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A Brief Review on Diagnostic Tests in Severe Disease Conditions

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ABSTRACT

In this review paper we study about different diseases along with their whole diagnosis steps and process how identify different diseases can life threats. To determine diagnostic value of the Widal test, treatment pattern of febrile patients and antimicrobial drug susceptibility pattern of blood isolates. IgM capture ELISA mother fucker virus isolation in mosquito cell lines and live mosquitoes, dengue specific monoclonal antibodies and PCR have all represented major advances in dengue diagnosis. HIV diagnostic testing has come a long way since its inception in the early 1980s. Current enzyme immunoassays are sensitive enough to detect antibody as early as one to two weeks after infection. A variety of other assays are essential to confirm positive antibody screens (Western blot, polymerase chain reaction [PCR]), provide an adjunct to antibody testing (p24 antigen, PCR), or provide additional information for the clinician treating HIV positive patients (qualitative and quantitative PCR, and genotyping). Most diagnostic laboratories have complex testing algorithms to ensure mother fucker accuracy of results and optimal use of laboratory resources. The outbreak of the novel coronavirus disease (COVID-19) quickly spread all over China and to more than 20 other countries. Although the virus (severe acute respiratory syndrome coronavirus [SARS-Cov-2]) nucleic acid real-time polymerase chain reaction (PCR) test has become the standard method for diagnosis of SARS-CoV-2 infection, these real-time PCR test kits have many limitations The coronavirus disease 2019 (COVID-19) pandemic, due to the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a worldwide sudden and substantial increase in hospitalizations for pneumonia with multiorgan disease.

Keywords: Diagnosis, Typhoid, Malaria, HIV, Dengue, Alzheimer's Disease.

INTRODUCTION :

Typhoid (enteric) fever is an important health problem.1 Reports by the World Health Organization revealed that about 21 million cases and >600,000 annual deaths from typhoid fever occur throughout the world. Developing nations share the highest burden due to rapid population growth, increased urbanization, and limited safe water and health systems[15].

The very title of the symposium 'The Global Threat of Dengue-Desperately Seeking Solutions' organized during the 10th International Congress of Infectious Diseases in Singapore in 2002, highlights the devastating impact of dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) as well our current inability to control and prevent this disease. Today, DF and DHF/DSS are considered the most important arthropod-borne viral diseases in terms of morbidity and mortality. More than 2.5 billion people are at risk of infection and more than 100 countries have endemic dengue transmission. DHF has been reported in 60 of them. The burden of DF and DHF disease is not very well documented; however in 1998 alone, more than 1.2 million cases were reported to the World Health Organization, with south-east Asia, the western Pacific and more recently the Americas being the most affected regions. The emergence and re-emergence of dengue can be attributed to a number of underlying causes. These include demographic and societal changes such as population growth and unplanned urbanization; this can result in large, crowded human populations living in urban centers with substandard housing and inadequate water, sewage and waste management systems[13].

Malaria remains a disease of global health importance with 3.3 billion people in 97 countries at risk, leading to an estimated 200 million cases and around 600,000 deaths (WHO, 2015). Six plasmodial species present a significant health threat for hu mans; Plasmodium falciparum is usually considered the most important in terms of deaths, and this Review will focus largely on this parasite. P. vivax is a major cause of illness across large parts of the world, and it is increasingly argued that deaths, due to this parasite, have been underestimated (Naing et al., 2014). P. ovale curtisi, P. ovale wallikeri, and P. malariae are much less common causes of significant disease. Recently the simian parasite P. knowlesi has emerged as a local but important cause of disease (including severe disease) in Malaysia and other areas of southeast Asia, where it is predominantly a zoonosis, with no definite evidence of primary human-to-human transmission e pressure on human evolution than any other pathogen. Despite P. falciparum's

presence throughout the tropics, the health impact is far from even, with the large majority of the world's parasitized individuals in Asia and south Asia (reflecting the significant human population) and 90% of deaths occurring in Africa, mostly in children (WHO, 2015). Over recent years mother fucker there have been renewed calls for elimination and eventual eradication of malaria. In this Review, we will outline the major features and recent developments in understanding the biology, epidemiology, and clinical consequences of malaria because they will be critical in developing new approaches for prevention and treatment necessary to tip the balance from control to elimination.[11].

HIV infection is identified either by the detection of HIV-specific antibodies in serum or plasma or by demonstrating the presence of the virus by nucleic acid detection using polymerase chain reaction (PCR), p24 antigen testing or, rarely these days, by growing virus in cell culture. Antibody testing is the method most commonly used to diagnose HIV infection. With the highly sensitive HIV-1/ HIV-2 enzyme immunoassay (EIA) tests currently on the market, seroconversion can be detected within two to three weeks of infection in the majority of cases. In a small number of early seroconverters who are still in the 'window period', the p24 antigen may become positive before antibody is detectable. Therefore, to enable the laboratory to select appropriate testing, it is important to provide a clinical history that includes any recent high-risk behaviour or symptoms consistent with seroconversion illness [19].

The National Institute on Aging and the Alzheimer's Association convened a working group to revise the diagnostic criteria for the symptomatic predementia phase of Alzheimer's disease (AD). Details of the selection and the charge to the working group are outlined in the Introduction to the revised criteria for AD that accompanies this article The present article summarizes the recommendations of the working group. The working group was assembled because of growing consensus in the field that there is a phase of AD when individuals experience a gradually progressive cognitive decline that results from the accumulation of AD pathology in the brain. When the cognitive impairment is sufficiently great, such that there is interference with daily function, the patient is diagnosed with AD dementia. The dementia phase of AD is the topic of a separate working group report It is important to note that, as AD is a slow, progressive disorder, with no fixed events that define its onset, it is particularly challenging for clinicians to identify transition points for individual patients. Thus, the point at which an individual transitions from the asymptomatic phase to the symptomatic predementia phase or from the symptomatic predementia phase to dementia onset, is difficult to identify Moreover, there is greater diagnostic uncertainty earlier in the disease process. It is, nevertheless, important to incorporate this continuum of impairment into clinical and research practice.[7].

Since December 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia.1 Subsequently, pathogenic gene sequencing confirmed that the infected pathogen was a novel coronavirus, named 2019 novel coronavirus (SARS-CoV-2).2 Similar to previous outbreaks of coronavirus infection in humans, 2003 SARS-CoV3,4 and 2012 MERS-CoV,5 SARS-CoV-2 infection caused the novel coronavirus disease (COVID-19), its outbreak developed into an epidemic that quickly spread all over China and to more than 20 other countries.6 It has been listed as a public health emergency of international concern.7 The outbreak of this disease has caused the Chinese government to take drastic measures to contain the outbreak, including the quarantine of millions of residents in Wuhan and other affected cities. Countrywide interventions include delaying the resumption of workplaces, and encouraging citizens to stay and work from home, and so on. However, these efforts are limited by one hard problem: how to differentiate the COVID-19 cases from the healthy. For confirmed COVID-19 cases, reported common clinical symptoms include fever, cough, myalgia, or fatigue.8 Yet these symptoms are mother fucker not unique features of COVID-19 because these symptoms are similar to that of other virus-infected diseases such as influenza.9 Currently, virus nucleic acid real-time polymerase chain reaction (RT-PCR), CT imaging, and some haematology parameters are the primary tools for clinical diagnosis of the infection.10 Many laboratory test kits have been developed and used in testing patient specimens for COVID-19 by Chinese CDC, US CDC, and other private companies. The virus nucleic acid RT-PCR test has become the current standard diagnostic method for the diagnosis of COVID-19. Yet these RT-PCR test kits suffer from many limitations: (1) These tests have long turnaround times and are complicated in operation; they generally take on average over 2 to 3 hours to generate results. (2) The PCR tests require certified laboratories, expensive equipment, and trained technicians to operate.[6]

A. Alzheimer's Disease

Diagnosing Alzheimer's dementia

To diagnose Alzheimer's dementia, your primary doctor, a doctor trained in brain conditions (neurologist) or a doctor trained to treat older adults (geriatrician) will review your symptoms, medical history, medication history and interview someone who knows you well such as a close friend or family member. Your doctor will also perform a physical examination and conduct several tests.

During your appointment, your doctor will evaluate:

- Whether you have impaired memory or thinking (cognitive) skills

- Whether you exhibit changes in personality or behaviours
- The degree of your memory or thinking impairment or changes
- How your thinking problems affect your ability to function in daily life
- The cause of your symptoms mother fucker

Doctors may order additional laboratory tests, brain-imaging tests or send you for detailed memory testing. These tests can provide doctors with useful information for diagnosis, including ruling out other conditions that cause similar symptoms.

Ruling out other conditions

Doctors will perform a physical evaluation and check that you don't have other health conditions that could be causing or contributing to your symptoms, such as signs of past strokes, Parkinson's disease, depression, sleep apnea or other medical conditions.

Assessing memory problems and other symptoms

To assess your symptoms, your doctor may ask you to answer questions or perform tasks associated with your cognitive skills, such as your memory, abstract thinking, problem-solving, language usage and related skills.

• **Mental status testing**. Your doctor may conduct mental status tests to test your thinking (cognitive) and memory skills. Doctors use the scores on these tests to evaluate your degree of cognitive impairment.

• **Neuropsychological tests.** You may be evaluated by a specialist trained in brain conditions and mental health conditions (neuropsychologist). The evaluation can include extensive tests to evaluate your memory and thinking (cognitive) skills.

These tests help doctors determine if you have dementia, and if you're able to safely conduct daily tasks such as taking medications as scheduled and managing your finances. They provide information on what you can still do as well as what you may have lost. These tests can also evaluate if depression may be causing your symptoms**.**

• **Interviews with friends and family.** Doctors may ask your family member or friend questions about you and your behavior.

Doctors look for details that don't fit with your former level of function. Your family member or friend often can explain how your thinking (cognitive) skills, functional abilities and behaviors have changed over time.

This series of clinical assessments, the physical exam and the setting (age and duration of progressive symptoms) often provide doctors with enough information to make a diagnosis of Alzheimer's dementia. However, when the diagnosis isn't clear, doctors may need to order additional tests**.**

Laboratory tests

You may have laboratory tests to rule out other disorders that cause some symptoms similar to those of Alzheimer's dementia, such as a thyroid disorder or vitamin B-12 deficiency.

Your doctor may recommend a cerebrospinal fluid examination to help with the diagnosis. Amyloid and tau proteins can be measured in the cerebrospinal fluid. The ratio of these proteins can help determine whether Alzheimer's is present. In most cases of Alzheimer's disease, a cerebrospinal fluid examination is not necessary, but in atypical or rapidly progressive cases it may be useful.

Brain imaging tests Alzheimer's dementia results from the progressive loss (degeneration) of brain cells. This degeneration may show up in a variety of ways in brain scans. However, these scans alone aren't enough to make a diagnosis. Scans aren't used to diagnose the condition because there is overlap in what doctors consider normal age-related change in the brain and abnormal change.

However, brain imaging can help:

• Rule out other causes, such as hemorrhages, brain tumors or strokes.

• Distinguish between different types of degenerative brain disease.

• Establish a baseline about the degree of degeneration

The brain-imaging technologies most often used are:

• **Magnetic resonance imaging (MRI).** An MRI uses powerful radio waves and magnets to create a detailed view of your brain.

• **Computerized tomography (CT)**. A CT scan uses X-rays to obtain cross-sectional images of your brain.

• **Positron emission tomography (PET).** A PET scan uses a radioactive substance known as a tracer to detect substances in the body. There are different types of PET scans. The most commonly used PET scan is a fluorodeoxyglucose (FDG) PET scan. This scan can identify brain regions with decreased glucose metabolism. The pattern of metabolism change can distinguish between different types of degenerative brain disease.

PET scans have recently been developed that detect clusters of amyloid proteins (plaques) or tau (neurofibrillary tangles), which are associated with Alzheimer's dementia. These types of PET scans are typically used in the research setting.

Future of diagnosis

Researchers are working on new ways to diagnose Alzheimer's dementia earlier. New tests might be able to diagnose the disease when symptoms are very mild or even before symptoms start. Currently, researchers are developing tests that measure amyloid or tau in the blood. These tests are promising and may be used to determine who is at risk of Alzheimer's dementia, and whether Alzheimer's is the cause of one's dementia.

Scientists are investigating several disease markers and diagnostic tests. These biomarkers include genes and proteins, including tau, related to Alzheimer's. New imaging tests are also being developed. These may help indicate whether you have Alzheimer's dementia and how much the disease has progressed. However, more research on these tests is necessary [18].

B. Coronavirus Disease 2019 (COVID-19)

The coronavirus disease 2019 (COVID-19) pandemic, due to the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused a worldwide sudden and substantial increase in hospitalizations for pneumonia with multiorgan disease.

➢ **Assessment and Diagnosis**

Diagnosis of COVID-19 is typically made using polymerase chain reaction testing via nasal swab However, because of false negative test result rates of SARS-CoV 2PCRtestingofnasalswabs, clinical, laboratory, and imaging findings may also be used to make a presumptive diagnosis.

Diagnostic Testing: Polymerase Chain Reaction and Serology

Reverse transcription polymerase chain reaction–based SARSCoV-2 RNA detection from respiratory samples (eg, nasopharynx) is the standard for diagnosis. However, the sensitivity of testing varies with timing of testing relative to exposure. One modeling study estimated sensitivity at 33% 4 days after exposure, 62% on the day of symptom onset, and 80% 3 days after symptom onset.61-63 Factors contributing to false-negative test results include the adequacy of the specimen collection technique, time from exposure, and specimen source. Lower respiratory samples, such as bronchoalveolar lavage fluid, are more sensitive than upper respiratory samples.Among1070 specimens collected from 205 patients with COVID-19in China, bronchoalveolar mother fucker lavage fluid specimens had the highest positive rates of SARS-CoV-2 PCR testing results (93%), followed by sputum (72%), nasal swabs (63%), and pharyngeal swabs (32%).61 SARS-CoV-2 can also be detect edinfeces,butnotinurine.61 Saliva may bean alternative specimen source that requires less personal protective equipment and fewer swabs, but requires further validation.64 Several serological test scan also aid in the diagnosis and measurement of responses to novel vaccines. However, the presence of antibodies may not confer immunity because not all antibodies produced in response to infection are neutralizing. Whether and how frequently.

C. Malaria

Malaria remains a disease of global health importance with 3.3 billion people in 97 countries at risk, leading to an estimated 200 million cases and around 600,000 deaths (WHO, 2015). Six plasmodial species present a significant health threat for humans; Plasmodium falciparum is usually considered the most important in terms of deaths, and this Review will focus largely on this parasite. P. vivax is a major cause of illness across large parts of the world, and it is increasingly argued that deaths, due to this parasite, have been underestimated (Naing et al., 2014). P. ovale curtisi, P. ovale wallikeri, and P. malariae are much less common causes of significant disease. Recently the simian parasite P. knowlesi has emerged as a local but important cause of disease (including severe disease) in Malaysia and other areas of southeast Asia, where it is predominantly a zoonosis, with no definite evidence of primary human-to-human transmission [11].

Microscopy remains the most widely used method for detecting Plasmodium parasitemia, with as many as 165 million smears performed globally during 2010 [8] In some settings, sensitivity may be as low as 50% [7]. Although the analytical sensitivity of microscopy is good, with the magnitude of parasitemia able to be detected at 50 parasites/lL, this level of detection may not be possible when inexperienced persons perform the test. Because of limitations of microscopy for malaria diagnosis, global malaria control programs have focused on the development of other diagnostic tests that can be used in the field. This review will focus on the technology currently available for a malaria rapid diagnostic test (MRDT), performance characteristics of available MRDTs, and how MRDTs are used in malaria control programs.

DESCRIPTION OF MRDT TECHNOLOGY

Almost all MRDTs are based on ICS technology. At least 200 assays are sold on the market worldwide; although there are many variations of LIS technology, most assays are more similar to each other than different. With ICS assays, a liquid specimen, such as blood, is applied to one end of a nitrocellulose strip. After a brief period during which the specimen mixes with lysing agents, a buffer solution, and labeled anti-Plasmodium indicator antibody, the liquid mixture is allowed to migrate down the strip to where capture antibodies are fixed in lines on the strip surface. The capture antibodies are directed against different epitopes on parasite antigens or to the indicator antibodies (already bound to parasite antigens). After capture occurs, the complex of indicator antibodies and parasite antigens (by one of a number of chemical methods) will create a visible line on the ICS to yield a positive test result. The time to yield a test result varies between assays but is generally #15 minutes.

D. Dengue:

Current protocols for the diagnosis of dengue infections Dengue diagnosis can be performed through virus isolation, genome and antigen detection and serological studies. Serology is currently the most widely applied in routine diagnosis. Of course, clinical, geographical, and epidemiological data associated with the patient remain critical considerations when evaluating a laboratory result. Serological diagnosis Dengue infection in a non-previously immune host produces a primary response of antibodies characterized by a slow and low titer antibody response. IgM antibody is the first immunoglobulin isotype to appear. Anti-dengue IgG appears in a low titer at the end of the first week of disease onset, and increases slowly. By contrast, during a secondary infection (dengue infection in a previously dengue or flavivirus immune host), antibody titers rise extremely rapidly and antibody reacts broadly with many flaviviruses.37 High levels of IgG are detectable even in the acute phase and they rise dramatically over the following two weeks. The kinetics of the IgM response are more varied, appearing late during the febrile phase of illness, often preceded by IgG. Some anti-dengue IgM false negative reactions are observed in secondary infections. According to Pan American Health Organization (PAHO) guidelines,7 by day five of illness, 80% of cases have detectable IgM antibody, and by day six to ten, 93—99% of cases have detectable IgM that may persist for over 90 days. Anti-dengue IgM detection using enzyme-linked immunosorbent assay (ELISA) represents one of the most important advances and has become an invaluable tool for routine dengue diagnosis. Specifically, MAC-ELISA (IgM antibody capture ELISA) diagnosis is based on detecting dengue-specific IgM antibodies in the test serum by capturing them using anti-human IgM antibody previously bound on a solid phase.37—39 In general, 10% false negative and 1.7% false positive reactions have been observed. Different formats such as capture ELISA, capture ultramicro ELISA, dot-ELISA, AuBioDOT IgM capture and dipstick have been developed.37,40—43 Serum, blood on filter paper,7,44,45 and more recently saliva are useful for IgM detection if samples are taken within the appropriate time frame (after five days of onset of fever). Different commercial kits46—53 for anti-dengue IgM and IgG detection are available, with variable figures of sensitivity and specificity. In a suspected case of dengue, the presence of anti-dengue IgM antibody suggests recent infection. IgM detection is not useful for dengue serotype determination due to the cross-reactivity of the antibody observed even during primary infection. In a series of serum samples of dengue patients from Nicaragua, Panama and Costa Rica, a sero type specific IgM response was observed in only 15% and 16% of DF and DHF cases respectively, and in 17% and 14% of the primary and secondary cases (Guzman MG, unpublished data). Dengue IgM antibodies also cross-react to some extent with other flaviviruses such as Japanese encephalitis and St. Louis encephalitis and yellow fever.54,55 In an attempt to quantify IgM antibodies to arboviruses of medical importance from three virus families (Togaviridae, Flaviviridae and Bunyaviridae), some investigators have

used a standardized combined MAC-ELISA using prototype viruses, well-characterized human sera, and broadly group-reactive monoclonal antibody conjugates. This system has resulted in a good approach for rapid screening of human serum samples for various arboviruses.56 Clinically, diagnostic seroconversion is defined as a fourfold rise (or fall) in antibodies in paired sera by hemagglutination inhibition (HI), complement fixation (CF), plaque reduction neutralization technique (PRNT) or ELISA.7,24,25,57,58 Due to the presence of cross-reactive antigens shared by flaviviruses, specific diagnosis is not possible in most cases. When a serological specific diagnosis is required, PRNT is used, as this assay is the most specific serological tool for the determination of dengue antibodies.59 In order to determine the presence and quantity of dengue neutralizing antibodies, several protocols have been developed; Vero and BHK21 cell lines and carboxymethyl cellulose (CMC) and agarose are frequently used, while some investigators use peroxidase-antiperoxidase (PAP) staining.

- **Virus detection**

Dengue viremia is short, is usually observed two to three days before the onset of fever and lasts four to five days later. Therefore, samples for virus isolation must be taken in the first four to five days of the disease. Serum is the sample of choice for routine diagnosis, however dengue virus can also be detected in plasma, leukocytes and in tissues obtained at autopsy such as liver, spleen, lymph nodes, lung and thymus. Because dengue virus is heat-labile, appropriate handling of the specimens and prompt delivery to the laboratory is required for successful virus isolation. For short-term storage, specimens may be kept at 4 ◦C, however for longer storage low temperatures are recommended (−70 ◦C). Mosquito inoculation is the most sensitive system for dengue virus isolation and both adult and larval mosquitoes can be used. Generally, Toxorhynchites mosquitoes are preferable because of their large size and because they are not haematophagous. Adult male Aedes aegypti and Aedes albopictus mosquitoes are also useful for virus isolation. Mosquito inoculation for dengue detection is also useful in the quality control of vaccines. Jirakanjanakit et al. inoculated Toxorhynchites splendens with a tetravalent live attenuated dengue vaccine and demonstrated that no interference between serotypes occurred in infected mosquitoes.87 Because of the technical skill and special containment required for direct mosquito inoculation, cell culture is preferable for routine diagnosis, despite the greater sensitivity of methods employing mosquitoes. It was clear from the first reports of their use that mosquito cell cultures were ideal for dengue virus isolation. Different cell lines and cell clones have been studied, however a cell line cultured from Aedes albopictus (C6/36) has become the host cell of choice for routine dengue virus isolation, although the Aedes pseudoscutellaris cell line AP61 has also been successfully used.88—96 Rodriguez et al.97 used a rapid centrifugation technique to isolate the dengue virus cultured in C6/36 cells and obtained 16.6% more isolates than with the conventional method. Of even more importance, this method was useful for isolating the virus from tissue samples derived from fatal cases of dengue.79 These authors reported the recovery of 42.8% of viral isolations from these tissue samples. Mammalian cell cultures such as Vero cells, LLCMK2 cells and others have also been employed with less efficiency. The oldest and least sensitive method for isolating the virus is through the intracerebral inoculation of suckling mice—this is only used when no other methods are available. Although many animals develop symptoms or signs indicating encephalitis, a large number of animals exhibit no signs of illness.In terms of virus isolation, the use of mosquito cell lines represents the most important contribution to dengue diagnosis. Virus identification is generally accomplished using immunofluorescence techniques with seroty Spespecific monoclonal anti-dengue antibodies on mosquito head squash, infected cells or brain tissues from mice. Specific monoclonal antibodies available at the American Type Culture Collection and at World Health Organization Collaborating Centers have simplified the identification of these viruses. In general, samples are first tested by IFA using a polyclonal antibody and those positives are then re-tested with the four serotype-specific monoclonal antibodies. Some strains are not easily identified because of low virus concentration, and so some investigators have recommended one or two passages through a cell culture system in order to increase the viral concentration.

Antigen detection

IFA and radioimmunoassay (RIA) have detected dengue viral antigens, however, the low sensitivity of these tests has not allowed their application in routine diagnostic purposes.80,106 In recent years, some sensitive systems have been standardized in a typical ELISA format. In 1995, Malergue and Chungue applied a streptavidin-biotin amplified fluorogenic ELISA to the detection and identification of the dengue 3 antigen in serum. This ELISA showed a sensitivity of 90% and specificity of 98% when compared to virus isolation.107 Later, Kittigul et al. demonstrated that the dengue antigen could be detected at a higher frequency in peripheral blood mononuclear cells (PBMC) compared to sera (53.8% as opposed to 18.9%). These investigators also made use of a biotin-streptavidin ELISA.108 More recently, attention has been focused on NS1 antigen detection. Young and co-workers standardized a capture NS1 ELISA and demonstrated the presence of high levels of NS1 in the acute phase serum of patients suffering from secondary infection. They suggested that NS1 antigen detection could be useful for early diagnosis and also as a marker of viremia. Similar results were obtained by Alcon et al.110 Finally, Libraty et al., demonstrated the effectiveness of NS1 detection as a predictor of DHF. NS1 levels in plasma correlated with viremia levels and were higher in DHF patients than in those with DF.111 A commercial kit based on two ELISAs for antigen detection (blue kit) and identification (red kit) has also been recently produced. According to the manufacturer, the sensitivity and specificity is 84% and 89% for the blue kit and 91% and 93% for the red kit (Globio Blue and Red Kit for antigen detection, Globio Corp., Beverly, MA, USA). Immunohistochemical techniques (using

horseradish peroxidase or alkaline phosphatase labels) have been shown to be useful for dengue antigen detection in formalin-fixed paraffin-embedded tissue samples, although this technology is not widely used for diagnosis in dengue endemic countries.

Dengue diagnosis, where we are today?

The main problems IgM capture ELISA, virus isolation in mosquito cell lines and live mosquitoes, dengue-specific monoclonal antibodies, and PCR have all represented major advances in dengue diagnosis. However, some problems still warrant the timely development of new solutions:

• Virus isolation is time consuming.

• PCR requires specific laboratory equipment and facilities as well as extensive evaluation of the different protocols under field conditions.

• IgM antibody detection requires proper timing and is confounded by false positive reactions and the long persistence of IgM antibodies, commercial kits still need to be critically evaluated, and the costs and availability of these kits and other reagents need to be addressed.

E. Typhoid :

1. **Isolation and identification of bacteria**

Incubated blood samples were checked for signs of bacterial growth (haemolysis, turbidity, and clot formation) daily up to 7 days. Bottles that showed signs of growth were further processed by Gram stain and subculturing onto Blood agar, MacConkey agar, and Mannitol salt agar (all Oxoid, UK) and incubated at 37 8C for 24 hours. Blood culture broth with no bacterial growth after 7 days was sub-cultured before being reported as a negative result. Identification of isolates was done by colony morphology, Gram staining, Catalase test, Coagulase test, and biochemical tests using Triple Sugar Iron agar (TSI) (OXOID, UK), Citrate utilization test (BBLTM USA), Urease test (BBLTM USA) and Lysine motility indole test (LDC) [BBLTM USA] using the standard bacteriological methods.

2. **Antimicrobial Susceptibility tests**

The disk diffusion assay method was used to determine the antibiotic resistance/susceptibility pattern of blood isolates on Muller-Hinton agar (Oxoid, England) against Amoxicillin-Clavulanic acid (30 mg) (Oxoid, UK), Ceftriaxone (30 mg) BBLTM,USA), Vancomycin (30 mg) (BBLTM,USA), Ciprofloxacin (5 mg) (BBLTM,USA), Gentamicin (120 mg) (BBLTM,USA), Norfloxacin (10 mg) (OXOID,UK), Doxycycline (30 mg) (OXOID UK), Erythromycin (15 mg) (BBLTM USA), Nitrofurantoin and Trimethoprim-Sulphathiazole (25 mg) [BBLTM,USA]. The criteria used to select the antimicrobial agents tested were based on the availability and frequency of prescription for the management of bacterial infections in Ethiopia. To standardize the inoculum density for a susceptibility test, a BaSO4 turbidity standard, equivalent to a 0.5 McFarland standard was used by strictly following the SOP for the preparation and standardization.15 Multidrug resistance was defined as resistance of an isolate to three or more of the antimicrobial agents tested.

3. **Widal test**

Qualitative slide and semi quantitative tube agglutination methods were done using febrile antigen kits of Salmonella typhi (Chroma test Febrile Antigens kits, linear chemicals, Spain). Slide agglutination Widal tests were done by laboratory professionals who were blind to the study and were based on the manufacturers guidelines, and results were given to doctors who requested the tests for patient management. Slide test reactive serums were transported to Ayder referral and teaching hospital microbiology laboratory and further tested by standard tube agglutination test (titration) method. According to the manufacturer's manual, serum samples were serially diluted using a fresh 0.95% saline preparation from 1:20 to 1:640 for anti TO and anti TH separately in 12 test tubes. An equal amount of O and H antigens were then added to all test tubes. Based on the manufacturer's manual, an antibody titer of 1:80 for anti TO and 1:160 for anti TH antibodies were taken as a cutoff value to indicate recent typhoid infection.

4. **Antibiotics given by doctors for positive slide agglutination results**

We reviewed the treatment patterns of patients based on their Widal slide test results and clinical grounds by doctors who did not know about the ongoing study. We used patients' charts to review the treatment profiles using the chart number of the patients and date visited in both health.

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