Comparison of Methods for the Analysis of Lysosomal Enzyme Activities in Quality Control Dried Blood Spot Specimens

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David Millington[1], Qun Shi[1], Gwen Dickerson[1], Hui Zhou[2], Victor DeJesus[2], Patrick Hopkins[3], Tracy Klug[3], Ramakrishna Sista[4], Vamsee Pamula[4] and Deeksha Bali[1]

[1]Duke Hospital Biochemical Genetics Laboratory, Durham, NC; [2]Centers for Disease Control and Prevention Newborn Screening and Mol Biology Branch, Atlanta, GA; [3]Newborn Screening, Missouri State Public Health Laboratory, [4]Advanced Liquid Logic, Inc., 615 Davis Drive, Morrisville, NC

Objective

To compare and assess new quality control (QC) materials prepared and circulated by Centers for Disease Control (CDC) Newborn Screening Section (Atlanta, GA) and by Advanced Liquid Logic, Inc. (ALL, Morrisville, NC) for lysosomal storage disease (LSD) enzyme activities in dried blood spots (DBS) using microtiter plate fluorometry (MTPF), digital microfluidic fluorometry (DMF) and tandem mass spectrometry (MSMS) assay methods.

Objective (contd)

The five enzymes targeted for the current study were selected as representative of the most treatable LSD conditions at the current time, according to a recent review (1):

Pompe:	**GAA (acid-α-glucosidase)**
Fabry:	**GLA (acid-α-galactosidase)**
Gaucher:	**GBA (acid-α-glucocerebrosidase)**
Hurler:	**IDU (α-iduronidase)**
Hunter:	**IDUS (α-iduronidate-2-sulfatase)**

1. Zhou H, Fernhoff P, Vogt RF. Newborn bloodspot screening for lysosomal storage disorders. J Pediatr 2011;159:7–13e1.

METHODS: PREPARATION OF QUALITY CONTROL (QC) MATERIALS

QC materials were prepared at Advanced Liquid Logic under a current good manufacturing practice environment and in similar fashion to those from the CDC (2) by mixing different ratios of two blood pools: the base pool (QC-BP), consisting of leuko-reduced, washed, red blood cells and the high pool (QC-H), consisting of packed cells from pooled, cord blood units.

During preparation, the hematocrit of both pools was adjusted to 50% with heat-inactivated, charcoal-stripped serum.

The low (QC-L) and medium (QC-M) pools were created by mixing ratios of 95:5 and 50:50 of QC-BP:QC-H, respectively.

Multiple aliquots (100 µL) of each pooled QC blood sample were spotted onto filter paper (Whatman 903®), then dried overnight at ambient temperature and stored in airtight bags with desiccant at -20 °C.

2. De Jesus VR, Zhang XK, Keutzer J, Bodamer OA, Muhl A, Orsini JJ et al. Development and evaluation of quality control dried blood spot materials in newborn screening for lysosomal storage disorders. Clin Chem 2009;55:158–64.

METHODS: PROTOCOL FOR ANALYSIS

The QC samples were distributed to the participating sites at Missouri State Newborn Screening Laboratory (MONBS) and Duke Biochemical Genetics Laboratory (DBGL) with instructions to analyze them in triplicate for five consecutive days (n=15 per specimen, 4 specimens) within the same 7-day period.

The MONBS and DBGL measured the activities of five lysosomal enzymes using Digital Microfluidic Fluorometry (DMF) and Microtiter Plate Fluorometry (MTPF) methods respectively, and the results compared with those determined for four of those enzymes by Tandem Mass Spectrometry (MS/MS) at CDC Laboratory.

METHODS: LSD Enzymatic Assays

MTPF: Diagnosis of alpha-L-iduronidase deficiency in dried blood spots on filter paper: the possibility of newborn diagnosis. NA *Chamoles, M Blanco, D Gaggioli.* Clin Chem 2001;47:780-781

Fabry disease: enzymatic diagnosis in dried blood spots on filter paper. NA *Chamoles, M Blanco, D Gaggioli.* Clin Chim Acta 2001;308:195-196.

A Comparison of Maltose and Acarbose as Inhibitors of Maltase-Glucoamylase Activity in assaying acid α-Glucosidase Activity in Dried Blood Spots for the Diagnosis of Infantile Pompe Disease. *H Zhang, H Kallwass, SP Young, et al*. Genet Med. 8(5):302-6, 2006.

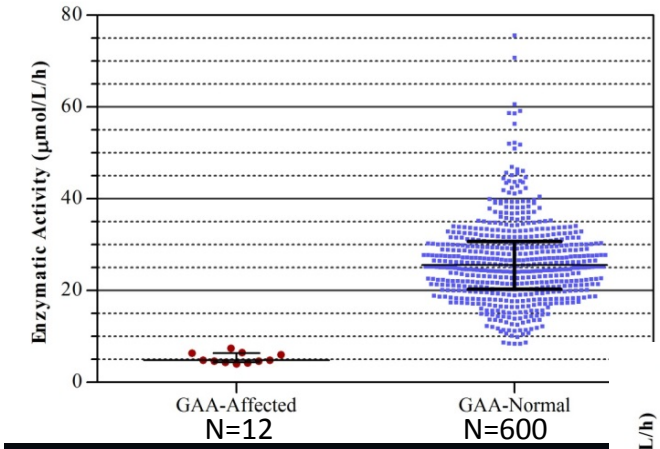
A novel fluorometric enzyme analysis method for Hunter syndrome using dried blood spots. *AA Tolun, C Graham, Q Shi, et al.* Mol. Gen. and Metab. 105 (2012) 519-521.

MSMS: Zhang XK, Elbin CS, Chuang WL, Cooper SK, Marashio CA, Beauregard C, et al. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. Clin Chem 2008;54:1725 – 8.

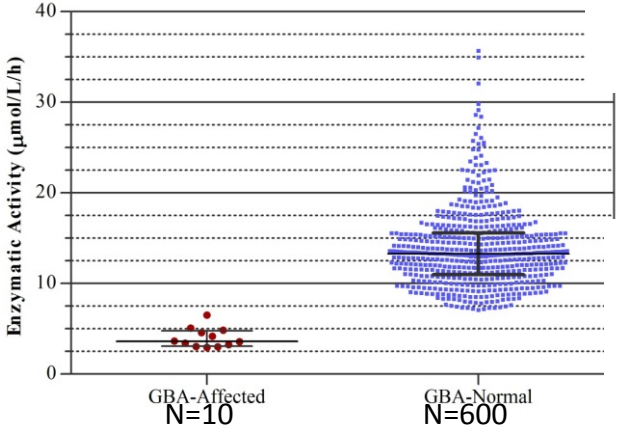
DMF: Multiplex Newborn Screening for Pompe, Fabry, Hunter, Gaucher, and Hurler Diseases Using a Digital Microfluidic Platform. *R. S. Sista, T, Wang, N. Wu, et al.* Clin Chim Acta 2013 (in press).

Performance of 5-Plex Newborn Screening for Pompe, Fabry, Hunter, Gaucher, and Hurler Diseases Using a Digital Microfluidic Platform: known affected (n = 7-12) vs random newborn DBS (n=600)

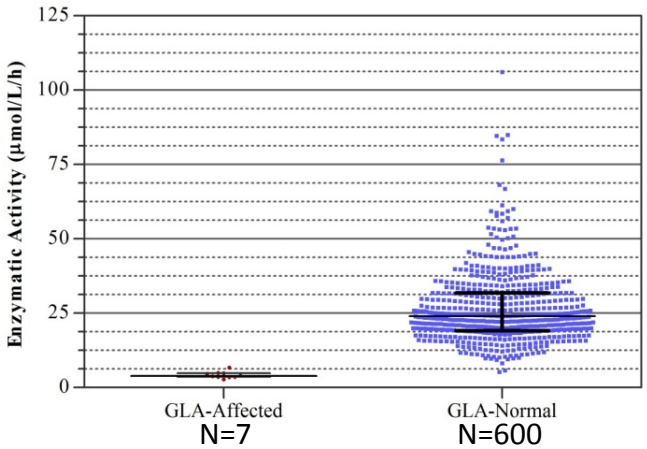
POMPE



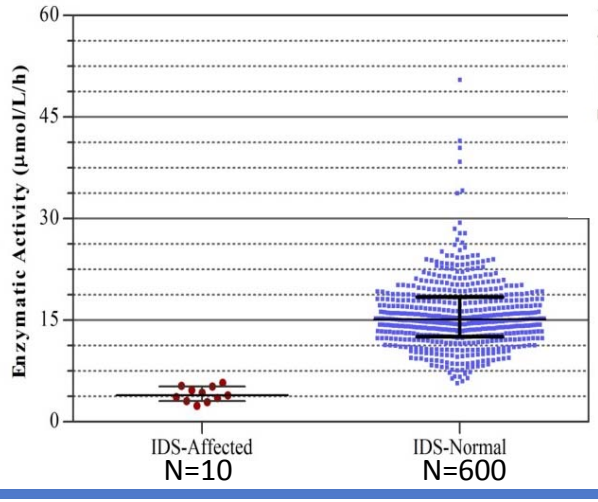
GAUCHER



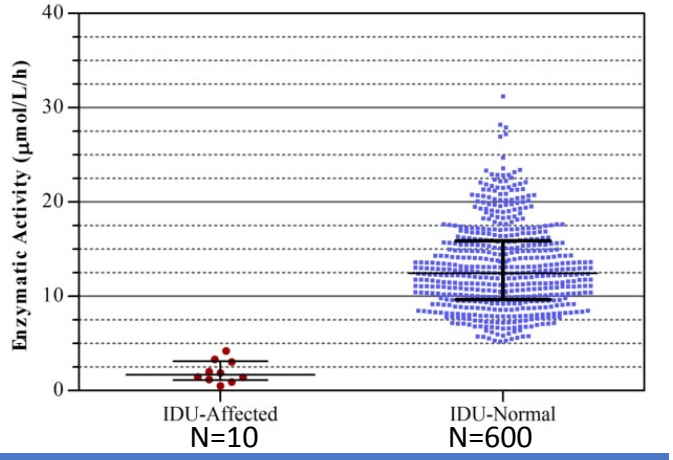
FABRY



HUNTER



HURLER

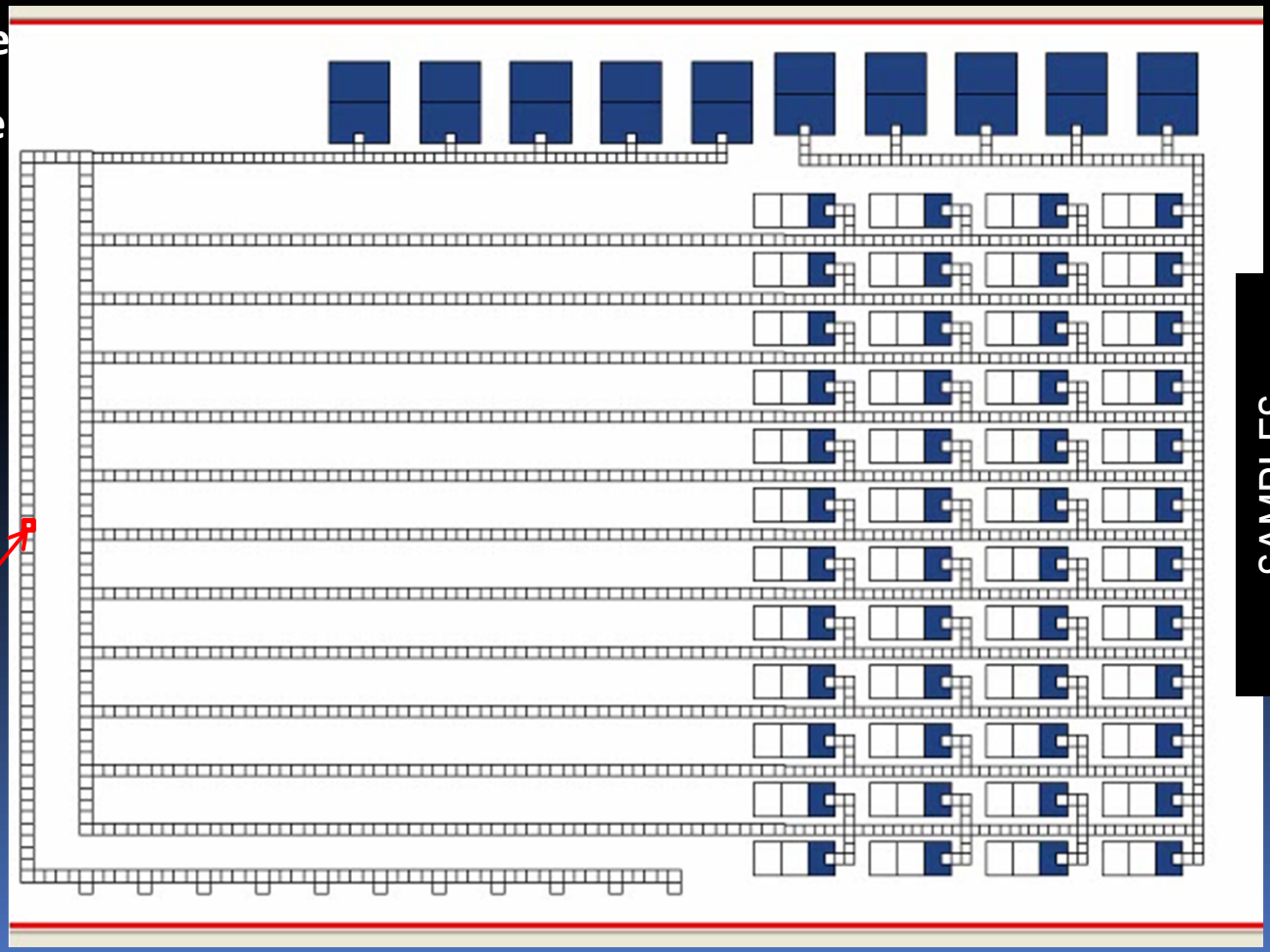


5-plex Panel of Enzymatic Assays for LSD

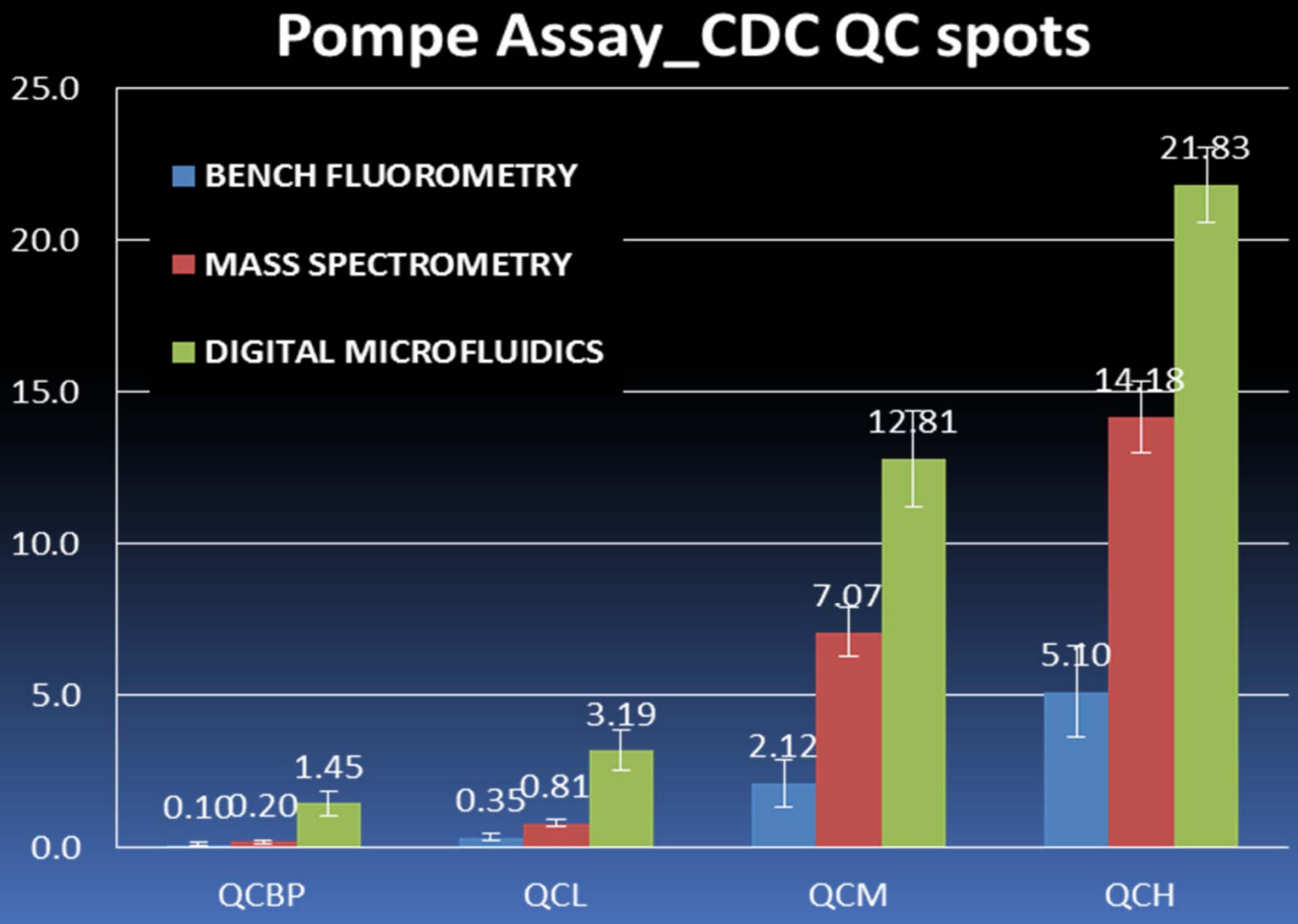
- 5-Plex: Pompe, Fabry, Gaucher, Hunter, Hurler
- 12 NBS DBS
- Droplet volume: 300 nanoliters
- Incubation time: 1hr
- Detection: 4-MU fluorescence
- Total 60 assays

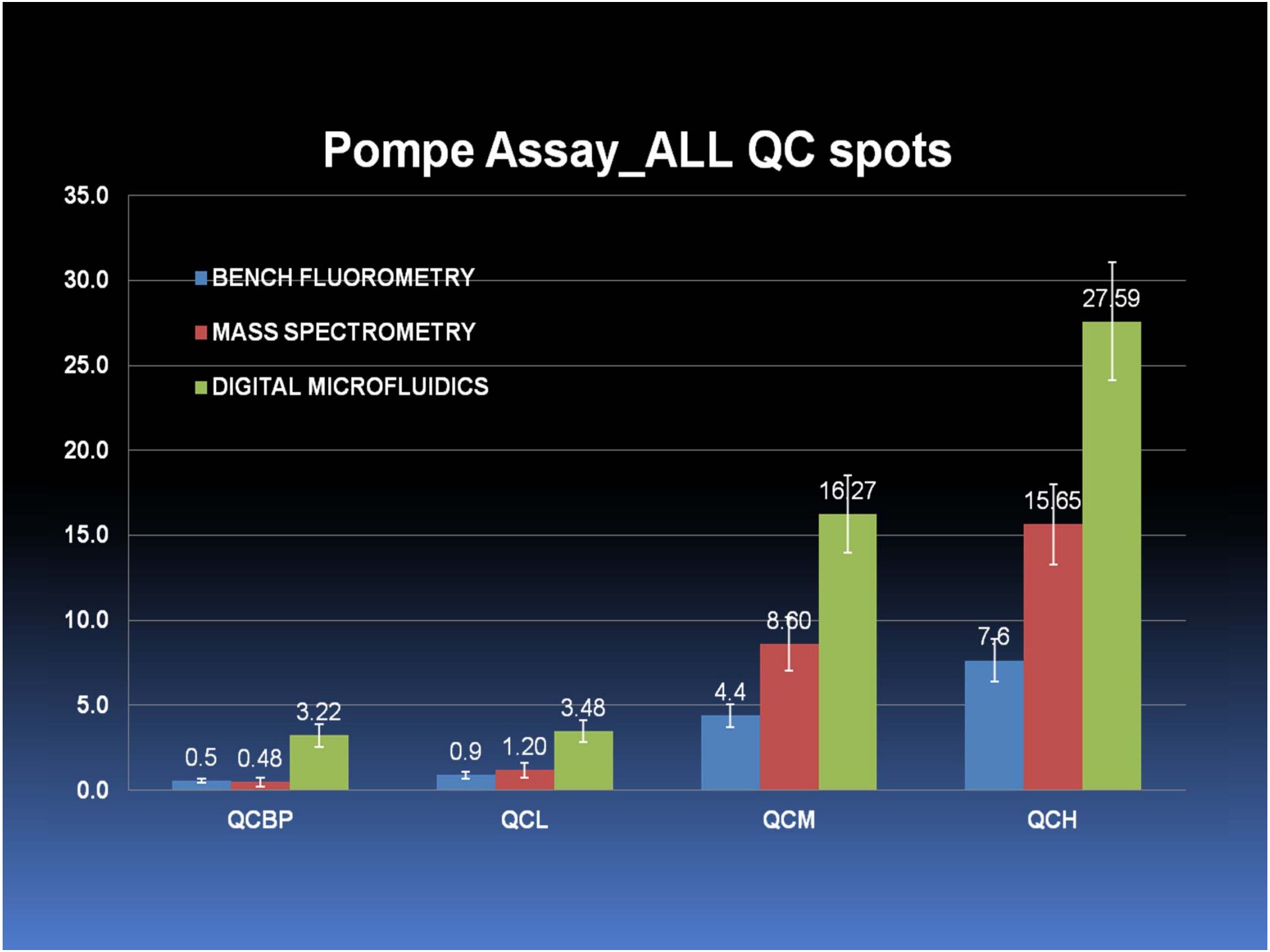
On-Chip Protocol for 5-plex enzymatic assay on High Throughput DMF Cartridge

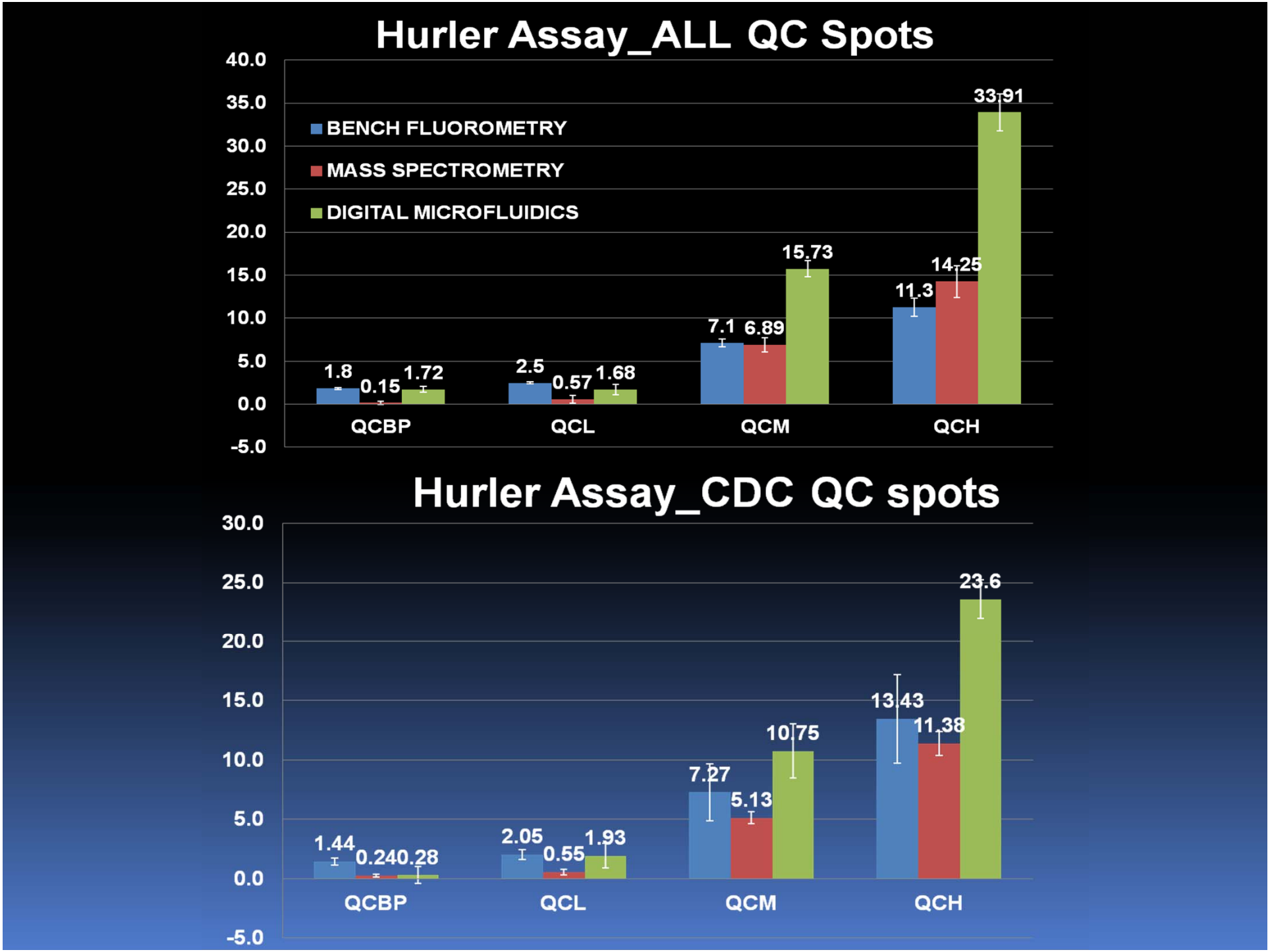
- 5 assays / sample
- 40 samples / cartridge
- 4 calibrants/ cartridge
- 4 controls
- 228 total reactions
- On Cartridge run time <3 hrs



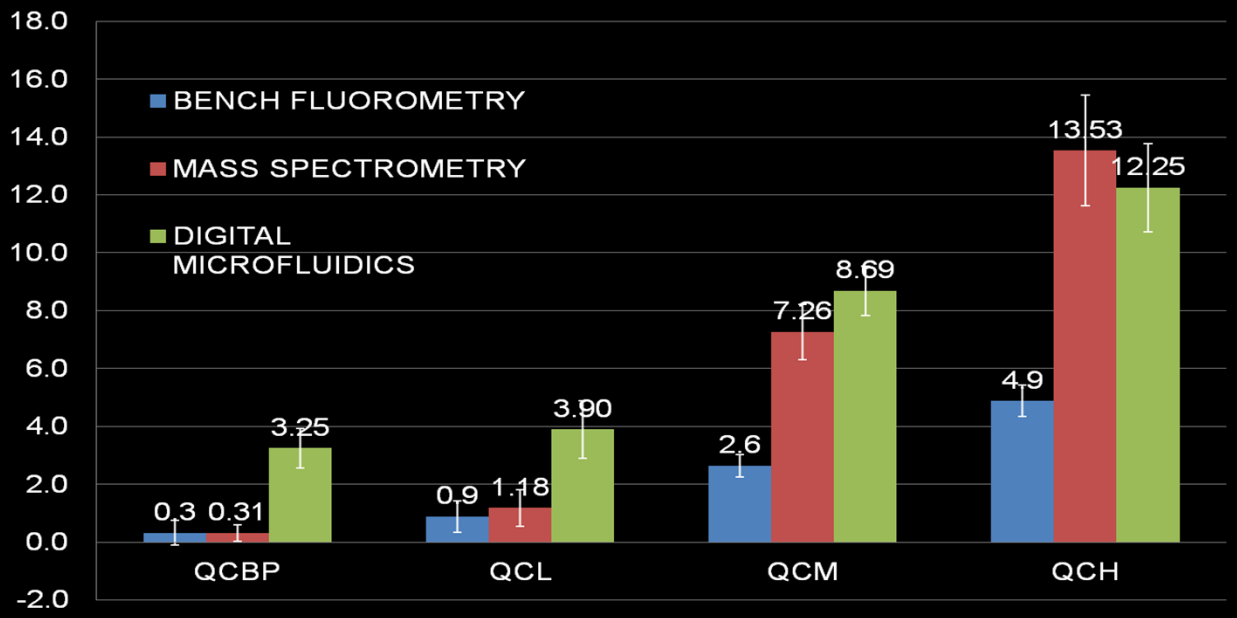
Enzyme activities are in µmol/L/hr: Mean (n=15) ± 95%



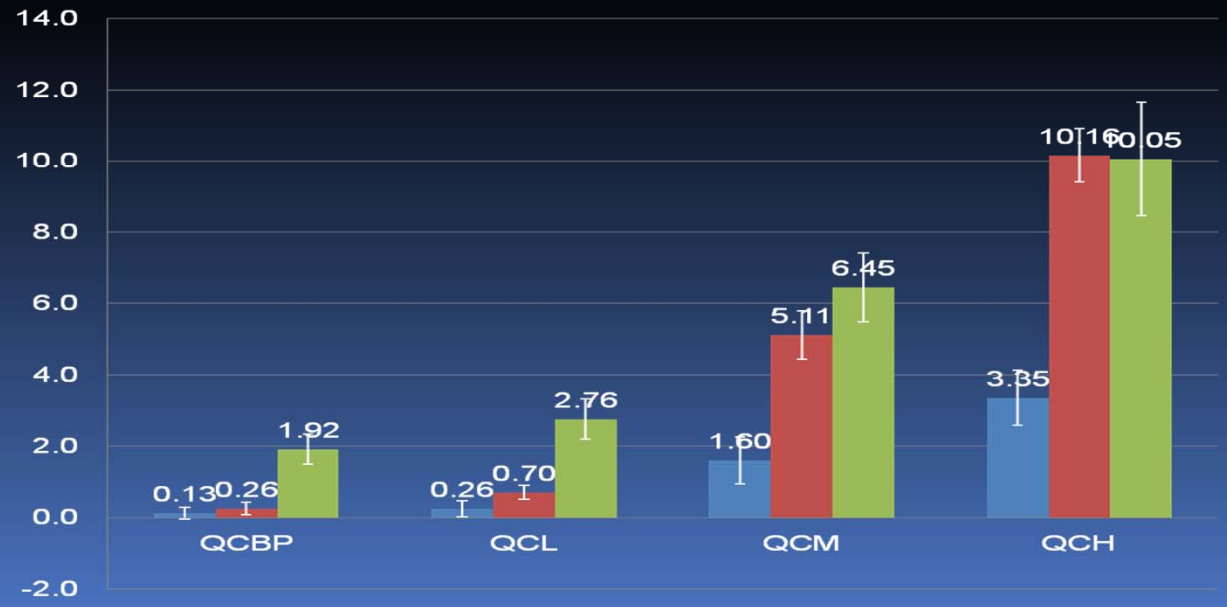

Pompe Assay_ALL QC spots
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
BENCH FLUOROMETRY
MASS SPECTROMETRY
DIGITAL MICROFLUIDICS
0.5
0.48
3.22
0.9
1.20
3.48
4.4
8.60
16.27
7.6
15.65
27.59
QCBP
QCL
QCM
QCH

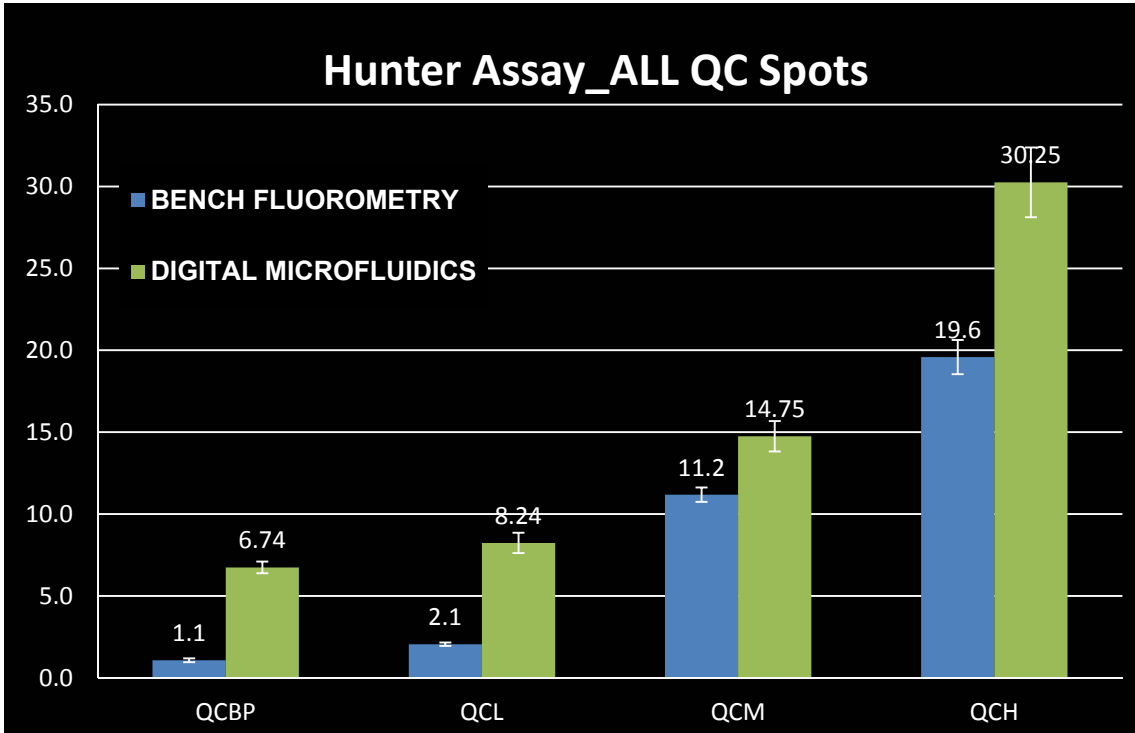

Hurler Assay_ALL QC Spots
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
BENCH FLUOROMETRY
MASS SPECTROMETRY
DIGITAL MICROFLUIDICS
1.8
0.15
1.72
2.5
0.57
1.68
7.1
6.89
15.73
11.3
14.25
33.91
QCBP
QCL
QCM
QCH
Hurler Assay_CDC QC spots
30.0
25.0
20.0
15.0
10.0
5.0
0.0
-5.0
1.44
0.24
0.28
2.05
0.55
1.93
7.27
5.13
10.75
13.43
11.38
23.6
QCBP
QCL
QCM
QCH

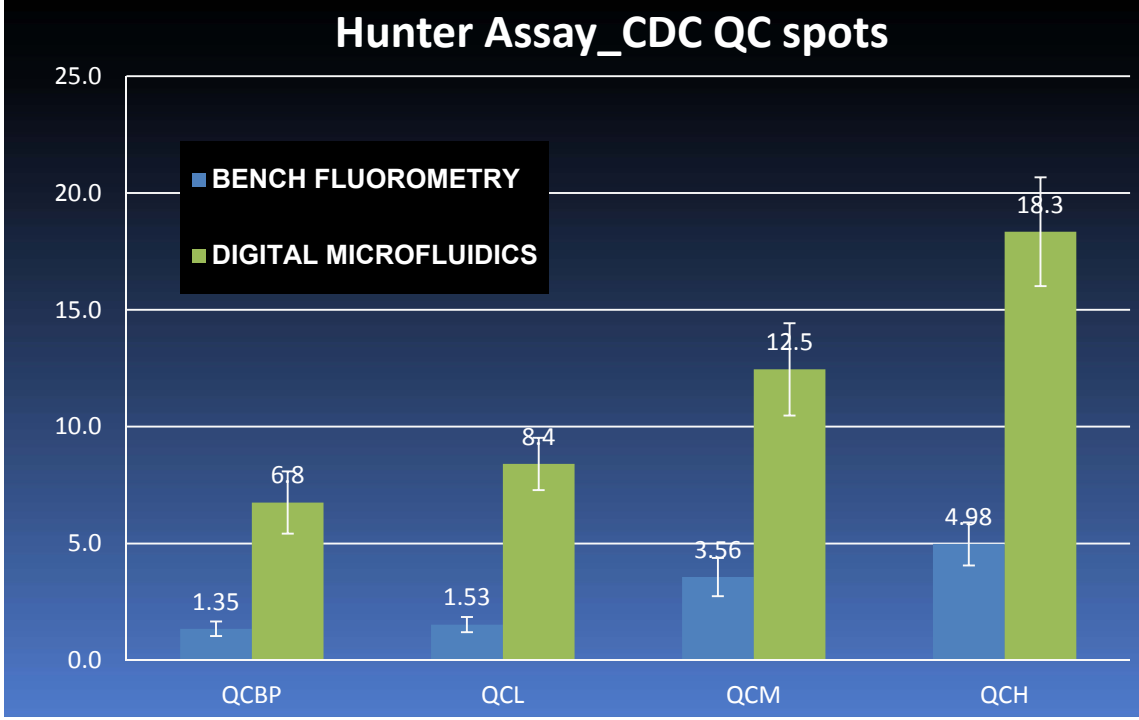
Gaucher Assay_ALL QC Spots



Gaucher Assay_CDC QC spots




Hunter Assay_ALL QC Spots
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
BENCH FLUOROMETRY
DIGITAL MICROFLUIDICS
1.1
6.74
2.1
8.24
11.2
14.75
19.6
30.25
QCBP
QCL
QCM
QCH


Hunter Assay_CDC QC spots
25.0
20.0
15.0
10.0
5.0
0.0
BENCH FLUOROMETRY
DIGITAL MICROFLUIDICS
1.35
6.8
1.53
8.4
3.56
12.5
4.98
18.3
QCBP
QCL
QCM
QCH

Summary

According to these results, both sets of QC samples are suitable for their intended purpose

Disregarding the Base Pool values, CVs for the MSMS and DMF assays were comparable and mostly well within 25%, while those for MTPF were generally higher

Quantitative differences between the activity values for the same enzyme determined by these methods are due to methodological differences

The overall performance of DMF in these assays is comparable with MTPF and MSMS

DMF satisfies the requirements of a high throughput multiplex assay and can be considered a viable platform for LSD NBS

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