A Brief History of Newborn Screening

The first 50 years.

Ken Pass

I didn’t do this alone…

Thanks to:

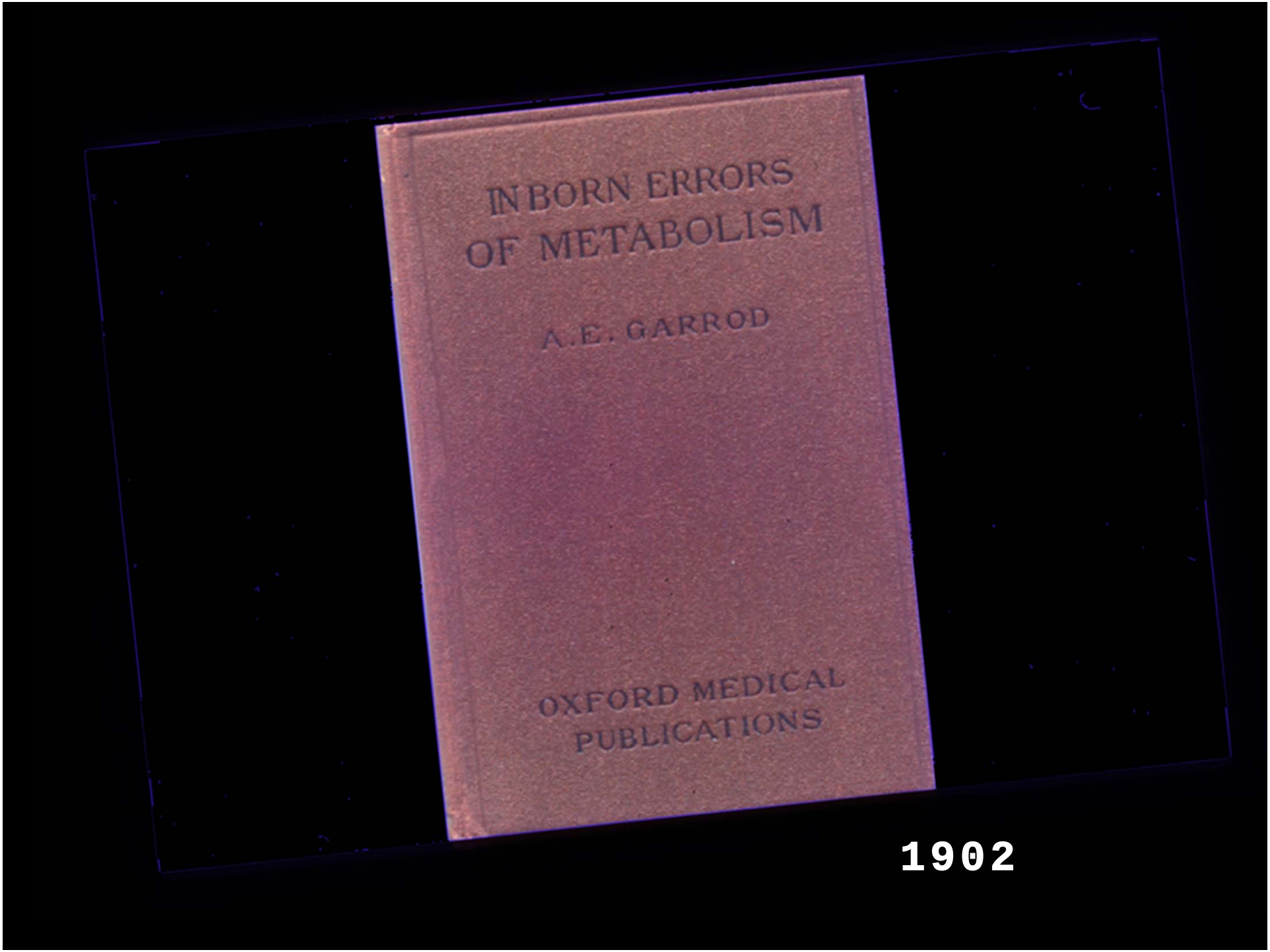
Amy Hoffman

Alex Kemper

Kathy Harris

Piero Rinaldo

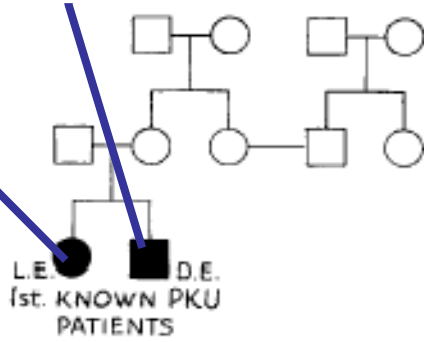
…and others


INBORN ERRORS
OF METABOLISM
A.E. GARROD
OXFORD MEDICAL
PUBLICATIONS
1902

Asbjorn Follin

Liv

Dag



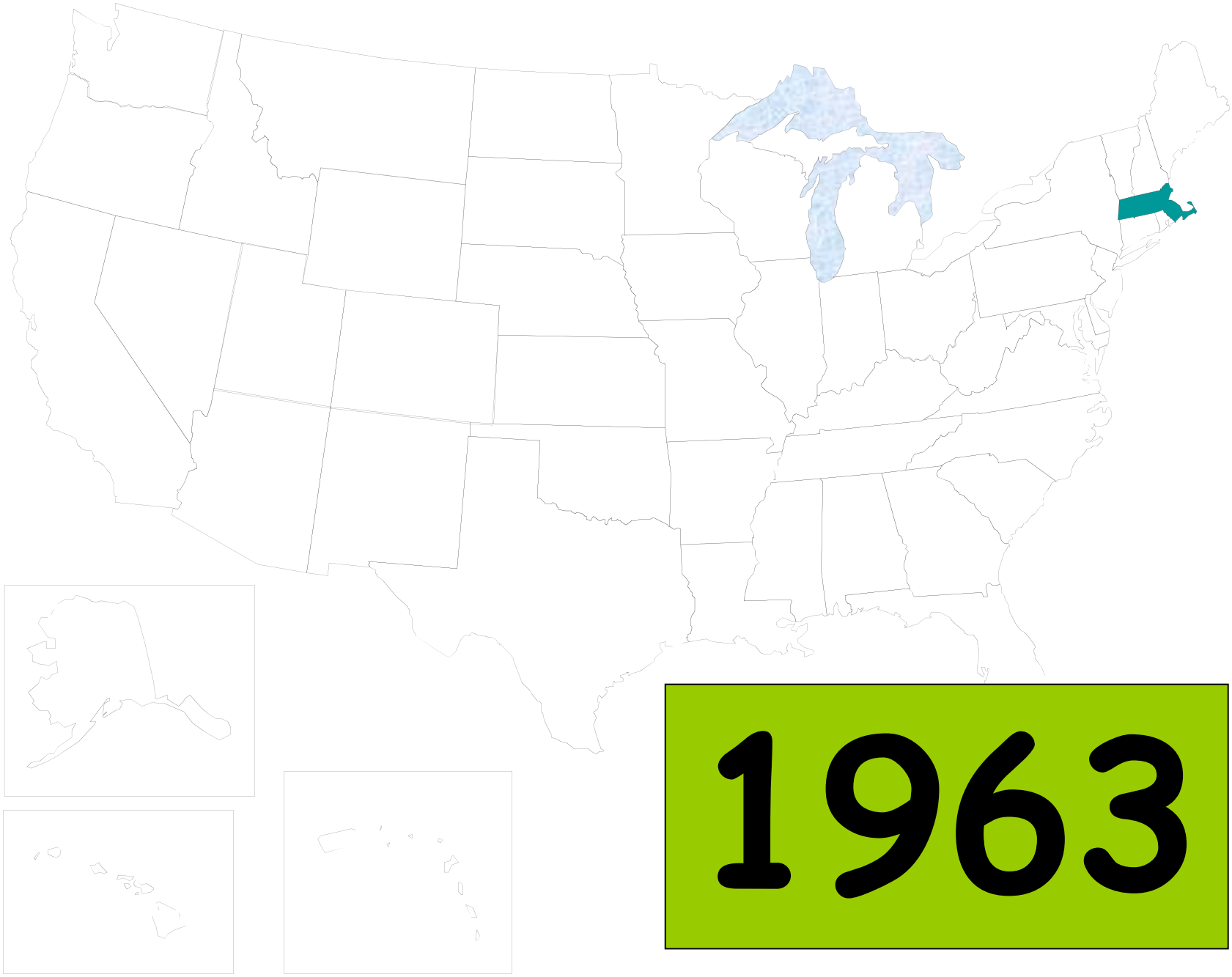
Borgny and Harry Egeland

Pediatrics 2000;105;89-103

"It all began with Johnny."
Screening 1992;1:1.

Dr. Robert Guthrie

1916-1995


1963

There were critics…

"At this time, the American Academy of Pediatrics favors neither the extension of current compulsory legislation nor passage of new legislation for the compulsory testing of newborn infants for the presence of congenital metabolic disease."

April 1967

And still today...

Newborn screening: A spot of trouble

By raising hell about newborn blood-spot screening, Twila Brase could jeopardize public-health programmes and derail research. The problem is, she has a point.

Nature 2011

Then….and….Now

1963

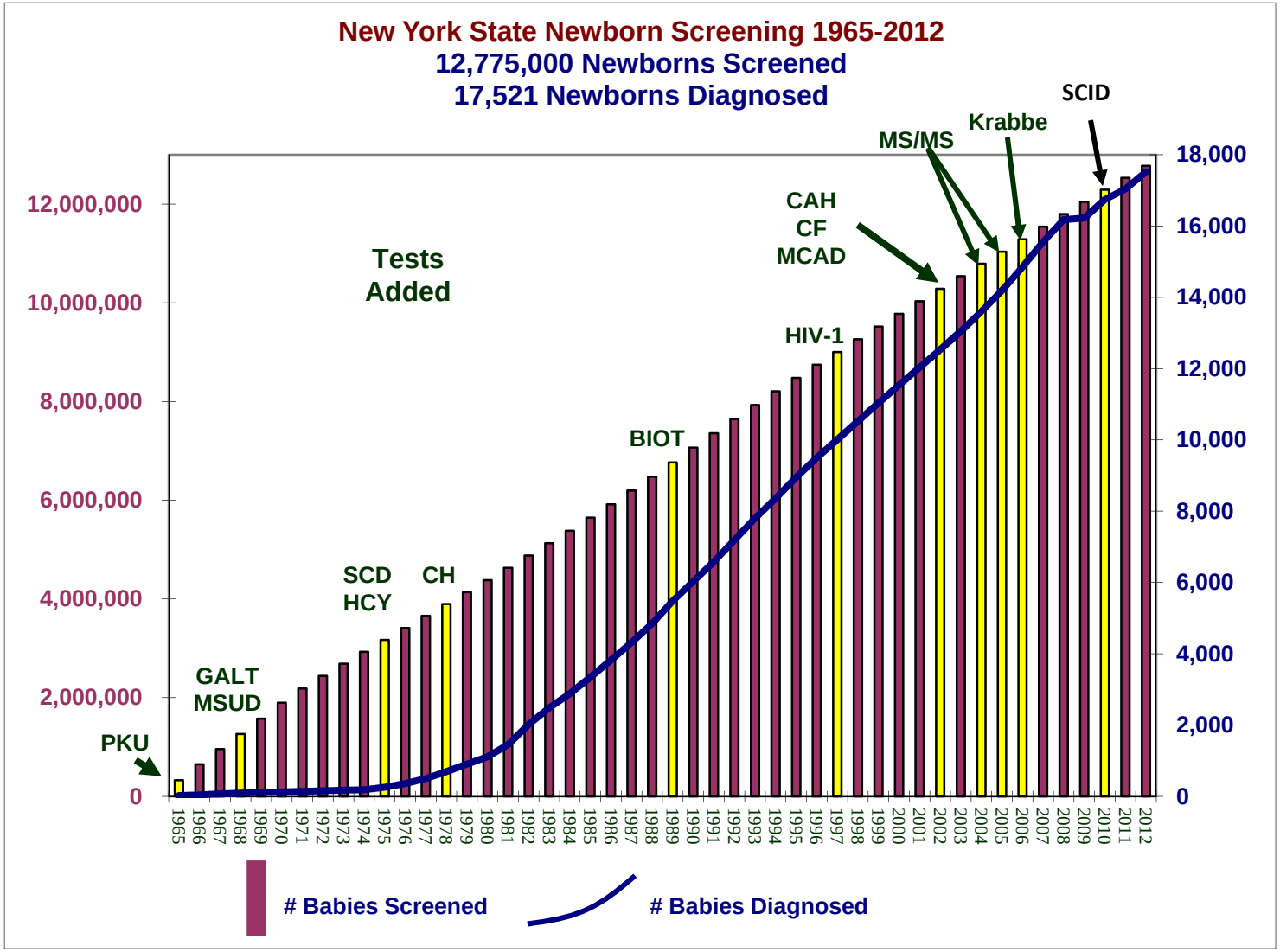
- **3-5 days**
- **PKU**
- **one lab test**
- **no DNA**
- **20 positive cases**

2013

- **24 hours**
- **PKU + 50 more**
- **8 tests**
- **DNA**
- **900^{+} cases**

Newborn screening today

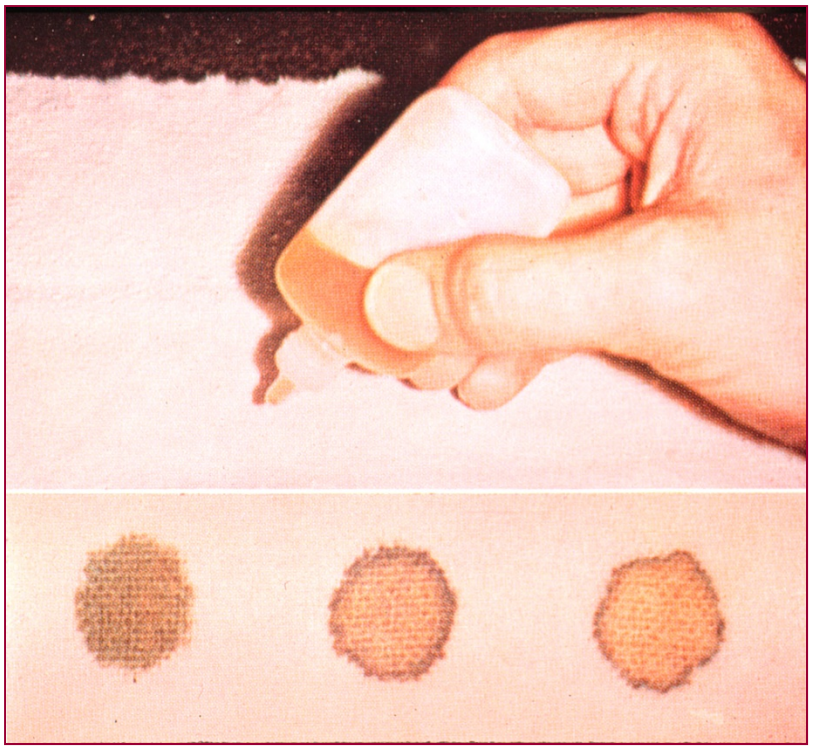
- every state provides a screening program
- 15,000 newborns tested daily
- 58 newborns referred daily
- 3 infants identified during this talk


New York State Newborn Screening 1965-2012
12,775,000 Newborns Screened
17,521 Newborns Diagnosed
SCID
Krabbe
MS/MS
CAH
CF
MCAD
HIV-1
BIOT
SCD
HCY
CH
GALT
MSUD
PKU
Tests
Added
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
1965 1966 1967 1968 1969 1970 1971 1972 1973 1974 1975 1976 1977 1978 1979 1980 1981 1982 1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012
Babies Screened
Babies Diagnosed

Specimen type

- **Diaper or urine**
- **Guthrie specimen**
- **Cord blood**
- **Archived specimens**

Early technology



A test for PKU

Basic Questions in Newborn Screening

Who should be tested?

When should the test be done?

How should the analysis be done?

What should be done with the results?

	CONC.	AMT IN
ANALYTE	RANGE	1/8 in. DBS
17-OHP	15-750 pmol/mL	23-1125 fmol
T4	26-400 pmol/mL	39-600 fmol
Carnitines	0.5-10 nmol/mL	2-30 pmol
Amino acids	60-900 nmol/mL	0.2-2.7 nmol
Galactose	0.3-3 umol/mL	1-8 nmol
TSH	1-36 ng/mL	2-50 pg
IRT	10-400 ng/mL	30-1200 pg
Hemoglobins	0.9-180 mg/mL	2.7-540 ug
DNA	6 pg/cell	190 ng
	10,000 cell/uL	

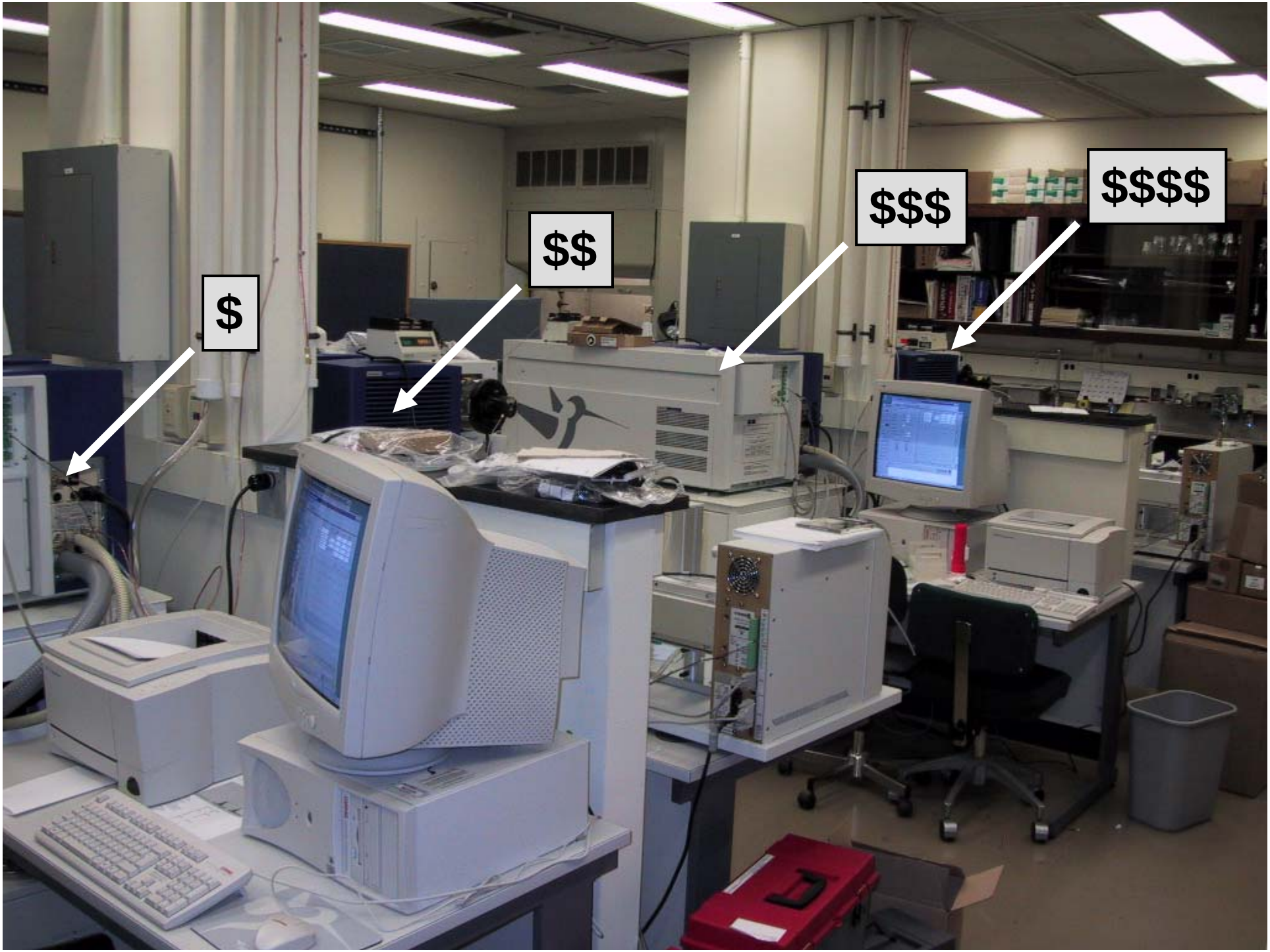
R Bellisario

Evolution of NBS

1957	**Diaper test in California**
1958	**Phenistix in Europe**
1963	**Guthrie & Susi publish: BIA for PKU and develop the use of dried blood specimens**
1963	**Massachusetts universal screening**
1975	**Electrophoresis for SSD – first multiplex test**
1994	**MS/MS – second multiplex test**
2000	**DNA analysis as second tier**
2006	**LSDs – first to challenge functioning of system**
2010	**SCID – DNA as first tier**
2020	**???**

NBS – A Slave of Technology

- Wet Diaper
- BIA
- Electrophoresis
- RIA
- EIA
- Isoelectric focusing
- msms
- PCR



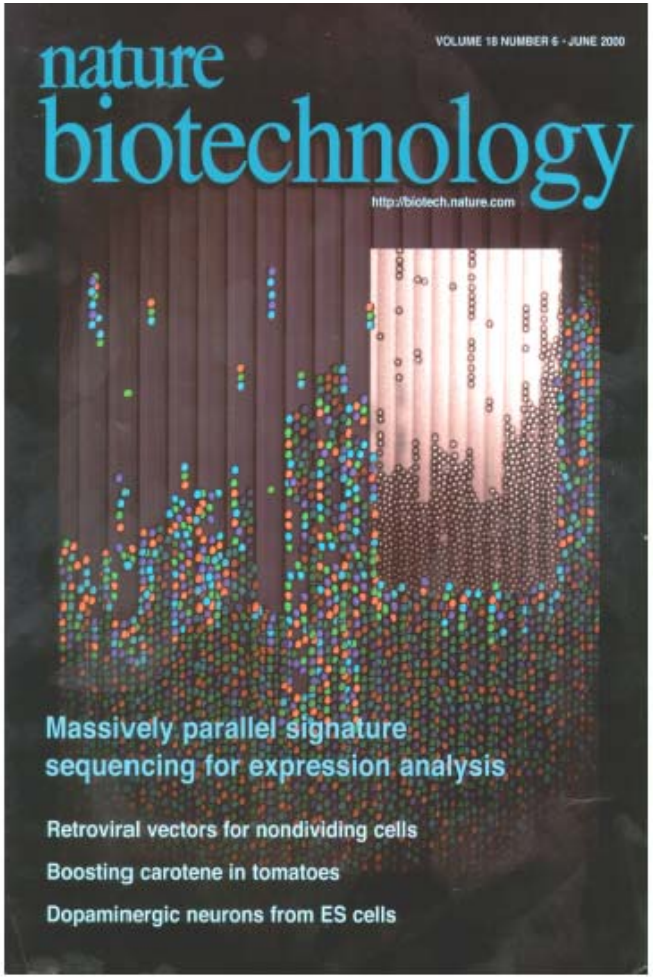
Some Disorders Detectable by MS-MS

- **Amino acidemias**
  - **Phenylketonuria**
  - **Maple syrup urine disease**
  - **Homocystinuria**
  - **Citrullinemia**
  - **Hepatorenal tyrosinemia**
- **Organic acidemias**
  - **Propionic acidemia**
  - **Methylmalonic acidemia**
  - **Isovaleric acidemia**
  - **3-Methylcrotonylglycinemia**
  - **Glutaric acidemia type 1**
  - **Hydroxymehtyglutaric acidemia**
- **Fatty acid oxidation disorders**
  - **SCAD deficiency**
  - **MCAD deficiency**
  - **VLCAD deficiency**
  - **LCHAD and trifunctional protein deficiency**
  - **Glutaric acidemia type II**
  - **CPT-II deficiency**

…and more

NBS – A Slave of Technology

- Wet Diaper
- BIA
- Electrophoresis
- RIA
- EIA
- Isoelectric focusing
- msms
- microarrays



Guthrie Card Data

Multiple DNA extraction methods examined from stored Guthrie Card specimens

Quality and quantity of DNA determined, successful extractions run on generic arrays

- Preliminary results indicate that the Guthrie spot will provide suitable DNA, both in quantity and quality, for better than acceptable performance on Affymetrix DNA chips

Collaboration: Robin Pietropaolo, Wadsworth Center; Michelle Caggana, Wadsworth Center; Kenneth Pass, Wadsworth Center; John Palma, Affymetrix; Janet Warrington, Affymetrix


AFFYMETRIX®
@520019000000000000SAMPLES41909
NBS Chip®
P/N: 520019
Lot #: 1
Exp. Date: 040125

Currently screened conditions

- **PKU**
- **BIOT**
- **HCY**
- **MSUD**
- **GAL**
- **CH** ✓
- **CAH**
- **CF**
- **SSD**
- **MCADD**
- **HIV** ✓
- **TYR**
- **G-6-PD**
- **TOXO** ✓

Organic Acidemias

GA-1
HMG
ICBD
IVA
MA
3-MCC
MMA
BKT
PA
2MBCD
MCD
3MGA
MHBD

Fatty Acid Oxidation Defects

CPT-1
CPT-2
CAT
CUD
LCH
[illegible]D
[illegible]CHAD
MCKAT
TFP
LCAD/VLCAD
2,4 Dienoyl-CoA Reductase Deficiency

Amino Acidemias U[illegible] Cycle Disorders

[illegible]A
CPS
CIT Type 1 or II
HHH
Nonketotic hyperglycinemia
5-oxoprolinuria
Tyrosinemia type I and II

Autosomal recessive

And in the future NBS panel?

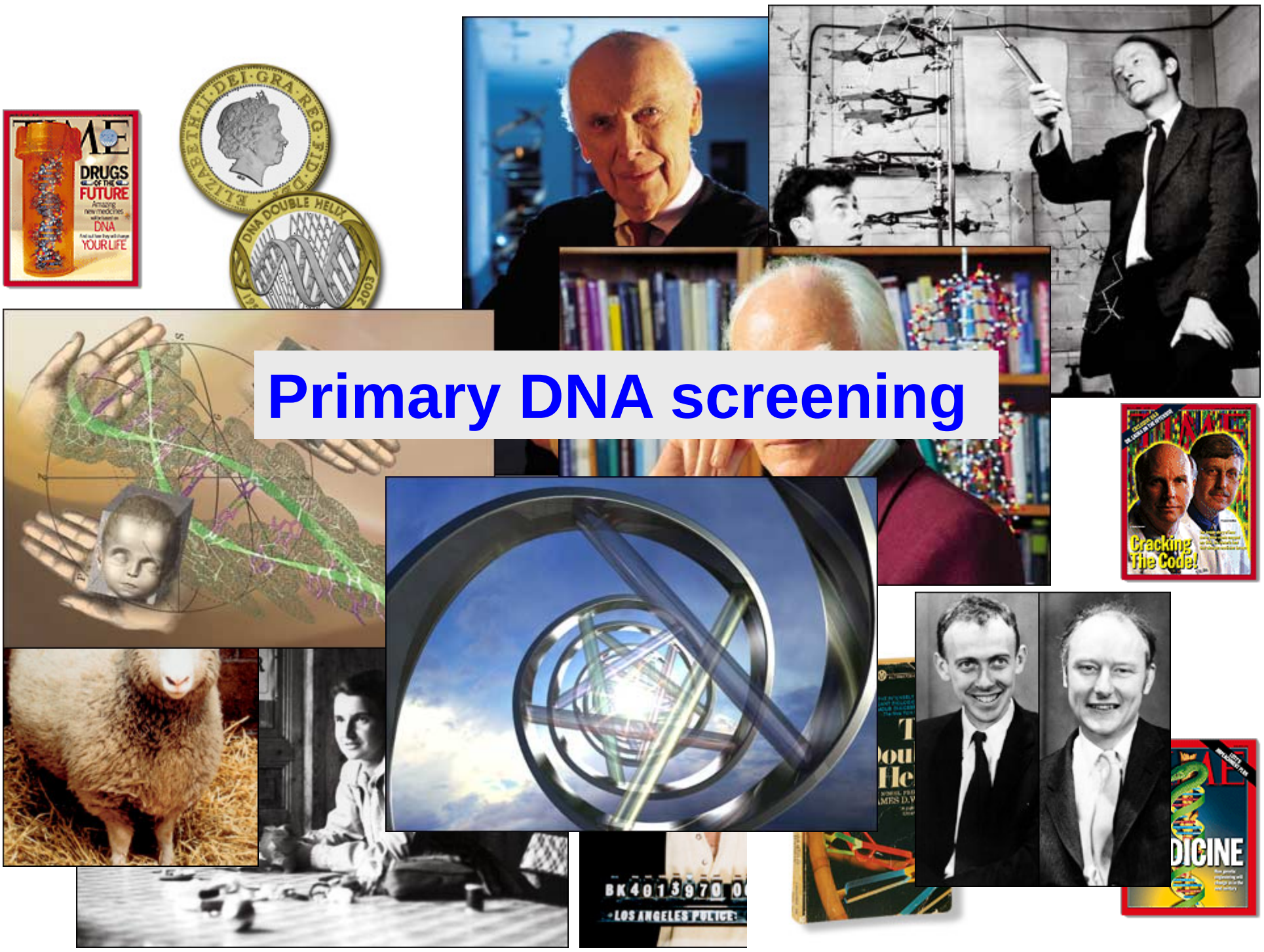
All identifiable by mutation analysis

Diabetes	Duchenne MD	GAMT deficiency
Cancer	Becker MD	AGAT deficiency
Hemochromatosis	SCID	Fabry
Asthma	Turner	Krabbe
Astrocytomas	Wi	Pompe
Neuroblastoma	terin defects	Hurler-Scheie
Hirschsprung	PS deficiency	Other LSD's
Lupus	OTC deficiency	GALK
Autism	Citrullinemia	GALE
Hemogl athies	Argininemia	E3 deficiency
Fragile	Hearing loss	CDG

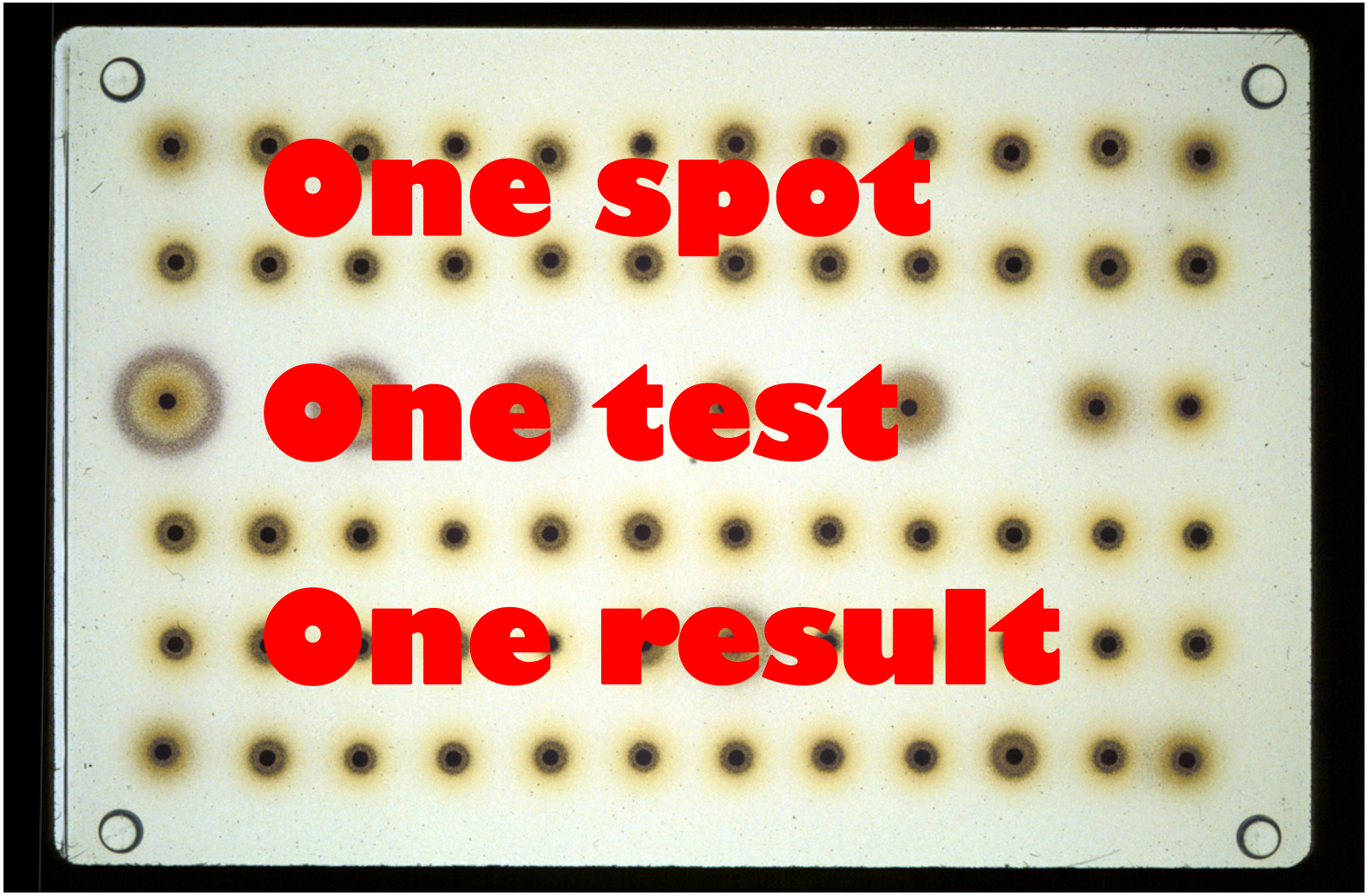
Issues to address

in DNA primary screening

- Genotype/phenotype correlations
- Private mutations
- Controls (CDC program? Private?)
- QA and QC (CDC program? Private?)
- Cost
- Others?



Primary DNA screening



Guthrie test for PKU

Each day:

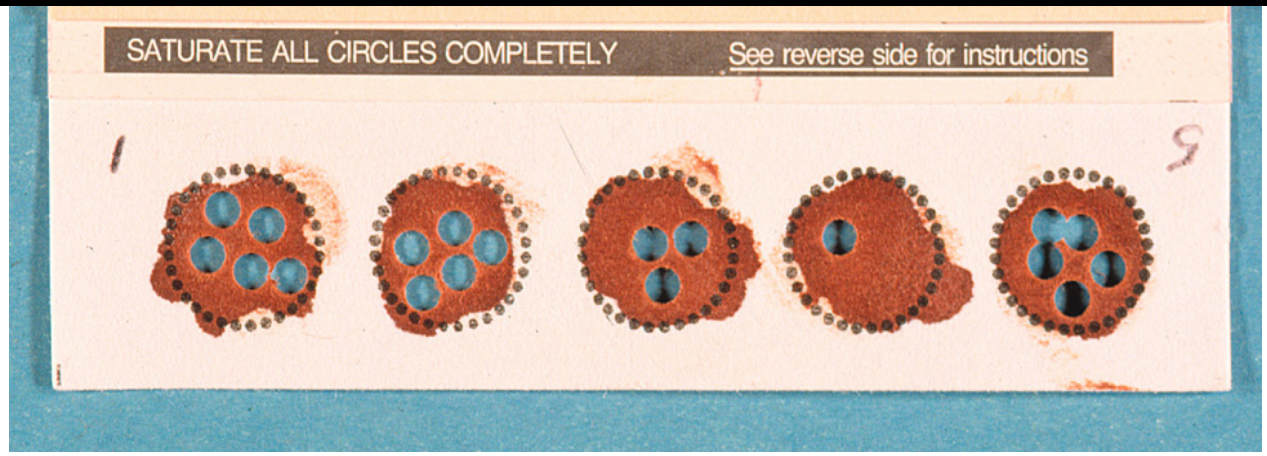
1000 specimens

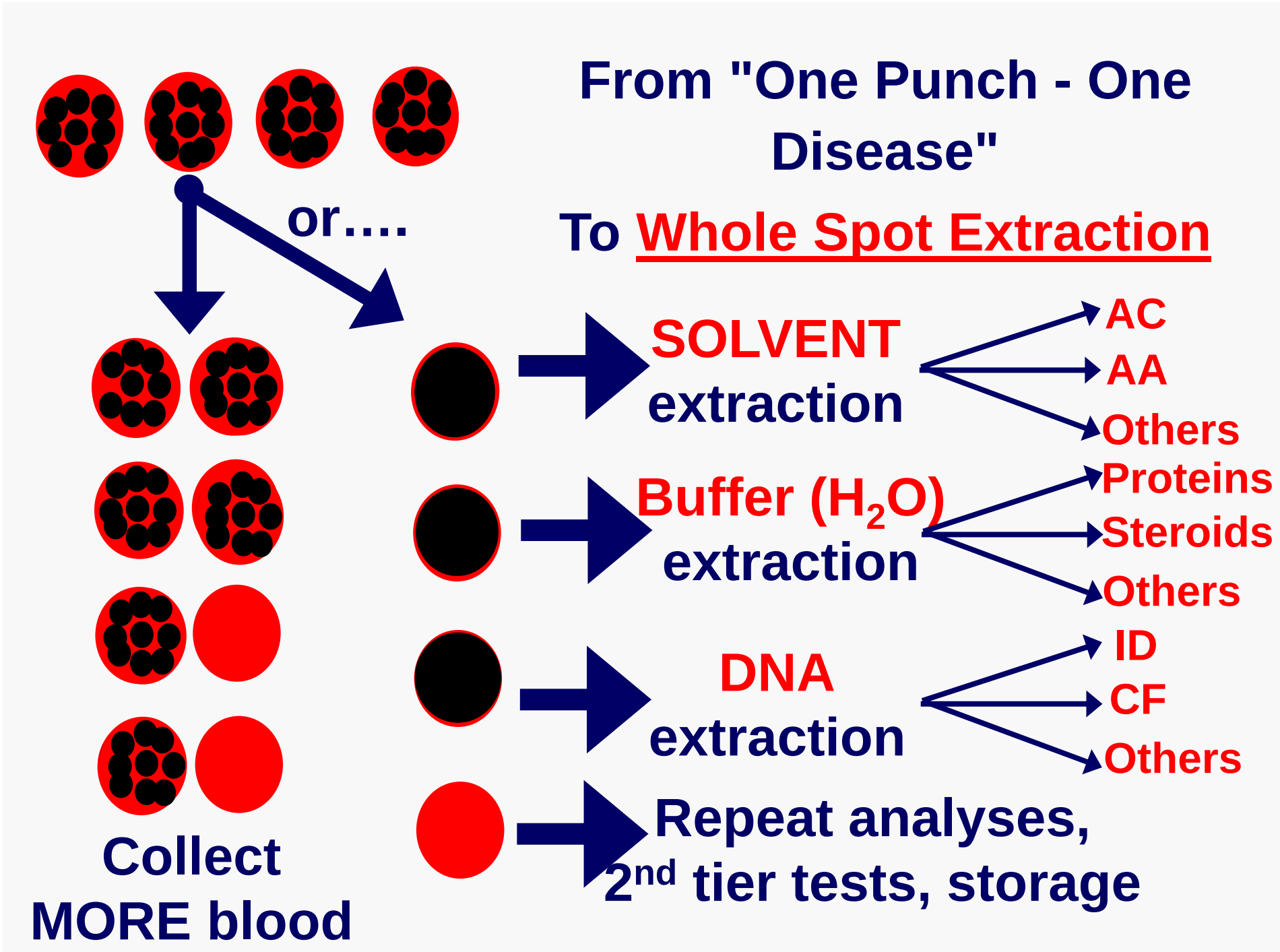
8 screening analyses

8 confirmatory analyses on 10%

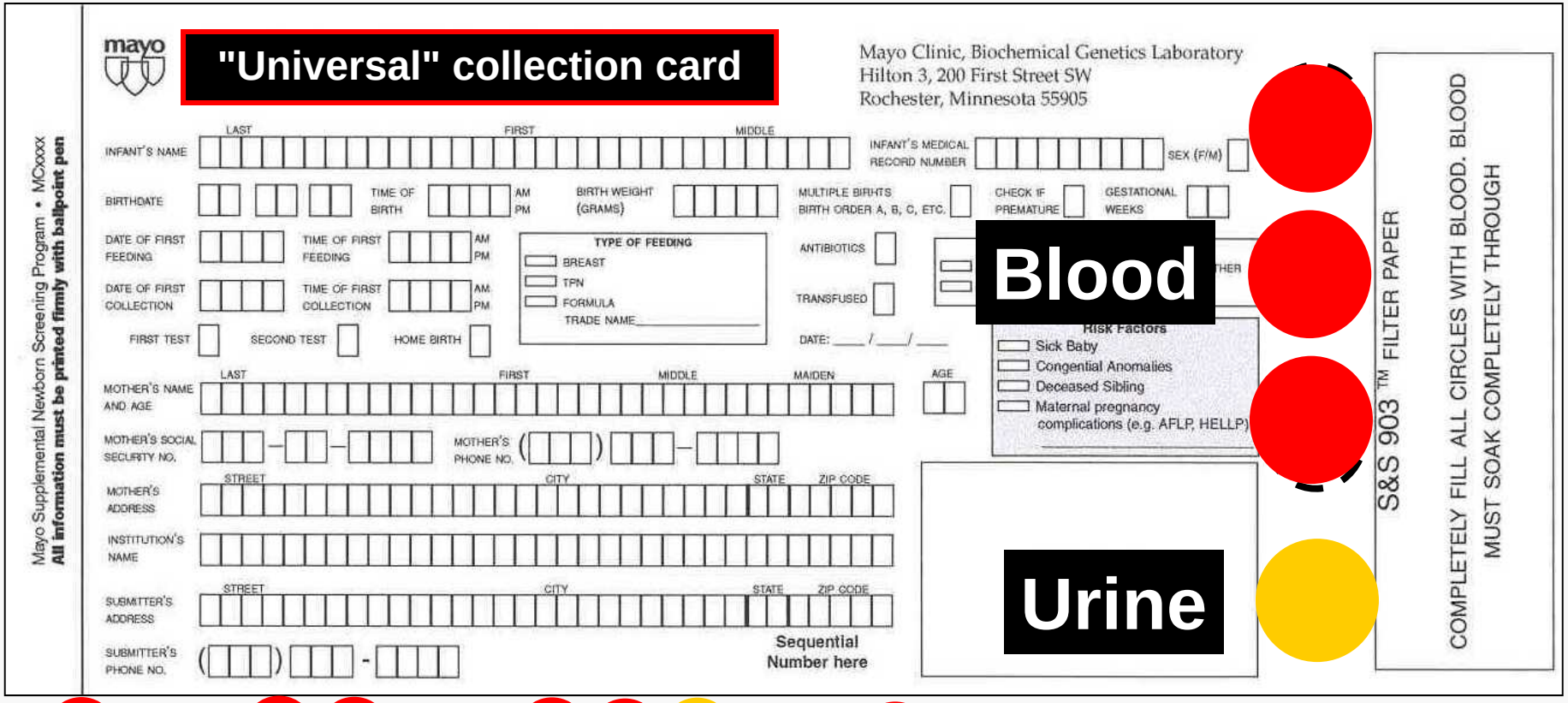
yields → 9000 spots used

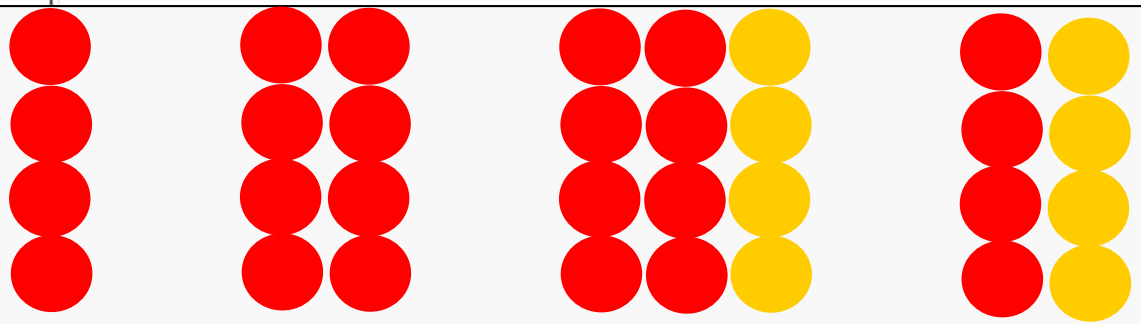
[redacted]


SATURATE ALL CIRCLES COMPLETELY
See reverse side for instructions


From "One Punch - One Disease"
To Whole Spot Extraction
or….
SOLVENT extraction
AC
AA
Others
Buffer (H_2O) extraction
Proteins
Steroids
Others
DNA extraction
ID
CF
Others
Repeat analyses, 2nd tier tests, storage
Collect MORE blood

Should We Collect More than Blood?

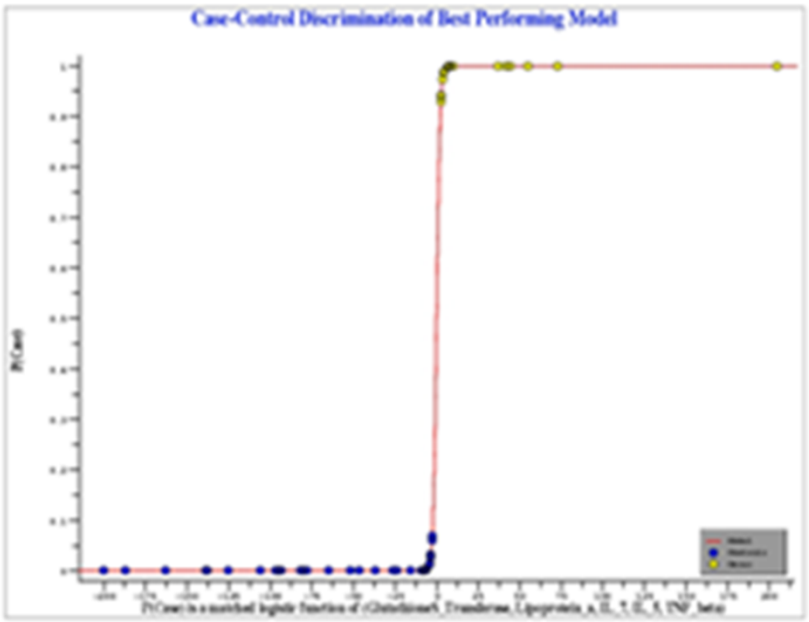




How many spots?

Multiplex Technology

- msms supreme example
- Hgb electrophoresis was first
- Targeted profile



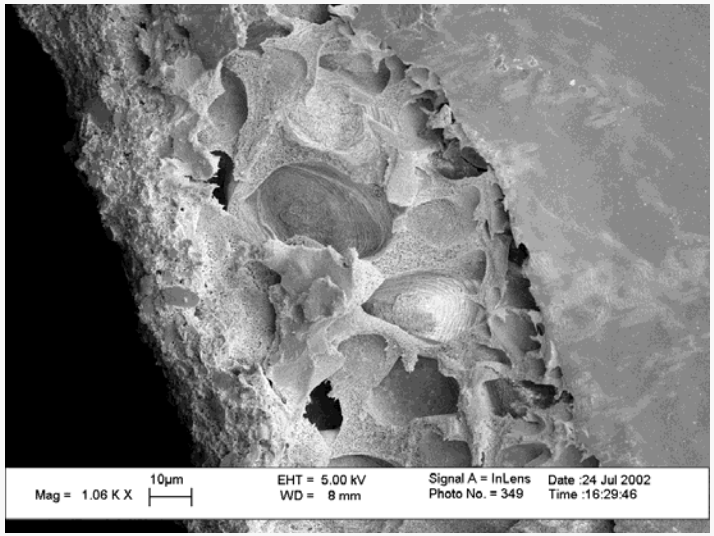
Newborn Screening for Autism. Search for Biomarkers. Mizejewski, Lindau-Shepard, Pass. Biomarkers in Medicine 7:247-260, 2013.

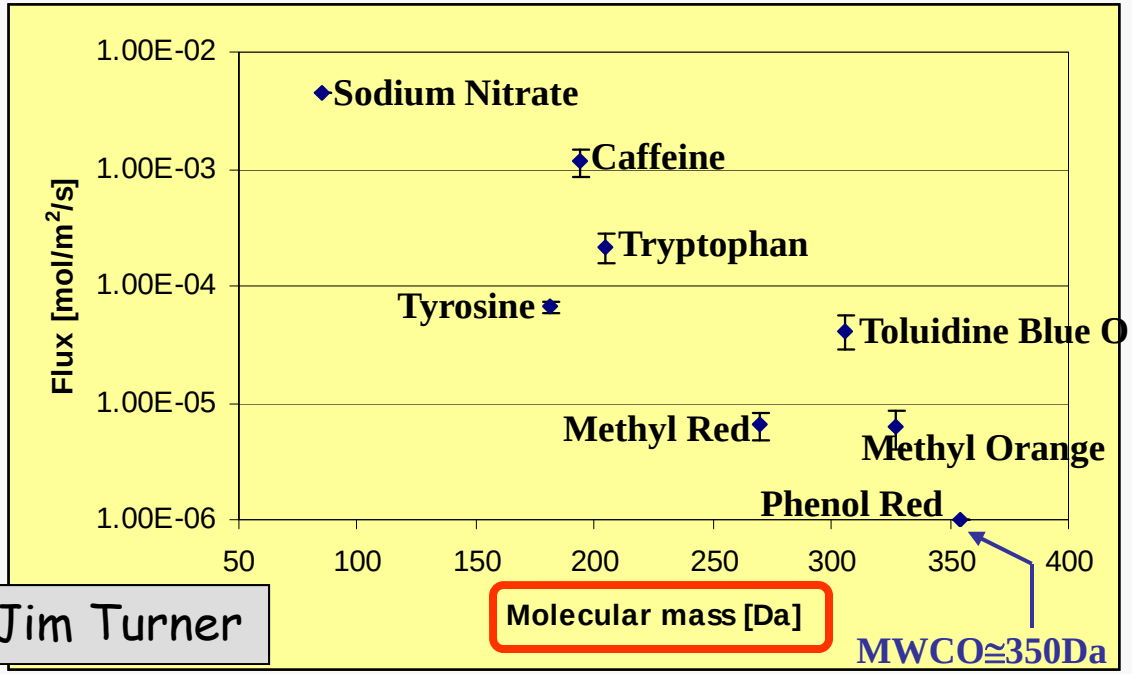
Time and Tide
Wait for No One

Time, Tide, and Tech
Wait for No One

Cellulose Acetate Membranes

- CA membranes can be cast directly onto silicon chips using a standard fabrication process.
- The membranes have good adhesion, good structural integrity, and are biocompatible.
- The membranes are made with variable rejection characteristics to restrict the passage of molecules as small as 300Da and up to 700Da (with current capabilities).
- Membranes' rejection characteristics, charge, thickness, and/or flux rate can be altered using different casting and treatment conditions.
- CA membranes, combined with electrophoresis can be used to purify DNA from PCR inhibitors such as heme.

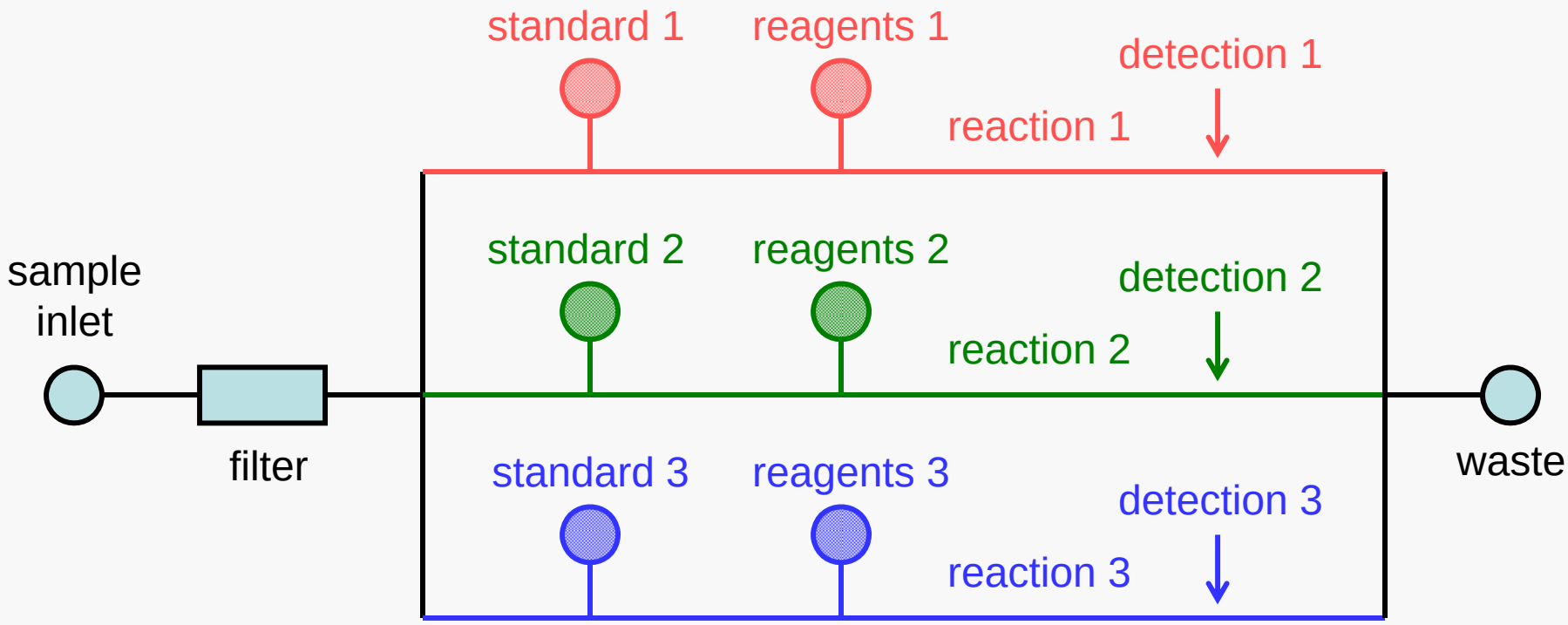




Jim Turner

Microfluidic Screening Assays

Device with Three Assays in Parallel



Assay 1: PKU
Assay 2: Galactosemia
Assay 3: CAH

lines: microfluidic channels
sample: drop of blood
filter: sample cleanup
standard: calibration or standard addition

The “Price” of Extra Tests

- More false positives - more parental anxiety
- Delayed Dx for false negatives
- Heterozygotes - what to with them?

4 Landmark Reports

1975 NAS - Proven public benefit, feasibility, consent, tests and follow-up, advisory committees

1994 IOM- Benefit to child, Dx, Rx and F/U

1997 Task Force on Genetic Testing - Direct benefit to child, mandatory screening

2006 Newborn Screening: Toward a Uniform Screening Panel and System.

N Green

ELSI Considerations

Premise: “Newborn screening should be conducted only when science and technology can serve both the individual and the public good.”

NBS Task Force Report 2000

ELSI-*type* Points to Consider

- **Do the diseases in the expanded panel meet the criteria for a screened disease?**

- **Diagnosing patients who would never present with a problem**

- **Diagnosing patients that may already be severely compromised or dead when the results of testing are available**

- **Identifying disease carrier status in children**

- **Screening for diseases where is no clear treatment protocol**

N Green

Traditional Screening Criteria

- Important health problem
- Natural history understood
- Detectable early stage
- Treatment at an early stage is [illegible]benefit
- A suitable test is availab[illegible]
- Test should be a[illegible]
- Intervals f[illegible]ng the test should be determined
- Ad[illegible]vices available to cover screening-induced need
- Ris[illegible]should be less than the benefits
- The costs should be balanced against the benefits

David and Patrick will expand on these.

Wilson, Jungner, World Health Organization, 1968

Charge of SACHDNC

- To make systematic evidence-based and peer-reviewed recommendations that include the heritable disorders that have the potential to significantly impact public health for which all newborns should be screened
- To develop a model decision-matrix for newborn screening expansion, including an evaluation of the potential public health impact of such expansion and periodically update the recommended uniform screening panel

SACHDNC Recommendations Adopted by the Secretary, HHS

Conditions recommended

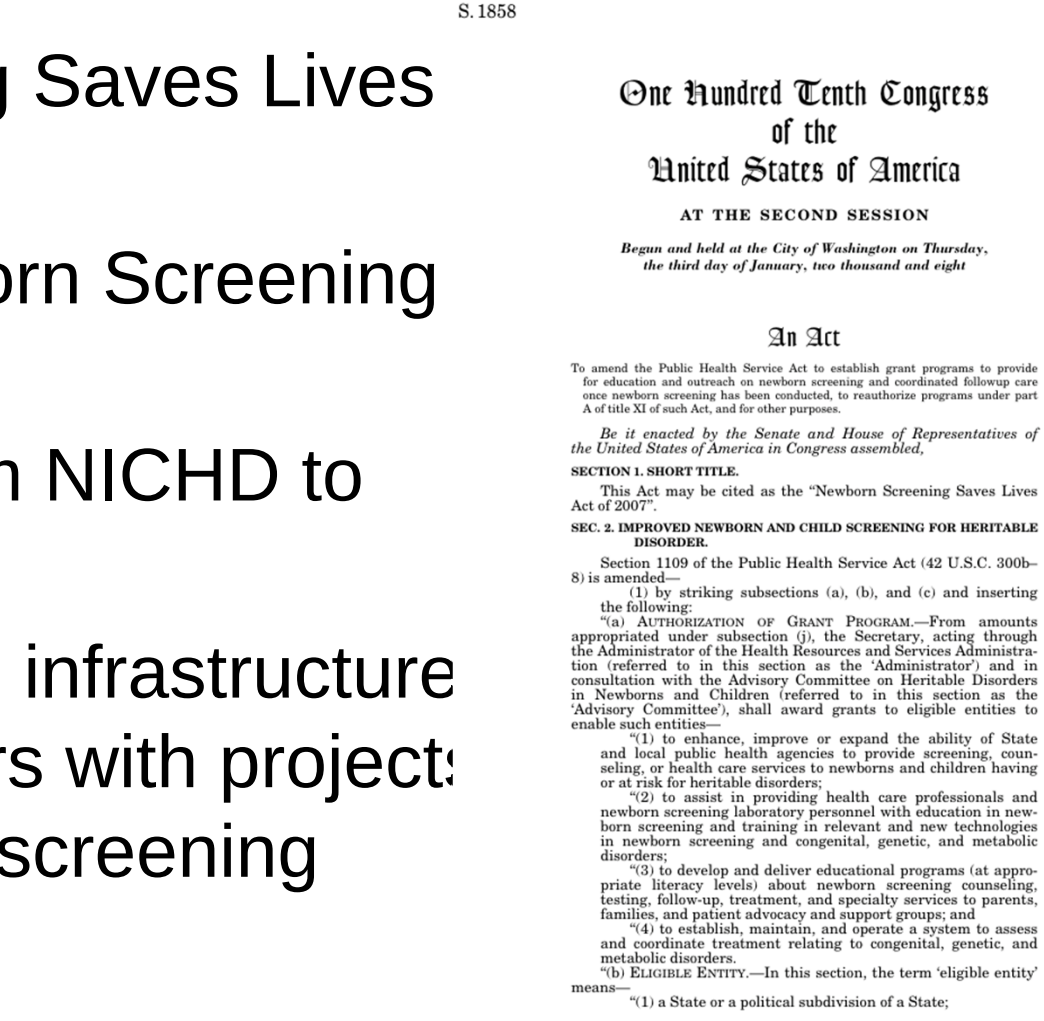
- Severe Combined Immunodeficiency
- Critical Congenital Heart Disease (CCHD)
- Pompe disease

Conditions considered but not recommended

- Hemoglobin H disease
- Krabbe disease
- Chronic Bilirubin Encephalopathy

The Newborn Screening Translational Research Network (NBSTRN)

- Newborn Screening Saves Lives Act of 2007
- Hunter Kelly Newborn Screening Research Program
- 5-year contract from NICHD to ACMG
- Develop a research infrastructure support investigators with projects related to newborn screening



S. 1858

One Hundred Tenth Congress
of the
United States of America

AT THE SECOND SESSION

Begun and held at the City of Washington on Thursday, the third day of January, two thousand and eight

An Act

To amend the Public Health Service Act to establish grant programs to provide for education and outreach on newborn screening and coordinated followup care once newborn screening has been conducted, to reauthorize programs under part A of title XI of such Act, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE.

This Act may be cited as the "Newborn Screening Saves Lives Act of 2007".

SEC. 2. IMPROVED NEWBORN AND CHILD SCREENING FOR HERITABLE DISORDER.

Section 1109 of the Public Health Service Act (42 U.S.C. 300b–8) is amended—

(1) by striking subsections (a), (b), and (c) and inserting the following:

"(a) AUTHORIZATION OF GRANT PROGRAM.—From amounts appropriated under subsection (j), the Secretary, acting through the Administrator of the Health Resources and Services Administration (referred to in this section as the 'Administrator') and in consultation with the Advisory Committee on Heritable Disorders in Newborns and Children (referred to in this section as the 'Advisory Committee'), shall award grants to eligible entities to enable such entities—

"(1) to enhance, improve or expand the ability of State and local public health agencies to provide screening, counseling, or health care services to newborns and children having or at risk for heritable disorders;

"(2) to assist in providing health care professionals and newborn screening laboratory personnel with education in newborn screening and training in relevant and new technologies in newborn screening and congenital, genetic, and metabolic disorders;

"(3) to develop and deliver educational programs (at appropriate literacy levels) about newborn screening counseling, testing, follow-up, treatment, and specialty services to parents, families, and patient advocacy and support groups; and

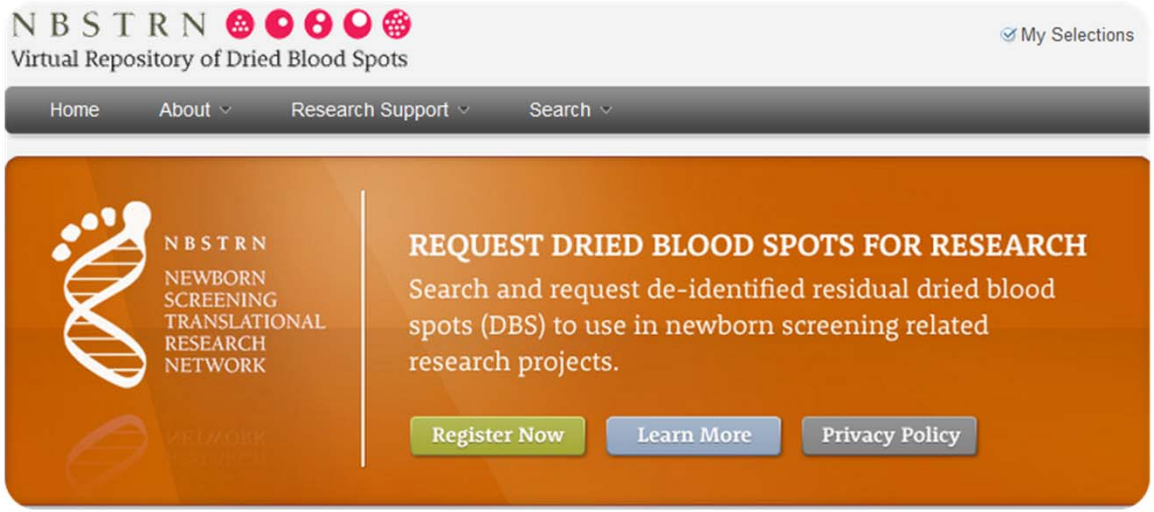
"(4) to establish, maintain, and operate a system to assess and coordinate treatment relating to congenital, genetic, and metabolic disorders.

"(b) ELIGIBLE ENTITY.—In this section, the term 'eligible entity' means—

"(1) a State or a political subdivision of a State;

Virtual Repository of Dried Blood Spots (VRDBS)

- Secure, centralized & web-based
- Pilot phase 6/12 to 9/12 – production date: 9/26/12
- Inventory of DBS samples – over 2.6 million
- Investigators can request letters of support, submit questions to participating states, browse & request specimens, track shipments & provide feedback
- States can respond to questions, review & manage requests, approve requests & control distribution



Unintended Consequences

- Carrier detection
  - Sickle cell trait
  - Cystic fibrosis
  - PKU?
- Late onset
  - Krabbe
- Affected mother
  - Maternal PKU

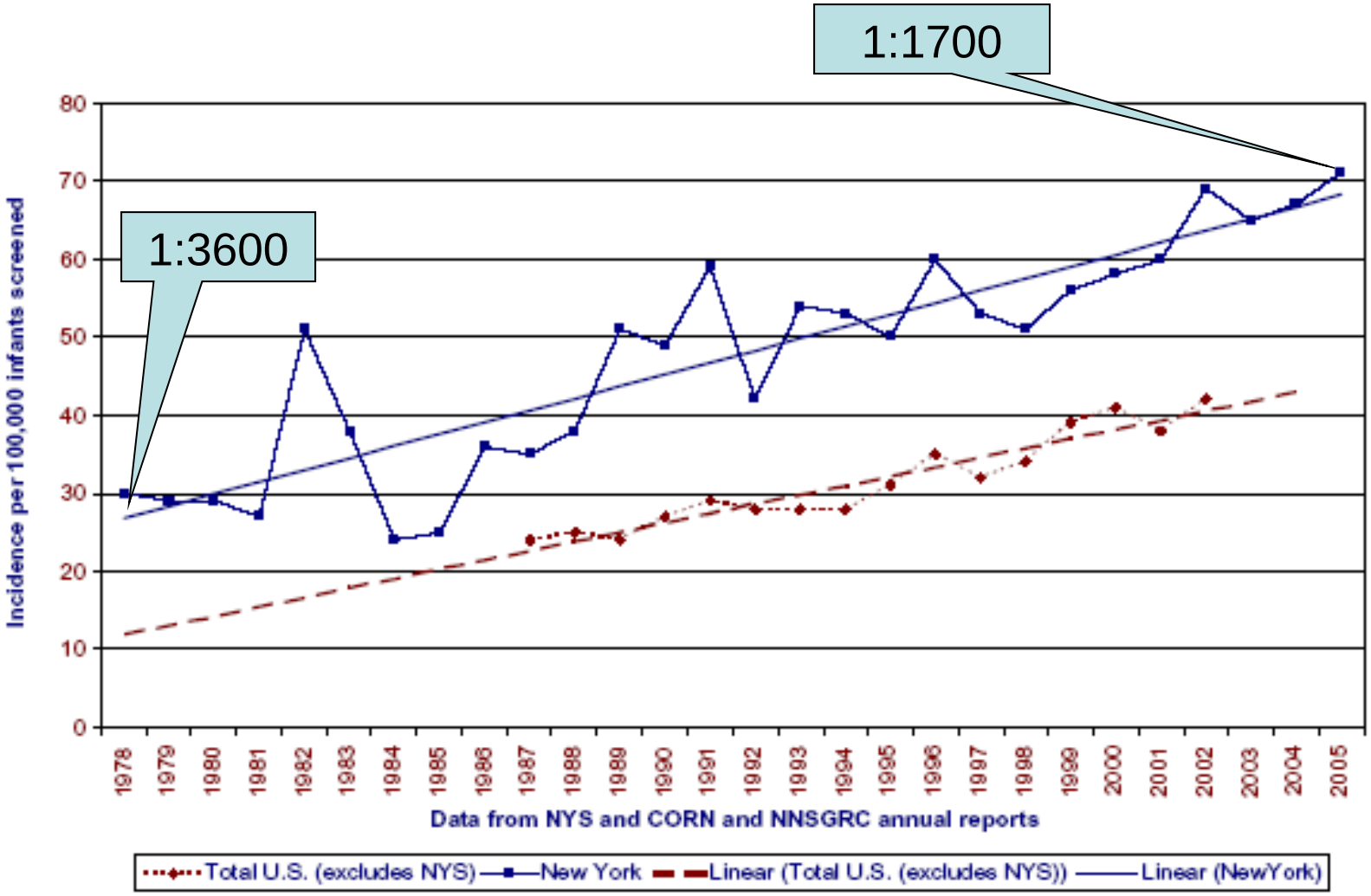


Fig. 1. Congenital hyponthyroidism U.S. (1987–2002) NYS (1978–2005).

Harris and Pass
Mol Gen and Metab, June 2007

And the DBS?

Better known as the Guthrie Specimen….

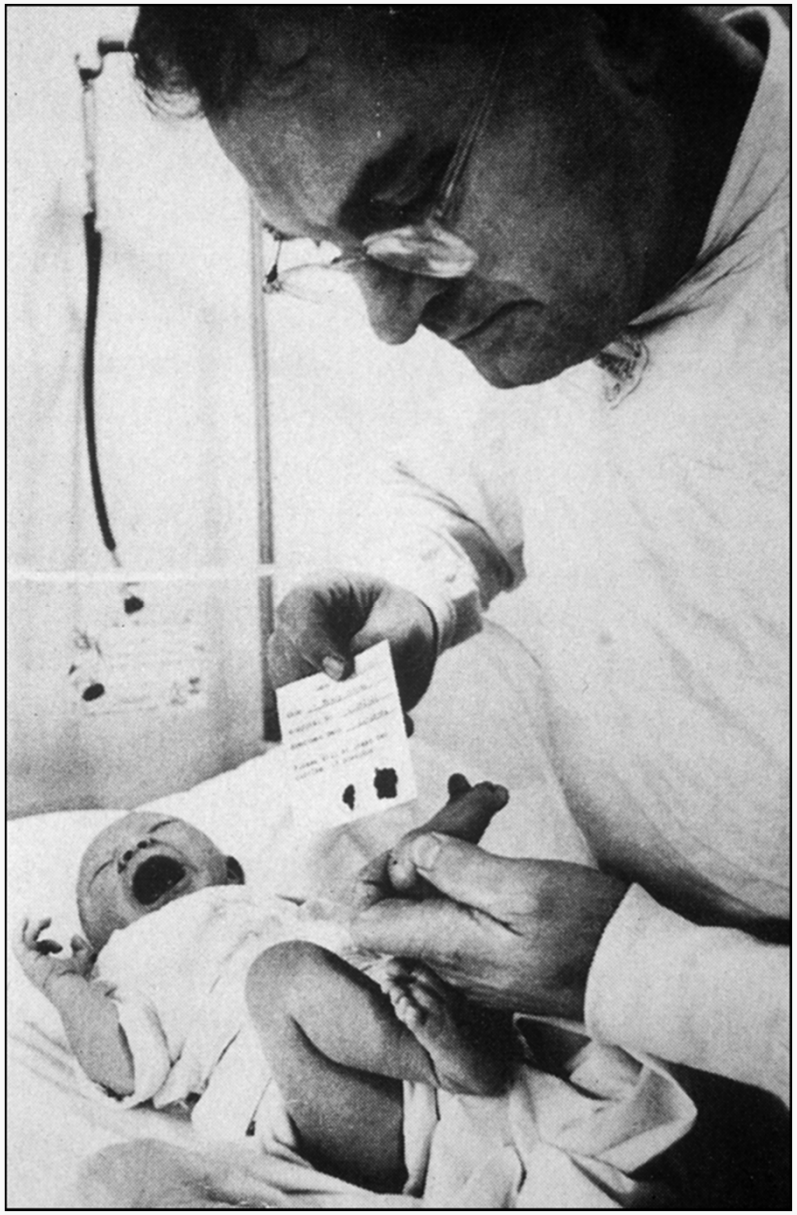
Analytes identified in DBS
Harry Hannon

Appendix 1. List of analytes that have been analyzed in dried blood spot (DBS) samples (not including analytes listed in appendix 2).

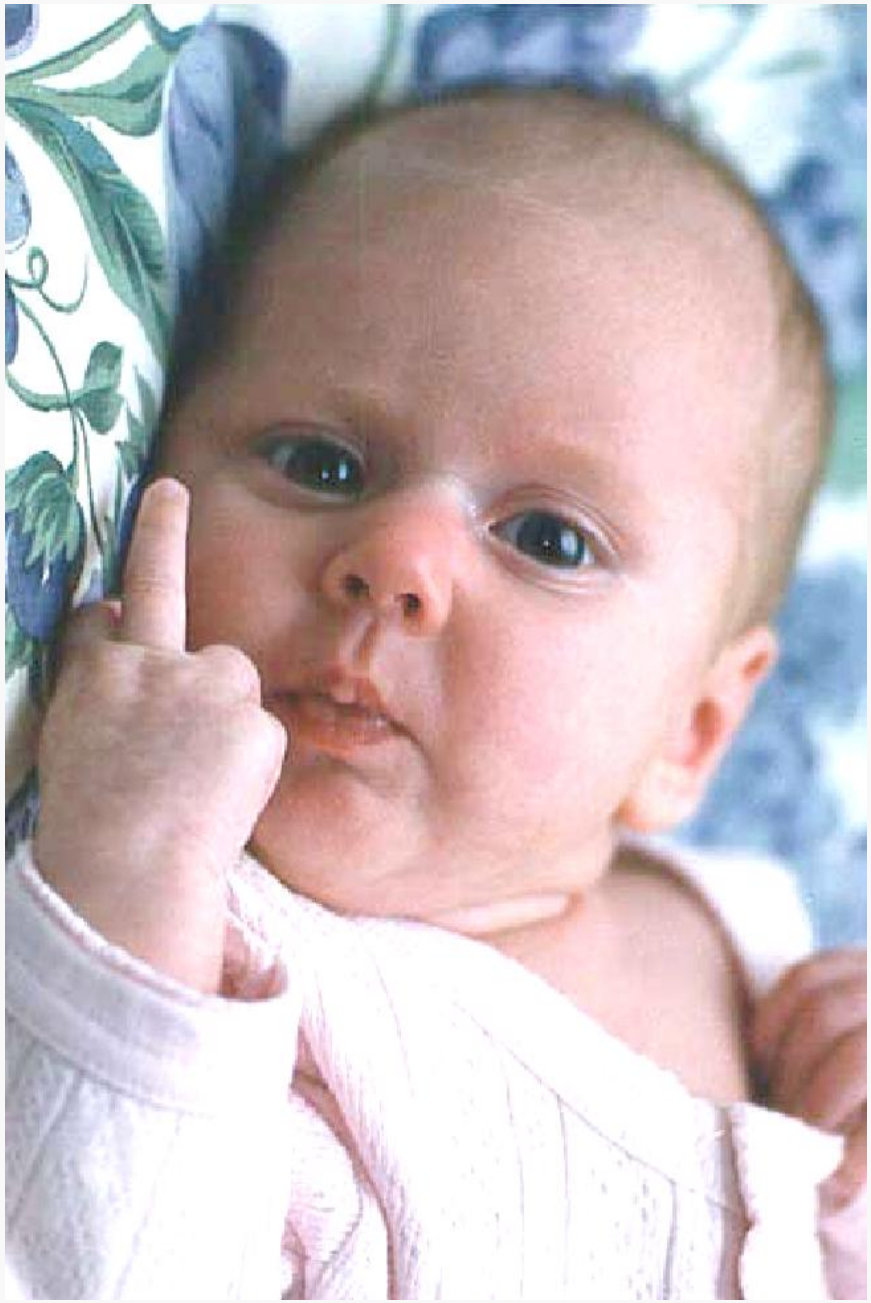
Analyte	Reference
Acylcarnitines/ Carnitine	(Chace, Hillman et al. 1997; Heinig and Henion 1999; Schulze, Schmidt et al. 2003)
Amino acids	(Zytkovicz, Fitzgerald et al. 2001; Deng, Deng et al. 2002; Deng and Deng 2003)
Alpha-fetoprotein	(Mizejewski, Bellisario et al. 1982; Parkinson, McMahon et al. 1996)
Amodiaquine/ Desethylamodiaquine	(Gitau, Mucholi et al. 2004)
Biotinidase	(Yamaguchi, Fukushi et al. 1987; Pettit, Amador et al. 1989; Broda, Baumgartner et al. 2001)
Brucella antibodies	(Takkoucha, Iglesias et al. 1995)
Ceruplasmin (Wilson's disease)	(Ohura, Abukawa et al. 1999)
Chloroquine/ Chlorpheniramine	(Okonkwo, Coker et al. 1999; Minzi, Rais et al. 2003)
Cocaine (Benzoylecgonine)	(Henderson, Powell et al. 1997)
Cysticercus antibodies	(Peralta, Macedo et al. 2001)
Cytokines (multiple)	(Phillips and Krum 1998; Nelson, Grether et al. 2003)
Dichlorodiphenyldichloroethylene	(Burse, DeGuzman et al. 1997)
Dihydropteridine reductase	(Jeaps, Silcox et al. 1986)
Diptheria/ Tetanus antitoxin	(Arya 1989; Hong, Ko et al. 1996)
Erythrocyte protoporphyrin	(Orfanos, Murphey et al. 1977)
Fatty acids/ Acylglycines	(Schmidt-Sommerfeld, Penn et al. 1993; Bennett, Ragni et al. 1994; Bonham Carter, Watson et al. 1996; Johnson 2000; Kimura, Yoon et al. 2002)
Filariasis antibodies	(Terhell, Haarbrink et al. 1996)
Galactose/ Galactose-1-phosphate	(Orfanos, Jinks et al. 1986; Hong, Yoon et al. 2001)
GALT	(Rhode, Elsi et al. 1998; Fujimoto, Okano et al. 2000)
Alpha-D-Galactosidase A	(Chamoles, Blanco et al. 2001)
Glutathione peroxidase	(Kelly and Schedlbauer 1978)
Halofantrine	(Mberu, Muhia et al. 1992)
Hemoglobin variants	(Henderson, Fishlock et al. 1991; Ros, Turner et al. 1993; Eastman, Wong et al. 1996; Hempe, Granger et al. 1997; Wild, Green et al. 2004; Fairhurst, Baruch et al. 2005)
Hepatitis C antibodies	(Judd, Parry et al. 2003)
Homocystine	(Acciani, Campolo et al. 2003)
Human chorionic gonadotropin hormone	(Macri, Anderson et al. 1996; Hallahan, Krantz et al. 2000)
Human Immunodeficiency virus	(Yoruno and Conroy 1992)
3-Hydroxybutyrate	(Burrin, Worth et al. 1981)
17-alpha-hydroxyprogesterone	(Arakawa, Maeda et al. 1985; Hofman, Klaniecki et al. 1985; Wallace, Beastall et al. 1986; Maeda, Arakawa et al. 1987; Tsuji, Maeda et al. 1987; Gonzalez, Maestrazzi et al. 1990; Xu, Pettersson et al. 1992; Erhardt, Solyom et al. 2000; Lai, Tsai et al. 2002)
Immunoreactive Trypsin	(Kirby, Applegarth et al. 1981; Cabrini, Pederzini et al. 1990; Xu, Pettersson et al. 1992)
Insulin-like Growth factor-1	(Mitchell, Hermos et al. 1998; Nindl, Kellogg et al. 2003)
Lactate	(Burrin, Worth et al. 1981)
Alpha-L-Iduronidase	(Chamoles, Blanco et al. 2001)
Lead	(Mehkeri, Romanowski et al. 1976; Wang and Demsher 1992; Srivuthana, Yee et al. 1996; Stanton, Maney et al. 1999; Shen, Zhang et al. 2003; Di Martino, Michniewicz et al. 2004)
Leishmania antibodies	(Rab and Evans 1997)
Beta-Lipoprotein	(Vladutin, Gluck et al. 1980)
Lysozomal enzymes	(Chamoles, Blanco et al. 2001)
Measles/ Rubella antibodies	(Smadman, Silva et al. 1986; Novello, Ridolfi et al. 1996; Helfand, Kayserling et al. 2001)
Mefloquine	(Bergqvist, Al Kabbani et al. 1993)
Oligosaccharides	(Ronaklis, Ramsay et al. 2002)
Onchocerca volvulus antibodies	(Rodriguez-Perez, Danis-Lozano et al. 1999)
Phytanic acid/ Pristanic acid	(ten Brink, van den Heuvel et al. 1993)
Prognanil	(Kolawole, Taylor et al. 1995; Bergqvist, Funding et al. 1998)
Pseudomonas aeruginosa	(Thanasekaran, Wiseman et al. 1989)
Pyrimethamine	(Minzi, Massele et al. 2005)
Quinine	(Rowell and Rowell 1987; Hellgren, Villen et al. 1990; Mberu, Ward et al. 1991; Dua, Sarin et al. 1993; Ericsson, Friden et al. 1993; Kolawole and Mustapha 2000; Jansson, Gustafsson et al. 2003)
RBC ([illegible]-infected erythrocyte surface antigen)	(Croes, McCarthy et al. 1994) (Duarte, Gyorkos et al. 2002)
Renin	(Parker and Cubitt 1999)
RSV antibodies	(Nielsen, Sieruma et al. 2003)
Rickettsial antibodies	(Fenollar and Raoult 1999)
Gentamicin/ Netilmicin	(Fujimoto, Tsuda et al. 1989)
Sisomicin	(Bergqvist, Hjelm et al. 1987; Fujimoto, Tawa et al. 1988; Tawa, Hirose et al. 1989)
Syphilis antibodies	(Stevens, Pass et al. 1992)
Theophylline	(Li, Lee et al. 1986; Watson, Oliveira et al. 2001)
Thyroid Stimulating Hormone	(Sullivan, May et al. 1997)
Thyroxine-binding globulin	(Dussault, Morissette et al. 1980)
Toxoplasma gondii antibodies (toxoplasmosis)	(Petersen and Eaton 1999; Sorensen, Spenter et al. 2002)
Treponema pallidum (syphilis)	(Backhouse, Lee et al. 1992)
Trichomonas vaginalis antibodies	(Mason, Fiori et al. 2005)
Trypanosoma cruzi antibodies	(Zicker, Smith et al. 1990)
Urea	(Plumbe and Worth 1985)
Wuchereria bancrofti antigen	(Itoh, Gunawardena et al. 1998)
Zinc Protoporphyrin	(Joselow and Flores 1976; Ho, Guthrie et al. 1987; Orfanos, Guthrie et al. 1989)
HPRT	(Jacomelli, Micheli et al. 2002)
Nandrolone	(Howe and Handelsman 1997)
Thyroxine	(Larsen and Brookin 1975)
Phenylalanine	(listed 1971; Newman and Starr 1971; Rudy, Rutledge et al. 1987; Rivero, Alba et al. 2000)
Reverse Triiodothyronine	(Filetti, Catanzso et al. 1980)
Echinococcus	(Coltorti, Guarnera et al. 1988; Kenny and MacCabe 1993)
Glucose 6-phosphate dehydrogenase	(Schoos-Barbette, Dodinval-Vercle et al. 1976; Nie and Zhao 1999; Simkins and Culp 1999)
Fumarylacetoacetase	(Laberge, Grenier et al. 1990)
21-deoxycortisol	(Arakawa, Maeda et al. 1985)
Succinylacetone	(Allard, Grenier et al. 2004)
Uroporphyrinogen-1-synthase	(Johansson, Thunell et al. 1984)
Glutathione	(Orfanos, Naylor et al. 1980)
Giardia antibodies	(Al-Tukhi, Ackers et al. 1993)
malaria antigen	(Jeffery, McWilson et al. 1975; Wirtz, Duncan et al. 1989; Biswas 2004)

And Next?

However, some things won’t change…

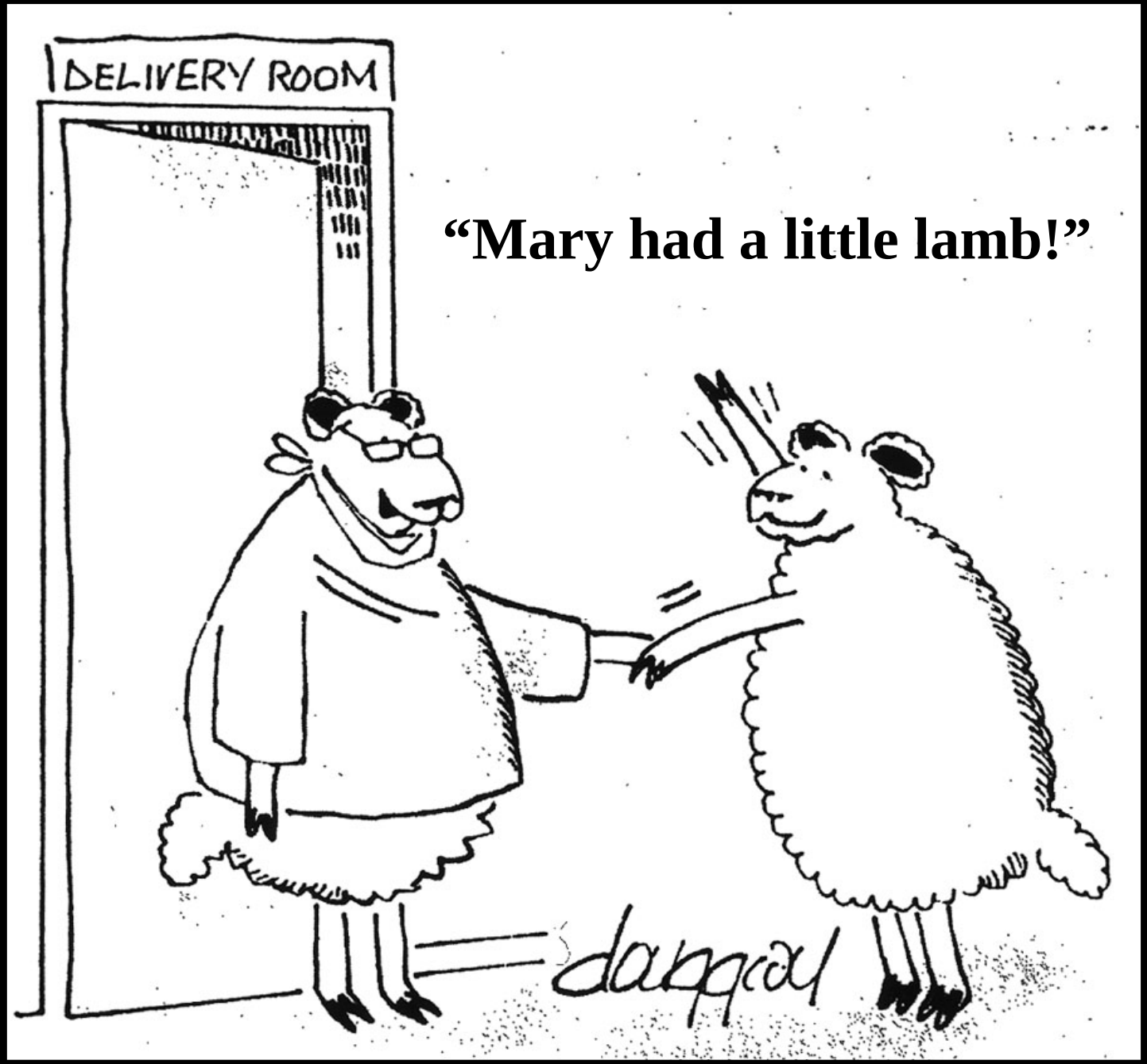


…despite some strong objections.

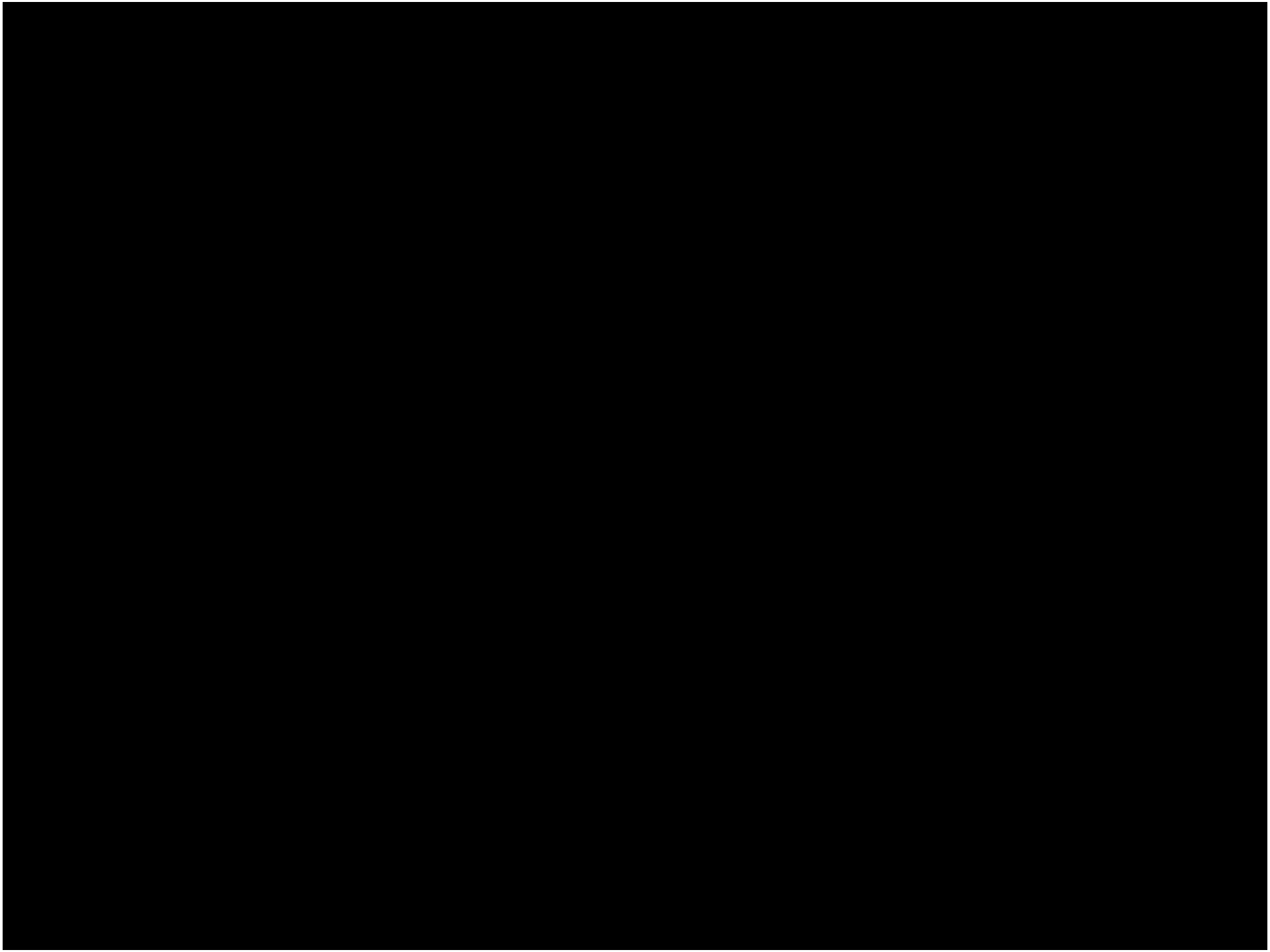


Thank you.

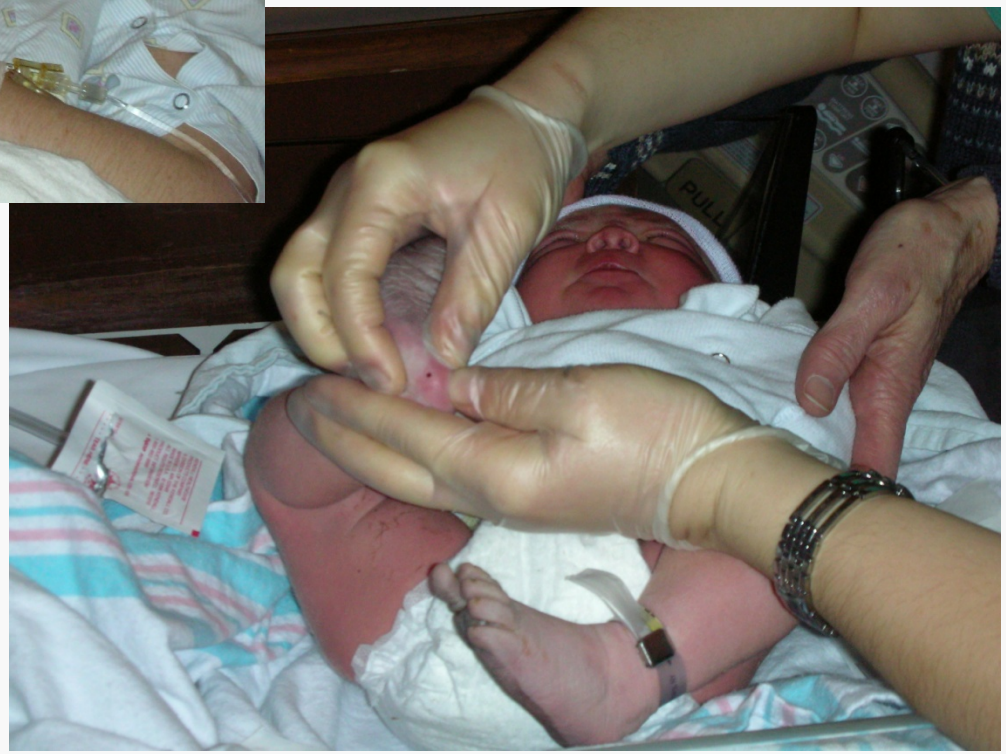
..and by the way…


DELIVERY ROOM
"Mary had a little lamb!"

No mas.

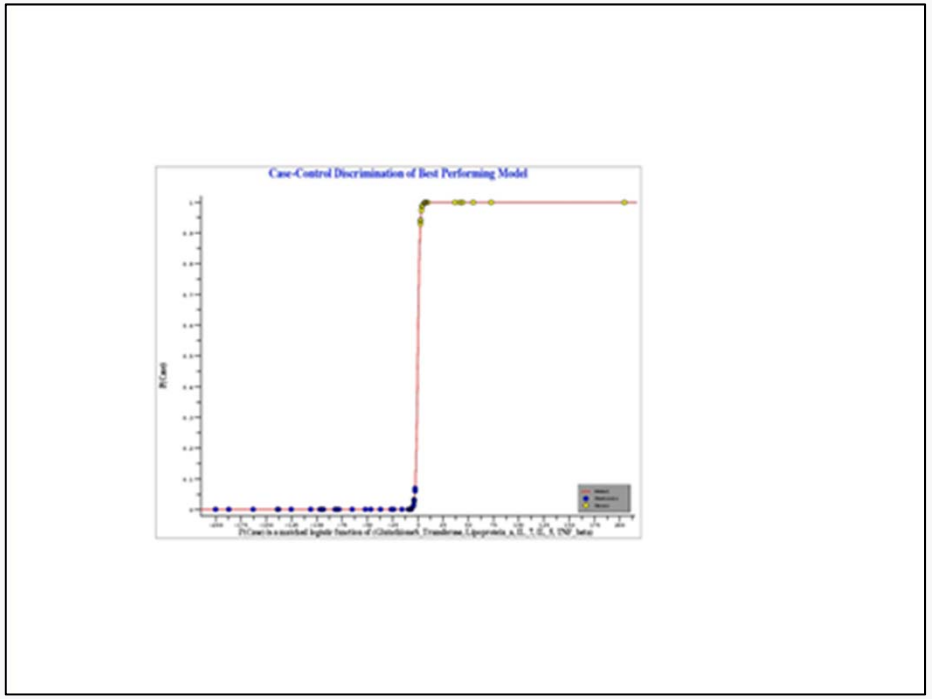


Clarrisa Ball
9lbs 8oz
Feb 16, 2006

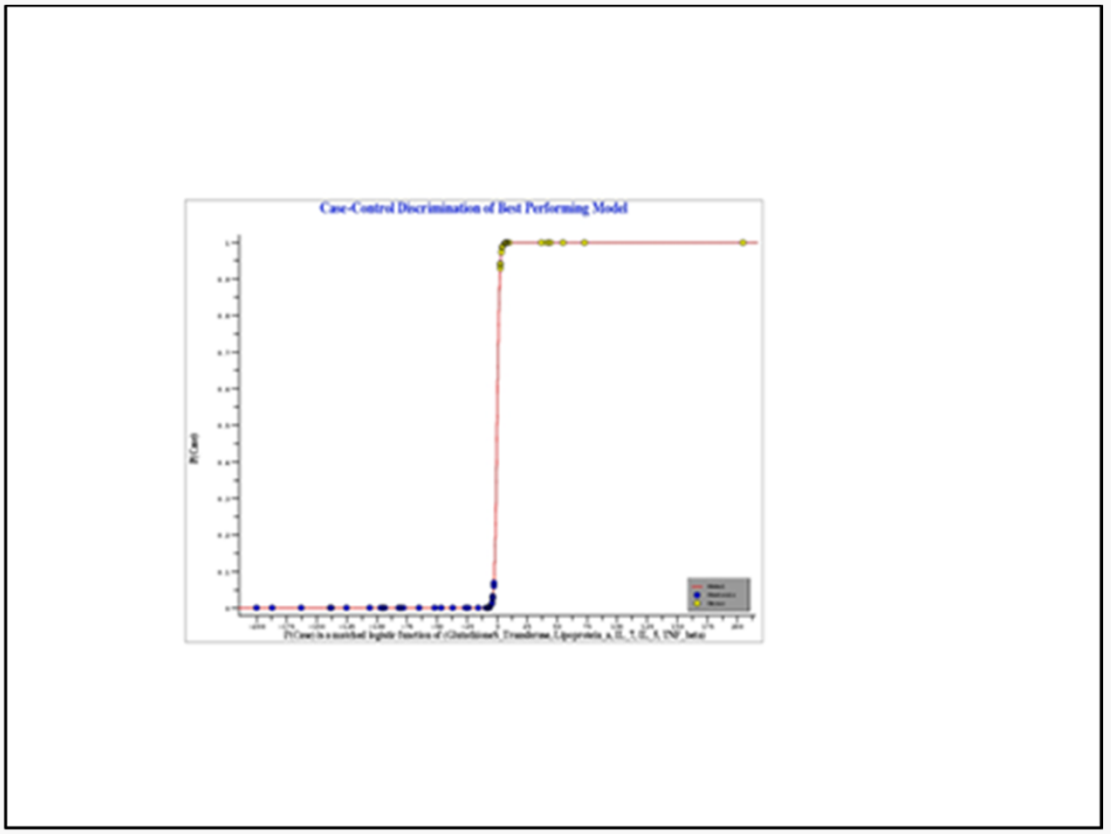


Multiplex Technology

- msms supreme example
- Hgb electrophoresis was first
- Targeted profile



Newborn Screening for Autism. Search for Biomarkers. Mizejewski, Lindau-Shepard, Pass. Biomarkers in Medicine 7:247-260, 2013.



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nepard, Pass. Biomarkers in Medicine 7:247-260, 2013.

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