare smears results to numbers of organism as opposed to Ct values.**

4. IMPACT OF EARLY *rpoB, katG* AND *inhA* PYROSEQUENCING

4a. Early Detection of Rifampin resistance (retrospective)
n=10

Pyrosequencing			Conventional Drug Susceptibility Testing					
			Liquid			Solid (2nd line)		
No mutations	Mutations	Average TAT (days)	Susc	Res	Average TAT (days)	Susc	Res	Average TAT (days)
5	5	7.2	4	6	23.1	5	5	48.3

	Pyrosequencing	Liquid	Solid
Specimen #	rpoB result	RIF result	RIF 1.0 result
11-33641	WT	R	S
11-36868	Asp516Tyr/Gln517Pro	R	R
12-01956	Ser531Leu	R	R
12-06405	Ser531Leu	R	R
12-08928	His526Tyr	R	R
12-21425	His526Tyr	R	R

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4a. Early Detection of Rifampin resistance (prospective)
n=50

Pyrosequencing			Conventional Drug Susceptibility Testing					
			RIF Liquid			RIF Solid (2nd line) (if applicable)		
No mutations	*Mutations	Average TAT (days)	Susc	Res	Average TAT (days)	Susc	Res	Average TAT (days)
49	1	7.0	49	1	24.5	N/A	1	49.3

* Mutations detected: Leu533Pro

4b. Early Detection of Isoniazid resistance (retrospective)

Conventional Drug Susceptibility Testing (n=10)									
Liquid					Solid (2nd line)				
INH 0.1		INH 0.4 (if applicable)		Average TAT (days)	INH 0.2		INH 1.0		Average TAT (days)
Susc	Res	Susc	Res		Susc	Res	Susc	Res	
1	9*	3	6	23.1	1	9	4	6	48.3

	Pyrosequencing		Liquid		Solid	
Specimen #	katG result	inhA result	INH 0.1 result	INH 0.4 result	INH 0.2 result	INH 1.0 result
11-33641	WT	C(-15)T	R	S	R	S
11-36868	Ser315Thr	ND	R	R	R	R
12-01956	Ser315Thr	ND	R	R	R	R
12-04677	WT	C(-15)T	R	S	R	S
12-06009	WT	C(-15)T	R	S	R	S
12-06405	Ser315Thr	ND	R	R	R	R
12-08928	Ser315Thr	ND	R	R	R	R
12-21425	Ser315Thr	WT	R	R	R	R
12-22515	Ser315Thr	ND	R	R	R	R

4b. Early Detection of Isoniazid resistance (prospective)

Pyrosequencing (n=50)				
katG gene		*inhA* gene		Average TAT (days)
No mutations	Mutation (Ser315Thr)	No mutations	*Mutations	
47	3	47	3	7.0

*Mutations detected: C(-15)T, T(-8)A

Conventional Drug Susceptibility Testing (n=50)									
Liquid					Solid (2nd line)				
INH 0.1		INH 0.4 (if applicable)		Average TAT (days)	INH 0.2		INH 1.0		Average TAT (days)
Susc	Res	Susc	Res		Susc	Res	Susc	Res	
40	10*	7	3	24.5	4	6	7	3	49.3

4b. Early Detection of Isoniazid resistance (prospective)

Specimen #	Pyrosequencing		Liquid		Solid	
	katG result	*inhA* result	INH 0.1 result	INH 0.4 result	INH 0.2 result	INH 1.0 result
12-28162	WT	WT	R	S	R	S
12-30443	WT	WT	R	S	S	S
12-32708	WT	WT	R	S	S	S
12-35358	WT	C(-15)T	R	S	R	S
12-36707	Ser315Thr	WT	R	R	R	R
12-37753	Ser315Thr	T(-8)A	R	R	R	R
12-41219	WT	C(-15)T	R	S	R	S
13-02707	WT	WT	R	S	S	S
13-05628	WT	WT	R	S	S	S
13-07153	Ser315Thr	WT	R	R	R	R

CONCLUSIONS-AIM 4

➢ **Presence of mutations in *rpoB katG* and *inhA* correlated at 100% in this study with resistance to RIF and/or INH.**

➢ **Absence of mutation correlated at 98% with susceptibility to Rifampin**

➢ **Absence of mutation in inhA and katG correlated at 93% with susceptibility to Isoniazid**

➢ **Conventional DST is still the gold standard as knowledge improves that will lead to next generation molecular DST assays**

➢ **TAT for molecular results is an average of 7 days vs. 23-24 days for conventional DST.**

➢ **Can be shortened. Our collaboration with Rhode- Island PHL has demonstrated that TAT for molecular DST could be decreased to 48-72 hours.**

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Kara Mitchell

Ann Trahn

NEW YORK STATE TB CONTROL BUREAU

Cheryl Kearns

Colleen Flynn

Margaret Oxtoby

NEW YORK CITY TB CONTROL BUREAU

David Quiros

Felicia Dworkin