LAL ASSAY OF BACTERIAL ENDOTOXIN ON CONTACT COMPONENTS: SUGGESTED MAXIMUM BACTERIAL ENDOTOXIN LIMIT CONCENTRATION ON RUBBER CLOSURES (AFTER SEPARATION, BEFORE WASHING)

M.B. Arambašić and B. Topalović

1) Pharmaceutical factory "GALENIKA a.d."; Quality Control Center; Dept. Biological Control; Batajniški put b.b.; YU-11.080 Beograd-Zemun; Yugoslavia

2) "Pharma-Gummi - Beograd"; Rimski Jarak b.b.; Yu-26.220 Kovin; Yugoslavia

INTRODUCTION

Bacterial endotoxins are, by chemical nature, lipopolysaccharides which are present in Gram-negative bacteria. During life-time, humans are exposed to minimal amounts of endotoxins which are released in the gastro-intestinal tract after bacteria are killed (mostly Escherichia coli Bergey). This small amount of endotoxins induces in the body the formation of moderate amount of antibodies to endotoxins. However, in case of gastro-intestinal crises (i.e. the obstruction of intestines with the occurrence of gangrene and rapid increase of the number of bacteria), a large amount of endotoxins can penetrate into blood inducing anaphylactic reaction resulting in severe shock (endotoxin shock) [1].

Due to the effect that bacterial endotoxins may have on human health, according to pharmacopoeic requirements, one of the conditions for release of drugs for parenteral use is their testing for bacterial endotoxins. Both compendial requirements (individual monograph in pharmacopoeias) and manufactures' requirements for specified products or components (i.e. contact components) prescribe maximum bacterial endotoxin limit concentration (ELC) (in: EU/g or EU/mg or EU/μl/m or EU/ml or EU/surface area/ml (for contact components) for contact components or a specified product. The ELC for rubber closures (after washing on the LF line, air class 2 100) is 0.125 EU/100 cm /100 ml [2].

If the ELC on rubber closures (after washing on the LF line, air class 100) exceeds the value of 0.125 EU/ 2 cm /100 ml, the closures should be re-washed and IAL re-tested (the requirement of "West Pharmaceutical Services, Werk Eschweiler", Deutschland, GmbH) [2]. The syntagma "after washing on the LF line, air class 100" refers to a process phase that is follower either by the sterilization of rubber closures and their further use for closing the finished the finished product or by their use for closing the finished product which is the subjected to sterilization, depending on the user.

In contrast with ELC requirements for rubber closure (after washing on LF line, air class 100), there are no literature requirements for ELC for rubber closures (after separation, before washing). The term "after separation, before washing" represents a process phase where the cut rubber closures are cleaned from impurities left during the cutting operation.

Since this is a very important phase in the process of rubber closure production, there should be a requirement for the accepted maximum ELC on rubber closures (after separation, before washing).

MATERIAL AND METHODS

The assay of bacterial endotoxin on rubber closures (after separation, both before and after washing on LF line, air class 100) was done using
LAL tests, gel-clot method (the method of hard gel formation). The rubber closure extracts were obtained according to the manufacturer's procedure [2].

RESULTS AND DISCUSSION

The assay bacterial endotoxin on rubber closures (after washing on LF line, air class 100) was done on 42 samples. Each of 42 samples (100%) met the requirement for bacterial endotoxin (less than 0.125 EU/100 cm² /100 ml).

The assay of bacterial endotoxin on rubber closures (after separation, before washing) was done on 16 samples. 9 samples (56.3%) showed the bacterial endotoxin level less than 0.125 EU/100 cm² /100 ml. 3 samples (18.8%) showed the bacterial endotoxin level less than 0.25 EU/100 cm² /100 ml, while that in 4 samples (25%) was higher than 0.25 EU/100 cm² /100 ml.

CONCLUSION

Based on the results we feel that the maximum acceptable bacterial ELC on rubber closures (after separation, before washing) should be set at 0.25 EU/100 cm² /100 ml, i.e. it should not be higher than 0.25 EU/100 cm² /100 ml.

ACKNOWLEDGEMENT

The authors would like to thank Mrs. Zlatica Milutinovic for translating the paper into English.

REFERENCES

[1] G.A. Guyton
Textbook of Medical Physiology
4th Ed., p. 346, W.B. Saunders

Band 3, Sektion 10: Prüfungen: LAL-Test für Gummiverschlüsse (Pyro 3 - PGWW). Ausgabe 8,
West Pharmaceutical Services,
Werk Eschweiler