# Buruli Ulcer RDT

## Instructions for Use

### INTENDED USE

The Buruli Ulcer Rapid Diagnostic Test (RDT) is intended to detect active infections of *Mycobacterium ulcerans*, the causative pathogen of Buruli ulcer. The RDT detects the presence of mycolactone (ML), a cytotoxic metabolite of *M. ulcerans,* and is designed to work with ulcer exudates collected with a swab.

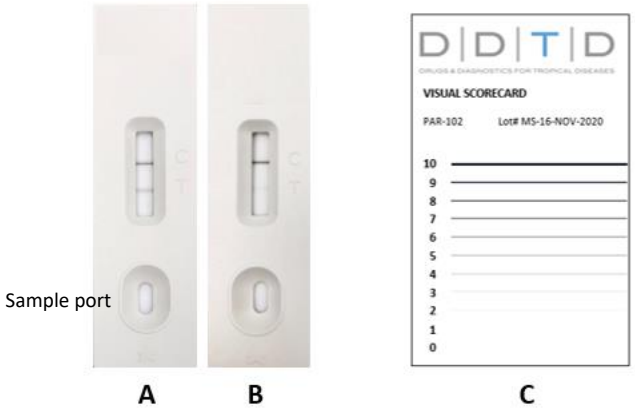


**Figure 1.** Examples of a negative (A) and a positive (B) Buruli ulcer test, T- test line, C-control line; (C) a visual scorecard, with test line shades ranging from 0 (not visible) to 10 (maximum).

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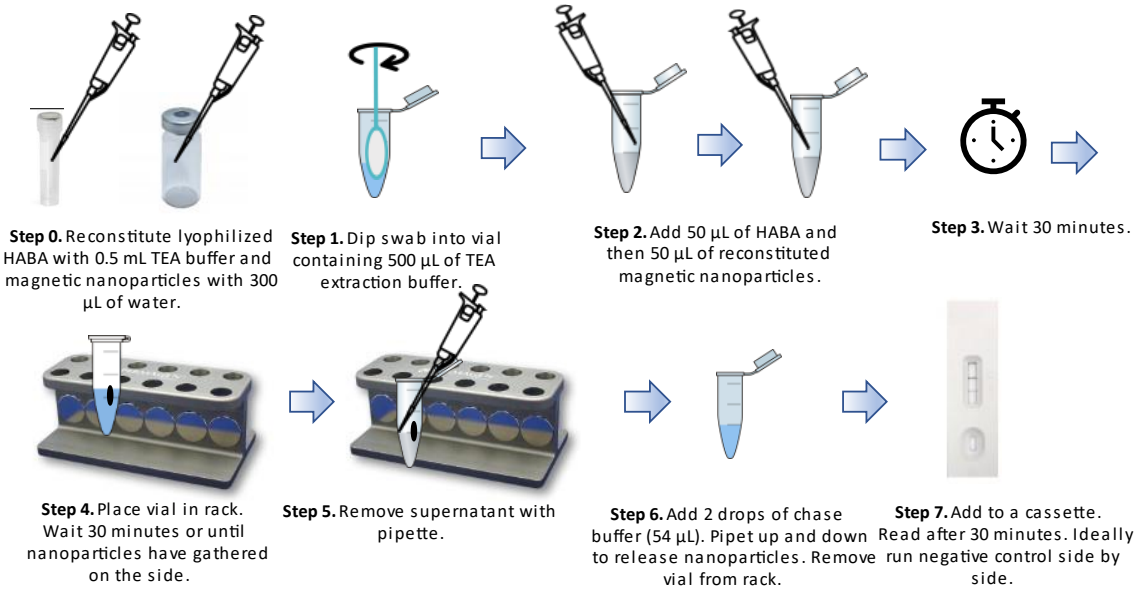


**Figure 2.** Process overview.

**PRINCIPLE**

The Buruli ulcer RDT is a competitive assay for the detection of mycolactone. Because of the competitive format, the assay produces a strong test line in the absence of mycolactone, and a weaker test line in the presence of mycolactone (Figure 1). To be considered positive, the test line intensity in the presence of mycolactone should be ≥3 shades weaker than in the negative control.

**PROCESS**

The process is summarized in Figure 2.

Step 0*:* The Heterophilic Antibody Blocking Agent (HABA) is reconstituted with TEA Extraction Buffer, magnetic nanoparticles are reconstituted with deionized water.

*Step 1*: The exudate specimen is collected from a suspected Buruli ulcer lesions with a swab and the mycolactone is extracted in TEA Extraction Buffer.

*Step 2:* Reconstituted HABA and magnetic nanoparticles conjugated to an anti-mycolactone antibody are added.

*Step 3*: The resulting mixture is allowed to incubate for 30 minutes. If mycolactone is present in the sample, it will bind to the antibodies present at the surface of the magnetic nanoparticles and saturate all their binding sites.

*Steps 4–5:* A magnet is used to retain the magnetic nanoparticles while discarding the excess of other substances. This is essentially a sample concentration step.

*Steps 6–7*: The magnetic particles are recovered and applied to the test strip, along with Chase Buffer. The test strip contains synthetic mycolactone immobilized at the test line. If the sample is mycolactone-free, the antibody conjugated to the magnetic nanoparticles will bind to the mycolactone in the test line. Because the magnetic nanoparticles have a deep black color, their accumulation at the test line will result in a line visible with the unaided eye. Conversely, if mycolactone is present in the sample, it will saturate the binding sites on the magnetic nanoparticles, and the nanoparticles will no longer be able to bind to the mycolactone immobilized on the test line, and thus will show no signal.

*Note*: A negative reference sample should always be run in parallel with the sample to be analyzed. The operator should compare the test line of the unknown sample with that of the negative reference. At this stage of development, relying on a single test, without comparative negative control, could be misleading.

**STORAGE AND STABILITY**

The devices should remain in the foil pouch with a desiccant until use and be stored at 4°C. Before opening the pouch, bring it to room temperature. Remove the devices from the pouch immediately before use. Do not freeze. Do not use after 6 months of the manufacture date.

**MATERIALS PROVIDED**

- Cassettes individually wrapped in sealed pouches.
- Lyophilized magnetic nanoparticles. One vial suffices for 5 tests.
- Lyophilized HABA (heterophylic antibody blocking agent). One vial for 10 tests.
- TEA extraction buffer, 250 mL bottle
- deionized water, 1 L bottle
- Chase buffer, 4 mL dropper bottle.
- 1.5 mL Axygen vials

- Mycolactone in ethanol, 7 ng/µL in Ethanol. Ampule of 60 µL
- Puritan swabs
- Magnetic rack

**MATERIALS NOT PROVIDED**

- deionized water
- normal human serum
- Pipettes (1–2 µL, 50–100 µL, and 500–1000 µL)
- pipette tips for all volumes
- 1.5- or 2-mL amber glass vial
- aluminum foil
- timer
- gloves
- marker pen

**INSTRUCTIONS**

**Reconstitute HABA**

1. Add 500 µL of TEA extraction buffer to a vial of lyophilized HABA.
2. Shake and leave for 5–10 minutes until all materials are dissolved.
3. Store in fridge (4 °C).

**Reconstitute Magnetic Nanoparticles**

4. Take vial of magnetic nanoparticle.
5. Remove aluminum seal and rubber stopper.
6. Add 300 µL of deionized water.
7. Shake gently 5–10 minutes until all materials are dissolved.
8. Store for up to 2 weeks in fridge (4 °C) but not in freezer (avoid freezing).

**Sample Preparation: negative and positive controls**

1. Take 4 Axygen vials.
2. Label 2 of them as positive and the other 2 as negative.
3. Place 500 µL of TEA extraction buffer in each vial.
4. For the negative controls, no further action.
5. For the positive controls, add 1 µL of mycolactone solution.
6. Cap and mix well, protect from light. Use within 1 hour.

**Sample Preparation: swab extract**

1. Label 1.5 mL Axygen vial.
2. Collect the ulcer exudate with a swab and place in vial with 500 µl TEA Extraction Buffer, soak for a few minutes, mix, squeeze swab against the wall to recover as much liquid as possible. Use within 1 hour.

**Run Test**

1. Place Axygen vial containing negative control, positive control, or swab extract on a non-magnetic rack.
2. Add 50 µL reconstituted HABA if using swab extract, mix. If using controls HABA is not required.
3. Add reconstituted magnetic nanoparticles.
    - If using positive and negative control, add 40 µL of reconstituted magnetic nanoparticles.
    - If using swab extract, add 50 µL of reconstituted magnetic nanoparticles
4. Incubate the mixture for 30 minutes by letting it sit on a regular rack, while protecting from light with aluminum foil or a dark box (mycolactone is light-sensitive). At this stage keep the magnet rack and any other magnets away to not interfere with the assay.
5. Place the vials on a magnetic rack for 30 minutes, protect from light. The nanoparticles will accumulate against the wall of the vial as shown in Figure 3. This should be obvious with negative and positive control, but may be barely visible or not visible at all with clinical specimen.

**Figure 3.** Magnetic nanoparticles accumulated after 10 minutes on the magnetic rack. The arrows pointed to the areas of maximum accumulation.

11. After 30 minutes, remove the liquid gently from the first vial with a pipet. When decanting, place the pipet tip closer to the wall opposite of the magnet to not disturb the particles.
12. Add 2 drops (= 54 µL) of Chase Buffer into the vial and remove the vial from magnetic rack. Wash out particles from the side wall of the vial and mix gently by pipetting several times.
13. Transfer the vial content to the sample port of the cassette, record start time.
14. Repeat steps 11–13 for all vials.
15. Read after 10 and 30 minutes from the start time. Bring the test as close as possible to the test card and score the test line intensity.
16. Take a picture.

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| Document Control | | | |
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| Amendment/<br>Change | 17 JUN 2021 | | |

Revision B was written with the intention to validate the test in a laboratory setting (Prof. Stinear, U. Melbourne) using magnetic nanoparticles provided in solution, and serum in negative/positive controls.

Revision C is written with the intention to validate the test in the field (Ivory Coast, Cameroon), using lyophilized magnetic nanoparticles, and no serum in negative or positive control.

Revision D includes revisions to get more consistent results with clinical specimen. (a) Use of HABA is introduced, (b) incubation time on rack changed from 10 to 30 minutes (c) first incubation changed from 25 to 30 minutes – does not change outcome, but easier to memorize that there are two incubation steps, each of 30 minutes.