

Serosurveillance to Assess Measles and Rubella Prevalence

Specimen Processing for Venous and Dried Blood Spot Specimens

Standard Operating Procedures

Version: October 20XX

1. Purpose/Background

- 1.1. The purpose of this document is to describe how to process liquid blood and dried blood spots (DBS) specimens from the MR (measles-rubella) serosurvey study at the DISTRICT laboratory. Liquid capillary or venous blood will be processed as sera within 24 hours of collection and stored in 2+ aliquots: first aliquot of 100 μ L, 2nd aliquot of 1 mL, and remainder in 3rd aliquot at -20 °C or colder until testing. DBS will be stored in individual Ziploc bags with a desiccant in a cool place in laboratory until testing.

2. Scope/Applicability

- 2.1. This SOP is intended for all individuals who will process and store DBS and liquid blood as part of study procedures.

3. Roles and responsibilities

- 3.1. Survey supervisors and laboratory staff are responsible for transferring blood specimens from the field to the DISTRICT laboratory and processing them.

4. Prerequisites / Supplies Needed

- 4.1. Ensure you have all the material that you need BEFORE beginning (see Appendix 5 for detailed supplies lists):
 - General lab supplies (gloves, laboratory coat etc.)
 - Centrifuge with adaptor for microtainer tubes
 - Thin permanent waterproof and smear-proof markers for labeling
 - Pre-printed cryolabels appropriate for -20 to -80 C storage
 - Extra blank labels
 - For liquid capillary or venous blood processing:
 - Pipettes and tips (P1000 and P100)
 - Cryovials (0.5mL and 2mL)
 - Vacutainer and cryovial tube racks
 - Cold/Freezer boxes, for storage
 - For dried blood spot processing (only for site doing serum vs DBS comparison)
 - Tupperware container to hold Ziploc plastic bags with DBS specimens
 - Paper-based lab log/register – record of specimen ID, time of collection, date of collection, presence of hemolysis (none/mild/moderate or severe) [sera only], estimated volume of serum and/or blood [sera only], size and quality of each spot

[DBS only], date and time of processing in the lab and location of freezer storage of each DBS specimen and aliquot of sera from liquid capillary blood (see Appendix 1). May print out copy of Lab Tally Sheet and paste into lab logbook.

- Freezer Box Map: Use to record where every sera aliquot and DBS card is stored in freezer; provided to testing laboratory when shipping specimens (see Appendix 3).
- Lab Tally Sheet (Electronic lab log) – record of serum ID, time of collection, date of collection, presence of hemolysis (none/mild/moderate or severe) [sera only], estimated volume of serum and/or blood [sera only], size and quality of each spot [DBS only], date and time of processing in the lab and location of freezer storage of each DBS specimen and aliquot of sera from liquid capillary blood, and any comments related to the specimen. This is an electronic (Excel) record of the paper lab log and to be uploaded to main server (see Appendix 2).

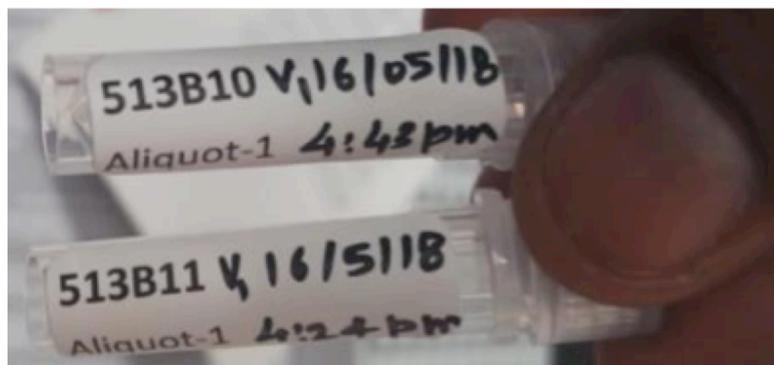
5. PROCEDURAL STEPS – FOR LIQUID BLOOD PROCESSING:

NOTE: Serum separator microtainer tubes or vacutainers of blood need to be kept in cold box at 4 °C and transferred to DISTRICT LAB within 24 hours for processing. Serum should be stored at -20 °C or colder.

- Put on gloves.
- Prepare and label freezer boxes:
 - One freezer box should be used for aliquot 1 (testing specimen; 0.5 mL cryovial) and a separate freezer box for aliquot 2 (2 mL cryovial). If three aliquots are obtained from a specimen, store the third aliquot in a separate freezer box. Samples should be placed into the cryobox in order based on participant ID number, leaving blank spaces for any samples that were not collected.
 - If specimens will be placed in a previously started freezer box, note the location of the last specimen in that freezer box (using the map). Set up dummy freezer boxes to use for organizing aliquoted specimens prior to transferring to the box currently in the freezer. Use an empty cryovial to mark the location where the first specimen should be placed. Reserve one blank space for every potential sample (13 per age group per cluster), and one blank space between the two clusters in the same box. Spaces for samples that were not collected will remain empty in the final freezer box.
 - Label boxes as follows:

STUDY NAME
Freezer Box __ [Serum Aliquot __]
Cluster ID: __ & __
SITE NAME
Month/Year: /2020

- As soon as the liquid blood samples arrive in the lab, take out the serum separator microtainer tubes or vacutainer from the cold box.
 - Check the number of specimens sent (per the field tally sheet) with the number in the freezer box.
 - Assess cold chain of the samples: Are ice packs frozen? Were tubes touching ice packs? If any problems, contact DISTRICT study coordinator immediately and record problems in lab register.
- If samples were not separated during field centrifugation, place the tubes in a centrifuge, taking care to balance the load.
- Check standard protocol at [NATIONAL LAB] on recommended revolutions per minute (RPM) and duration of centrifuging for venous blood. Centrifuge for 10 minutes at 3000 rpm. Do not open lid during spinning. Note: Speed in revolutions per minute (rpm) may be related to relative centrifugal force (g) by the following formula: $RCF \text{ (in g forces)} = 1.12 * 10^5 * (\text{rpm})^2 * r \text{ (in cm)}$. “r” is the radial distance from the center of the centrifuge head to the bottom of the tube.
- Using pre-printed labels, add the following information to each label with a sharpie pen:
 - Three labels should be prepared for each sample: Aliquot-1, Aliquot-2, and Register
 - Add specimen type to end of participant ID (V = venous blood, D = dried blood spot or S – capillary serum from heel or finger prick).
 - To the aliquot labels, add aliquot number after specimen type code. The 0.5 mL cryovial should be labeled aliquot 1 (e.g.: 112A04S1 or V1). The 2ml cryovial should be labeled aliquot 2 (112A04S2 or V2).
 - Example: The participant ID is 112A04V (112= Cluster; A= Age Group (A: 9 m - < 5 years; B: 5 - < 15 years; C: adult women); 04= 4th randomly selected individual in the age group; V= venous sample), then label the first aliquot by adding “1” to participant ID (112A04V1), and similarly, the 2nd aliquot 2 (112A04V2). Do not use a pen that will smear or rub off in freezer.



- Add date (DD/MM/YY) and time of collection on each label (should match date of collection from the specimen collection tube). Recommend copying the date and time of collection from the specimen collection tube onto one of the pre-printed lab labels prior to placing the specimen in the

centrifuge, then copy information onto other tubes while samples are being spun.

- Remove tube from centrifuge and record hemolysis level (none, mild, moderate/severe) in lab register. Organize the vacutainer and cryovial tubes by cluster, age group and number, leaving spaces for specimens that were not collected. For each cluster, organize A01-A13, B01-B13, and C01-C13.
- Transfer 100 uL of serum to the 0.5 ml cryovial labelled as S1 and up to 1 mL in the 2ml cryovial labelled as S2 (or V1 and V2, depending on specimen type). If there is any remaining serum, transfer to a third tube (write the specimen ID and related details on a blank label). Carefully pipette the serum without touching the pellet at bottom of the microtainer tube. Record estimated volume, date and time of processing on the lab register.
- Place labeled cryovials in freezer boxes, either newly started freezer boxes or the dummy freezer boxes for organizing specimens prior to transferring to the box in the freezer. Place aliquot 1 (testing aliquot) into one box and aliquot 2 (storage aliquot) into a separate box but in the same location (e.g., Box A6 location 7 [aliquot 1] and Box B6 Location 7 [aliquot 2]). Place any third aliquots into a separate box and record the location in the Comments of the lab register (one box should be reserved for any third aliquots obtained at the site).
- Record specimen location (box number and position location) of each aliquot of sera in the lab log/ register (Appendix 1) and Freezer Box map (Appendix 3). Record this information before placing aliquots in freezer.
- Place freezer boxes at -20 °C or colder for storage until testing.
 - If transferring tubes to a previously started freezer box, remove freezer box from freezer and quickly transfer the tubes from the dummy freezer box used to the organize specimens into the real freezer box. Do not leave frozen boxes with samples at room temperature for more than 3 minutes.
 - Freezer must stay at -20 C at all times. If electricity supply is interrupted, turn on back-up generator within 1 hour.
 - Freezer should be connected to a temperature monitoring system that is regularly monitored; any fluctuations in temperature of more than ± 5 degrees C should be reported to the site coordinator and central investigators.
- Ensure lab log/register contains all the required details (see Appendix 1):
 - Affix the Specimen ID label ('Register' label) with handwritten time and date of collection (recorded from original specimen label)
 - Aliquot 1 ID
 - Aliquot 2 ID
 - Aliquot 3 ID (report in Comments)
 - Specimen receipt date
 - Estimated volume of serum and/or blood (estimated by lab technician)
 - Presence of hemolysis (none/mild/moderate or severe)
 - Date and time of processing in the lab

- Freezer box number and position for each aliquot (report in Comments for Aliquot 3)
- Comments (may be left blank; record here if there were 3 aliquots obtained)
- Dispose of empty microtainer tubes, cryovials, contaminated pipette tips and other contaminated supplies in a biohazard waste container.
- Confirm information recorded in lab log/ register and copy into electronic Lab Tally Sheet (*electronic Lab Tally Sheet should be populated within 1 day of processing*).
- Upload electronic Lab Tally Sheet to main server (*may be done later*).
- Unit lab technician must discuss plans (method and timing) for shipment of specimen with the site coordinator and PI and central investigators. There are two options (see below). Refer to Shipment SOP for more details.
 - Hand-carried by air to NATIONAL LAB
 - Transport via commercial carrier

6. PROCEDURAL STEPS – FOR DRIED BLOOD SPOTS (only for site doing serum vs DBS comparison)

ORGANIZING AND LOGGING DBS CARDS

1. If using **Whatman 903 cards**:
 - Upon receipt at the DISTRICT LAB, DBS cards should be removed from their Ziploc bag and placed on a drying rack in a dust-free area, such as inside a fume hood (vents should not be turned on), to dry overnight. Individually labelled Ziploc bags with desiccant packs should be kept next to/near the cards; cards will be returned to their labelled Ziploc bags after drying and logging.
2. If using **HemaSpot HF devices**:
 - Ensure all DBS devices collected from cluster are placed in a large Ziploc bags containing additional desiccants and labelled with the cluster ID. No overnight drying step required.
3. Using the pre-printed ‘Register’ labels, prepare an additional label for each card collected.
 - Using waterproof pen, append the letter “D” on corresponding label.
 - Write date/time of collection from the DBS card label onto the Register label
4. Locate the partially filled plastic Tupperware box where the DBS cards will be stored and note down the box number (to be entered in lab log/register). If starting a new Tupperware box, prepare a label for the top of the box as pictured below. Record the cluster number of the first cluster to be placed into the box and the month and year of collection. Leave the last cluster number box blank until the box is filled.

STUDY NAME

Freezer or DBS Box Number: ___

DBS

Cluster ID: ___ & ___

SITE NAME

Month/Year: /2020

5. If using **Whatman 903 cards**: After drying overnight, record information about each DBS card into lab log/register and freezer box map. If using **HemaSpot HF devices**, this can be done immediately upon receipt. *Set Ziploc bag aside momentarily.*
 - Affix the Specimen ID label ('Register' label) with handwritten time and date of collection (recorded from original specimen label)
 - Specimen receipt date
 - If using **Whatman 903 cards**, also record number of spots by size and quality. How to categorize size of each spot (Appendix 4):
 - i. 100% full spot: Circle filled completely. No white visible inside the dotted lines of circle. *A complete spot must be the size of the circle but it can be off center.*
 - ii. 75-99% complete: At least 75% of circle is filled but less than 100% full.
 - iii. 50-74% complete: At least 50% of circle is filled but less than 75% full.
 - iv. Less than 50% complete: Less than 50% of spot filled
 - Freezer or DBS box number (where cluster-specific bag will be placed)
 - Comments (may be left blank)
6. After recording information into lab log/register (Appendix 1), staff will return DBS cards to the same individually labelled Ziploc bag with original desiccant pack (1 card per Ziploc bag) and carefully seal to close. Avoid switching bags between DBS cards; may lead to cross-contamination of specimens. *If no desiccant packet, put in new packet.*
7. Place individually labelled Ziploc bags with DBS cards into a gallon-sized Ziploc bag labelled with the cluster ID (1 gallon-sized Ziploc bag per cluster; 'cluster packs'); may be the same larger cluster-specific Ziploc bag that was used to transport the DBS cards from the field to the DISTRICT LAB.
8. Place the cluster-specific gallon-sized Ziploc bag into the plastic Tupperware box. Ensure box number is recorded in the lab log/register (see above).
9. When freezer or DBS box is full, write the cluster number of the last cluster placed into the box on the top of the freezer box.
 - Place cluster-specific gallon-sized Ziploc bags in order of Cluster ID
 - Ensure all DBS cards belonging to same cluster are stored in same freezer or DBS box, and within the box, stored within the same gallon-sized Ziploc bag.
10. Storage:
 - In case of **Whatman 903 cards**, place top on Tupperware plastic box and store in a cool place in laboratory - until testing.
 - i. Note: If room temperature is above 25 °C then DBS specimens if there are no options for a cool dry place, or there are concerns about temperature fluctuations during the day, a -20 C freezer could be used for storage (where available). Always transition specimens from warmer to colder temperatures (e.g., room temperature to 4 °C to -20 °C). Moving a specimen from a colder temperature to a warmer temperature may expose specimen to moisture.
 - In case of **HemaSpot HF device**, place DBS box at room temperature in an airconditioned room.

11. Confirm information recorded in lab log/ register and copy into electronic Lab Tally Sheet (*electronic Lab Tally Sheet should be populated within 1 day of processing*) (Appendix 2). Confirm storage location information for DBS cards recorded in freezer box map.
12. Upload electronic Lab Tally Sheet to main server (may be done later).
13. Unit lab technician must discuss plans (method and timing) for shipment of specimen with the site coordinator and PI and central investigators. There are two options (see below). Refer to Shipment SOP for more details.
 - Hand-carried by air to NATIONAL LAB
 - Transport via commercial carrier

Appendix 1. Lab Register

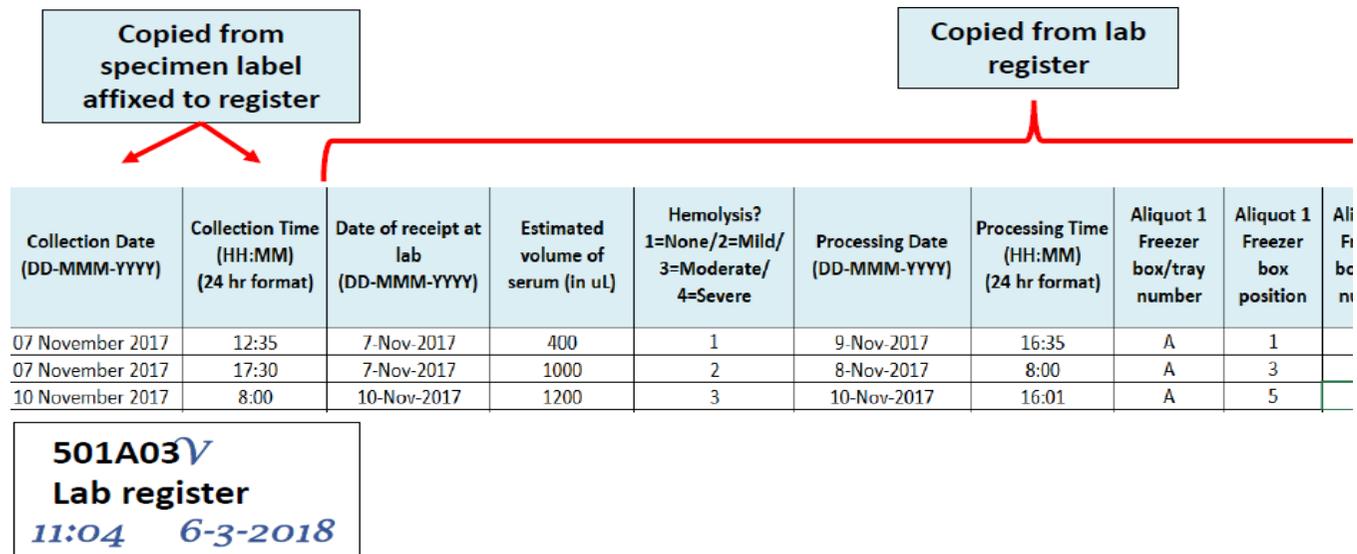
SERIAL #	PARTICIPANT ID	ALIQVOT 1 ID	ALIQVOT 2 ID	LABEL	SPECIMEN RECEIPT DATE DD-MM-YY	ESTIMATED SFAA VOL (uL)	HELYSIS 1-NONE 2-MILD 3-MODERATE OR SEVERE	PROCESSING DATE DD-MM-YY	PROCESSING TIME HH:MM	ALIQVOT 1 FREEZE BOX #	ALIQVOT 1 BOX POSITION	ALIQVOT 2 FREEZE BOX #	ALIQVOT 2 BOX POSITION	COMMENTS
1	513A02	513A02 V1	513A02 V2	513A02 14/5/18 Lab Register 4:05 pm	16/05/18	550	1	16/5/18	18:03	A	1	B	1	
2	513A03	513A03 V1	513A03 V2	513A03 17/5/18 Lab Register 11:57 pm	17/5/18	200	1	17/5/18	13:30	A	2	B	2	
3	513A04	513A04 V1	513A04 V2	513A04 16/5/18 Lab Register 3:24 pm	16/5/18	1100	1	16/5/18	19:03	A	3	B	3	
4	513A06	513A06 V1	513A06 V2	513A06 16/5/18 Lab Register 5:29 pm	16/5/18	400	1	16/5/18	19:03	A	4	B	4	
5	513A07	513A07 V1	513A07 V2	513A07 16/5/18 Lab Register 5:44 pm	16/5/18	400	1	16/5/18	19:03	A	5	B	5	
6	513A08	513A08 V1	513A08 V2	513A08 14/5/18 Lab Register 3:58 pm	16/5/18	200	3	16/5/18	13:03	A	C	B	C	
7	513A09	513A09 V1	513A09 V2	513A09 16/5/18 Lab Register 4:03 pm	16/5/18	500	1	16/5/18	19:03	A	7	B	7	
8	513A10	513A10 V1	513A10 V2	513A10 16/05/18 Lab Register 04:51 pm	16/5/18	400	1	16/5/18	19:03	A	8	B	8	
9	513A11	513A11 V1	513A11 V2	513A11 16/5/18 Lab Register 5:32 pm	16/5/18	500	1	16/5/18	19:03	A	9	B	9	
10	513A13	513A13 V1	513A13 V2	513A13 16/5/18 Lab Register 5:58 pm	16/5/18	400	1	16/5/18	19:24	A	10	B	10	
11	513B01	513B01 V1	513B01 V2	513B01 16/5/18 Lab Register 5:57 pm	16/5/18	400	1	16/5/18	19:24	A	11	B	11	
12	513B02	513B02 V1	513B02 V2	513B02 14/5/18 Lab Register 1:29 pm	16/5/18	1100	2	16/5/18	19:24	A	12	B	12	
13	513B03	513B03 V1	513B03 V2	513B03 16/5/18 Lab Register 3:29 pm	16/5/18	800	1	16/5/18	19:24	A	13	B	13	
14	513B04	513B04 V1	513B04 V2	513B04 17/5/18 Lab Register 11:20 am	17/5/18	300	1	17/5/18	13:30	A	14	B	14	
15	513B05	513B05 V1	513B05 V2	513B05 14/5/18 Lab Register 11:24 pm	16/5/18	1100	2	16/5/18	19:24	A	15	B	15	

Appendix 2: Lab Tally Sheet (Sera)

Information from the paper lab register will be data entered into the electronic version of the Lab Tally Sheet where the calculated fields will be populated. The completed Lab Tally Sheet will be uploaded or submitted to national/central lab.

ClusterID	HH NO	Name	Head	Sex	Sno	AgeGroup	Participant ID	Enter '1' if Aliquot 1 Collected. Leave blank if not collected	Enter '1' if Aliquot 2 Collected. Leave blank if not collected	Aliquot 1 ID (autopopulated)	Aliquot 2 ID (autopopulated)	Confir Aliquot
114	1	Aryan	Vihaan	Male	1	A	114-A-1	1	1	114A1V1	114A1V2	Yes
114	7	Anaya	Muhammad	Female	1	C	114-C-1	1		114C1V1		Yes
114	12	Krishna	Aarush	Male	1	B	114-B-1	1	1	114B1V1	114B1V2	Yes
115	57	Prajeet	Arun	Male	2	A	115-A-2	1	1	115A2V1	115A2V2	Yes
115	57	Prajeet	Arun	Male	3	A	115-A-3	1	1	115A3V1	115A3V2	Yes

Green = Copied from the Field Tally Sheet Excel file (sort Field Tally Sheet by Age Group and Sno prior to copying to match order specimens were organized in)
Blue = Entered by lab technician (using Lab Register)
Gray = Autopopulated based on prior columns



Appendix 2: Lab Tally Sheet (DBS [if using Whatman, add columns to the lab tally sheet to record size and quality of each spot])

ClusterID	Sno	AgeGroup	Participant ID	Hemolysis? Enter None, Mild, Moderate or Severe	Estimated volume of serum (in uL)	Aliquot 1 collected? (Tick 1 [Y] or leave blank [N])	Aliquot 2 collected? (Tick 1 [Y] or leave blank [N])	DBS collected? (Tick 1 [Y] or leave blank [N])

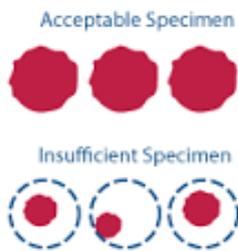
Green = Copied from the Field Tally Sheet Excel file (sort Field Tally Sheet by Age Group and Sno prior to copying to match order specimens were organized in)

Blue = Entered by lab technician (using Lab Register)

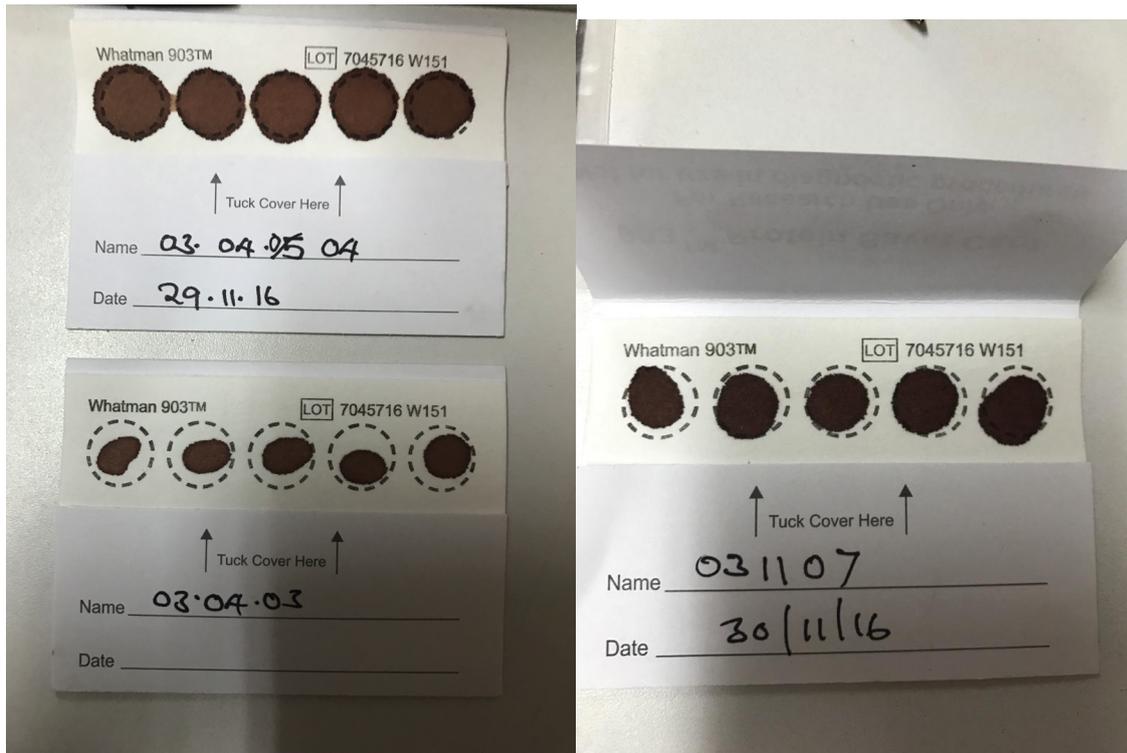
Gray = Autopopulated based on prior columns

Sno with leading zero (autopop)	Aliquot 1 ID (autopopulated)	Aliquot 2 ID (autopopulated)	DBS ID (autopopulated)	Aliquoting Date (DD-MM-YYYY)	Aliquoting Time (HH:MM AM/PM)	Aliquot 1 Freezer box/tray number	Aliquot 1 Freezer box position	Aliquot 2 Freezer box/tray number	Aliquot 2 Freezer box position	Comment
0										
0										

Appendix 4. Appropriate blood collection on a DBS card



Left card - comparison of acceptable and insufficient DBS. Right card – all spots are acceptable (100%)



Left photo – top card shows 100% for spots 1-4 and 75-99% for spot 5. Bottom card shows <50% for all spots

Right photo - card shows 50-74% for spot 1 and 75-99% for spots 2-5.

Appendix 5: Blood Collection and Processing Supplies list

Supplies list for 1500 participants in 1 site

#	Material	Quantity for 2 teams
Field (Specimen Collection)		
1	DispoVan 5ml syringe with needles 23/24G	1000
2	DispoVan 2ml syringe with needles 23/24G	500
3	Butterfly scalp vein with 23-gauge needles	200
4	Needle Destruction unit	2
5	BD vacutainer SST Tubes (Gel, yellow top vacutainer tubes) (Product number: 367984)	1500
6	BD Microtainer SST (500ul Gel, yellow top) tubes	100
7	Hard shell Vaccine Carrier (Standard size)	4
8	Vacutainer/ gel tube rack (should hold up to 40 tubes)	2
9	Ice/ gel packs	16 (4 in each vaccine carrier plus extras)
10	500ml Beakers (for transportation of tubes from field to site and overnight storage in fridge)	4
11	Cotton rolls (for packing samples during transport)	10 rolls
12	Sharps Container	35- 40 (if single use)
13	Band-aid	1700
14	Cold Box (small-medium size: to be used for storing extra ice packs)	2
15	Alcohol Wipes	1700
16	Tourniquets	2
17	Gloves (<i>check with site on preferred sizes</i>)	2500
18	Hand sanitizer (Sterilium 50 ml)	4
19	Thin waterproof markers	5
20	Bio Hazard Bag (Red) (thicker, higher quality)	100
21	Garbage bags (black) (thicker, higher quality)	100
22	Gauge Swab	1700
23	Cotton Swab Sterile	1700
24	Rubber bands	450

25	Cello tape	2 rolls
26	Scissor	2
27	BD retractable lancet (Product number: 366594)	800
28	HemaSpot HF Device	500
29	Desiccant (size of a postage stamp)	600
30	Small Ziploc bags (1 DBS device should fit in this bag)	500
31	Medium Ziploc bags (Glad Freezer Bags 30 units (gallon size))	2
32	Tote bags (to carry supplies to field) (1/ team)	2
33	Laboratory coat (<i>check with site on preferred sizes</i>)	2
34	Portable centrifuge	2
Site Lab (Specimen Processing)		
35	Pipette 1000ul	2
36	Pipette 200ul	2
37	Filter tips 200ul	10 boxes
38	Filter tips 1000ul	10 boxes
39	Sterile Screw cap vial 0.5ml	1250
40	Sterile Screw cap vial 1.8ml	1250
41	Vacutainer/ gel tube rack (should hold up to 40 tubes)	2
42	Cryovial tube rack	2
43	Cryoboxes (capacity 9x9) for smaller screw cap vial 0.5mL	15
44	Cryoboxes (capacity 9x9) for larger screw cap vial 1.8 mL	15
45	Large Ziploc bags (to carry samples from site to national lab)	100
46	Large Tupperware (to carry from site to national lab)	4
47	500ml Beaker (to be used in lab)	2
48	Laboratory coat (<i>check with site on preferred sizes</i>)	1
49	Scissor	1