

# A 5 Minute Extraction Protocol for MSMS: STAT Reporting of Medical Emergency MSMS Profiles

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# Symptomatic Before Results Reported

## MI Experienced MSMS Diseases

- CIT I
- PA/MUT/Cbl
- MCD
- LCHAD
- MSUD

## Literature/Personal Communication Diseases

- MCAD
- VLCAD

## Michigan Initiatives

- Courier Pickup (2007)
- Courier Delivery before 7:00 a.m.
- Saturday laboratory operations (2008)
- Courier Pickup - Sunday p.m. (2012)
- 24-30 hour specimen collection age (2014)
  - ❖ 2014: 98.1% collected before 36 hr.
- Evaluating transit time using hospital-specific cutoffs (2014)

P17

- 2014: 96.1% specimens received within 3 days of collection



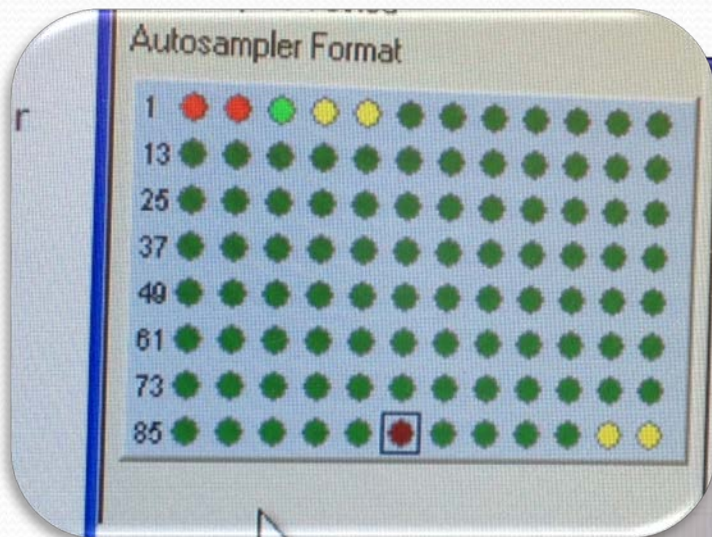
## Michigan Initiatives



- P.M. interpretation of MSMS results
- Early MSMS plate processing
- STAT Confirmation/Reporting of Medical Emergency MSMS Profiles



# P.M. Plate Review



Report Scheme: Neolbase\_AAAC\_HD.nrs

Target Mass#1	Target Mass#2	Result	Calculated Conc	High Conc	Low Conc
90.0	94.0		513	1000	0.00
94.0	0.0		1.35e+5	1.00e+8	4.58e+4
175.1	180.1		13.33	68.00	0.00
180.1	0.0		3.62e+5	1.00e+8	2.00e+5
291.1	180.1		0.14	0.70	0.00
176.1	175.1	Ct high	517	45.00	5.25
178.1	0.0		6.27e+4	1.00e+8	2.79e+4
76.0	78.0		633	3000	0.00
78.0	0.0		6.12e+4	1.00e+8	4.25e+4
132.1	135.1		108	225	0.00
135.1	0.0		1.09e+6	1.00e+8	3.13e+5
150.1	153.1		47.17	56.00	11.00
✓ NB_MET IS_int	153.1	0.0	2.44e+5	1.00e+8	6.39e+4
✓ NB_ORN	133.1	139.1	55.62	1000	0.00
✓ NB_ORN IS_int	139.1	0.0	1.59e+5	1.00e+8	7.13e+4
✓ NB_PHE	166.1	172.1	77.90	120	0.00
✓ NB_PHE IS_int	172.1	0.0	7.33e+5	1.00e+8	2.94e+5
✓ NB_PRO	116.1	121.1	370	1000	0.00
✓ NB_PRO IS_int	121.1	0.0	2.16e+5	1.00e+8	9.04e+4
✓ NB_SA	155.1	160.1	0.56	1.40	0.00
✓ NB_SA IS_int	160.1	0.0	5.14e+5	1.00e+8	1.64e+5
✓ NB_TYR	182.1	188.1	189	450	0.00
✓ NB_TYR IS_int	188.1	0.0	9.65e+4	1.00e+8	5.97e+4
✓ NB_VAL	118.1	126.1	109	340	0.00

(Time: 0.37) Combine (6:16)

1. MRM of 25 Channels EB+ 1.1e+005



## Why Confirmation x2 Analysis?

- Verify
  - ❖ True Elevations - Not contamination

# Analytes affected by contaminants

- Citruline

Citrullus vulgaris (watermelon)

Watermelon rind: Cit = 2200  $\mu$ M, Arg = 302  $\mu$ M





## Analytes affected by contaminants

- C8, C6, C10, C10:1, C6DC\_C7OH
  - Anti-static agent LDEA (used in plastic bags etc.)
    - Non-derivatized: C8
    - Derivatized: C4

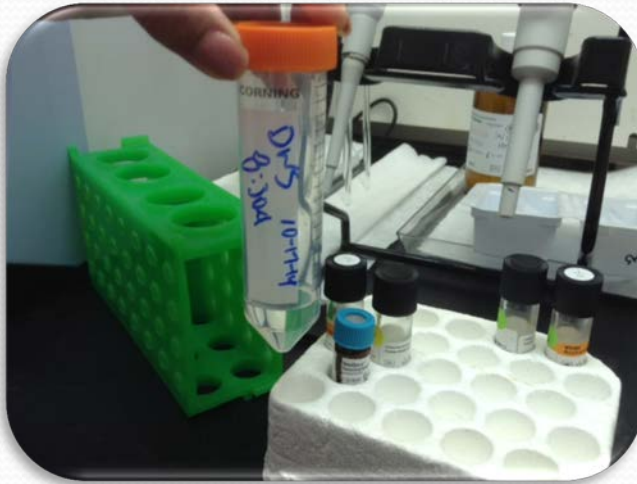


## Confirmation x2 Analysis

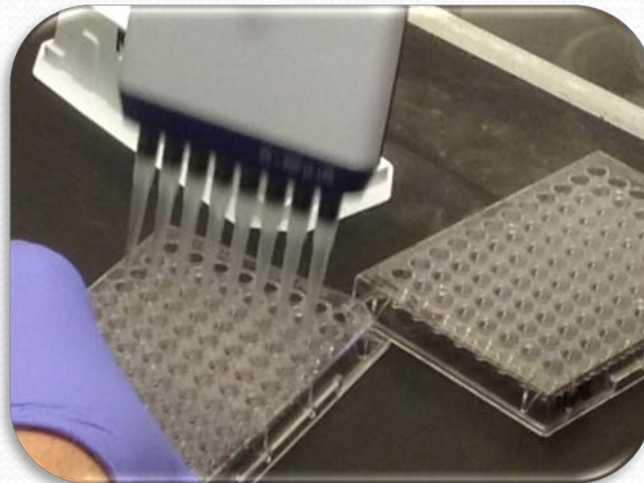
- Verify
  - ❖ True Elevations - Not contamination
  - ❖ Specimen ID

## Routine

## STAT



- Make Daily Working Solution (15 minutes) NA



- Elute Plate (< 5 minutes) 5 minutes

## Routine

## STAT



- Extraction (45 minutes)

5 minutes (?)



- Transfer (< 5 minutes)

5 minutes

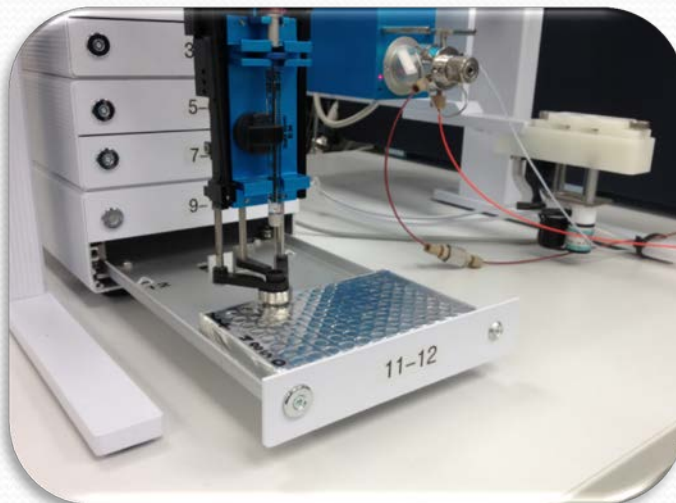
## Routine

## STAT



- Incubate - SUAC derivatization (2 hours)

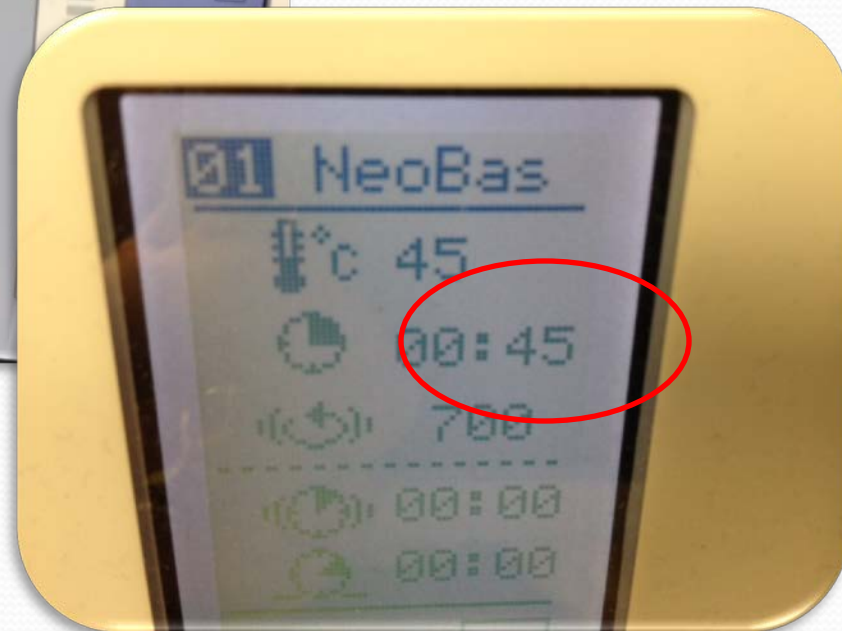
NA



- MSMS analysis (2 minutes)

4 minutes

**Rate Limiting Step = 45 minutes Incubation  
Is 45 minutes needed?**



# Extraction Time 5 vs. 45 minutes Citruline ( $\mu\text{M}$ )

	HI Control	Cutoff $\geq 50 \mu\text{M}$
Mean	45 minutes	296
Mean	5 minutes	230
	% Extraction	78

# EP Evaluator®

MDCH -- Newborn Screening

Cit

Instrument Marge/Homer  
Sample Name High Control

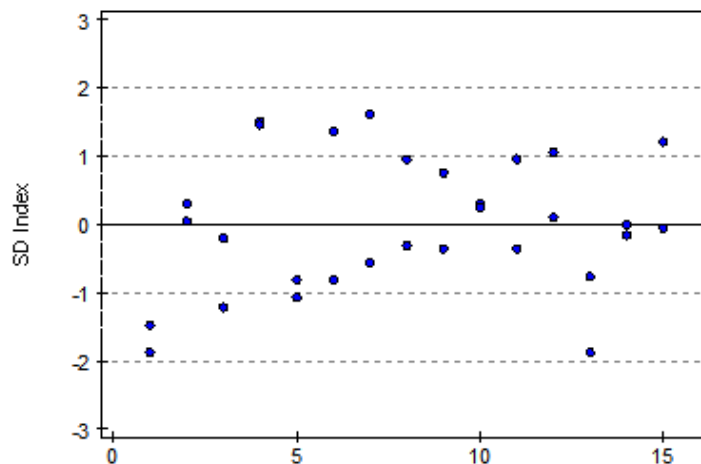
## Alternate Precision

### Precision Estimate

User's Concentration: 231.1

	Within Run	Between Run	Between Day	Total	Medically Required	Verification Value (95%)	Pass/Fail
Std. Dev	15.8	0.0	11.9	19.8	20.8	25.5	Pass
% CV	6.8	0.0	5.1	8.5	9.0		
df	15	--	--	25			

### Precision Plot



### Outlier Rejection Criteria

SD	15.3 (calculated)
Multiplier	5.5
Max difference between duplicates	84.15

### Preliminary estimate of precision

Mean	215.9	CV	7.1%
SD	15.3	N	12

Results				
204	202	213	218	222
227	211	210	209	202
258	215			



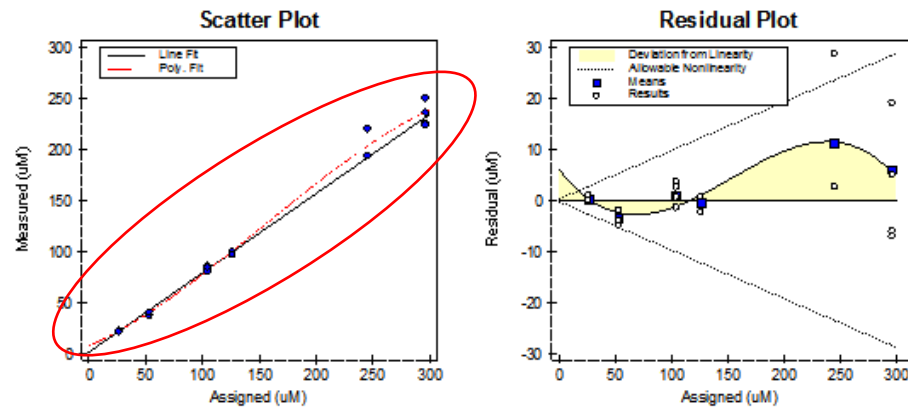
# EP Evaluator®

MDCH -- Newborn Screening

Cit

Instrument Marge/Homer

## CLSI EP6 Linearity



**Data IS linear within allowable nonlinearity of 12.25%**  
 Fit of polynomial to data is acceptable ( $p=0.052$ )  
 Power of test to detect nonlinearity is acceptable (ratio=5.4)

### Statistical Analysis

Specimen	Assigned Value	Mean	Poly. Fit	Line Fit	Deviation from Linearity	Deviation Percent
CAA101421	26	22.3	22.3	21.9	0.3	1.5
CAA201422	53	39.3	40.4	42.8	-2.4	-5.6
CLK628139	104.1	83.2	81.1	82.3	-1.2	-1.5
CAA301423	126	99.0	100.2	99.3	1.0	1.0
CAA401424	245	202.7	202.7	191.3	11.4	6.0
CHK628139	296.2	237.0	236.5	230.9	5.6	2.4

\*\*\*: Absolute value > 99% x: Excluded o: Exceeds allowable nonlinearity

## True Positive Comparison - CITI

	Cit (cutoff $\geq 50\mu\text{M}$ )
5 minutes	341
45 minutes	537
% Extraction	64

## Extraction Time: 5 vs. 45 minutes – All Analytes

Analyte	% Extraction	
	HI Control	Low Control
C0	83	80
C10	88	84
C12	91	86
C14	82	77
C16	76	71
C18	70	65
C2	93	90
C3	95	93
C4	94	91
C5	89	86
C5DC_C6OH	89	84
C6	91	89
C8	89	85
Ala	84	80
Cit	78	75
Leu	85	82
Met	86	84
Phe	86	83
SA	18	24
Tyr	78	75
Val	86	84

## True Positive Comparison - MSUD

	Leu (cutoff $\geq 225$ )	L/P (cutoff $\geq 4.5$ )
5 minutes	465	11.9
45 minutes	648	11.5
% Extraction	72	104

## True Positive Comparison – LCHAD/TFP, GA II, IVA

LCHAD/TFP	C16-OH (0.09)	C18-OH (0.04)	C18:1-OH (0.07)
5 minutes	0.23	0.23	0.27
45 minutes	0.27	0.27	0.33
% Extraction	85	83	80

GA II	C4 (1.40)	C5 (0.60)
5 minutes	1.8	1.06
45 minutes	2.8	1.64
% Extraction	64	65

IVA	C5 (0.60)
5 minutes	12.4
45 minutes	13.5
% Extraction	91.5

## True Positive Comparison – VLCAD, CPT IA, CACT/CPTII

VLCAD	C14:1 (0.65)
5 minutes	1.5
45 minutes	2.2
% Extraction	68

CPT IA	C0 (80)	C0/(C16+C18) (50)
5 minutes	108	290
45 minutes	139	220
% Extraction	78	132

CACT/CPTII	C16 (7.40)
5 minutes	9.3
45 minutes	14.2
% Extraction	65

## True Positive Comparison – MCAD, MMA

MCAD	C8 (0.65)
5 minutes	16.6
45 minutes	26.1
% Extraction	64

MMA	C3 (8.0)	C3/C2 (0.35)
5 minutes	11.1	1.3
45 minutes	14.3	1.3
% Extraction	77.3	98.2

## True Positive Comparison – GAI, MCD, BKT/2M3HBA

GAI	C5DC_C6OH (0.35)
5 minutes	4.5
45 minutes	6.3
% Extraction	71.6

MCD	C3 (6.0)	C4DC_C5OH (1.5)
5 minutes	9.7	2.3
45 minutes	11.5	2.8
% Extraction	84.7	81.4

BKT/2M3HBA	C5:1 (0.1)
5 minutes	0.22
45 minutes	0.26
% Extraction	84.6



## True Positive Comparison - TYRI

TYRI	SA (1.4)
5 minutes + 0 incubation	0.65
45 minutes + 2hr incubation	6.4
% Extraction	10

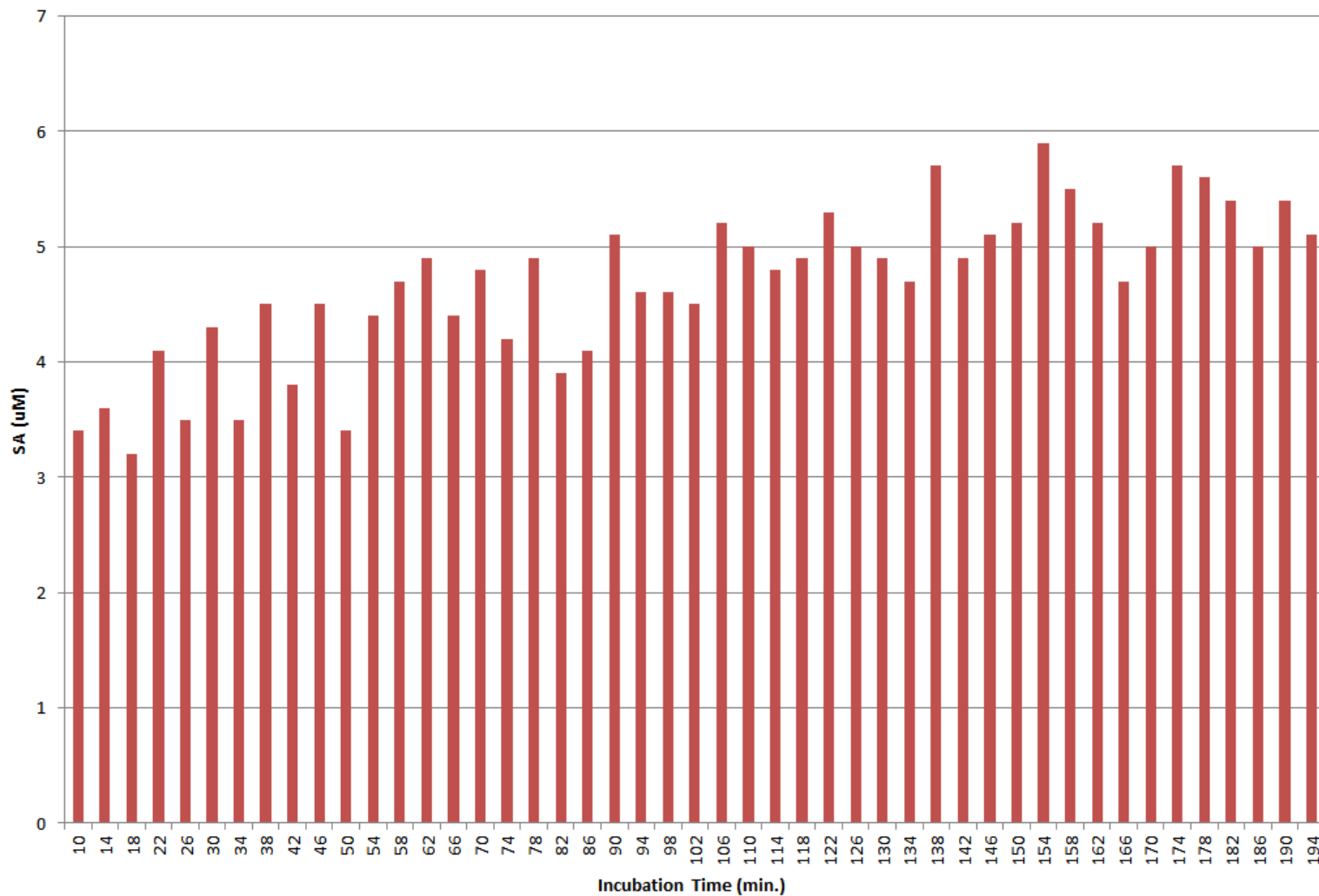
## Conclusions

- An incubation time of only 5 minutes allows for sufficient extraction efficiency for Disease Profile identification.
  - Except TYR I
- Medical Emergency MSMS Profiles can be identified and reported on the day of accession.

## Future

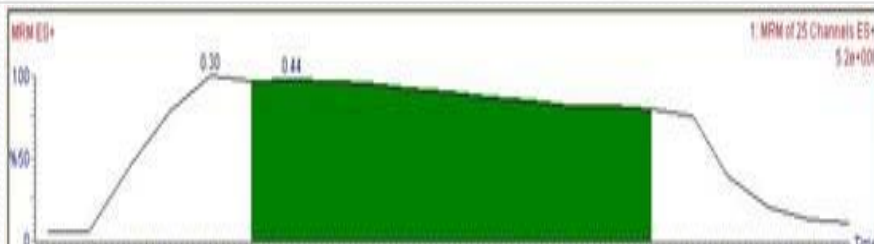
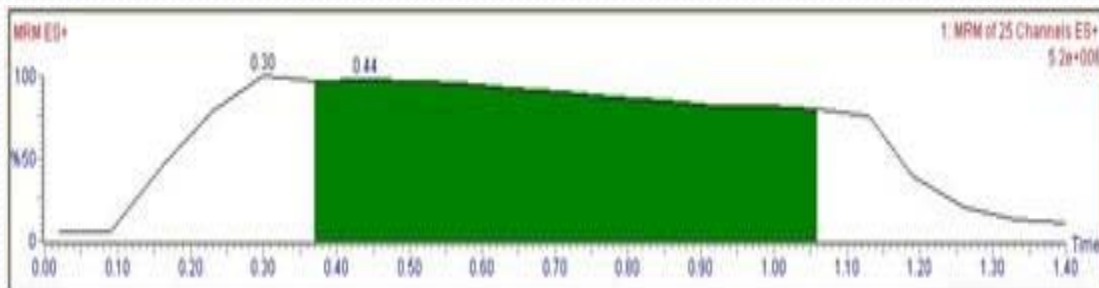
- Reduce the SUAC incubation time?
  - ❖ 2 hours = 120 samples

### [SUAC] vs Incubation Time - Low Control



## Future

- Reduce the SUAC incubation time?
  - ❖ 2 hours = 120 samples
- Increase the MSMS throughput?
  - ❖ 2 minutes → 1.5 minutes
  - ❖ 300 samples/workday → 400 samples/workday



# Acknowledgements

Michigan Department of Community Health  
Newborn Screening Team

- Hospitals
- Laboratory
- Follow-up
  - Clinic