Mentorship on malaria microscopy diagnostic service in Ethiopia: Baseline competency of microscopists and performance of health facilities

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Abstract

Background: In Ethiopia, malaria cases are declining as a result of proven interventions and in 2017, the country launched a malaria elimination strategy in targeted settings. Accurate malaria diagnosis and prompt treatment are the key components of the strategy to prevent morbidity and stop the continuation of transmission. However, the quality of microscopic diagnosis in general is deteriorating as malaria burden declines. Therefore, this study was carried out to evaluate the competency of microscopists and the performance of health facilities on malaria microscopic diagnosis.

Methods: A cross-sectional study was conducted from August 1st to September 30th, 2019 in nine regional states and one city administration. A standard checklist was used for on-site evaluation, archived patient slides were re-checked, and proficiency of microscopists was tested using WHO certified slides from the national slide bank at the Ethiopian Public Health Institute (EPHI). The strength of agreement, the sensitivity, the specificity, and the positive and negative predictive values were calculated.

Results: In this study, 102 health facilities (84 health centers and 18 hospitals) were included; from which, 202 laboratory professionals participated. In slide re-checking, moderate agreement (Agreement: 76.0%; Kappa: 0.41) was observed between experts and microscopists on malaria detection in all health facilities. The sensitivity and specificity of routine slide reading and the re-checking results were 78.1% and 80.7%, respectively. Likewise, positive predictive value of 65.1% and negative predictive value of 88.8% were scored in the routine diagnosis. By panel testing, a substantial overall agreement (A: 91.8%; K: 0.79) was observed between microscopists and experts in detecting malaria parasites. The sensitivity and specificity in the detection of malaria parasites was 92.7% and 89.1%, respectively. Furthermore, in identifying species, a slight agreement (A: 57%; K: 0.18) was observed between microscopists and experts.

Conclusion: The study found significant false positive and false negative results in routine microscopy on slide re-checking of Plasmodium parasites. Moreover, reduced grade in parasite species identification was reported on the panel tests. Therefore, implementing comprehensive malaria microscopy mentorship, in-service training, and supportive supervision are the key strategies to improve the overall performance of health facilities in malaria microscopy.

Background

Malaria remains a major public health challenge. In 2019, an estimated 229 million cases of malaria and 409,000 deaths were reported [1] compared with 238 million cases and 736,000 deaths in 2000 which shows a significant decline. In Ethiopia, around 52% of the country's population is at risk of the disease. Generally, areas that lay below 2000 meters above sea level are considered malarious. Plasmodium falciparum accounts for nearly 70% of all malaria cases while the remaining cases are due to P. vivax but malaria prevalence is collectively declining from 2011(4.5%) to 2015 (1.2%) onwards [2- 3].
The malaria elimination program in Ethiopia planned to eliminate malaria through a step-wise and sub-national approach targeting specific adjacent areas in order to shrink the country's malaria map by 2030 [2]. Accurate diagnosis and prompt treatment are core strategies in the elimination of malaria [4]. The diagnosis of malaria is carried out by detecting evidence of parasites or parts of parasites. Microscopic examination of Giemsa-stained blood film is still the gold standard for malaria diagnosis [5]. Due to a lack of sustainable quality assurance program and trained laboratory technicians, this method has many setbacks in detecting and identifying malaria species correctly in Ethiopia [6-9]. Additionally, inaccuracies in diagnostic testing can lead to potentially devastating outcomes for the patient and public health, compromising the quality of surveillance data at national level, and ultimately affecting public health policy [10].

An effective malaria diagnosis practice has to be put in place to implement a quality management system that is in line with international quality standards [11]. Strong laboratory capacity with full equipment, reagents, and competent professionals ensures better curative interventions and influences treatment-seeking behavior [9] by attracting a higher patient flow than facilities with a weak laboratory service. Therefore, incorporating a mentoring approach into supervision can transform the traditional supervision into a more effective intervention to improve the quality and delivery of patient care [16]. Mentoring typically includes a continued relationship and a broad skills transfer from an individual with more experience in an area to a less experienced mentee to improve the performance of laboratory personnel in a health facility [12-15]. An acceptable malaria microscopy service should provide results that are consistently accurate and timely enough to have a direct impact on treatment [14]. This requires a comprehensive mentorship and active quality assurance scheme. Therefore, this study aimed to evaluate the competency of microscopists and the performance of health facilities on malaria microscopy in Ethiopia. A comprehensive mentorship was provided based on the identified gaps.

**Methods**

**Study area, design and period**

A health facility based cross-sectional study was conducted from August 1\textsuperscript{st}, 2019 to September 30\textsuperscript{th}, 2019, at 102 health facility laboratories in nine regional states and one city administration in Ethiopia.

**The distribution of study sites in Ethiopia**

The spatial distribution of study sites is depicted in figure 1.

**Sample size and selection procedure**

In this study, about 102 *Woredas (district)* were selected according to the malaria reporting health facilities proportionally from each region based on Annual Parasite Incidences (national malaria program report, 2017). The mentors’ team in coordination with the *Woreda* health offices selected one health
facility per Woreda based on their malaria burden report, the presence of medical laboratory professionals and active malaria microscopy diagnostic service.

**Expert selection**

Twenty-six experts, two from each respective regional Public Health Institutes were selected. Experts were selected based on their experience on malaria microscopy, training on malaria diagnosis and quality assurance, demonstration ability to transfer knowledge, commitment and willingness to mentor a facility for three days. Five days standardization training was provided on malaria microscopy and mentorship.

**Data collection process**

Thirteen malaria microscopy expert teams, each team with two laboratory experts were formed. The experts conducted interviews using a standardized questionnaire using the Open Data Kit (ODK) software programmed on a Smartphone. Panel slides were distributed for proficiency testing. Blindly re-checking slides were checked at the health facilities level and the discordant slides were referred to the national laboratory for confirmation. Real time data were sent to the EPHI data server immediately after data collection completion at each health facility.

**On-site evaluation**

A standard supervisory checklist was used to assess the status of availability of documents and records of quality indicators, quality of smear slides, microscopy diagnosis methods and safety practice, equipment and supply management, capacity building issue and training related to malaria microscopy in each health facility.

**Proficiency testing**

A set of four standard reference slides from the national malaria slide bank at EPHI (one slide Pf, one Pv, one mixed (Pf+Pv) and one negative) were used to test the proficiency of microscopists for parasite detection and species identification.

**Slide re-checking**

Expert microscopists (the mentors) selected ten stained slides randomly and systematically from the archived of each participant laboratory (five negative and five positive stained slides) as per the national EQA guideline [9] and assessed the performance of health facilities in malaria microscopy diagnosis. The slides were read by experts independently at health facility level and the results were compared immediately.
a. Criteria for assessing quality of malaria blood film slide according to national malaria laboratory diagnosis EQA scheme guideline [9].

| Grading | Criteria |
|---------|----------|
| Excellent | Gross appearance: Both thin and thick film prepared on the same slide, thick film 10 mm diameter, newsprint read under thick film before staining, 10 mm from frosted end and thick film and between thick and a thin film with distinct head, body and tail. Microscopic appearances: Demonstrates RBCs lysed in thick film and a monolayer of RBCs, with normal and abnormal morphology in thin film. Staining allows the trophozoites, gametocytes and/or schizonts and the white blood cells to be clearly distinguished against the background. |
| Good | Gross appearance: Film with uneven tail, too thick, too wide or too long with uneven thickness. Microscopic appearance: Demonstrates a monolayer of RBCs, and fixed RBCs. Staining allows the trophozoites, gametocytes and/or schizonts malaria parasites and the white blood cells to be clearly distinguished against the background. |
| Poor | Gross appearance: Film with ragged tail, too thick, too wide or too long with uneven thickness. Microscopic appearance: Distorted appearance of the RBCs, malaria parasite and the white cells. It is difficult to spot fields with monolayer of cells and distorted appearance of the RBCs, malaria parasite, and the white cells. |

Data management and statistical analysis

The real time data were downloaded from the server, cleaned in Microsoft Excel, and imported to SPSS V20 for analysis. Descriptive statistics were used to determine quality monitoring indicators, training, the performance of health facilities in malaria microscopy, and competence of the laboratory professional. Sensitivity, specificity, percent agreement, and kappa values were used to determine the competency of laboratory personnel against the expert reader in routine diagnosis. Kappa value was calculated to see the strength of agreement. Strength of agreement was classified as: Kappa of $<0.20$ is slight agreement, $0.21–0.40$ is fair agreements, $0.41–0.60$ is moderate agreement, $0.61–0.80$ is substantial agreement, and $0.81–0.99$ is almost perfect agreement [17].

Ethical consideration

Ethical approval was obtained from the Ethiopian Public Health Institute (EPHI) Institutional Review Board (IRB) (Protocol number: EPHI-IRB-197-2019). An official cooperation letter was written to the health facilities. Administrative approvals were obtained from the directors of the participating health facilities.

Results
Demographic characteristics

A total of 102 health facilities were enrolled; eighteen (17.6%) hospitals and 84 (82.4%) health centers from the nine regional states and one city administration, in Ethiopia.

Of 202 laboratory professionals were included as participants in the competence assessment. The median age of the study participants was 29 years (range: 20 – 55 years). Most of the participants (68.5%) were male and about three in four participants 154(76.2%) reported that they had a diploma in laboratory. Out of the 202 laboratory personnel, 158 (78.2%) were working at health centers. More than half 107 (53.0%) of the laboratory personnel had five years or more work experience in malaria microscopy (Table 1).

Table 1: Demographic characteristics of Health facilities and laboratory personnel in Ethiopia, 2019

| Characteristics                  | Variable        | Frequency | Percentage (%) |
|----------------------------------|-----------------|-----------|----------------|
| Health facilities enrolled 102  | Hospitals       | 18        | 17.6           |
|                                  | Health centers  | 84        | 82.4           |
| Age of laboratory personnel (Years) | 20 – 30       | 150       | 74.2           |
|                                  | 31 – 40         | 44        | 21.8           |
|                                  | ≥41             | 8         | 4.0            |
| Total                            |                 | 202       | 100            |
| Sex                              | Male            | 139       | 68.8           |
|                                  | Female          | 63        | 31.2           |
| Type of college attended         | Government      | 121       | 59.9           |
|                                  | Private         | 81        | 40.1           |
| Education level                  | Diploma         | 154       | 76.2           |
|                                  | BSc             | 48        | 23.8           |
| Work Experience (in Years)       | < 2 Yrs         | 38        | 18.8           |
|                                  | 2-5 Yrs         | 57        | 28.2           |
|                                  | >5Yrs           | 107       | 53.0           |
| Place of Work                    | Health center   | 158       | 78.2           |
|                                  | Hospital        | 44        | 21.8           |

Proficiency Testing: Detection of malaria parasites and Species Identification

Overall performance on detection of parasites

A substantial percent agreement (91.8%; Kappa 0.79) was observed between study participants and expert references in detecting malaria parasites. The overall sensitivity of malaria parasites detection by the participants’ microscopy was 92.7% while the specificity was 89.1%. In the study, 92.7% positive predictive value and 89.1% negative predictive values were observed.

Almost perfect agreement (92.87%; Kappa: 0.81) was observed at the health centers and substantial agreement (88.1%; Kappa: 0.71) was observed at hospitals in detecting malaria parasites. The overall
score for sensitivity of malaria detection in health centers was 94.5% which was higher than in hospitals (86.4%). On the other hand, the specificity of participants in detecting malaria parasites was higher in hospitals (93.2%) than in health centers (87.9%) (Table 2).

Table 2: Detection performance of malaria parasites between participants and experts in Ethiopia, 2019.

| Variables   | Expert reading/PT | Agreement (%) | Kappa       | PPV (%) | NPV (%) | Sensitivity (%) | Specificity (%) |
|-------------|-------------------|---------------|-------------|---------|---------|-----------------|-----------------|
|             | Positi ve          | Negati ve     | Total       |         |         |                 |                 |
| Participant | Positive          | 562           | 22          | 474     | 91.4    | (89.18-93.28)   | 0.79            | 96.23          | (94.51-97.43)  | 80.56          | (75.40-84.52) | 92.7            | (90.38-94.66) | 89.1            | (83.98-93.05)  |
|             | Negative          | 44            | 160         | 204     | 90.0    | (88.01-91.99)   | 0.76            | 89.36          | (85.37-93.35)  | 84.72          | (79.63-89.71) | 85.43           | (81.66-89.20) | 87.9            | (85.78-90.04)  |
| Total       | 506               | 184           | 688         |         |         |                 |                 |                 |                 |                 |                 |                  |                  |                  |                  |
| Health Centers | Positive          | 448           | 19          | 467     | 92.87   | (90.59-94.76)   | 0.81            | 95.94          | (93.92-97.30)  | 84.24          | (78.55-89.64) | 94.5            | (92.7-96.39)   | 87.9            | (85.85-90.02)  |
|             | Negative          | 26            | 139         | 165     | 90.57   | (88.57-92.57)   | 0.76            | 89.54          | (86.56-92.54)  | 79.27          | (75.39-83.15) | 84.67           | (81.74-87.60) | 87.9            | (85.85-90.02)  |
| Total       | 474               | 155           | 629         |         |         |                 |                 |                 |                 |                 |                 |                  |                  |                  |                  |
| Hospitals   | Positive          | 114           | 3           | 117     | 88.06   | (82.34-92.46)   | 0.71            | 97.44          | (92.71-99.13)  | 69.49          | (59.54-77.90) | 86.4            | (79.31-91.71) | 93.2            | (81.34-95.77)  |
|             | Negative          | 19            | 41          | 59      | 78.39   | (70.21-86.57)   | 0.60            | 97.44          | (92.71-99.13)  | 69.49          | (59.54-77.90) | 86.4            | (79.31-91.71) | 93.2            | (81.34-95.77)  |
| Total       | 132               | 44            | 176         |         |         |                 |                 |                 |                 |                 |                 |                  |                  |                  |                  |

PPV=Positive Predictive value, NPV=Negative Predictive Value, PT=proficiency testing

Species identification

Overall percent agreement on species identification

Slight agreement (57%; Kappa=0.18) was observed between participants and expert readers in identifying Pf from non-Pf parasites. The overall percent agreement in identifying Pf and non-Pf was 49% (Kappa=0.04) at hospital level and 59% (Kappa= 0.22) at the health center level. The percent agreement in species identification at health centers was slightly higher than the agreement at the hospitals despite low overall agreement at all facilities (Table 3).

Table 3: Identification performance of Pf against non-Pf species in Ethiopia, 2019.

| Variables   | Expert reading | Agreement (%) | Kappa |
|-------------|----------------|---------------|-------|
|             | Pf             | Non-Pf        | Total |       |
| Participants result | 147            | 205           | 352   | 0.18  |
| Non-Pf      | 55             | 199           | 254   | (0.11-0.26) |
| Total       | 202            | 404           | 606   |       |
| Health Center | Pf             | 119           | 154   | 0.22  |
| Non-Pf      | 39             | 162           | 201   | (0.15-0.30) |
| Total       | 158            | 316           | 474   |       |
| Hospital    | Pf             | 28            | 51    | 0.04  |
| Non-Pf      | 16             | 37            | 53    | (-0.1-0.19) |
| Total       | 44             | 88            | 132   |       |

Performance of health facilities in malaria microscopy diagnosis

Slide Re-checking
In 102 assessed health facilities, 750 blood film slides (maximum 10 blood film slides) from each health facility of routine activities were re-checked by experts. Comparing with expert readers, percent agreement of the experts and facilities result in malaria parasite detection was 76.0% (K: 0.41) which was at the base boundary of moderate agreement. The sensitivity and specificity were 78.1% and 80.7%, respectively. Similarly, the positive and negative predictive values were 65.1% and 88.8%, respectively (Table 4).

Table 4: Detection of the malaria parasites in slide re-checking in Ethiopia, 2019.

| Slides from health facilities | Expert reading | Agreement | Kappa | Sensitivity | Specificity | PPV | NPV |
|------------------------------|----------------|-----------|-------|-------------|-------------|-----|-----|
|                              | Positi ve      | Negative  | Total |             |             |     |     |
| Health Facilities result     | 185            | 99        | 284   | 76.0%       | 0.41        | 79.1%| 80.7%|
| Negative                     | 52             | 414       | 466   |             |             |     |     |
| Total                        | 237            | 513       | 750   |             |             |     |     |

On-site evaluation

Only 72(70.5%) health facilities were performing thick and thin smears on a single slide for every patient. Three in ten health facilities did not use thin smear for species identification. Less than half of health facilities 46(45.1%) monitor the quality of prepared blood film slides regularly. Only one in three health facilities 38(37.3%) perform regular internal quality control (IQC) to check the quality of Giemsa solution and more than two in three health facilities 65(64.7%) did not store or archive examined slides properly. As part of quality assurance, one of the observed major challenges in most facilities was failure to archive and store examined slides properly except in only 37(37.3%) of health facilities and only half of 51(50%) health facilities were participating regularly in any method of external quality assurance (Table 5). Sixty-one (59.8%) health facilities were supervised by national and/or regional health offices in the preceding year. Regular training on malaria diagnosis and quality assurance was reported on one-fourths of 26(25.5%) health facilities. However, about 32(31.4%) health facilities diagnosed malaria using both RDTs and microscope (Table 5).
Discussion

In the routine microscopic diagnosis, moderate performance agreement (A: 76.0%; K: 0.41%) was observed between study facilities and expert microscopists in parasite detection in all health facilities. This study showed lower performance agreement as compared to study reported from the West Amhara Region of Ethiopia [22], [23] and Hawassa, Southern of Ethiopia [24]. The discrepancy may be due to the big number of health facilities used in the current study and may be because of the capacity of experts. The sensitivity and specificity at detecting malaria in the peripheral blood stained slides were 78.1% and 80.7%, respectively. Similarly, the positive and negative predictive values were 65.1% and 88.8%, respectively. Therefore, high false positive and false negative results were found on the assessed health facilities which showed poor performance of the health facilities service in the routine diagnosis of malaria at large. This result was lower than the findings reported from other parts of Ethiopia [22], [23][25] and, similar findings were also reported in Pakistan [26]. However, our finding was in line with a study performed in the Democratic Republic of Congo which showed the performance of routine malaria microscopy remains inaccurate with large variations among different health centers [27]. Accurate microscopy results depend on the availability of a competent microscopist using good-quality reagents.
for examining well-prepared slides and with a low-to-moderate workload [21]. In this finding, in slide re-checking, almost half of the assessed health facility laboratories did not prepare both thick and thin blood films as per the standards. Consequently, a low sensitivity in the detection of malaria parasite indicated that there were many false-negative results, i.e. missed diagnosis of true infection. This can lead to the delayed treatment, the development of serious complications, and the death or exposure to unnecessary treatment with other drugs due to suspecting other fever-like diseases.

Regarding the proficiency testing of the current finding, the overall percent agreement of the malaria microscopists in the current study was 91.8% (K: 0.79) in parasite detection which is relatively higher when compared with similar findings at elimination targeted districts of Ethiopia where performance agreement was 84.6% (K: 0.6) [28]. A study conducted in Hawassa town, Ethiopia, reported an agreement of 88% (k: 0.67) [24] and similar findings was reported from a study conducted in Bahirdar, Ethiopia where the agreement was 88.5% (k: 0.78) [29]. Another study conducted in Tigray, Ethiopia, reported an agreement of 79% (k: 0.62) [30]. A concordant result reported from Addis Ababa public health facilities showed a performance agreement of 91.7% [19]. But the agreement of the current study was relatively lower when compared with findings of similar studies conducted in Ethiopia where the agreement was 96.8% (k: 0.9) [30]. The reason for this deviation may be because of the difference in malaria prevalence which can affect microscopists’ ability to detect, but also the lack of mentorship, training, consistent supervision, and capacity building used to develop detecting skills and standardizing malaria parasite detection. Overall, sensitivity and specificity of laboratory personnel in detecting malaria parasites were 92.7% and 89.1%, respectively. Again, these results overlapped with positive predictive value (92.7%) and negative predictive value (89.1%). These findings were almost in agreement with a sensitivity 88% and specificity 91% from a study conducted in Zambia [31]. The sensitivity but not the specificity of this study was higher than the sensitivity 83.2% and specificity 90.1% in a study conducted elsewhere in Ethiopia [28], and sensitivity 63% and specificity 97% in a study reported in Tigray, Ethiopia [30]. The sensitivity and specificity of this study was lower than the sensitivity 96.8% and specificity 96.7% of malaria detection in a study conducted in another place in Ethiopia [30]. The relative lower specificity than sensitivity in the current study at detecting malaria parasites showed that high rate of false positive results of malaria were reported which led to the misdiagnosis of malaria when there was no true infection of malaria parasite in the provided slides to the mentee.

Overall, the performance agreement on the identification of malaria species was 57% (K: 0.18) which showed a slight agreement between participants and malaria microscopy experts. This result was higher than in a similar study conducted in elimination targeted districts in Ethiopia with an agreement of 43.8% (k: 0.11) [28] while it was lower than in a study conducted in Tigray, Ethiopia with an agreement of 76% (k: 0.61) [30] and a study reported from Bahirdar, Ethiopia where the agreement was 72% (k: 0.47) [29]. The reason for the low identification of species by the microscopists in the current study may be due to the fact that the microscopists prepared only a thick film and were unable to differentiate the morphology of the parasites. It may also due to the lack of training on how to differentiate the species, in addition to the poor staining of reagents found on the on-site evaluation.
In the on-site evaluation, we identified that only 72(70.5%) health facilities were performing thick and thin smears on a single slide for every patient but the recommended blood film preparation for diagnosis of malaria parasite is doing both thin and thick films on the same slide using 2µL and 6µL of whole blood, respectively [18]. Three in ten health facilities were not using thin smears which can be used for species identification. The result of the current study is greater than a study conducted in Addis public health facilities [19]. Moreover, blood films performed in 43 (42.2%) health facilities did not meet the quality of a good blood film for malaria microscopy diagnosis in this study. Internal quality control, used to check the quality of Giemsa stains, was performed only by 38(37.3%) health facilities. In addition, only 37(37.3%) of health facilities stored and archived slides properly. Moreover, in the previous year, the study identified in 41 (40.2%) and 76 (74.5%) health facilities with no supervision and refresher training, respectively. This study was in line with a study conducted in Addis Ababa public health facilities [19] and other study conducted in the Asia-Pacific [20]. This result showed that less attention is given to the quality of malaria diagnosis in the health facility level. It may be due to the lack of re-fresher trainings and regular supervisions provided to laboratory professionals at the health facility level.

**Limitation of the study:** There were some limitations where the study health facilities were incorporated purposely based on their high malaria load and presence of microscopic service; and hence, the finding may not be inferred to all health facility performance in the country. The health facilities with no or lower malaria reporting were not included. In addition, a low numbers of slides (three malaria positive slides & one negative slide) were used in the panel testing which is below the WHO standard of ten slides.

**Conclusion**

Most of the malaria microscopists in the current study achieved a good grade agreement in parasite detection. However, a poor grade was obtained in parasite species identification by the panel tests. Moreover, high false positive and false negative results were seen on slide re-checking which showed the poor performance of the health facilities in routine malaria microscopy. Poor quality control indicators and follow up gaps were reported by a significant number of health facilities in this study. Consequently, an improvement in the quality and accuracy of microscopic diagnosis of malaria is urgently needed. Therefore, a strong commitment from the National Malaria Elimination Program (NMEP) and a commitment from stakeholders are a vital step to accomplish the mentoring approach at all level of the health facilities.

**Abbreviations**

EPHI: Ethiopian public health institute; EQA: external quality assurance; EQAS: External quality assessment; NMEP: National Malaria Elimination Program; GPS: global positioning system; IQC: internal quality control; IRB: institutional review board; ODK: open data kit; PT: proficiency/panel testing; RDTs: rapid diagnostic tests; SERO: scientific and ethical review office; PPV: positive predictive value; NPV: negative predictive value.
Declarations

Author contribution

All authors contributed in conceptualization, study design, protocol development, training, and field data collection. BG, DN, AdA, AbA, MH, DD, DM, AW, EW analyzed the data and wrote the result, BG drafted the manuscript and all authors read, commented and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The dataset and materials used for the study are kept in a safe place on the EPHI server.

Consent for publication

All authors have read and agreed to publish this article.

Ethical approval and consent to participants

The study protocol was reviewed and approved by the Ethiopian Public Health Institute -IRB Office, Addis Ababa, Ethiopia.

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Figures

Figure 1

Spatial distribution of participating health facilities (N=102) in Ethiopia, 2019.
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