The Feasibility of Modified HIV and Antiretroviral Drug Testing using Self-collected Dried Blood Spots from Men Who Have Sex with Men

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Disclaimer: This evaluation was conducted using a non-FDA approved indication for use.
Tradenames are used for informational purposes and do not constitute an endorsement by CDC.
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Background

- Currently, an estimated 1.1 million people with HIV infection are living in the United States(1)
- In 2015, 82.9% of all HIV infections among men were caused by sexual contact between men(1)
- In 2015, 17% of MSM in U.S. recruited into RCT of HIV self-testing had never been tested for HIV(2)

(1) CDC. Estimated HIV incidence and prevalence in the United States, 2010-2016. HIV Surveillance Supplemental Report; 2018
(2) MacGowan R, Chavez PR, Borkowf C, et al. A randomized controlled trial: the impact of HIV self-testing among internet-recruited men who have sex with men, eSTAMP, 2015-2016. 2017 IAS Conference, Paris, France

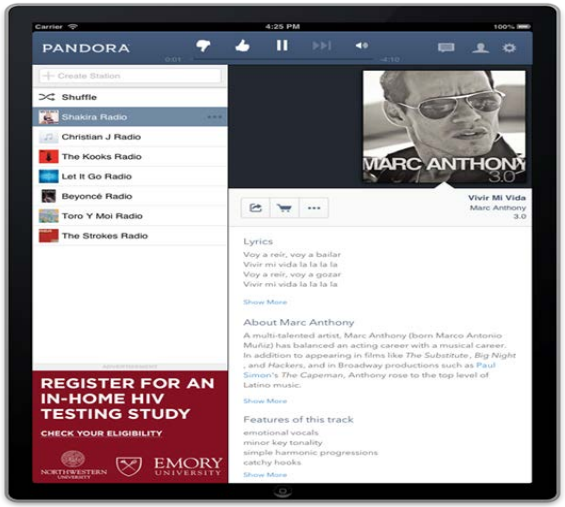
HIV testing

- First step in awareness of a person's infection status
- Travel, wait times, work schedules, and concerns about confidentiality are the most common barriers to testing(3)
- Alternative testing strategies, such as HIV self-testing and the use of self-collected dried blood spots (DBS) for lab-based testing may facilitate access to regular testing
- Persons aware of their HIV infection reduce risk behaviors, access HIV treatment, resulting in prevention of HIV transmission.

(3) MacKellar DA, Hou SI, Whalen CC, et al. Reasons for not HIV testing, testing intentions, and potential use of an over-the-counter rapid HIV test in an internet sample of men who have sex with men who have never tested for HIV.Sex Transm Dis 2011; 38 419-28.

Objective

To assess the feasibility of self-collected DBS for HIV serology, HIV-1 viral load (VL), and antiretroviral (ARV) drug testing from MSM who participated in the “Evaluation of Rapid HIV Self-testing among MSM project” (eSTAMP) through internet recruitment.



Methods

eSTAMP

Formative Parts

Part 1. Qualitative Assessment of Study Materials (e.g. DBS kit, placemat)

Part 2. Observed Assessment of User Proficiency when Self-Testing and Self-Collecting DBS card

Part 3. Cross-sectional Field Performance Evaluation of HIV Self-Tests

Part 4. 12-Month Longitudinal Randomized Control Trial

CDC funded a multipart study to evaluate the use and impact of providing HIV self-tests to internet-recruited MSM.

Part 3 and 4 Eligibility Criteria

- Male at birth and identify as male
- ≥18 years old and residing in the US
- HIV-negative or unaware of HIV status
- Able to read in English
- Anal sex with ≥1 man, past 12 months
- Not currently on PrEP
- Never in an HIV vaccine trial
- No bleeding disorder

Participants received a package with:

- **OraQuick In-home HIV Test**
- **Sure Check At-Home test (SC)**
  - Sure Check HIV 1/ 2 Assay – under IDE
- **DBS specimen collection kit**
  - Written instructions and video
  - 2 Whatman 903 protein saver DBS cards
  - 2 BD Microtainer contact-activated lancets
  - 2 Gauze sterile pads, alcohol swab, bandage
  - 3 Desiccants and a humidity indicator card
  - 2 Ziploc plastic bags
  - A pre-paid return envelope

Who received the package?

- **Part 3 – Field Performance Evaluation**
  - All Participants were mailed a package

- **Part 4 – Randomized Control Trial with surveys every 3 months**
  - Participants were mailed a package
    - After reporting an HIV positive result on quarterly surveys or
    - At end of study, after completing 12-month survey

DBS Flow Chart

Perform self-collection of blood sample on filter paper card using contact-activated lancets and dry the card for 4 to 12 hours at ambient temperature

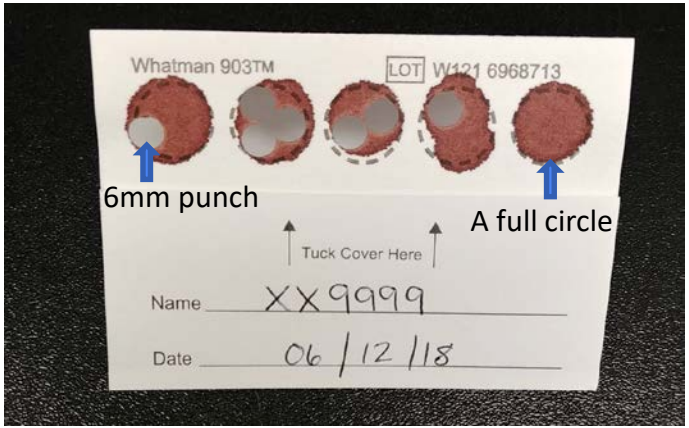
Place dried DBS card in plastic bag with desiccants and humidity indicator card and mail to Emory University in a pre-paid envelop within 48 hours

Upon receipt of DBS from Emory University, CDC lab inspects DBS for quality and stores specimens at -20 °C until tested for HIV and ARVs

Laboratory Testing: how far can a spot go?

FDA–approved protocols:

- Avioq HIV-1 Microelisa System (Avioq)
- Bio-Rad GS HIV-1 Western Blot (WB)



Modified protocols for use with DBS:

- Bio-Rad GS HIV Combo Ag/Ab EIA (Ag/Ab)[5]
- Bio-Rad HIV-1/2 Geenius Supplemental assay (Geenius)[5]
- Abbott *Real*Time HIV-1 viral load assay (VL) when needed to confirm HIV infection status
- Levels of six extracellular ARV drugs measured by mass spectrometry:
  - Emtricitabine (FTC)
  - Tenofovir (TFV)
  - Lamivudine (3TC)
  - Abacavir (ABC)
  - Efavirenz (EFV)
  - Raltegravir (RAL)

(5) Luo W, Davis G, Li L, et al. Evaluation of dried blood spot protocols with the Bio-Rad GS HIV combo Ag/Ab EIA and Geenius HIV1/2 supplemental assay. JCV 91:84-9, 2017

Modified Protocol for Use of DBS with the Abbott RealTime HIV-1 Viral Load Assay (m2000)

Elution of DBS:

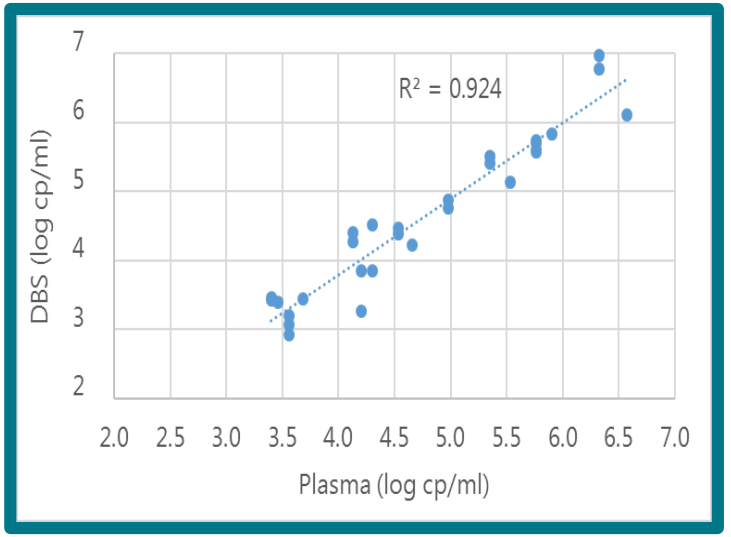
- Thaw DBS cards at room temperature for 30 min
- Add four 6mm punches (~50 µl of whole blood) into 1.3 ml of DBS lysis buffer in MM tube
- Incubate at 55˚C for 30 min
- Mix, spin, and place in the m2000

Test Protocol:

- 1.0ml HIV-1 RNA DBS IUO US II V11/HIV-1 DBS VL IUO US 1 spot in Transport Tube V11

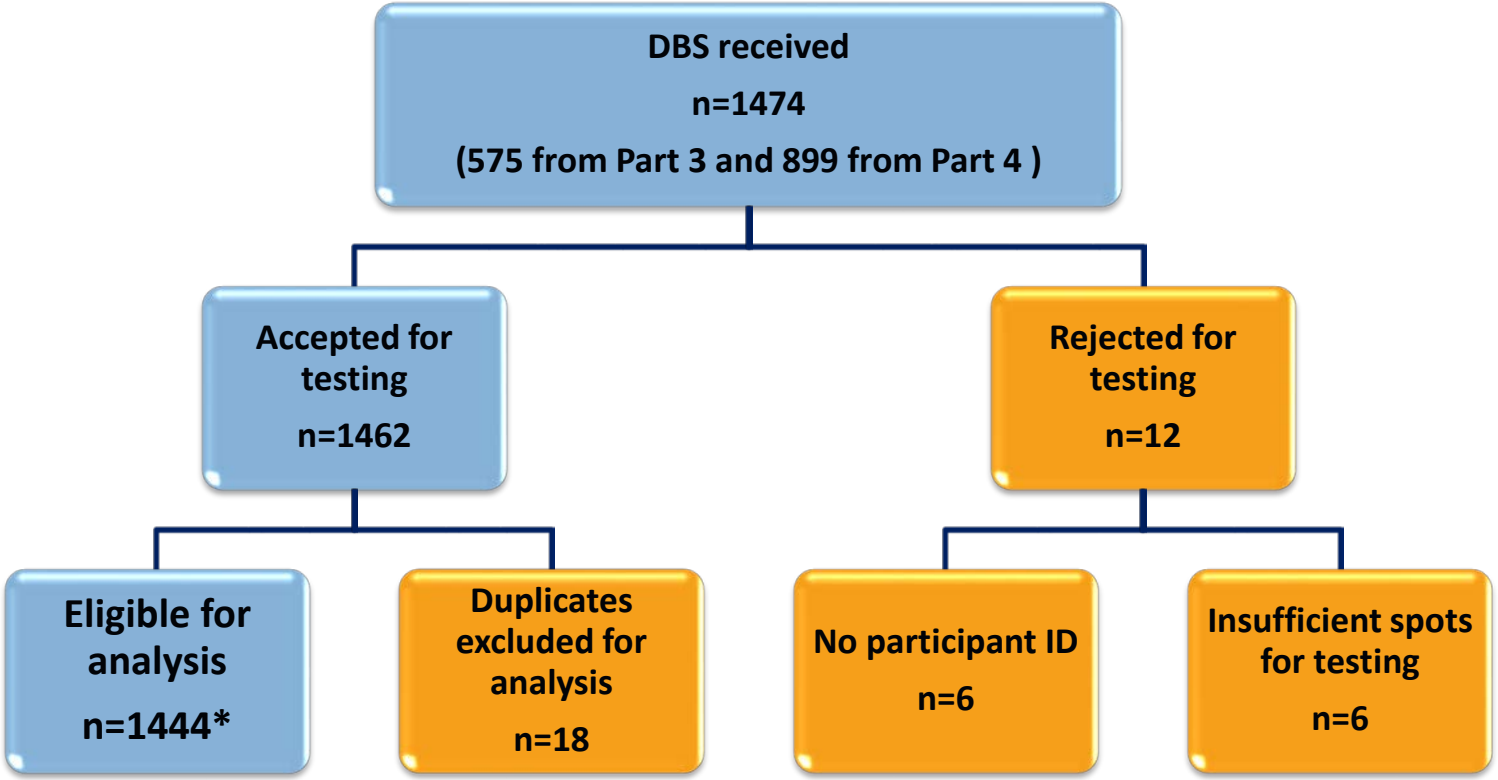
Results:

- Automatically reported
  - LOQ of 2.92 log (copies/ml)



- ✓ **Good agreement between four 6 mm punches and plasma**
- ✓ **LIMITATION: 66% of 3 log (copies/ml) are detected using four punches instead of 70 µl whole blood (a full circle of DBS)**

Results



*71 were not properly shipped: not in zip-lock plastic bag, without desiccants, or without humidity indicator card

Notes from the CDC lab: shipping

- **The median time at ambient temperature from DBS self-collection to arrival at the Emory lab was 8 days**

Days from collection to Emory lab	N=1444	%
≤ 7 days	589	40.8
8 to 14 days	711	49.2
14 to 21 days	93	6.5
> 21 days	51	3.5

- **The median time at ambient temperature from Emory to CDC lab was 4 days (range from 0 to 15 days)**

Notes from the CDC lab: quality of the DBS cards

- **89% DBS received had 5 spots, not necessarily a full circle**

Number of filled spots	N=1444	%
1	2	0.14
2	1	0.07
3	58	4.2
4	97	6.7
5	1286	89.0

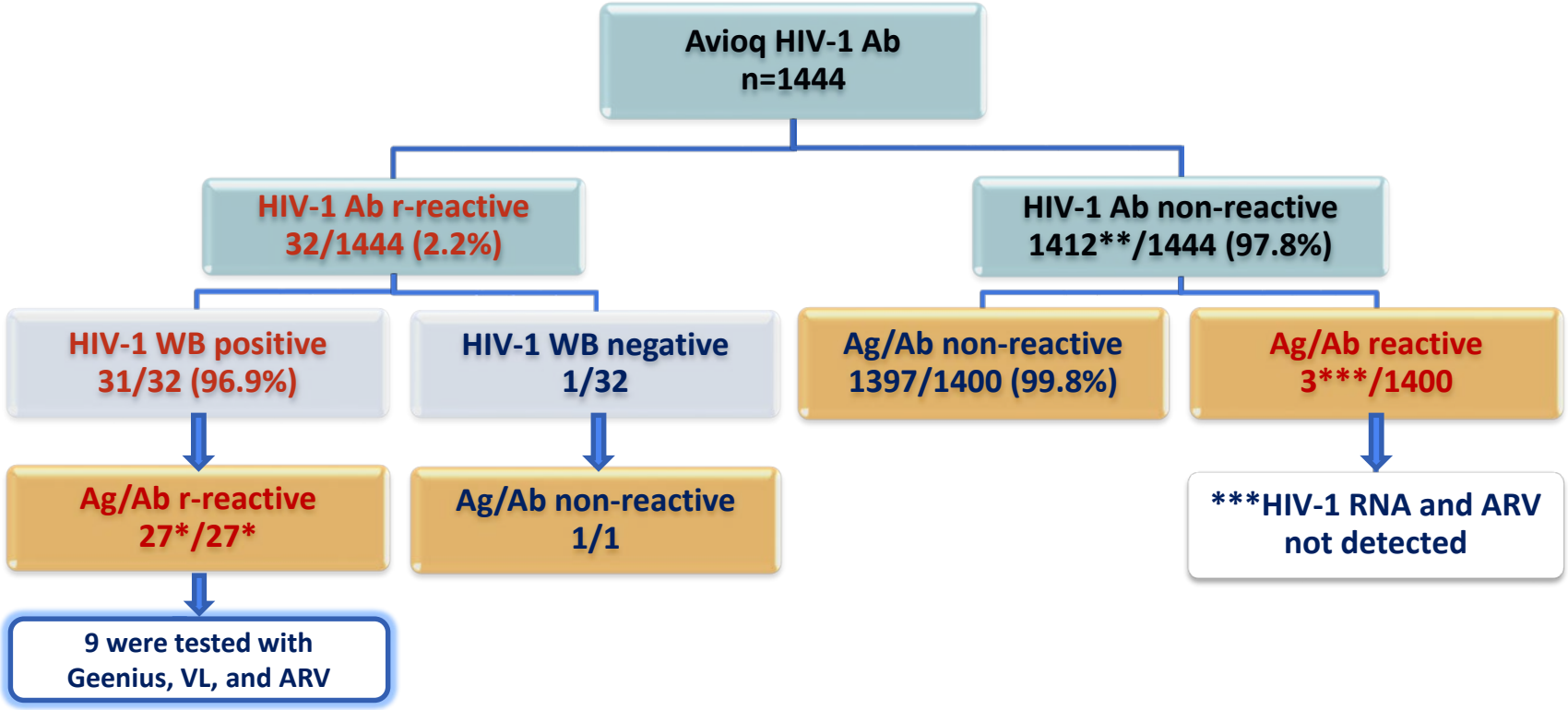
- **46.7% ≥ 50% humidity or did not have a humidity indicator card**

Humidity level checked at CDC	N=1444	%
30%	244	16.9
40%	525	36.4
50%	599	41.5
>50%	38	2.6
No indicator card	38	2.6

Characteristics of the participants (Part 3 & 4)

- **Participants were from all across the U.S.**
  - 38.7% South, 23.5% West, 19.3% Midwest, 18.5% Northeast
- **69.5% were male between 18-34 years old**
  - 21.7% 35-50yr, 8.8% ≥ 50yr
- **64.4% were Non-Hispanic-white**
  - 19.6% Hispanic, 7.4% Black, 5.1% Asian

Lab-based Testing Results



*4 HIV-1 WB positive samples had insufficient quantity for Bio-Rad Ag/Ab combo (Ag/Ab) testing

** 12 HIV-1 Avioq non-reactive samples had insufficient quantity for Ag/Ab testing

***Due to limited quantity these samples were tested in singlet with Ag/Ab, VL, and for ARV levels (No HIV-1 WB or Geenius results)

Nine Ag/Ab-reactive Geenius HIV-1 positive samples

- Two samples with mean VL 5.3 log (cop/ml) had no ARVs detected
- Seven samples with presence of at least one ARV, five had undetectable VLs and two had a mean VL of 3.4 log (copies/ml)

Viral load log (cop/ml)	Humidity (%)	Days from collection to CDC	Ral	ABC	3TC	EFV	TFV	FTC
5.17	40	21	-	-	-	-	-	-
5.4	40	15	-	-	-	-	-	-
3.29	40	13	-	-	-	-	+	+
3.58	50	20	-	-	-	-	-	+
TND	50	13	-	-	-	-	-	+
TND	50	48	-	-	-	-	+	+
TND	40	15	-	-	-	-	+/-	+
TND	No card	11	-	-	-	+	+	+
TND	50	13	-	-	-	-	+	+

The limit of detection for ARV drug is 20 ng/ml

Limitations

- Small number of infected participants with DBS cards in the study
- DBS Ag/Ab is less sensitive than plasma protocol, thus low p24 levels may have been missed
- DBS VL LOQ is less sensitive than plasma protocol and RNA integrity could have been compromised in DBS that were not stored and shipped properly or by several freeze/thawed cycles due to sequential testing

Conclusions

- **High percentage of self-collected DBS had enough quantity for lab testing with the FDA-approved HIV tests**
- **Deviations from optimal storage and shipping conditions were observed**
  - Serological and ARV tests may not be greatly affected, but further evaluation of impact on HIV-1 RNA detection is needed
- **In a small number of HIV-1 positive samples, the modified Ag/Ab protocol performed similarly to the FDA-approved Ab protocol**
- **Self-collected DBS is a feasible alternative for HIV testing, monitoring viral load and drug adherence**

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Disclaimer

The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Thank you!

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