Results from Tandem Mass Spectrometry (MS/MS) Ratios Pilot Proficiency Testing Program

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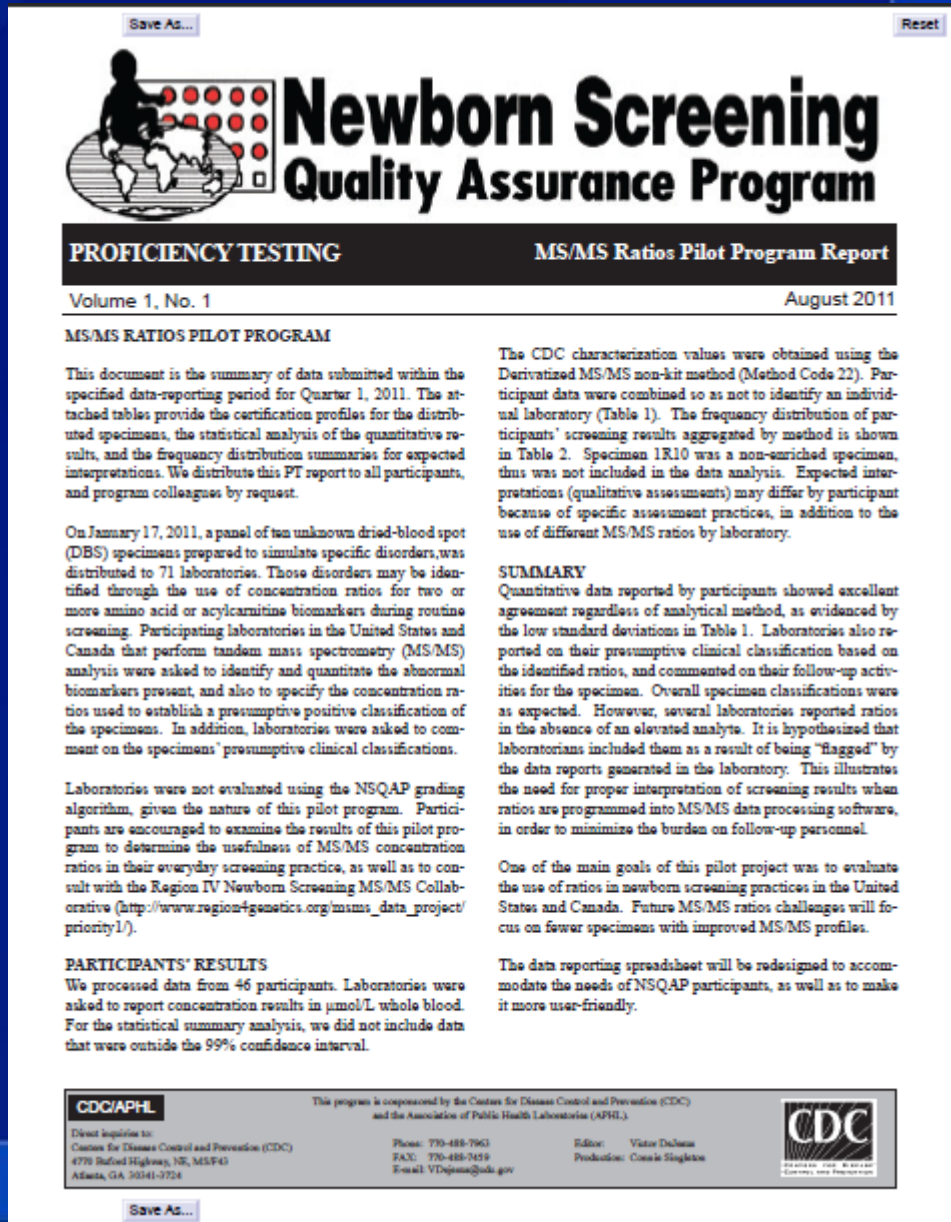
National Center for Environmental Health
Division of Laboratory Sciences

MS/MS Ratios Pilot Program

- Program designed to meet needs of those laboratories that use ratios
  - More labs adopting this practice
  - Region 4 Score Cards, training
- Focus: analytical proficiency
- Initial questions:
  - How many labs?
  - Which ratios?



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Newborn Screening Quality Assurance Program

PROFICIENCY TESTING — MS/MS Ratios Pilot Program Report

Volume 1, No. 1 — August 2011

MS/MS RATIOS PILOT PROGRAM

This document is the summary of data submitted within the specified data-reporting period for Quarter 1, 2011. The attached tables provide the certification profiles for the distributed specimens, the statistical analysis of the quantitative results, and the frequency distribution summaries for expected interpretations. We distribute this PT report to all participants, and program colleagues by request.

On January 17, 2011, a panel of ten unknown dried-blood spot (DBS) specimens prepared to simulate specific disorders,was distributed to 71 laboratories. Those disorders may be identified through the use of concentration ratios for two or more amino acid or acylcarnitine biomarkers during routine screening. Participating laboratories in the United States and Canada that perform tandem mass spectrometry (MS/MS) analysis were asked to identify and quantitate the abnormal biomarkers present, and also to specify the concentration ratios used to establish a presumptive positive classification of the specimens. In addition, laboratories were asked to comment on the specimens' presumptive clinical classifications.

Laboratories were not evaluated using the NSQAP grading algorithm, given the nature of this pilot program. Participants are encouraged to examine the results of this pilot program to determine the usefulness of MS/MS concentration ratios in their everyday screening practice, as well as to consult with the Region IV Newborn Screening MS/MS Collaborative (http://www.region4genetics.org/msms_data_project/priority1/).

PARTICIPANTS' RESULTS

We processed data from 46 participants. Laboratories were asked to report concentration results in µmol/L whole blood. For the statistical summary analysis, we did not include data that were outside the 99% confidence interval.

The CDC characterization values were obtained using the Derivatized MS/MS non-kit method (Method Code 22). Participant data were combined so as not to identify an individual laboratory (Table 1). The frequency distribution of participants' screening results aggregated by method is shown in Table 2. Specimen 1R10 was a non-enriched specimen, thus was not included in the data analysis. Expected interpretations (qualitative assessments) may differ by participant because of specific assessment practices, in addition to the use of different MS/MS ratios by laboratory.

SUMMARY

Quantitative data reported by participants showed excellent agreement regardless of analytical method, as evidenced by the low standard deviations in Table 1. Laboratories also reported on their presumptive clinical classification based on the identified ratios, and commented on their follow-up activities for the specimen. Overall specimen classifications were as expected. However, several laboratories reported ratios in the absence of an elevated analyte. It is hypothesized that laboratorians included them as a result of being "flagged" by the data reports generated in the laboratory. This illustrates the need for proper interpretation of screening results when ratios are programmed into MS/MS data processing software, in order to minimize the burden on follow-up personnel.

One of the main goals of this pilot project was to evaluate the use of ratios in newborn screening practices in the United States and Canada. Future MS/MS ratios challenges will focus on fewer specimens with improved MS/MS profiles.

The data reporting spreadsheet will be redesigned to accommodate the needs of NSQAP participants, as well as to make it more user-friendly.

CDC/APHL

This program is cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL).

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CDC

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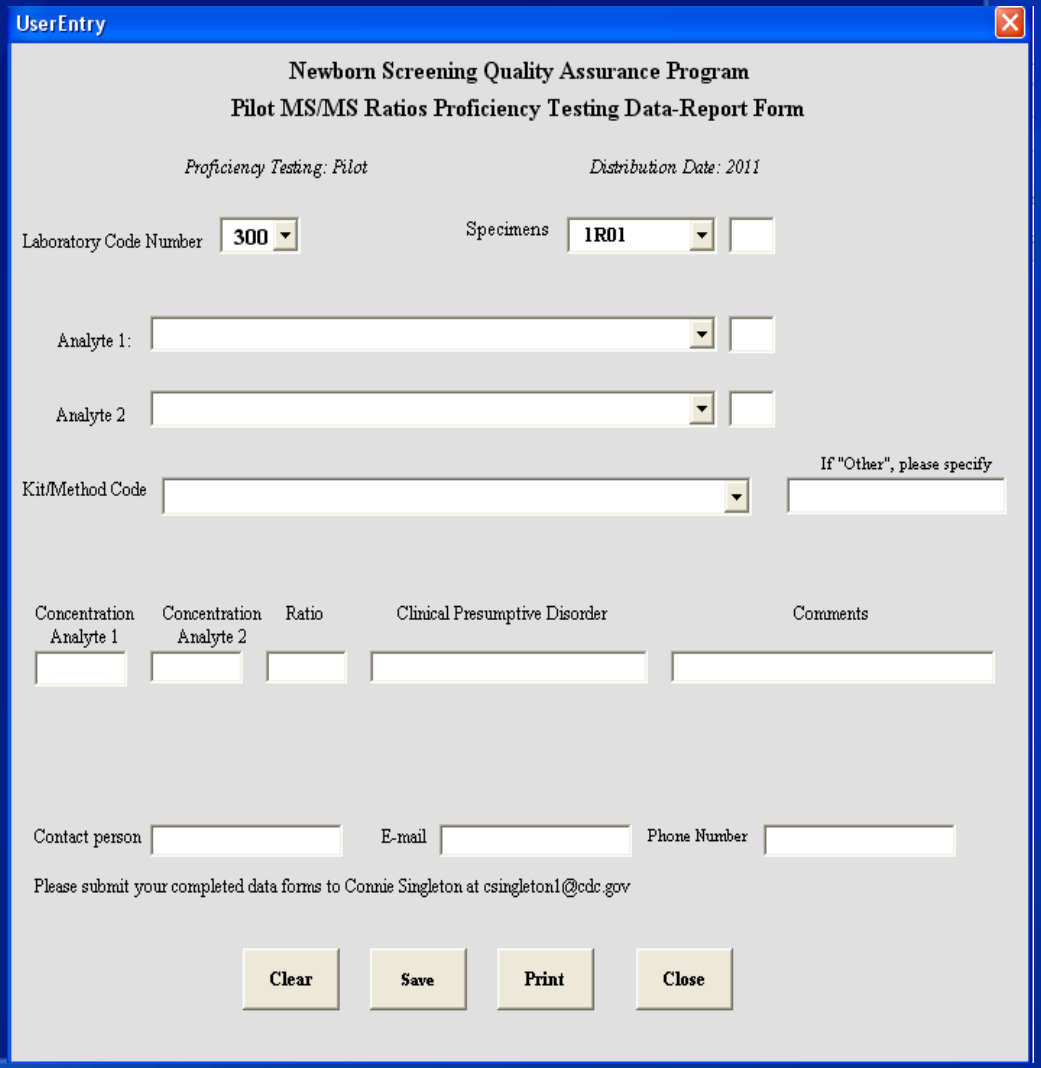
Targeted Ratios

- **Amino acids**
  - PKU
    - Phe/Tyr
  - MSUD
    - Leu/Phe
  - HCY
    - Met/Phe
  - Cit-1
    - Cit/Phe
  - Normal
    - Unenriched
- **Acylcarnitines**
  - Cbl C,D
    - C3/C2
  - IVA
    - C5/C3
  - MCADD
    - C8/C10
  - VLCADD
    - C14:1/C16
  - LCHADD
    - C16OH/C16

2011 MS/MS Ratios Pilot Launched!

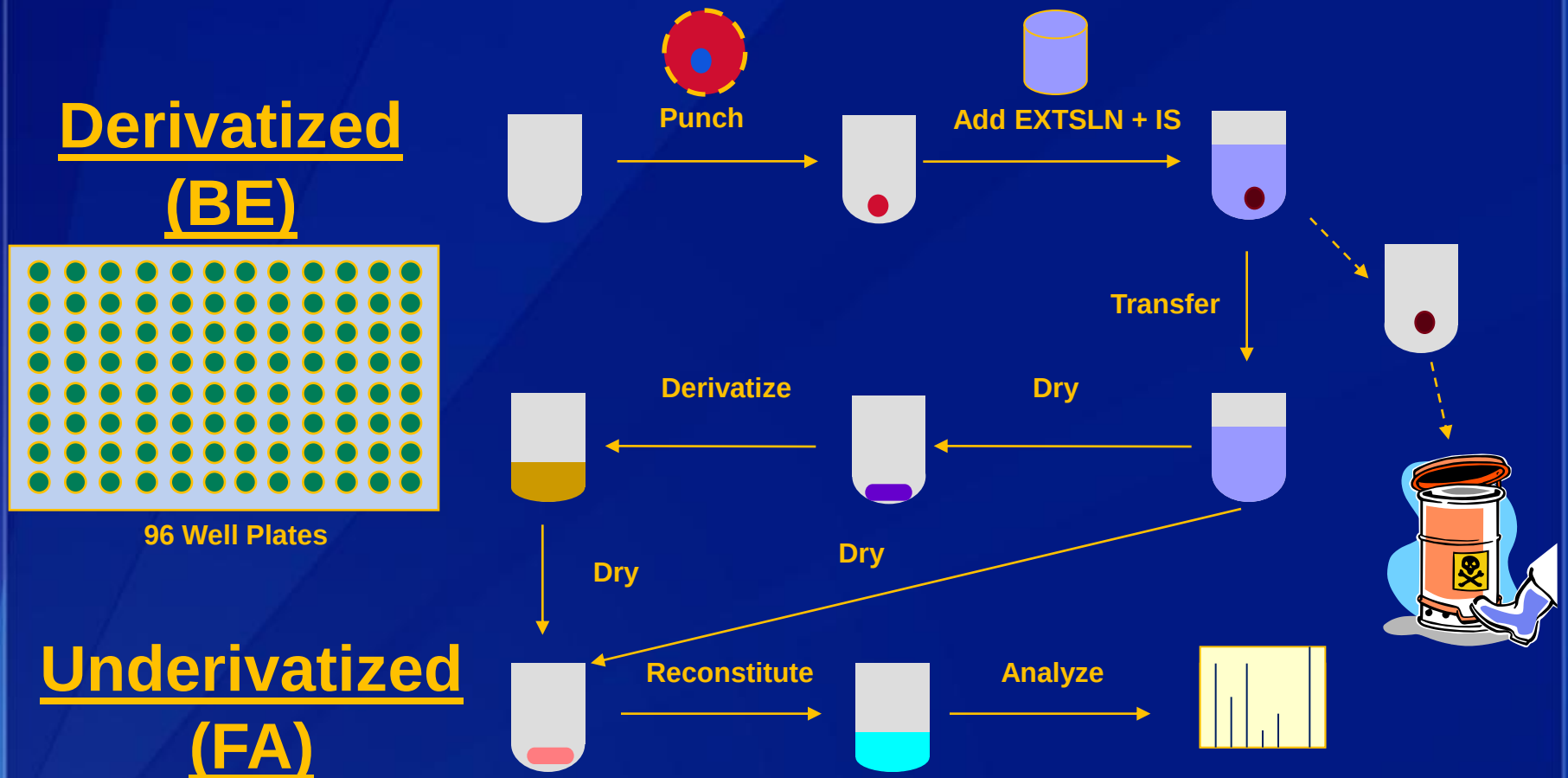
- **Panel sent to US & Canadian laboratories (N=71) in Q1 2011**
  - UDOT mailing list
  - Specimen enrichment
- **6 weeks to complete**
- **Data reporting**
  - Limited programming
  - Pre-loaded analytes
  - Comments field



Results

- **Laboratory response: N=46**
  - BUT – only 60/71 perform MS/MS – **77% response rate**
    - Positive feedback received from several laboratories
  - All participating labs responded within allotted reporting time
  - Report issued August 2011 by email
    - Both DER, UND assays reported

MS/MS NBS Assay Scheme



De Jesús VR, Chace DH, Lim TH, Mei JV, Hannon WH. Comparison of Amino Acids and Acylcarnitines Assay Methods Used in Newborn Screening Assays by Tandem Mass Spectrometry. Clinica Chimica Acta 2010; 411: 684-689.

Representative Results

1R01	CDC Characterized Values	Participant Average (N=46)	STDEV	MIN	MAX
1) Phe (μM)	283.5	307.1	61.8	199.3	622.6
2) Tyr (μM)	14.6	19.1	4.2	12.4	34.0
Ratio	19.43	16.36	2.43	10.12	21.50
1R04	CDC Characterized Values	Participant Average (N=10)	STDEV	MIN	MAX
1) Cit (μM)	159.2	203.5	46.8	138.0	271.6
2) Phe (μM)	44.0	44.7	4.5	36.6	49.6
Ratio	3.62	4.54	0.74	3.40	5.54
1R07	CDC Characterized Values	Participant Average (N=23)	STDEV	MIN	MAX
1) C8 (μM)	1.6	1.6	0.2	1.3	2.1
2) C10 (μM)	0.6	0.6	0.1	0.3	0.8
Ratio	2.67	2.95	0.55	2.10	4.39
1R09	CDC Characterized Values	Participant Average (N=39)	STDEV	MIN	MAX
1) C16OH (μM)	1.0	1.2	0.2	0.8	1.9
2) C16 (μM)	2.8	2.7	0.7	0.0	4.5
Ratio	0.39	0.41	0.05	0.32	0.53

Method-Specific Results

1R01	Average (N=46)	MIN	MAX	DER Non-Kit (N=22)	DER PE (N=13)	UND PE (N=11)
1) Phe (µM)	307.1	199.3	622.6	323.5	291.5	292.6
2) Tyr (µM)	19.1	12.4	34.0	18.3	19.1	20.6
Ratio	16.36	10.12	21.50	17.84	15.64	14.26
1R04	**Average (N=10)**	**MIN**	**MAX**	**DER Non-Kit (N=6)**	**DER PE (N=2)**	**UND PE (N=2)**
1) Cit (µM)	203.5	138.0	271.6	174.4	260.1	234.5
2) Phe (µM)	44.7	36.6	49.6	42.9	48.4	46.5
Ratio	4.54	3.40	5.54	4.08	5.37	5.07
1R07	**Average (N=23)**	**MIN**	**MAX**	**DER Non-Kit (N=13)**	**DER PE (N=7)**	**UND PE (N=2)**
1) C8 (µM)	1.6	1.3	2.1	1.6	1.5	1.6
2) C10 (µM)	0.6	0.3	0.8	0.6	0.4	0.5
Ratio	2.95	2.10	4.39	2.69	3.46	3.06
1R09	**Average (N=39)**	**MIN**	**MAX**	**DER Non-Kit (N=14)**	**DER PE (N=15)**	**UND PE (N=10)**
1) C16OH (µM)	1.2	0.8	1.8	1.2	1.2	1.0
2) C16 (µM)	2.7	0.0	4.5	3.0	2.4	2.7
Ratio	0.41	0.32	0.53	0.40	0.43	0.36

Salient Points

- **Excellent analytical performance**
  - Semi-quantitative values agreement
- **Widespread use of ratios**
  - Real field practice or just PT?
- **Several ratios reported for each specimen**
  - Ratios reported in absence of elevated analyte
  - No profile interpretation?
- **Can ratios be used in everyday practice?**
  - **Yes!** Ask Fred Lorey (CA) and Piero Rinaldo (MN)

NSQAP adapts to ensure high-quality screening

- **PT Testing**
  - NSQAP new category: C3DC + C4OH
  - Allows for reduced corrective action reports
  - On-line reporting category: live in January 2012 (as of 11-07-2011)
  - Instructions will be provided as soon as web site changes are completed
- **MS/MS Ratios Challenges**
  - Better "patient" profiles for improved challenges
  - Improved data-reporting form that automatically calculates ratios

Summary

- **Newborn screening by tandem mass spectrometry is a successful public health program**
  - >95% of newborns screened in US
- **Many challenges remain for MS/MS ratios screening**
  - Understanding assay and ratios significance is key
  - Profile interpretation is very important – ratios alone?
- **NSQAP is a comprehensive resource for laboratory services**
  - New PT programs reflect current practices in the field

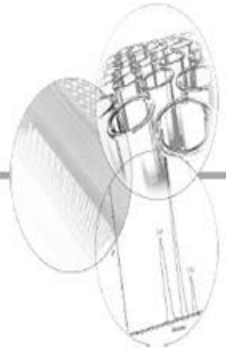
NSQAP Web Site: http://www.cdc.gov/labstandards/nsqap.html

Why Must We Assure Assay Quality in Newborn Screening Labs?

- ✓ Early and accurate detection of congenital disorders saves lives!

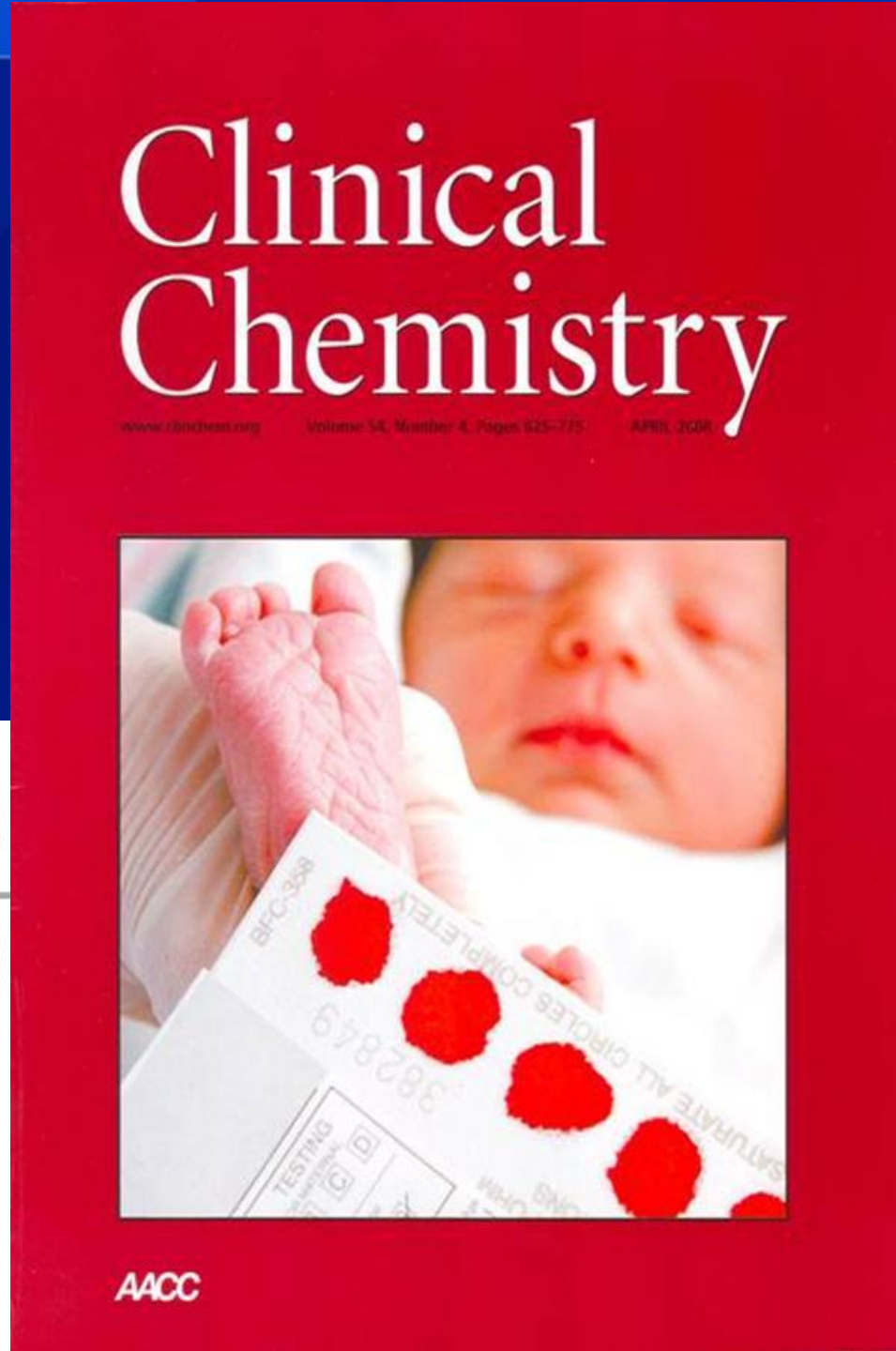
FOREWORD

SPECIAL FOCUS: DRIED BLOOD SPOTS



A glowing future for dried blood spot sampling

"...a number of factors have recently come together to encourage this industry to break out of its shell and look for suitable alternatives to traditional plasma sampling."



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  - Association of Public Health Laboratories
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Thank you for your attention!