Systematic Analysis of *Plasmodium Falciparum* Histidine Rich Protein-2 (*Pf* HRP-2) Based Rapid Diagnostic Test for Malaria

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Abstract: - The Philippines is known to have malaria as an endemic infection primarily affecting people in areas like Palawan and Mindoro. With the lack of equipment such as microscopes for accurate diagnosis, rapid diagnostic tests (RDTs) have been widely used for the initial diagnosis of infected people in remote areas. However, only limited studies are published locally that compile and summarize related studies about *Plasmodium falciparum* Histidine Rich Protein-2 (*Pf*HRP-2) based RDTs. Through a systematic review of relevant literature, two *Pf*HRP-2-based RDTs were compared in terms of their sensitivity and specificity with reference to microscopy as the gold standard method. The journals and articles were systematically searched, screened through various stages for relevance, and assessed for quality. Following that, statistical data were extracted, gathered, and analyzed. The meta-analysis showed that Paracheck-*pf* ® performed better than Parahit-*f* ® in terms of its pooled sensitivity (91.8% and 59.9%, respectively) and specificity (85.0% and 98.1%, respectively). Consequently, Paracheck-*pf* ® demonstrated greater accuracy than Parahit-*f* ® based on the pooled DOR (91.184 and 42.013, respectively) and AUC (0.956 and 0.843, respectively). These RDTs were greatly influenced by factors such as parasitemia levels, kit quality, storage requirements and temperature, performance of consumers, etc. With this, the use of RDTs may be utilized, as an initial diagnosis for the disease, as there is still a need to use the gold standard microscopy to confirm the diagnosis.

Key Words: — ***malaria, microscopy, rapid diagnostic test, Plasmodium falciparum histidine-rich protein-2, sensitivity, specificity, paracheck-pf ®, Parahit-f ®***

I. INTRODUCTION

A mosquito-borne disease referred to as Malaria is caused by the Plasmodium parasites namely Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae. This Plasmodium spp. are vectors of the infected female Anopheles minimus flavirostris mosquito that feed on humans [1]. This mosquito-borne disease if left untreated will most likely lead to an increased rate of fatality due to the flu-like symptoms, fever, and severe body chills that it brings [2].

The rate of Malaria in the Philippines has rapidly increased over the years, with 92% and deaths by 98% [3]. Evolving cases of malaria remain to be highly concentrated in remote and far-flung areas like Palawan.

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As for malaria prevention and awareness allocated by the Philippine government, the Department of Health (2019) has established its goal of reducing the incidence rate of malaria to 90% [4]. Key measures are continually being protocolled on categorized endemic areas; however, local transmission may not be prevented. The diagnosis of malaria is achieved through a blood smear preparation which is the primary gold standard. This gold standard for the laboratory diagnosis of malaria includes a thick and thin blood smear preparation and its microscopic examination. In a microscopic examination, the laboratory personnel may observe the presence of erythrocytes infected with the Plasmodium spp. through its cell morphology, cell membrane rigidity, ring forms, permeability, and adhesiveness to endothelial surfaces [5].

Despite the preferred use of the gold standard, restrained healthcare circumstances in tropical endemic areas like the Philippines, especially the provinces of Palawan and Mindoro, limit the access of this gold standard in the diagnosis of malaria. Far-flung areas that demand rapid turnaround time and the lack

of local health services such as increased equipment cost, unstable reagents, and the necessity for skilled personnel result in the use of Rapid Diagnostic Tests (RDTs) as a presumptive clinical basis for patients who experience malaria symptoms in endemic areas [6].

Malaria Rapid Diagnostic Tests (RDTs) or Malaria Rapid Diagnostic Devices (MRDDS) are designed as dipsticks, cards, or cassette devices that are immunochromatographic lateral flow tests that identify specific antigens released by malaria parasites in the blood. The RDTs are designed to detect the Histidine-rich protein II of *Plasmodium falciparum (Pf*HRP-2*)*, parasite-specific plasmodium lactate dehydrogenase (pLDH) of *Plasmodium vivax* and Aldolase present in all malaria parasites [7].

A variety of studies assess the two *Plasmodium falciparum* Histidine Rich Protein-2 (*Pf*HRP-2) Based Rapid Diagnostic Test. However, there are only limited studies evaluating and summarizing all these relevant individual researches about their performance in reference to the gold standard diagnosis. The problem of the study asked which of the two RDTs gives a better performance based on specific parameters. Primarily, the study aimed to determine which of the two *Pf*-HRP2-based malaria RDTs is better in terms of pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and area under the curve (AUC), assess the presence of heterogeneity between the included studies, and lastly, to specify if there is a significant difference between the two *Pf*-HRP2-based malaria RDTs in terms of sensitivity and specificity.

The provision of better knowledge and understanding of the different rapid diagnostic tests for malaria here in the country is an essential contribution of this study. This study can also be beneficial to the areas where malaria is constantly present, specifically in the endemic areas of Mindoro and Palawan. Lastly, this paper will serve as a possible reference material for future research that would be conducted that is in the same inclination as that of this paper.

II. METHODOLOGY

A. Research Design

This research would follow the research design of a systematic review and meta-analysis in order to be able to do a comparative study of the two RDTs mentioned. Meta-analysis of the two different commercially available rapid diagnostic tests for malaria will be done by selecting and identifying relevant literature and studies about the said RDTs. Collection and analysis of data gathered from existing studies would be assessed for bias risk. The relevant findings from these existing studies would be extracted and analyzed without any manipulation. Figure.1. illustrates the steps that will be taken in conducting the study.

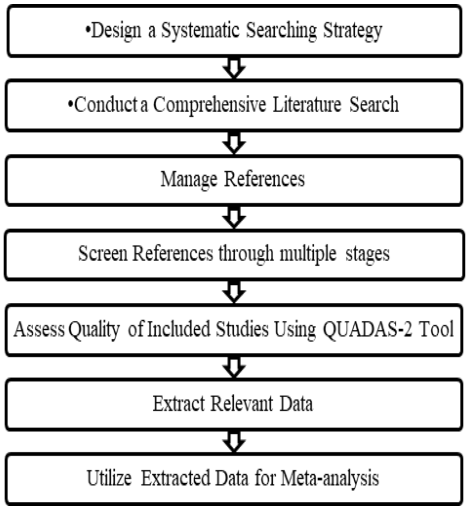

Fig.1. Schematic Diagram of the Study

B. Data Gathering Techniques

The PRISMA guidelines for systematic reviews and meta-analyses were used for the study to be done.

C. Literature Search

Electronic databases would be utilized in searching for relevant literature to be used in this research. The following electronic databases were used: Proquest, Google Scholar, PubMed, Biomed Central, JID, Europe PMC, and AJOL.

In order not to miss articles which could be substantial for data analysis, a structured approach of a searching strategy was designed through the systematic way of inputting searching terms, i.e. "[Brand name]" + "malaria" + "performance". Moreover, retrieved journals would be compiled and managed using Excel to check and avoid duplication.

D. Screening Studies

The different literature retrieved from the electronic databases are to be screened to assess whether their content and data are useful, relevant, and significant for the comparative study. Screening would be done in different stages: title screening,

abstract review, and full-text review. The flow of literature review is shown in Figure.2. Literature passing all the review stages would only be the ones used for the meta-analysis.

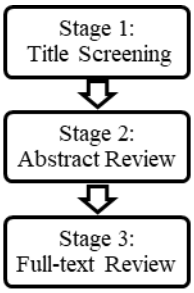

Fig.2. Flow of Literature Search

Scholarly journals, articles, and theses in English that were published in the last one to two decades would be covered. The inclusion criteria for this study are as follows: (1) original research articles evaluating either one or both of the RDTs mentioned; (2) microscopical examination of thick and thin blood smear as the gold standard; and (3) sufficient data to construct a 2x2 table in order to calculate the parameters mentioned in the objectives. Meanwhile, exclusion criteria are as follows: (1) publications that were repeated or articles that used the same patient population; and (2) journals published as reviews, case reports, or comments. Screening of journals was assessed by all the reviewers, and the votes of the majority resolved disagreements.

E. Assessing Evidence

The QUADAS checklist would be used to assess the risk of bias for all the studies to be used.

F. Data Analysis

All statistical analyses created out of the data were performed using Review Manager (RevMan) 5.2 and Meta-DiSc 1.4 software. Forest plots and receiver operating characteristic curves (SROC) were presented using RevMan 5.2; Meta-DiSc 1.4 was used to calculate for the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and heterogeneity analysis. The calculated Spearman correlation coefficient was used to test for the presence of the threshold effect. The presence of apparent heterogeneity resulted in using a random-effects model, and a fixed-effects model was utilized when insignificant heterogeneity existed.

III. RESULTS AND DISCUSSION

A. Results of the Search

A total of 124 journal articles were collected from databases such as Proquest, Google Scholar, and PubMed with a designed searching strategy of [brand name] + "malaria" + "performance." Afterwhich, the journal articles underwent a three-stage screening process of title screening, abstract review, and full text review. Fifty-two articles were excluded after examining the title or its duplicity on the researchers' record database, leaving a total of seventy-two journal articles left. The screening of abstracts resulted in twenty-five journal articles being excluded mainly due to irrelevant cases and data to the study, such as the wrong brand of RDTs and the difference of gold standard used. After a full-text review of the remaining forty-seven journal articles, twenty journal articles were excluded. Consequently, the difficulty in data extraction resulted in eighteen journals being excluded. A total of nine journal articles met the inclusion criteria and were included for meta-analysis: 3 for Parahit-*f* ® [8,9,10] and 6 for Paracheck-*pf* ® [11,12,13,14,15,16].These included a total population of 89,201 and 1,463 patients who underwent the Parahit-*f* ® test, while 1,524 patients from a total population of 3,312 underwent the Paracheck-*pf* ® test. The flow chart of the searching strategy used in this study is shown in Figure.3.

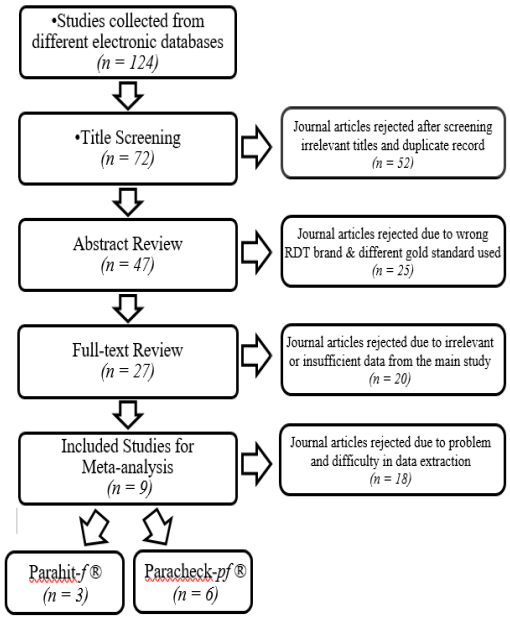

Fig.3. Flow Chart of the Study Selection Process

All nine journal articles were published in English and dated back from two decades ago. Table.1. Summarizes the study characteristics of each journal. All subjects in the study are from malaria-endemic areas. Subjects in the 5 articles are predominantly from Eastern Africa [9,10,13,15,16], whereas two were from Western Africa [12,14], and one was from Central Africa [11]. Also, there is one journal from South Asia—Odisha State, India [8]. The subjects included were primarily children; however, some studies included middle-aged to elderly patients [8,9,10,13,16]. Overall, the total sample size from the nine publications covered is 2,987. For the gold standard, all studies utilized microscopy as their reference method. The studies used capillary blood obtained from a finger prick for both RDT and gold standard microscopy. Furthermore, all journals screened for *Plasmodium falciparum* infection.

Table.1. Characteristics of the Included Studies

First Author	Year	State/Country	Sample Size	Study Design	RDT Used	Blood Source
Sahu	2013	Odisha State, India	1030	Cross-sectional Study	Parahit-*f* ®	**Finger-prick**
Buhalata	2011	North-Western Tanzania	243			
Komlosi	2017	Northern Burundi	190			
Swarthout	2007	Congo	358		Paracheck-*f* ®	
Rabiu	2012	Southwest Nigeria	140			
Mohammed	2012	South Ethiopia	158			
Iwauafor	2013	Calabar, Nigeria	167			
Kamugisha	2008	North-Eastern Tanzania	301			
Tekeste	2012	Ethiopia	400			

B. Methodologic Assessment of Included Studies

The included studies were assessed for quality and risk of bias using the QUADAS-2 tool, with the results shown in Figure.4.

Five of the studies (56%) had low concerns on the patient selection domain in the risk of bias section. These studies were able to enroll a consecutive or a random sample of eligible patients. One study [11] was evaluated as unclear as it did not clearly and explicitly stated how the patients who met the inclusion criteria were recruited or enrolled. Three studies were considered as inappropriate as these studies included inappropriate exclusion criteria that may have introduced bias in patient selection.

Three studies (33%) could not state if "blind" interpretation were done on the results of index tests with the reference standard and vice versa, thereby judging it as unclear. Lastly, the study of Iwuafor et.al. (2018) [14] was of high concern on flow and timing domain, as "not all the participants recruited into the study were matched for both microscopy and RDT testing."

Thus, according to the evaluation criteria, a low risk of bias was found in 56% of the studies in the patient selection domain, 67% of the studies in the index test & reference standard domains, and 89% of the studies in flow and timing domain.

In the applicability section, no studies were evaluated as high concern in all of the domains. Five studies in the patient selection domain (56%) had unclear concerns, mainly due to unclear and insufficient data of these articles in order to match it with the study's review question, in terms of demographic features, presence of comorbidities, study setting, as well as previous testing protocols**.**

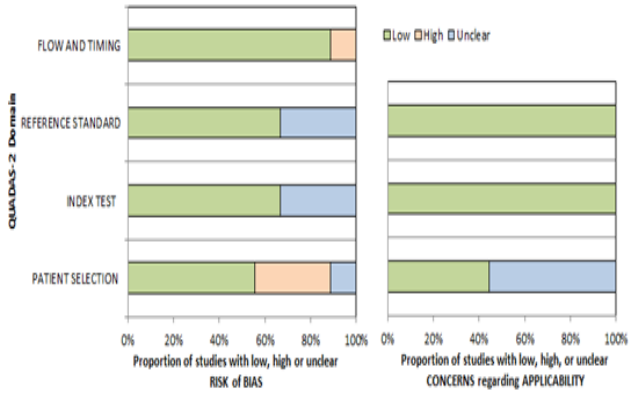

Fig.4. Risk of bias and applicability concerns summary: review authors' judgments about each domain of the QUADAS-2 checklist for each study

C. Heterogeneity Analysis and Meta-Analysis

Using Meta DiSc 1.4, the Spearman correlation coefficient was used to assess whether the threshold effect is present in the studies. Studies about Parahit-*f* ® suggest that there is a threshold effect (rs = 1.000, p-value = 0.000). A threshold effect is possibly due to the minimal number of included studies (n = 3) or the different cut-offs or thresholds used in different studies to define a positive or negative test result [17]. On the other hand, Paracheck-*pf* ® studies showed no threshold effect (rs = 0.143, p-value = 0.787).

Aside from the variations brought by the threshold effect, heterogeneity due to other factors was also calculated using Cochrane's Q test and X^2 test under the same software. Results show that there is obvious heterogeneity in the DOR of both Parahit-*f* ® (Cochrane Q = 29.84, p = 0.000, I2 = 93.3%) and Paracheck-*pf* ® (Cochrane Q = 101.28, p = 0.000, I2 = 95.1%) studies.

Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were calculated using the random effects model (REM) due to the apparent heterogeneity between the studies. The forest plots were generated using RevMan 5.2 software shown in Figure.5.

Similarly, the summary receiving operating characteristics (SROC) curve is also drawn using RevMan 5.2 software showing the sensitivity and specificity of Paracheck- p*f* ® and Parahit-*f* ® in Figure.6.

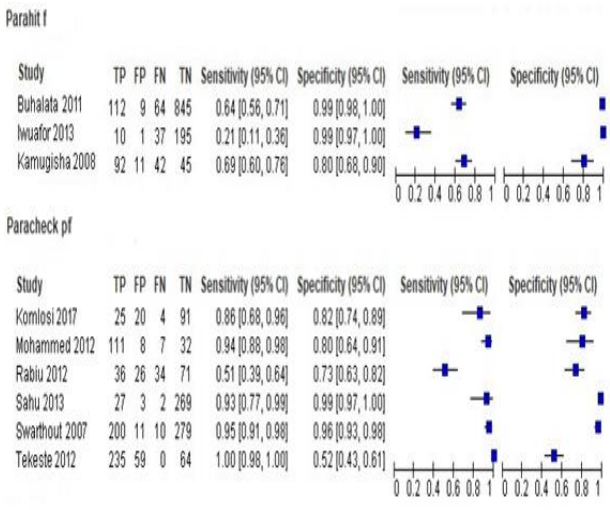

Fig.5. Forest plot of pairs of sensitivity and specificity in each study

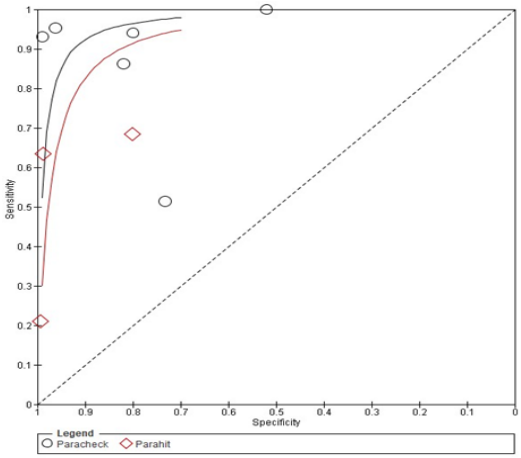

Fig.6. SROC for Parahit-f ® and Paracheck-pf ® tests

Table.2. Pooled values of Parahit-*f* ® and Paracheck-*pf* ® tests

Parameters	**Parahit-*f* ®**	**Paracheck-*pf* ®**
Pooled Sensitivity (95% CI)	**0.599** (0.547 - 0.651)	**0.918** (0.894 - 0.937)
Pooled Specificity (95% CI)	**0.981** (0.971 - 0.988)	**0.850** (0.824 - 0.873)
Pooled PLR (95% CI)	**19.487** (2.041 - 186.042)	**6.473** (2.722 - 15.393)
Pooled NLR (95% CI)	**0.486** (0.255 - 0.928)	**0.083** (0.016 - 0.433)
Pooled DOR (95% CI)	**42.013** (4.648 - 379.75)	**91.184** (11.627 - 715.08)
Pooled AUC	**0.8430**	**0.9562**

The meta-analysis showed that Parahit-*f* ® tests demonstrated a 59.9% pooled sensitivity and a 98.1% pooled specificity. Meanwhile, Paracheck-*pf* ® tests showed a pooled sensitivity of 91.8% and a pooled specificity of 85.0%. It suggests that Parahit-*f* ® demonstrated a lower sensitivity but better specificity compared to the Paracheck-*pf* ®. The SROC showed an AUC (area under the curve) of 0.843 in the Parahit-*f* ® tests and 0.956 in the Paracheck-*pf* ® tests, suggesting good discriminative abilities for both brands. DOR estimates the odds of positive test results between diseased and non-diseased groups; Parahit-*f* ® and Paracheck-*pf* ® tests had a pooled DOR of 42.013 and 91.184, respectively thereby concluding that the latter had greater accuracy compared to the former.

The low sensitivity and high specificity for pooled Parahit-*f* ® tests is consistent with the results of all the included studies assessing the said RDT, as all of them reported to have low performance as compared to microscopy. Moreover, the included studies also mentioned the strong association between parasite density and sensitivity, with the sensitivity significantly increasing with increasing parasitemia. It echoes the need to further improve the efficiency of the said kit in order for *actual* infections to be detected, especially at low parasitemia levels.

Meanwhile, Paracheck-*pf* ®, suggesting a higher pooled sensitivity, agrees with the assessment of included studies for the said RDT brand since it is considered sensitive to microscopy based on Swarthout's concluded study et al.[11]. Moreover, the relatively high pooled specificity of Paracheck-*pf* ® concurs with the assessment of included studies as seen in the study of Mohammed et al, accounting for 80%, deeming it comparable to the gold standard microscopy [13]. Mohammed et al. (2012) also mentioned that the patients who have been successfully treated with antimalarial drugs might be a factor for decreased false positive rate [13].

The pooled sensitivity of Paracheck-*pf* ® shows contradictory results to the study of Tekeste et al. (2011) [16], which was able to fulfill the WHO recommendation requiring a sensitivity of RDTs greater than 95%. However, the same study stated that these contradictory results prove that further investigation is necessary.

Another study for Paracheck-*pf* ® by Iwuafor et al. (2018) [14] also obtained consistent results from this meta-analysis. Both presented a lower RDT performance as compared to the gold standard, microscopy. The performance of the said RDT was affected by factors such as parasite density, antigen expression, temperature, storage, and transport conditions. The varying transmission levels also influence clinical sensitivity of this RDT among different populations. Moreover, the majority of false-negative results obtained from this journal are due to the cross-reactivity in the blood specimen. This predominantly affects the overall performance of the said RDT. Thus, diagnosis of malaria using RDTs only can be challenging and in need of further validation.

The performance of RDTs is influenced by many factors such as the quality of the kit, storage temperature and humidity and end users' performance [13]. In the study conducted by Sahu et.al. (2013) [8], the poor performance of Parahit-*f* ® test was possibly attributed to defects in the manufacturing of the test strips and other problems such as transport, utilization, and storage. On the other hand, the study conducted by Mohammed et al. (2012) [13] presented that the Paracheck-*pf* ® kit they used was in a well-controlled condition, a longer shelf-life and kept in the recommended temperature by the manufacturer. This may reduce the false-positive or false-negative rate as it was less affected with the said factors. The frequent presence of false-positive results limits the monitoring of treatment conditions of malaria as concluded by Mohammed et al.; Hence, this RDT only serves as a mere diagnostic tool in detecting *Plasmodium falciparum* infections.

An independent t-test was done to test our hypothesis on whether there is a significant difference in the mean sensitivities and specificities between the two brands. Results showed a significant difference ($p = 0.044$) between the mean sensitivities of Parahit-*f* ® and Paracheck-*pf* ® when equal variances are assumed but demonstrate otherwise ($p = 0.124$) when equal variances are not assumed. Meanwhile, the mean of the specificities between the two brands has no significant difference when equal variances are both assumed ($p = 0.274$) and not assumed ($p = 0.215$).

IV. Conclusion

The study was designed to provide data on the two RDTs to know which is more effective when microscopy is not available. Paracheck-pf ® showed significantly higher sensitivity but lower specificity, whereas the Parahit-f ® showed a better specificity and lower sensitivity. Parahit-f ® has shown a greater positive and negative likelihood ratio compared to Paracheck-pf ®. Paracheck-pf ® showed a greater accuracy than Parahit-f ® as proved by the pooled DOR and AUC. Hence, it can be generally concluded that Paracheck-pf ® has better performance compared to Parahit-f ® given the pooled results. However, the performance of rapid diagnostic tests may be varied due to the heterogeneity present in the included studies. Although both RDTs may have shown remarkable performance in the diagnosis of Plasmodium falciparum infections, neither can fully replace the use of microscopy. Therefore, the use of RDTs may only serve as a tool in the initial diagnosis of malaria, which then still requires the utilization of microscopy as the gold standard to confirm diagnosis.

V. Recommendation

The reviewers would like to recommend future researchers to perform a meta-regression and subgroup analysis (e.g.,

according to parasitemia levels) to determine the possible source/s of heterogeneity among the studies since the collected journal articles were insufficient and/or inconsistent of essential data needed for the analysis. Lastly, increasing the number of journal articles to be included to increase the sample size per RDT brand is advised to strengthen and limit the bias within the results and make the study more robust.

The cost-effectiveness of a RDT should be taken into consideration in future research, not just the effectiveness of the RDT. The reviewers suggest conducting an economic evaluation on the patients who would be using the said RDTs. Different countries were also involved in the study in which the journals were collected from. The heterogeneity in the study areas makes it difficult to accurately compare the result with external factors. Whether the results can be applied to other countries was not elaborated in the study. Thus, caution is advised when applying the results to other settings.

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