SOP FOR MALARIA DIAGNOSIS

RDT AND MICROSCOPY (THICK & THIN BLOOD SMEAR)

QUALITY ASSURED DIAGNOSIS | PROMPT REPORTING | TIMELY TREATMENT | MALARIA ELIMINATION

1. WHEN TO TEST FOR MALARIA?

- Fever or history of fever with / without chills.
- No obvious alternative cause.
- All suspected cases (regardless of age).
- High-risk groups and vulnerable populations.
- People from malaria endemic areas with travel history.
- Test household members, neighbours and epidemiologically linked persons.
- Test in low transmission settings and during elimination activities.

Test, Treat and Help Eliminate Malaria

2. DIAGNOSTIC ALGORITHM

Suspected malaria
(Fever / history of fever / chills)
No obvious alternative cause

↓

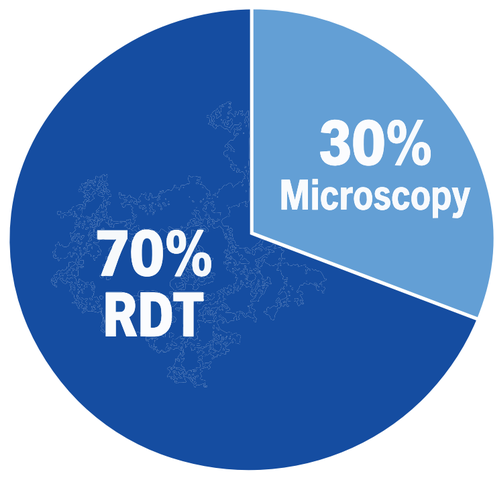
Parasitological confirmation
Preferred: Microscopy – Gold Standard
OR
Quality-assured RDT
(when microscopy not available within 24 hours)

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Positive for malaria → **Start treatment as per national guidelines**

Negative but clinical suspicion high → **Repeat test / Do microscopy**

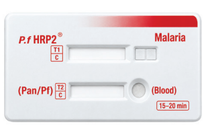
3. DIAGNOSTIC MIX
(Programme Level)



- ✓ Facilities with laboratory services should routinely use microscopy.
- ✓ RDT useful during emergencies / odd hours.
- ✓ RDT to be used when microscopy result cannot be provided within 24 hours.

4. RAPID DIAGNOSTIC TEST (RDT) – PROCEDURE (as per NCVBDC)

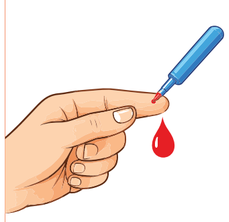
1 Check kit



- Use approved RDT kit.
- Check expiry date and kit condition.
- Keep test kit at room temperature

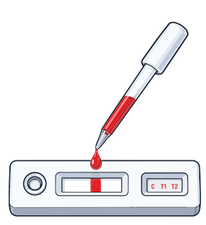
2 Collect blood

- Clean fingertip with spirit and allow to dry.
- Prick with sterile lancet.
- Wipe first drop of blood.



3 Add blood

- Add required volume of blood (usually 5 µl) as per kit instruction.



4 Add buffer

- Add specified number of buffer drops (usually 3–4 drops) as per kit instruction.



5 Read result

- Read within 15–20 minutes as per kit instruction.
- Do not read after 30



15–20 min

5. RDT – INTERPRETATION (Pf HRP2 & Pan Antigen)

Negative	Pf Positive (T1)	Non-Pf Positive (T2)	Pf + PV Positive (T1 & T2)	Invalid
				
Only control line (C). No line in T1 or T2 **No malaria infection detected.**	Control line (C) and line in T1. P. falciparum malaria **detected.** (Test is positive even if T1 line is faint.)	Control line (C) and line in T2. Non-falciparum malaria (P. vivax, P. ovale, P. malariae) detected.	Control line (C), line in T1 and line in T2. mixed infection detected.	No control line (C), with or without T1/T2. **Test is invalid. Repeat with a new kit.**

6. BLOOD FILM PREPARATION

A. Collect blood sample



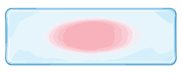
- Use middle/ring finger.
- Puncture the side of fingertip.
- Do not puncture too close to nail bed.
- Wipe first drop with clean cotton.
- Gently squeeze to get a large drop of blood.

B. Prepare thick & thin smear
(on the same slide)

Thick smear
(Parasite Detection)



Thin smear
(species identification)



THICK FILM
- Place 2–3 large drops of blood on the slide.
- Using the corner of another slide/applicator, spread in a small circular area (≈1 cm diameter).
- Make a thick, even film.
- Air-dry completely. Do NOT fix with methanol.

THIN FILM
- Place 1 small drop of blood near one end of the slide.
- Hold a clean spreader at 30–45° and push smoothly forward.
- Prepare a feather-edged thin film.
- Air-dry and fix with absolute methanol for 1–2 seconds.

7. STAINING OF BLOOD FILMS
(as per NCVBDC guidelines)

A. JSB STAINING (JSB-I & JSB-II)
(Programme method)

- Prepare thick & thin blood films and air-dry.
- Fix thin film only with absolute methanol for 1–2 sec.
- Do not fix thick film.
- Staining JSB-II → 1–2 sec
- Wash 2–3 times with buffered water (pH 6.2–6.8)
- JSB-I → 45 sec
- Wash 3–4 times with buffered water
- Air-dry → Examine under 100× oil immersion
- Key point: JSB-II is applied first, followed by JSB-I.



B. GIEMSA STAINING
(Alternative / Reference method)

1. Prepare 3% Giemsa stain with buffered water (pH 7.2).
2. Fix thin smear with methanol for 1–2 seconds.
3. Blood slide with 3% Giemsa stain.
4. Stain for 30–45 minutes (as per SOP).
5. Wash gently with clean water (pH 7.2).
6. Allow to stand for 10 minutes.
7. Air dry in upright position and examine.



8. EXAMINATION & REPORTING



1. Use 100× oil immersion.
2. Examine at least 100 high power fields (HPF) for thick film before reporting negative (as per SOP).
3. For thin film: examine minimum 100 HPF before reporting negative.
4. Identify species and report parasite density as per standard method (if required).
5. Record species, date & time, name and ID of microscopist.
6. Report results immediately (within 24 hours) for all positives.

9. QUALITY ASSURANCE (QA)

- Daily check with known positive & negative slides / RDT controls.
- Check quality of reagents, stains and pH.
- Maintain microscope, slides, stains and equipment.
- Maintain register for QA, results and corrective actions.
- Ensure staff competency through training and proficiency testing.
- Participate in external quality assurance (EQA) / slide rechecking.

10. BIOSAFETY



- Treat all blood as potentially infectious.
- - Wear gloves and lab coat.
- - Use clean and disinfected slides.
- - Dispose sharps in puncture-proof container.
- - Decontaminate surfaces and spills with 1% sodium hypochlorite.
- - Wash hands after work.

11. WHEN TO REPEAT / REFER?

- High clinical suspicion but test negative.
- Poor quality slide / RDT error.
- Early in illness (<5 days).
- After 24–48 hours if fever persists.
- Mixed infection suspected.
- If RDT invalid → repeat with new kit.
- Refer to higher laboratory if required.

राष्ट्रीय स्वास्थ्य मिशन
हिमाचल प्रदेश

NATIONAL HEALTH MISSION

DEPARTMENT OF HEALTH AND FAMILY WELFARE, H.P.

मानव सेवा

TOGETHER WE CAN END MALARIA

HEALTHY HIMACHAL
MALARIA FREE HIMACHAL