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Detection of Aspergillosis

Background

Aspergillosis is a fungal infection caused by species of the *Aspergillus* genus, most commonly *A. fumigatus*, *A. flavus*, *A. niger* and *A. terrus* of which *A. fumigatus* accounts for 50% of infections. These fungi are found in the environment but are also common in the home. Aspergillosis can be categorised into three main forms: allergic, chronic and invasive.

Infection often starts in the lungs due to the inhalation of airborne spores from the environment but this rarely causes serious disease in healthy individuals. Some people with asthma or cystic fibrosis can have an allergic reaction to *Aspergillus* presenting with fever, wheezing and coughs, bringing up plugs of mucus. Individuals with chronic pulmonary conditions can develop aspergillomas, fungal balls that have formed in the air spaces in the lungs. If left untreated, aspergillomas can worsen underlying chronic lung conditions.

Invasive Aspergillosis (IA) is the most severe form of aspergillosis, with a high mortality rate of 50-80% (1). IA occurs when infection spreads rapidly from the lungs to the brain, heart, kidneys or skin, symptoms depending on which organ system(s) are involved. IA is predominantly reported in those undergoing chemotherapy and stem-cell or organ transplants. Hence, those with weakened immune systems and low white blood cell counts are at particularly high risk.

Diagnosis

The non-specific signs and symptoms of IA along with difficulty distinguishing *Aspergillus* from some other fungal species poses a problem in diagnosis of IA. Approaches to making a specific diagnosis generally involve a combination of direct imaging/radiographic evidence, culture, microscopy, DNA detection and antigen detection.

Imaging can aid the identification of aspergillomas in the lungs as well as identification of characteristic ‘halo’ signs of IA via CT scans. Testing of respiratory secretions can also aid diagnosis through identifying *Aspergillus* filaments by staining and microscopy, followed by culture to confirm findings. However, the sensitivity of culture is relatively low, ranging from 30-60% in bronchoalveolar lavage (BAL) fluid (1). Definitive diagnosis of IA requires histopathological evidence of deep-tissue invasion or a positive culture, however these specimens are often difficult to obtain due to the critically ill nature of the patient.

The BioRad® PLATELIA Aspergillus Ag serological test used at Micropathology Ltd. has been approved by the FDA for the detection of galactomannan (GM), a component of the fungal cell wall which is released during growth of the fungus.

Serum and lower respiratory bronchoalveolar lavages are validated sample types. Other samples may be tested and reported along with an appropriate caveat. The assay is also included in annual quality assurance schemes.

Galactomannan detection

Due to the large size of GM, the antigen cannot freely enter the blood stream – angio-invasion is needed for it to reach the circulation (2, 3). Serum GM can be detected 7-14 days before other diagnostic evidence is apparent, thus GM detection may allow for earlier diagnosis confirmation and the assessment of infection progression if serial GM levels are obtained (1). GM levels typically decline with response to effective therapy. Patients at risk of IA should be monitored regularly for GM levels. GM can also be detected in BAL, and has demonstrated higher sensitivity than in serum (4).

The test uses antibodies which are directed against *Aspergillus* GM, these antibodies coat the wells of a microplate and bind to the GM if present in the patient sample. An enzyme-linked antibody is then used to detect if GM is bound to the microplate via a colour change reaction (Fig. 1).

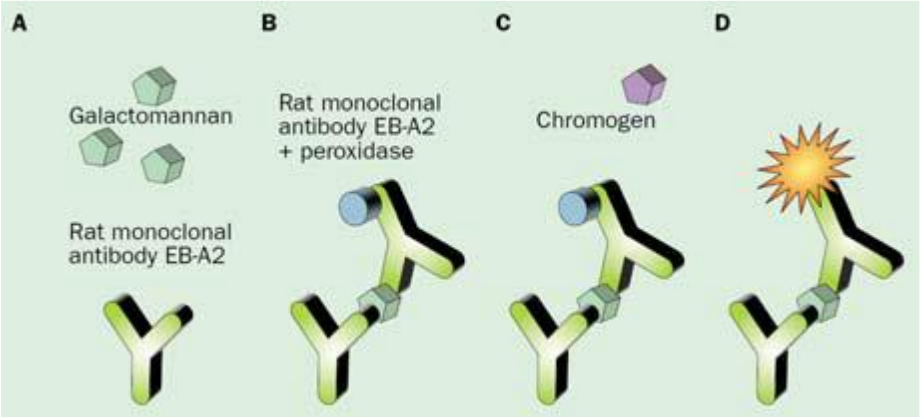

Figure 1. Mechanism of action of the GM detection assay.

A number of factors should be considered when interpreting results from GM detection. For example, there have been reports of reduced sensitivity of galactomannan detection in patients with chronic granulomatous disease and Job's syndrome as well as use of mould-active anti-fungal therapy in some patients with IA (5-7).

Positive GM test results can be obtained without clinical signs; however, some factors should be taken into consideration when interpreting these test results;

- Consumption of various foods, especially cereals, cream deserts and ice-pops containing cow's milk contain high concentrations of galactomannan, such dietary factors should be taken into consideration in patients with altered intestinal barriers (11).
- Some antibiotics (e.g. piperacillin/tazobactam) and semi-synthetic β-lactams have been found to contain GM antigen and so results from patients taking these agents should be treated with caution (8).
- There have also been some reported cross-reactions of the test with specimens containing *Cryptococcus, Geotrichum, Histoplasma*, *Alternaria, Penicillium* and *Paecilomyces* species (9,11).
- BAL results in non-immunocompromised patients should be interpreted with caution (10).

For more information and full limitations of the procedure please refer to the PLATELIA™ ASPERGILLUS Ag assay insert (11).

Aspergillus spp. DNA Detection

In contrast to other infections, there are only limited diagnostic tools for the early detection of invasive aspergillosis, often with poor sensitivity and reliability. More sensitive and rapid detection assays have been developed, utilising PCR, which can be designed to encompass a range of *Aspergillus* species. Such PCR-based detection assays are now included in the disease-defining criteria (12); however, alone, they cannot differentiate between infection and colonisation.

A combination of both GM assay and PCR-based detection assay has been supported for years, meta-analyses indicating that positive results from both tests is highly suggestive of an active infection (13), with concurrent use yielding high sensitivity (82%) (14) and increasing diagnosis (15, 16). High specificity for both assays would prevent the unnecessary use of antifungal treatment. Hence, the use of GM assay alone should be discouraged due to the potential for false positives.

At Micropathology Ltd., in addition to the Galactomannan assay, we offer a PCR-based *Aspergillus* genus assay targeting the 18S rRNA gene which will detect *Aspergillus* spp. including but not limited to *A. flavus, A. fumigatus, A. niger, A. restrictus, A. ustus, A. versicolor and A. wentii;* and most strains of *A. terreus*.'

The UKAS accredited specimen types for the *Aspergillus* spp. DNA assay are EDTA whole blood, BAL and sputum. **Other samples may be tested and reported along with an appropriate caveat. The assay is also included in annual quality assurance schemes.**

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