European Journal of Clinical and Biomedical Sciences
2021; 7(2): 33-36
http://www.sciencepublishinggroup.com/j/ejcbs
doi: 10.11648/j.ejcbs.20210702.14
ISSN: 2575-4998 (Print); ISSN: 2575-5005 (Online)

Establishment of Microbial Limit Test for Hospital Preparation 'Compound Alum Powder'

Fangming Tan, Minna Huang*, Suishan Qiu, Zhuanzhi Liang

Department of Medicine, The First Affiliated Hospital of Jinan University, Guangzhou, China

Email address:
583869064@qq.com (Fangming Tan), 418553103@qq.com (Minna Huang), 494490817@qq.com (Suishan Qiu), 1252617671@qq.com (Zhuanzhi Liang)

*Corresponding author

To cite this article:
Fangming Tan, Minna Huang, Suishan Qiu, Zhuanzhi Liang. Establishment of Microbial Limit Test for Hospital Preparation 'Compound Alum Powder'. *European Journal of Clinical and Biomedical Sciences*. Vol. 7, No. 2, 2021, pp. 33-36. doi: 10.11648/j.ejcbs.20210702.14

Received: April 22, 2021; **Accepted**: May 11, 2021; **Published**: May 21, 2021

Abstract: Background: the assessment of microbiological quality of medicinal products is one of control stages in medicinal products saft, because the holder of a manufacturing authorization must drug so as to ensure that they are fit for their intended use, comply with the requirements of the Marketing Authorization and do not place patients at risk due to inadequate safety, quality or efficacy. Objective: To assess microbial limit test for hospital preparation 'compound alum powder'. Methods: The results of strain culture and analysis were used to evaluate the drug quality of 'compound alum powder'. The objective is 'compound alum powder' (approval number: Z20140229) was made by the First Affiliated Hospital of Jinan University. Their batch numbers include: 201019, 200818, 200701. Validation tests were carried out in accordance with the standards of the Chinese Pharmacopoeia (2020 edition). Three parallel independent validations were conducted to calculate the recovery rate of each test bacterium and investigate whether each test bacterium was detected in the control bacterial inspection. Result: The recovery ratio of the total number of aerobic bacteria, molds and yeast in the control group was in the range of 0.5 ~ 2, and the results of the control group were positive, and the results of each control group met the requirements. The results show that the membrane filtration method is suitable for the microbial limit test of 'compound alum powder'. Conclusion: this microbial limit test method control and ensure the quality of drug production by solving the problem of interference of antimicrobial components in drugs. It can eliminate the bacteriostasis of boric acid, phenol and alum.

Keywords: Microbial Limit Test, 'Compound Alum Powder', Boric Acid, Phenol, Membrane Filtration

1. Introduction

the assessment of microbiological quality of medicinal products is one of control stages in medicinal products saft, because the holder of a manufacturing authorization must drug so as to ensure that they are fit for their intended use, comply with the requirements of the Marketing Authorization and do not place patients at risk due to inadequate safety, quality or efficacy [1-3]. Based on the fourth part of the Chinese Pharmacopoeia 2020 edition, when testing the microbial limit of the tested product, the applicability of the microbial count method and the control bacteria test method of the tested product shall be verified to ensure that the established method can be used for the colony count and control bacteria test of the product [4, 5]. 'Compound alum powder' is an in-hospital preparation of the First Affiliated Hospital of Jinan University. It has been used in clinic for many years and has a clear effect. It is composed of boric acid, phenol, menthol and alum, because boric acid, phenol and alum have strong antiseptic effect [6-8]. To assess the degree of contamination of a drug, hospitals need to establish effective microbial limit testing methods, that the sample was rinsed by membrane filtration.

The purpose of this study was to establish microbial limit test for hospital preparation 'compound alum powder'. Because microbes presented in drugs not only makes them hazardous from the infectious stand point, in addition may change the chemical, physical and organoleptic properties of the drugs or change the contents of active ingredients [9-11].

2. Methods

2.1. Objective and Methods

The results of strain culture and analysis were used to evaluate the drug quality of 'compound alum powder'. The objective is 'compound alum powder' (approval number: Z20140229) was made by the First Affiliated Hospital of Jinan University. Their batch numbers include: 201019, 200818, 200701. Validation tests were carried out in accordance with the standards of the Chinese Pharmacopoeia (2020 edition). Three parallel independent validations were conducted to calculate the recovery rate of each test bacterium and investigate whether each test bacterium was detected in the control bacterial inspection.

The passage times of the test strains were 3-4 generations. They included: Staphylococcus aureus [CMCC(B)26003], Bacillus subtilis [CMCC(B)63501], Pseudomonas aeruginosa [CMCC(B)10104], Candida albicans [CMCC(B)98001], Aspergillus Niger [CMCC(B)98003], Escherichia coli [CMCC(B)44102]. Also, the medium included: Pancreatine Soy Peptone Agar Medium, Pancreatine Soy Peptone Liquid Medium, Scharloy Glucose Agar Medium, Scharloy Glucose Liquid Medium, pH 7.0- Sodium Chloride Peptone, McConkey Agar Medium, McConkey Liquid Medium, Cetyltrimethylammonium Bromide Agar Medium, Mannitol Sodium Chloride Agar Medium.

2.2. Bacteria Solution

The fresh cultures of Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis were inoculated into the liquid medium of peptone from pancreatic cheese and soybean. The culture medium was incubated at 35°C for 24 hours and then diluted to 50-100 CFU/mL with sterile normal saline at a 10-fold rate. Also, the fresh culture of Candida albicans was inoculated into Schachfeldt-glucose liquid medium, which was incubated at 25°C for 48 hours, and then diluted to 50-100 CFU/mL with sterile normal saline at a 10-fold rate. The culture of Aspergillus Niger was inoculated on Scharbella glucose AGAR medium, which was incubated at 25 ° C for 5 days, and then 5ml [Buffered Sodium Chloride - Peptone Solution with 0.05% (ml/ml) Polysorbate80] buffer was added. The spore suspension was sucked out of a tube filled with cotton into a sterile tube and diluted to 50-100 CFU/mL with the previously prepared buffer solution.

2.3. Preparation of Test Solution

We added 10g of 'compound alum powder' to 100 mL of Buffered Sodium Chloride - Peptone Solution, then mixed well and let it stand for 3 minutes. Finally, we obtained the supernatant as the test solution, which is 1:10 test solution.

2.4. The Operation of the Test Group

We injected 10ml of the above test solution into the sterilized filter cup of the bacteria collector, washed it with 200ml of sterile normal saline, and added the solution of Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans and Aspergillus Niger containing bacteria number of 50-100CFU. Then we mix and strain it. We took out the filter membrane and pasted it face to face on a sterile plate filled with 20 mL of buttermilk soybean peptone AGAR medium. The medium was cultured at 35°C for 3 days. 10 mL of the above test solution was injected into the sterilized filter cup of the bacteria collector, and 400 mL of sterile normal saline was rinsed. Then, Candida albica and Aspergillus Niger solution containing bacteria number of 50-100 CFU were added respectively and mixed. After filtration, the filtration membrane was removed and pared face to face on the sterile plate with 20 mL of Scharbella AGAR culture medium poured. We then cultured this medium at 25°C for 5 days. We prepared two plates in parallel and measured the number of all the colonies on the plates. The average number of colonies was taken as the counting result.

2.5. Preparation of Sample for Test

We inject 10ml of each of the above test solution into the filter cup of the bacteria collector. This operation is the same as the operation of the test group, and it is used to determine the number of colonies in the test products.

2.6. Preparation of Bacteria Solution

We took 1 mL of the diluent of culture solution of the five representative strains above and injected them into a sterile plate with a diameter of 90mm. This operation was the same as that of the test group, and was used to determine the number of colonies in the liquid group.

2.7. Feedback Ratio

Feedback ratio of the test group = [(the average number of colonies in the test group - the average number of colonies in the test solution group)/the average number of colonies in the bacteria liquid group]. The Feedback ratio was in the range of 0.5 ~ 2, and the colony morphology was the same as that in the control medium

2.8. Validation of the Inspection Method for Controlling Bacteria

Base on the requirements of the 2020 edition of the Chinese Pharmacopoeia, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans shall not be detected in the control bacteria inspection after the use of 'compound alum powder'.

2.9. Staphylococcus Aureus, Pseudomonas Aeruginosa and Candida Albicans

10ml of the previously manufactured test solution was filtered through the membrane filtration method. It was then rinsed with 100 mL sterile normal saline, and the film was removed and inoculated into 100 mL Sacherloin glucose liquid medium. Then 50 to 100 CFU of Candida albicans were added and cultured at 35°C for 48 hours. The above cultures were crossed and inoculated on Sacherloin

glucose AGAR medium plate and cultured at 35°C for 72 hours.

10ml of the previously manufactured test solution was filtered through the membrane filtration method. Then, the film was rinsed with 100 mL sterile normal saline, removed and inoculated into 100 mL liquid culture medium of peptone of pancreatic cheese soybean. Then Staphylococcus aureus bacterial solution with colony number of 50 ~ 100 CFU was added respectively. Cultured at 35°C for 24 hours. The above cultures were crossed and inoculated on mannitol sodium chloride AGAR medium plate and cultured at 35°C for 24 h.

We inoculated 10ml of each test solution previously made into 200ml liquid culture medium of tryptone and added Pseudomonas aeruginosa bacterial solution with colony number of 50 ~ 100cfu, respectively. Cultured at 35°C for 24 hours. The above cultures were crossed and inoculated on cetyltrimethylammonium bromide AGAR medium plate and cultured at 35°C for 24 h.

2.10. Negative Control Group and Positive Control Group

10 mL of pH 7.0- Sodium Chloride Peptone was inoculated into 100 mL of peptone liquid culture medium. Negative control group did not add test bacteria, other operations were the same as each test group. On the other hand, in the positive control group, diluent was used instead of the test solution, and other operations were the same as those in the test groups.

3. Result

Table 1, Table 2, Table 3 and Table 4 shown that feedback results of aerobic bacteria total count method were collected from test results. The recovery ratio of the total number of aerobic bacteria, molds and yeast in the control group was in the range of 0.5 ~ 2, and the results of the control group were positive, and the results of each control group met the requirements. The results show that the membrane filtration method is suitable for the microbial limit test of 'compound alum powder'.

Table 1. *Feedback results of aerobic bacteria total count method (1).*

Item		Staphylococcus aureus		E. coli		Bacillus subtilis		Candida albicans		Aspergillus Niger	
bacteria	Colonies (case)	116	125	132	128	69	75	98	92	72	72
solution group	Mean	121		130		72		95		72	
sample for test group		0						0			
test group	Colonies (case)	137	116	111	127	81	68	77	104	75	69
	Mean	127		119		75		91		72	
Result collection rate		100.0%		91.5%		100.0%		95.8%		100.0%	

Table 2. *Feedback results of aerobic bacteria total count method (2).*

Item		Staphylococcus aureus		E. coli		Bacillus subtilis		Candida albicans		Aspergillus Niger	
bacteria	Colonies (case)	51	66	103	96	71	76	41	46	50	60
solution group	Mean	59		100		74		44		55	
sample for test group		0						0			
test group	Colonies (case)	39	58	95	81	73	79	43	41	55	58
	Mean	49		88		76		42		57	
Result collection rate		100.0%		83.1%		88.0%		100.0%		95.5%	

Table 3. *Feedback results of aerobic bacteria total count method (3).*

Item		Staphylococcus aureus		E. coli		Bacillus subtilis		Candida albicans		Aspergillus Niger	
bacteria	Colonies (case)	51	66	96	103	71	76	41	46	50	60
solution group	Mean	59		100		74		44		55	
sample for test group		0						0			
test group	Colonies (case)	63	54	86	49	44	49	44	41	55	58
	Mean	59		90		63		47		62	
Result collection rate		100.0%		100.0%		90.0%		85.1%		100.0%	

Table 4. *The suitability test results of the control bacteria inspection method in 'compound alum powder'.*

tester strain	test group	sample for test group	Negative control group	Positive control group
Candida albicans	+	−	−	+
Staphylococcus aureus	+	−	−	+
Pseudomonas aeruginosa 100ml	−	−	−	+
Pseudomonas aeruginosa 200ml	+	−	−	+

4. Discussion

To assess microbial limit test for hospital preparation 'compound alum powder', we used validation tests and analyze the result from feedback results of aerobic bacteria total count method and the suitability test results of the control bacteria inspection method in 'compound alum powder'. As shown in Table 1, Table 2, Table 3 and Table 4, the test group had most positive results in microbial limit test. These results

showed that the membrane filtration method eliminated the bacteriostasis of boric acid, phenol and alum by rinsing with sterile normal saline, effectively solved the problem of the interference of bacteriostasis components in the drug, and ensured the scientific nature of the microbial limit test method and the accuracy of the test results. Pseudomonas aeruginosa has strong antibacterial ability, so it can be tested by medium dilution method (200ml).

Several studies in related fields clearly demonstrated that the microbial limit test method can control and ensure the quality of drug production, that it can effectively solve the problem of interference of antimicrobial components in drugs [12-15]. Likewise, this study had similar result and data, this microbial limit test method also controls drug quality by affecting interference of antimicrobial components in drugs.

Therefore, this microbial limit test method is effective for 'compound alum powder', the microbial limit test method is an effective tool for control medical quality of 'compound alum powder'.

In limitation, our subject was 'compound alum powder', so it is not known whether our results are application to other drugs.

5. Conclusion

In conclusion, this microbial limit test method control and ensure the quality of drug production by solving the problem of interference of antimicrobial components in drugs. It can eliminate the bacteriostasis of boric acid, phenol and alum.

References

[1] Tyski S. How to manufacture a pure and safe drug. Pharm. Ind. 2017; 5: 57–61.

[2] Guide to good manufacturing practice for medicinal products, 2017. Pharmaceutical Inspection Convention Pharmaceutical Inspection co-operation scheme, PE 009–10 (Part I) 1 January 2017.

[3] Długaszewska J, Muszyn´ski Z, Ratajczak M. Microbiological purity of oral drugs manufactured in industrial and pharmacy environment. In: 26th Congress of the Polish Society of Microbiologists: Microbes –Challenges and Hopes. Conference Materials. Szczecin. 2018: 4–7.

[4] National Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China: four parts of the 2020 edition. Beijing: China Medical Science and Technology Press. 2020: 160-171.

[5] Yang XL, Li H, Ma YY, et al. Chinese Pharmacopoeia 2015 edition of the microbial limit test of non-sterile products: the introduction and countermeasures of bacterial control test [J]. Chinese Pharmacist. 2016; 19 (4): 748-752.

[6] You SX, Wu DK, Liu SJ, et al. Research on processing of Alumen. Joumal of Chinese Medicinal Materials. 2018; 33 (7): 1192-1194.

[7] She F, Xu ZG. Influencing factors and countermeasures of microbial limit testing error for drugs. China Health Standards Management. 2018; 9 (18): 100-102.

[8] Ma SH, Liu P, Yang LH, et al. Experimental study on adding bacteria in the applicability test of microbial limit test method for drug. Journal of Pharmaceutical Analysis. 2018; 38 (5): 877-882.

[9] Yang YR, Shen QL, Wang QF. Western China Journal of Traditional Chinese Medicine. 2017; 30 (11): 59-61.

[10] Wang ZJ, Mei LQ, Wang XJ, et al. Study on the applicability of microbial limit test for six traditional Chinese medicine preparations. Pharmaceutical Care and Research. 2018; 18 (4): 300-303.

[11] Min H, Tang N, Yi LJ. Microbial limit and control method of traditional Chinese medicine preparation. Northwestern Pharmaceutical Journal. 2016; 28 (3): 301-303.

[12] Wang J, Zhang XH, Ren ZL, Sheng CL, Yan SS. Study on the applicability of the microbial limit test method of Yangyin Qingfei pill. China Contemporary Medicine. 2020; 27 (36): 29-32.

[13] Gong Y, Li M, Cao J, Zhang R. Establishment of four kinds of microbial limit test methods of hospital Chinese medicine preparation containing Chinese herbal powder. Evaluation and Analysis of Hospital Drug Use in China. 2020; 20 (10): 1197-1199+1203.

[14] Wei YY, He K, Wu YM, Wang XX, Pang YJ. Guangzhou Chemical Industry. 2020; 48 (19): 89-91.

[15] Zhang XH, Liu YP, Qu GJ. Establishment of microbial limit test method for compound Danshen tablets. China Pharmaceutical Standard. 2020; 21 (05): 464-471.