PURPOSE: This SOP applies to the use of WHO International Standard for Plasmodium vivax lactate dehydrogenase (PvLDH), 19-116 (NIBSC, Hertfordshire, UK), in antigen detection assays. The purpose of preparation of standards for antigen testing is to test WHO-prequalified or investigational malaria rapid tests for detection of *Plasmodium vivax* antigen *Plasmodium vivax* lactate dehydrogenase *(PvLDH)* for quality checking of new lots and at specific study timepoints, training, and proficiency testing. Creating the dilution series of the standards followed by aliquoting and freezing will preserve the standards at relevant concentrations to be used as needed.

RESPONSIBILITIES:

1. The Project lead has the authority to establish this procedure.
2. The Scientific lead is responsible for the control of SOP documentation.
3. Laboratory staff are responsible for the implementation of this procedure and for ensuring that all appropriate personnel are trained.

MATERIALS REQUIRED:

- Cryovials suitable to hold 50-500 µL volume for aliquoting. (minimum of about 60 tubes)
- Titer tubes, microcentrifuge tubes, or cryotubes capable of holding volumes up to 2 mL, minimum of 7, for preparing dilutions.
- 15 mL vial for freezing of donor whole blood.
- Refrigerator (4°C) for storing dilutions, or wet ice if refrigerator not available.
- P-200 and P-1000 calibrated pipettors and pipette tips. Low-retention tips should be used, if available.
- Labels and labeling printer or markers.
- Whole blood diluent*: Plasmodium*-negative healthy universal (O+) donor whole blood, venous draw of K_2EDTA (see preliminary procedures), minimum volume of 9.5 mL for preparation of dilutions and additional sufficient volume to screen by microscopy and extract for PCR confirmation of negativity.
- Materials to conduct microscopy screening and PCR of donor whole blood diluent.

1. Specimen Handling

1.1 Consider all human specimens as capable of transmitting infectious agents. Use Blood borne pathogen precautions for all samples. Personal Protective Equipment (PPE) must be used for handling specimens and reagents. PPE includes:

- 1.1.1 Laboratory coat or gown
- 1.1.2 Eye protection
- 1.1.3 Latex or nitrile gloves, non–powdered preferred

1.2 Dispose of all specimens and used materials in accordance with local applicable guidelines and/or regulations.

2. Specimen Rejection

2.1 Quality of specimens must be evaluated at the point of delivery.

2.2 Unlabeled or mislabeled specimens must be rejected.
2.3 Clotted specimens must be rejected.

***WHO standards*:** *Plasmodium vivax 19/116* standard is available at NIBSC for procurement. Shipment requires import permit.

WHO standard *NIBSC* material	Description	Intended Use	Unit	Link
19/116	Lyophilized red blood cell (RBC) lysates from *P. vivax* infected donors from Peru.	Standardization, and evaluation of performance and sensitivity of *P. vivax* antigen detection tests that detect *P. vivax* lactate dehydrogenase (PvLHDH).	1000 IU of PvLDH per ampoule	NIBSC 19-116 https://www.nibsc.org/documents/ifu/19-116.pdf

3. Preliminary procedures to be completed before preparing standards set:

a. Whole blood diluent is prepared from a venous draw of K2EDTA whole blood from a healthy, universal donor (O+), negative for any *Plasmodium* infection. Ideally, the donor can be confirmed negative by microscopy upon day of draw when fresh, and by PCR to confirm negativity, prior to use to prepare standards. Following any confirmation by microscopy, the full volume (minimum 7 mL) blood should be frozen, reserving approximately 150 µL for PCR to confirm Plasmodium negative.

PROCEDURES:

Preparation of the dilution series

The dilution series will support preparation of the standards for use with RDT quality assessment. Concentration of the *PvLDH19/116* standard: 1000 IU of PvLDH per vial.

Reconstitute the lyophilized product within the vial by adding 250 ul of whole blood diluent. The whole blood diluent will be used to prepare the dilution series. Concentration of reconstituted vial: 1000 IU/250 µL or 4.00 IU/ µL or 4000 IU/mL.

- Gently tap down the vial before opening, to ensure all lyophilized material is near the bottom of the vial.

- After adding the 250 µL of whole blood diluent to the lyophilized material, wait for 5 minutes before mixing and pipetting. Mix the reconstituted stock thoroughly through pipetting and stirring with pipette tip, but mix carefully, avoiding bubbles.
- Pipette slowly since whole blood may be viscous. Try to avoid bubbles when pipetting.
- Mix each dilution gently, but thoroughly. Each dilution step should be mixed at least 20 times by gently pipetting up and down throughout the full volume, before proceeding to next dilution.
- Reconstituted stock and dilutions should be placed in the refrigerator (4°C) or on wet ice until aliquoted and frozen. Dilutions should be aliquoted and frozen within approximately 2 hours of reconstituting the stock.

Table 1: Dilution Series

Label ID	*Volume Stock or Previous dilution, uL*	*Diluent blood, uL*	*Total Volume*	*Remaining Volume*	***Concentration IU/mL***	*Number of aliquots of minimum 100 uL each*
PvLDH19/116_stock	Vialx1	**250**	250	175	**4000**	1
PvLDH19/116 - RDT A	**75**	**1425**	1500	750	**200**	7 or 8
PvLDH19/116_RDT B	**750**	**750**	1500	750	**100**	7 or 8
PvLDH19/116_RDT C	**750**	**750**	1500	800	**50**	7 or 8
PvLDH19/116_RDT D	**700**	**700**	1400	800	**25**	7 or 8
PvLDH19/116_RDT E	**600**	**600**	1200	800	**12.5**	7 or 8
PvLDH19/116_RDT F	**400**	**400**	800	800	**6.25**	7 or 8
PvLDH19/116_RDT G	**0**	**800**	800	800	**0**	9 or 10

Immediately after preparing, aliquot the resulting dilutions PvLDH19/116 - RDT A-G into 7 to 8 labeled cryovials, each with 100 µL minimum. Freeze immediately after aliquoting at -20°C or lower, preferably -80°C.

- Label tubes with Label ID and date prepared.
- Mark any last aliquot tubes which are over or under 100 µL volume.

For RDT testing:

Malaria RDTs should be tested using the controls, according to instructions for use. Aliquots of the dilutions are single-use only and should be used within 2 hours of thawing and be kept on ice until 10 minutes before use when they can be brought to room temperature and mixed before using. For new lot testing or quality checks at a specific study timepoint, it is recommended that each RDT is tested in duplicate with each of the control dilutions, including the negative, for a total of 14 RDTs per testing. Each aliquot can be used with up to 8 different

RDTs, tested in duplicate. If disagreement in result, error in run, or other problem is noted, then an additional replicate should be run as needed and volume is available. Expected positivity with each control dilution may depend on which RDT is used, although some dilutions must have specific results for the lot to pass.

Data Collection:

Site information should be completed with each RDT testing to record location, user, date, purpose of testing, and test information. Results for each RDT should be recorded, as shown in the table below. If replicate 1 and 2 have disagreement in test line results or if there is an invalid or error/unreadable, a repeat test should be run with the same aliquot of control. If repeat due to invalid, error, unreadable, and repeat result should be used to determine passing. For repeats due to disagreement in result, should be noted as partial positivity. These will not meet criteria for pass unless control is listed with criteria of "Positivity depends on RDT".

Result choices:

Overall:

☐ Valid: Control line present and result area is readable
☐ Invalid (no control line): Present/absent test line, NO control line visible.
☐ Unreadable/error: unreadable, severe background with control line, user error or other problem with test

Test line for PvLDH

☐ Positive for PvLDH: test line visible and control line visible
☐ Negative for PvLDH: NO test line visible and control line visible

Other test lines (for non-Pv targets, if present). Note the test line target in results: HRP2, PfLDH, or N/A if not present.

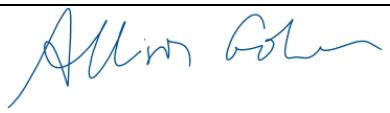
☐ Positive: test line visible and control line visible
☐ Negative: NO test line visible and control line visible

NIBSC *P. vivax* 19-116 control for rapid antigen testing

SITE:	
USER:	
DATE:	
Purpose of testing	☐ New lot ☐ Proficiency ☐ Training ☐ Time point quality check
Test Name	
Reference and Lot Number	
Test Line(s)	

Control Dilution	**Results for Replicate 1**	**Results for Replicate 2**	**Repeat Test Result note reason**	**Criteria for PvLDH**	**Criteria for any non-Pv test line**	**Pass/ Fail**
EXAMPLE	**Overall:** *valid* **PvLDH:** *positive* **Other:** *negative*	**Overall:** *Error-forgot buffer, invalid* **PvLDH:** *n/a - invalid* **Other:** *n/a invalid*	**Reason:** *error* **Overall:** *valid* **PvLDH:** *positive* **Other:** *negative*	Positive	Negative	*Pass*
PvLDH19/116 - RDT A	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Positive	Negative	
PvLDH19/116_RDT B	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Positive	Negative	
PvLDH19/116_RDT C	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Positive	Negative	
PvLDH19/116_RDT D	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Positivity depends on RDT	Negative	
PvLDH19/116_RDT E	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Positivity depends on RDT	Negative	
PvLDH19/116_RDT F	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Positivity depends on RDT	Negative	
PvLDH19/116_RDT G	**Overall:** **PvLDH:** **Other:**	**Overall:** **PvLDH:** **Other:**	**Reason** **Overall:** **PvLDH:** **Other:**	Negative	Negative	

4. Approval

Author	Signature	Date
Allison Golden		4 May 2022