# Challenges in Diagnosis of Febrile illnesses in Tanzania in the Era of Declining Malaria Epidemiology

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**Abstract**

Malaria and other febrile illnesses are very common especially in children in developing countries. Due to reliance on clinical algorithms for diagnosis in resource-poor settings, most febrile episodes have always been attributed to malaria. However, continuous malaria monitoring and recent improvements in malaria diagnosis have revealed a progressive decline in malaria and significant involvement of non-malarial etiologies in most febrile cases. This paper highlights the situation of malarial and non-malarial fevers, challenges facing the health sector, and possible approaches to addressing these challenges for better diagnosis of non-malarial febrile illnesses in Tanzania.

***Key words:*** Non-malarial fevers, febrile illness, malaria, diagnostic challenges, Tanzania

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**Introduction**

Malaria is ranked among the leading causes of morbidity and mortality in Africa and other malarial endemic regions with fever being the predominant clinical manifestation (Chandramohan *et al.* 2002; Luxemburger *et al.* 1998; Muhe *et al.* 1999; Snow *et al.* 2005; WHO 2009). Diagnosis of febrile episodes using fever as the sole criteria is somewhat cumbersome as many illnesses are accompanied by elevated axillary body temperatures. Studies conducted over 5—10 years ago have estimated the number of annual fever episodes among African children to be as high as 870 million (Gething *et al.* 2010b; Gething *et al.* 2011; Snow *et al.* 2003; Snow *et al.* 2005), a large proportion of which has been associated with malaria. However, these data have been found to overestimate malaria burden due to the lack of specificity of a purely clinical diagnosis. Studies have revealed a diminishing contribution of malaria to the febrile illnesses whose prevalence remains high (D'Acremont *et al.* 2010; Bisoffi & Buonfrate 2013; Crump *et al.* 2013; ,D'Acremont *et al.* 2014a). With the current decline in malaria transmission in many of the malaria endemic regions, the preponderance of febrile episodes could therefore be attributed to non-malarial febrile illnesses (NMFIs) (Acestor *et al.* 2012; Bisoffi & Buonfrate 2013; Okiro & Snow 2010). Since most fevers without any other obvious cause were in the past almost considered malarial, identification of alternative causes of fever has always been perceived as unnecessary and causes of NMFIs have remained mostly unknown. Today, the world has made a significant step in terms of malaria diagnosis. With deployment of malaria rapid diagnostic tests (mRDTs), over-diagnosis, and consequently over-treatment of malaria has been reduced and the contribution of non-malarial etiologies to febrile illnesses has now been recognized (D'Acremont *et al.* 2010). Some studies reported a proportion of non-malarial fevers ranging between 57% to above 90% (D'Acremont *et al.* 2010; Gething *et al.* 2010a). With malaria having been ruled out and without sufficient means to specifically reach

etiologic diagnosis of the febrile conditions (Petti *et al.* 2006), clinicians often find themselves in a dilemma under such circumstances. Consequently, clinical management is often driven by syndrome-based guidelines employing empiric treatment (Baltzell *et al.* 2013;WHO 2011). In the next sections, we discuss the situation of malarial and non-malarial fevers, challenges facing the health sector, and possible approaches to addressing these challenges for better diagnosis of NMFIs in Tanzania.

**Febrile illnesses among children and adults in Tanzania**

Febrile illnesses among Tanzanian children and adults are due to a wide range of etiologies including malaria and other non-malarial causes. A recent study on malaria passive case detection (PCD) in communities of Korogwe District in Tanga, north-eastern Tanzania, has revealed that despite a significant decline in malaria prevalence in these communities, fever prevalence remains high (Rutta *et al.* 2012). In this study, the malaria positivity rate in 15,729 febrile cases was 20% in 2006, but progressively declined to below 10% in 2009 while fever cases remained unchanged (over 40% for children under five, and over 20% for children older than five (Rutta *et al.* 2012). This study did not identify the causes of non-malarial fevers, but its findings underscore the fact that even though malaria has declined, the prevalence of fever cases remains high. For example, a 2013 hospital-based study in the same district investigating causes of fever in children aged 2 to 59 months showed multiple etiology of febrile illnesses with respiratory infections occurring in more than 60% of cases (Mahende *et al.* 2014). Other syndromes include gastroenteritis (>21%), bacteremia and urinary tract infections (>21%) and malaria (8%). More than 26% of patients were found to be co-infected with more than one pathogen in this study (Mahende *et al.* 2014).

A separate study in rural and urban Tanzania investigated causes of fevers in children up to 10 years of age. As in other studies, acute respiratory infections were by far the most

common etiology (62%) followed by malaria (11%), gastroenteritis (10%) and urinary tract infection (6%). Others included typhoid fever, mucosal infections, meningitis, and some with no known causative organism (D'Acremont *et al.* 2014b). Furthermore, hospital-based studies investigating all cases admitted as severe malaria cases at Bombo (Tanga), Magunga (Korogwe), and KCMC (Kilimanjaro) hospitals revealed that a considerable proportion of admissions were malaria negative on microscopy (Reyburn *et al.* 2004; Msangeni *et al.* 2011). Specific tests on bacteremia patient samples revealed infection with *Salmonella typhi*, *Streptococcus s*pp, *Staphylococcus spp,* non typhi Salmonella, brucellosis, rickettsiosis, leptospirosis and others (Biggs *et al.* 2011; Biggs *et al.* 2013; Biggs *et al.* 2014; Crump 2012; Crump *et al.*, 2013; D'Acremont *et al.* 2014a; D'Acremont *et al.* 2014b; Mahende *et al.* 2014; Msangeni *et al.* 2011; Prabhu *et al.* 2011). It should also be noted that not all cases were positive on blood culture, indicating that most of the patients were infected with other groups of pathogens such as viruses, protozoans, fungi as well as other bacteria that do not grow well on blood cultures and whose detection methods were beyond the scope(s) or objective(s) of these studies (Msangeni *et al.* 2011). It is apparent that febrile illnesses are still highly prevalent in Tanzania despite the reported decline in malaria. However, unlike the situation in the past, frequent causes of current febrile illnesses are non-malarial.

**Diagnostic dilemma of non-malarial fevers**

Accurate diagnosis is a prerequisite for appropriate management of any disease or condition. Therefore, diagnostic facilities should be strengthened in order to make sure that appropriate treatment is instituted for the right disease. Failure to meet the above standards usually leads to wrong treatments, poor outcomes, and an increased risk for development of drug resistance. While the above is true, the Tanzanian health sector is still facing a challenge of poor quality diagnostic facilities, which are contributing to difficulties in disease management

(Ishengoma *et al.* 2009; Ishengoma *et al.* 2010). Some of the ongoing challenges facing health systems in Tanzania with respect to diagnosis of malarial and non-malarial fevers are discussed below.

***Submicroscopic malaria infection***

Microscopic examination of Giemsa-stained blood slides remains the gold standard and the most widely-used laboratory tool for diagnosis of malaria. Although mRDTs provide a more convenient option at point-of-care, and nucleic acid-based detection and identification techniques offer more sensitivity and specificity, it is microscopy that has continued to be the mainstay for malaria diagnosis in settings where resources are available. This is because, while mRDTs are rapid and user-friendly and require no specialized training, equipment, or power to read the results, they are relatively new in application and some of the original versions lacked the ability to distinguish different species of plasmodium. Most mRDTs also are qualitative, and products from different suppliers vary in sensitivity and specificity (Forney *et al.* 2003; Wongsrichanalai *et al.* 2003a; Wongsrichanalai *et al.* 2007). Nucleic acid-based detection methods are more sensitive and specific than microscopy, but require expensive and highly specialized equipment, highly trained personnel and continuous power supplies (Coleman *et al.* 2006; Santana-Morales *et al.* 2012; Nicastri *et al.* 2009). Most of these essential conditions are limited or missing in most low-resource (developing) countries where malaria is endemic.

Despite the usefulness of microscopy as a tool for malaria diagnosis, its relatively high limit of detection is a concern. The established theoretical limits of detection of malaria parasites by microscopy lie between 5—20 parasites per microliter of blood (Babiker & Schneider 2008; Bruce-Chwatt 1984; Dowling & Shute 1966; Payne 1988). However, due to a number of factors including sample collection, processing, slide reading, equipment, and malaria

incidence and prevalence, the practical limit of detection is over 100 parasites per microliter of blood (WHO 1988). This detection limit implies that microscopy may miss lower density infections, especially in endemic areas and in areas where malaria is declining. For example, it has been found that about 88% of all infections are missed in areas where malaria prevalence measured by PCR is less than 10% (Okell *et al.* 2009). This proportion of patients with parasite levels below the limit of detection by microscopy are referred to as patients with submicroscopic malaria infection (parasitemia).

Studies have demonstrated that submicroscopic parasites contribute substantially to malaria transmission (Karl *et al.* 2011; Babiker *et al.* 2008). Although the proportion of febrile infections as a result of submicroscopic parasite density has not been well characterized, the possibility has been highlighted (Snounou 1993; Cohee *et al.* 2014). The contribution of submicroscopic parasites to transmission intensity as well as febrile episodes is likely to significantly increase in areas of declining malaria burden as a result of diminishing naturally acquired immunity to malaria (Doolan *et al.* 2009). It has also been shown that, apart from acting as reservoirs of transmission, submicroscopic infections could have an impact on hemoglobin levels (Mockenhaupt *et al.* 2000; Cohee *et al.* 2014). Febrile cases due to submicroscopic parasites are expected to be few, but whenever they occur, they are likely to be missed by conventional microscopic diagnosis. Such cases will be considered to be non-malarial and patients will receive inappropriate treatment, hence minimizing their chances of timely and complete recovery, and thus further propagating not only the malaria transmission cycle, but also promoting antimicrobial resistance.

***The use of malaria rapid diagnostic tests (mRDTs)***

Along with microscopic examination of Giemsa-stained thick and thin blood smears, mRDTs are currently the best available and affordable alternative means of diagnosing malaria,

especially in resource-poor settings (Minja *et al.* 2012; Ishengoma *et al.* 2011; Mubi *et al.* 2011; Santana-Morales *et al.* 2012; Ochola *et al.* 2006). The use of mRDTs significantly facilitates targeted malaria treatment and reduces over-treatment with anti-malarials (Ishengoma *et al.* 2011; Mubi *et al.* 2011; Rutta *et al.* 2012). These tests are useful because they do not require sophisticated equipment or complicated procedures or training, and can be stored at room temperature, which enables their use in areas where refrigeration is lacking.

However, most mRDTs are not ideal. Their sensitivity and specificity under different manufacturing, storage, and operating conditions vary (Iqbal *et al.* 2003; Ishengoma *et al.* 2011). Varying performance implies that under certain circumstances, mRDTs may fail to accurately diagnose malaria, thus compromising the choice of treatment. Low sensitivity results in failure to detect malaria parasites in an infected patient (false negative), while low specificity results into over-diagnosis, hence overtreatment of malaria. It is paramount that mRDTs are sufficiently accurate for proper diagnosis, which is crucial in effecting optimal management of fever patients. Fortunately, in most cases their sensitivity and specificity are sufficiently high for *Plasmodium falciparum* (Pattanasin *et al.* 2003; Iqbal *et al.* 2003).

Some brands of mRDTs are designed to detect only one Plasmodium species and may be the best choice in areas where that species is the predominant cause of malaria. For example, most cases in Sub-Saharan Africa are caused by *Plasmodium falciparum* (Gething *et al.* 2011; Snow *et al.* 2005; Hay *et al.* 2009). In other parts of the world like Asia, North and South America, the Middle East, North Africa, and the South Pacific, *P. vivax* is the predominant malaria parasite (Li *et al.* 2001; Oliveira-Ferreira *et al.* 2010). However, between these extremes, other minor species such as *P. ovale,* and *P. malariae* also are present and cannot be ignored. For this reason, some of the mRDTs being developed today are capable of detecting non-falciparum malaria (Ashton *et al.* 2010). This diversity in malaria etiology poses a challenge to health care workers in developing countries, and

sometimes leads to improper diagnosis. In Tanzania for instance, mRDTs are usually provided by the Ministry of Health and Social Welfare (MoHSW) through the Medical Stores Department (MSD) (Chipwaza *et al.* 2014a). If the stock delivered to health facilities is capable of detecting *P. falciparum* only, then any non-falciparum malaria will be missed and may be treated symptomatically as a non-malarial febrile case. Therefore, an ideal mRDT would be highly sensitive, specific, and capable of detecting all common causes of malaria in a particular epidemiological and clinical setting (Mouatcho & Goldring 2013).

Despite favorable mRDT performance parameters, malaria diagnosis still poses a substantial challenge resulting from potential concomitant infection with other causes of fever (Chipwaza *et al.* 2014a). When other pathogens are involved, mere demonstration of malaria parasites may not be conclusive as to what treatment should be applied to the patient (Wongsrichanalai *et al.* 2003b; Agwu *et al.* 2009; Brouqui *et al.* 2005; Keong & Sulaiman 2006; Uneke 2008). In malaria endemic countries, infection with *Plasmodium spp* does not necessarily result in malaria, since individuals may harbor malaria parasites without becoming ill. Therefore, for every febrile case, a thorough check-up should be performed to confirm malaria or rule out non-malaria causes of fever. Unfortunately, in low-resource countries like Tanzania, healthcare facilities are ill-equipped to make this distinction.

***Diminishing naturally acquired immunity to malaria and its potential role in fever of malarial origin***

Naturally acquired immunity (NAI) to malaria has been associated with non-sterile protection against malaria disease in individuals who have been infected by malaria parasites (Doolan *et al.* 2009; Pinkevych *et al.* 2012; Richards *et al.* 2010; Douglas *et al.* 2011). This immunity may last for years, but can be overcome. For example, exposure to high infectious doses of

the parasites, changes in the genetics of the parasite resulting in more virulent forms, and compromised immune systems all may overcome and thus result in diminished naturally acquired immunity.

In these situations, endemic stability is likely to be disrupted. In recent years, the world has observed a substantial drop in malaria incidence and prevalence across most of the malaria-endemic regions (Mmbando *et al* 2010; O'Meara *et al.* 2010; WHO 2012; Ishengoma *et al.* 2013; Mboera *et al.*), with the exception of malaria hotspots where no decline has been observed or reported. In certain areas, this decline has been occurring for more than 15 years (Ishengoma *et al.* 2013). If withdrawal of continuous exposure to low-dose infective bites is sustained over a long period, then NAI subsequently diminishes, which will render adults susceptible to malaria (Doolan *et al.* 2009). Unpublished data from Korogwe, Tanzania show a time lag in acquisition of natural immunity to malaria in children from the age of 5 to the age of 10 years and above.

Apart from predisposing the population to rebound epidemics (Doolan *et al.* 2009; Ursing *et al.* 2014), diminished immunity will shift the endemic stability balance. The level of parasite dose to which the population is tolerant will drop in accordance with diminishing natural immunity. As a consequence, certain levels of parasites that previously resulted in asymptomatic infection could eventually cause clinical cases of fever (Doolan *et al.* 2009). Some fever cases may even be caused by parasite levels below detection limits of current rapid diagnostics and microscopy. Although greatly confounded by the non-malarial scenario, this is a possibility that needs to be investigated, and which should be addressed by developing more sensitive tests for both malarial and non-malarial febrile illnesses.

***Knowledge, attitudes and practices of the community and health workers regarding non-malaria fevers***

Although the prime clinical manifestation of malaria is fever, malaria is not the only disease that presents with fever (D'Acremont *et al.* 2014b; D'Acremont *et al.* 2014a; Mayxay *et al.* 2013; Ishengoma *et al.* 2013; Punjabi *et al.* 2012; Baltzell *et al.* 2013; Acestor *et al.* 2012; Bisoffi & Buonfrate 2013). It is therefore important to distinguish all cases of fever and correctly diagnose them to their respective etiologies or groups in order to institute appropriate treatment.

However, the current state of affairs attributes most febrile conditions as malaria. A significant proportion of community members in Tanzania and other countries do not have appropriate knowledge about febrile conditions and most believe that fever and malaria are synonymous (Baltzell *et al.* 2013;Chipwaza *et al.* 2014a;Chipwaza *et al.* 2014b). Only a small proportion were able to associate fever with true causes such as respiratory and urinary tract infections (Chipwaza *et al.* 2014a). Due to this lack of knowledge, most febrile patients or parents and guardians of febrile children expect a positive malaria test. When they are given negative results, sometimes they do not accept them. They would rather have their children given antimalarial drugs even if not diagnosed with malaria. This situation also leads to self-medication with antimalarial drugs, with or without consulting healthcare professionals (Chipwaza *et al.* 2014b; Kunda *et al.* 2007; McCombie 2002). In studies involving community members, most admitted that they did not know other causes of fever apart from malaria (Crump 2012; D'Acremont *et al.* 2014a).

Healthcare workers face a dilemma in diagnosis and management of febrile illnesses. Clinical diagnosis of fever based on symptoms and signs is not sufficient to render appropriate treatment. The use of mRDTs helps in definitive identification of positive malaria cases, but

has no use for negative cases. Non-malarial febrile illnesses due to bacteria, protozoa, fungi and viruses, for example typhoid fever, urinary tract infections (UTI), acute respiratory tract infections (ARIs), rotavirus infection, brucellosis, tick-borne relapsing fever, leptospirosis, dengue fever, and Chikungunya virus infection have been reported in Tanzania (D'Acremont *et al.* 2014b; Crump *et al.* 2013; Mahende *et al.* 2014; Prabhu *et al.* 2011; Schoonman and Swai 2009), however there is limited diagnostic capacity for non-malarial fever etiologies. Thus, healthcare workers always resort to clinical diagnosis or symptomatic treatment of febrile cases (Chipwaza *et al.* 2014a).

**What should be the way forward?**

***Create community awareness on the existence of non-malarial febrile etiologies***

It is important for patients and the general community to understand the existence of febrile etiologies other than malaria. Healthcare providers should play a leading role in this education. Patients and the general community also should be made aware that a negative malaria test of a febrile person does not necessarily mean that the method, device, or personnel doing the testing is incompetent. It also does not mean that the patient 'is not ill'. Instead, a negative test creates the need for further testing to establish the real cause of the febrile illness in the patient. Such awareness can be accomplished at different levels. First, healthcare providers could educate patients during consultation sessions after receiving test results regardless of the status of the test. Second, the health system should offer alternative diagnosis for non-malarial fever cases. It is certainly not assuring when, having all indications of febrile illness, a patient is told to go home without being treated simply because *only one* test was found to be negative. To meet this requirement, researchers and scientists must continue to explore all possible causes of febrile disease in different epidemiological settings. Third, mass education through local and regional media should be

employed to strengthen awareness and knowledge about the existence of non-malarial febrile illnesses, and the approaches for diagnosing and treating them.

***Strengthen diagnostic systems for malaria and create options for diagnosis of other causes of fever***

Most of what appears to be the non-malarial fever dilemma in Tanzania and other low-resource countries has gradually resulted from over-dependence on clinical algorithm for malaria diagnosis (Craig *et al.* 2010;Font *et al.* 2001;Petti *et al.* 2006;Reyburn *et al.* 2004). Lack of diagnostic infrastructure or resources, trained personnel, instruments such as microscopes, and accompanying reagents has always made it impossible to correctly diagnose malaria in the developing world. Slight improvements in malaria diagnostics coupled with recent declines in malaria infections revealed that not all febrile cases are due to malaria (Mouatcho & Goldring 2013; Murray *et al.* 2003; Reyburn 2010; Snounou *et al.* 1993; Van Den Ende *et al.* 2010; Wongsrichanalai & Miller 2002; Wongsrichanalai *et al.* 2007; Yukich *et al.* 2010). It is therefore proposed that improved diagnostics be extended to remote areas where such capacity is not available. Building malaria diagnostic capacity in health facilities will help discriminate true malarial and non-malarial fevers, thereby enabling accurate, effective and optimal malaria case management.

However, improved malaria diagnostics will be only one step towards success. Capacity needs to be built or strengthened to enable diagnosis of alternative causes of fever in malaria-negative febrile patients (Chappuis *et al.* 2013; Pang & Peeling 2007). To ensure that these tests are useful to those living in remote, low-resource areas in developing countries, universal tests must conform to the requirements set by the World Health Organization (WHO). WHO has developed the ASSURED criteria for ideal diagnostic tests. The test must be ***A****ffordable* by those at risk of infection, ***S****ensitive* (few false-negative results), ***S****pecific*

(few false-positive results), ***U**ser-friendly* (simple to perform by persons with little training), **R**apid and robust, **E**quipment-free and **D**eliverable to those who need it (Urdea *et al.* 2006; Peeling *et al.* 2006). Current research is directed towards understanding the etiology of non-malarial febrile illnesses in different epidemiological settings. Most of these studies employ advanced molecular and serological techniques that are expensive, time-consuming, requiring trained personnel and in most cases, electric-powered equipment (D'Acremont *et al.* 2014b; Mahende *et al.* 2014). Although these techniques are useful in pathogen identification, most of them are impractical to be used at point-of-care. This underscores the need to develop simple, highly sensitive, and specific tests that meet the WHO ASSURED criteria in order to reach and serve resource-limited parts of the world where they are needed most. An example of such efforts employs the use of microfluidic paper-based analytical devices (μPADs) (Chin *et al.* 2007; Mabey *et al.* 2004; Martinez *et al.* 2010; Yager *et al.* 2006). Using paper ensures cost reduction and flexibility for patterning where photolithography (Martinez *et al.* 2008), plotting (Bruzewicz *et al.* 2008), ink etching (Abe *et al.* 2008), plasma etching (Li *et al.* 2008), cutting (Fenton *et al.* 2009), and wax printing (Carrilho *et al.* 2009; Lu *et al.* 2009) have potential as patterning methods for μPADs. Paper has potential as a diagnostic platform because it is readily available and inexpensive, can wick and distribute aqueous fluids, and has been in use for a long time as an analytical platform. Paper can be modified to incorporate functional groups, can easily be transported and stored, is compatible with most printing technologies, and can easily be disposed by incineration. Paper is therefore a potential starting material for making ASSURED diagnostics (Martinez *et al.* 2010; Phillips & Lewis 2014).

***Develop multiplex rapid diagnostic tests for alternative causes of febrile illness***

The WHO recommends rapid diagnostics that are affordable. This means that they should be made using cheap, readily available materials and simple technology so that the overall cost

of a finished product should not deter the poor from using the service. Multiplexing the tests will also help. Even in cases where simple and cheap diagnostic tests for unconventional fever etiologies are available, most of these diseases are so unknown or overlooked in the developing world that it may imply a trial-and-error approach to arrive at the right diagnosis taking one disease at a time after malaria has been excluded. This act of sequential testing may be slow, and during this time the patient's condition may deteriorate. It may also become prohibitively expensive to most people with limited resources, since the cost of each test will quickly reach an unaffordable range. These setbacks may be overcome if multiplexed, low-cost, rapid diagnostic tests that are capable of simultaneous detection of multiple pathogens are developed. Prototype µPADs have shown potential for multiplexing and also may have favorable performance. This type of flexibility should be utilized in designing multiplex tests for common causes of febrile illnesses in Tanzania and other low-resource countries where malarial and non-malarial febrile illnesses are common. Ongoing research provides crucial information about common causes of fever in children and adults and in different geographical and epidemiological settings. This information should be taken into consideration while planning any multiplex prototype. With all other WHO guidelines met, a diagnostic that is capable of multiplexing will greatly enhance affordability by cutting the overall cost of otherwise sequential multiple testing in non-malarial febrile patients.

## Conclusion

Febrile illnesses continue to cause significant problems in developing countries, especially in children under five years of age. Despite its historical dominance, malaria parasites are not the only cause of febrile conditions; the actual contribution of malaria has been diminishing over the past decade. Continuous malaria monitoring and improvement in malaria diagnosis

has uncovered significant contributions from non-malarial causes of febrile illnesses. This observation has created a new challenge since most healthcare outlets in low-resource countries, like Tanzania, are not equipped with the capacity to accurately diagnose non-malarial febrile illnesses at point-of-care. Instead, they usually rely on patient or parent history and presenting clinical signs and symptoms. Therefore, while there is still a need to develop sensitive diagnostics for malaria in order to meet new challenges resulting from low parasitemia and decline in naturally acquired immunity to malaria, there also is an urgent need to (i) create knowledge and awareness of the community regarding non-malaria fevers, and (ii) intensify efforts to develop point-of-care diagnostics for non-malarial febrile illnesses in order to improve diagnosis and management of these diseases in developing countries.

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**Competing Interests**

We declare no competing interests.

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