UDC: 001.891:615.015.32

MICROBIOLOGICAL RESEARCHES OF THE HOMEOPATHIC MEDICINE LILIUM D3

Yuryeva G.B., Yarnykh T.G.
National University of Pharmacy, Kharkiv

Introduction. An important place in the evaluation of the quality of medicine is microbiological control. Contamination by microorganisms in the process of production, storage or use can lead to apparent loss of the stability of the agent, and to reduce the therapeutic efficacy of the medicine without explicit visual changes [1].

As you know, medicines from a microbiological point of view are divided into two groups: sterile medicines (injections, ophthalmic medicinal forms) and medicines for which sterility is not required (powders, granules, pills, tinctures, extracts etc.).

Medicinal forms may contain a large number of the most different microorganisms entering into them during the technological process (primary contamination), storage and use (secondary contamination). Sources of pollution can be plant raw materials, water, air, packaging, equipment, clothing personnel, etc.

From auxiliary substances most often pollute those that themselves can be as a contamination factor (sucrose, lactose, etc.). Water, as a solvent, is also an enabling environment for the growth of microorganisms [2].

Basic homeopathic medicine *Lilium Ø* was prepared according to method 1a in the SPU using all flowering plant [3].

Homeopathic granules *Lilium D3* were prepared by saturation method.

Aim. Microbiological analysis of the homeopathic medicine Lilium D3 for the purpose of development and analysis of a new medicine.

Methods. In our work the microbiological researches of homeopathic medicine Lilium X3 have been used.

For the study of microbiological purity, one series of homeopathic medicine Lilium D3 was analysed [4].

All media were prepared in accordance with the requirements of the manufacturer (amount of powder per liter, pH of the medium, autoclaving conditions, etc.). Each medium which was used in the experiment was tested for growth qualities according to regulatory documents.

For testing the homeopathic medicine Lilium D3 on the microbiological purity, Thyoglycollate semi-liquid medium, liquid Sabouraud, solid nutrient media (nutrient agar and Sabouraud medium) were used.

A Chistovich medium, blood agar-based nutrient agar with added defibrinated blood or red blood cell, Endo medium are used for identification of pathogenic *Staphylococcus*, *Pseudomonas aeruginosa* and various types of *Enterobacteria*.

Before checking of microbiological purity, a test for compliance with the growth properties of nutrient media was conducted. Nutrient media were inoculated with a small amount of appropriate test-strains of microorganisms ($10\text{-}10^2$ colony forming units per ml of medium - CFU/ml).

The growth of the microorganism test culture on this medium in 18-20 hours confirms its suitability for research work. *Candida albicans* was seeded on the Sabouraud medium, *Pseudomonas aeruginosa* and *Bacillus subtilis* - on the nutrient agar, *Staphylococcus aureus* on the Chistovich medium, *Escherichia coli* - on the Endo medium. Thyoglycollate medium was kept in a thermostat at a temperature of 35ºC for three days [1, 2]. Results are represented in table 1.

Table 1

Growth properties of nutrient media when inoculated with test microorganisms before testing for microbiological purity

Indicator microorganisms	Culture media	Culture conditions		Conclusion
		Temperature	Culture duration	
Staphylococcus aureus ATCC 6538	Chistovich medium	35ºC	24-72 hours	The morphology of the colonies and cells is typical
Escherichia coli ATCC 25922	Endo medium	35ºC	24-72 hours	The morphology of the colonies and cells is typical
Bacillus subtilis ATCC 6633	Nutrient agar	35ºC	24-72 hours	The morphology of the colonies and cells is typical
Pseudomonas aeruginosa ATCC 9027	Nutrient agar	35ºC	24-72 hours	The morphology of the colonies and cells is typical
Candida albicans ATCC 885/653	Sabouraud	25ºC	24-120 hours	The morphology of the colonies and cells is typical
x	Thyoglycollate medium for sterility test	35ºC	24-72 hours	No microbial growth

Note: x – microorganisms are not seeded.

All cultures of microorganisms corresponded to the taxonomic designation of the strain, and the morphology of the colonies during cultivation on the medium and the morphology of the cells under microscopy was typical. Thyoglycolate medium is corresponds to requirements for sterility - the growth of microorganisms was absent; the medium is transparent.

The test of the homeopathic medicine Lilium D3 for microbiological purity was carried out by the method of direct seeding on the liquid accumulation media. In this case, the Thyoglycollate medium and Sabouraud liquid medium were poured into test tubes 10.0 ml each in sterile conditions. Then, 1 ml (1 g) of the test medicine was added to each tube. The culture was incubated for 28 days in a Thyoglycollate

medium in a thermostat at the temperature of 35 ° C, and seeding in a liquid Sabouraud at 25 ° C. The data presented in table 2.

Table 2

Test for the microbiological purity of the homeopathic medicine Lilium D3 by the method of direct seeding on liquid media

Medicine	Culture media and conditions	
	Thyoglycollate medium 28 days at 35ºC	Sabouraud liquid 28 days at 25ºC
Lilium D3	No microbial growth	No yeasts and moulds growth

As shown in table 2, after 28 days of incubation on the Sabouraud medium, a growth of yeasts and moulds was not recorded and growth of microorganisms on the Thyoglycollate medium was not observed.

In the study by the method of deep seeding, which consisted in adding the medicine in the amount of 1 g (1.0 ml) to agar, and surface seeding (1 g or 1.0 ml) – the number of viable cells of microorganisms and fungi was determined on the agar. A study of deep and surface inoculation of medicines on Sabouraud cups showed no growth of yeasts and moulds. When cultivated on nutrient agar - the growth of microorganisms was not observed. Results are represented in table 3.

Table 3

Research on the microbiological purity of the homeopathic medicine Lilium D3 by the method of direct seeding on cups

Medicine	The number of microorganisms for the decimal logarithm of the degree of growth while cultivating on solid nutrient media			
	Deep seeding method 1 g (ml) medicine (x10)		Surface seeding method 1 g (ml) drug (x10)	
	Nutrient agar 35ºC 3 days	Sabouraud medium 25ºC 5 days	Nutrient agar 35ºC 3 days	Sabouraud medium 25ºC 5 days
Lilium D3	No microbial growth	No fungi growth	No microbial growth	No fungi growth

As shown in table 3, the growth of fungi and microorganisms was absent in the study of the medicine.

The criterion for evaluating the effectiveness of the homeopathic medicine Lilium D3 was a decrease in the number of viable microbial cell colonies over a certain period after contamination. In accordance with the requirements of SPU, the logarithm of reducing the number of viable bacterial colonies after 6 hours should be at least 2, after 24 hours - at least 3, in the future, the number of viable bacteria cells should not increase. The logarithms of reducing the number of viable fungal cells after 7 days is not less than 2. These indicators meet the criterion "A".

In accordance with criterion "B", the logarithm of the number of viable microbial colonies after 24 hours must be at least 1, after 7 days - at least 3, in the future the number of viable colonies should not increase. The logarithm of reducing the number of viable fungi in 14 days should be at least one and not increase further.

After contamination by microorganisms, the medicine was sown on agar at regular intervals to determine the number of viable cells. No growth on agar or no increase in the number of colonies after 14 days of incubation indicated that the medicine meets the requirements of SPU [4].

The presence of viable cells of microorganisms and fungi on the 28th day of research indicates that the medicine does not meet the criteria "A" or "B" and does not meet the requirements of the SPU. The results of the study are shown in table 4.

Table 4

The results of determining the effectiveness of the homeopathic medicine Lilium D3

Exposition	Requirements of the SPU		Логарифм числа микроорганизмов (КОЕ/мл)		
	The number of bacteria CFU/ml Lg decrease	The number of fungi CFU/ml Lg decrease	*Staphylococcus aureus* ATCC 6538	*Pseudomonas aeruginosa* ATCC 9027	*Candida albicans* ATCC 885/653
Microbial challenge test	10^6	10^6	2.2×10^5 (5.34)	2.3×10^5 (5.36)	2.5×10^5 (5.39)
Primary isolation Lg	-	-	5.1×10^4 (0.64)	5.2×10^4 (0.76)	5.3×10^4 (0.67)
6 hours	2	-	2.2×10^3 (2.0)	1.7×10^3 (2.13)	2.3×10^4 (2.03)
24 hours	3	-	0.6×10^2 (3.57)	1.1×10^2 (3.32)	2.1×10^3 (3.07)
7 days	-	2	NS	NS	0.3×10^2 (3.92)
14 days	-	-	NS	NS	NS
28 days	NI	NI	NS	NS	NS

Notes: NI – Microorganisms are not increase;

NS – Microorganisms or fungi are not secreted.

Results. As we can see (table 4), after 6 hours of cultivation, the logarithm of the number of viable fungal cells was 2.03 for *Candida albicans*. After 24 hours of contamination, the logarithm of the number of viable cells for *Candida albicans* was 3.07, on the 7th day - 3.92. *Candida albicans* cells do not stand out after 14 days of culture. After 6 hours of cultivation, the logarithm of the number of colonies of microorganisms was more than 2 for *Staphylococcus aureus* - 2.0 and for *Pseudomonas aeruginosa* - 2.13. After 24 hours, these numbers were 3.57 and 3.32, respectively. On the 7th and 14th day of incubation, the colonies of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were not recorded. Studies of this sample showed that it corresponds to criterion "A" according to the SPU [4].

Conclusion

1. Viable cells of fungi and microorganisms in the test homeopathic medicine was not found.
2. The investigated homeopathic medicine Lilium D3 corresponds to the criterion "A", according to the requirements of microbiological purity of the SPU.

Literature

1. Безуглая Е.П. Методологический подход к фармацевтической разработке лекарственных препаратов и ее стандартизация / Е.П. Безуглая, Н.А. Ляпунов, В.А. Бовтенко // Фармаком. – 2008. - №4. – С. 75-82.
2. Настанова 42-3.6:2004. – Настанови з якості. Лікарські засоби. Допоміжні речовини / М. Ляпунов, В. Георгіївський, О. Безугла та ін. – Київ, МОЗ України, 2004. – 12 с
3. Державна Фармакопея України : в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид. – Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2014. –Т. 3. – С. 507-527.
4. Державна Фармакопея України : в 3 т. / Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид. –Харків: Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2015. – Т. 1. – С. 251.