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Comparison of Microscopy and Rapid Diagnostic Techniques in Malaria Detection Among Children Attending Federal Medical Centre, Keffi, Nasarawa State, Nigeria

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Abstract

Malaria is a mosquito-borne disease of humans and other animals caused by eukaryotic protist of the genus plasmodium. Malaria and anemia among children, especially those that are ≤ 5 years of age are still diseases of public health concern. This study was designed to compare microscopy and rapid diagnostic techniques in detecting malaria among children attending Federal Medical Centre Keffi, Nasarawa State, Nigeria. Blood samples were collected from out-patients under five years reporting at the paediatric unit of the hospital. The blood samples were screened for the presence of malaria parasites using Microscopy and Rapid Diagnostic Test methods while packed cell volume status was determined by microhaematocrit centrifuge technique. The prevalence of malaria is calculated. The level of significance was determined using chi-square statistics. 150 samples collected, 21 (14%) were positive for malaria using microscopy while 7 (4.7%) were positive for Rapid Diagnostic Test. It was recorded that female had the highest prevalence of 52.4% (11) compared to 47.6% (10) recorded for male children. Children of 1year age had the highest prevalence of 61.9% (13) with lowest packed cell volume range of 14 – 26. Children of 2 and 5 years had the prevalence of 9.5% (2) with packed cell volume range of 26 - 28. 4.8% (1) prevalence was recorded among children of 3 years, this recorded the least prevalence and highest PCV level of 32 – 35. It was observed that the malaria prevalence has an effect on the packed cell volume level among the children. There was significant difference in the age and sex relationship with the Packed Cell Volume using microscopy and rapid diagnostic techniques (as $P<0.05$). Sensitivity of 75% and specificity 47.4% in microscopy was higher than 25% sensitivity and 52.6% in Rapid Diagnostic Test. The study showed that Microscopy performed better than Rapid Diagnostic Test in the diagnosis of malaria in children under the age of <5 years. Malaria remains a common

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infection among under five children in the tropics and predisposes them to having effect on packed cell volume. There should be improvements in the uptake of control interventions against malaria.

Keywords: Malaria, Microscopy, Children, Rapid Techniques

INTRODUCTION

Malaria is a mosquito-borne disease caused by the *Plasmodium* genus, which consists of eukaryotic protists. This disease primarily affects humans and other animals, and it remains one of the most pervasive health challenges globally. Malaria is especially common in tropical and subtropical regions, where conditions like consistent rainfall, high temperatures, and warm humidity provide the perfect environment for the *Anopheles* mosquitoes, the primary vectors of the disease. In countries like Nigeria, malaria is responsible for over 90% of the reported cases of tropical diseases (World Malaria Report, 2019), making it a leading cause of morbidity and mortality.

Globally, malaria continues to be one of the foremost causes of illness and death. It is estimated that malaria leads to 300 to 500 million clinical cases annually, contributing to about 1 to 3 million deaths each year (Keiser *et al*., 2017). These deaths occur at a staggering rate, with one life lost to malaria approximately every 30 seconds (WHO, 2019). By the end of 2004, malaria transmission posed a risk to about 3.2 billion people across 107 countries and territories, making it a widespread global threat. Most malaria cases, approximately 90%, occur in sub-Saharan Africa, which is the epicenter of the disease (Odo *et al*., 2015). The remaining 10% of malaria cases are distributed across Southeast Asia (SEA), which accounts for 70% of this fraction, and the West Pacific region, which represents the remaining 30%. Between 1999 and 2003, SEA reported 2.7 million confirmed malaria cases and 22 million probable cases, with a staggering 83.5% of the population in the region being at risk of infection. Despite the efforts to combat malaria, SEA still accounts for 30% of the global morbidity and 5% of the mortality from the disease (WHO, 2019).

The diagnosis of malaria involves several laboratory methods, which include microscopy, immunological tests, and molecular techniques (Khan *et al*., 2020). Microscopic examination of blood films remains the gold standard for diagnosing malaria, as it directly identifies malaria parasites within blood samples (Kyalo *et al*., 2016). In this method, blood is stained and examined under a microscope to detect the presence of *Plasmodium* species. Microscopy is widely regarded as the most reliable method due to its ability to identify the species of malaria parasite and quantify parasitemia (Mahgoub *et al*., 2019). However, microscopy requires skilled personnel and significant resources, such as electricity, which may not be available in rural or resource-limited settings.

In contrast, rapid diagnostic tests (RDTs) have become increasingly popular due to their convenience and ease of use. RDTs are based on the detection of specific antigens from the malaria parasites, such as Histidine Rich Protein 2 (HRP2), Aldolase, and parasite lactate dehydrogenase (Keiser *et al*., 2017). These tests use immunochromatographic methods to detect these antigens in lysed blood samples, typically yielding results in 5 to 20 minutes. RDTs are advantageous because they do not require electricity, are easy to perform, and the results are easy to interpret (Nkrumah *et al.*, 2019; Opoku Afriyie *et al.*, 2023). Moreover, commercial RDT kits are widely available and often use dipsticks bearing monoclonal antibodies that target specific parasite antigens. These test kits are available in various

formats, including cassettes, dipsticks, and card-flaps, providing a range of options to suit different diagnostic needs (Coleman *et al.*, 2020).

Children are particularly vulnerable to malaria due to their natural immune immaturity and lack of acquired immunity, which makes them more susceptible to severe cases of the disease (Fievet *et al.*, 2007; Chitre *et al*., 2024). In the World Health Organization's African Region, it is estimated that about 25% of all malaria cases occur in Nigeria (WHO, 2019). This highlights the urgent need for effective diagnostic methods, especially for vulnerable populations such as children. Given the widespread nature of malaria and the challenges associated with diagnosing the disease, it is crucial to compare the effectiveness of different diagnostic methods, such as microscopy and RDTs, in detecting malaria.

The purpose of the study outlined here is to compare the effectiveness of microscopy and rapid diagnostic techniques in detecting malaria among children attending the Federal Medical Centre (FMC) Keffi, located in Nasarawa State, Nigeria. This comparison will provide valuable insights into the reliability, speed, and practicality of these diagnostic methods, especially in settings with limited resources. By evaluating these methods, the study aims to contribute to improving malaria diagnosis and, ultimately, the management of this deadly disease in Nigeria and similar regions.

MATERIALS AND METHODS

Study Area

The study was conducted at the Federal Medical Centre (FMC) in Keffi, located in Keffi Local Government Area (L.G.A), Nasarawa State, Nigeria. Keffi is situated at a geographical coordinate of Latitude 8° 50' 47.44" North and Longitude 7° 52' 24.74" East. The area covers a total land mass of approximately 138 square kilometers. Keffi experiences a tropical climate with an average temperature of 32°C, characteristic of the region's warm and humid environment, which is conducive to the breeding of *Anopheles* mosquitoes, the primary vectors of malaria. This environmental context provides a relevant setting for studying malaria prevalence and diagnostic methods, particularly in regions where the disease burden is significant(Figure 1).

Figure 1: Map of Nasarawa State showing the location of Keffi Local government Area (NPC, 2010)

Ethical Consideration
Ethical approval for the study was obtained from the Federal Medical Centre, Keffi. The consent of the parents or legal guardians of the children were first obtained in written form before the commencement of the research.

Sample Size
A total of 150 blood samples were collected from children attending the Federal Medical Centre (FMC) Keffi who presented with a history of malaria symptoms such as fever, malaise, headache, and vomiting. Blood samples were taken from children aged ≤ five years for the purpose of testing for malaria parasites (NMCP *et al.*, 2016).

The sample size was determined using the formula for estimating the minimum number of samples needed to obtain reliable results in studies of disease prevalence. The sample size was calculated based on the expected prevalence of malaria and the desired confidence level. The formula used to estimate the required sample size is as follows:

$$n = \frac{Z^2 \cdot P \cdot (1 - P)}{d^2}$$

The required sample size (nnn) is calculated using the following parameters: ZZZ represents the Z-value corresponding to the desired confidence level, which is 1.96 for a 95% confidence level; PPP denotes the estimated prevalence rate of malaria in the population, assumed to be 0.5 to account for maximum variability when the prevalence is unknown, thereby ensuring a conservative estimate; and ddd is the margin of error, set at 0.08 or 8%, reflecting the desired level of precision in the estimate.
Calculation: Substituting the values into the formula:

$$n = \frac{(1.96)^2 \cdot 0.5 \cdot (1 - 0.5)}{(0.08)^2} = \frac{3.8416 \cdot 0.25}{0.0064} = 150.06$$

Thus, the sample size required for the study is approximately 150 participants. This ensures that the study has sufficient statistical power to detect malaria parasitemia and to make valid comparisons between microscopy and rapid diagnostic tests (RDTs) in detecting malaria among children at FMC Keffi.

Sample Collection and Processing
Sample collection was performed using a sterile lancet. The fingertip of each child was first cleaned, and then pierced with the lancet to obtain blood. The first drop of blood was discarded, and a pipette was used to collect a sufficient amount of blood for the preparation of both thick and thin blood films, as well as for the rapid diagnostic test (RDT) method.

The collected samples were processed immediately: the RDT was performed on-site, providing rapid results, while the blood films were prepared for microscopic examination. The thin blood film was stained with Giemsa stain, and the thick film was prepared to concentrate the parasites for easier detection. Both film preparations were examined under a microscope for the presence of malaria parasites. The samples were analyzed by trained laboratory personnel to ensure accurate diagnosis and comparison between the RDT and microscopy methods.

Microscopy Analysis
Microscopic examination of blood films was used as the gold standard for the diagnosis of malaria. For each child, both thick and thin blood films were prepared on labeled slides. Each slide was tagged with a unique barcode and recorded in the biomarker questionnaire along

with the corresponding blood sample form (Gilles, 2020). After preparation, the slides were air-dried in the laboratory, with thin smears being fixed with methanol at the end of each day to ensure the preservation of the samples.

The prepared slides were stained using Giemsa stain following established protocols. Initial examination focused on the thick blood smears, which are used to detect the presence of malaria parasites, as they concentrate the parasites in the sample. For any slides that tested positive for malaria on the thick smear analysis, the corresponding thin smears were further examined to identify the specific malaria species present, as this allows for species-level identification of *Plasmodium* (NMCP *et al.,* 2016).

To ensure accuracy and reliability of results, 10% of all slides underwent re-examination by an independent quality control microscopist. This re-evaluation process helped validate the findings of the primary microscopist and ensured high diagnostic precision and consistency (WHO, 2019). This quality control procedure also served to reduce the likelihood of errors and improve the overall reliability of the study's malaria diagnoses.

Rapid Diagnostic Test (RDT) Analysis:
The Rapid Diagnostic Test (RDT) utilized in this study was the SD Bioline Malaria Ag P.f. Histidine-Rich Protein II (HRP-II). The test was conducted strictly following the manufacturer's instructions to ensure accuracy and consistency. This RDT is highly reliable for the detection of *Plasmodium falciparum* malaria, with a reported sensitivity of 96.66% and specificity of 98.42%**,** making it a robust diagnostic tool for field and clinical applications.

Statistical Analysis
Statistical analysis of the data was conducted to evaluate the significance of variables, with the Chi-square test employed to assess relationships between categorical variables. Descriptive statistics, including percentages, were used to summarize the collected data effectively.

Prevalence data were analyzed using Statistical Package for the Social Sciences (SPSS) version 17.0 for Windows**.** A p-value of ≤0.05 was considered statistically significant, providing a threshold for identifying meaningful differences or associations. Additionally, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulae to evaluate the diagnostic performance of the methods used.

RESULTS
Table 1 presents the monthly prevalence of malaria among children tested using both microscopy and Rapid Diagnostic Test (RDT) techniques over a three-month period. A total of 150 blood samples were collected and analyzed during the study. The data outlines the differences in diagnostic outcomes across months and sexes, providing insights into the performance of both diagnostic methods. In January, microscopy results revealed a malaria prevalence of 4% among males and 6% among females, while negative results were 50% for males and 40% for females. No positive cases were detected using RDT, with negative RDT results at 60% for males and 40% for females. This discrepancy highlights the potential limitations of RDT sensitivity in detecting malaria cases during this period. In February, microscopy indicated a 4% positivity rate for both males and females. Negative microscopy results were observed in 52% (26 samples) of males and 40% (20 samples) of females. RDT detected a single positive case (**2**%) among females, while negative results accounted for 52%

of males and 44% of females, further underscoring differences in detection capabilities between the two methods. In March, microscopy results showed a higher prevalence, with 14% (7 samples) positivity among males and 10% (5 samples) among females. Corresponding negative microscopy results were 45% (23 samples) for males and 30% (15 samples) for females. RDT, however, detected 8% (4 samples) positivity among males and 4% (2 samples) among females, with negative results recorded at 52% for males and 36% for females.

Table 2 explores the relationship between malaria prevalence, age, sex, and Packed Cell Volume (PCV) levels among malaria-positive children, as identified using microscopy and RDT. The analysis reveals significant variations in prevalence across age groups and highlights the impact of malaria on PCV levels. Females demonstrated a higher prevalence of malaria at 52.4% (11 cases) compared to males at 47.6% (10 cases). Age group analysis showed that children aged 1 year had the highest prevalence at 61.8%, accompanied by the lowest PCV range of 14–26. In contrast, children aged 2 years and 5 years each exhibited a 9.5% prevalence, with a PCV range of 26–28. The lowest prevalence of 4.8% was recorded among 3-year-olds, who also had the highest PCV range of 32–35. The data underscores a clear relationship between malaria prevalence and PCV levels. Younger children and those with higher malaria prevalence tend to experience significantly lower PCV levels, indicating a notable impact of the disease on overall health and hematological parameters.

Table 3 compares the diagnostic performance of microscopy and RDT techniques by analyzing their sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). This comparison sheds light on the relative strengths and limitations of both methods in malaria diagnosis. Microscopy demonstrated a sensitivity of 75% and a specificity of 47.4%, reflecting its ability to detect most true positive cases but a lower ability to correctly identify non-malarial cases. The Positive Predictive Value (PPV) was 81.8%, indicating a high likelihood that a positive result was accurate, while the Negative Predictive Value (NPV) was exceptionally high at 99.2%, ensuring confidence in negative results. RDT, on the other hand, showed a sensitivity of 25% and a specificity of 52.6%, suggesting a lower capability to detect true positive cases but slightly better performance in ruling out non-malarial cases compared to microscopy. The PPV was 81.8%, similar to microscopy, while the NPV was also high at 99.2%, indicating reliability in negative diagnoses. While microscopy displayed superior sensitivity, both methods exhibited comparable PPV and NPV values. These findings emphasize the need for complementary use of both diagnostic tools to enhance accuracy in malaria detection and treatment decision-making.

Table 1: Monthly Prevalence of Malaria among children attending Federal Medical Centre (FMC) Keffi, using microscopy and rapid diagnostic techniques

Months	Number Examine	Gender		Microscopy Test Positive (%)		Microscopy Test Negative (%)		Rapid Diagnostic Test Positive (%)		Rapid Diagnostic Test Negative (%)	
		Male	Female	Male +Ve (%)	Female +Ve (%)	Male -Ve (%)	Female -Ve (%)	Male +Ve (%)	Female +Ve (%)	Male -Ve (%)	Female -Ve (%)
January	50	30	20	2(4)	3(6)	25(50)	20(40)	0	0	30(60)	20(40)
February	50	28	22	2(4)	2(4)	26(52)	20(40)	0	1(2)	28(56)	21(42)
March	50	30	20	7(14)	5(10)	23(46)	15(30)	4(8)	2(4)	26(52)	18(36)
Total	**150**	**88**	**62**	**11(7.3)**	**10(6.7)**	**74(49.3)**	**55(36.7)**	**4(2.7)**	**3(2)**	**84(56)**	**59(39.3)**

χ^2 = 5.429 (Microscopic Test) and 3.571 (RDT)
P – value = 0.066 (Microscopic Test) and 0.59 (RDT)

Table 2: Microscopic and Rapid Diagnostic Test (RDT) Positive sample with their Packed Cell Volume (PCV).

Year	Microscopy Test +Ve		RDT +Ve		PCV %	Standard PCV Range (%)
	Male	Female	Male	Female		
1	5(23.8)	8(38)	2(28.5)	1(14.3)	14 – 36	
2	1(4.8)	1(4.8)	1(14.3)	0	26 – 28	
3	0	1(4.8)	0	0	32 – 35	
4	2(9.5)	1(4.8)	1(14.3)	1(14.3)	20 – 31	
5	2(9.5)	0	0	1(14.3)	26 – 28	Children 33 – 64
	10(47.6)	11(52.5)	4(57.1)	3(42.9)		
Total (%)	**21(100)**		**7(100)**			

χ^2 = 16.143 (Microscopic Test) and 2.000 (RDT)
P – value = 0.001 (Microscopic Test) and 0.369 (RDT)

Table 3: Showing the sensitivity, specificity, PPV and NPV standard values.

	Microscopy test	**RDT Test**
Sensitivity	75%	25%
Specificity	47.4	56.6
PPV	92.3%	81.8%
NPV	50.0%	99.2

Key:
PPV = Positive predictive value
NPV = Negative predictive value
RDT = Rapid diagnostic test

DISCUSSION.

Malaria and anemia remain significant public health concerns among children, particularly those aged five years and below, in Nigeria. This study aimed to compare microscopy and rapid diagnostic techniques (RDTs) in detecting malaria among children attending the Federal Medical Centre (FMC) Keffi, Nasarawa State, Nigeria. The findings highlight important trends in malaria prevalence, diagnostic efficiency, and associated hematological impacts.
The percentage of children testing positive for malaria via RDT or microscopy in this study was below 15%, a considerably lower figure compared to prior local studies. For instance, Oladeinde *et al.* (2019) reported an 81.9% prevalence in Edo State, Abah and Temple (2015) found 63.3% in Bayelsa State, Alain *et al.* (2017) recorded 66.3% in Cross Rivers, and Simon-Oke *et al.* (2019) reported 63% in Ekiti State. The disparities in prevalence may stem from variations in climatic and atmospheric conditions across regions, which influence mosquito breeding. Mordi and Borke (2020) emphasize that environmental factors play a critical role in mosquito reproduction and malaria transmission.

This study recorded a prevalence of 14% using microscopy, higher than the 4.7% recorded for RDTs. These results align with a Nigerian study by Oladosu and Oyibo (2021), which reported a prevalence of 16.9% in children under five years using rigorous diagnostic methodologies. However, the findings contrast sharply with other studies, such as Nakakana *et al.* (2020) in Sokoto (60.4%), Onyishi *et al.* (2018) in Nsukka (72.8%), and Ukwubile *et al.* (2018) in Taraba (64%). Additional studies have reported varying prevalence rates, including Adedotun (2013) in Oyo (29.7%), Umaru and Uyaiabasi (2015) in Kaduna (35.7%), and Ocheje and Dogara (2016) in Jigawa (51%). These variations highlight the influence of local environmental, socio-economic, and healthcare factors on malaria prevalence.

The relatively lower prevalence rates of 14% (microscopy) and 4.7% (RDT) in this study reflect a downward trend in malaria infections across Nigeria, consistent with global reports (WHO, 2020). This decline may be attributed to enhanced malaria prevention initiatives, including widespread use of insecticide-treated nets, indoor residual spraying, and improved diagnostic and treatment strategies (Jemimah *et al.*, 2019). Despite these efforts, environmental conditions in rural areas and the limited ability of children to protect themselves from mosquito bites make them more vulnerable to malaria compared to their urban counterparts, who often have better access to protective measures.

Malaria prevalence between males and females in this study was not statistically significant ($P < 0.05$), though females showed slightly higher prevalence rates. This finding aligns with studies by Amadi *et al*. (2021), Chijoke-Nwauche and Sam-Ozini (2017), Egbom and Nzeako (2017), and Okonko *et al*. (2021), which also reported higher prevalence in females. However, it contrasts with studies by Abah (2015) and Wokem *et al.* (2020), which observed higher rates in males. The differences in gender-based prevalence could be due to variations in exposure, immunity, or behavior patterns that influence susceptibility to mosquito bites.

The study found that children with malaria parasitemia had significantly lower mean packed cell volumes (PCVs) compared to their non-infected counterparts. This observation is consistent with reports from several sub-Saharan African countries, where malaria infection is a leading cause of anemia among children (Geldsetzer *et al.*, 2014; Adigun *et al*., 2015). PCV is a critical hematological index for diagnosing anemia, and the results underscore the impact of malaria on the hematological health of children. The link between malaria and anemia highlights the need for effective interventions to prevent malaria-related complications in vulnerable populations.

Numerous studies have compared the efficacy of microscopy and RDTs in diagnosing malaria, with most concluding that both methods are sensitive and valuable tools (Ojurongbe *et al*., 2017; Nyenke, 2024). The sensitivity of RDTs in this study aligns with findings from Lagos (69.6%) and Enugu (82.0%) reported by Ben Edet *et al.* (2014) and Adesanmi *et al*. (2011), respectively. However, a study by Elechi *et al*. (2015) reported an RDT sensitivity of just 8.3% in children under five with acute uncomplicated malaria, a result comparable to the low sensitivity observed in this study.

The low sensitivity of RDTs in this context may be influenced by storage and handling conditions. RDTs are temperature-sensitive, and improper storage could degrade their performance. In contrast, microscopy remains the gold standard for malaria diagnosis due to its ability to differentiate and quantify parasites (Obimakinde and Simon-Oke, 2017). Although microscopy requires expertise and is time-intensive, its superior diagnostic accuracy makes it indispensable for malaria control programs.

RDTs offer significant advantages, including speed, ease of use, and minimal need for specialized training (Onyishi *et al*., 2018). These features make RDTs ideal for field settings and areas with limited laboratory infrastructure. However, their reliance on specific antigen detection, which may decline as parasite densities drop, limits their sensitivity in some contexts. Studies recommend using RDTs as a complementary tool alongside microscopy rather than as a standalone diagnostic method (Nas *et al*., 2017).

CONCLUSION

This study demonstrated that microscopy is superior to rapid diagnostic tests (RDTs) in diagnosing malaria in children aged 0–5 years. The findings reinforce the reliability of microscopy as the gold standard for malaria diagnosis, particularly in settings where accurate detection and species differentiation are critical. Although RDTs are faster and more convenient, they have limitations, particularly in detecting low parasite densities. A negative RDT result should not be regarded as conclusive evidence of malaria absence, and microscopy should be employed wherever possible to confirm results, particularly in cases of suspected malaria.

Malaria prevalence was slightly higher in females than males in this study, although the difference was not statistically significant. This aligns with broader findings that gender does not play a major role in malaria susceptibility but suggests a need for universal prevention strategies that address all children equally. Importantly, the study also highlighted a significant relationship between malaria prevalence and packed cell volume (PCV) levels, underscoring the role of malaria in contributing to anemia among children. Anemia remains a serious health concern in malaria-endemic regions and can have long-term implications for physical and cognitive development in children.

Given these findings, it is recommended that microscopy remains a cornerstone of malaria diagnosis, particularly in healthcare settings where expertise and resources are available. Where microscopy is not feasible, RDTs can be used for initial screening, but a confirmatory test should follow negative RDT results to ensure accurate diagnosis.

To enhance understanding and improve diagnostic approaches, further research with larger sample sizes is recommended to compare the performance of microscopy and RDTs across diverse populations and settings. Studies should also investigate factors that affect the sensitivity and specificity of RDTs, such as storage conditions and the operational environment. Additionally, improving RDT technology to enhance its sensitivity for low parasite densities would make it a more reliable tool in resource-limited settings.

Thus, the accurate diagnosis of malaria, particularly in children, remains vital to effective management and control of the disease. Continued investment in diagnostic tools, coupled with preventive measures such as the use of insecticide-treated nets and community education, is essential to reduce malaria prevalence and its associated complications in vulnerable populations.

To improve malaria diagnosis accuracy, it is recommended that RDTs be used as a preliminary diagnostic tool, followed by confirmatory microscopy testing in healthcare facilities where resources and expertise are available. This dual approach can help mitigate the limitations of RDTs, particularly their reduced sensitivity in cases of low parasite density. It will ensure more accurate malaria detection and better treatment outcomes, especially in pediatric populations.

Our future studies should involve larger sample sizes and broader geographical coverage to account for variability in malaria prevalence due to environmental, socioeconomic, and climatic factors. Such research could provide a more comprehensive understanding of the comparative effectiveness of RDTs and microscopy, thereby guiding tailored diagnostic strategies in different settings.

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