Melioidosis, an infectious disease caused by *Burkholderia pseudomallei*, remains an important public health problem in the endemic Southeast Asian countries and northern Australia. Identification of the causative agent by conventional bacterial culture and biochemical tests is still used as gold standard for definitive diagnosis.\(^1\)\(^-\)\(^2\) However, misidentification of *B. pseudomallei* using the API 20NE tests (BioMerieux, Marcy l’Etoile, France) has been noted.\(^3\) Generally, it takes at least 3–4 days before the result is obtained and by that time it may be too late for acutely ill septicemic patients.\(^4\)\(^-\)\(^5\) To speed up the diagnosis, several methods based on immunologic and molecular approaches have been developed, but most of them have never been critically evaluated in an actual clinical situation.\(^6\)\(^-\)\(^8\) Antibody detection assays are not very useful, particularly in the endemic areas where subclinical infection exists.\(^6\)\(^-\)\(^8\) In our hands, the detection of IgM antibody does not give results any better than the detection of IgG antibody.\(^7\) Thus, antigen detection assays have been developed, but the limited data currently available suggest that further refinement is needed.\(^9\)\(^-\)\(^12\)

Using a specially designed ELISA system, Desakorn and others were able to detect antigen in the urine in 80% of patients with culture-proven melioidosis.\(^10\) A few years ago, we produced monoclonal antibodies (MAbs) that could agglutinate specifically only with the *B. pseudomallei* isolates of the arabinose-negative biotype.\(^8\)\(^-\)\(^11\) One of these MAbs was used for antigen detection, but the sensitivity of the method was only 75%.\(^9\) A molecular approach using DNA hybridization and the polymerase chain reaction has been reported with satisfactory results in laboratory tests, but in the most recent communication, the results from a clinical setting were rather disappointing, particularly with regard to sensitivity, which was reported to be only 67%.\(^14\) The main objective of the present study was to compare the MAAbased latex agglutination test (MAb-LA) in actual clinical settings with the conventional biochemical test for the identification of *B. pseudomallei* in hemoculture fluid.

The method described herein was based on the use of MAbs previously shown to be reactive with a 200-kD surface antigen present only in the arabinose-negative *B. pseudomallei*. One such antibody (5F8) is an IgM antibody and has already been described in detail.\(^3\)\(^-\)\(^13\) The other 2 MAbs, 4B11 (IgG\(_1\)) and 6G9 (IgG\(_2\)), reacted with different epitopes on the 200-kD component (Sirisinha S, unpublished data). All 3 MAbs did not react in the ELISA and immunoblot analyses with crude extracts or with purified lipopolysaccharide prepared from other gram-negative bacteria or with purified *B. pseudomallei* capsular polysaccharide CP-1a, CP-1b, and CP-2 (kindly provided by Dr. K. Kawahara, Department of Bacteriology, Kitasato Institute, Tokyo, Japan).\(^15\) Competitive ELISA analysis using the IgG1 MAb 3015, which reacted with high molecular weight exopolysaccharide (kindly provided by Dr. I. Steinmetz, Institute of Medical Microbiology, Hannover Medical School, Hannover, Germany),\(^16\) showed that our MAbs most probably react with different epitopes present on the same high molecular weight component (Anuntagool N, unpublished data). The MAb mixture consisting of 5F8, 4B11, and 6G9 at a ratio of 1:2:2 by weight was used for the sensitization of latex particles (0.81-\(\mu\)m diameter; Interfacial Dynamics Corporation, Portland, OR). The sensitized latex particle suspension reacted positively with more than 100 human, animal, and environmental *B. pseudomallei* strains of the arabinose-negative biotype from Thailand, Malaysia, Singapore, Cambodia, Laos, and Hong Kong.\(^13\) It did not react with 70 arabinose-positive environmental *B. pseudomallei* and 13 other gram-negative and 4 gram-positive bacteria relevant in clinical situations. The reagent is stable for more than 1 year when kept at 4°C.

Blood specimens were collected from patients admitted to 2 provincial hospitals in the area of Thailand endemic for melioidosis during May and October 1998. These specimens were subjected to routine hemoculture using automatic culture systems. The Bactec 9240\(^®\) system (Becton Dickinson, Sparks, MD) was used at the Srinagarind University Hospital (Khon Kaen, Thailand), while the BacT/Alert\(^®\) system (Organon Belgie NV, Brussels, Belgium) was used at the Khon Kaen Hospital. The semiautomated method (30 ml for Bactec\(^®\) and 40 ml for BacT/Alert\(^®\) bottles) was inoculated with 5 ml of blood specimens and in most cases loaded in the machine within 2 hr of collection. The MAb-LA tests were performed directly on the samples taken from the hemoculture fluids, again within 2 hr after the alarm of the machine sounded (when detectable bacterial growth occurs), which for *B. pseudomallei* varied between 25 and 30 hr. The hemoculture fluid was simultaneously subcultured in brain heart infusion broth and subjected to conventional biochem-
TABLE 1
Comparative study on the detection and identification of Burkholderia pseudomallei by conventional biochemical tests and monoclonal antibody–based latex agglutination (MAb-LA)

<table>
<thead>
<tr>
<th>Culture system</th>
<th>Culture positive for B. pseudomallei</th>
<th>MAB-LA positive for B. pseudomallei</th>
<th>Others</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>BacTec®</td>
<td>22</td>
<td>98</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>BacT/Alert®</td>
<td>53</td>
<td>223</td>
<td>49</td>
<td>0</td>
</tr>
<tr>
<td>Both (total)</td>
<td>75</td>
<td>321</td>
<td>71</td>
<td>0</td>
</tr>
</tbody>
</table>

The overall performance of the MAB-LA test was highly satisfactory, with a sensitivity of 95%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 99%. This test was highly specific because there were no false-positive results noted among the 321 hemocultures that showed other microbial growth, including gram-positive (e.g., Staphylococcus, Streptococcus, and Bacillus spp.) and gram-negative (e.g., enteric, Pseudomonas, Burkholderia cepacia, Acinetobacter, and Xanthomonas spp.) bacteria, yeasts, and fungi. Another advantage is that after the incubation for bacterial growth, the additional time required for species identification can be reduced from 1–2 days to only 1 min. This point is very critical in the management of patients with acute septicemia because the earlier the diagnosis is made, the higher the chance of success. It has been reported that about 50% of the patients with septicemic melioidosis die within 48 hr after admission. Based on the data presented, we recommend that this combined method be used in conjunction with the more time-consuming biochemical method so that antibiotic treatment can be initiated as early as possible.

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