Case study: De-masking of endotoxin in pharmaceutical antibody formulations down to 5 EU/ml using EndoRS®

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Introduction

Low endotoxin recovery or "masking effect" describes the phenomenon occurring in many pharmaceutical formulations where spiked endotoxin is no longer detectable after a certain period of time. Accordingly, contaminations occurring in the process of purification and formulation could not be identified during final product release as demanded by the regulatory bodies. EndoRS® comprises a screening kit for the development of demasking protocols based on the combinatory use of various demasking agents. It is assumed, that for a given drug formulation, a specific set of conditions has to be optimized in a screening approach. In the present study, EndoRS® was applied to explore the possibility to demask down to 5 EU/ml of endotoxin in two different commercial antibody drug formulations.

Materials and methods

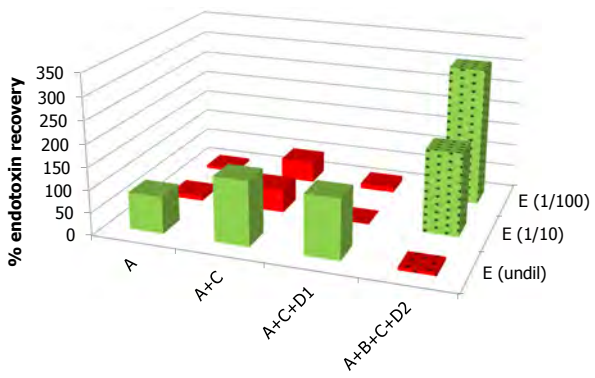
EndoRS® and EndoLISA® were obtained from Hyglos GmbH, Germany. RSE was used for spiking. All used materials were endotoxin-free.

Masking of LPS was achieved by spiking antibody with the desired concentration of RSE and storage at 2-8°C for 7 days (example 1) or at room temperature for 24 hours (example 2, experiments II and III). Endotoxin-free water was treated in parallel as control (water control). For demasking, 1 ml aliquots spiked with LPS in drug formulation and water control were prepared. Demasking components (Component A-E) provided in the kit were added sequentially in alphabetical order with subsequent mixing for 2 minutes after each addition according to the instruction manual. For measurement with EndoLISA® samples were diluted 1/10 in water and analysed according to EndoLISA® instruction manual. Demasking was defined to be successful when LPS recovery was ≥ 50 %.

Results

Example 1: 10 mg/ml antibody in a formulation based on citrate and polysorbate 80.

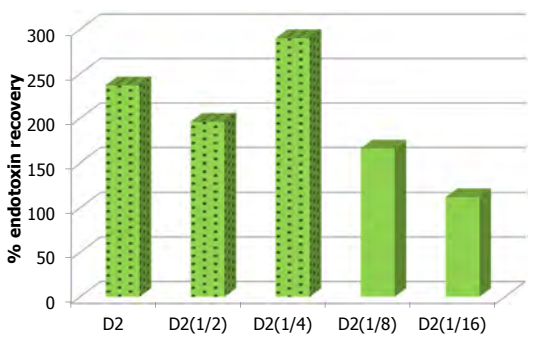
I. Initial screening with 100 EU/mL



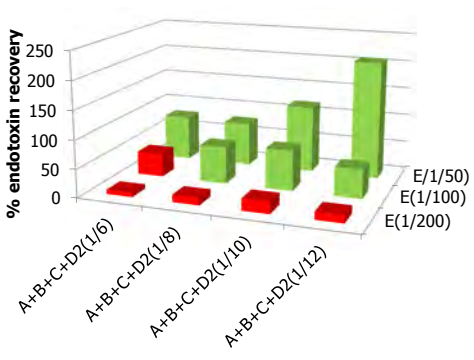
Initial Screening was performed according to EndoRS® instruction manual. 70 % of endotoxin were shown to be masked prior to the procedure. Water control yielded 126 % recovery.
Dotted columns: samples showed precipitation after 1/10 dilution for EndoLISA® measurement. The highest recoveries were achieved by A+B+C+D2+E(1/10) and (1/100) and were chosen for fine titration.

To overcome the precipitation of antibody in the sample, component D2 was titrated down to 1/16 within the combination A+B+C+D2+E(1/100) for demasking of 50 EU/mL. Dotted columns: Samples showed precipitation after 1/10 dilution for EndoLISA® measurement. Samples containing D2 in a 1/8 dilution or less did not precipitate and showed good recovery. 69 % of endotoxin were masked , but the water control yielded only 22%. Nevertheless it was descided to go on with this combination.

II. Fine Titration of component D2



III. Fine Titration at 10 EU/mL

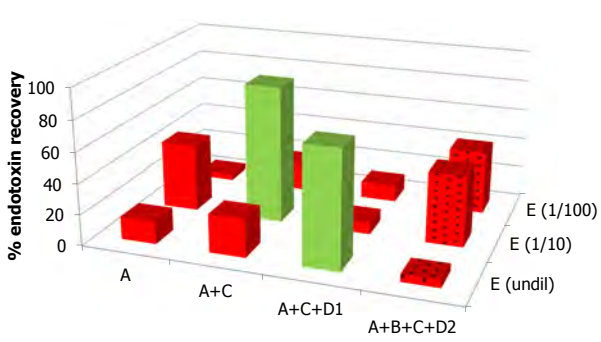


Component D2 was further fine titrated from 1/6 to 1/12 against component E. As a result very good recoveries were achieved at 10 EU/mL for combinations containing E(1/50). Water control was 107 % and 82% of spiked endotoxin were masked in advance.

The best combination **A+B+C+D2(1/12)+E(1/50)** was also capable to demask endotoxin with only 5 EU/mL in this formulation (data ot shown).

Example 2: 25 mg/ml antibody in a formulation based on phosphate and polysorbate 20.

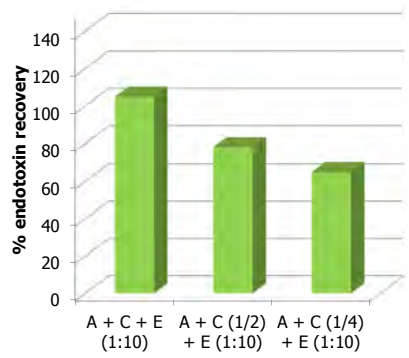
I. Initial screening with 100 EU/mL



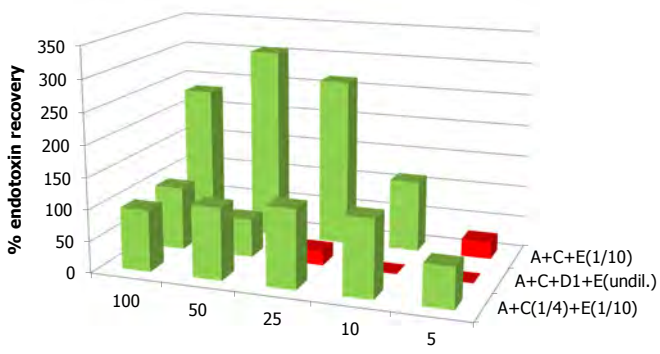
Initial Screening was performed according to EndoRS® instruction manual. 68 % of endotoxin were shown to be masked prior to the procedure. Water control yielded 120 % recovery.
Dotted columns: samples showed precipitation after 1/10 dilution for EndoLISA® measurement. The combination providing the highest recovery (A+C+E(1/10)) was selected for further fine titration.

Component C was titrated for the demasking of 100 EU/mL. Recovery slightly decreased by the decrease of C, but all three combinations resulted in recoveries between 60 and 100 %. 69% of endotoxin was shown to be masked, water control yielded 136%.

II. Fine Titration of component C



III. Reduction of endotoxin concentration



The three most promising combinations resulting from exeriments I and II were compared in their ability to demask concentrations of 100 to 5 EU/mL endotoxin. Water control yielded 97 %, masking control indicated that 85 % of endotoxin were masked.

The combination **A+C(1/4)+E(1/10)** showed > 50 % demasking down to 5 EU/mL.

Conclusion

In an exploratory study, EndoRS® approach was applied to two different therapeutic antibody formulations to develop a protocol for the recovery of masked endotoxin in low concentrations. Two different buffer formulations containing 10 or 25 mg/ml antibody were used. Initial screening at 100 EU/ml resulted in different combinations of components that were subjected to fine titration and reduction of endotoxin concentration. For both antibodies combinations were identified that were capable to recover > 50 % at 5 EU/mL.
Endotoxin masking can be simulated by incubation at 2-8°C for 7 days, but was more significant when incubation was at room temperature for 24h.
As a summary, this study shows that it is possible to find a promising combination of demasking components within a few experiments. However, for the development of a valid demasking protocol, further fine titration and optimization is necessary.

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