

Original Research Article

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## Enzyme-Linked Immunosorbent Assay for Detection of *Aspergillus fumigatus* Antibodies

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### ABSTRACT

#### Keywords

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*Aspergillus* species are ubiquitous fungi, commonly found in soil, water and decaying matter. Generally humans get infection through inhalation of spores. The spores come in lung and then disseminate to cause systemic aspergillosis. This prospective and experimental study was carried out at Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai. Total 86 blood samples were included in this study. ELISA test was done for detection of IgM antibodies of *A. fumigatus*. IgM antibody of *A. fumigatus* was detected by ELISA test in patient serum was 18 out of 86 (20.93%). ELISA method found more sensitive and specific method for detection of invasive aspergillosis.

### Introduction

Invasive pulmonary aspergillosis (IPA) is a major threat to leukemic patients with cytotoxic therapy-induced neutropenia and transplant recipients receiving high-dose corticosteroid therapy. Since the diagnosis of IPA in an early stage is seldom possible and the mortality rate is very high (Bodey *et al.*, 1992), successful treatment is directly related to early diagnosis (Aisner *et al.*, 1977).

Serological tests are useful in the diagnosis of allergic and mycetomal forms of

aspergillosis in immunocompetent patients. The usefulness of these tests in invasive forms of aspergillosis in immunocompromised patients is less well established. Agar gel double diffusion (AGDD) is the principal serological method used at present (Coleman *et al.*, 1972; English *et al.*, 1967; Longbottom *et al.*, 1964; Schaefer *et al.*, 1976). Precipitins to *Aspergillus fumigatus* can be detected in the serum of almost all patients with the mycetomal form of aspergillosis. In contrast, serum from patients with allergic

aspergillosis often has to be concentrated before precipitins can be detected. Other less time-consuming methods that have been evaluated for the rapid detection of *A. fumigatus* antibodies, included counter-immunoelectrophoresis (Dee, 1975; Warnock, 1977), passive haemagglutination (Gold *et al.*, 1980), radioimmunoassay (Bardana *et al.*, 1975; Marier *et al.*, 1979) and indirect immunofluorescence (Warnock *et al.*, 1974, 1981).

A recently developed sandwich enzyme-linked immunosorbent assay (ELISA) allowed the detection of low levels of circulating *Aspergillus* galactomannan (GM) in sera from patients at high risk of IPA, but the increase in sensitivity was also associated with false-positive results in up to 8% of the serum samples. Since IPA is predominantly a pulmonary infection in immunocompromised patients, detectable antigen levels may be present in BAL fluid samples from patients suspected of having IPA and therefore may be of use for the diagnosis of IPA.

## Materials and Methods

**Study type:** Prospective and experimental.

**Period of study:** The study was carried out over a period of 12 months with effect from January 2015 to December 2015.

**Place of study:** Department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai.

**Number of samples:** 86.

**Study group:** 86 Patient attending IPD and OPD, MGM Medical College and hospital, Navi Mumbai.

**Inclusion criteria:** Blood samples of patients with chronic purulent exudates in sputum samples.

**Exclusion criteria:** Blood samples of patients which do not show any pus cells in sputum samples were excluded from studies.

**Statistical analysis:** Chi Square test, Fischer's (F) test and t test was used for testing the hypothesis.

## Ethical Clearance

The study was cleared by Institutional Ethics committee of MGM Institute of Health Sciences, Navi Mumbai and written consent from the patients was taken prior to collection of samples.

## Sample Collection

Blood samples from patient attending tertiary care hospital were collected in plain sterile tube by taking all aseptic precautions and properly labelled. After 30 minutes of collection the samples were centrifuged at 3000 RPM for 5 minutes using centrifuge machine and serum separated and stored at 5-8°C in refrigerator.

## Results and Discussion

Sensitivity and specificity of ELISA test was 100%, however sensitivity- 66.67% and specificity 100% for culture.

ELISA test for *Aspergillus* IgM antibodies were carried out on 86 blood samples of those patients, whose sputum samples showed purulent exudates. This means that there is lower respiratory tract infection and there is presence of IgM antibody in 18 blood samples (20.93%) which is indicative of sensitization or exposure to *Aspergillus* fungus elements.

SDA culture on same number of sputum samples with purulent exudates, showed culture positivity in 12 out of 86 sputum

samples (13.95%). IgM Antibody present but no fungal growth on SDA in 6 sputum

samples could be because of prior treatment which will inhibit growth.

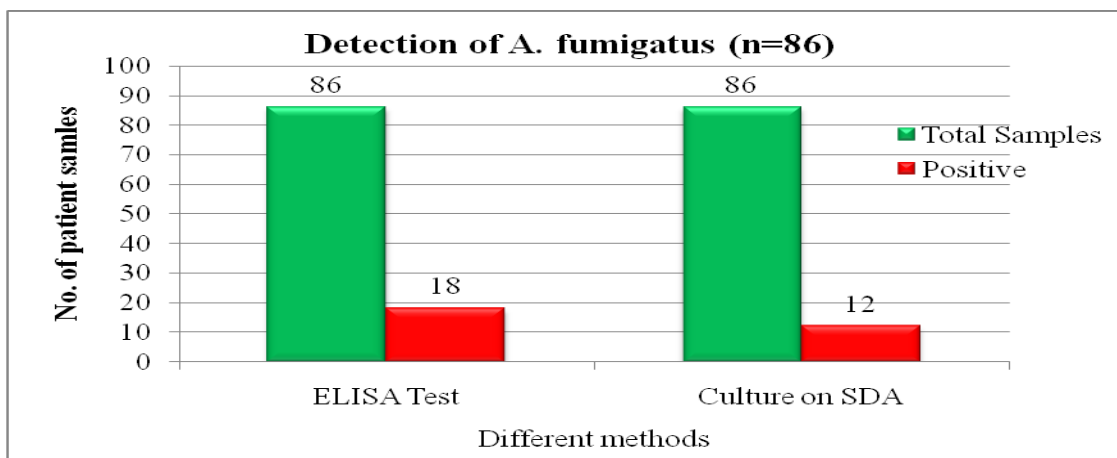
**Table.1** Showing comparison of two methods for detection of *A. fumigatus*.

Methods	No. of samples	Positive
ELISA test	86	18(20.93%)
Culture on SDA	86	12(13.95%)
Chi square =1.02, dg=1, P value >0.05, Not significant		

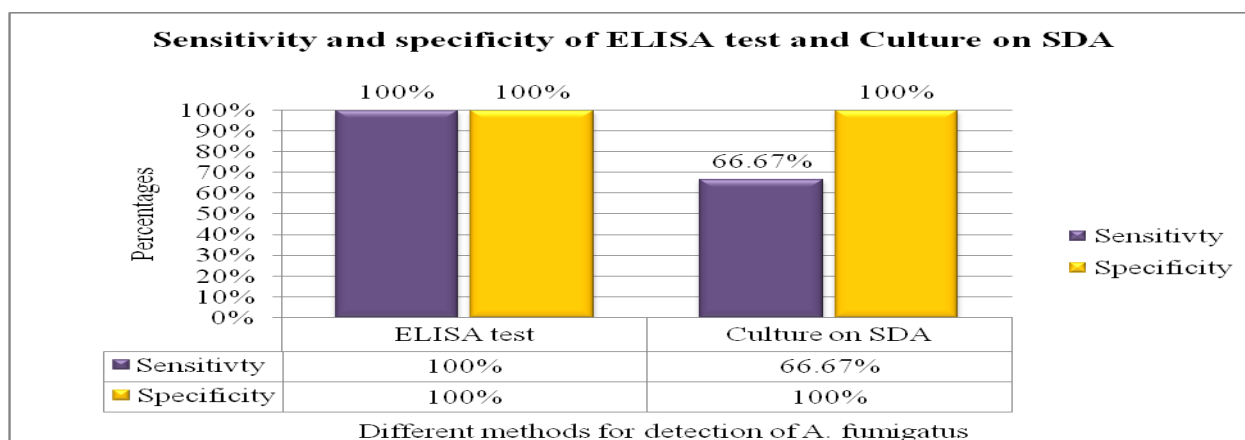
**Table.2** Calculation of sensitivity and specificity of two methods for detection of *A. fumigatus*

Methods	Positive	Negative
ELISA test	18	68
Culture on SDA	12	74

**Fig.1** Showing methods for detection of *A. fumigatus*.



**Fig.2** Showing sensitivity of different methods for detection of *A. fumigatus*.



Sensitivity of ELISA was 100% but that of culture was 66.67% and specificity of both ELISA and Culture was 100%. Our IgM positivity results (20.93%) compared well with study of Kumar *et al.*, (1989) from Manipal, India reported 20% positivity. Shahid *et al.*, (2001) in 2001 reported 30.6% positivity. Similarly our culture positivity (13.95%) compares well with that of Shahid *et al.*, (2001) (14.7%).

Findings of foreign studies was Richardson *et al.*, (1982) (53.57%), Florent *et al.*, (2006) 63.6% and 89.7%, Sabetta *et al.*, (2000) reported 57.89%, Maesaki S *et al.*, (1999). Higher antibody percentages were reported by foreign workers from different parts of world. There can be higher chance of subclinical exposure to aspergilli.

In conclusion, this ELISA is a sensitive, specific, and easily performed assay for circulating *Aspergillus* antibody that should facilitate early diagnosis of invasive aspergillosis. It is concluded that prevalence of Aspergillosis is quite high. Culture and serological test should be performed in conjunction to establish the diagnosis. Isolation of aspergilli by culture from lesion (pulmonary and other lesion) is desirable but may not be possible in each and every case. Sample /exudates collection may be improper/ inadequate. Prior treatment can inhibit growth on culture media. Deep seated lesions may not yield sample. Hence for diagnosis of aspergillosis all diagnostic methods are necessary but more wattage should be given serology (Ig M) antibodies for ongoing infection.

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