Evaluation of a Commercial Latex Agglutination Test Kit for Cryptococcal Antigen

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Two dozen Crypto-LA kits for detecting Cryptococcus neoformans capsular polysaccharide antigens were evaluated. Ten kits proved reliable for detecting and titrating antigen in clinical materials. Fourteen kits were found to be inadequate.

In 1971 a Crypto-LA kit (Canalco Diagnostics, Rockville, Md.) for the detection of Cryptococcus neoformans capsular polysaccharide antigen was marketed. Preliminary evaluations carried out at the Center for Disease Control (CDC) prior to the marketing date indicated that the kit was satisfactory for diagnostic application. In the past year we have received numerous inquiries as to the quality of the kit and some reports as to its being inadequate. The latter inadequacy was attributed at times to (i) faulty control antigen, (ii) C. neoformans antilobulins of little or no avidity, or (iii) vague performance instructions. Because of the serious nature of cryptococcal meningitis and other forms of cryptococcosis, rapid diagnosis and treatment are necessary. Many physicians rely upon serologic test results as a basis for diagnosis when attempts to isolate the fungus are unsuccessful. The serious consequences of failure to treat or of unnecessary treatment demand that only a kit of unquestionable quality be commercially available. To ascertain the quality of the LA kits, we evaluated them in parallel with the CDC reference reagents.

Twenty-four kits for detecting cryptococcal antigen were investigated. Kits 1 to 12 were purchased, and kits 13 to 24 were provided by the manufacturer. Tests were performed according to instructions provided with the kits. A kit was not used unless its reagent could agglutinate 0.06 μg of the polysaccharide control antigen per ml; this was the prescribed validation test. The CDC reference method was carried out by a previously described procedure (2). Two laboratory workers independently performed tests with the kits on coded specimens while a third worker tested the same specimens by the CDC reference procedure. Nineteen antigen-positive sera or cerebrospinal fluids (CSF) from culturally proven cases of cryptococcosis, 18 negative sera and CSF specimens from humans free of cryptococcosis, and 5 sera positive for rheumatoid factor (RF) were included in the study. The undiluted specimens were screened for antigen by all three workers. It was our plan to screen each specimen four times with the kit and reference reagents. This was accomplished with the reference reagents but, because of numerous unexpected faulty kits, not with all kit reagents. Because of a limited supply of reagents in the kits, only randomly selected specimens positive in the screening test were titered.

The results of this evaluation revealed that 14 of the 24 kits (kits 4, 6, 13–24) were unsatisfactory. In each case the unsatisfactory rating was due to the failure of the latex suspension sensitized with anti-C. neoformans globulin (LA) to agglutinate the polysaccharide control necessary to validate the test. The LA reagents from 12 of these 14 kits gave no reaction with the positive controls, whereas two gave slight but unacceptable agglutination reactions. The remaining 10 kits contained satisfactory reagents.

It is apparent from the results shown in Table 1 that all of the 10 satisfactory kits gave screening reactions with the positive cryptococcosis case sera and CSF specimens which were the same as those obtained with the CDC reference procedure. When positive specimens were titrated, titers obtained with the kits were within fourfold dilutions of the reference titers. In six cases specimens negative by the reference procedure were identified as positive with kits 11 and 12. Four of these results were actually from a serum and CSF of a patient who had been treated over a year ago for cryptococcosis.

The importance of an RF control in the latex test for cryptococcosis has been reported (1). Of the five sera positive for RF included in this study, one was incorrectly identified three times as positive for cryptococcal antigen with kits 5 and 11. In four trials, the reference reagents identified this same specimen as positive for cryptococcal antigen twice and negative for RF
Table 1. Screening test results obtained on serum and spinal fluid specimens with 10 acceptable Crypto-LA kits and the CDC reference procedure

| Kit no. | Positive* specimens | Negative* specimens | RF-positive* specimens |
|---------|---------------------|---------------------|------------------------|
|         | Technician 1 | Technician 2 | Technician 1 | Technician 2 | Technician 1 | Technician 2 |
| 1       | 3/3          | 3/3         | 4/4          | 4/4          | 1/1         | 1/1         |
| 2       | 3/3          | 3/3         | 3/3          | 3/3          | 1/1         | 1/1         |
| 3       | 3/3          | 3/3         | 3/3          | 3/3          | 1/1         | 1/1         |
| 4       | 3/3          | 3/3         | 4/4          | 4/4          | 1/1         | 1/1         |
| 7       | 2/2          | 2/2         | 3/3          | 3/3          | 1/1         | 1/1         |
| 8       | 4/4          | 4/4         | 3/3          | 3/3          | 1/1         | 1/1         |
| 9       | 3/3          | 3/3         | 1/1          | 1/1          | 1/1         | 1/1         |
| 10      | 4/4          | 4/4         | 2/2          | 2/2          | 1/1         | 1/1         |
| 11      | 2/2          | 2/2         | 2/2          | 2/2          | 1/1         | 1/1         |
| 12      | 3/3          | 3/3         | 1/1          | 1/1          | 1/1         | 1/1         |
| Total kit results | 31/31* | 31/31 | 29/31 | 27/31 | 7/8 | 6/8 |
| Reference procedure results | 76/76* | 72/72 | 15/20 |

* Classification of specimens is based on results obtained with CDC reference procedure prior to this study.
* Number of specimens correctly identified.
* Number of specimens tested.
* Reported positive for cryptococcal antigen.
* Two of five sera reported positive for cryptococcal antigen.

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