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 titering 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 antiglobulins 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 titer. 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         | 0/1         |
| 4       | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 5       | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 6       | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 7       | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 8       | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 9       | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 10      | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 11      | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |
| 12      | 3/3         | 3/3         | 3/3         | 3/3         | 1/1         | 1/1         |

Total kit results | 31/31c | 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.

twice. An additional RF-positive specimen was considered negative once with the reference reagents.

The false positives encountered with the RF specimen with the kit and reference reagents might have been avoided if the latex controls had been sensitized with preimmune globulin taken from the same rabbit used to produce the anti-C. neoformans globulin. This should provide rabbit preimmune and anti-C. neoformans globulin containing identical allotypic determinants (1).

Control polysaccharide antigen preparations from the unsatisfactory kits reacted satisfactorily with the CDC reference LA reagents. However, LA reagents from the unsatisfactory kits did not react with CDC antigen preparations.

Further studies with the faulty LA reagents indicated that they reacted with the sera positive for the rheumatoid factor, thus indicating that either the latex particles were sensitized with anti-C. neoformans globulin which lost potency and had become nonreactive for the cryptococcal antigen or that normal globulin had been used to sensitize the particles.

The package inserts stated that kit reagents that failed to agglutinate the 0.06 µg of polysaccharide per ml should be considered unsatisfactory. It is our belief that such unsuitable reagents may be used by some diagnostic laboratories because those performing the test fail to fully comprehend the importance of the titration procedure. Also, if interaction time between reagents is prolonged, false-positive reactions could result and the reagents could be considered acceptable for diagnostic use.

A major point of concern for us was the failure of the package insert to stress the necessity of properly performing the control procedure. The meaning of "complete agglutination" and the significance of reactions where specimens react with both the LA and the latex sensitized with normal globulin should be elaborated more fully.

A summary of our data has been made available to Canalco Diagnostics, and they have indicated that they are acting to remedy the deficiencies. The need for both the producer and the consumer to use quality control procedures cannot be overemphasized. It is mandatory that only a kit of the highest quality be made available for the diagnosis of cryptococcosis.

ADDITION IN PROOF

Subsequent to submission of this paper, we received and tested five additional Crypto-LA kits (lot C-101). These kits with improved packaging and extensively revised package inserts were found acceptable.

LITERATURE CITED

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