Method Performance for Measuring Immunoreactive Trypsinogen (IRT) in Dried Blood Spots

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NSQAP Prepares IRT QC and PT Materials

- **Whole blood is enriched with commercial IRT (human)**
  - IRT reconstituted according to package insert
  - >95% cationic form of IRT (IRT1)
  - IRT is labile and susceptible to proteolysis
  - CDC assayed values used
- **Quality Control Materials**
  - Certified values
  - QC used to track method performance over time
  - 6 Month expiration
- **Proficiency Testing Materials: Blind-coded**
  - IRT "within normal limits"
  - IRT "outside normal limits"
  - Cutoffs variable

http://www.cdc.gov/labstandards/nsqap.html

QC Data sent to NSQAP

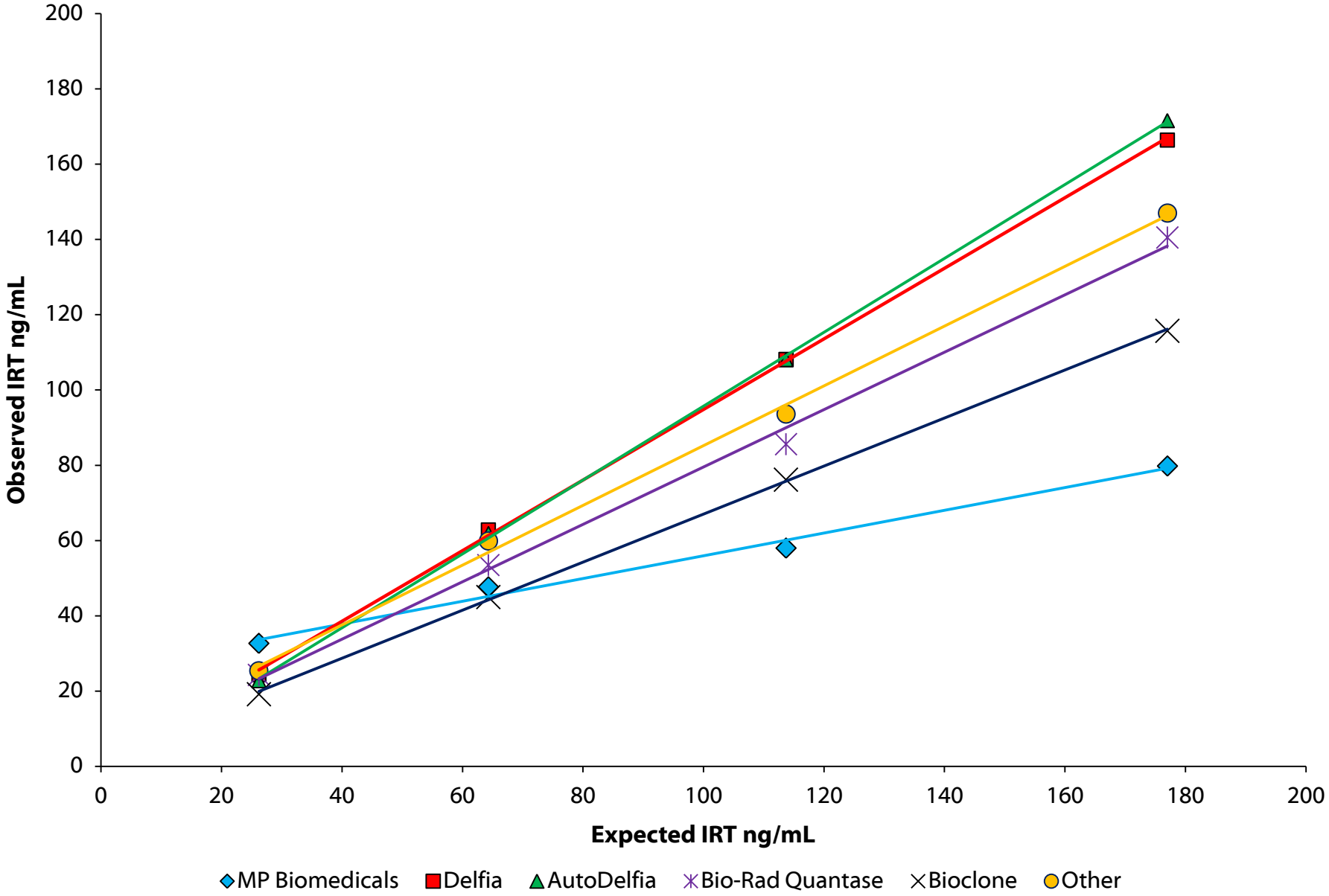
- **Dose-response QC DBS sent 2x per year**
  - Four levels are made for IRT
  - Labs report data from 5 independent runs
    - Two replicates per run
  - Data is compiled by method
  - Weighted linear regression examines the comparability by method of reported versus enriched concentrations
  - Parameters summarized
    - Mean, within-lab and total std, y-intercept, slope
  - Method biases reflected in slopes
    - 1.0 = ideal slope
    - Deviations from 1.0 indicate bias
- **Summary QC results published 2x per year**
  - http://www.cdc.gov/labstandards/pdf/nsqap/nsqap_qcmidyearreport_2010.pdf
  - http://www.cdc.gov/labstandards/pdf/nsqap/nsqap_summaryreport_2010.pdf

IRT Immunoassays Reported to NSQAP

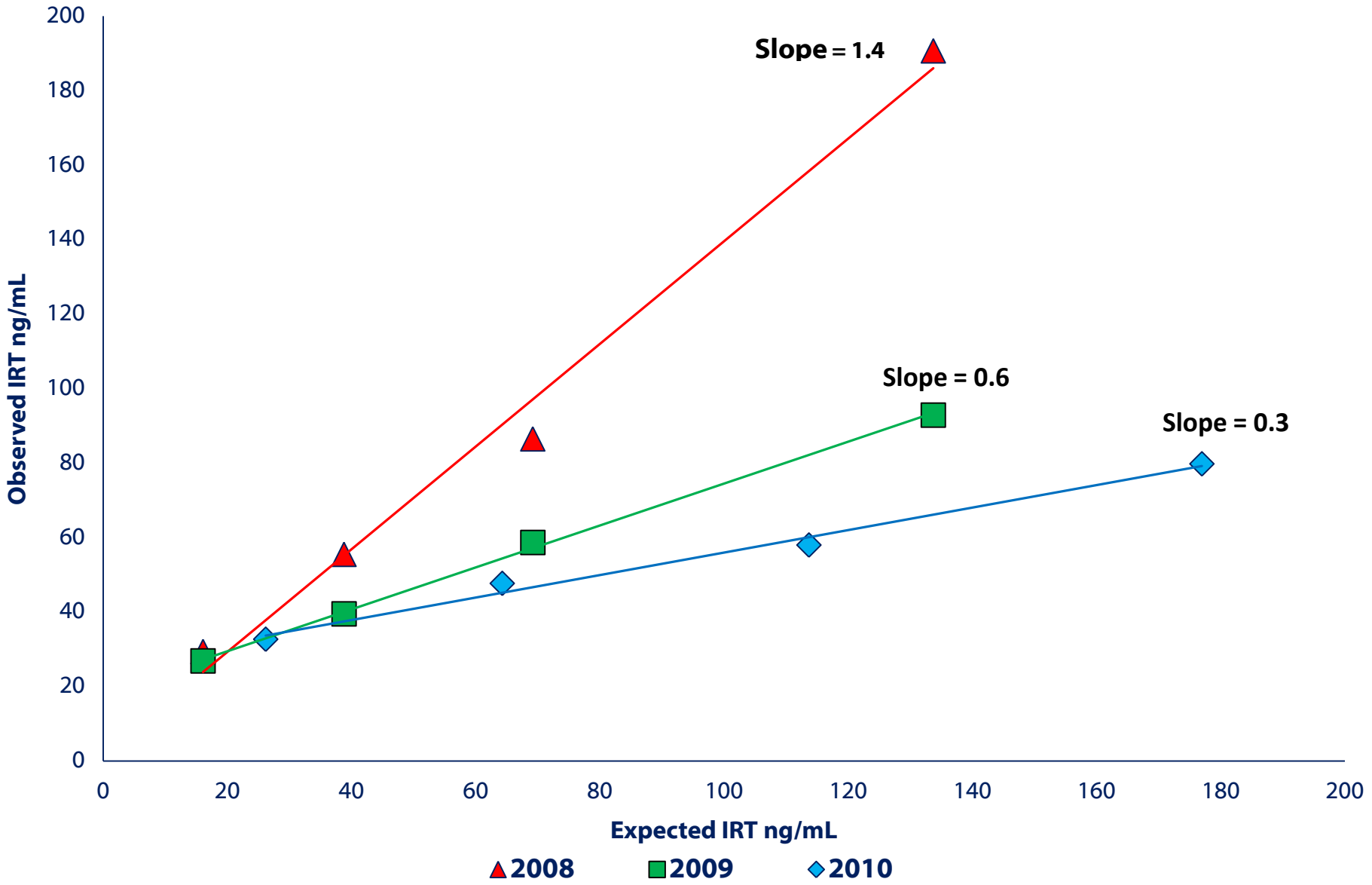
- **Time Resolved Fluorescence**
  - Perkin Elmer assays – Delfia* and AutoDelfia*
    - Lanthanide fluorescence – Europium-labeled antibodies
- **Enzyme Immunoassays**
  - MP Biomedicals*
  - Bioclone
  - Bio-Rad Quantase
- **Other** (less than 3 participants/method)
    - Ani Labsystems
    - CIS Bio International - RIA
    - GSP - Perkin Elmer
    - IBL International
    - Immunotech Beckman Coulter
    - Interscientific
    - TecnoSuma Umelisa

*Available in the US

Recovery of IRT in QC Materials 2010



Recovery of IRT in QC Materials by MP Biomedicals 2008-2010

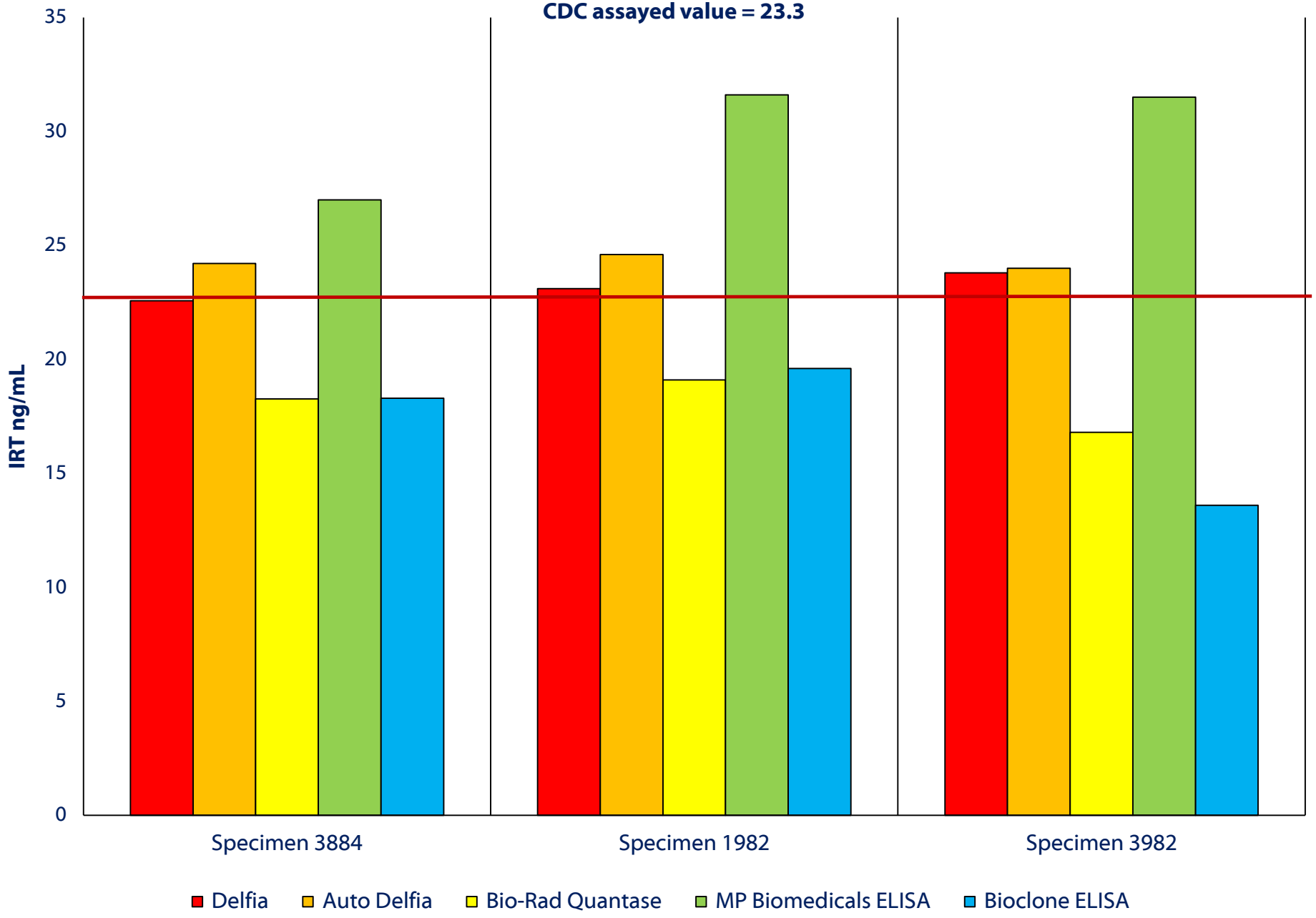


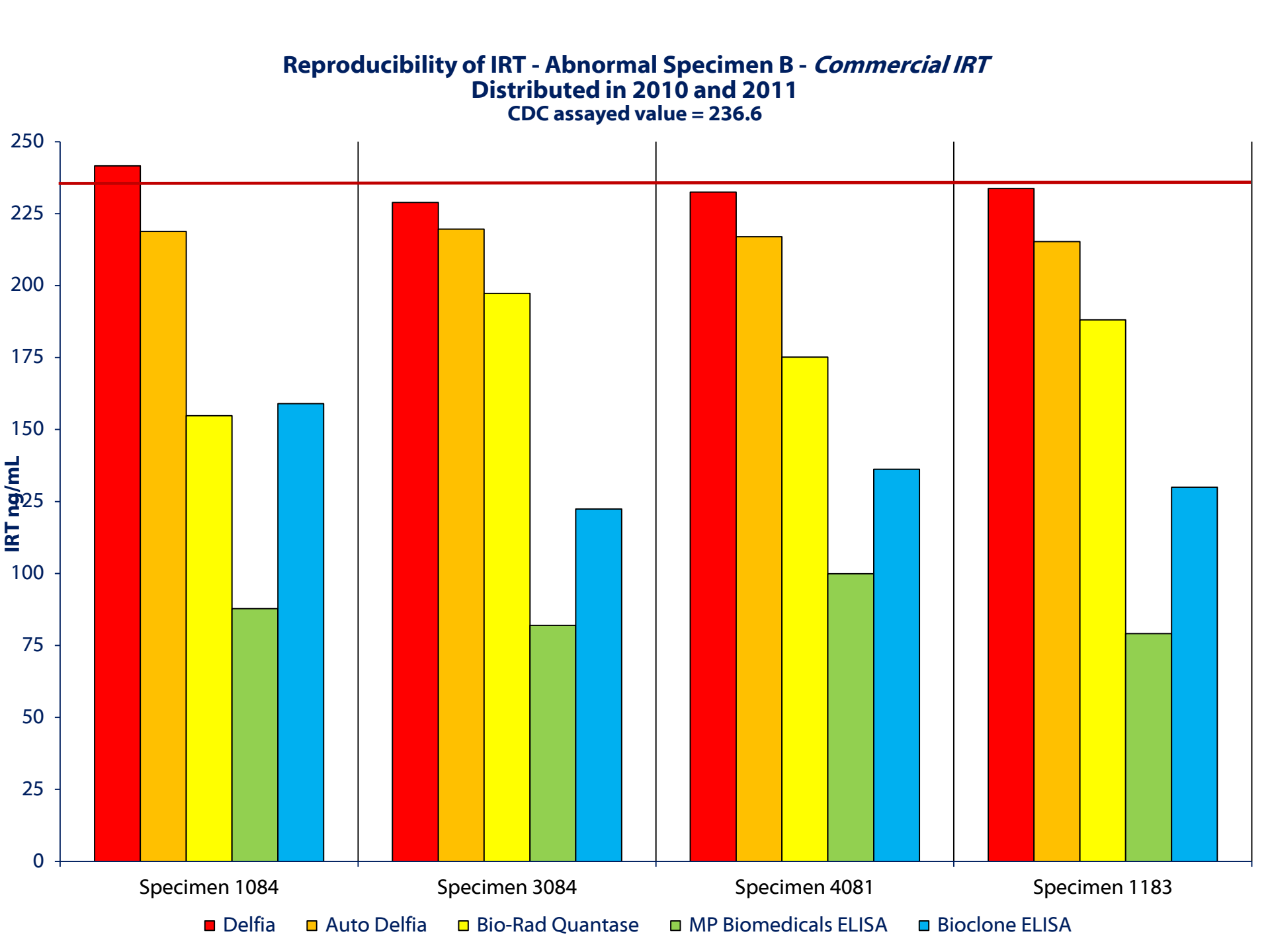
Proficiency Testing Materials

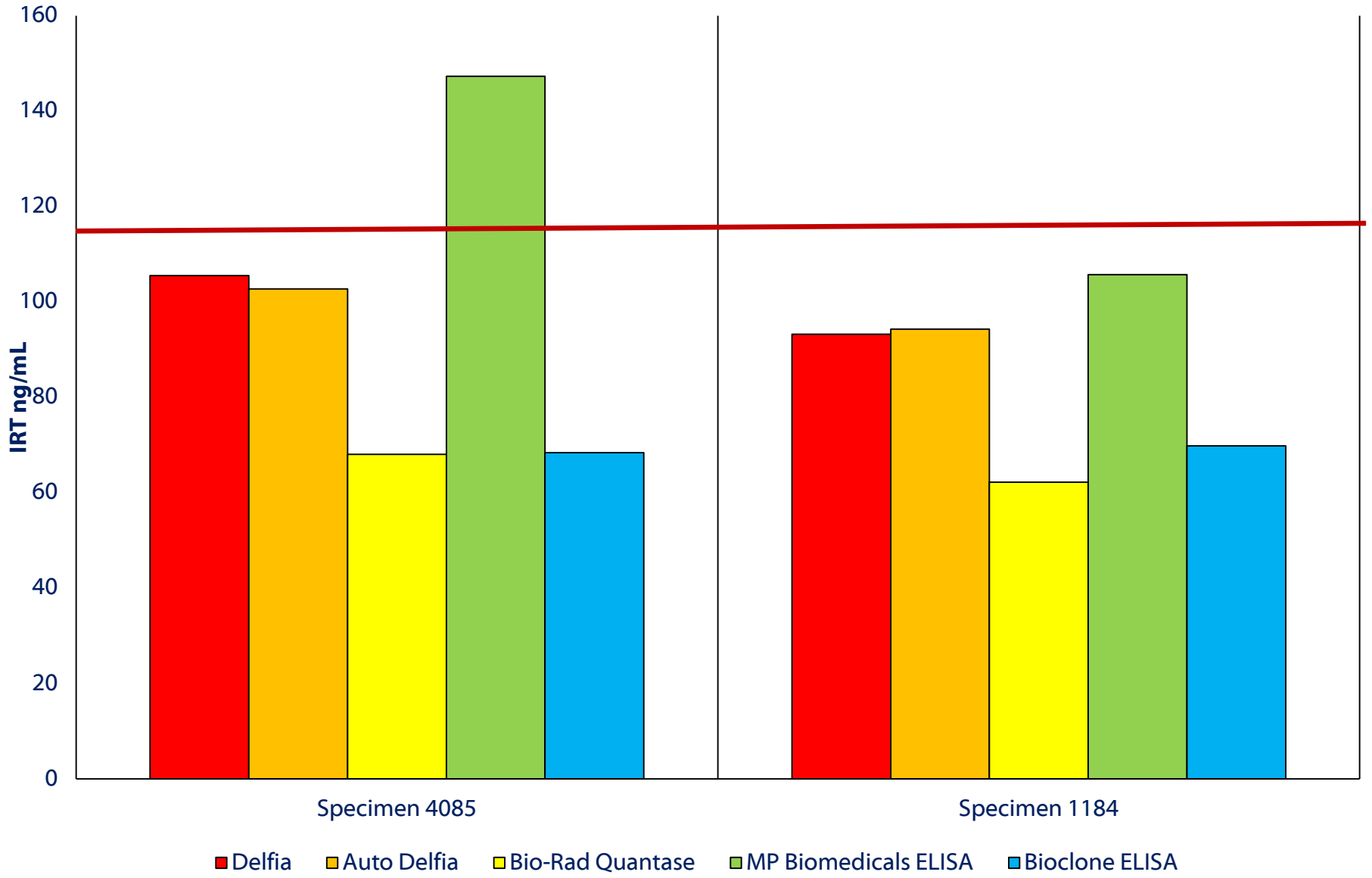
- Distributed 4 times per year (US and Canada)
- Data reported within 4 weeks of distribution date
- Analytical values, presumptive clinical assessments, and cutoffs values reported

Domestic IRT Cutoff Values: 2003-2010

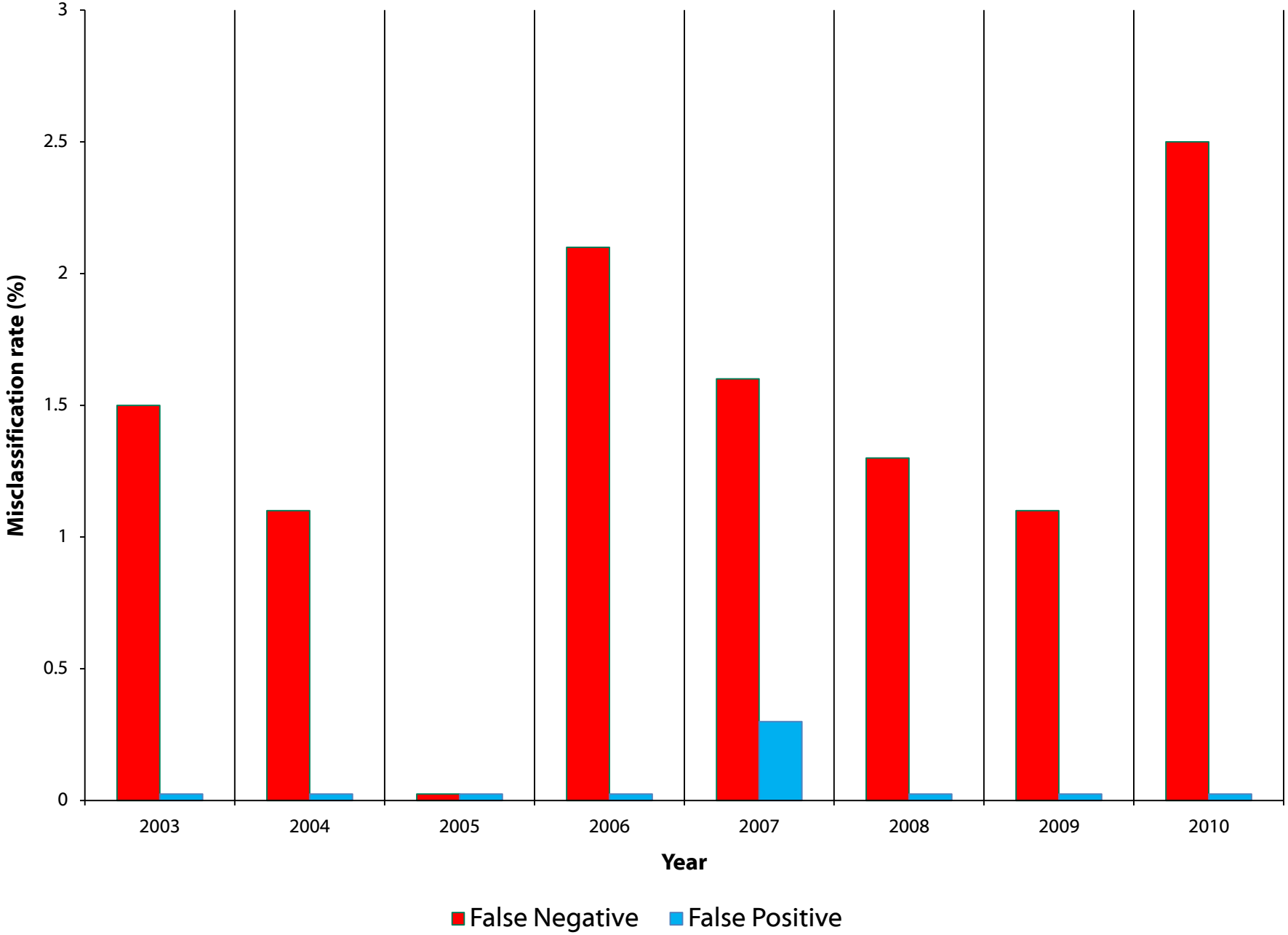
Year	N	Mean (ng/mL)	Median	Mode	Min/Max
2003	7	93.6	NA	90.0	66-114
2004	8	97.3	92.5	90.0	58-170
2005	9	95.3	105.0	105.0	63-170
2006	14	106.2	100.0	100.0	57-170
2007	30	89.0	80.0	62.0	62-170
2008	34	85.0	67.5	100.0	32-170
2009	42	79.9	65.0	62.0	39.6-170
2010	48	76.6	62.0	62.0	48.8-170


Reproducibility of IRT- Normal PT Specimen A – Commercial IRT
Distributed in 2008 and 2009
CDC assayed value = 23.3
IRT ng/mL
35
30
25
20
15
10
5
0
Specimen 3884
Specimen 1982
Specimen 3982
Delfia
Auto Delfia
Bio-Rad Quantase
MP Biomedicals ELISA
Bioclone ELISA


Reproducibility of IRT - Abnormal Specimen B - *Commercial IRT*
Distributed in 2010 and 2011
CDC assayed value = 236.6
IRT ng/mL
250
225
200
175
150
125
100
75
50
25
0
Specimen 1084
Specimen 3084
Specimen 4081
Specimen 1183
Delfia
Auto Delfia
Bio-Rad Quantase
MP Biomedicals ELISA
Bioclone ELISA


Reproducibility of IRT - Abnormal Specimen C - *Native IRT*
Distributed in 2010 and 2011
CDC Assayed value = 113.5
IRT ng/mL
160
140
120
100
80
60
40
20
0
Specimen 4085
Specimen 1184
Delfia
Auto Delfia
Bio-Rad Quantase
MP Biomedicals ELISA
Bioclone ELISA

IRT Domestic Misclassification Rates: 2003-2010.



IRT False Negative Results: Quarter 1, 2011[a]

Specimen Number	Method	N	CDC Assayed Value	Reason for False Negative
1181	AutoDelfia[b]	2	164.8	Low quantitative value
	MP Biomedicals[c]	4		Low quantitative value
	Bio-Rad Quantase	1		Low quantitative value
	IBL International	1		Low quantitative value
1183	MP Biomedicals[c]	2	236.6	Low quantitative value
1184[d]	AutoDelfia[b]	5	113.5	Low quantitative value
	Delfia[b]	5		Low quantitative value
	Bio-Rad Quantase	3		Low quantitative value
	Bioclone ELISA	1		Low quantitative value
	ILMA Beckman Coulter	1		Low quantitative value

[a]Domestic and Foreign combined; [b]Unusual number of FNs for the AutoDelfia and Delfia methods; [c]Current method cannot detect commercial IRT in CDC blood spots; [d]Native IRT specimen; N=25 FNs.

Domestic Labs with IRT FN* Cutoffs were higher than Mean Cutoff

CDC Cutoff = 80 ng/mL

Domestic Lab	IRT Cutoff (ng/mL)	Mean Domestic Cutoff	Median	Mode	Min/Max
1	100	76.6	62.0	62.0	48.8-170
2	100				
3	140				
4	100				
5	114				
6	90				
7	90				
8	100				
9	89				

*Quarter 1, 2011

IRT False Negative Results: Quarter 3, 2011[a]

Specimen Number	Method	N	CDC Assayed Value	Reason for False Negative
3183	MP Biomedicals[b]	3	143.5	Low quantitative value
	Bio-Rad Quantase	1		Low quantitative value
3185	MP Biomedicals[b]	2	206.1	Low quantitative value
	CIS Bio International RIA	1		Wrong Assessment Code

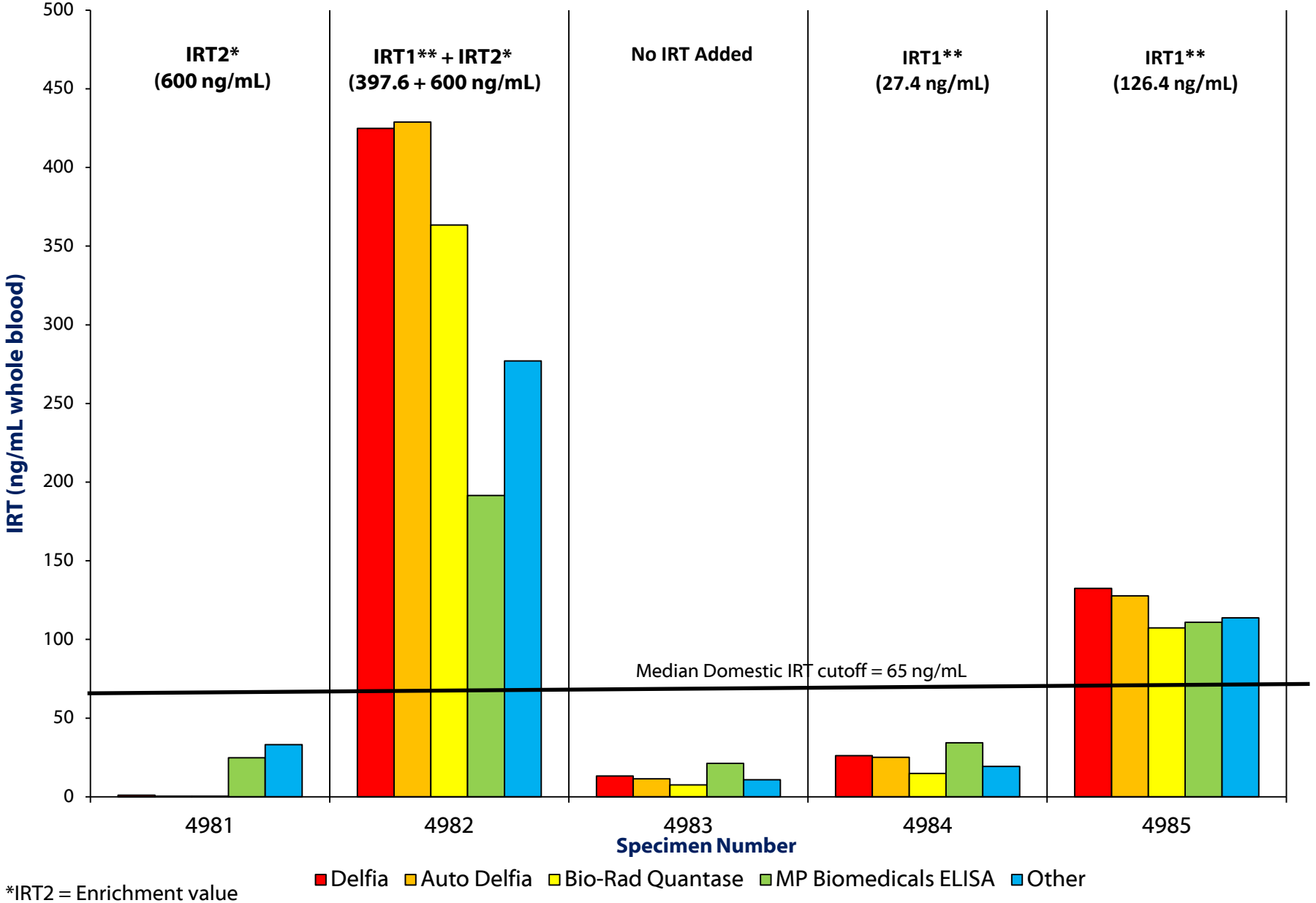
[a]Domestic and Foreign combined
[b]Current method cannot detect commercial IRT in CDC blood spots.

Can Commercial IRT kits distinguish between IRT 1 and IRT2?

- **Objective:**
  - To ascertain if methods reported to the CDC's Newborn Screening Quality Assurance Program (NSQAP) can detect the anionic and cationic forms of Immunoreactive Trypsinogen, IRT1 and IRT2, respectively, in dried blood spots prepared for proficiency testing (PT).

Mei JV, et.al. Recovery of Anionic and Cationic Immunoreative Trypsinogen (IRT1 and IRT2) by Methods Reported to the Newborn Screening Quality Assurance Program. Proceedings of the 2010 Newborn Screening and Genetic testing Symposium. Orlando, FL. May 2010.

Mean recovery of IRT1 and IRT2 by method
Quarter 4, 2009 PT panel.



*IRT2 = Enrichment value

**IRT1 = CDC Assayed value

Conclusions

- IRT methods detect IRT1 (cationic form) only
- IRT method performance can be variable; dependent on epitope recognition of kit antibodies
- External QC materials provide valuable data for dose-response method performance
- Proficiency testing highlights method and laboratory differences in performance over time
- Low IRT recovery and higher-than-average IRT cutoff contributed to false-negative errors.

Future IRT Material Preparation

- Pilot IRT DBS with and without protease inhibitor sent to several participants
- Improved recovery seen for MP Biomedicals
- *BUT* other methods called normal specimens abnormal
- Continued research using protease inhibitors needed
- No change in how current IRT QC DBS made
- Protease inhibitor may be added to PT specimens

Acknowledgements

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