Indication for use of molecular diagnostics and interpretation of results

Aspergillus Galactomannan
A carbohydrate antigen, galactomannan (GM), is secreted from actively growing hyphae of most Aspergillus species and other fungi.
Galactomannan detection in body fluids using the Platelia ELISA format is more sensitive than culture in the diagnosis of invasive aspergillosis.

In serum/plasma a cut off ratio \( \geq 0.5 \) is used.
In other specimens an accurate cut off is unclear although in a bronchoalveolar fluid GM detection at an cut off ratio of \( \geq 3.0 \) corresponds to a 100% specificity whereas a cut off of \( <0.5 \) had a high sensitivity in ruling out invasive pulmonary aspergillosis.

Aspergillus PCR
The Aspergillus specific PCR has been independently validated through the European Aspergillus PCR initiative (EAPCRI), but has also had extensive clinical validation in haematology patients.
Results are reported as positive or negative. All positive specimens are repeated. Some positive s will be non-reproducible and this could represent contamination or early infection with low fungal load, and/or the effect of antifungal therapy.

Interpretation

Serum/Plasma

PCR and EIA negative: Invasive aspergillosis extremely unlikely anti-fungal treatment not indicated.

Single positive EIA Index : \(<0.7\): Request a repeat specimen.

Single positive PCR: Request a repeat specimen.

Single positive PCR, or a single positive GM index of \( \leq 0.7 \) or two consecutive GM indices of 0.5-0.6 may indicate false positivity.
It is not an indication for commencement of anti-fungal therapy in patients without clinical signs but should prompt repeat specimens and further investigation.

Repeat PCR positives: Consider further investigations and anti-fungal treatment.

Repeat EIA positives: Consider further investigations and anti-fungal treatment.

Whether repeat positivity is sequential or intermittent anti-fungal treatment and further investigations are warranted, particularly if EIA index is increasing.

Concomitant Single positive ELISA + Single positive PCR: Antifungal treatment and further investigations probably indicated.
**BAL fluid**

EIA cut off <0.5 excludes invasive pulmonary aspergillosis

EIA cut off >3.0 diagnoses invasive pulmonary aspergillosis

**Explanatory notes**
Both tests have high sensitivity and specificity but clinical utility varies according to the patient population and the prevalence of disease. These tests are best used for their negative predictive value to RULE–OUT invasive aspergillosis. As individual assays both PCR and EIA provide excellent sensitivity (>92.5%) and NPV (>98.5%) for proven/probable invasive aspergillosis enabling empiric antifungal therapy to be withheld with confidence.

The evidence of clinical utility is greatest when used together as a twice weekly screening strategy in high risk adult patients (allogeneic stem cell transplants, acute myeloid leukaemia/myelodysplastic syndromes, with a high prevalence of invasive fungal infection (>7%). In these populations twice weekly testing can be used to drive a diagnostically driven approach that replaces empiric therapy.

The NPV in patients consistently negative by PCR and EIA is 99.6%. A single positive result (either PCR or EIA) is not necessarily an indication for antifungal therapy in patients without clinical signs of fungal infection but can be used to trigger an intensive diagnostic workup including repeat testing, HRCT and BAL as clinically indicated. Multiple positive biomarkers show good diagnostic performance with a positive PCR plus positive EIA showing 90.6% sensitivity and 84.4% specificity for proven/probable IA.

In populations where the prevalence of fungal infection is less than 7% or less or in those in whom mould active prophylaxis is used, a twice weekly screening strategy is unlikely to be cost-effective and a fever driven testing regimen is recommended.

**Bronchoalveolar fluid samples**
BALF is generally performed in patients who already have clinical signs of pulmonary infection and as such the pre-test probability of disease (and hence prevalence) will be high. BAL is an invasive procedure and not suitable for regular screening. Consequently testing BALF should be considered a diagnostic specimen and although an EIA cut off <0.5 excludes invasive pulmonary aspergillosis a cut off >3.0 should be used for positivity. PCR on BALF has been shown to be more sensitive but clinical utility is not fully evaluated.