Adenosine deaminase severe combined immunodeficiency (ADA-SCID)

Christopher A. Haynes, PhD
Service Fellow, CDC

National Conversation: Tandem Mass Spectrometry in Newborn Screening
February 5-6, 2015

National Center for Environmental Health
Division of Laboratory Sciences

Severe combined immunodeficiency (SCID)

- **Disturbed development of B- and T-cells**
  - Mutations in at least 15 different genes can lead to SCID
  - Overall prevalence is 1:50,000 live births (may be higher)
  - Diagnosis typically uses T-cell receptor excision circles (TRECs); a DNA and PCR-based assay
  - Treatment is typically a bone-marrow transplant (BMT); enzyme replacement & gene therapies have also been used
  - Mouse models of SCID have been created

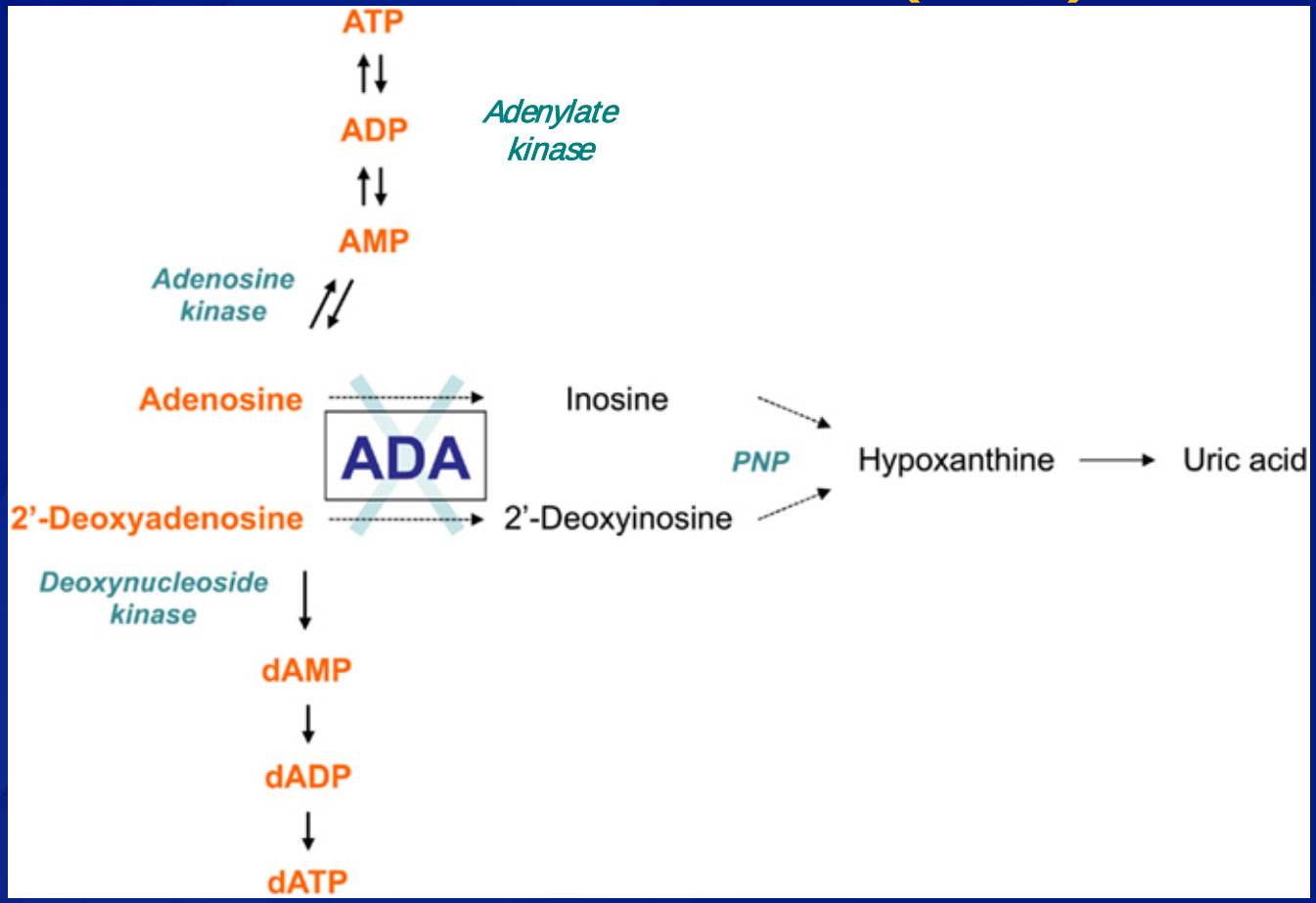
Severe combined immunodeficiency (SCID)

- Different types of SCID
  - X-linked SCID (mutations in interleukin receptor gamma chain)
    Shared by IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21
  - Adenosine deaminase deficiency
    Impaired purine catabolism
  - Purine nucleoside phosphorylase deficiency
    Impaired purine catabolism
  - Reticular dysgenesis (mutations in adenylate kinase 2)
    ATP + AMP ↔ 2 ADP in granulocyte mitochondria
  - Omenn syndrome (mutations in RAG1 or RAG2)
    V(D)J recombination
  - Bare lymphocyte syndrome (mutations in MHC II regulatory genes)
  - JAK3 (mutations in Janus Kinase 3)
    Downstream transducer of IL gamma chain signaling
  - Artemis / DCLRE1C (most common in Navajo and Apache)
    V(D)J recombination and DNA repair

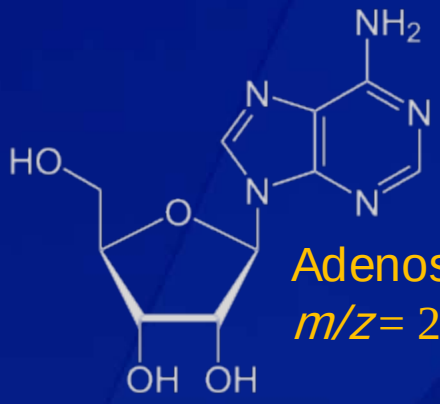
ADA-SCID (~15% of all SCID cases)

- **Mutations in adenosine deaminase (ADA)**
  - ADA catabolizes adenosine and deoxyadenosine to form inosine and deoxyinosine
  - Lack of ADA activity results in accumulation of deoxyadenosine
  - Deoxyadenosine is phosphorylated to generate dATP
  - Accumulation of dATP inhibits ribonucleotide reductase
  - Biosynthesis of deoxyribonucleotides is decreased
  - Lowered dNTP concentrations inhibit DNA synthesis
  - Impaired proliferation of lymphocytes
  - Immune system is compromised

Purine Catabolism (ADA)



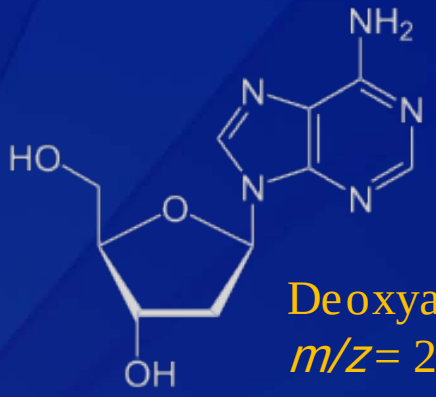
Adenosine deaminase (ADA)



Adenosine
m/z = 268.2

ADA

Inosine
m/z = 269.2



Deoxyadenosine
m/z = 252.2

ADA

Deoxyinosine
m/z = 253.2

Neonatal screening for severe combined immunodeficiency caused by an adenosine deaminase defect: A reliable and inexpensive method using tandem mass spectrometry

Chiara Azzari, MD, PhD,[a,b] Giancarlo la Marca, PharmSc,[b,c] and Massimo Resti, MD[a,b] *Florence, Italy*

J. Allergy Clin. Immunol., v127 (6), 2011

- Sample: 3.2 mm punch of DBS specimen
- Extraction: 66:33 methanol / water containing hydrazine and internal standards (stable-isotope labeled AA, AC, SUAC, Ade, dAde), 30 min @37ºC shaking
- Analysis: Flow-injection tandem mass spectrometry

  Positive ion mode

  Ade: 268.2→136.1 (ribose-^{13}C-Ade 269.2→136.1)

  dAde: 252.2→136.1 ($^{13}C_5$-dAde 257.2→136.1)

J. Allergy Clin. Immunol., v127 (6), 2011

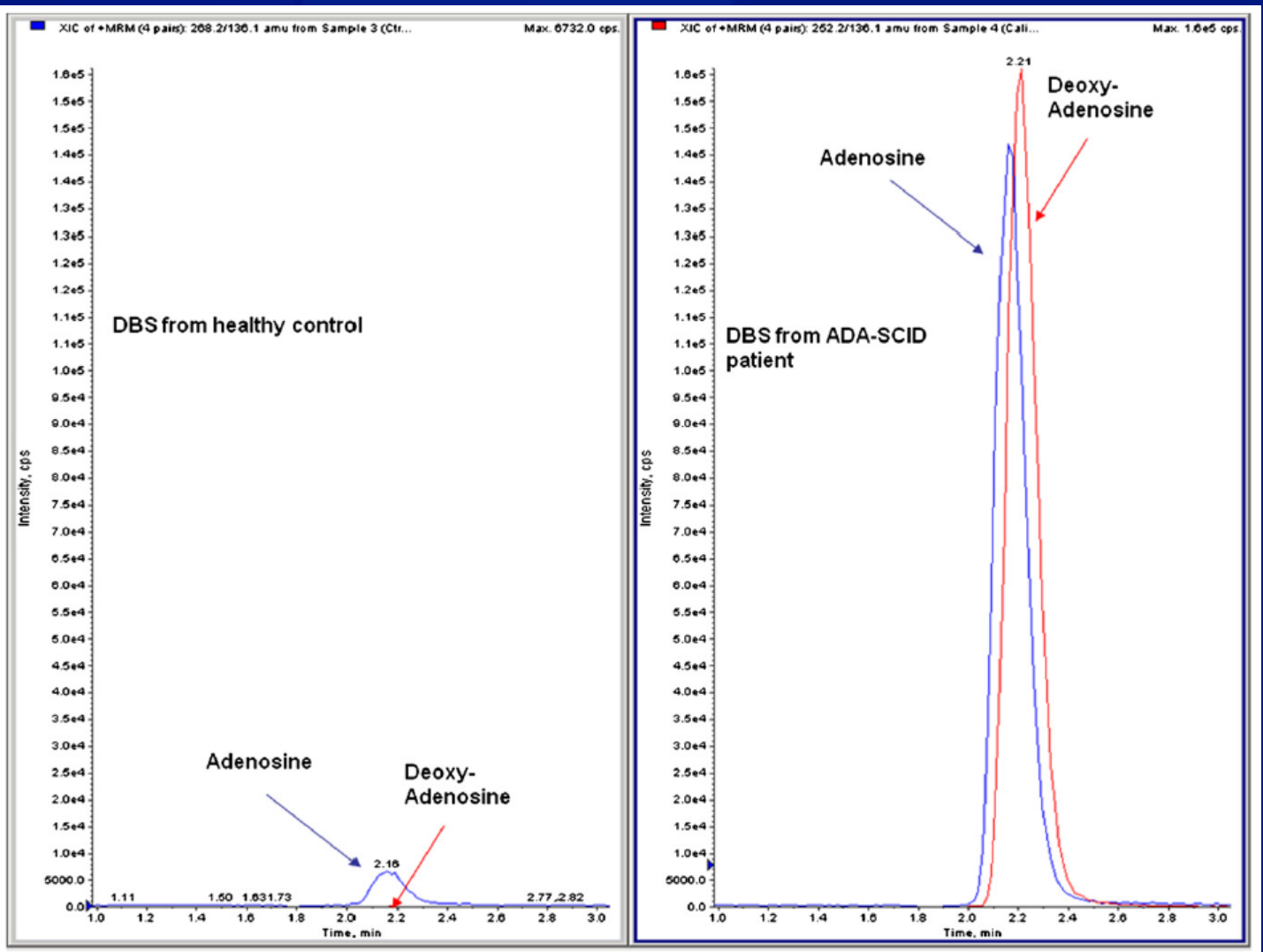


FIG 1. Histograms of metabolite (adenosine and 2′-deoxyadenosine) concentration in a patient with ADA-SCID and a representative control subject. Adenosine and 2′-deoxyadenosine levels of a patient with ADA-SCID *(right)* and a representative healthy control subjects *(left)*, as obtained from DBS samples taken at birth, are shown. Adenosine is shown in *blue*, and 2′-deoxyadenosine is shown in *red*.

Neonatal screening for severe combined immunodeficiency caused by an adenosine deaminase defect: A reliable and inexpensive method using tandem mass spectrometry

Chiara Azzari, MD, PhD,[a,b] Giancarlo la Marca, PharmSc,[b,c] and Massimo Resti, MD[a,b] *Florence, Italy*

J. Allergy Clin. Immunol., v127 (6), 2011

Reference standard blood (whole blood) spots were prepared by using a pooled whole-blood sample obtained from 5 subjects. The blood was processed by adjusting the hemoglobin concentration to 17 mg/dL and adding adenosine and deoxyadenosine at known concentrations. The processed blood was dispensed onto filter paper cards to form blood spots on the filter paper matrix. Each blood spot was generated by dispensing 25 μL of processed blood. The blood spots were allowed to dry overnight.

J. Allergy Clin. Immunol., v127 (6), 2011

TABLE I. Results of the imprecision assay for adenosine and deoxyadenosine detection in DBS samples: imprecision within run, between runs, within day, and between days

Investigated metabolite	Spiking (nmol/L)	Intraday precision (%), n = 6	Interday precision (%), n = 6	Average readings (nmol/L)	Accuracy (n = 6)
Adenosine	0	0	0.0	0.0	
Adenosine	33	3.5	3.1	34.0	103.1
Adenosine	165	4.9	3.7	158.0	95.8
Adenosine	330	7.8	6.0	336.2	101.9
Adenosine	3300	3.8	4.8	3299.7	100.0
Adenosine	6600	2.1	2.6	6594.2	99.9
Adenosine	9900	2.3	2.0	9899.9	100.0
Deoxyadenosine	0	0	0.0	0.0	
Deoxyadenosine	33	19.6	16.9	32.8	92.9
Deoxyadenosine	165	6.6	4.8	169.6	100.3
Deoxyadenosine	330	5.2	3.6	325.4	100.6
Deoxyadenosine	3300	5.6	6.7	3300.2	100.3
Deoxyadenosine	6600	3.4	3.4	6599.2	100.0
Deoxyadenosine	9900	3.1	3.1	9904.9	100.0

10 µM

10 µM

J. Allergy Clin. Immunol., v127 (6), 2011

	N	Ade, µM	dAde, µM
Normal	12,020	0.23 ± 0.09 (0.01 – 0.81)	< LOD (< 0.005)
Patients	4	7.8 ± 3.1 (4.4 – 11.8)	8.5 ± 6.0 (2.5 – 16.2)
Ratio of Patient / Normal		34	1700

- No overlap between normal and patient for analytical values
- No indeterminate or false-negative results
- Uses same DBS punch as AA, AC, SUAC so almost no extra cost per sample

BMSL's Goal:

- Produce quality control and proficiency testing materials for ADA-SCID
- Enrich blood with Ade and dAde at concentrations similar to patients
- Analyze DBS using published / patented method of La Marca, et al.

Methods and Results:

- DBS prepared with 0, 10, and 100 µM enrichments of Ade and dAde
- Analyzed as described using published / patented method
- Very small peaks of similar intensity in all DBS specimens
- "Where's the beef?"
- Same results from repeat preparations, whole blood, lysed blood
- Results suggested enriched analytes were being enzymatically degraded
  (previously observed with L-Arginine and Argininosuccinic acid)

Journal of Pharmaceutical and Biomedical Analysis 88 (2014) 201–206

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

The inclusion of ADA-SCID in expanded newborn screening by tandem mass spectrometry

Giancarlo la Marca[a,b,*], Elisa Giocaliere[a], Sabrina Malvagia[a], Silvia Funghini[a], Daniela Ombrone[a], Maria Luisa Della Bona[a], Clementina Canessa[c], Francesca Lippi[c], Francesca Romano[c], Renzo Guerrini[a,b], Massimo Resti[d], Chiara Azzari[c]

Calibrators were prepared at 0.1, 1, 10 and 100 μmol/L of dAdo and 0.2, 1, 10 and 100 μmol/L of Ado as follows: a 3.2 mm disk from a DBS was punched and extracted by dispensing 300 μL of a mixture of methanol and water (2:1, v/v) containing ribose-1-^{13}C-adenosine and $^{13}C_5$ deoxyadenosine (10 μmol/L) as internal standard and different concentrations of Ado and dAdo. Calibrators were shaken on a vortex system for 25 min at 37 °C and then transferred to a 96-well plate.

A Synergi® fusion RP column (150 mm × 2 mm i.d.; 4 μm particle size) was used for the analysis. The mobile phase composed

J. Pharm. Biomed. Anal., v88, 2014

Calibrators were prepared at 0.1, 1, 10 and 100 μmol/L of dAdo and 0.2, 1, 10 and 100 μmol/L of Ado as follows: a 3.2 mm disk from a DBS was punched and extracted by dispensing 300 μL of a mixture of methanol and water (2:1, v/v) containing ribose-1-^{13}C-adenosine and $^{13}C_5$ deoxyadenosine (10 μmol/L) as internal standard and different concentrations of Ado and dAdo. Calibrators were shaken on a vortex system for 25 min at 37 °C and then transferred to a 96-well plate.

Adenosine deaminase is present into the cells as well as on membranes and it catalyzes the irreversible deamination of Ado and dAdo to inosine and deoxyinosine. For this reason standards were not added to the whole blood to prevent ADA metabolism. Calibrators, prepared using a pooled whole blood obtained from healthy donors spotted on filter paper, were extracted as above described. The extracts were enriched of different Ado, dAdo and their internal standard concentrations and were analyzed to test validation parameters.

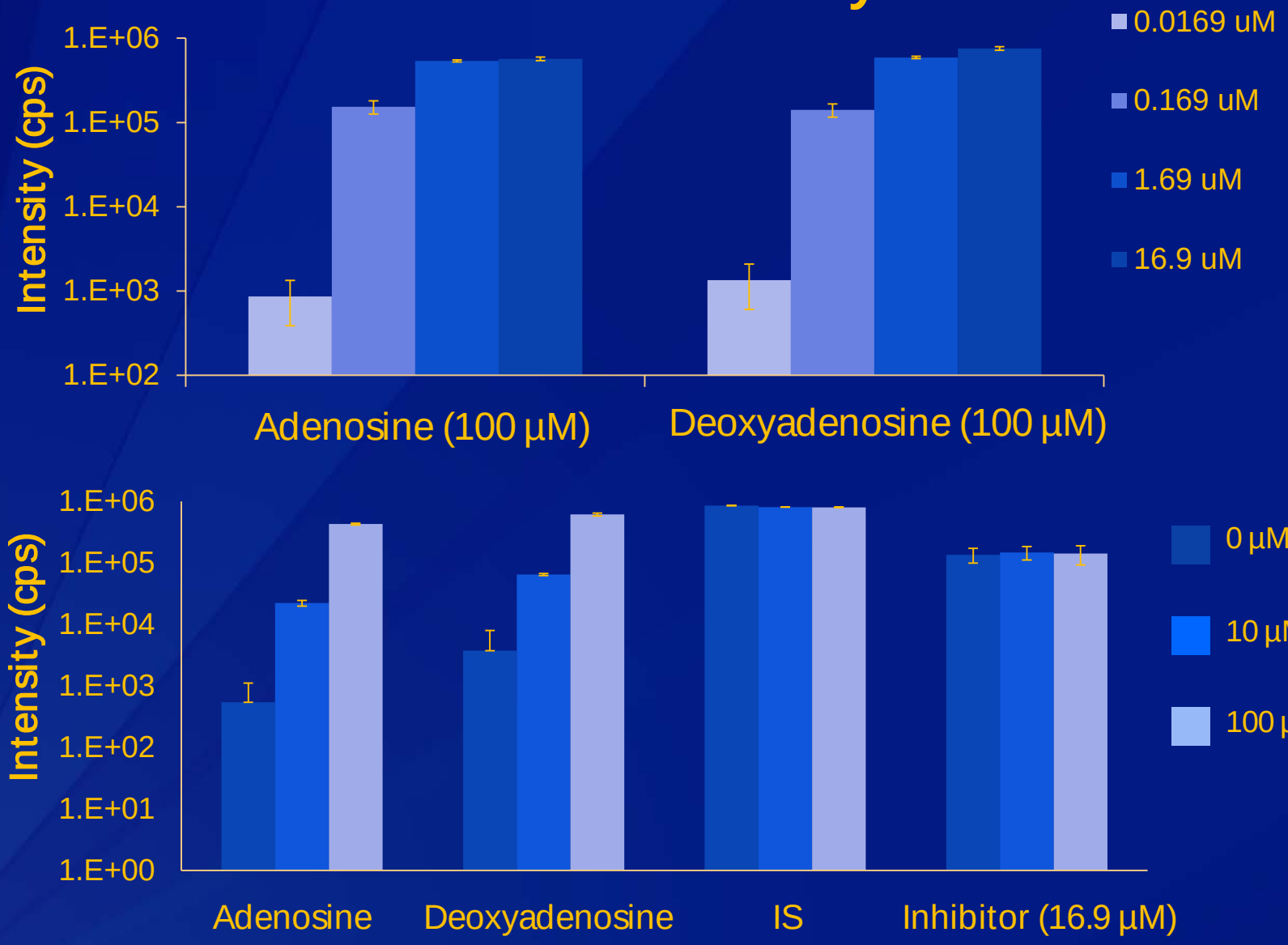
BMSL's Goal:

- Produce quality control and proficiency testing materials for ADA-SCID
- *Inhibit ADA activity in the blood used to prepare DBS*
- Enrich blood with Ade and dAde at concentrations similar to patients
- Analyze DBS using published / patented method of La Marca, et al.

Methods and Results:

- *Stir blood with an inhibitor of ADA activity*
- DBS prepared with 0, 10, and 100 µM enrichments of Ade and dAde
- Analyzed as described using published / patented method
- Peaks of increasing intensity in DBS specimens with increasing enrichment
- Approximately 80% recovery of dAde between 1 and 100 µM enrichment
- Results suggested Ade was still being enzymatically degraded at lowest concentrations

Inhibition of ADA activity in blood



ADA-SCID Summary

- ~ 15% of all SCID cases
- Biochemical markers (adenosine & deoxyadenosine) detectable by MS/MS
- Simple extraction of DBS punches, multiplex analysis with AA, AC, SUAC
- Can be incorporated into routine newborn screening (Tuscany)
- Can be used as second-tier test to identify SCID sub-type as ADA deficiency
- ADA activity must be inhibited to produce QC and PT DBS enriched with adenosine and deoxyadenosine
- Availability of QC and PT DBS will support the expansion of ADA-SCID detection by MS/MS analysis

Acknowledgments

Candice Hovell (ORISE)
David Foreman (ORISE)
Victor R. De Jesus
Carla Cuthbert

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: http://www.cdc.gov