Dried Blood Spots: A Convenient and Easy-to-Collect Sample for Large-Scale Immunological Surveillance

Linda Styer, Jean Rock, Erica Yauney, Katherine Nemeth, Amanda Damjanovic, Rachel Bievenue, Eli Rosenberg, James Tesoriero, Colleen Flanigan, Michele Caggana, Monica Parker

New York State Department of Health: Wadsworth Center, AIDS Institute, Office of Public Health
Department of Biomedical Sciences, SUNY Albany School of Public Health

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National Cancer Institute: SeroNet U01CA260508
How many were infected?

IgG antibodies in blood are a marker of infection

Antigens

Antigen-binding site

Antibody

From: Wikimedia Commons
### Venipuncture
- Requires trained health professional
- Performed in lab/clinic
- Fear of needles

### Dried Blood Spots (DBS)
- Minimally trained staff or self-collected
- Community sites or at home
DBS Collection and Storage

- Wipe with alcohol wipe
- Poke with safety lancet
- Wipe away first drop
- Drop blood onto card
- Dry for 3 hours
- Send in regular mail
- Newborn DBS use heel sticks
- DBS stable for days to weeks

https://www.wadsworth.org/programs/newborn/screening/providers/specimen-collection
Previous experience with DBS serology

2010 to Present:
HIV and HCV DBS referral testing

2017:
Developed HIV Luminex assay for newborn screening program

2018:
Implemented HT HIV testing on newborn DBS (1000-2000/day)
High-throughput HIV IgG microsphere immunoassay (MIA)

3mm punch from DBS into 96 well plate

Add buffer, elute 1hr @ room temp

Add eluate & antigen-coated magnetic beads 30 min @ 37°C

Wash plate

Liquid handler or by hand using 12-channel pipette

Add secondary anti-IgG antibody 30 min @ 37°C

Wash plate

Read on FlexMAP 3D

Output: MFI

Magnetic Bead 30

Magnetic Bead 15
Objective: Modify HIV MIA to detect antibodies to other pathogens for large-scale surveillance projects

2019: Hepatitis C Virus (HCV) seroprevalence study of women recently giving birth using newborn DBS

SARS-CoV-2 Seroprevalence Studies
• Apr 2020 - NYS serological testing survey
• July 2020 – Newborn DBS Nov 2019-Nov 2021
• Feb 2021 – Self-collected DBS for vaccine Ab response
2019: Hepatitis C Virus (HCV) seroprevalence among women recently giving birth

Cases include those from New York State (excluding New York City)
Source: NYS DOH Communicable Disease Electronic Surveillance System, 2019
Data current as 8/7/2019. Data preliminary and subject to change
Actively infected women (HCV RNA positive) can transmit HCV to their babies

- 6% with active infections will transmit HCV to baby
- 11% will transmit if co-infected with HIV

**Approach:** Modify MIA to test remnant newborn DBS for HCV antibodies

Babies passively acquire maternal IgG antibodies, including antibodies to HCV

Study Methods

1) 3mm punch from all DBS received in 6 wks
   29,323 DBS in 353 96-well plates

2) Obtain demographic data
   Age at collection; mom’s county of residence -> 7 regions;
   birthweight: < or ≥ 2500g; gestational age: < or ≥ 37 wks;
generic patient ID to identify repeat samples

3) Blind sample plates, de-identify & de-link data

4) Test 18,581 on HCV MIA
HCV seroprevalence in Central and Western NYS was 3-4 times higher than the rest of the state

<table>
<thead>
<tr>
<th>NYS Region</th>
<th># DBS in 6 wks</th>
<th># tested</th>
<th># HCV Ab reactive</th>
<th>Seroprevalence % (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>2,132</td>
<td>1,419</td>
<td>30</td>
<td><strong>2.1</strong> (1.5-3.0)</td>
</tr>
<tr>
<td>Western/Finger Lakes</td>
<td>3,481</td>
<td>2,380</td>
<td>36</td>
<td><strong>1.5</strong> (1.1-2.1)</td>
</tr>
<tr>
<td>Hudson/Northeast</td>
<td>4,398</td>
<td>3,459</td>
<td>23</td>
<td><strong>0.7</strong> (0.4-1.0)</td>
</tr>
<tr>
<td>Long Island</td>
<td>3,277</td>
<td>2,676</td>
<td>17</td>
<td><strong>0.6</strong> (0.4-1.0)</td>
</tr>
<tr>
<td>New York City</td>
<td>12,283</td>
<td>8,647</td>
<td>42</td>
<td><strong>0.5</strong> (0.4-0.7)</td>
</tr>
<tr>
<td>Total NYS</td>
<td>25,571</td>
<td>18,581</td>
<td>148</td>
<td><strong>0.8</strong> (0.7-0.9)</td>
</tr>
</tbody>
</table>

* Overall $\chi^2 = 57.43$, p value < .0001

Source: Wadsworth Center
HCV seroprevalence in Central and Western NYS was 3-4 times higher than the rest of the state.
2020 - 2021: Detection of antibodies to SARS-CoV-2 for serological surveillance

3/1/2020 – 1st COVID-19 case in NYS

3/20/20 – “Let’s develop a SARS-CoV-2 Ab assay using DBS”

4/19/20 – New York State Serological Testing Survey using DBS – ‘Grocery Store Study’

July 2020 – Begin testing 400,000+ newborn DBS from Nov 2019 to Nov 2021 for serological survey of women recently giving birth in NYS

Feb 2021 – Study of post-vaccination antibody response using self-collected DBS
April 2020 – NYS SARS-CoV-2 Serological Testing Survey

• 15,101 DBS collected at 99 grocery stores in 26 counties in NYS over 10 days
• Tested using MIA that detected Ab to nucleocapsid (N) antigens of SARS-CoV-1 (and then SARS-CoV-2)

Figure 1.

SARS CoV-2 Structure

- Spike (S)
- Nucleocapsid (N)
- Membrane (M)
- Envelope (E)
- RNA viral genome

Figure 1.

- Long Island
- Rest of State
- New York City
- Westchester/Rockland
### Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted estimated cumulative incidence</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Overall</td>
<td>14.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14.8</td>
</tr>
<tr>
<td>Female</td>
<td>13.3</td>
</tr>
<tr>
<td>Race and Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>29.2</td>
</tr>
<tr>
<td>NH-White</td>
<td>8.1</td>
</tr>
<tr>
<td>NH-Black/African American</td>
<td>20.2</td>
</tr>
<tr>
<td>NH-Asian</td>
<td>12.4</td>
</tr>
<tr>
<td>Multiracial/Other</td>
<td>11.9</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
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<tr>
<td>18-34</td>
<td>14.6</td>
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<tr>
<td>35-44</td>
<td>15.3</td>
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<tr>
<td>45-54</td>
<td>16.0</td>
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<tr>
<td>55+</td>
<td>12.1</td>
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<tr>
<td>Region</td>
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<tr>
<td>New York City b</td>
<td>22.7</td>
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<tr>
<td>Westchester/ Rockland Counties</td>
<td>16.1</td>
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<tr>
<td>Long Island c</td>
<td>13.2</td>
</tr>
<tr>
<td>Rest of NYS d</td>
<td>3.6</td>
</tr>
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Seroprevalence rates were 10 times higher than lab-confirmed COVID-19 cases.
July 2020 – SARS-CoV-2 Serosurvey of Women Recently Giving Birth in NYS

- Tested ALL newborn DBS Nov 2019 to Nov 2021 (>400,000 DBS)
- Questions:
  - Earliest COVID-19 Ab+?
  - Seropositivity over time by region
- Tested using MIA that detects Ab to SARS-CoV-2 S & N antigens

Note: Time lag between maternal infection and infant birth
Earliest SARS CoV-2 antibody positive infants week of March 29, 2020

- 6 babies
- NYC and a nearby downstate county

<table>
<thead>
<tr>
<th>Year and Month of Birth</th>
<th>2019</th>
<th>2020</th>
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<tbody>
<tr>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
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<tr>
<td>1</td>
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<td>6</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td></td>
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</tr>
</tbody>
</table>

- S reactive
- S non-reactive
- N reactive
- N non-reactive
COVID-19 vaccination detectable starting in Feb 2021
Seropositivity increased after rise in cases and vaccines in NYS

Source: Damjanovic et al 2022 JAMA Network Open
High incidence regions had early peak in cases and Abs

Low/moderate incidence regions had low cases and Abs thru Fall 2020

Source: Damjanovic et al 2022 JAMA Network Open
Feb 2021– Post-Vaccination IgG Antibody Response Study Using Self-Collected DBS

- Wadsworth Center employees self-collected DBS before & after COVID-19 vaccination (n=175 enrolled, 168 complete sets)
- 7 previously COVID-19 infected, rest naïve
- Of 509 DBS, only 2 were unsatisfactory (99.6% success rate)
Vaccination induces higher Ab levels than natural infection.

Recovered had higher Abs after 1st dose than naïve.

Abs peaked ~60 days post Dose 1 and declined.

Source: Nemeth et al 2023 Microbiology Spectrum
DOI: https://doi.org/10.1128/spectrum.01336-22
Four serological surveys using DBS and MIA

• Serosurvey of HCV among women giving birth in NYS
  – High HCV seroprevalence (~2%) in Central/Western/Finger Lakes; NYS = 0.8%

• NYS SARS-CoV-2 Serological Testing Survey
  – Overall 14% in NYS; 23% in NYC; 4% rest of state

• SARS-CoV-2 among women giving birth, Nov 2019-2021
  – First Ab positives in late March 2020 in NYC area
  – Regional correlation between cases and seropositivity
  – Detect uptake of vaccines

• Post-vaccine study using self-collected DBS
Large-scale testing of DBS using a high-throughput, flexible MIA is an effective way to assess the prevalence of infectious diseases in the population.
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