

# Quality assurance protocol for dried blood spots

# Standard operating procedure 3.0

Subtitle: Laboratory protocol for TPPA testing using Serodia Fujirebio test kit

Protocol ID: 3.0

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**Purpose of protocol:**

Serodia TPPA test kit from Fujirebio inc. for qualitative detection of Treponema Pallidum antibodies is used for the detection of syphilis on DBS samples.

This protocol describes in detail the testing procedures when using DBS samples. The procedure is divided into two stages. The first stage explains the creation of a masterplate which needs to be incubated overnight. The second stage describes the TPPA test kit protocol using the masterplate as sample material. Test result interpretation is qualitative and described at the end of this SOP.

TPPA testing should be performed after DBS card quality evaluation (protocol ID 2.0). Printed 96 wells templates are needed to record test results. If bar-coded stickers on the DBS cards are used the printed template might be exchanged for an excel using a bar-code scanner device. The template to be printed can be found at the global health diagnostic website.

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HEALTH AND SAFETY INFORMATION

CAUTION:

**You are working with potentially infective materials. Read the manufactures manual for safety regulation.**

**Procedure (1):**

**Materials needed:**

- Specimen DBS cards + empty DBS

- Punch machine or punchers

- Disposable gloves

- Disposable tips

- Pipette’s (multi-pipette)

- PBS buffer (96\*100ul = 9,6ml for 1 plate)\*

- 96 flat wells plate + cover

- Printed 96wells template

- Plate shaker

*\* see page 4 (bottom) for detailed PBS-buffer requirements.*

**Operations:**

* Collect (sample) DBS cards
* Set up 96 wells plate (number the plate for reference)
* Leave well A1 empty for positive control
* Punch blank card at B1 for blank control
* Punch samples into the well’s starting at C1 (followed by D1 etc)
* For each punch, write down card-number on printed template
* For each punch, write down what punch (number) you used in the small square (optional)
* Add **100µl of PBS** buffer to each well (using multi-pipette)
* Tap the plate from the side (3x), the spots will turn
* Shake plate for ~30sec on a shaker
* Cover wells and place in a refrigerator (4 degrees Celsius) overnight to elute

This is your master plate.

Do not store the plate longer than 2 days (at 4 degrees Celsius)

**Procedure (2):**

Serodia TPPA test (following within 2 days after creating master plate).

**Materials needed:**

- (Multichannel) Pipette’s

- Timer

- Gloves

- Disposable tips

- U-shaped 96 wells plate (2x per one fully filled masterplate)

- 1.5ml vial

- Pen

**Pre-test**

- Prepare reagents **30min** in advance.

- Bring TPPA test kit at room temperature.

- Bring the Master plate at room temperature.

- Every test requires 2 wells, so for a full master plate, 2 u-shaped 96wells plate are needed.

**Operations**

* Number the U-shaped 96 wells plate 1 and 2 if applicable and mark the plate’s with numbers as on the masterplate. U-shaped 96 wells = U96W
* Pipette **25µl** of sample diluents into the whole first column (A1-H1)
* Repeat this step for column 3; 5; 7; 9 and 11 (A3-H3; A5-H5; A7-H7 etc) and go on, on the 2nd U96W plate if necessary.
* Elute **25µl** of positive control (from kit) and **190µl** sample diluents into a vial and mix thoroughly, with pipette.
* Pipette **25µl** of the positive control mix into well A1 of the U96W plate

**Read the following 4 steps first before proceeding**

* Pipette **25µl** of each well from the first column of the master plate into the first column of the U96W plate
* Mix specimen solution with sample diluent in U96W plate with pipette, thoroughly.
* Without changing tips, pipette **25µl** of the diluted specimen solution into the column next to it (first column A1-H1 🡪 A2-H2)
* Repeat for the other columns of the master plate. Column two of master plate must be pipetted into column 3 of the U96W plate, column 3 of the master plate into column 5 of the U96W plate etc. *(column 7 of the master plate must be pipetted into column 1 of the second U96W plate*.)
* Pipette **25µl** unsensitized particles with dropper or multi-pipette in every ***even*** column (A2-H2; A4-H4..etc)
* Pipette **25µl** sensitized particles in each well of every first (***odd***) column (A1-H1; A3-H3; A5-H5 etc)
* Cover the U96W plate’s and shake the plate’s with shaker for about 20sec
* Incubate at room temperature for at least **2 hours**.
* **Keep the plate free from vibrations**

\*if you are using the multichannel pipette instead of the provided droppers to add the unsensitized and sensitized particles, make sure that you place the pipette tips high on the wells to minimize contamination (see figure below). This is only of relevance when you are not changing tips.



**Test interpretation:**

* After incubation period, make a photo of the plate’s
* Write down the results on the printed template (P = positive N = negative I = Indetermined)
* Every odd column of the U96W plate corresponds with consecutive columns on the printed template
	+ Fill in results Column 1 of 1st U96W on column 1 of the printed template
	+ Fill in results Column 3 of 1st U96W on column 2 of the printed template
	+ Fill in results Column 5 of 1st U96W on column 3 of the printed template
	+ Fill in results Column 7 of 1st U96W on column 4 of the printed template
	+ Fill in results Column 9 of 1st U96W on column 5 of the printed template
	+ Fill in results Column 11 of 1st U96W on column 6 of the printed template
	+ Fill in results Column 1 of 2nd U96W on column 7 of the printed template
	+ Fill in results Column 3 of 2nd U96W on column 8 of the printed template
	+ Fill in results Column 5 of 2nd U96W on column 9 of the printed template
	+ Fill in results Column 7 of 2nd U96W on column 10 of the printed template
	+ Fill in results Column 9 of 2nd U96W on column 11 of the printed template
	+ Fill in results Column 11 of 2nd U96W on column 12 of the printed template
* Particles are concentrated in the shape of a button at the center of the well with a smooth (sharp) round outer margin. **Read:** **(-)** Non-reactive
* Particles are concentrated in the shape of a compact ring with a ***very small*** ‘hole’ in the middle and a smooth (sharp) round outer margin. **Read: (-)** Non-reactive
* Particles are concentrated in the shape of a compact ring with a ‘hole’ the middle and a smooth round outer margin. **Read: (+/-)** Inconclusive
* Defined large ring with a rough multiform outer margin and peripheral agglutination. **Read: (+)** Reactive.
* Agglutinated particles spread out covering the bottom of the *Well* uniformly. Edges sometimes folded. **Read: (++)** Reactive.

**Interpretation criteria:**

Check if all unsensitized particles are negative

Positive: specimen is (-) with unsensitized particles, but (+) with sensitized particles

Negative: (-) or (+) with unsensitized particles, if sensitized particles is (-)

If unsensitized and sensitized are both positive, the sample must be re-tested.

**Test validity**

The test is valid when the positive control is reactive.

**Limitations of procedure**

* Alterations in the physical appearance of the test kit materials may indicate instability or deterioration
* All highly sensitive assays have the potential for non-specific reactions
* Adaptations have been made to the manufacturers’ protocol.
* This protocol is established and validated within our laboratory setting. A test validation within your laboratory setting is highly recommended.

**PBS-buffer**

Needed: PBS buffer with 0.05% tween20

Example:

80ml PBS

40ul tween

Use ‘positive displacement’ pipette tips to add the tween20 to the PBS. This is a very thick soap, and normal tips will not work.