



Accuracy of immunoglobulin M and immunoglobulin A of saliva in early diagnosis of dengue: Systematic Review and Meta-analysis

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ABSTRACT

The objective of the study was to conduct a systematic review to synthesize the current evidence on the accuracy of IgM and IgA to early diagnosis the dengue virus. The review protocol was registered at PROSPERO (CRD 42015024808). We searched for studies in the following electronic database from 1990 to January 2018. The search identified 3507 studies. Five studies were included for quantitative analysis. Three studies included evaluations of salivary IgM provided a sensitivity of 86% and specificity of 93%. Two studies included evaluating of IgA salivary showed a combined sensitivity of 69% and a combined specificity of 98%. Despite the results found and the low methodological quality of the studies included in the meta-analysis it is still soon to claim that IgA is better than IgM to diagnosis Dengue.

Key words: Immunoglobulin M, Immunoglobulin A, antibodies, dengue virus, diagnosis.

INTRODUCTION

Dengue virus is an arbovirus, the disease characterized by acute febrile illness with good prognosis in classical form. When severe it is also called dengue hemorrhagic. It is transmitted by two types of mosquitos *Aedes aegypti* and *Aedes albopictus*. The virus that causes dengue belongs to the Flaviviridae family, genus flavivirus, its genome consists of single-stranded RNA positive polarity. The dengue virus has four different serotypes: DENV-1, DENV-2, DENV-3 and DENV-4 (Vasilakis and Weaver 2008). According to data published by the World Health Organization

dengue fever reaches over 100 countries on all continents and it infects between 50 and 100 million people each year, with approximately 500,000 severe cases around the world. The World Health Organization reported an increase from 2.4 million for 3 million cases in the Americas, Western Pacific and South-East Asia (WHO 2012). Globalization, urbanization, demographic changes and warming temperatures are some of the causes associated with the increased number of mosquitoes *Ae. aegypti* and *Ae. albopictus* (Murray et al. 2013). The clinical manifestations of dengue depends on the infecting serotype and may be presented asymptomatic or symptomatic, with moderate fever, pain in the orbital region, muscle and joint pain, among others.

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These symptoms will arise during 4 to 7 days after infection (WHO 2009).

The dengue diagnosis is performed through specific tests, with serological test being the most used method for confirmation of diagnosis of dengue (Guzmán and Kouri 2004). The serological tests may be performed using hemagglutination inhibition (HI), complement fixation (FC), neutralization test (TN), and enzyme-linked immunosorbent assay for IgM - (MAC ELISA). The first three techniques require the collection of paired samples and exhibit high cross reactivity, factors that hinder the specificity of the diagnosis. Thus, the most commonly used tests are IgM, IgG or antigen capture immunoenzymatic nonstructural protein 1 (NS1), which require only one serum sample (De Paula and Fonseca 2004).

IgA and IgM are immunoglobulins sensitive to infections such as Dengue and they have been used as a marker to diagnose Dengue infection (Guzmán and Kouri 2004). Due to the need for rapid and sensitive tests for early diagnosis, the objective of the study was to conduct a systematic review to synthesize the current evidence on the accuracy of IgA and IgM for early diagnosis of the dengue virus.

MATERIALS AND METHODS

DATA SOURCES AND SEARCHES

We performed a systematic review following the PRISMA–statement guidelines (Liberati et al. 2009). The review protocol was registered at PROSPERO (International prospective register of systemic reviews, <http://www.crd.york.ac.uk/prospéro>; CRD 42015024808). We searched for studies in the following electronic database from 1990 to January 2018: MEDLINE via PubMed, EMBASE, Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), Biomed Central, Web of Science, IBECs and LILACS. We used the terms “IgM” and “IgA”, “antibodies in saliva”

and “dengue virus infection” to search for relevant studies. The search strategy use both standardized subject headings (i.e., MeSH and Emtree) and text words, with adjustments made to account for differences in indexing across databases. An additional search using the same terms was carried out on Google Scholar. The search was limited to human studies and had no language restrictions. Reference lists of all primary studies were reviewed to identify additional relevant citations. We included study with primary diagnostic, prospective or retrospective cohort, and cross-sectional designs that evaluated dengue virus infection IgM and IgA antibodies in saliva. We included studies of patients with suspected Dengue (moderate fever, pain in the orbital region, muscle and joint pain, among others) who were tested for IgM and IgA antibodies in saliva with ELISA or RT-PCR for detection of antibody response in serum (reference standard).

STUDY SELECTION

Two review authors (MIR, BE) independently assessed all studies identified from the database searches by screening titles and abstracts using the Review Management website Covidence (<http://www.covidence.org>). We separated potential studies which presented the inclusion criteria for full-text reading. A third review author (AJG) resolved any disagreements in selection of included studies.

DATA EXTRACTION AND QUALITY ASSESSMENT

Two review authors (MIR, TC) independently extracted data from the selected studies using a standard data extraction form. We extracted information regarding study design, participants, index test, reference test, dengue classification and total number of participants. We developed 2x2 tables for each individual study in which both tests were compared. All included studies were assessed for their methodological quality using the quality

assessment tool for diagnostic accuracy studies (QUADAS-2). A figure representing each study was provided in the results section.

DATA SYNTHESIS AND STATISTICAL ANALYSIS

The primary analysis made assessed the pooled sensitivity, specificity, likelihood ratios (positive and negative), diagnostic odds ratio (DOR) and ROC curves. Meta-analysis was conducted using random effects model implemented with Metadisc (Zamora et al. 2006). We calculated the point estimate and 95% confidence interval of pooled sensitivity and pooled specificity data.

RESULTS

The search identified 3507 studies, after screening 3294 were irrelevant. For the full text, 213 studies were included, of which 208 were irrelevant for inclusion criterion. This 208 excluded for not evaluate IgM or IgA in saliva but in other fluid such as blood or urine and did not have a comparison to the standard gold test. Five studies were included for quantitative analysis (Figure 1). Five primary studies, published from 1999 to 2011, involving 698 patients, met the inclusion criteria and were analyzed. The studies were conducted in four different countries: Brazil, India, Nicaragua and Singapore. The age of the participants ranged between 0-58 years. The data collection ranged from 1 to 78 days (Table I). The studies of Vazquez et al. 2007 and Andries et al. 2016 assessed IgA and IgM samples in the saliva, but did not present a control group because of this we chose not included in the meta-analysis.

METHODOLOGICAL QUALITY OF INCLUDED STUDIES

The risk of bias for patient selection, index test, reference standard, and flow and timing as well as the concerns for applicability related to the first three domains are shown in Figure 2. The QUADAS-2

items were evaluated, and four studies received a positive assessment in all domains (Balmaseda et al. 2003, 2008, Yap et al. 2011, Oliveira et al. 1999). One study were analyzed as having unclear risks of bias in their selection of patients (Chakravarti et al. 2007). With regard to applicability concerns, all studies showed an unclear risk in the index test because of the unclear cut-off.

SALIVARY IgM X IgM SERUM (ELISA)

Three studies (Balmaseda et al. 2003, Chakravarti et al. 2007, Oliveira et al. 1999) included evaluations of salivary IgM and serum IgM for ELISA. Pooled estimates provided a sensitivity of 86% (95% CI, 80% - 91%) and specificity of 93% (95% CI, 86% - 97%) (Figure 3). The Diagnostic Odds Ratio (DOR) for identification of dengue infection using salivary IgM was 103.38 (CI 95%, 38.03 - 281.03) (Figure 3). ROC curves were formed to evaluate the performance of IgM detection in saliva samples collected from patients with dengue which demonstrated an Area under ROC (AUC) of 0.96 (Figure 4). The study conducted by Balmaseda et al. 2008 also evaluated salivary IgM but using RT-PCR method for comparison. We performed a sensitivity analysis including this study and showed an increase in the heterogeneity and reduction in sensitivity (83%), specificity (91%) and DOR (55.3).

SALIVARY IgA X RT-PCR

Two studies included evaluating of salivary IgA (Balmaseda et al. 2008, Yap et al. 2011) showed a combined sensitivity of 69% (95% CI, 58% - 78%) and a combined specificity of 98% (95% CI, 93% - 100%) (Figure 5). DOR was 89.6 (CI 95%, 22.35 - 359.44) (Figure 5). AUC cannot be performed for two studies in statistics software. Balmaseda 2003 also evaluated salivary IgA, but comparing to serum IgM. We included and after the analysis we observed reduction of specificity (86%) and DOR

TABLE I
Characteristics of included studies.

Study/year	Country	Design	N	Age	Comparator	Time of data collection	TP	FP	FN	TN	
Balmaseda et al. 2003	Nicaragua	RCT-Crossover	147	not specified	IgM serum	1-78 day	IgM	65	7	6	69
							IgA	51	21	24	51
Balmaseda et al. 2008	Nicaragua	RCT-Crossover	356	0-14	RT-PCR	1-4 day	IgM	122	7	33	46
							IgA	34	0	16	26
Chakravarti et al. 2007	India	RCT-Crossover	80	not specified	IgM serum	4-8 day	IgM	60	0	6	14
Oliveira et al. 1999	Brazil	RCT-Crossover	46	5-58	IgM serum	1-33 day	IgM	25	0	13	8
Yap et al. 2011	Singapore	RCT-Crossover	69	not specified	RT-PCR	1-3 day	IgA	23	2	10	73
Total			698								

TP: True Positive; FP: False Positive; FN: False Negative; TN: True Negative; RCT: Randomized Controlled Trial.

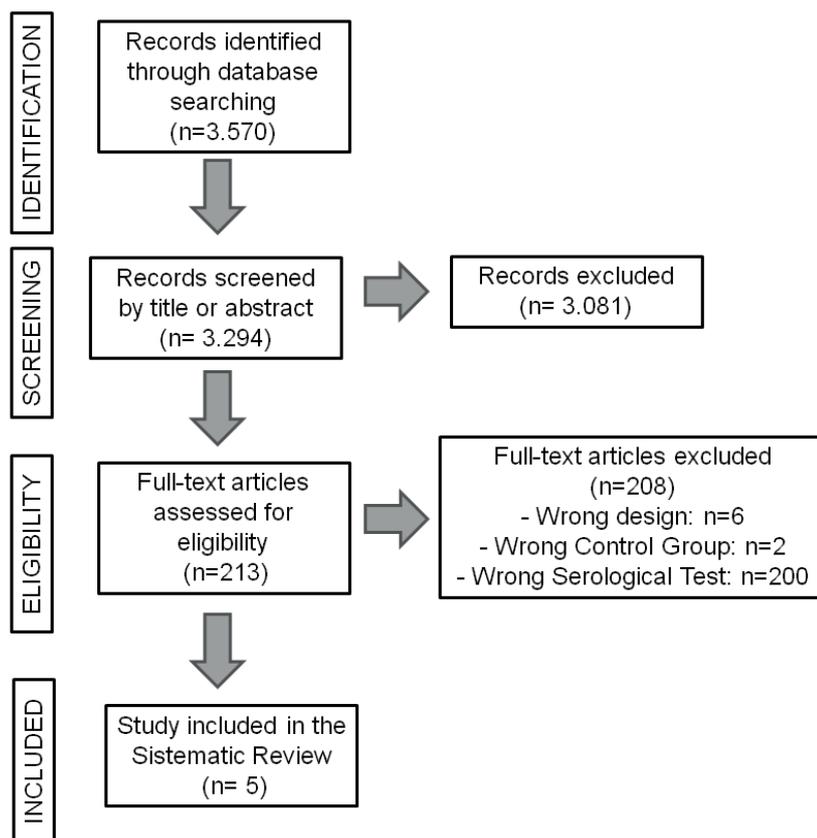


Figure 1 - Flow chart of study selection.

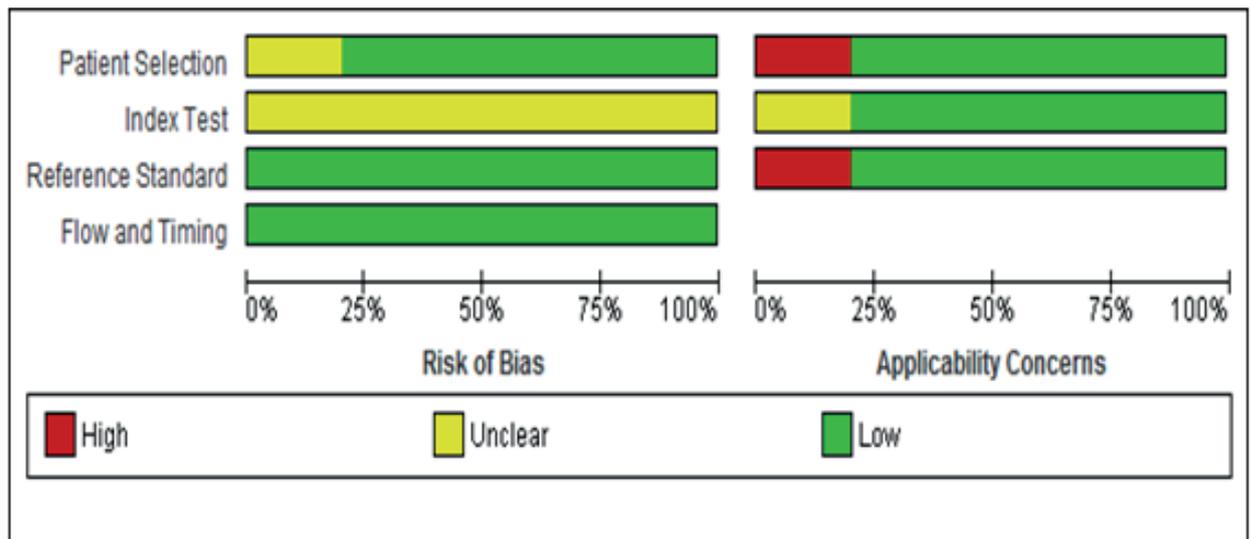


Figure 2 - QUADAS-2 – Risk of Bias.

(29.78) and small reduction of sensitivity (68%). As well as increasing the heterogeneity of the studies in the analysis of the specificity (I^2 93%), because of this we chose not to include this study in the metanalysis.

DISCUSSION

Dengue fever is a viral disease with higher prevalence in tropical and subtropical countries. The ideal period for the diagnosis of dengue infection is approximately from the onset of fever up to 10 days after infection. However, not all patients are diagnosed within this time, so an optimal diagnostic test should be sensitive regardless of the stage of infection (Vorndam and Kuno 1997).

The results show that salivary IgM sensitivity is 86% and specificity 93%; while salivary IgA presents a better specificity 93% than sensitivity for the diagnosis of dengue. Recently, Alagarasu et al. 2016 performed a meta-analysis of the diagnostic accuracy of serum IgA-based tests for the diagnosis of dengue infections was evaluated. The results revealed that IgA-based tests had a sensitivity of 73.9%, specificity of 95.2% and DOR of 66.7. It is important to mention that Dengue diagnosis false positive is widely discussed, since the same

mosquito transmits Zika virus, yellow fever and chikungunya. The different viruses can generate the same immunological response to test which could result in false positive events. The IgA presented specificity of 98%, showing to be an exclusion factor to those not having Dengue.

Yap et al. 2011 shows that salivary IgA are more elevated in the first three days of infection than plasma IgA and IgM during the same period, salivary IgA can be used to detect infection in the first day, providing early diagnosis. Salivary IgA during days 3-5 still provide better scores than plasma IgA and IgM, showing that salivary IgA is better to early detect Dengue virus.

The early detection of dengue results in allows us to identify and put in place management and observation algorithms that may increase survival. It also help to decide who can go home and who needs to stay in hospital for accompaniment. A study by Koka et al. 2008 evaluated the preferences of adult patients to give saliva, urine or blood for clinical trials. In total 413 surveys were completed. The questionnaire contained specific questions about which fluids (saliva, urine and blood) is the most comfortable and convenient to give in the doctor's and which one is the easiest to collect at

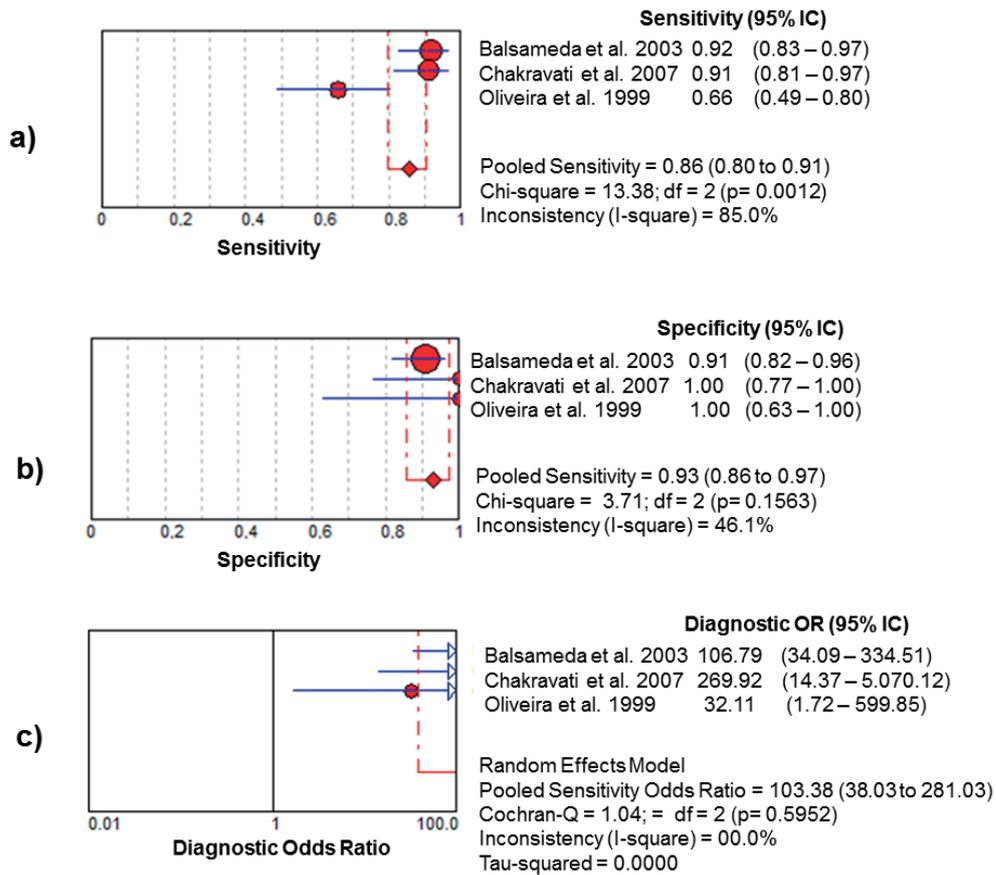


Figure 3 - Forest plot of sensitivity, specificity and diagnostic odds ratio of IgM.

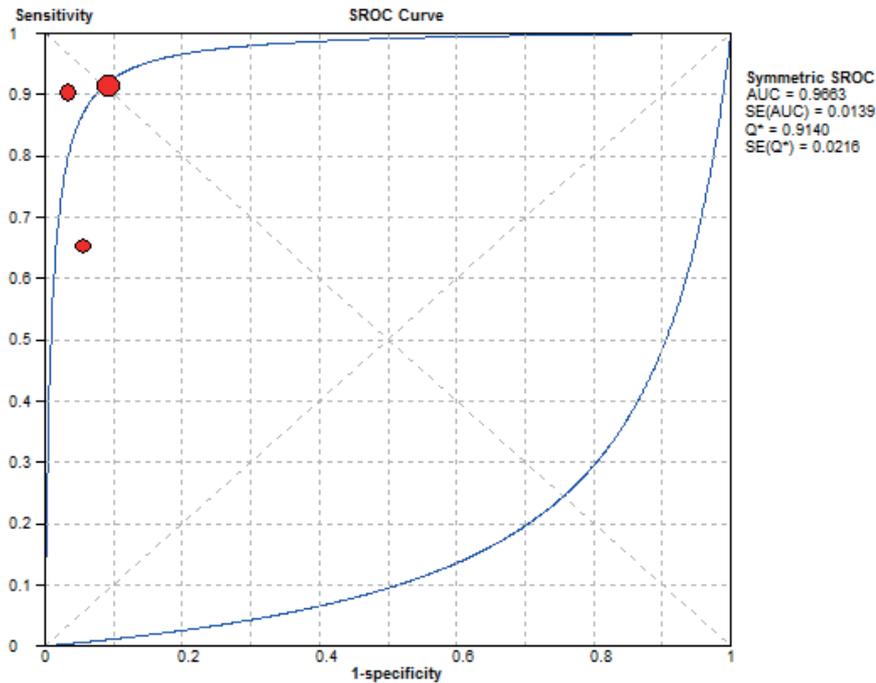


Figure 4 - SROC Curve of IgM.

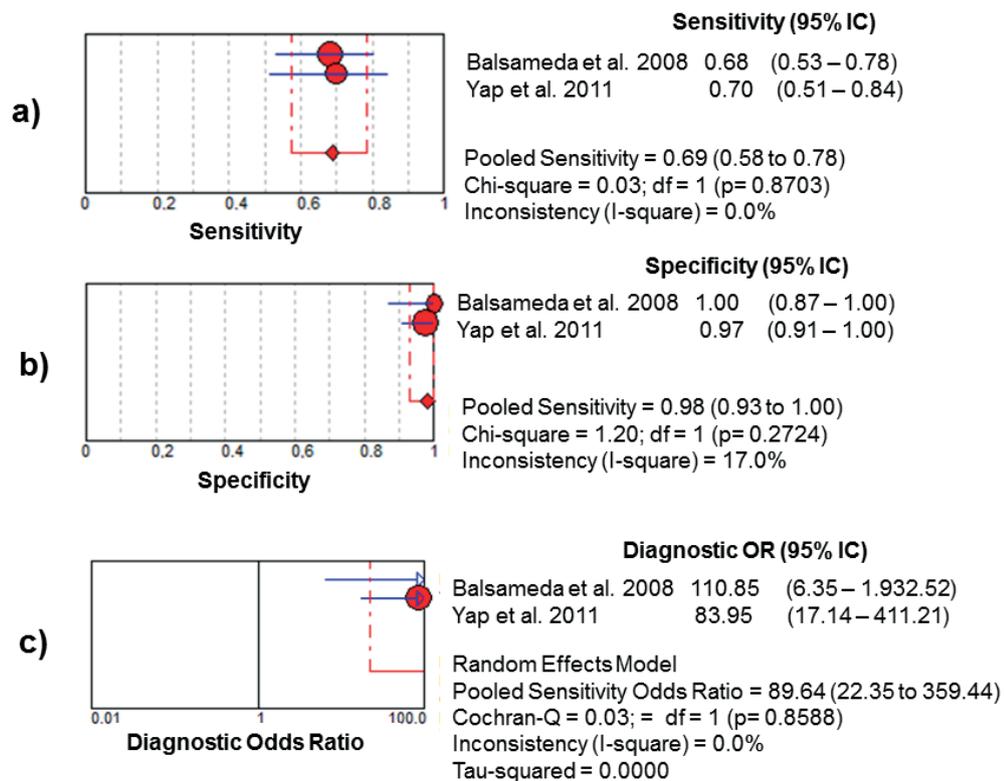


Figure 5 - Forest plot of sensitivity, specificity and diagnostic odds ratio of IgA.

home. The results presented for authors affirm that in terms of convenience and comfort, patients prefer to donate saliva rather than blood and urine for diagnostic testing in clinical practice and research. With the development of accurate, inexpensive and accessible tests, saliva may become the fluid of choice for patient-centered diagnostic testing (Koka et al. 2008). Also, the detection of dengue through saliva would help the early diagnosis, since it is a non-invasive method and easy to collect.

It is recommended that all systematic review produced assess the quality of evidence generated. Thus, considering the studies included in this systematic review and the evidence summarized, we can classify it as low, which means that “further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate or any estimate of effect is very uncertain”, the evidence was downgraded due to imprecision (wide confidence intervals) and

inconsistency (widely different estimates) across the included studies.

The limitations of the study consist on the data currently available in the literature, as new research on the topic is being published, this review should be updated. Inconsistencies in data reporting in the primary study datasets could have also be a limitation.

Here, we have assessed and presented the best available evidence for use of the Saliva (IgM and IgA) in Dengue diagnosis. Dengue diagnosis is challenging as the disease presentation is almost indistinguishable to many other tropical and subtropical infections. New rapid tests are being developed, but attention must be paid in its accuracy with gold standard tests. The accuracy of IgM in the saliva for dengue diagnosis was greater than IgA, however, salivary IgA presents high specificity demonstrating to be an important test to early Dengue diagnosis, being easy to collect data.

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