Diagnostic Tests Guidance

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TAS Workshop
Abidjan, Cote d’Ivoire
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### GPELF recommended diagnostic tests

<table>
<thead>
<tr>
<th>Field assay</th>
<th>Detection target</th>
<th>Recommended for use during</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood smear</td>
<td>Microfilariae (Mf)</td>
<td>Mapping, sentinel site and spot-check site monitoring</td>
</tr>
<tr>
<td>Abbott Filarisis Test Strip (FTS)</td>
<td>Filarial <strong>antigen</strong> (Ag)</td>
<td>Mapping, sentinel site and spot-check site monitoring, <strong>TAS</strong></td>
</tr>
<tr>
<td>Brugia Rapid™ test</td>
<td>Antifilarial <strong>antibody</strong> (Ab)</td>
<td><strong>TAS</strong></td>
</tr>
</tbody>
</table>
Planning
Filariasis Test Strip Subsidy

- FTS needed for surveys now available through WHO
- Countries submit the following to WHO country office (cc RO & HQ):
  - Letter of request from MOH
  - “No objection certificate” for importation
  - If TAS, completed WHO TAS Eligibility and Planning Form
  - FTS for pre-TAS are available and requires a letter of request and pre-TAS plans (e.g., indicate which IUs meet the criteria).
  - FTS also available for re-mapping
- Expected time from cleared request to delivery is **12 weeks** *(submit request as early as possible)*
- FTS has **12-month shelf life** from production
- Currently, donated FTS cannot be used for post validation surveillance activities
TAS eligibility and planning

- **TAS Eligibility Form** to be submitted to WHO
- Allows multiple evaluation units
- Reviewed independently by RPRG
- Submit early (preferably 6 months prior to planned TAS or immediately after eligibility criteria met)
- Estimates required diagnostic tests

http://www.who.int/entity/lymphatic_filariasis/resources/WHO_TAS_EPF.xlsm
Positive Control
Using positive control

- Use of positive control ensures that between the manufacturing site and delivery, we can have confidence that the test is working correctly and able to detect circulating antigen.

- Test FTS **from each lot** using positive control.
  A. Upon receipt of FTS order at national level
  B. Immediately prior to use in surveys
  C. Recommended to test two FTS per lot

- 1 vial contains enough positive control to test 5 strips

- Recommended to refrigerate positive control upon receipt.
How to test FTS using positive control

**Testing with a positive control** – Before a field survey is begun, two tests from each lot should be tested with a weak positive control, which can be obtained from WHO. With this control, the test line may be very faint.

*Do not* use the lot of strips that give a negative result when tested with the control.
How to get positive control

1. Country submits a letter of request (WHO template) and signed agreement through WHO country office (CO)

2. WHO CO forwards to WHO Regional Offices and WHO HQ for review

3. Name and address of consignee provided by WHO CO [WHO Country Representative]

4. DHL delivery from WHO HQ (Geneva) to consignee
Filariasis Test Strip (FTS)
Filariasis Test Strip (FTS)

- Sensitive for detecting *Wuchereria bancrofti* antigen
- Does not require laboratory equipment and can be processed quickly
- Positive result indicates the presence of adult worm antigen; proxy for infection

**Training**
- Adequate training is necessary to reduce inter-observer variation and to reduce misreading of tests, which can lead to false-positive results
- Limit technicians to only those who demonstrate efficiency in blood collection and test function
FTS: Preparation

- **Storage** – tests have a limited shelf-life at ambient temperature (12 months). Tests should not be frozen or used past the printed expiration date. They should be stored in a cool, dry place out of direct sunlight. Ambient temperature should be between 2-37° Celsius. Tests also should not be exposed to extreme heat for prolonged periods of time. Report any irregularities with the tests using the new form that will be posted on the NTD Toolkit website.

- **Transport** – A cool box is not required for transporting tests for use in the field; however, care should be taken not to expose tests to extreme heat for long periods.

- **Adequate light** – tests must be read under adequate lighting, as faint lines can be difficult to see. This is especially important when reading tests at night.
Materials

- Expiration date and kit lot number on outside of box
- Each kit contains 30 sealed test strips and fixed volume (75ul) micropipettes
- If using blood collection tubes, must contain heparin anticoagulant (NOT EDTA tubes)
- FTS should be used immediately upon opening
Where do you find the expiration date?

- Countries may consider reserving tests close to expiry date for training purposes
FTS Design

1. Sample pad
2. Conjugate pad
3. Absorbent pad
4. Nitrocellulose strip

FLOW DIRECTION
Blood Collection
### Procedure (1)

<table>
<thead>
<tr>
<th>Adhere label to test strip and tray.</th>
</tr>
</thead>
<tbody>
<tr>
<td>If outside – windy, tests blow away!</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Collect 75ul of fingerstick blood using the supplied micropipette. <strong>DO NOT</strong> squeeze the bulb end when collecting the sample. (field team in home)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternatively, pipette 75ul of blood from heparin capillary tube. (lab team in central location)</td>
</tr>
</tbody>
</table>
**Procedure (2)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add the blood sample slowly to the lower half of the sample pad.</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Wait for the sample to migrate to the reading window.</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Record reading time onto the tray.</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Read the results exactly 10 minutes after the sample is visible in the reading window.</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Record result directly on the tray</td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Blood collection tips

- Ensure you have sufficient quantities of blood collection supplies each day before going to the field
  - Pack supplies in advance
- Always practice universal precautions when handling blood
- Prick side of finger, not tip
  - It bleeds more and hurts less
- Change materials (e.g. absorbent pad) if they are visibly dirty
- Do not perform more than 2 finger sticks on the same person
  - Always ask the child for permission to perform a 2nd finger stick
FTS training

How to Use the Alere Filariasis Test Strip

- Training video available for streaming on YouTube ([https://www.youtube.com/watch?v=oB04WgBrGdo&list=PL2kJmo93WRTKkIcavVSH3uixHh21oUVOQ](https://www.youtube.com/watch?v=oB04WgBrGdo&list=PL2kJmo93WRTKkIcavVSH3uixHh21oUVOQ))
## Results

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Weak positive</th>
<th>Negative: Control line only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive: <strong>Any</strong> visible pink line in the reading window</td>
<td>![Positive Image]</td>
<td>![Weak Positive Image]</td>
<td>![Negative Image]</td>
</tr>
</tbody>
</table>

**Positive**
- Any visible pink line in the reading window.

**Weak positive**
- Specific indicative lines.

**Negative**: Control line only.
### Invalid Tests

<table>
<thead>
<tr>
<th>Sample has not migrated ALL the way up the strip</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image of a test strip with no control line" /></td>
</tr>
<tr>
<td>No lines appear</td>
</tr>
<tr>
<td><img src="image2.png" alt="Image of a test strip with no control line" /></td>
</tr>
<tr>
<td>Test line only (no control line)</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image of a test strip with no control line" /></td>
</tr>
</tbody>
</table>
When should a diagnostic test be repeated?

Repeat the test when

- 1st test is positive
- 1st test is invalid

Best practice

- Take a photo of positive and invalid results
- Repeat a test no more than once per person
# Interpreting the result of a repeat test

<table>
<thead>
<tr>
<th>1st Result</th>
<th>2nd Result</th>
<th>Interpretation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Provide treatment</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Indeterminate</td>
<td>Exclude from sample and treat</td>
</tr>
<tr>
<td>Positive</td>
<td>Invalid</td>
<td>Positive</td>
<td>Provide treatment</td>
</tr>
<tr>
<td>Invalid</td>
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<tr>
<td>Invalid</td>
<td>Negative</td>
<td>Negative</td>
<td>-</td>
</tr>
</tbody>
</table>
In the event of a positive test

- Confirm the positive test result with another team member/supervisor as soon as possible

- All children with a positive FTS results should be tested a second time as soon as possible
  - Positive – Record as « positive »
  - Negative – Record as « indeterminate »
  - Not able to retest – Record as « positive »
FTS Operational Notes

- Do not add any water or buffer to facilitate movement of the sample

- The micropipette does not contain any anticoagulant, the capillary collection tube does – lithium heparin

- Add sample slowly so it doesn’t spill off the pad, but fast enough so the blood doesn’t start to flow through the reading window before all the sample is added

- Results must be read at exactly 10 minutes and recorded on the tray
Questions?