



Median Normalization of Newborn Screening Analyte Data to Improve Screening Performance

T Henry¹, C Wolf², M McCann², and S Berberich¹

¹Iowa Newborn Screening Program

²Minnesota Newborn Screening Program

APHL Newborn Screening and Genetic Testing Symposium

New Orleans, LA

September 11, 2017



m DEPARTMENT OF HEALTH





Median Normalization

- Inherent variability in screening programs/labs (ethnicity, reagents, instruments, etc) makes it difficult to compare data across laboratories to improve screen performance



Median Normalization

- Maternal/Prenatal Screening has successfully utilized median normalization, Multiples of the Median (MoM), to standardize analyte data among testing laboratories
- Median normalization:
 - May provide a method for standardization of NBS data
 - May provide a means to apply a uniform cut-off



Project Overview

- Collaborative effort between the Minnesota and Iowa Newborn Screening Programs
- Analyte data before and after median normalization was compared for each state program
- Specimens ordered by date and time received by the laboratory
- Median for each analyte was calculated using a 3000 specimen window; raw analyte value divided by median
- Minnesota dataset
 - 7 months of data (Jan to Jul 2016)
 - total n: 42,176
- Iowa dataset
 - 12 months of data (Jan to Dec 2016)
 - total n: 38,888



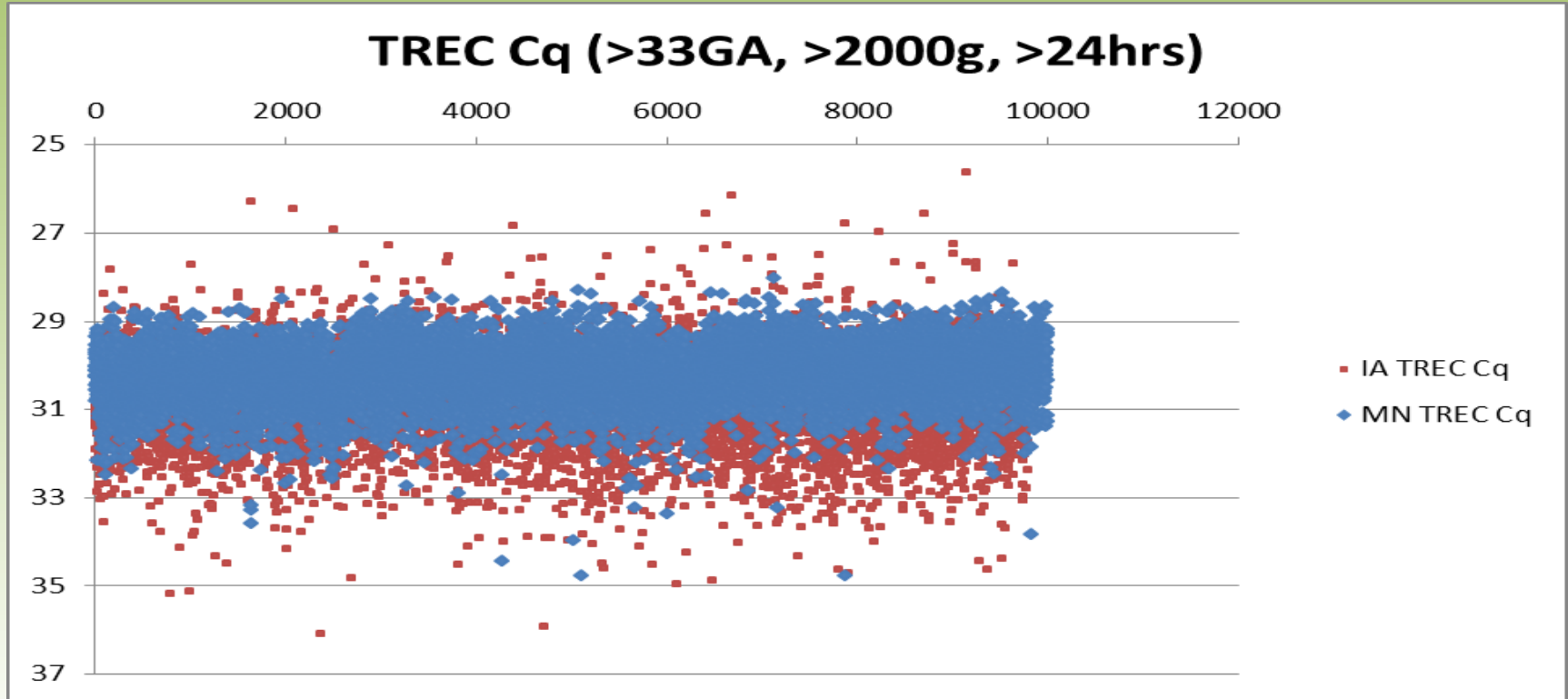
Pilot Study

- Disorders/Analytes
 1. Severe Combined Immunodeficiency (SCID)/
T-cell Receptor Excision Circles (TREC)
 2. Congenital Hypothyroidism (CH)/
Thyroid Stimulating Hormone (TSH)
 3. Cystic Fibrosis (CF)/
Immunoreactive Trypsinogen (IRT)

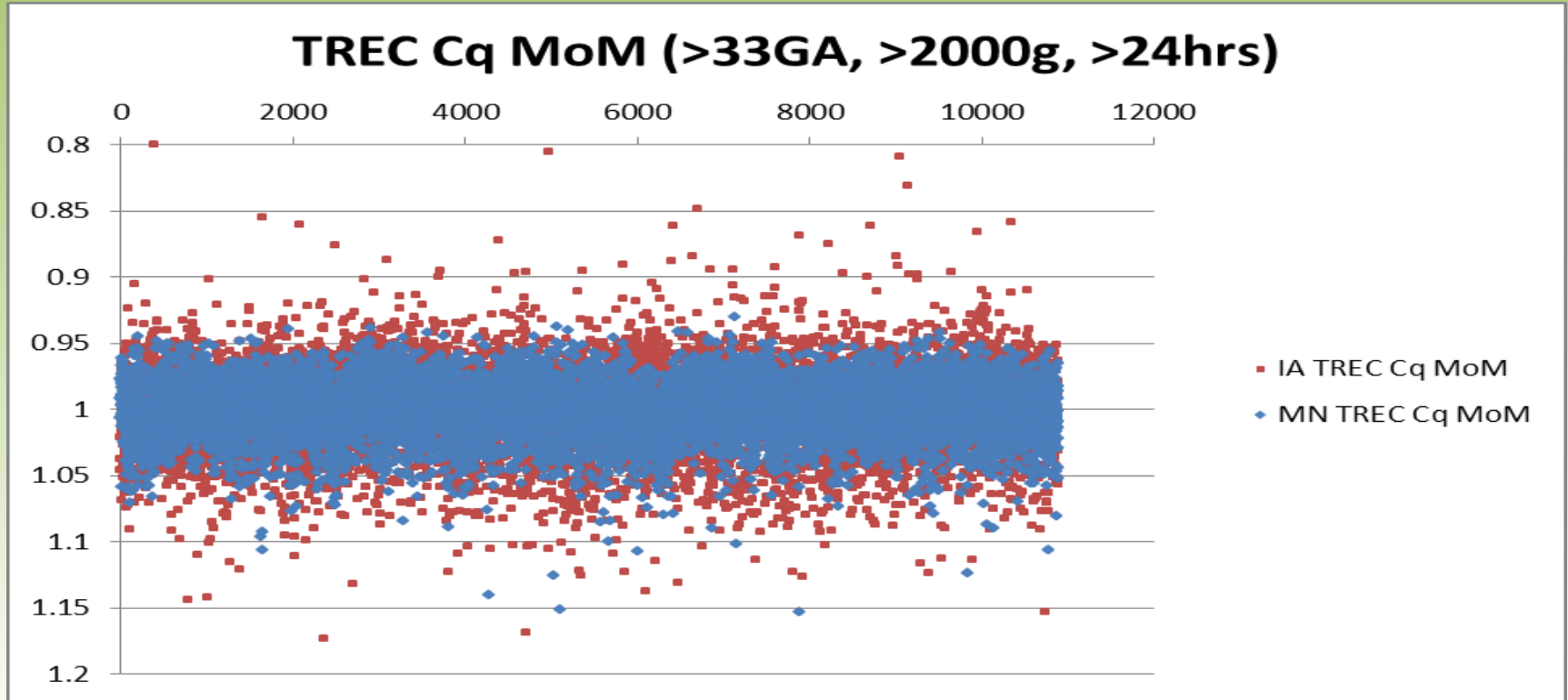
SCID/TREC

- Severe Combined Immunodeficiency/T-cell Receptor Excision Circles
 - MN: DNA extraction assay, 384 well format, ViiA7 real time PCR instrument
 - MN cut-off: 1.065 and 1.100 TREC Cq MoM (daily median)
 - IA logistics – *in situ* assay, 96 well format, ViiA7 real time PCR instrument
 - IA cut-off: 1.100, 1.132, and 1.151 TREC Cq MoM (population median)
- Data analysis
 - Exclude GA < 34 wks, < 2000g birthweight, < 24 hrs collection post-delivery

SCID/TREC



SCID/TREC





SCID/TREC

- Statistical analysis of TREC Cq values:

TREC Cq

Column B	IA TREC Cq
vs.	vs.
Column A	MN TREC Cq
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	1180911332 , 1745634434
Mann-Whitney U	422223751
Difference between medians	
Median of column A	30.14, n=38953
Median of column B	30.76, n=37540
Difference: Actual	0.617

TREC Cq MoM

Column D	IA TREC Cq MoM
vs.	vs.
Column C	MN TREC Cq MoM
Mann Whitney test	
P value	0.4282
Exact or approximate P value?	Approximate
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column C,D	1492488370 , 1434057395
Mann-Whitney U	728962267
Difference between medians	
Median of column C	0.999, n=38953
Median of column D	0.9993, n=37540
Difference: Actual	0.0002795

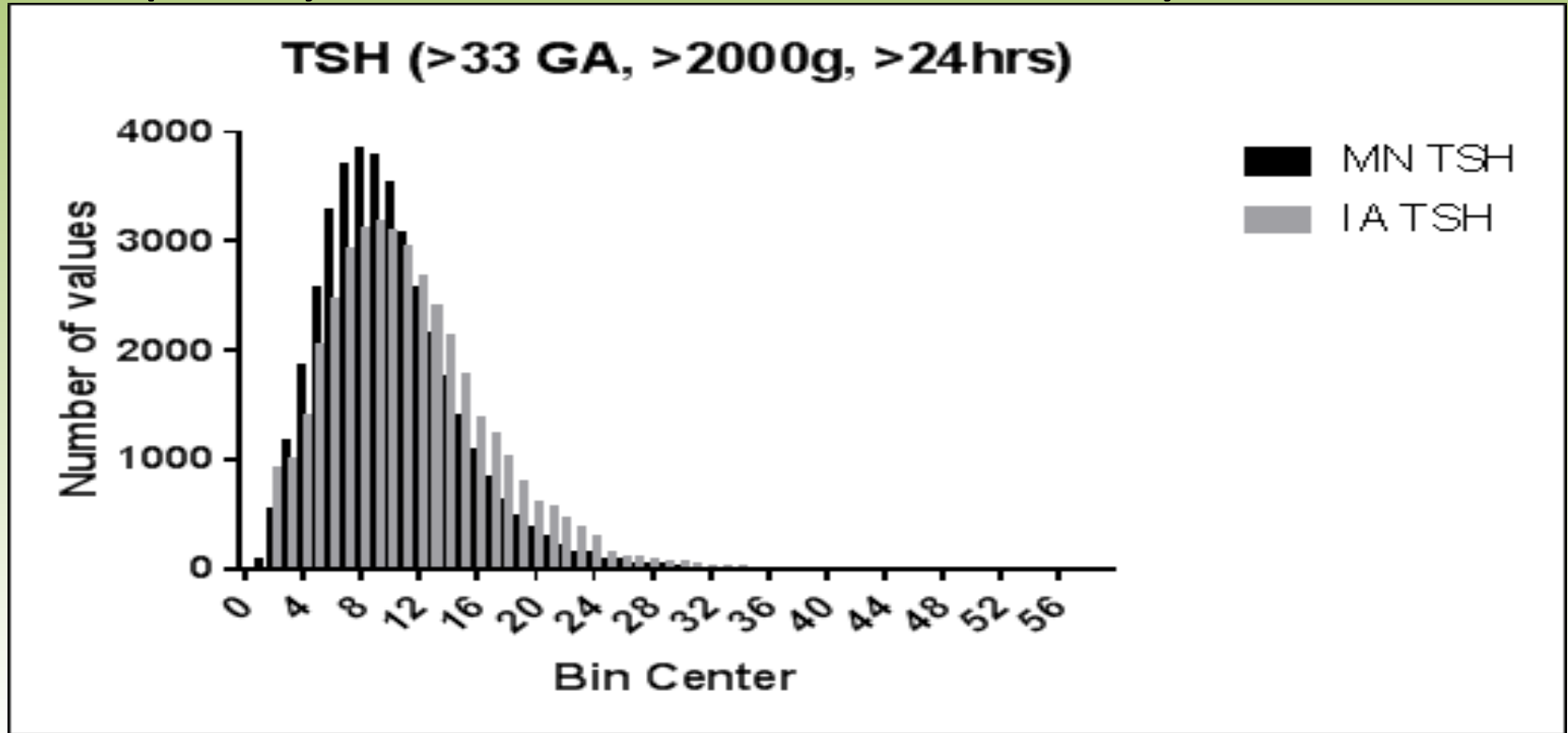


CH/TSH

- Congenital Hypothyroidism/Thyroid Stimulating Hormone
 - Minnesota
 - Perkin Elmer GSP instrument
 - Age at time of collection cut-offs
 - Iowa
 - Perkin Elmer AutoDELFIA instrument
 - Single fixed cut-off
- Data analysis
 - Exclude GA < 34 wks, < 2000g birthweight, < 24 hrs collection post-delivery

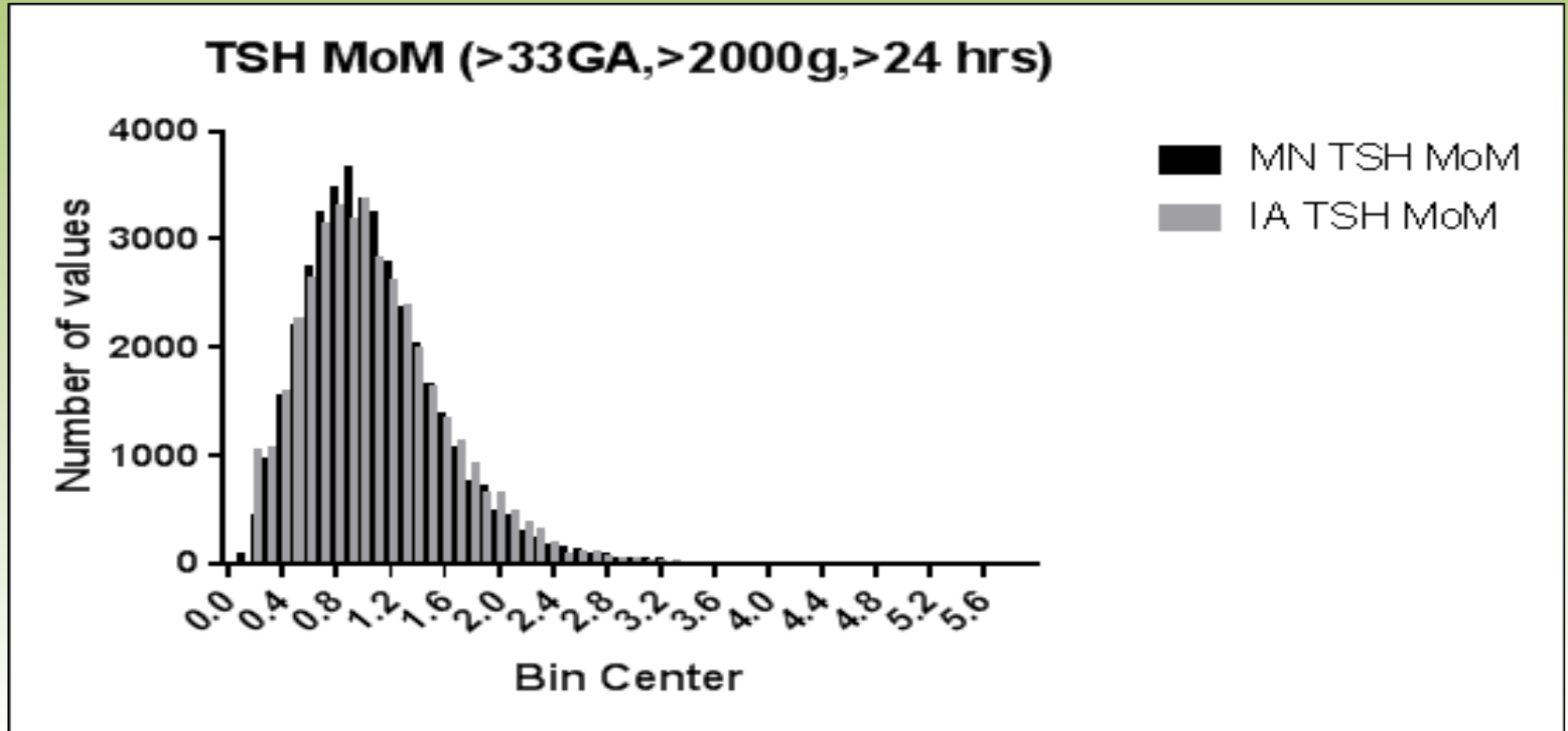
CH/TSH

- Frequency distribution for raw TSH analyte values:



CH/TSH

- Frequency distribution for median normalized TSH analyte values:





CH/TSH

- Statistical analysis of TSH values:

TSH raw

Column B	IA TSH raw
vs.	vs.
Column A	MN TSH raw
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	1501022800 , 1686229921
Mann-Whitney U	697599145
Difference between medians	
Median of column A	9.2, n=38953
Median of column B	10.3, n=37540
Difference: Actual	1.1

TSH MoM

Column D	IA TSH MoM
vs.	vs.
Column C	MN TSH MoM
Mann Whitney test	
P value	0.295
Exact or approximate P value?	Approximate
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column C,D	1603623330 , 1583629390
Mann-Whitney U	793379500
Difference between medians	
Median of column C	1, n=38953
Median of column D	1, n=37540
Difference: Actual	0



CH/TSH

- Comparative TSH presumptive positive rates:

Age at collect	TSH BORD	TSH PP
0 to 26 hrs	30 to 49.9 uIU/ml	>= 50 uIU/ml
MN	51 (0.13 %)	15 (0.04 %)
IA	166 (0.42 %)	16 (0.04%)
27 to 48 hrs	25 to 49.9 uIU/ml	>= 50 uIU/ml
MN	58 (0.15 %)	9 (0.02 %)
IA	98 (0.26 %)	7 (0.02 %)
49 to 168 hrs	18 to 49.9 uIU/ml	>= 50 uIU/ml
MN	2 (0.005 %)	0 (0.00 %)
IA	7 (0.02 %)	1 (0.01 %)
> 168 hrs	NA	>= 10 uIU/ml
MN	NA	11 (0.03 %)
IA	NA	3 (0.01 %)

CH/TSH

- Comparative TSH MoM presumptive positive rates:

Age at collect	TSH BORD	TSH PP
0 to 26 hrs	>/= 3.00 MoM (~30 uIU/ml)	>/= 5.00 MoM (~50 uIU/ml)
MN	115 (0.30 %)	18 (0.05 %)
IA	131 (0.33 %)	14 (0.04 %)
27 to 48 hrs	>/= 2.50 MoM (~25 uIU/ml)	>/= 5.00 MoM (~50 uIU/ml)
MN	119 (0.31 %)	9 (0.02 %)
IA	88 (0.22 %)	6 (0.02 %)
49 to 168 hrs	>/= 2.00 MoM (~20 uIU/ml)	>/= 5.00 MoM (~50 uIU/ml)
MN	2 (0.01 %)	0 (0.00 %)
IA	6 (0.02 %)	3 (0.01 %)
> 168 hrs	NA	>/= 1.00 MoM (~10 uIU/ml)
MN	NA	14 (0.04 %)
IA	NA	3 (0.01 %)

CF/IRT

- Cystic Fibrosis/Immunoreactive Trypsinogen

- Minnesota

- Perkin Elmer GSP
 - Fixed and floating cut-offs

- Iowa

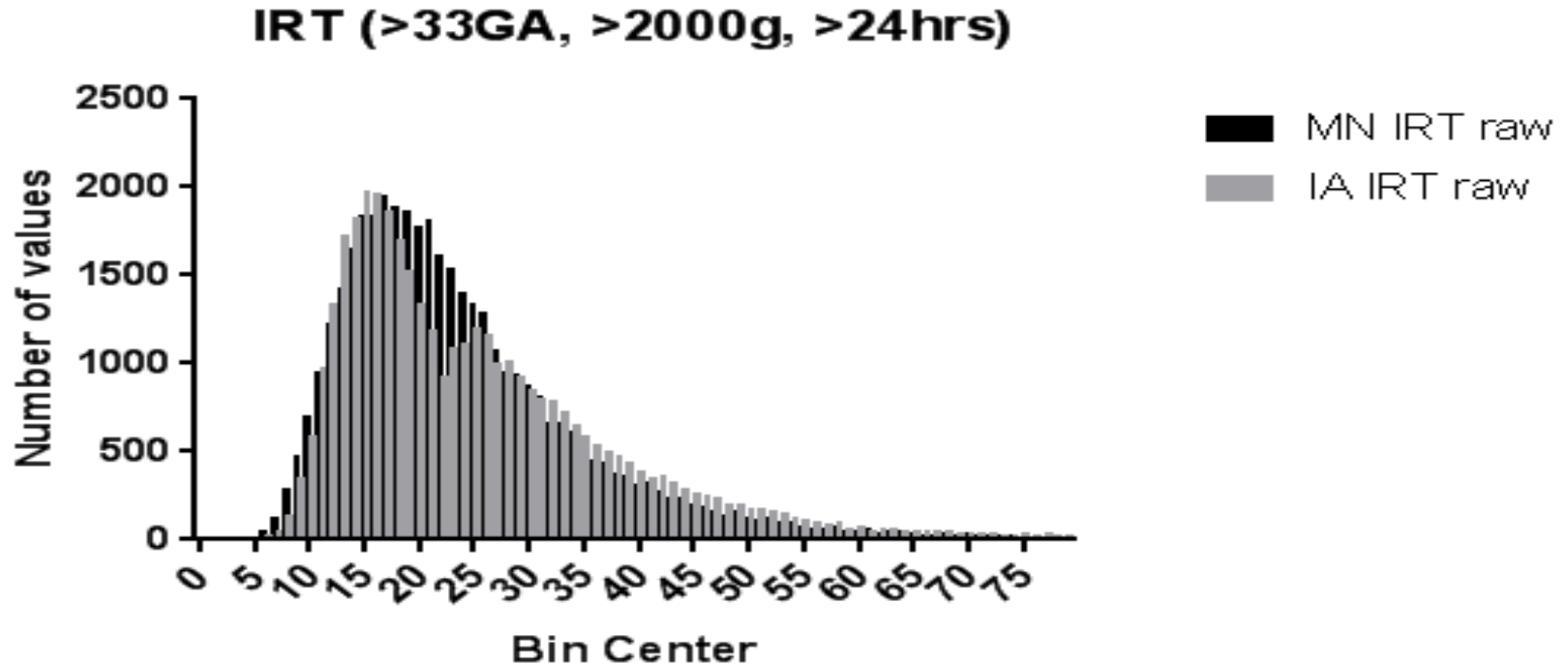
- Perkin Elmer AutoDELFIA
 - Fixed cut-off

- Data analysis

- Exclude < 34 weeks GA, < 2000 grams, and < 24 hours age at collection

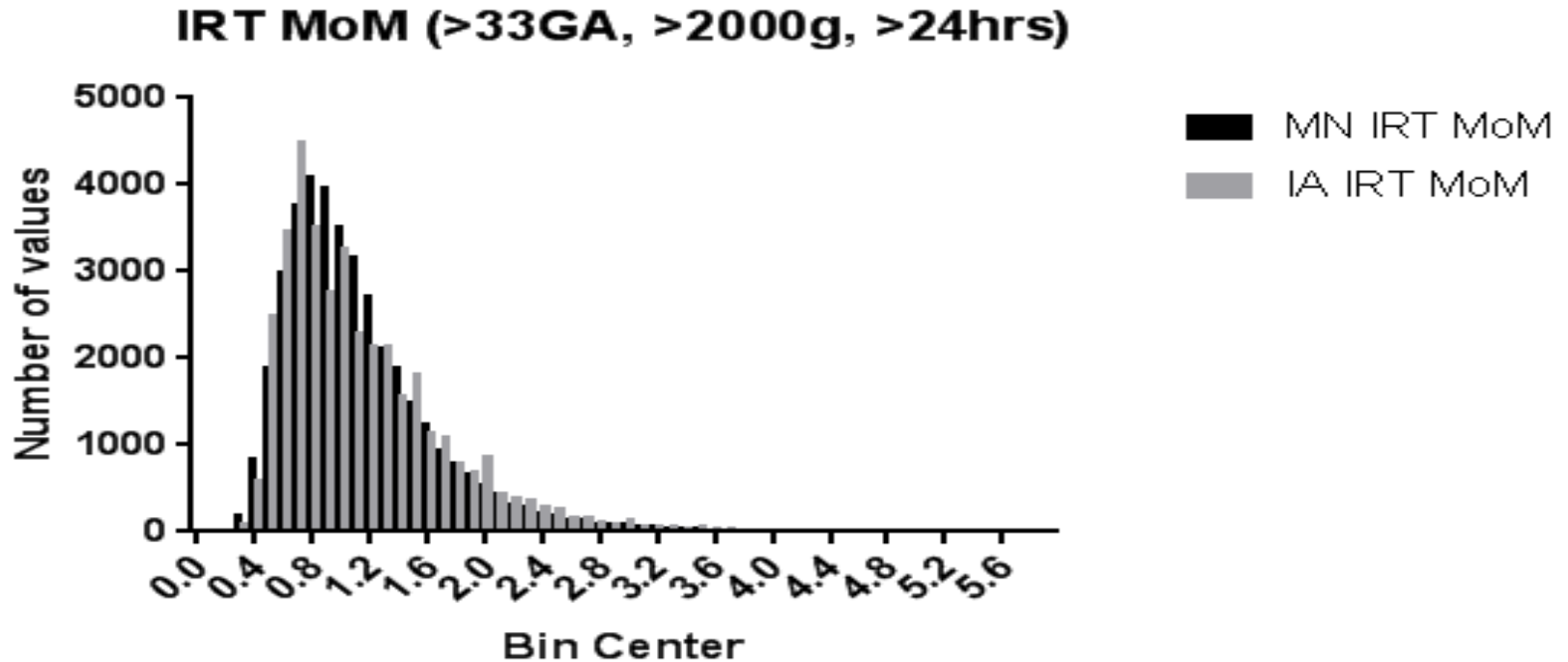
CF/IRT

- Frequency distribution for raw IRT values:



CF/IRT

- Frequency distribution for median normalized IRT values:



CF/IRT

- Statistical analysis of IRT values:

IRT raw

Column B	IA raw IRT
vs.	vs.
Column A	MN raw IRT
Mann Whitney test	
P value	<0.0001
Exact or approximate P value?	Approximate
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	1459500219 , 1475850292
Mann-Whitney U	701124234
Difference between medians	
Median of column A	21.3, n=39189
Median of column B	22, n=38887
Difference: Actual	0.7

IRT MoM

Column D	IA IRT MoM
vs.	vs.
Column C	MN IRT MoM
Mann Whitney test	
P value	0.8525
Exact or approximate P value?	Approximate
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column C,D	1492571616 , 1442778895
Mann-Whitney U	733057245
Difference between medians	
Median of column C	1, n=39189
Median of column D	1, n=38887
Difference: Actual	0

CF/IRT

- Comparative IRT presumptive positive rates:

state	IRT \geq 55.0 ng/ml	total n	%
MN	1369	38953	3.51
IA	2123	37540	5.66
state	IRT \geq 65.0 ng/ml	total n	%
MN	409	38953	1.05
IA	687	37540	1.83
state	IRT \geq 75.0 ng/ml	total n	%
MN	211	38953	0.54
IA	336	37540	0.90

CF/IRT

- Comparative IRT MoM presumptive positive rates:

state	IRT MoM ≥ 2.50 (approx. 55 ng/ml)	total n	%
MN	1028	38953	2.64
IA	1315	37540	3.50
state	IRT MoM ≥ 3.00 (approx. 66 ng/ml)	total n	%
MN	439	38953	1.13
IA	568	37540	1.51
state	IRT MoM ≥ 3.50 (approx. 77 ng/ml)	total n	%
MN	187	38953	0.48
IA	201	37540	0.54

Summary

- Prior to median normalization, statistically significant differences in raw analyte values were observed between the MN and IA NBS labs
- Following median normalization, no significant differences were observed
- Following median normalization, borderline and presumptive positive rates were similar for the MN and IA labs for disorders analyzed

Conclusions

Median normalization:

1. may provide a method to compare data across labs (regardless of instrumentation, reagents, etc)
 2. may provide a method to analyze data using a uniform cut-off
 3. may provide a method to aggregate data for rare disorders
- Aggregation of data may provide improved screen performance through better characterization of affected versus unaffected populations

Future Directions

- Much more to be done
- Analysis of data from more disorders
- Inclusion of corrections for demographic variables with known impact to screen performance
 - example: age at time of specimen collection for TSH

Acknowledgements

- Minnesota Department of Health
- Iowa Department of Public Health

