

## Diagnostic Value of Indirect Hemagglutination Test in Melioidosis

Amorn Leelarasamee, M.D.\*

In this issue, Prapit Teparrugkul reported the result of her evaluation of the indirect hemagglutination test (IHA) for diagnosis of melioidosis in 325 cases with culture-proven melioidosis at Sapprasithprasong Hospital in Ubon Ratchathani (1). Two control groups were carefully selected to critically evaluate the test. The first group comprised of patients with other febrile illnesses proven to be due to other causes. The second group was the patients who sought medication for non-febrile illnesses. The comparable mortality rates of melioidosis and non-melioidosis febrile groups (46% vs. 44%) confirmed how well the matching was done for the febrile control group. The study took place at Sapprasithprasong Hospital where melioidosis has been well recognized. The hospital is also known to be among the top community-based hospitals in Thailand where annual case report of active melioidosis is high and facilities to isolate and identify *Burkholderia pseudomallei* are far complete than other hospitals. The result of the study revealed values for sensitivity and specificity of the IHA at various cut-off titers and the suitable single cut-off titer was chosen to be  $\geq 1:160$  to give the best combined results for both the sensitivity (70%) and specificity (67%). At this cut-off titer, the positive predictive value was 80 percent and negative predictive value was 55 percent. Since melioidosis is perceived there as the most challenging illness because it is a major cause of community-acquired septicemia with current mortality rate of 40-50 percent, she concluded that the result of IHA test should be carefully interpreted and decision to initiate an antimicrobial active for *B. pseudomallei* should also take severity of individual patient into account.

At present, the IHA test is still the only practical serological test available in Thailand since the report of superior property of IHA over CF test (2). Evaluation of the IHA test in the American soldiers in Vietnam war disclosed a sensitivity of 96 percent and specificity of 95 percent at a cut-off titer at 1:40 (2). Later studies revealed that such interpretation of IHA titer at the cut-off point is seriously confound by the presence of high "background" IHA antibody in asymptomatic people living in endemic area such as northeast of Thailand (3-6) where *B. pseudomallei* is found widely distributed in the soil and water. Frequent exposures to the micro-organism in this area can be anticipated and thus boost the antibody production though severe infection may never occur. In addition, the IHA titer also rises and remains positive for 4-9 months after the apparent infection (2). The report of Prapit Teparrugkul on the result of her evaluation of the indirect hemagglutination test added to the existing evidence that the suitable cut-off titer is no more 1:40 as previously reported if the test is to be used in endemic area.

When the result of her study was compared with another study that was also performed in the northeast region (5), the values for sensitivity from her study are higher and for specificity are lower at each cut-off titer. We do not have good explanation for the discrepancy but severity of melioidosis, number of cases enrolled in the study and type of selected control, may affect the results. Fifteen years ago when melioidosis was only recognized at University Hospitals in Bangkok, our study with only 19 cases of melioidosis, revealed the higher value for sensitivity than hers. Our cases also came from northeast region and perhaps only those

\* Division of Infectious Diseases, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

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Reprint Request : Amorn Leelarasamee, M.D., Division of Infectious Diseases, Department of Medicine, Faculty of Medicine Siriraj Hospital Mahidol University, Bangkok 10700, Thailand.

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who survived the initial attack of septicemic melioidosis, reached our hand. Hence there was ample time for IHA antibody to develop before their blood were collected for titer determination. This phenomenon may explain a higher sensitivity in our study. To determine the true specificity in endemic area, the control group must be appropriately selected as done in her study (1).

In general, when using an appropriate dilution scheme, a 2-dilution incremental rise or greater in IHA titer is accepted as serologic confirmation of a recent infection. However, there were times when single blood specimens could only be obtained and physicians were compelled to interpret the titer due to severity of illness. Under this condition, a common practice is to interpret a single titer above the cut-off titer as evidence of recent infection. As can be seen from Table 1, the values for sensitivity and specificity of the IHA test gradually changed when the cut-off titer varied. At each cut-off titer, although a single titer can be classified as positive and negative, it is by no mean that a negative titer can definitely rule out active melioidosis and vice versa. What we realize is that the higher the titer is, the likelihood of active infection is also high. The chance may be highest or near 100 percent if the IHA titer reaches 1:20,480 or over. This titer was ever obtained from a non-fatal case with subacute but extensive and severe pneumonitis due to *B. pseudomallei* infection. Other patients with sepsis yield IHA titers around 1:40-1:1,280. Thus we feel that the dichotomous method fails to quantify the likelihood that individual IHA titer results reflect recent infection because no matter their magnitude, all titers above the cut-off titer are classified in the same fashion. Therefore we prefer a likelihood ratio method of interpreting a single IHA titer. The likelihood ratio method offers a continuum of risk estimation, based upon the magnitude of the test result. This method was IHA value specific, more consistent with clinical judgement, and better emphasized the caution clinicians must use in interpreting a single titer. If one wants to avoid the likelihood ratio method, we would suggest that interpretation is done with three ranges of IHA titers and their implications for investigation and antimicrobial administration against *B. pseudomallei* (3). If IHA titer is less than 1:80, antibiotic therapy is not given in the absence of firm evidence of active infection. For IHA titers between 1:80-1:320, antibiotic therapy is not given unless the patient is moderately to severely ill. Further investigations for concealed intra-abdominal abscesses

**Table 1. The values for sensitivity and specificity of the IHA test at various cut-off titers for melioidosis by various studies in Thailand (1,3,5).**

Cut-off titer	Sensitivity(%)			Specificity(%)		
	T*(1) n = 325	P*(5) n = 47	L*(3) n = 19	T*(1) n = 177	P*(5) n = 318	L*(3) n = 611
≥ 1:40	86	83	95	38	90	74
≥ 1:80	81	74	90	51	96	88
≥ 1:160	70	49	79	67	97	95
≥ 1:320	56	30	63	79	99	97
≥ 1:640	39	19	47	85	100	98
≥ 1:1,280	20	6	ND	95	100	ND

ND = not done

\*Values from references no. 1, 3 and 5

and deep-seated lesions are warranted in patients with no identifiable site of infection. Alternatively, ceftazidime or co-trimoxazole may be used with an aminoglycoside as empirical treatment in patients with titers in this range. If IHA titer is over 1:320, empirical antibiotic therapy against *B. pseudomallei* is given, and definite evidence of active melioidosis is extensively sought. In the near future, we believe that the IHA test will be refined or even replaced by an immunoturbidimetric technique. This technique employs latex particles coated with antigen which binds to serum antibody. The change of turbidity of the latex suspension after incubation with serum sample is measured by degree of absorbance using light. The method is rapid, truly quantitative i.e. reported in term of U/mL, automated applicable and highly reproducible though the equipment is rather expensive at the beginning.

Up to now, there has not been a perfect serologic technique to discriminate past or asymptomatic from current *Burkholderia pseudomallei* infection or sensitive enough to detect active melioidosis. The IFA-IgM was reported in a study to be more successful than IHA test but the authors concluded that it was at best only supplementary to the "gold standard" bacteriological culture (4). Newer methods are now being evaluated (7). DNA amplification techniques seem very promising but

they are still at a developmental stage. The PCR method has been successfully applied to detect genetic materials of various pathogenic and difficult-to-isolate microorganisms. For example, the detection of *Mycobacterium tuberculosis* DNA in clinical specimens using a microwell hybridization assay was recently reported (8). The test can be performed in two hours, is much faster and less laborious than Southern blot hybridization and interpretation of the results is objective. While we are waiting for these newer diagnostic tests to be fully developed and feasible to use in clinical practice, presumptive laboratory diagnosis is often based on the microscopic observation of the characteristic safety-pin, short and thin gram-negative rods by means of gram-stain method. This method is always appreciated by anyone who are familiar with its morphology and have gained a lot of experience in doing gram stain of clinical specimens. Then an antimicrobial can be almost immediately chosen with good accuracy in many cases. A major remaining obstacle to the development of a successful diagnostic test is how to lower the current mortality rate of 40 percent in septicemic melioidosis once the correct diagnosis could be reached by that method in no time. This is also a challenging area to chemotherapists who are fascinated by melioidosis.

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