Pre-test and Method Validation of Microbial Limit Test for Drugs Based on Predictive Microbiology

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Abstract: Microbial limit test of drugs is an important inspection item to control the quality of drugs. The microbial limit validation method has been greatly revised, and all the drugs that need microbial limit test should be re-validated in accordance with the requirements of the current Pharmacopoeia. The special role of specific spoilage bacteria in microbial prediction, the application of traceability technology, temperature synthesis function and biological indicators in microbial prediction. Select the method of predictive microbiology to improve the microbiological detection rate of the drug, and ensure the correctness and credibility of the test results. The operating environment, standards, cleanliness test and other indicators for microbiological limit inspection and verification are clarified to ensure the inspection method, Verify the reliability of the results. the feasibility of application in predictive microbiology and molecular prediction were also studied in order to provide theoretical reference for promoting the progress of microbial limit test of drugs in an all-round way.

1. Introduction

In recent years, with the development of new drugs in China, it is often found that the microbial indicators of new drugs can not be effectively controlled by the inspection method contained in the Pharmacopoeia. The growth of microorganisms in medicines is inhibited. The results obtained by routine methods are not credible. Appropriate test methods must be adopted to inactivate the microorganisms so that they can grow normally [1]. In the microbial limit test of supervised sampled drugs, the microbial limit test verification data of relevant pharmaceutical manufacturers were collected. However, the verification data collected were messy and much was omitted [2]. When performing a drug microbial limit test, the method used should be verified to confirm that the method used is suitable for the microbial limit test of the drug, and for the bacteriostatic product. Since there is interference with the test bacteria under the test conditions, the test results cannot truly reflect the amount of contaminated microorganisms in the drug, and certain measures must be taken. Some designs are not rigorous, some are not standardized, and cannot reflect the integrity and feasibility of the test method [3]. Predictive microbiology is a quantitative prediction method for the growth, survival and death of microorganisms in medicines based on computer. It combines drug microbiology with engineering and statistics [4]. Its development direction is to study and design a series of models that can describe and predict the growth and decline of microorganisms under specific conditions. It also helps to improve the microbiological risk assessment system and reduce the risk of pathogenic microorganisms, which is of great significance for ensuring quality and safety and improving public health [5].

The microbial limit test methods of drugs need to be verified to determine that the method used is suitable for microbial limit test of the sample, and can be used only after passing the test, so as to ensure the reliability of the test results. These techniques are not only efficient and fast, but also can provide information that can not be quantified by traditional culture methods, which makes up for the shortcomings of traditional predictive microbial models [6]. At present, the predictive microbial model has been approved by domestic and foreign experts in predictive microbiology, and has great potential for development and application prospects. The validation of microbial limit test method ensures that the test method used is suitable for the determination of bacteria, fungi and yeast counts of the drug and for the control of bacterial examination [7]. Baydoun S et al. put forward the
concept of "specific spoilage bacteria". In the study of microbiology, it was found that compared with the dynamic response of pathogenic bacteria, the dynamic response of spoilage bacteria was complex and varied due to the different product characteristics and storage conditions [8]. Jarrad A M et al. validated the microbial limit methodology when reviewing the quality standards of new drugs, and proposed that the validation method would be effective only when the recovery rate of control bacteria was above 70% [9]. To determine the correctness and credibility of the inspection method, it is first necessary to conduct a preliminary experiment to determine whether the sample has antibacterial activity in order to determine the test method and to verify the test method used. The bacteriostatic effect of the drug is eliminated or negligible under the test conditions, thereby smoothly detecting various microorganisms contaminated by the drug, improving the detection rate of the microorganism, better controlling the quality of the drug, and ensuring the medication of the people Security [10].

2. Materials and Methods

2.1. Necessity of Methodological Verification of Biological Limit Check

Before considering various environmental condition detection methods, we must recognize the importance of databases such as networks, which can allow us to do related work from point to surface, and transmit discrete data from a single point to aggregate. The first-order model is mainly used to describe the functional relationship between the number of microorganisms and time under specific environmental conditions. Because microorganisms usually show a S-shaped growth trend with time, the S-shaped function is most often used to fit the growth kinetics of microorganisms. The antimicrobial or bactericidal components in drugs interfere with the test; the purification level of key operating points or purification workbenches in the sterile room is less than 100, and the hanging height and wattage of the ultraviolet lamp are not uniform; the sterile concept is weak and the sterile operation is not standardized; the sensitivity of the culture medium decreases; the sampling of the test solution is not uniform; the growth of bacteria is small and dense, and the counting error is easy to occur. The design of the recovery test in microbiological limit test method validation is critical because the bacteria recovery test is a key difficulty, except that it follows from simple to complex, from single to combined. According to the method provided by the Chinese Pharmacopoeia, the number of bacteria was verified and the control bacteria were verified. Check and verify coliform or Salmonella. Therefore, the route of drug use affects whether or not the control bacteria need to be verified and tested, and the determination of the number of bacteria, the number of molds, and the number of yeasts in the microbial limit test.

2.2. Design of Recovery Rate Test

In application, different inoculation dosage can be used, such as the initial content of indicator bacteria is 100 cfu/mL, when the temperature is 4 °C, the growth of drug microorganisms will take about 7 generations and 39 hours to reach this level, and the predicted time is 80% of the shelf life. A computer software with database is established to make reasonable suggestions on formulation, process and packaging mode through computer, at least in theory, to make the product. Microbial stability is guaranteed. Appropriate test methods can be used according to the nature of the sample, but the reliability of the method can only be proved by method verification. Verification records should be detailed, appropriate and clear, and the numbers should be detailed and specific. In the collected verification data, there is almost no mention of the verification basis, and the preparation and processing of the equipment, the culture medium, the strain and the bacterial liquid are long and cumbersome. "take the prepared test solution, add the test bacteria, and mix". The bacterial liquid is directly mixed with the test solution during the preparation process, fully simulating the sample contamination state, which can more accurately reflect the sample dyeing. Bacterial status. Through the parallel test, the result of detecting the control bacteria is obtained, and if the control bacteria cannot be detected by the conventional method, the verification test is repeated. Really and effectively reflect the number of bacteria in the drug contamination, the detection rate is high, but
the operation should be carried out according to the specified speed and time during centrifugation, and the conditions cannot be changed arbitrarily, otherwise the test results will be affected.

Prescriptions or main ingredients of drug varieties and preparation methods of products are indispensable elements for validation data of microbial limit methods. Whether there are active antimicrobial ingredients in the prescription is very important to verify the design and work, and it can reduce a lot of unnecessary pre-test process. In the research of predictive microbiology, different methods should be selected according to different situations, and traditional models and molecular models should be combined organically, so that various methods and technologies can complement each other and corroborate each other. If the minimum dilution level test solution cannot meet the requirement of recovery rate by various methods to eliminate the Bacteriostasis of the sample, it can be determined that the sample can meet the minimum dilution level test solution of recovery requirement according to the limit requirement of the sample, and the results determined under this dilution level can judge whether the number of impurities in the test product meets the requirements of drug microbial limit standard. The distribution of the number of bacteria and the recovery of the number of molds of the chemical and Chinese patent medicines in the variety was statistically determined to determine the effectiveness of the method for determining the number of bacteria provided by the pharmacopoeia. The results are shown in Table 1. The sample to be tested must be verified by pre-experiment and method using the recovery rate or proliferation value when performing microbial limit examination, so as to ensure the correctness and credibility of the test result by using the correct test method.

Table 1 Distribution Table of Recovery Rate for Bacterial Number Measurement.

<table>
<thead>
<tr>
<th>Category</th>
<th>Project</th>
<th>0%~ 20%</th>
<th></th>
<th>20%~ 50%</th>
<th></th>
<th>50%~ 70%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variety number</td>
<td>Proportion</td>
<td>Variety number</td>
<td>Proportion</td>
<td>Variety number</td>
<td>Proportion</td>
<td></td>
</tr>
<tr>
<td>Chemical medicine</td>
<td>Bacterial count</td>
<td>8</td>
<td>0.5</td>
<td>5</td>
<td>0.3125</td>
<td>3</td>
<td>0.1875</td>
</tr>
<tr>
<td></td>
<td>Mold count</td>
<td>6</td>
<td>0.6</td>
<td>3</td>
<td>0.3</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Chinese patent medicine</td>
<td>Bacterial count</td>
<td>13</td>
<td>0.325</td>
<td>15</td>
<td>0.375</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Mold count</td>
<td>18</td>
<td>0.72</td>
<td>3</td>
<td>0.12</td>
<td>4</td>
<td>0.16</td>
</tr>
</tbody>
</table>

3. Result Analysis and Discussion

3.1. Methodological factors of microbial limit test

The influence of culture medium, the error of counting and observation, the influence of equipment and personnel, the error of sampling and sampling; the factors affecting the test error include culture medium, preparation of sample solution, testing equipment and equipment, colony counting error, drug properties, operating environment, etc. When temperature, pH, Aw (NaCl concentration), additives and initial bacterial count are input, important parameters of microbial growth, such as delay time, generation time, growth curve and growth data, can be obtained. Therefore, labor productivity can be improved by reducing the cost of steps and time in current microbial experiments. Predictive microbiology will also provide a reasonable basis for drafting guidelines, standards and rules concerning microorganisms in pharmaceuticals. Accurate determination of bacterial count is the most critical step in recovery determination. The prepared bacterial liquid should not be placed for too long, otherwise the recovery rate will be affected by the physiological state of the bacteria. The recovery rate is based on the number of bacteria tested test requires 30 to 80 cfu to reduce the operational error.

3.2. Prediction of Microbial Growth

The growth kinetics of microorganisms can be used to simulate the survival of microorganisms
in the process of pathogenic bacteria "from intake to pathogenicity", to make up for the blank of this link, so as to construct more accurate quantitative microbial risk assessment. Correct preparation of test solution and true reflection of microorganisms in drugs are important conditions to ensure the correct and reliable test results. For those varieties that can not be checked by routine methods, the pretreatment method of test solution provided by pharmacopoeia can be used to detect them effectively. The analysis may be related to the rapid propagation of the bacteria in the process of placing and testing. It also indicates that attention should be paid to the time control of placing bacteria after adding bacteria in the validation test. Fresh cultures are generally used within 1 week, and will be re-cultured after next week, and must be ensured that the number of passages must not exceed 5 generations. In order to ensure the biological characteristics of the test strains, targeted biochemistry and identification are also required. Wait for the test. By adjusting the initial level of bacteria in the indicator, various points in the shelf life of the product can be predicted. The study identified the most important single factors affecting bacterial growth and the interaction of each factor. The probability of microbial growth quickly determines the guiding direction for changing product formulations or storage conditions.

Molecular prediction model can provide some information that traditional prediction model can not provide. If it is combined with traditional prediction model organically and serves the quantitative microbial risk assessment system together, it can greatly improve the accuracy of quantitative microbial risk assessment. The quantitative relationship of bacterial liquid is different from that of chemical reference substance, the latter is more stable, but the content of bacterial liquid is not fixed, and it is also a metabolic process of proliferation and death itself. Therefore, fresh cultures should be counted every time they are cultured. The count of bacterial liquid should be made as the predicted number of fresh cultures and the synchronous count at each test. It is proved that it has antimicrobial activity. The microbial limit test was carried out by the routine method prescribed in the pharmacopoeia. The detection rate of microorganisms was low and the results were unreliable. In microbial limit test, samples must be inactivated to obtain accurate and reliable results. The microbial lethal curve shows the initial shoulder and the final tail. Applying the concept of accuracy objectively assessing the ratio between predicted and actual values, establishing a database of microbial growth, forming expert systems and application software packages to quickly estimate specific spoilage organisms. Determination by the Pharmacopoeia conventional plate counting method, the correct result of the number of contaminating bacteria in the sample cannot be obtained, but the methodological study should be carried out, and the antibacterial activity or other interference should be excluded by predicting the microbiological method.

4. Conclusion

In this paper, the preliminary experiment and method validation of microbial limit test of drugs based on predictive microbiology were studied. The vigorous development of predictive microbiology provides a powerful tool and excellent opportunity to further promote the progress of this discipline. Further research should consider evaluating the recovery rates of each control bacteria on the premise of reasonable selection of representative control bacteria. It is beneficial to control the safety of non-sterile preparations from the point of view of microorganisms and ensure their effectiveness. In order to ensure the integrity of the method and the reliability of the test results, it is necessary to carry out the verification and standard formulation of a factory, a product and a routine microbial limit inspection. Relevant departments shall make a thorough investigation on the actual situation of the units requiring microbial limit inspection and verification, issue certificates of certification to those units with certification qualification, and conduct regular assessment. The methodological study of microbial limit inspection for different drug varieties, and the establishment of inspection methods under various varieties can ensure the accuracy of the test results. The mutual cooperation between microbiologists and mathematicians helps to overcome difficulties and facilitates the description of the model of microbial growth state development in ecosystems. In order to better carry out strategic research on microbial prediction, new technologies
should be introduced at the same time, and it is predicted that microbes will have great development prospects.

References


