

# Rapid Detection of *Aspergillus Fumigatus* Growth Percentage by Microplate Reader

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**Abstract:** In this experiment, the OD value of different concentrations of *Aspergillus fumigatus* cultured for 48 hours was measured on a microplate reader using a 96-well plate. The absorbance-concentration curve was plotted according to the results, and the absorbance value was used instead of visual observation to quickly detect the growth of *Aspergillus fumigatus*. situation. The results showed that the OD value was between 0.05 and 0.06 when visually 50%, and 100% when the OD value was greater than 0.17. The use of the OD value can be a rapid method for observing the growth of *Aspergillus fumigatus*, which has the advantages of high efficiency, accuracy and high throughput compared with the visual method.

**Keywords:** *Aspergillus fumigatus*, Microplate Reader, OD, Value Growth Status

## Introduction

*Aspergillus fumigatus* is an important conditional pathogenic fungus that causes invasive aspergillosis (IA) in immunocompromised hosts [1-2]. When patients with immunocompromised inhalation of *Aspergillus* spores, spores invade the lungs through the trachea, bronchi, and alveoli, causing initial invasive infections, often accompanied by dissemination of distant organs, in view of clinically not directly effective for *Aspergillus fumigatus* The method of determination, therefore, the study of the growth changes of *Aspergillus fumigatus* will become the first element of the treatment of *Aspergillus fumigatus* [3-4]. At present, the research of fungi directly uses visual inspection to directly observe its morphology. The method is simple and cannot be concluded quickly and effectively, and there is a certain error. In other literatures, since the number/concentration of the test bacteria is proportional to the optical density (OD) value of the bacterial suspension, the *Aspergillus fumigatus* suspension is measured by a spectrophotometer, but the method is also obvious. Disadvantages: the experimental operation is complicated, the cycle is long, and the bacteria are easily infected during repeated feeding. The fungus in the bacterial suspension will precipitate and the OD value will change, and the result is unstable [5-6]. The purpose of this paper is to measure the OD value of *Aspergillus fumigatus* in the state of bacterial suspension by using the microplate reader, draw the absorbance-concentration curve according to the OD value, and use a safe and reliable method to express the growth status of *Aspergillus fumigatus* quickly and accurately for later detection. The antibacterial

ability of antimicrobial peptides provides a reliable method [7-8].

## MATERIALS AND METHODS

### Strain activation

The AMM medium suitable for the growth of *Aspergillus fumigatus* is configured as follows:

Reagent	dose
glucose	2g
Salt solution	4ml
Add water to 200ml, 1N NaoH adjusts ph to 6.5	
Agar powder	3g(1.5%)
Autoclave (121°C 20min)	
Ammonium tartrate	2ml
Supp solution	1ml

Shake well, pour into the plate, and cool for use. WT was inoculated into AMM medium, incubated in a 37 ° C incubator for 72 hours, and spores were collected in an ultra-clean bench in an autoclaved EP tube for a concentration of 10<sup>7</sup> cells/ml.

### Visible absorption curve of bacterial suspension

The prepared spores were dissolved in 1640 medium, and the concentration was adjusted to 10<sup>5</sup> cells/ml. The 1640 medium without the bacterial solution was used as a blank control, and the configured bacterial suspension was placed under a microplate reader to measure the OD value.

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### Preparation of 96-well plate suspension and measurement of corresponding OD value

The freshly collected *Aspergillus fumigatus* spores were subjected to gradient dilution, and the concentration of each gradient was  $1 \times 10^2$ ,  $2 \times 10^2$ ,  $3 \times 10^2$ ,  $4 \times 10^2$ ,  $5 \times 10^2$ ,  $6 \times 10^2$ ,  $7 \times 10^2$ ,  $8 \times 10^2$ ,  $9 \times 10^2$ ,  $1 \times 10^3$ ,  $2 \times 10^3$ ,  $3 \times 10^3$ ,  $4 \times 10^3$ ,  $5 \times 10^3$ ,  $6 \times 10^3$ ,  $7 \times 10^3$ ,  $8 \times 10^3$ ,  $9 \times 10^3$ ,  $1 \times 10^4$ ,  $2 \times 10^4$ ,  $3 \times 10^4$ ,  $4 \times 10^4$ ,  $5 \times 10^4$ ,  $6 \times 10^4$ ,  $7 \times 10^4$ ,  $8 \times 10^4$ ,  $9 \times 10^4$ , and each concentration was used as a standard comparison (addition of the same medium and bacterial solution in the well), and the 1640 medium was used as a blank control. The 96-well plate was wrapped in aluminum foil and placed in an incubator at 37 ° C to prevent evaporation of water in the plate. The OD value of each well was measured after 48 hours of incubation.

### Data analysis

All experiments were repeated three times. The results were expressed as mean  $\pm$  standard error (mean  $\pm$  SEM). Data were analyzed and plotted using GraphPad Prism5 software.  $P < 0.05$  indicated that the difference was statistically significant.

## RESULTS

### Measurement wavelength selection

The reason why the spectrophotometer can measure the concentration of a liquid is because of the application of Lambert Beer's law, which describes the relationship between the intensity of light absorption at a certain wavelength and the concentration of the light absorbing material and the thickness of its liquid layer. Since the experiment uses a bacterial suspension, there is no strict limit on the wavelength for determining the optical density of the suspension. For convenience, we chose 620 nm as the measurement wavelength.

### Test plate observation

From Figure 1, we can clearly see the change of turbidity of *Aspergillus fumigatus* wild type before and after culture. From A1 to A9, it is  $1 \times 10^2$ ,  $2 \times 10^2$ ,  $3 \times 10^2$ ,  $4 \times 10^2$ ,  $5 \times 10^2$ ,  $6 \times 10^2$ ,  $7 \times 10^2$ ,  $8 \times 10^2$ ,  $9 \times 10^2$ , A11 is the standard control, A12 is the reference, only equal volume of 1640 medium is added, each concentration Repeat three times, followed by 103 and 104. It can be seen from Fig. 1A that all the wells before the culture are transparent liquids. After the culture, according to the B chart, the standard control wells (A11) are the most turbid, and the blank control wells (A12) are still transparent liquids. As the concentration of the bacteria increases, the turbidity of the medium is higher until the bottom of the hole is covered.

### Test OD value

According to the microplate reader, the OD value of all the wells of the 96-well plate was measured, and the OD value-visual scatter plot was drawn according to the commonly used visual method, as shown in Fig. 2. As can be seen from the figure, the OD value of

the well to which only the medium is added is about 0.05, corresponding to Figure 3A. The OD value also increases as the concentration increases. When visually 50%, the OD value is between 0.05 and 0.06, corresponding to Figure 3B. When the OD value is greater than 0.17, the visual inspection is 100%, corresponding to Figure IIC. D and E are the positive control and the negative control, respectively.

### Discussion

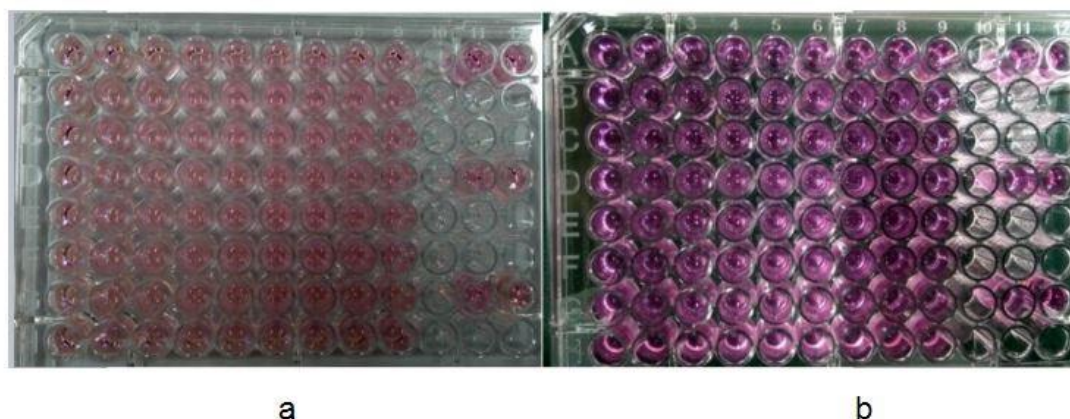
*Aspergillus fumigatus* is an important pathogenic fungal pathogen of immunodeficient hosts. It is ubiquitous in people's periphery. Its spores are extremely small, floating in the air, and can enter the lungs with breathing. In the presence of moisture and sufficient nutrients, such as in mammalian lungs, dormant conidia disrupt metabolism and dormancy of the cell cycle. After a period of nuclear fission and isotropic growth (expansion), each conidia establishes a polar axis and then develops into invasive hyphae, causing invasive aspergillosis, which is also the current death of invasive aspergillosis. The highest rate [9-11].

At present, the clinical evaluation of the growth state of *Aspergillus fumigatus* is mostly by visual inspection, and the proportion of *Aspergillus fumigatus* in 96-well plates is directly observed [12-13]. The method is easy to understand and simple to operate, but there are also many problems. For example, the difference of the observers may result in different readings, resulting in a certain error range of the results. In addition, the visual methods are mostly read only 0%, 20%, 50% and 100%, and can not accurately and effectively distinguish smaller intervals. The existence of these shortcomings also makes the visual method only a basic method auxiliary use [14-15]. In this experiment, the OD value is read by the microplate reader, and the percentage read by the visual method is replaced by the OD value, so that the whole experimental process is simpler in operation, the result is more accurate, and the human error is reduced as small as possible. Exist, and the value is more visualized. At the same time, since the culture identification in this experiment is carried out in a 96-well plate, it is not necessary to remove the culture solution and is not easily contaminated by bacteria, and is less interfered by environmental factors. Compared with the visual method, the microplate reader can simultaneously detect the OD value of 96 samples in a short time, which has the advantages of fast, accurate and high throughput [16-18].

There are also corresponding deficiencies in this experiment. The various experimental data obtained by using the microplate reader to read the OD value are limited to the liquid medium 1640. If other medium is used, the OD value should be re-measured and determined. However, the results of this

experiment show that it is feasible to use the microplate reader to read the OD value of the 96-well plate instead of the visual method, which provides a

new method for the next step to identify the antibacterial effect of the antibacterial substance.



a:Growth of *Aspergillus fumigatus* in 96-well plates before culture; b:Growth of *Aspergillus fumigatus* in 96-well plate after culture.

Figure 1: Comparison of the growth status of *Aspergillus fumigatus* before and after 48 hours of culture.

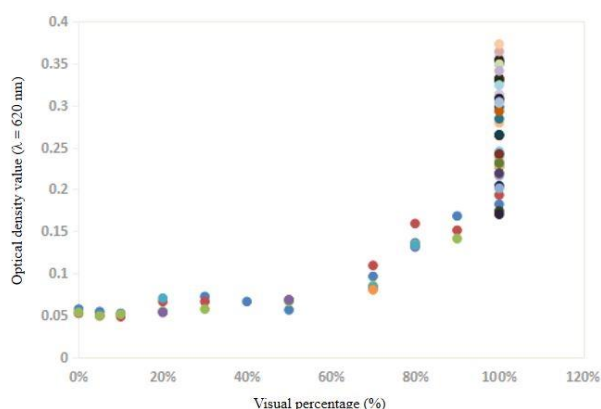


Figure 2: Correlation between visual and OD values after 48 hours of culture with *Aspergillus fumigatus* at different concentrations.

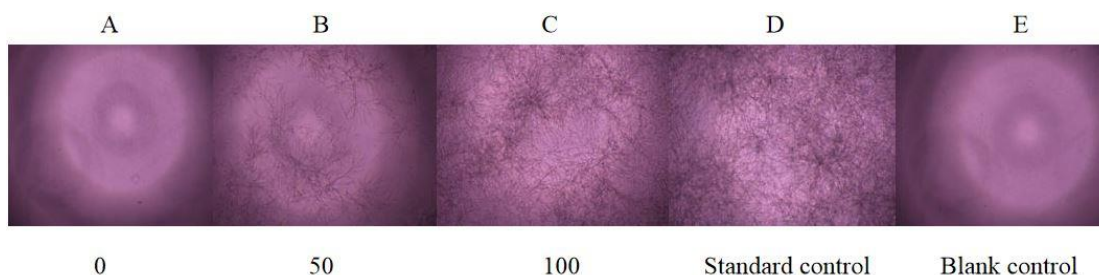


Figure 3: Observation of colony growth under an inverted microscope. A: Microscopic image when the visual value is 0% B: Microscopic image when the visual value is 50% C: Microscopic image when the visual value is 100% D: Microscopic image of the standard control well E: blank control Microscopic image of the hole.

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