

Vol. 17, No. 2 Maret 2011

ISSN 0854-4263

INDONESIAN JOURNAL OF  
**Clinical Pathology and  
Medical Laboratory**

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

IJCP & ML (Maj. Pat. Klin. Indonesia & Lab. Med.)	Vol. 17	No. 2	Hal. 57-126	Surabaya Maret 2011	ISSN 0854-4263
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Diterbitkan oleh Perhimpunan Dokter Spesialis Patologi Klinik Indonesia

*Published by Indonesian Association of Clinical Pathologists*

Terakreditasi No: 43/DIKTI/Kep/2008, Tanggal 8 Juli 2008



INDONESIAN JOURNAL OF  
**CLINICAL PATHOLOGY AND  
 MEDICAL LABORATORY**

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# DIAZO TEST AS A SCREENING TEST OF TYPHOID FEVER A PRACTICAL APPROACH

*(Uji Diazo sebagai Penyaring Demam tifoid; Sebuah Pendekatan Praktis)*

**J. Nugraha, Meiti Muljanti**

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## ABSTRACT

Typhoid fever represents an endemic acute infection with a high mortality, in this case a laboratory test is needed to establish the early diagnosis. For this study the researchers prefer urine diazo test which is a relatively easy, simple and inexpensive test. The aim of this study is to know whether the urine diazo test can be used for screening typhoid fever and whether there is a concordance with the Widal and TUBEX<sup>®</sup> TF serological tests. Forty patients aged 2–18 years, attending to the Jeremy Medical Service Clinic, Surabaya, suffering of fever more than 3 days were studied from June up to August 2010. Urine samples were tested by diazo test, while blood samples as comparison were tested by Widal and TUBEX<sup>®</sup> TF tests, and blood culture was carried out as the gold standard. The sensitivity and specificity of those tests were then recorded. From the 40 samples, 12 patients showed positive *Salmonella typhi* blood cultures, 26 diazo positive, 22 Widal positive and 14 TUBEX<sup>®</sup> TF positive. Of the 12 positive blood cultures, 10 (83%) diazo positive, 7 (58%) Widal positive and 9 (75%) TUBEX<sup>®</sup> TF positive were found. The sensitivity and specificity of diazo test was 83% and 43%, Widal test 58% and 46%, TUBEX<sup>®</sup> TF test 75% and 82%. It is shown that the Diazo test had a higher sensitivity value, while the TUBEX<sup>®</sup> TF test showed a higher level of specificity. In conclusion, so far it can be concluded that the Diazo test is quite reliable in aiding the diagnosis of typhoid fever and can be considered as a screening test for typhoid fever.

**Key words:** Typhoid fever, Diazo test, screening test

## ABSTRAK

Demam tifoid merupakan penyakit infeksi akut endemis dengan angka kematian cukup tinggi. Sehingga, dibutuhkan uji laboratorium untuk menegakkan diagnosis secara dini. Uji diazo air kemih untuk menunjang diagnosis demam tifoid merupakan pemeriksaan yang relatif mudah, murah dan sederhana. Penelitian ini untuk mengetahui uji diazo air kemih sebagai uji penyaring awal pada penderita demam tifoid kemudian dilihat kesesuaiannya dengan uji serologis Widal dan TUBEX<sup>®</sup>. Penelitian terhadap 40 penderita usia 2–18 tahun, yang datang ke klinik Jeremy Medical Service, Surabaya, Indonesia, bulan Juni sampai Agustus 2010, mengalami panas lebih dari 3 hari. Sampel air kemih dilakukan pemeriksaan uji diazo, sedangkan sampel darah dilakukan pemeriksaan biakan darah sebagai standar baku emas dan pemeriksaan serologis uji Widal dan TUBEX<sup>®</sup>. Kemudian dilihat kepekaan dan kekhasannya. Dari 40 sampel diperoleh 12 biakan darah positif *Salmonella typhi* dan 28 diazo positif, 28 Widal positif dan 11 TUBEX<sup>®</sup> positif. Dari 12 biakan darah positif, 9 (75%) diazo positif, 7 (58%) Widal positif dan 5 (42%) TUBEX<sup>®</sup> positif. Kepekaan dan kekhasan spesifisitas uji diazo 75% dan 32%, uji Widal 58% dan 25%, uji TUBEX<sup>®</sup> 42% dan 78%. Uji diazo memiliki kepekaan lebih tinggi sedangkan uji TUBEX<sup>®</sup> memiliki kekhasan yang lebih tinggi. Uji diazo cukup handal dalam membantu menegakkan diagnosis demam tifoid sehingga dapat dipertimbangkan uji diazo sebagai uji penyaring penderita demam tifoid.

**Kata kunci:** Demam tifoid, Diazo tes, uji saring

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## INTRODUCTION

Typhoid fever represents an acute systemic infectious disease of the reticuloendothelial system (RES), and this fever is caused primarily by *Salmonella enterica subtype enterica serotype typhi* (*Salmonella typhi*) which could be found widely in many developing countries particularly located in the tropical and subtropical regions.<sup>1</sup>

Typhoid fever in Indonesia still represents as endemic disease with a high mortality rate. It is spread all over the provinces in rural areas with an incidence of 358/100.000 cases/year and in urban areas of 760/100.000 cases/year. In 91% patient's age cases is reported between 3–19 years.<sup>2</sup>

Clinical appearance of typhoid fever in children is often not specific and varies from fever symptoms, abdominal pain, diarrhea, delirium, rose spots and splenomegaly, with complications such as gastrointestinal haemorrhage and perforation. The clinical symptoms may look like other infection disease such as dengue haemorrhagic fever or malaria.<sup>2</sup>

*Salmonella typhi* enters by ingestion of contaminated food and water into the gastrointestinal and attach to the intestinal wall. Then invade the small intestinal mucosa towards lamina propria and ingested by the macrophage cell. Further on, the bacteria which was ingested by macrophage will

be transported to the Plaques of Peyer, then pass through the thoracic duct and enter the blood stream (bacteriemia stage I) as well. Then they metastasize to the network of the reticuloendotelial system all around the body, especially in the liver and spleen. In those organs *Salmonella typhi* multiply and generate inflammation. This process takes place during 7–10 days. Then the bacteria will metastasize to the blood stream and generate twice bacteriemias (bacteriemia stage II).<sup>3</sup>

Systemic symptoms of typhoid fever are caused by *Salmonella typhi* endotoxin and material mediator which is released by macrophage. In the bacteriemia stage, some bacteria will enter the gall bladder and are secreted into the intestine along with the bile. Bacteria in the intestinal lumen will be excreted along with faeces and partly will invade again and return to the intestinal wall. The result by bacteria damaging in the process of phagocytosis, will happen at the end of second week. In this case there are no *Salmonella typhi* found in the blood, but they are still in the bone marrow.<sup>3</sup>

It is difficult to establish the diagnosis typhoid fever based on some clinical symptoms only, because of the variation and unspecific clinical appearance of typhoid fever. Therefore, reliable laboratory tests are essential to establish early diagnosis of typhoid fever, so that appropriate treatment can be applied.<sup>4</sup>

The laboratory tests to establish diagnose typhoid fever consist of: urine, stool, besides serologic and bacteriologic tests. The urine diazo test preferred to support the diagnosis of typhoid fever is relatively easy, simple and inexpensive, so it can used in a health centre with simple laboratory facilities. Positive diazo reaction is shown in the urine by a phenol ring containing substance. This substance is the result of decomposition of protein in the intestine absorbed into the blood stream, detoxified in the liver and excreted through the kidney. Diazo test usually gives a positive result at the 5–14<sup>th</sup> day of typhoid fever and becomes negative if the fever disappears.<sup>3</sup>

Some diseases often give a false positive diazo test result such as morbilli, acute miliary tuberculosis, and sometimes even dengue haemorrhagic fever. At the relapse of typhoid fever, diazo test will again become positive. Antipyretic drugs and sulpha antibiotic group also give a false positive result. In order to obtain a good result in this case a new diazo reagent must be used.<sup>3</sup>

The current standard diagnostic test for typhoid fever is culture of the blood, bone marrow or other body fluids. This procedure is often difficult to perform in a simple laboratory, because it needs a long time (4–7 days) and has a low sensitivity (40%). When blood culture is taken at the first week, positive results of more than 80% can be found. After this week the positive possibility decreases. Failure to

isolate *S.typhi* bacteria can be caused by limitation of the media, prior usage of antibiotics, very small numbers of bacteria in blood, and insufficient amount of the specimen. It is suggested to draw 10–15 mL of blood for older children and adults, while for the younger children about 2–4 mL.<sup>2,4</sup>

Bone marrow results in a much higher positive possibility 80–95% are not affected by the period of the specimen taken, or prior curative antimicrobial medicines. However, retrieval of bone marrow specimen requires a skilled and special experience operator, because it is invasive, and it is difficult to apply routinely in the suspected typhoid fever patients.<sup>4</sup>

Serology test is carried based on the finding of antibody to diagnose typhoid fever has been used widely, it was introduced by Widal test since 1896. Serology Widal test is often used because it is practical, and does not require a long time for the result, but has a low sensitivity and specificity. The factors which affect the Widal test are for example: the time period of the disease, usage of antibiotics, usage of antigen type and the history of prior vaccination. The antibody of Salmonella usually will be found in the blood one week after the fever exerted with a peak at 5–6<sup>th</sup>, week, therefore a negative Widal serology test result shown early in the disease is not be able to exclude diagnosis.<sup>4-5</sup>

TUBEX<sup>®</sup> TF test specifically detects IgM antibodies to the *S.typhi* O9 lipopolysaccharide antigen. In this case the researchers use an efficient mixture analyte with a reagent of latex particle, and the result is read visually. To increase sensitivity and specificity, TUBEX<sup>®</sup> TF test uses special V form of tubes and two kinds of particle colour indicators and one magnetic particle. This test has a sensitivity of 75–85% and specificity of 75–90%.<sup>5-6</sup>

The aim of this study is to know whether the urine diazo test can be used for screening typhoid fever especially during the bacteraemia period and whether it is in concordance with the Widal and TUBEX<sup>®</sup> TF serological tests.

## METHODS

Forty patients aged 2–18 years, attending the Jeremy Medical Service Clinic, Surabaya, with fever for more than 3 days were studied, from June up to August 2010. The urine samples were tested by diazo test, while blood samples were tested by Widal and TUBEX<sup>®</sup> TF tests, and the blood culture was used as the gold standard.

The urine diazo test was performed by adding diazo A reagent (Sulphanilic acid 0.5 gram, Hydrochloric acid conc 5 mL, distilled water ad 100 mL) and diazo B reagent (Nitrite sodium

0.5 gram, distilled water ad 100 mL), it was mixed using 40 parts of diazo A and with 1 part diazo B into 2 ml urine, and then 1–2 drops of 30% ammonium hydroxide was added. The presence of a red coloured ring was considered as a positive result.<sup>3</sup>

The principle of Widal test is an agglutination test between insoluble bacteria suspension as antigen and a specific antibody existing in the patient's serum. The Widal test is considered as positive if the O titre  $\geq 1/160$ .<sup>7</sup>

The TUBEX<sup>®</sup> TF test is a semiquantitative diagnostic serum assay for the detection of current typhoid fever caused by *Salmonella typhi*. TUBEX<sup>®</sup> TF test detects the present of anti -O9 antibodies in the patient's serum by assessing their ability to inhibit the binding between coloured indicator particles that are coated with a monoclonal antibody (mAb) specific for the *S. typhi* O9 lipopolysaccharide (LPS) antigen and magnetic particles that are coated with *S. typhi* LPS. The results are read visually against a colour scale, and considered as positive if the colour reading is  $\geq +4$ .<sup>6</sup>

## RESULTS AND DISCUSSION

The results of 40 samples showed that 12 have *Salmonella typhi* positive in the blood culture and 26 diazo tests positive, 22 Widal positive and 14 TUBEX<sup>®</sup> TF positive. The sensitivity, specificity, positive predictive value, as well as the negative predictive value and efficiency were then recorded.

**Table 1.** Urine diazo test from blood culture

		Blood culture	
		+	-
Diazo	+	10 (83%)	16 (57%)
	-	2 (17%)	12 (43%)

The sensitivity was 83%, specificity 43%, positive predictive value 39%, negative predictive value 86%, and the efficiency was 63%

**Table 4.** Comparison of the sensitivity and specificity

	Diazo	Widal	TUBEX <sup>®</sup> TF
Sensitivity	83% (95%CI=0.55–0.95)	58% (95%CI=0.32–0.81)	75% (95%CI=0.47–0.91)
Specificity	43% (95%CI=0.27–0.61)	46% (95%CI=0.30–0.64)	82% (95%CI=0.64–0.92)
Positive predictive value (PPV)	39% (95%CI=0.22–0.58)	32% (95%CI=0.16–0.53)	64% (95%CI=0.39–0.84)
Negative predictive value (NPV)	86% (95%CI=0.60–0.96)	72% (95%CI=0.50–0.88)	88% (95%CI=0.50–0.88)

**Table 2.** Widal test from blood culture

		Blood culture	
		+	-
Widal	+	7 (58%)	15 (54%)
	-	5 (42%)	13 (46%)

The sensitivity was 58%, specificity 46%, positive predictive value 32%, negative predictive value 72%, and the efficiency was 52%

**Table 3.** TUBEX<sup>®</sup> TF test from blood culture

		Blood culture	
		+	-
TUBEX <sup>®</sup> TF	+	9 (75%)	5 (18%)
	-	3 (25%)	23 (82%)

The sensitivity was 75%, specificity 82%, positive predictive value 64%, negative predictive value 88%, and the efficiency was 78.5%

The diagnosis of typhoid fever is established based on the clinical examination and laboratory tests. The clinical diagnosis of typhoid fever is frequently imprecise because no specific clinical symptoms can be found or the same symptoms of other diseases are found, especially in the first week. This shows the importance of the laboratory testing to confirm typhoid fever diagnosis. Typhoid fever in Indonesia represents an endemic disease with a high mortality; therefore, reliable laboratory tests are essential to establish early diagnosis of typhoid fever, so that appropriate treatment can be applied.<sup>1,4</sup>

A rapid diagnostic method, easy to perform, as well as inexpensive, with a high sensitivity and specificity is very much needed to assist the therapy. In order to support the possibility of establishing early diagnosis, an early appropriate therapy can be given to decrease complications and possibility of death, as well as to prevent the spreading of the disease by the carriers.<sup>4</sup>

Isolating the causative organism by blood culture is still the gold standard in establishing the diagnosis

of typhoid fever. However, this takes a long time, and can only be done in a hospital with adequate laboratory facilities and show only in a few positive results.<sup>4</sup>

This study was performed by initial screening test to establish early diagnosis of typhoid fever by urine diazo test and also to find a concordance with the serological Widal and TUBEX<sup>®</sup> TF.

The urine diazo test for supporting the diagnosis typhoid fever is relatively easy, simple and inexpensive, so it can be used in a place with simple laboratory facilities.

There was a high concordance between positive diazo test with positive Widal test but false negative diazo (17%) was less than the Widal test (42%). This is affected by the period of the disease or the possibility of relapse.

Two patients with positive blood culture were detected by diazo, but not by Widal or TUBEX<sup>®</sup> TF. So the possibility of positive diazo was due to the phase of bacteriemia where no antibody had been formed. A considerably high false positive diazo test (57%) could be caused by the existence of other diseases or drugs causing to form a phenol ring in the urine.

The results of diazo test showed a high sensitivity (83%) but a low specificity (43%) while the Widal test showed a lower sensitivity and specificity. TUBEX<sup>®</sup> TF test had a considerably high sensitivity (75%) and better specificity (82%) compared to diazo test but is more expensive. Furthermore, 2 (two) patients with a positive blood culture were detected by diazo but not by Widal or TUBEX<sup>®</sup> TF.

## CONCLUSION

Diazo test is quite reliable in aiding the diagnosis of typhoid fever and may be considered as a screening test for typhoid fever.

## ACKNOWLEDGEMENT

The authors would like to thank PT. Pacific Biotekindo Intralab for supporting this study and dr. M.Y. Probohesodo, SpPK (K) on the advice and assistance in preparing the paper as well as correcting it into the English version.

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