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Short communication

Validation of analytical method for determination of microbiological quality of concentrated solution for hemodialysis

Nadica Gjurichikj*, Sanja Trajchevska Kovilovska, Tatjana Gjurichikj, Filip Godzo

Alkaloid AD, Aleksandar Makedonski 12,1000 Skopje, R.N. Macedonia

Introduction

Hemodialysis is a form of renal replacement therapy in which waste solutes are removed primarily by diffusion from blood flowing on one side of a membrane into dialysis fluid flowing on the other side (ISO 23500-4:2024). Concentrated solutions containing electrolytes are diluted prior to administration to the required concentration to obtain the final or ready-to-use solutions for hemodyalisis. These solutions contain electrolytes with a concentration close to the electrolytic composition of plasma. (Ph. Eur 01/2026:3206). As a medical device, class II b, (Regulation (EU) 2017/745), they must meet the required microbiological quality criterion in order to be safe for the patient. (Law on medicines and medicinal devices, official gazette of R. Macedonia Nº 106/07). The European Pharmacopoeia requirement for Microbial contamination is Total Aerobic Microbial Count (TAMC): acceptance criterion 10^2 CFU/mL (Ph. Eur. 01/2026:3206; 2.6.12).

Chapter 2.6.12 of the European Pharmacopoeia provides tests for the quantitative estimation of aerobic mesophilic bacteria (TAMC) that might be present in the product. These tests are designed primarily to verify whether a sample complies with the established specification for microbial quality. The method must be validated/qualified prior to use. The ability of the compendial method to detect microorganisms that might be present in the product being tested must be established. Method suitability studies should be performed prior to the examination of a product for its microbial content. Adequate recovery of the test organism confirms the suitability of the test method (Clontz, 2009).

The aim of this study was to determine the suitability of the method for testing microbiological quality of a concentrated solution for hemodialysis. The purpose of this test was to establish a reliable method for routine testing of the microbiological purity of the product.

Materials and methods

Materials

An acidic concentrated solution for bicarbonate hemodialysis was used for the method suitability test. Three commercial batches were used for the purpose of determining the suitability for each cycle. The following media and reagents manufactured in Biomerieux, France were used: Tryptic soy agar - non – selective medium used for the cultivation and enumeration of bacteria, buffered peptone water used for preparation of initial suspension as well as preparation of microbial suspension of test microorganisms, Nitrocellulose membrane filter, 0,2µm (Sartorius, Germany) was used for filtration of the samples.

The test microorganisms used in this study, manufactured in Microbiologisc, USA were:

*nlisickova@alklaoid.com.mk

Pseudomonas aeruginosa ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis ssp. spizizenii* ATCC 6633, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404. The laboratory equipment used was: Biosafety cabinet, class II (Telstar, Spain), Laboratory incubator set at 33 ºC (Binder, Germany), Membrane filtration system (Satrorius, Germany), Vacuum pump (Satrorius, Germany), Bunsen burner (Integra, USA), High pressure autoclave (Panasonic, Japan), Laboratory balance (Satrorius, Germany), Sterile forceps (Iso Lab, Germany), Sterile individually wrapped pipets (Thermo Fisher, USA).

Method

The method suitability tests were conducted on three commercial batches of the product according to the European Pharmacopoeia chapters 2.6.12 Microbiological examination of non-sterile products: microbial enumeration tests for the TAMC. Two test groups were prepared for each batch: test group with product (diluted in buffered peptone water in ratio 1:10) and test group without product (peptone water). Each group was inoculated with less than 100 CFU of each of the test microorganisms. The test group without product (positive control group) was used for determination of the referent microorganism count. A volume of 1 mL was applied on TSA agar from each of the test groups. The test was conducted in duplicate. Additionally, negative control of 1 mL buffered peptone water was inoculated on two TSA plates. The plates were incubated 5 days on 33 ºC. After the incubation period, CFU on all agar plates were counted and recovery rate was calculated using the equation:

$$r = b/a \times 100$$

where r = recovery rate, a = CFU count of the positive control test group, b = CFU count of the test group with product. A mean count of any of the test organisms not differing by a factor greater than 2 from the value of the control in the absence of the product must be obtained.

Results and discussion

The recovery results obtained for the tests of each microorganism in each of the three cycles for TAMC ranged from 73 % to 127 % and the count was not differing by a factor greater than 2. The results for each of the test microorganism in the three cycles is as follows:

- *Bacillus spizizenii ATCC 6633*: 73 % - 90 %
- *Staphylococcus aureus* ATCC 6538: 88 % - 100 %
- *Pseudomonas aeruginosa* ATCC 9027: 94 % - 127 %
- *Candida albicans* ATCC 10231: 100 % - 112 %
- *Aspergillus brasiliensis* ATCC 16404: 92 % - 101 %.

The plates of the negative control group did not have any growth.

Conclusion

The results from the method suitability test showed that the acceptability factor for all test microorganisms in each of the three performed cycles of three industrial batches of the product does not exceed the factor 2 and complies with the requirements of the European Pharmacopoeia. Therefore, this method is considered suitable for routine testing of TAMC of concentrated solution for hemodialysis.

References

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