# Results

*Sample Selection*

Of 2228 total blood smears taken by Mae Tao Clinic staff between March 5-31, 2007, 845 came from eligible patients. Of these patients, 786 consented to participate in the study. Of these, 762 had blood smears and PCR samples recovered from them successfully. Of the 762 smears, 136 were interpreted as positive, and 626 were interpreted as negative by Mae Tao Clinic staff. All 136 positive smears, (100.0%) were submitted for quality control analysis. Of the 626 negative smears, 203 (32.4%) were randomly selected for quality control analysis. Therefore, a total of 339 blood smears and their corresponding PCR samples were included in the quality control analysis (Figure 2).

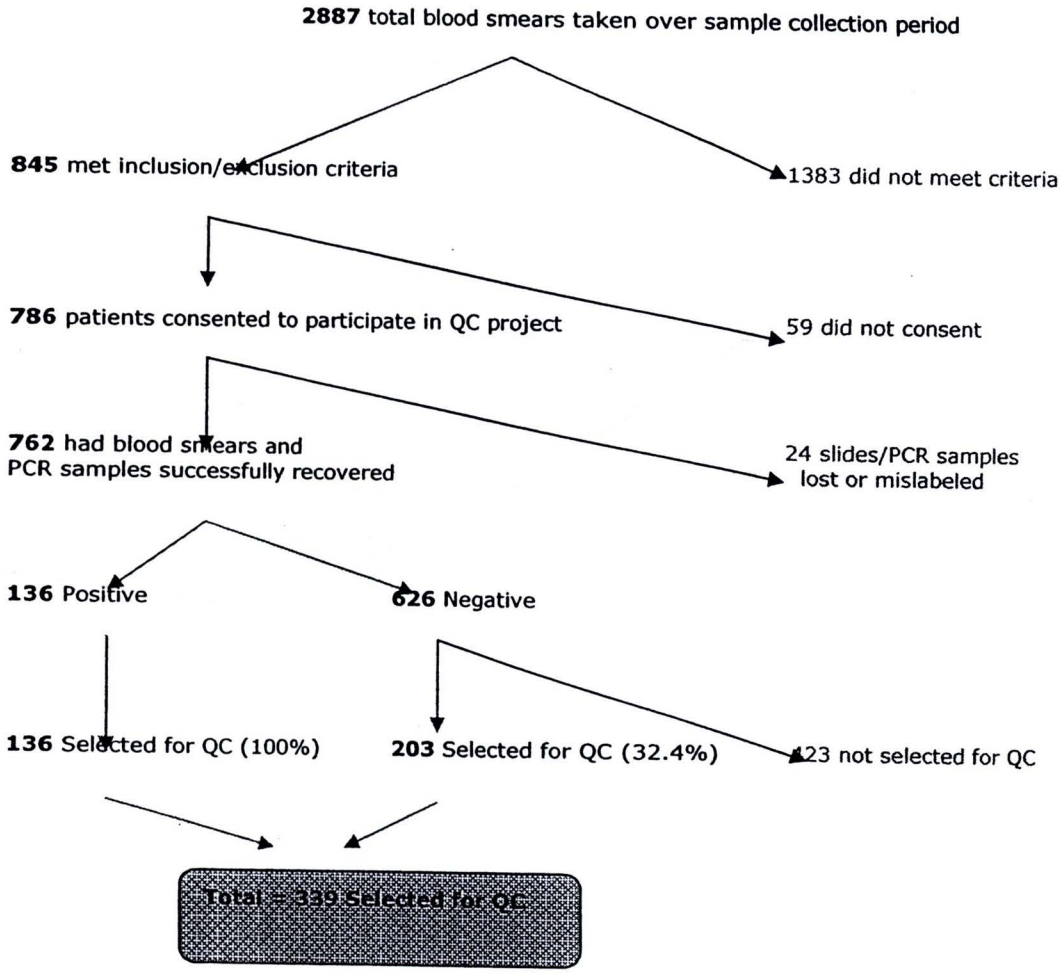


**Figure 2:** Flow chart of sample selection

*Sample Concordance and Errors*

A summary of frequencies for each of the three diagnostic modalities (MTC microscopy, MoPH microscopy, PCR) can be found in Table 2. There was high concordance in sample interpretation among Mae Tao Clinic staff, Ministry of Public staff, and PCR results ($p = 0.48$). More detailed frequency tables are provided in Appendix 1. Even though the final MoPH and final PCR interpretations were the results used for statistical analysis, it is worth noting that MoPH staff had the opportunity to re-evaluate 38 slides that were originally discordant with MTC results, while 91 PCR samples with interpretations originally discordant with microscopy (27% of total samples) underwent PCR amplification a second time, and 49 PCR samples (12.1% of total samples) with interpretations discordant with previous PCR results underwent amplification a third time.

**Table 2**: *Plasmodium* species identification of all 339 selected blood samples

| Dx Method | Neg | Pf | Pv | Pf + Pv | Pf + Pv + Pm | Pf + Pv + Po | Pm | Po |
|---|---|---|---|---|---|---|---|---|
| MTC Microscopy | **203** 59.9% | **67** 19.8% | **59** 17.4% | **9** 2.7% | **0** | **0** | **1** 0.3% | **0** |
| MoPH Microscopy | **199** 58.7% | **64** 18.9% | **73** 21.5% | **3** 0.9% | **0** | **0** | **0** | **0** |
| PCR | **191** 56.3% | **65** 19.2% | **71** 20.9% | **9** 2.7% | **1** 0.3% | **1** 0.3% | **0** | **1** 0.3% |

***MTC = Mae Tao Clinic microscopy interpretation***

***MoPH = Ministry of Public Health final microscopy interpretation***

***PCR = polymerase chain reaction final interpretation***

***Neg*** *= negative;* ***Pf*** *= Plasmodium falciparum;* ***Pv*** *= Plasmodium vivax;* ***Pm*** *= Plasmodium malariae;*

***Po*** *= Plasmodium ovale*

Overall, 40 out of 339 (11.8%) MTC results cross-checked with PCR were incorrect, compared with 22 (6.5%) of MoPH results that were incorrect ($p = 0.02$) Of the 40 MTC errors, 16 were false-negative or false-positive (negative-positive errors) and 24 were species-specific errors (e.g. *P. falciparum* misdiagnosed as *P. vivax*). In comparison, the MoPH made 12 negative-positive errors and 10 species-specific errors. There were a total of 11 mixed infections detected by PCR (7.4% of all 148 positive infections detected by PCR). The Mae Tao Clinic misdiagnosed 7 (63.6%) of these infections as either negative or a single infection ($p = 0.001$ for MTC vs. PCR). In comparison, the MoPH misdiagnosed 8 (72.7%) of these infections as either negative or a single infection ($p = 0.0004$ for MoPH vs. PCR; $p = 0.64$ for MTC vs. MoPH). A summary of the different types of errors made by both groups of microscopists can be found in Table 3.

**Table 3:** Types of Errors (compared with final PCR interpretation)

| Diagnostic Method | Total # Neg/Positive Errors | Total # Species-Specific Errors | Total # missed mixed infections (out of 11) | Total # Errors (out of 339) |
|---|---|---|---|---|
| MTC Microscopy | 16 | 24 | 7 (63.6%) | 40 (11.8%) |
| MoPH Microscopy | 12 | 10 | 8 (72.7%) | 22 (6.5%) |

*Clinical Consequences of Errors*

As a result of Mae Tao Clinic diagnostic errors, 13 of 76 *P. falciparum* (*Pf*) cases (17.6%) went untreated, and 8 of 82 *P. vivax* (*Pv*) cases (9.8%) went untreated. 13 of 263 patients (4.9%) received unnecessary treatment for *Pf*, and one patient out of 257 (0.4%) received unnecessary treatment for *Pv*. In comparison, if treatment outcomes had been dictated by MoPH diagnostics, MoPH errors would have resulted in 10 untreated *P. falciparum* cases

(13.1%), 5 untreated *P. vivax* cases (6.1%), one patient unnecessarily treated for *Pf* (0.3%) and 2 patients unnecessarily treated for *Pv* (7.8%). (Comparing consequences of MTC errors versus MoPH errors, $p = 0.50$ for missed *Pf*; $p = 0.39$ for missed *Pv*; $p = 0.001$ for unnecessary *Pf* treatment; $p = 0.56$ for unnecessary *Pv* treatment). Please see Table 4 for more details.

**Table 4:** Clinical Consequences of Errors

| Diagnostic Method | Untreated Pf cases (out of 76 Pf) | Unnecessary treatment of Pf (out of 163 non-Pf) | Untreated Pv cases (out of 82 Pv) | Unnecessary treatment of Pv (out of 257 non-Pv) |
|---|---|---|---|---|
| MTC Microscopy | **13** (17.6%) | **13** (4.9%) | **8** (9.8%) | **1** (0.4%) |
| MoPH Microscopy | **10** (13.1%) | **1** (0.3%) | **5** (6.1%) | **2** (7.8%) |

Of the 20 patients who went untreated due to MTC diagnostic errors, 12 had untreated *Pf*, 7 had untreated *Pv*, and one had an untreated mixed *Pf* + *Pv* infection.[§] A summary of the follow-up and treatment profiles of these patients can be found in Table 5.

Of the untreated *P. falciparum* cases, seven patients (#'s 2, 3, 5, 7, 12, 17, 19) never received appropriate treatment for their infections; three (#'s 9, 15, 18) presented again to the clinic later that week, were correctly diagnosed with *Pf* the second time, and received appropriate treatment; and two untreated *Pf* patients (#'s 13, 14) were mistakenly diagnosed and treated for *P. vivax*, but their follow-up visits to the clinic later that week showed negative blood smears. One patient (#8) with a mixed *Pf* + *Pv* infection was treated for *Pv* only, but his

[§] One Pf patient (#'s 3 and 5 in Table 4) who had a negative smear returned to the clinic 4 days later, had another negative smear, and therefore did not receive any malaria treatment. Since both his visits fell within the sample-collection period and both his smears were randomly selected for QC analysis, he is counted as 2 patients for the purposes of this study.

follow-up visit later in the week showed a negative smear. Of the untreated *P. vivax* cases, 6 patients (#'s 1, 4, 7, 11, 16, 20) never received appropriate treatment for their infections. One untreated *Pv* patient (#6) presented to the clinic later that week, was diagnosed with *Pf*, and received appropriate treatment for *Pf*, which should have eradicated the *Pv* infection. One untreated *Pv* patient (#10) presented again to the clinic later that week, was correctly diagnosed with *Pv* the second time, and received appropriate treatment. Two triple infections (*Pf*+*Pv*+*Pm* and *Pf*+*Pv*+*Po*) were present among this patient group, but the *P. malariae* and *P. ovale* infections are not considered separately in the results analysis because there was only one case of *Pm* and 2 cases of *Po*, and treatment for these species is the same as for *Pv*. The *Pm* infection (while misdiagnosed as *Pv* only) was adequately treated with the *Pv* regimen, whereas the *Po* infection was untreated.

**Table 5**: Treatment and follow-up profiles for missed malaria cases

| # | Age | Sex | MTC Result | MoPH result | PCR Result | Treatment Outcome |
|---|---|---|---|---|---|---|
| 20 | | F* | Neg | Pv | Pv | No malaria tx; presented again 30 d later w/ fever/headache/dysuria → neg smear, tx for UTI. Delivered healthy 2.4 kg baby 69 d later. |
| 12 | | M | Pm, g | Pv | Pf + Pv + Pm | Tx for Pv and Pm only. No follow-up. |
| 35 | | M | Neg | Pf | Pf | No malaria tx; presented 4 d later w/ 103 F fever and neg smear → dx w/ appendicitis. No planned follow-up. |
| 7 | | F | Neg | Pv | Pv | No malaria tx. No follow-up |
| 35 | | M | Neg | Pf | Pf | See # 3 (same patient) |
| 26 | | M | Neg | Pv | Pv | No malaria tx; presented 2 d later & dx'd w/ Pf → treated for Pf. Follow-up visit 6 d later showed neg smear. |
| 40 | | F | Neg | Pv | Pf + Pv + Po | No malaria tx. No follow-up |
| 15 | | M | Pv | Pv | Pf + Pv | Tx for Pv only. Follow-up visit 4 d later showed neg smear. |
| 28 | | M | Neg | Neg | Pf | No malaria tx; presented 4 d later & dx'd w/ Pf → treated for Pf. Follow-up visit 5 d later showed neg smear. |
| 13 | | F | Neg | Neg | Pv | No malaria tx; presented 6 d later & dx'd w/ Pv → treated for Pv. Follow-up visit 3 d later showed neg smear. |
| 40 | | F | Neg | Neg | Pv | No malaria tx. Did not deliver baby @ MTC; no follow-up. |
| 22 | | F* | Neg | Neg | Pf | No malaria tx; presented 6 d later w/ neg smear. |
| 73 | | M | Pv | Pf | Pf | Tx for Pv. Follow-up visit 4 d later showed neg smear. |
| 36 | | M | Pv | Pf | Pf | Tx for Pv. Follow-up visit 4 d later showed neg smear. |
| 27 | | M | Pv | Pf, g | Pf | Tx for Pv; presented 4 d later & dx'd w/ Pf → treated for Pf. No follow-up. |
| 11 | | F | Neg | Neg | Pv | No malaria tx. No planned follow-up. |
| 26 | | M | Pv | Pf | Pf | Tx for Pv. No follow-up. |
| 47 | | M | Neg | Pf, g | Pf | No malaria tx; presented 3 d later and dx'd w/ Pf → treated for Pf. F/u visit 5 d later showed neg smear. |
| 38 | | M | Neg | Neg | Pf | No malaria tx. No planned follow-up. |
| 38 | | F | Neg | Neg | Pv | No malaria tx. No planned follow-up. |

*Pregnant

*Main Quality Indicators*

Using PCR as the reference standard and the presence of malaria as the dependent variable (i.e., reflecting each group's ability to discern positive from negative blood smears and not taking species-specific errors into account) sensitivity and specificity of Mae Tao Clinic microscopy were 90.5% (95% CI, 85.8-95.2) and 98.9% (95% CI, 97.4-100.0), respectively; positive predictive value [PPV] and negative predictive value [NPV] were 98.5% (95% CI, 96.5-100.0) and 93.1% (95% CI, 89.6-96.6) respectively. ($p = 0.0001$ for sensitivity and NPV; $p = 0.16$ for specificity and PPV). In comparison, MoPH microscopy exhibited greater sensitivity (93.2%; 95% CI, 89.2-97.2), the same specificity (98.9%; 95% CI, 97.4-100.0), and improved positive and negative predictive values (98.6%; 95% CI, 96.6-100.0 and 95.0%; 95% CI, 91.97-98.03 respectively). ($p = 0.001$ for sensitivity and NPV; $p = 0.16$ for specificity and PPV; Tables 6a and 6b.)

**Table 6a:** Mae Tao Clinic vs. PCR positive/negative cross-tabulation

| | | PCR Control | | |
|---|---|---|---|---|
| | | + | - | Total |
| MTC | + | 134 | 2 | 136 |
| | - | 14 | 189 | 203 |
| | Total | 148 | 191 | 339 |

True positives = 134

True negatives = 189

False positives = 2

False negatives = 14

**Sensitivity:** 134/(134+ 14) = **90.5%** (95% CI, 85.8-95.2)

**Specificity:** 189/(189+2) = **98.9%** (95% CI, 97.4-100.0)

**Positive Predictive Value:** 134/(134 + 2) = **98.5%** (95% CI, 96.5-100.0)

**Negative Predictive Value:** 189/(189 + 14) = **93.1%** (95% CI, 89.6-96.6)

**Table 6b:** Ministry of Public Health vs. PCR positive/negative cross-tabulation

| | | PCR control | | |
|---|---|---|---|---|
| | | + | - | Total |
| M | + | 138 | 2 | 140 |
| O | - | 10 | 189 | 199 |
| P | Total | 148 | 191 | 339 |
| H | | | | |

True positives = 138

True negatives = 189

False positives = 2

False negatives = 10

**Sensitivity:** 138/(138+ 10) = **93.2%** (95% CI, 89.2-97.2)

**Specificity:** 189/(189+2) = **98.9%** (95% CI, 97.4-100.0)

**Positive Predictive Value:** 138/(138 + 2) = **98.6%** (95% CI, 96.6-100.0)

**Negative Predictive Value:** 189/(189 + 10) = **95.0%** (95% CI, 91.97-98.03)

Most real-world quality control studies compare results from the group of microscopists in question with those of expert microscopists. Using MoPH results as the reference standard (instead of PCR), MTC sensitivity was 94.3% (95% CI, 90.5-98.1), specificity 98.0% (95% CI, 96.1-99.9), positive predictive value 97.1% (95% CI, 94.3-99.9), negative predictive value 96.1% (95% CI, 93.4-98.8). ($p = 0.004$ for sensitivity and NPV; $p = 0.04$ for specificity and PPV). See Appendix 2 for calculations and cross-tabulations.

When assessing how well the MoPH microscopists would have performed with only one chance to interpret blood smears, disregarding results that were discordant with MTC interpretations, their sensitivity, specificity, positive predictive value, and negative predictive value were 91.9% (95% CI, 87.6-96.2), 98.4% (95% CI, 96.1-99.9), 97.8 % (95% CI, 95.4-

100.0), and 94.0 % (95% CI, 90.7-97.3) respectively. See Appendix 2 for calculations and cross-tabulations.

*Agreement*

Sensitivity and specificity measure overall group test performance but do not isolate individual disagreements among cases. To evaluate the degree of agreement among positive and negative results from each of the three groups, unweighted kappa statistics were calculated. Kappa agreement between MTC and PCR results was 0.799 (95% CI 0.723-0.874). Kappa agreement between MoPH and PCR results was 0.889 (95% CI 0.831-0.947). Kappa agreement between MTC and MoPH results was 0.827 (95% CI 0.758-0.896). More detailed calculations and cross-tabulations are presented in Appendix 3.

*Slide Quality (Table 7)*

Of 339 thick smears, 99 (29.3%) were classified as "good" quality, 34 (10.0%) average quality, and 206 (60.8%) poor quality. For thick smear color, 78 slides were graded as having "good" color (23.0%), 147 "average" color (43.4%), 83 (24.2%) "poor" color, and 32 (9.4%) received no comments. Among the 339 thin smears, 110 (32.4%) were "good" quality, 88 (26.0%) "average" quality, 139 (41.0%) "poor" quality. Two (0.6%) received no comments.

**Table 7:** Blood Smear quality and color *(n = 339)*

| | Thick Smear Quality | Thick Smear Color | Thin Smear Quality |
|---|---|---|---|
| [redacted] | 99 (29.3%) | 78 (23.0%) | 110 (32.4%) |
| [redacted] | 34 (10.0%) | 147 (43.4%) | 88 (26.0%) |
| [redacted] | 206 (60.8%) | 83 (24.2%) | 139 (41.0%) |
| [redacted] | 0 | 32 (9.4%) | 2 (0.6%) |

In addition to basic smear evaluation, supplemental, miscellaneous comments were provided for certain slides. Of the 339 slides, 137 (40.4%) were classified "dirty," 48 (14.2%) "very dirty," and 17 (5.0%) contained precipitins from oxidized Giemsa stain.

When correlating slide quality comments with errors made, 27.5% of the 40 MTC misdiagnosed slides had "good" thick smears, 10.0% "average" thick smears, and 62.5% "poor" thick smears. In comparison, 27.4% of the 22 MoPH misdiagnosed slides had "good" thick smears, 9.1% "average" thick smears, and 63.5% "poor" thick smears. ($p = 0.99$). Similarly, 27.5% of MTC misdiagnosed slides had "good" thin smears, 35.0% "average" thin smears, and 37.5% "poor" thin smears, versus MoPH misdiagnosed slides, 18.2% of which had "good" thin smears, 27.3% "average" thin smears, and 54.5% "poor" thin smears. ($p = 0.05$). In terms of additional comments, 45% of MTC misdiagnosed slides were considered "dirty," 12.5% were considered "very dirty," and 2.5% had Giemsa precipitins, compared with MoPH misdiagnosed slides, 36.4% of which were considered "dirty," 18.2% "very dirty," and 9.1% of which had Giemsa precipitins.