Study on Drug Sensitivity Test for Detection of Mycoplasma Infection by Computer Aided Biological Immune Mechanism

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Abstract. Mycoplasma microbium infection is one of the main causes of morbidity and mortality. Rapid screening of a large number of pathogenic agents by multiple pathogen detection has become an important technique for mycoplasma infection detection. In this paper, the effects of diffusion factors on the kinetics of pathogens interacting with host immune cells were studied. A pathogen - host immune response diffusion model with homogeneous Neumann boundary conditions was established. Using the theory of partial differential equations, we discuss the distribution of eigen roots of linearized systems at the positive equilibrium solution, and obtain the critical condition for the model to undergo Turing instability at the positive equilibrium solution. It is found that multiple detection technology platform has practical value for rapid detection of pathogenic microorganisms in acute infection diseases. In particular, it is of great significance in solving the problem of epidemiological etiological analysis.

Keywords: computer; biology; immune mechanism; microbial detection; mycoplasma drug susceptibility test.

1. Introduction

In recent years, people begin to study the restriction law of immune factors to pathogens based on immunological principles. Some scholars consider the interaction between pathogens and immune cells from the microscopic level, establish mathematical models to depict the dynamic behavior of pathogens and immune cells, assist in predicting the change rule of pathogens in the host body, and provide some theoretical guidance for controlling pathogen infection. In recent years, a lot of research results have been produced on the risk assessment of pathogen transmission infection. Some scholars have proposed that the risk of human infection will be increased by diseases transmitted in hospitals depends on three factors: surface materials in hospitals, ventilation volume and human direct contact behavior, and concluded that direct contact behavior will greatly increase the risk of infection. Some scholars have studied the indoor transmission of pathogens and put forward the susceptibility index to evaluate the risk of infection. Some scholars combined the Wells-Riley model with computational fluid dynamics to assess the risk of pathogen infection in the built environment [1]. Numerous data show that pathogenic microorganisms that can cause acute infection are very complex, including bacteria, mycoplasma microbium, mycoplasma eigen roots, chlamydia, fungi, etc., which cause great difficulties in disease diagnosis and treatment, and new pathogens are still being discovered, such as Mycoplasma boca, Mycoplasma human metapleuron, Mycoplasma SARS, etc. In this paper, the effects of diffusion factors on the kinetics of pathogens interacting with host immune cells were studied. A pathogen - host immune response diffusion model with homogeneous Neumann boundary conditions was established. Using the theory of partial differential equations, we discuss the distribution of eigen roots of linearized systems at the positive equilibrium solution, and obtain the critical condition for the model to undergo Turing instability at the positive equilibrium solution.

2. Detection technology of Mycoplasma microbiome infection

The isolation and culture of mycoplasma is still the gold standard for the diagnosis of mycoplasma infection, but the traditional culture of mycoplasma has some problems, such as long cycle, difficult to maintain mycoplasma infection during transportation and storage [2]. The slide culture method

realized the rapid isolation and culture of mycoplasma microbe, and the detection period was shortened to 1 ~ 3d. For example, the R-MixReadyCells multi-immunofluorescence culture system based on SV technology, consisting of a mixture of cells sensitive to two mycoplasma microorganisms, detects seven common mycoplasma microorganisms simultaneously by using a pool of mycoplasma fluorescent antibodies. In vitro, the sensitivity of R-Mix system was roughly similar to that of traditional mycoplasma culture, while the sensitivity of freeze-preserved R-MixReadyCells was significantly higher than that of traditional mycoplasma culture.

Simultaneous detection of mycoplasma microbe by multiplex PCR has been widely reported in the literature. For example, Biradicals et al. designed three multiplex semi-nested PCR methods, which can simultaneously detect 12 common microbial mycoplasma and subtypes. In recent years, through the specific design of primers and/or the improvement of detection methods after multiple PCR, there have been some PCR product detection technology platforms with high degree of automation and easy operation:

2.1. Gel detection technology

Both Selector and Selector test kits can identify multiple PCR amplification products by gel electrophoresis. Seepiest technology is a multiplex PCR system designed based on double initiation oligonucleotide primers, which ensures high specificity and has a wider range of suitable annealing temperature. SeeplexTMRV-12 kit can detect 12 species of human microbial mycoplasma. The later development SeeplexTMRV-15 is EU certified. See plex is simple to use and can be used to quickly, sensitively and specifically detect most common microbial mycoplasma [3]. However, see plex detects PCR products by gel or capillary electrophoresis, and the multiple detection capability is not as good as other high-throughput detection platforms. Labioplasty can detect 12 common microbial mycoplasmas, including Mycoplasma boca. Figure 1 shows the gel detection technology platform.

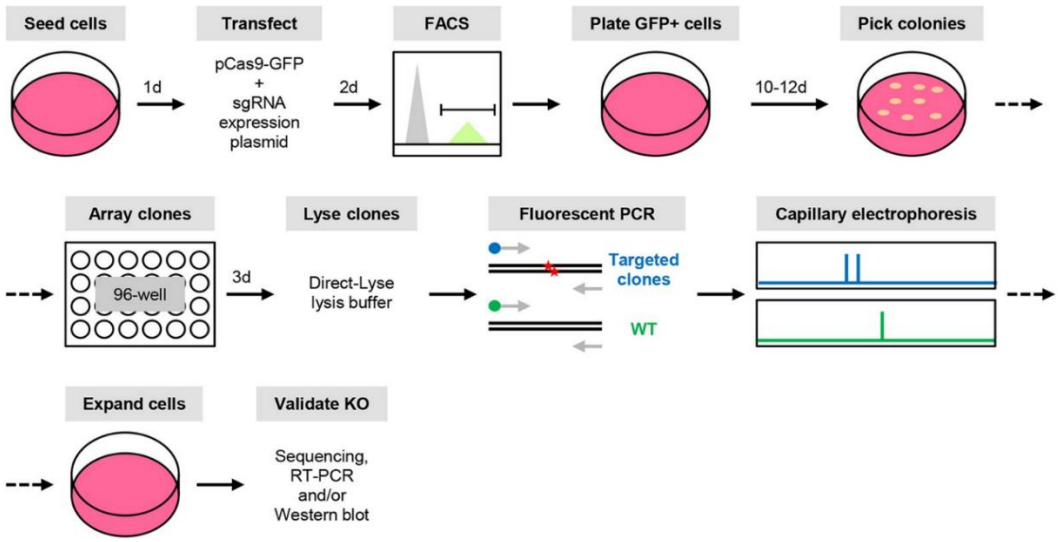


Figure 1. Technical flow of gel testing

2.2. Liquid chip testing technology platform

Liquid chip is also called microsphere suspension chip. It is a new biochip technology platform based on xMAP technology developed by Luminex [4]. The liquid chip system consists of a number of uniforms sized round microspheres, each of which is fixed with a different probe molecule, corresponding to a unique fluorescence code. Then the microspheres were detected and the results were analyzed.

2.3. Solid phase chip detection technology platform

The solid-phase chip uses a single fluorescent, enzyme or nano-coupled probe to complete the detection, thus avoiding the spectral overlap of multiple fluorescent probes in liquid-phase detection

systems. Solid chip technology is integrated with multiple PCR technology to produce a variety of fast automated detection platforms [5]. The Arcatured oligonucleotide assay platform is a new non-fluorescent (enzyme-labeled), low-density, low-cost chip. Probes can be designed according to researchers' needs and chips can be made using AT patented technology. The AT system is characterized by the need for no special hybridization device, only the need for a simple, inexpensive scanner. Although the system cannot detect some clinically suspicious specimens, it is beneficial to detect pathogens with significant clinical significance at infection levels. Figure 2 shows the solid chip detection technology platform.

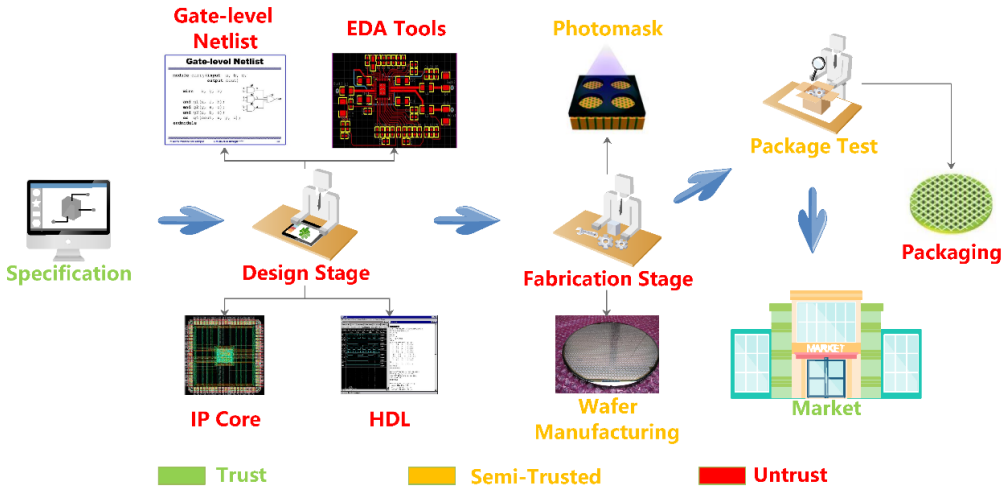


Figure 2. Solid chip detection technology platform

2.4. Microfluidic chip

Microfluidic chip, also known as "laboratory on chip", refers to the integration of the whole laboratory function including sample pretreatment, separation, dilution, mixing, reaction and detection on the microchip. Microfluidic chip and microarray chip are two kinds of parallel microchip systems. Compared with microarray, it has the features of function integration and multiple use. At present, microarray has been deeply industrialized, while microfluidic chips are still in the initial stage. However, microfluidic chip has the unique advantages of micro, portable, integrated and multidisciplinary, which shows a broad application prospect [6]. It is believed that with the maturity of technology and the reduction of cost, it will replace the complex and heavy testing instruments in clinical testing. Verulam, a portable "lab-on-a-chip" test that rapidly detects all major subtypes of Mycoplasma influenza, has been developed by Verde's Laboratories Singapore and STMicroelectronics. It can identify and distinguish all human flu strains in a single test. Figure 3 shows the microfluidic chip detection technology.

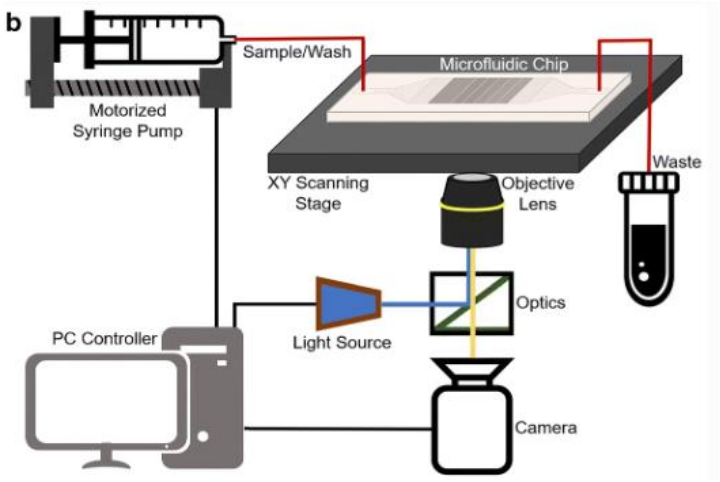


Figure 3. Microfluidic chip detection technology

3. Pathogen transmission model

Euler model focuses on the point in space, is to investigate the physical quantity and its change at a point in space. In Euler's model, FLUENT processes different phases into a continuous medium that penetrates each other, which can be applied to a variety of steady and unsteady airflow models [7]. The Euler model regards the pathogen and the air released by the sick passengers in the cabin as two kinds of fluids. By observing the interaction between the pathogen and the air in the Euler coordinate system, the concentration distribution of the pathogen in the cabin and its propagation rule can be obtained. In this paper, N_2O is used as tracer gas to replace pathogen exhaled by sick passengers.

$$\frac{\partial \rho C}{\partial t} + \nabla \cdot [\rho C(\vec{u} + \vec{v}_s)] = \nabla \cdot (\Gamma \nabla C) + S_C \tag{1}$$

Where: t is time; C is the concentration of N_2O; ρ is cabin air density; ∇ is a Hamiltonian operator; u is the average component of air velocity in all three directions; Γ is the diffusion coefficient of N_2O; S_C is the source of N_2O; v_s is the settling velocity of N_2O. Γ is the diffusion coefficient of N_2O:

$$\Gamma = \rho(v_p + D) \tag{2}$$

Where, v_p is the turbulence diffusion coefficient of N_2O; D is the Brownian diffusion of N_2O.

Suppose (i) that when pathogen density is particularly high, the growth rate of specific immune cells is higher than their decay rate, i.e., $k/k_m > \delta$. It is assumed that the specific (ii) immune response is stronger than the non-specific immune response to the high-density pathogen, i.e., as < au. The dimensionless transformation of model (1) was performed to obtain the condition that the dimensionless model has a unique positive equilibrium point, the condition that the positive equilibrium point is stable and the condition that the model experiences Hopf branch at the positive equilibrium point, and the equilibrium state and periodic change of pathogens and immune cells in the body over time were simulated [8]. However, in practice, some pathogens and immune cells will randomly spread into the host body with the flow of blood or body fluids in the host body, thus inducing the production of diseases.

This paper refers to the two-dimensional diffusion model of HIV infection dynamics in the host established by Stankevich, and introduces the spatial effect into the pathogen-host immunity model (1) to obtain a diffusive pathogen-host immunity model in the following form

$$\left.\begin{aligned}
&\frac{\partial P}{\partial t} = \tilde{d}_1 \Delta P + rP - \frac{c_s P}{1 + a_s P} I - \frac{c_u P}{1 + a_u P} M, (x, y) \in |\, \Omega, t > \\
&\frac{\partial I}{\partial t} = \tilde{d}_2 \Delta I + \frac{kP}{1 + k_m P} I - \delta I + h, \quad (x, y) \in \Omega, t > 0 \\
&\frac{\partial P(t, x, y)}{\partial n} = \frac{\partial I(t, x, y)}{\partial n} = 0, \quad (x, y) \in \partial\Omega, t > 0 \\
&P(0, x, y) = P_0(x, y), I(0, x, y) = I_0(x, y), (x, y) \in \bar{\Omega}
\end{aligned}\right\} \tag{3}$$

Where: $\Omega = (0, \pi) \times (0, \pi)$ represents a bounded open set with a smooth boundary $\partial\Omega$ on a two-dimensional space. n represents the out-of-unit normal vector on the boundary $\partial\Omega$. $\tilde{d}_1$ and $\tilde{d}_2$ represent the spread rate of the pathogen and specific immune cells. The homogeneous Neumann boundary conditions indicate that model (2) is a closed system, with no change in pathogen and specific immune cells at the boundary.

By dimensionless transformation.

$$u=\frac{kP}{\delta} \quad v=\frac{c_s I}{\delta} \quad \tau=\delta t$$

And τ is still represented by u, then model (2) can be simplified

$$\left.\begin{aligned}
&\frac{\partial u}{\partial t}=d_1\Delta u+\alpha u-\frac{uv}{1+\beta_s u}-\frac{mu}{1+\beta_u u},(x,y)\in\Omega,t>0\\
&\frac{\partial v}{\partial t}=d_2\Delta v+\frac{uv}{1+\gamma u}-v+\eta,\ (x,y)\in\Omega,t>0\\
&\frac{\partial u(t,x,y)}{\partial n}=\frac{\partial v(t,x,y)}{\partial n},\quad (x,y)\in\partial\Omega,t>0\\
&u(0,x,y)=u_0(x,y),v(0,x,y)=v_0(x,y),(x,y)\in\bar{\Omega}
\end{aligned}\right\} \tag{4}$$

$$\alpha=\frac{r}{\delta},\beta_s=\frac{a_s\delta}{k},\beta_u=\frac{a_u\delta}{k},\gamma=\frac{k_m\delta}{k},\eta=\frac{c_s h}{\delta^2},$$
$$m=\frac{c_u M}{\delta},d_1=\frac{\tilde{d}_1}{\delta},d_2=\frac{\tilde{d}_2}{\delta}$$

$\alpha=\frac{r}{\delta},\beta_s=\frac{a_s\delta}{k},\beta_u=\frac{a_u\delta}{k},\gamma=\frac{k_m\delta}{k},\eta=\frac{c_s h}{\delta^2},$ $m=\frac{c_u M}{\delta},d_1=\frac{\tilde{d}_1}{\delta},d_2=\frac{\tilde{d}_2}{\delta}$. From hypothesis (i) and hypothesis (ii), we know that $\gamma<1,\beta_u>\beta_s\geq 0$. Some clinical data show that some pathogen infections can be suppressed by immune cells under some conditions, but the pathogen cannot be completely suppressed by immune cells under other specific conditions. In this case, the pathogen and immune cells often coexist.

4. System inspection

After diseased passengers release pathogens into the air through breathing in different seats, pathogen transmission rules and simulation data collection, 30 seats in the cabin are numbered in order of 1 ~ 30. The cross section of the breathing area of all passengers in the cabin was intercepted and the pathogen concentration was detected [9]. The height of the cross section was 110cm from the cabin floor. 30 sampling points were selected from each cross section 5cm from the passenger's mouth to collect the pathogen concentration of the passenger's breathing area. When the sick passengers were in different seats from No. 1-30, the simulated passengers released droplets containing pathogens through breathing and coughing to solve the average infection risk probability of other passengers when the sick passengers were in different positions in the cabin. When Q was 750 and 900m3/h, the average infection risk probability of other passengers was shown in Figure 4.

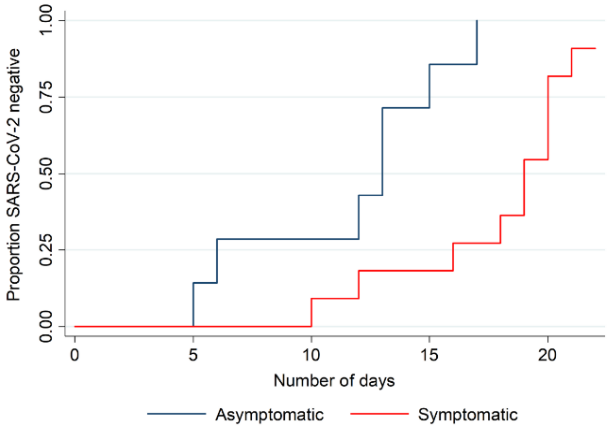


Figure 4. Average risk of infection for remaining passengers

It can be seen from Figure 4 that when Q is 750 and 900m3/h and the sick passenger is in position 3, the average risk of infection of other passengers is the lowest. By increasing Q, the average risk of infection of other passengers can be effectively reduced. When the sick passenger was in seat 25, Q was 750m3/h, the other passengers had the highest average risk of infection.

5. Conclusion

Mycoplasma microbium is the main pathogen of infection, and the co-infection of mycoplasma microbium, the variation of mycoplasma microbium and the emergence of new mycoplasma microbium are the great challenges facing the detection technology of mycoplasma microbium. It is particularly important to understand and master the new techniques and methods of mycoplasma microbium detection for disease diagnosis, treatment and epidemic prevention and control. It can be seen from theoretical analysis and numerical simulation that the diffusion ratio directly affects the generation of Turing instability phenomenon, and too large ratio cannot generate Turing instability. This means that if the spread rate of mycoplasma microbiome is too fast, immune cells will not be able to control the pathogen well in space, which will result in a certain imbalance between the pathogen and the host immune cell population, resulting in the generation of disease. According to the simulation, under different diffusion ratios, the plaques formed by pathogens in space are different, and the plaques formed by slow diffusion of immune cells are larger than those formed by fast diffusion of immune cells in the same time.

Acknowledgments

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References

[1] Qu Teng, Ju Ying, Liu Chunmei, et al. Correlation between RNA of Mycoplasma pneumoniae and levels of immunoglobulin M and immunoglobulin G of Mycoplasma pneumoniae. Chinese Journal of Medicine, vol. 16, pp.14-24, May 2021.

[2] Jin Haiyong, Shen Guanqiao, Lu Yaling. Value of chemiluminescence immunoassay to detect antibodies against Mycoplasma pneumoniae in the diagnosis of Mycoplasma pneumoniae infection in children. Chinese Journal of Health Inspection, vol. 32, pp.74-81, August 2022.

[3] Shen Weirong, Hu Bingyu. Level and clinical significance of immune function indexes in children with mycoplasma pneumonia complicated with EB positive. China Maternal and Child Health Care, vol. 36, pp.33-39, December 2021.

[4] Zhang Yi, Zhou Bin, Zhang Jue, et al. Establishment and preliminary application of time-resolved fluorescence immunochromatography for IgM and IgG antibodies of Mycoplasma pneumoniae. Chinese Journal of Nuclear Medicine and Molecular Imaging, vol. 42, pp.51-57, April 2022.

[5] Chen Jiayi, Zhang Han, Shang Yunxiao. Clinical and bronchoscopic characteristics of children with positive 23S rRNA resistance gene in bronchoalveolar lavage fluid of mycoplasma pneumoniae pneumonia. Chinese Journal of Practical Pediatrics, vol. 37, pp.63-66, December 2022.

[6] Liu Liyan, Zhang Xueyun, Wang Yan. Leukotriene receptor antagonists mediated NLRP3/Caspase-1/IL-1β signaling pathway to improve Th1/Th2 immune imbalance in mice with mycoplasma pneumonia. Advances in Anatomical Science, vol. 28, pp.42-46, March 2022.

[7] He Jing, Lu Liqun, Wang Yan, et al. Expression of inflammatory cytokines in children with Mycoplasma pneumoniae pneumonia and its role in immunological pathogenesis. International Journal of Respiratory Science, vol. 41, pp.55-60, March 2021.

[8] Yu Mo-chi, WANG Jia-xin, Chen Yu. Research progress on immune regulation and mechanism of damage caused by Mycoplasma infection. International Journal of Respiratory Science, vol. 41, pp.41-45, March 2021.

[9] Tian Zengchun, Liang Lu, Liu Yang, et al. Relationship between Mycoplasma pneumoniae -DNA levels in throat swabs and alveolar lavage fluid and inflammatory response and immune function in community-acquired mycoplasma pneumoniae pneumonia. Journal of Anhui Medicine, vol. 26, pp.51-53, August 2022.