Assay of Dried Blood Spots using the SD BIOLINE Onchocerciasis IgG4 Rapid Test

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Abbreviations

DBS	dried blood spot
ELISA	enzyme-linked immunosorbent assay
HRP	horseradish peroxidase
ICS	immunochromatographic strip
LOD	limit of detection
MSDS	material safety data sheet
MF	microfilaria
Oncho	onchocerciasis
Ov16	a protein expressed by the filarial parasite *Onchocerca volvulus*
RDT	rapid diagnostic test

Introduction

Dried blood spot (DBS) specimens have been collected as part of many onchocerciasis control and elimination program activities. These specimens have utility in laboratory analysis where testing can be done in batches under controlled settings. More than one test can typically be performed from a DBS specimen, allowing comparison of test methods or testing for multiple analytes. Additional benefits of DBS specimens are that they are easy to collect, store, and transport and often exhibit excellent stability for serological testing.

The SD BIOLINE Onchocerciasis IgG4 Rapid Test utilizes whole blood (fingerstick or venipuncture), plasma, or serum as validated specimens supported by the manufacturer. In order to be able to utilize DBS specimens collected by researchers and onchocerciasis control and elimination program activities, PATH developed and tested procedures to elute the specimen from the DBS and use on the rapid test. This document describes the methods and procedures that were determined to provide the optimal results. The methods and procedures described in this document have been utilized by PATH and other researchers with favorable results that correlate well with ELISA results and/or microfilaria staus. The goal of this document is to broadly disseminate these methods and procedures as best practices for utilizing DBS specimens with the rapid tests to the onchocerciasis community.

Materials

- TropBio or Whatman 903 DBS specimens (see method for note about quality)
- Punch, scissors, and/or forceps for cutting and transferring DBS specimens (considering best practices to reduce contamination and cross-contamination)
- Elution plate map
- Elution data recording form
- RDT results form
- 96 well elution plate (Corning 3788 or similar) and plate cover
- Adjustable volume pipette(s) and tips capable of delivering 10µL, 60µL, 70µL, and 100µL
- DBS Elution Buffer:
    - Sodium borate, decahydrate, reagent grade
    - Sodium chloride, reagent grade
    - Tween® 20, reagent grade
    - ProClin™ 950
    - Sodium caseinate, reagent grade
    - Water, high purity
- Refrigerator maintaining 2-8°C temperature
- SD BIOLINE Onchocerciasis IgG4 Rapid Tests, including assay diluent included in the kit
- Timer
- Thermometer (for measuring laboratory temperature)

Method

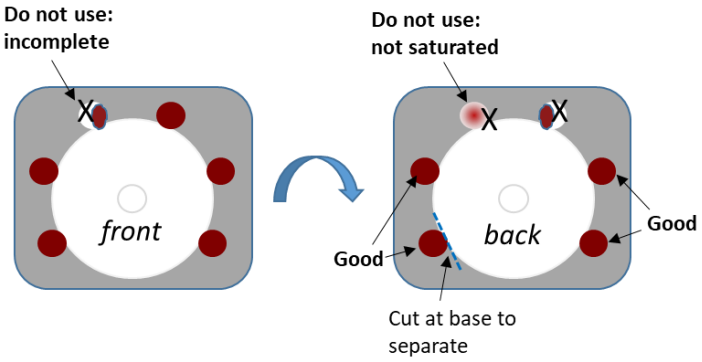
Training, proficiency, and quality

It is recommended that all operators are trained and proficient on the procedures of this method prior to testing clinical DBS specimens.

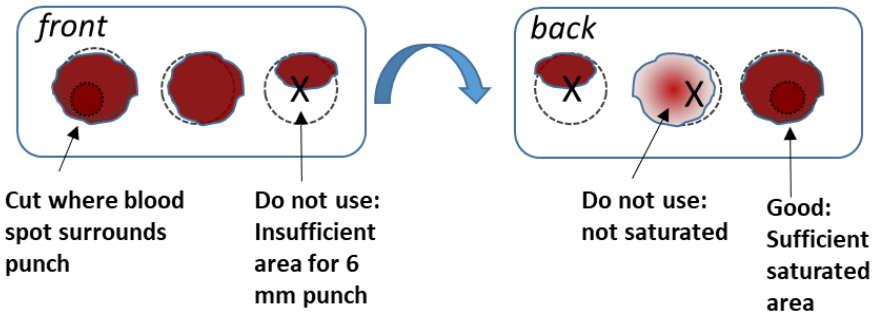
It is recommended that appropriate controls be included when testing clinical DBS specimens.

Dried blood spot elution – DAY 1

1. Assess the quality of DBS samples. Examine both the front and back of the DBS paper. There should be complete saturation of the TropBio 'petal' or saturation of the Whatman 903 paper that allows for a full punch to be taken from the saturated area. Do not use when incomplete in fill or not completely saturated.
   Examples of TropBio DBS quality are shown below:



   Examples of Whatman 903 DBS quality are shown below:



2. Cut (TropBio 'petal') or punch out 6 mm disc (Whatman 903) DBS samples, taking care not to contaminate or cross-contaminate DBS samples. Place DBS sample in a 96-well elution plate (Corning 3788 or similar).
3. Record the DBS sample ID in the elution plate map.

4. **For TropBio 'petal' add 60 µL of DBS Elution Buffer** to each well containing a DBS punch. Push the DBS down with the pipet tip to ensure that the punched spot in completely submerged in the buffer, with no air bubbles surrounding the spot.

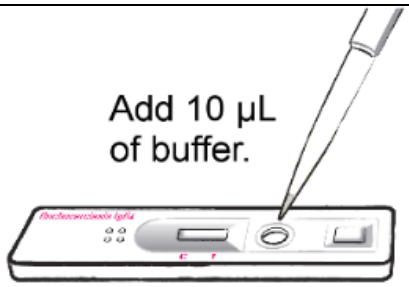
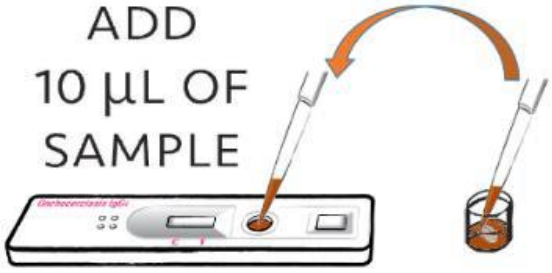
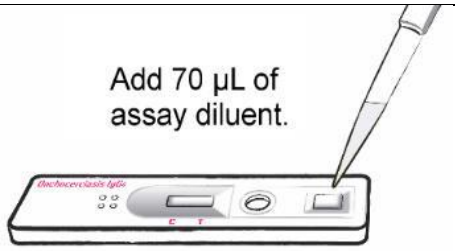
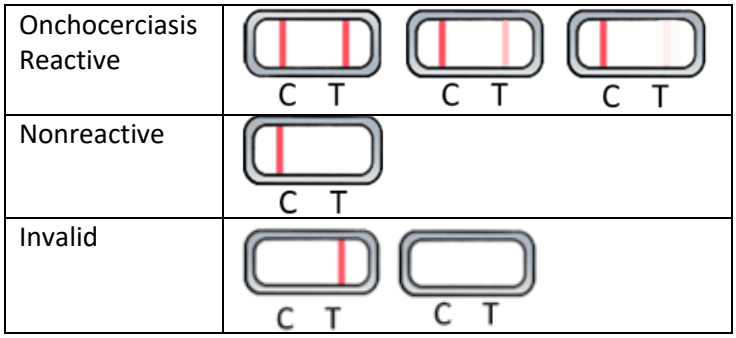
   OR

   For 6mm Whatman 903 punch add 100 µL of DBS Elution Buffer to each well containing a DBS punch. Push the DBS down with the pipet tip to ensure that the punched spot is completely submerged in the buffer, with no air bubbles surrounding the spot.

5. Cover the plate and incubate in the refrigerator overnight (12-24 hours) at 2-8°C.
6. Update the "elution data recording form."

Running DBS on the RDT – DAY 2

1. Prepare your workbench: bring the protocol, elution plate map, elution data recording form, RDT results form, timer, pen, marker, pipettes, tips, DBS Elution Buffer, RDTs, and the eluted samples.
2. Record the RDT lot number in the RDT results form. Record lab temperature (for monitoring only).
3. Open the RDT pouch and check the desiccant color. Only use RDT if color is yellow.
4. Write on the RDT the DBS ID, the date, and initials.
5. Using the elution plate map run the eluted DBS according to the following method:

1. **Add 10 µL of DBS Elution Buffer to the sample well (round).** Wait for the buffer to be absorbed.	
2. **Add 10 µL sample of DBS eluate to the same sample well (round)**. Wait for the sample to be absorbed.	
3. **Add 70 µL of assay diluent to the assay diluent well (square).**	
4. Set timer for 1 hour after adding assay diluent.	***1:00***
5. Read test result after 1 hour for preliminary results and record them in the RDT results form.	
6. Read test result after 24 hours for **final result** and record them in the RDT results form. **If results disagree from 1-hour result, 24-hour result should be used.**	

Elution plate map

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

Elution data recording form

Elution date	Eluted by	Number of DBS eluted	From well	To well	From DBS ID	To DBS ID
4.19.18	Jane Doe	4	A1	A4	4774	4778

RDT results form

RESULT INTERPRETATION OF EACH DBS SAMPLE
1=Positive, 0=Negative

Well	DBS ID	1-hour Result	24-hour Result	Comments	RDT lot	Room temperature
A1						
B1						
C1						
D1						
E1						
F1						
G1						
H1						
A2						
B2						
C2						
D2						
E2						
F2						
G2						
H2						
A3						
B3						
C3						
D3						
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H12						

Elution buffer formula

The final formula for the DBS Elution Buffer for the SD BIOLINE Onchocerciasis Rapid Test is:

50mM sodium borate

30mM sodium chloride

0.2% (v/v) Tween® 20

0.2% (w/v) sodium caseinate

0.05% (v/v) ProClin™ 950

The elution buffer is stable for 12 months from the data of manufacture when stored at 2-8°C. The buffer can be kept at room temperature during use.

Contamination of bulk solutions should be avoided.

ProClin 950 is added as a preservative.

A material safety data sheet (MSDS) for the buffer is not proposed or included in this document. When handling materials use appropriate precautions based on the chemical profile and safety requirements of individual components, as well as appropriate local, national, or international requirements.

Elution buffer work instruction

Preparation of DBS Elution Buffer

Calculate and record the batch volume to be prepared: _____ Liters

Assign a unique lot number to the buffer: _____

Composition: 50mM sodium borate, 30mM sodium chloride, 0.2% (v/v) Tween® 20, 0.2% (w/v) sodium caseinate, 0.05% (v/v) ProClin™ 950.

Bill of Materials

Description	Lot Number	Quantity Per Liter	No. Liters	Quantity Required	Quantity Used
Sodium borate, decahydrate, reagent grade		19.07 g			
Sodium chloride, reagent grade		1.75 g			
Tween® 20, reagent grade		2 mL			
ProClin™ 950		0.5 mL			
Sodium caseinate, reagent grade		2 g			
Water, high purity		0.8 L Additional as needed			

1. Purpose

This document describes the preparation of the DBS elution buffer for use with the SD BIOLINE Onchocerciasis IgG4 Rapid Test.

2. Health and Safety Information

2.1. Exercise care when handling all components. Observe protective equipment and clothing requirements when handling these materials. Refer to specific chemical MSDS for more information.

3. Required Equipment

3.1. Serological pipette and pipette tips.

3.2. Positive displacement pipette and pipette tips.

3.3. Analytical balance, capable of mg range.

3.4. Weigh paper or weigh boats.

3.5. Spatula.

3.6. Stir plate.

3.7. Magnetic stir bar.

3.8. Compounding vessel, appropriate to batch volume.

3.9. Graduated cylinder, appropriate to batch volume.

3.10 KimWipes.

3.11 pH meter.

3.12 0.45 µM cellulose acetate, surfactant-free, membrane filter.

3.13 Storage bottle, appropriate to batch volume.

4. Preliminary Operations

4.1. Ensure that the analytical balance has been calibrated and that the calibration date is current.

4.2. Calibrate the pH meter.

4.3. Label all components during the manufacturing process with the contents and date.

5. Procedure

5.1. Calculate and record the batch volume to be prepared.

Batch volume: _____ L Operator: _____ Date: _____

5.2. In the Bill of Materials, calculate and record the quantity required of each material.

Operator: _____ Date: _____

Verified by:_____ Date:_____

5.3. Add the required amount of high purity water to the compounding vessel.

Operator: _____ Date: _____

5.4. Place the vessel on the stir plate. Add a clean magnetic stir bar and begin stirring.

5.5. Add the required amount of sodium borate, decahydrate to the compounding vessel. Record the actual measured quantity used in the Bill of Materials. Mix until all salt crystals are dissolved and incorporated. (Note, this may take a long period of time.)

Operator: _____ Date: _____

5.6. Add the required amount of sodium chloride to the compounding vessel. Record the actual measured quantity used in the Bill of Materials. Mix until all salt crystals are dissolved and incorporated.

Operator: _____ Date: _____

5.7. Add the required volume of Tween® 20 to the compounding vessel. Record the actual measured quantity used in the Bill of Materials. Note: This volume should be measured and delivered using a positive displacement pipette. Wipe the outside of the pipette tip clean with a KimWipe before adding the Tween® 20. Slowly add the Tween® 20 to the compounding vessel and pipette several times to dispense the entire volume.

Operator: _____ Date: _____

5.8. Add the required volume of ProClin™ 950 to the compounding vessel. Record the actual measured quantity used in the Bill of Materials.

Operator: _____ Date: _____

5.9. Add the required amount of sodium caseinate to the compounding vessel. Record the actual measured quantity used in the Bill of Materials. Mix until all the sodium caseinate is completely dissolved and incorporated. (Note, this may take a long period of time.)

Operator: _____ Date: _____

5.10. Adjust the volume to the planned batch volume with high purity water.

Operator: _____ Date: _____

5.11. Mix until the solution is homogeneous.

Operator: _____ Date: _____

5.12. Measure the final pH and record below. Final pH should be between 9.1 and 9.3. consult supervisor if pH is outside this range.

Final pH: _____ Operator: _____ Date: _____

5.13. Set up filtration apparatus (0.45 µM) and filter batch into a clean storage bottle.

5.14. Confirm the final volume of the batch.

Final Batch Volume: _____ Operator: _____ Date: _____

Note: If final batch volume is not within ± 5% of the planned batch volume, consult supervisor.

6. Labeling and Storage

6.1. Assign a lot number per SOP Q0016 Assignment of Lot Numbers and label the bulk with the following:

DBS elution buffer for Oncho RDT	
Lot number:	Amount:
Store at: 2-8°C	Expiration Date:
Prepared by:	Date:

6.2. Shelf life: One year from date of manufacture.

6.3. Storage conditions: 2-8°C.

Evidence – Limit of Detection study

PATH conducted a LOD: study where procedures for specimen types (whole blood, plasma, and DBS) were compared on the RDT and using reference ELISA methods. The study concluded that all sample types (whole blood, DBS on RDT and plasma) can be used with results similar to the reference Ov16 HRP ELISA method (Standard Diagnostics). Additionally, it was found that DBS on RDT had the highest sensitivity of all sample types, especially at 24 hours.

Limit of detection

	Limit of detection of recombinant Ov16 IgG4 positive control, ng/mL in plasma[a]			***Highest dilution factor of MF+ clinical positive pools detected as positive[b]***		
	Whole blood	**DBS**	**Plasma/ Serum**	**Whole blood**	**DBS**	**Plasma/ serum**
Ov16 monoplex RDT						
30 min	50 ng/mL	25 ng/mL	50 ng/mL	MF+ 1:100	MF+ 1:200	MF+ 1:100
24 hr	50 ng/mL	15 ng/mL	15 ng/mL	MF+ 1:100	MF+ 1:400	MF+ 1:200
Ov16/Wb123 Biplex RDT (Ov16 results)						
30 min	25 ng/mL	15 ng/mL	15 ng/mL	MF+ 1:50	1:400	1:400
24 hr	25 ng/mL	15 ng/mL	15 ng/mL	MF+ 1:100	1:400	1:400
SD Ov16 HRP ELISA[c]	N/A	100 ng/mL	50 ng/mL	N/A	MF+ 1:100	MF+ 1:200
Ov16 AP ELISA[d]	N/A	2500 ng/mL	2000 ng/mL	N/A	MF+ 1:5	MF+ 1:10

[a] Tests were run in 10 replicates. Limit of detection is defined as the concentration of true positives at which ≥90% test results are positive.
[b] Tests were run in 5 replicates. All replicates were required to be positive to be considered a detectable dilution.
[c] Positivity based on 0.4 ratio to the kit calibrator control.
[d] PATH method adapted from Oguttu, et al 2014 AJTMH with cut-off guidance from Tom Unnasch.

References

The following peer-reviewed journal articles have utilized the materials and methods described in this document.

1. Kamgno J, Pion SD, Chesnais CB, Bakalar MH, D'Ambrosio MV, Mackenzie CD, Nana-Djeunga HC, Gounoue-Kamkumo R, Njitchouang GR, Nwane P, Tchatchueng-Mbouga JB, Wanji S, Stolk WA, Fletcher DA, Klion AD, Nutman TB, Boussinesq M. A Test-and-Not-Treat Strategy for Onchocerciasis in Loa loa-Endemic Areas. N Engl J Med. 2017 Nov 23;377(21):2044-2052. doi: 10.1056/NEJMoa1705026. Epub 2017 Nov 8. PubMed PMID: 29116890; PubMed Central PMCID: PMC5629452. https://www.nejm.org/doi/10.1056/NEJMoa1705026
2. Dolo H, Coulibaly YI, Dembele B, Guindo B, Coulibaly SY, Dicko I, Doumbia SS, Dembele M, Traore MO, Goita S, Dolo M, Soumaoro L, Coulibaly ME, Diallo AA, Diarra D, Zhang Y, Colebunders R, Nutman TB. Integrated seroprevalence-based assessment of Wuchereria bancrofti and Onchocerca volvulus in two lymphatic filariasis evaluation units of Mali with the SD Bioline Onchocerciasis/LF IgG4 Rapid Test. PLoS Negl Trop Dis. 2019 Jan 30;13(1):e0007064. doi: 10.1371/journal.pntd.0007064. eCollection 2019 Jan. PubMed PMID: 30699120; PubMedCentral PMCID: PMC6370230. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007064