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Galactomannan Antigenemia Detection for Diagnosis of Invasive Aspergillosis, Part I*

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Abstract

Until recently, the diagnosis of invasive aspergillosis was often made based on clinical and radiologic findings, which are insensitive and non-specific. The Platelia *Apergillus* galactomannan enzyme immunoassay, which has been available in Europe for nearly a decade and in the United States since 2003, provides an aid to the diagnosis of invasive aspergillosis. Clinicians should be familiar with the uses and limitations of the assay to use it effectively in patient management. Part I of this two-part series of articles describes the test methodology, its application for use, and those factors that may affect test performance.

Introduction

Invasive aspergillosis (IA) occurs in 8 to 15% of patients who undergo allogeneic stem cell transplantation (SCT) and in a smaller proportion of solid-organ allograft recipients; mortality ranges from 50 to 90%. Although radiographs or CT scans may suggest the diagnosis, their findings are neither specific nor sensitive. Diagnosis by histopathology or culture is limited by the need for invasive procedures and low sensitivity. For example, cytology was positive in only 23% and culture in 17% of patients with IA who underwent bronchoalveolar lavage (1).

Antigen detection for diagnosis of IA was first reported in 1978 (2), and early reports were encouraging. The Pastorex *Aspergillus* galactomannan latex agglutination kit, introduced in the early 1990s (3), was largely replaced in Europe by the Platelia *Aspergillus* galactomannan enzyme immunoassay in 1995 (4,5), which was first offered in the U.S. in 2003. To date, however, publications about its accuracy report inconsistent findings and conclusions, making it difficult to determine its role in patient care. This report will describe the causes for these inconsistencies and propose guidelines for use of the test in patient care.

Description of Test Methodology

The test is an enzyme immunoassay that uses rat monoclonal antibodies, which recognize β (1→5)-linked galactofuranose (6). The antibody reacts with galactomannan moieties of *Aspergillus* and several other molds: *Penicillium, Trichophyton, Botrytis, Wellemia,* and *Cladosporium* (6); *Acremonium, Alternaria, Fusarium, Wangiella,* and *Rhodotorula* (7); and *Paecillomyces* (8). The test kit includes 96-well microtiter plates pre-coated with the monoclonal antibody and blocking agent and necessary reagents and controls. Equipment required to perform the test includes a water bath or heating block to heat the specimens at 100°C, a centrifuge for polypropylene tubes capable of 10,000 × *g*, a microplate incubator, and a dual-wavelength microplate reader.

Care is required to avoid contamination of specimens with mold during collection, processing, shipment, and testing. Unopened specimens may be stored at 2 to 8°C for 5 days prior to testing, but for only 48 h after they are opened: longer storage requires freezing at -70°C. Specimens should be packaged in cold packs to avoid warming during shipping.

The specimen is pre-treated to inactivate interfering substances. Three hundred µl of test specimen is mixed with 100 µl of EDTA, and the mixture is heated at 100°C for 3 min, producing a coagulum. Then, the treated specimen is centrifuged at 10,000 × *g* for 10 min, after which the supernatant is aspirated and tested for galactomannan or stored at 2 to 8°C for up to 72 h before testing. Fifty microliters of the peroxidase-labeled monoclonal antibody is added to the test strip, followed by 50 µl of the specimen, and the strip is incubated

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at 37°C for 90 min. After thorough washing, the chromogenic substrate is added, followed by incubation at room temperature for 30 min. The reaction is stopped by addition of 100 µl of stopping solution.

In the presence of galactomannan, the chromogenic substrate produces a yellow color, which is measured in the microplate reader at 450/620 to 630 nm. The test includes a high- and mid-range "cutoff" control that contains about 1 ng galactomannan/ml, as well as a negative control. The optical density (OD) of the sample is divided by the mean OD of the cutoff control, and the results are expressed as a galactomannan index. In the kit used in the U.S., an index of 0.5 is reported as positive, versus 1.5 in Europe. All positive results should be verified by retesting a new aliquot of the specimen that was originally positive and by demonstration of reproducibility in a new specimen.

Experience in a Reference Laboratory

This test has been performed each weekday at Miravista Diagnostics since July 2003. During the first 9 months of 2004, 2 of 189 assays failed to meet the acceptance criteria recommended by the manufacturer. Of 5,011 specimens tested, 5.8% were positive, including 2.4% with indices between 0.5 and 0.9 and 3.1% with indices of 1.0, or greater. For specimens with results of >1.0, 96% were reproducible upon re-extraction the following day compared to 87% for those between 0.5 and 0.9 units. Unfortunately, in our experience, less than 20% of physicians submit a follow-up specimen to validate the initial positive result.

Considerations before Offering the Test In House or Selecting a Reference Laboratory

The assay is labor-intensive, susceptible to error, and relatively expensive. A few designated employees should be selected to perform the test, and they should exhibit ongoing proficiency. Care must be taken to avoid false-positive results caused by contamination with molds containing galactomannan. The test should be performed daily to assure that the results are timely. If the laboratory performs the test only twice weekly, delays of up to 4 days may occur in cases with positive results. Technician time averages about 4 h/assay.

Factors Affecting Interpretation of Published Studies

Several factors must be considered in analysis of literature on rapid diagnostics in IA. First, the criteria used for diagnosis must be considered. Most studies use the consensus definition recommended by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infection Cooperative Group (EORTC/IFICG) and the National Institutes of Allergy and Infectious Diseases Mycoses Study Group (NIAID MSG) (9). Cases are classified as proven, probable, and possible, based upon the level of certainty of the clinical, radiographic and laboratory findings. As only 15 to 25% of "possible" cases are found to have IA at autopsy, they are typically excluded from analysis.

Second is the study design. Was the study prospective or retrospective? Were the tests performed real-time or in batch after completion of the study? And were the results used in classification of the cases, or was classification based solely upon histopathology and culture results? Ideally, cases should be enrolled prospectively into a protocol designed to assess the accuracy of the test using accepted criteria for diagnosis other than the test under evaluation. Less rigorous design allows for bias that may affect the outcome of the analysis.

Third, the cutoff for positivity must be considered. The European kit recommends a cutoff index of 1.5, and the U.S. kit recommends 0.5. Several reports have analyzed the data at cutoffs different from those recommended by the manufacturers.

Fourth, the type of population studied affects the performance of the test. The incidence of IA is highest in SCT patients, followed by neutropenic patients with underlying hematologic malignancy, and then solid-organ transplantation (SOT). The accuracy of the test is generally greatest in populations with the highest rate of IA, namely, allogeneic SCT recipients. The age of the patient also may impact the performance of the assay. Others reported reduced specificity during the first few weeks following allogeneic SCT and reduced sensitivity for the late cases occurring during immunosuppression for chronic graft versus host disease (CGVHD).

Fifth, the purpose of testing must be considered. Most studies report experience using the test to monitor patients with underlying hematologic malignancy or SCT for development of IA, while only a few report its use for evaluation of suspected cases.

Sixth, in studies using the assay to monitor patients for the development of IA, the frequency of screening affects its performance. Sensitivity is improved by more frequent screening, typically twice weekly during the period of greatest risk.

Seventh, several medications may affect the accuracy of the test. Mold-active antifungal prophylaxis or empiric therapy may reduce its sensitivity. If the lower (0.5) cutoff is used, prior antifungal therapy appears to have a minor effect on the sensitivity of the test (10,11). Conversely, antibiotics produced by *Penicillium* may contain galactomannan and cause false-positive results (discussed below).

This review will focus on the studies

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