A Microsphere Immunoassay (MIA) to Screen Newborn Dried Blood Spots for HIV Antibodies and Conduct Serosurveys for Hepatitis C Virus & COVID-19

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Talk Outline

- HIV screening of pregnant women in New York state, use of microsphere immunoassay (MIA)

- Alternative uses of dried blood spot (DBS) MIA protocol to conduct serosurveys of Hepatitis C virus and SARS-CoV-2

- Operational use of HIV MIA in Newborn Screening Program

Preventing mother-to-child transmission of HIV is most effective during pregnancy and up to 12 hours after birth

- HIV transmission to baby: 25-40% if untreated, <1% if treated

- In NYS, all pregnant women offered HIV test in 1st trimester

- HIV testing offered at delivery if no prior HIV testing

- If HIV+, antiretrovirals for mom and baby

- Mom transfers IgG antibodies to infant, so need HIV RNA/DNA testing to diagnose HIV infection in baby

HIV antibody screening of newborn DBS is a 'Safety Net' to prevent mother-to-child transmission of HIV

- HIV screening of newborn DBS mandated by NYS regulation
- Test all infant DBS to detect HIV IgG antibodies from mother
- Follow up for HIV Ab+ DBS - from infants of known HIV+ mothers?
- Very few cases of unknown HIV-exposed infants in NYS
    - Usually women infected late in pregnancy
- Benefits of newborn DBS HIV screening
    - **All** HIV-exposed babies are identified in NYS and provided care
    - Prevent HIV transmission by breastfeeding

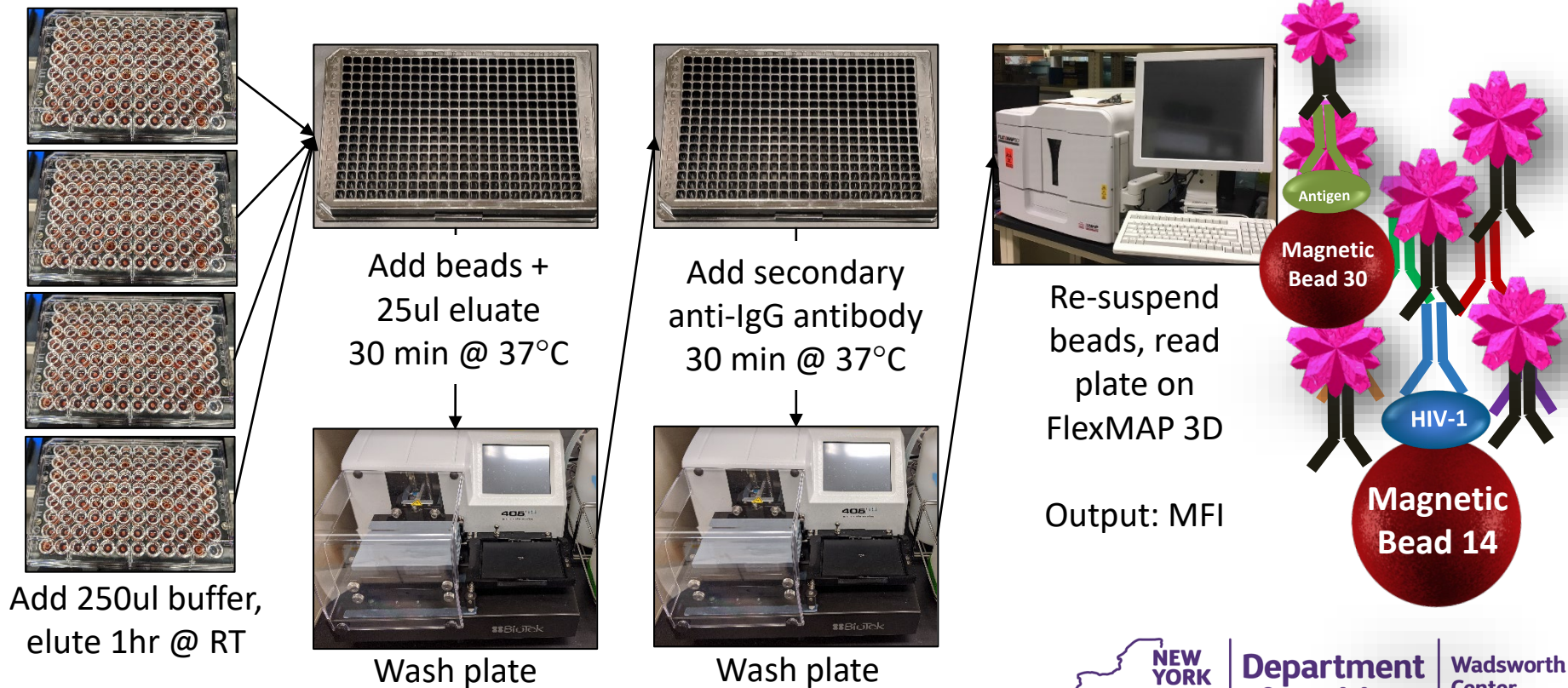# In 2018, transitioned from modified FDA-approved method to lab-developed MIA for increased flexibility

Test	Avioq HIV-1 Microelisa	HIV MIA
Dates in Use	1/7/11-11/30/18	12/3/18-current
Approval	NYS CLEP (Modified FDA)	NYS CLEP (LDT)
Target	HIV-1 (IgG)	HIV-1/HIV-2→HIV-1 (IgG)
Plate & Method	96 well; manual	384 well; semi-automated
Cost	~$1/well	~$0.90/well
TAT	Elute overnight + 3 hrs	1 hr elution + 4 hrs

High-throughput HIV IgG MIA assay for DBS

Add 250ul buffer, elute 1hr @ RT

Add beads + 25ul eluate 30 min @ 37°C

Wash plate

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

Wash plate

Re-suspend beads, read plate on FlexMAP 3D

Output: MFI

Antigen

Magnetic Bead 30

HIV-1

Magnetic Bead 14

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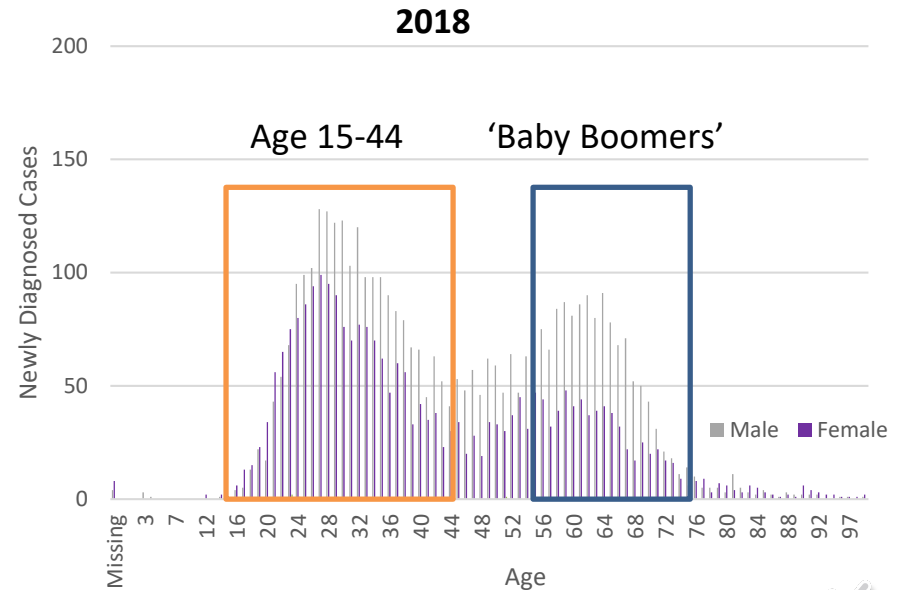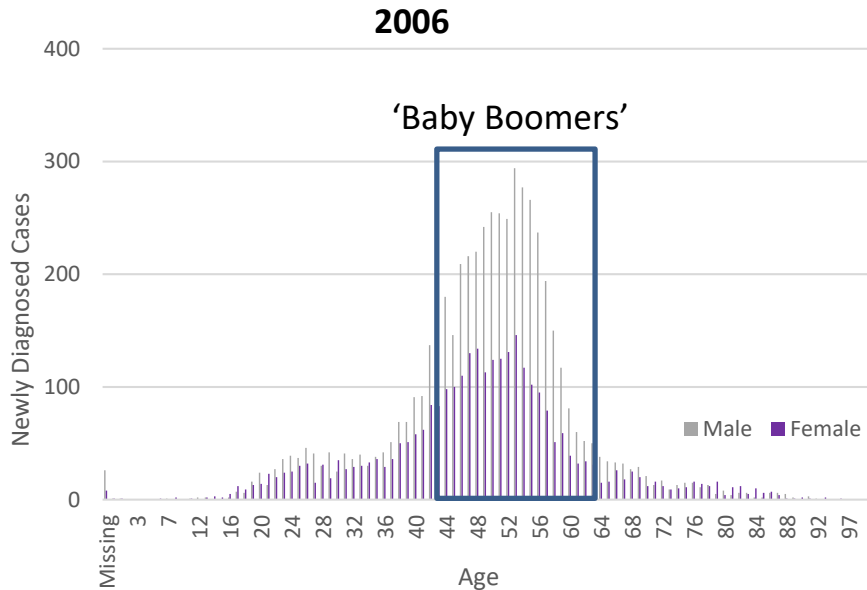# Use of DBS MIA protocol for other large serosurveys

- 2019 – Hepatitis C Virus (HCV) serosurvey of newborn DBS to assess prevalence in NYS pregnant women (n=~18,000)

- 2020 – SARS CoV-2 serosurveys
  - NYS general population and selected cohorts (n=~57,000)
  - Newborn DBS from Nov 2019 to Oct 2020 (n=~250,000)

HCV cases in New York State are shifting toward younger age groups and more women
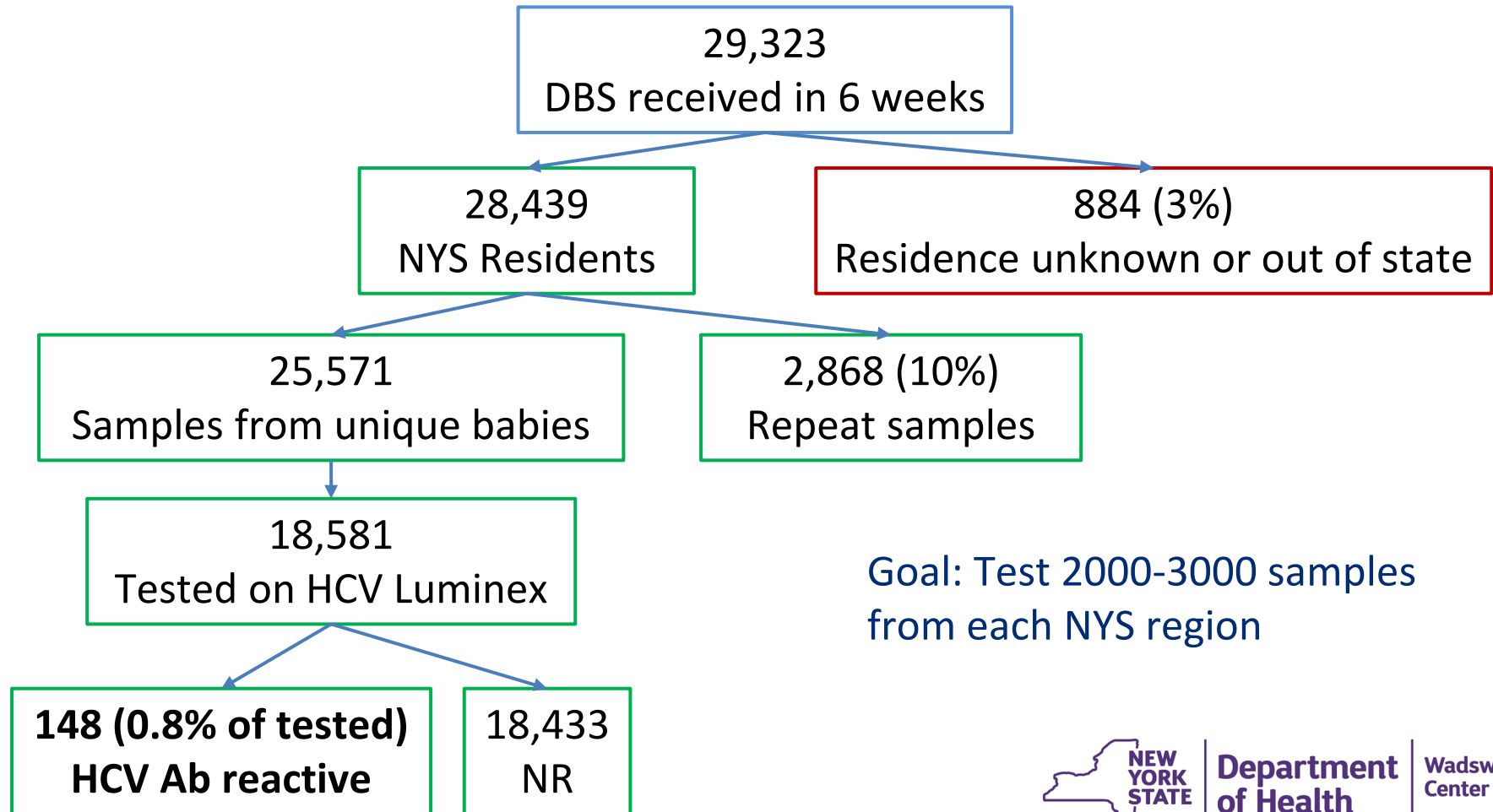

Cases include those from New York State (excluding New York City)
Source: NYS DOH Communicable Disease Electronic Surveillance System, 2019
Data current as 8/7/2019. Data preliminary and subject to change

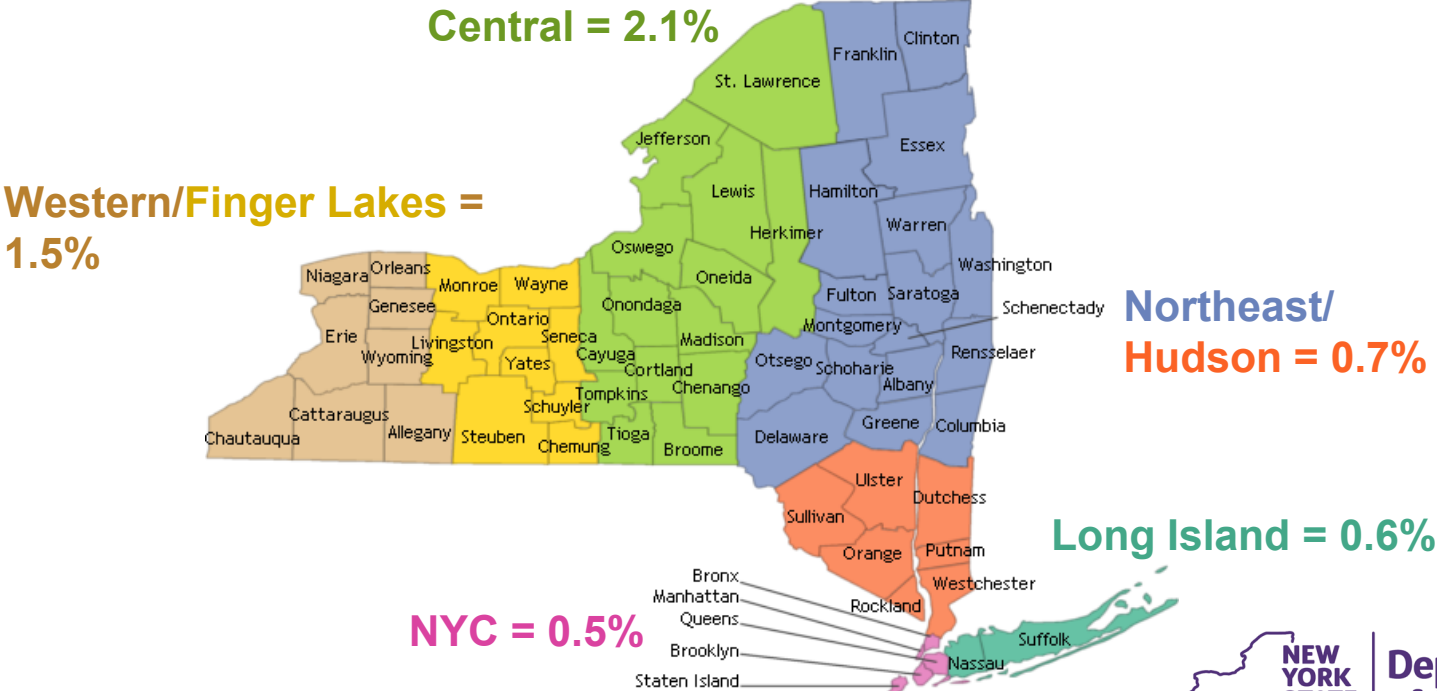# HCV seroprevalence in pregnant women in Central and Western NYS was 3-4 times higher than the rest of the state

Central = 2.1%

Western/Finger Lakes = 1.5%

Northeast/Hudson = 0.7%

Long Island = 0.6%

NYC = 0.5%

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# Use of DBS MIA protocol for other large serosurveys

- 2019 – Hepatitis C Virus (HCV) serosurvey of newborn DBS to assess prevalence in NYS pregnant women (n=~18,000)

- 2020 – SARS-CoV-2 serosurveys
  - NYS general population and selected cohorts (n=~57,000)
  - Newborn DBS to assess serostatus of pregnant women (n=~280,000)

SARS CoV-2 serosurvey of pregnant women using newborn DBS

- Test ALL newborn DBS Nov 2019 to Dec 2020

- Test ~2700 DBS/day, 2 bead sets (Nucleocapsid and Spike)

- Questions:

   - Earliest COVID-19 Ab+?

   - Seropositivity over time by region/zip code

   - Association with birth weight, gestational age, maternal age

- Note: Lag time between mom's infection & baby's birth

SARS CoV-2 Structure

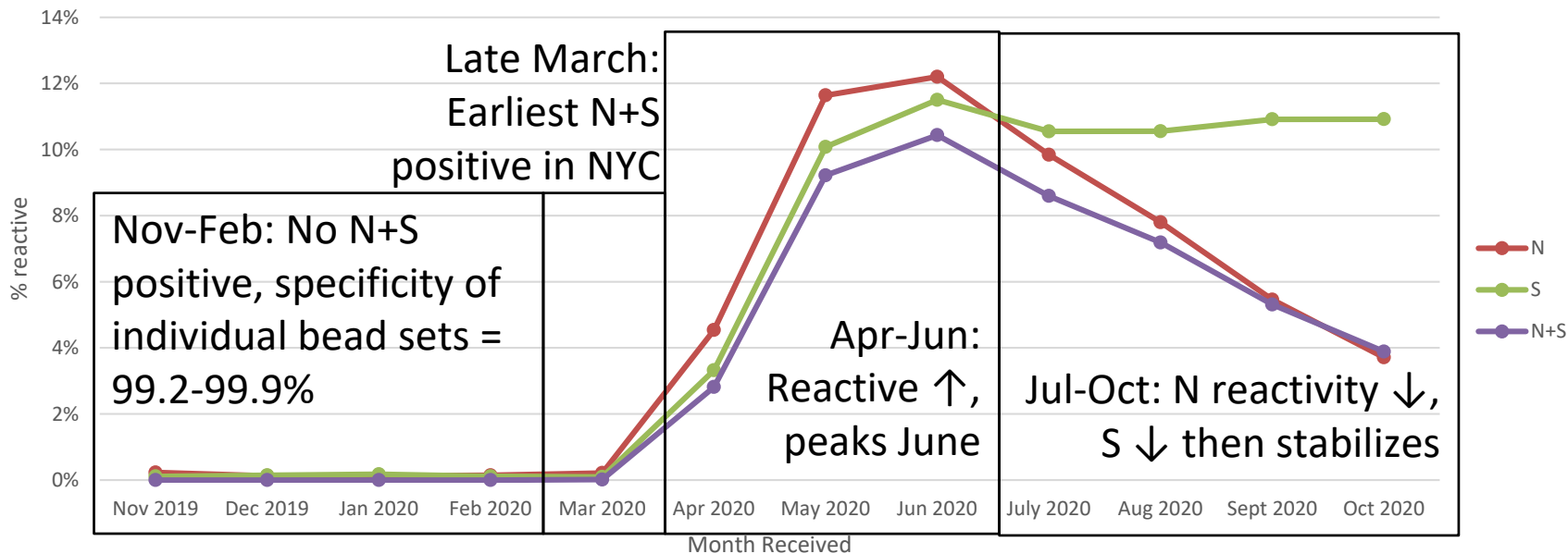Spike (S)

Nucleocapsid (N)

Membrane (M)

Envelope (E)

RNA viral genome
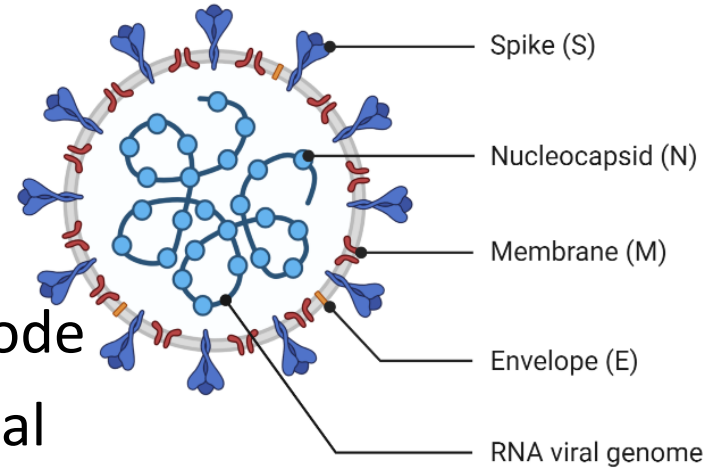
Created with BioRender.com

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SARS CoV-2 reactivity in pregnant women peaked in June, first reactive in late March

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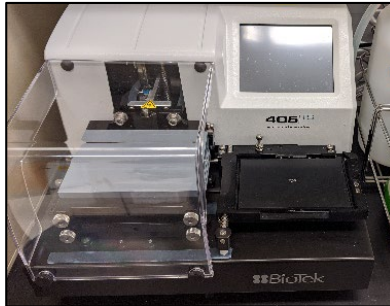- Operational use of HIV MIA in Newborn Screening Program

Scaling up HIV IgG MIA DBS assay for newborn screening

- Receive ~1,000 DBS/day
- Start eluting @ 11am, load on FlexMAP @ 2pm, analyze data next AM
- One FTE technician

Two Hamilton MicroLab
STARlet liquid handlers

Two BioTek 405 TSUS
magnetic plate washers

Four Luminex
FlexMAP 3D

Quality Control for HIV IgG MIA DBS assay

On each 96 well plate:
- CDC Neg Ctrl (Neg Calibrator)
- CDC HIV-1 Pos Ctrl
- In-House Neg Ctrl
- Background (Buffer)

Day: 351

	NC	1POS	NEG	BG		1POS	NEG
Upper Limit	230	4256	189	32		300	4
Lower Limit	32	402	39			4	0
Lot #	A1807-3	D1807-3	ExtNeg081920				

HIV 1 NC Average: 114

MFI values divided by average Neg Calibrator for day to calculate S/CO value

QC -- MFI

	NC	1POS	NEG	BG
Plate 1	149	2395	124	5
	98	1889	153	
Plate 2	169	1648	124	21
	136	1863	171	
Plate 3	152	1581	121	21
	77	2034	107	
Plate 4	81	1348	171	7
	84	1781	110	

QC -- S/CO

1POS	NEG
21.0	1.1
16.6	1.3
14.4	1.1
16.3	1.5
13.9	1.1
17.8	0.9
11.8	1.5
15.6	1.0

S/CO value > 4.0 = reactive

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Testing algorithm for HIV IgG MIA DBS assay

Test DBS (1x) on HIV-1 Luminex

Low bead count or low MFI value?

→ Yes

No ↓

Is S/CO value > 4.0?

→ Yes

No ↓

End Testing Result = "Screen Negative"

Repunch/retest in duplicate

Is S/CO value > 4.0 in 2/3 reps?

No

Yes ↓

Repeat specimen with previous HIV-1 positive result?

→ No

Yes ↓

End Testing Result = "Repeat Referral"
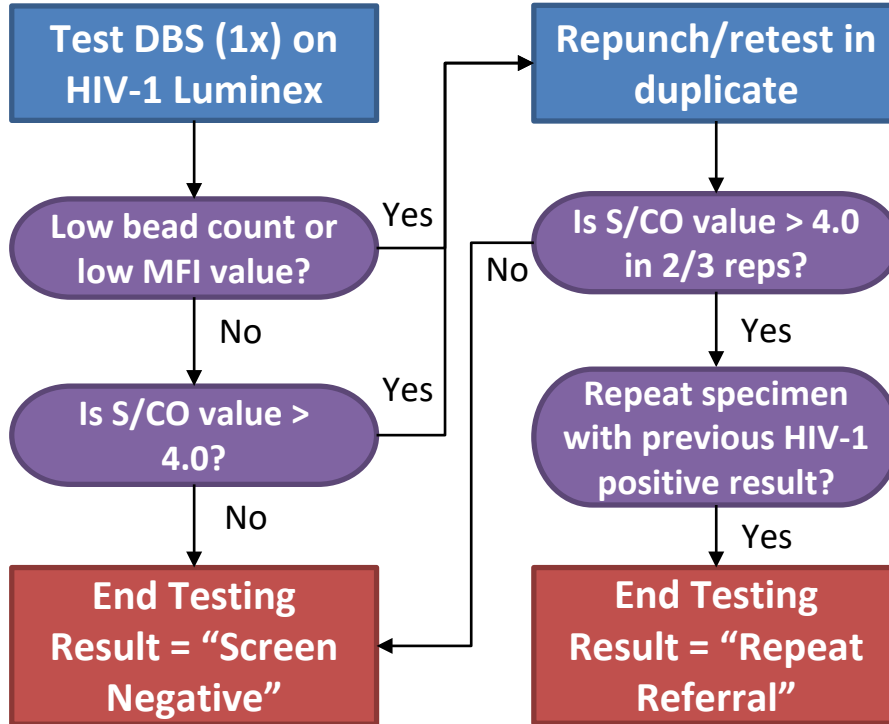
Test with Geenius
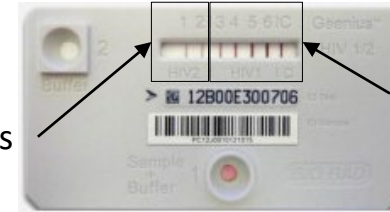
2 HIV-2 bands

4 HIV-1 bands
1 control band

Geenius Result	Overall Result
HIV-1 Reactive	**HIV-1 Reactive**
Indeterminate	**Indeterminate**
Non-reactive; S/CO >10	
Non-reactive; S/CO <10	**Screen Negative**

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Two years of HIV IgG MIA DBS: Dec 2018-Dec 2020

Result	Specimens	Babies
HIV-1 Reactive	919 (0.18%)	684 (0.16%)
Indeterminate	113 (0.02%)	105 (0.02%)
Screen Negative	511,869 (99.8%)	434,031 (99.8%)
Total	512,901	434,820

Identified 1 unexpected HIV- exposed baby
- Mother tested HIV negative first trimester
- Baby is HIV negative

4 false HIV-1 reactive results
- Luminex low reactive, odd Geenius 'HIV-1 Positive' banding pattern

5 false negative results
- Luminex low reactive; Geenius negative (1)
- Luminex low reactive on initial; retests negative (2)
- Luminex non-reactive on initial (2); reactive when retested

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Issues with HIV IgG MIA DBS testing

- **Limitation**: Cannot detect HIV infection in mom before she develops IgG antibodies; early HIV treatment can affect antibody production

- Variability of background and cutoffs with reagent lots

- Low bead counts, low MFI values

- Maintaining adequate stock of supplies and reagents

- FlexMAP 3D instrumentation issues

- Transition dates:

  – March 2, 2020– Dropped HIV-2 bead set

  – August 3, 2020 - Millipore custom assay design - HIV Kits

Conclusions

- HIV antibody screening mandated in NYS, serves as safety net to prevent mother-to-child transmission of HIV

- In 2018, switched to lab-developed Luminex HIV assay for flexibility

  - Used same protocol to conduct large-scale serosurveys of Hepatitis C virus (2019) and SARS CoV-2 (2020) in newborn DBS

- Two-test algorithm (Luminex-Geenius) provides sensitivity & specificity

- Detected one unknown HIV-exposed baby; 4 false pos, 5 false neg

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Acknowledgements

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