The global outbreak of aseptic peritonitis observed with icodextrin-based peritoneal dialysis solutions as discussed above serves as a sentinel example of how contemporary parenteral products with microbial, non-endotoxin contaminants may be considered safe under Pharmacopoeia standards but provoke adverse clinical effects. Therefore, a need exists to provide improved standards for parenteral products that employ detection procedures to better ensure that the parenteral products are effectively free of contaminating substances.

SUMMARY

In a first aspect, there is provided a method for manufacturing a dialysis solution, the method comprising: a) providing a glucose polymer; b) performing a bioburden test on the glucose polymer in an acidic medium and at an elevated temperature to detect contaminating *Alicyclobacillus acidocaldarius*; c) preparing a glucose polymer solution using the glucose polymer; d) adding a reagent derived from silkworm larvae plasma to the glucose polymer solution, wherein the reagent is reactive with peptidoglycan to determine a peptidoglycan concentration; and e) using the glucose polymer solution to prepare the dialysis solution if the peptidoglycan concentration is determined to be about 10 ng/ml or less.

In a second aspect, there is provided a method of testing a glucose polymer for peptidoglycan contamination, the method comprising: a) providing a glucose polymer; b) performing a bioburden test on the glucose polymer in an acidic medium and at an elevated temperature to detect *Alicyclobacillus acidocaldarius* which is a source of peptidoglycan; c) preparing a glucose polymer solution using the glucose polymer; d) adding a reagent derived from silkworm larvae plasma to the glucose polymer solution, wherein the reagent is reactive with peptidoglycan to determine a peptidoglycan concentration in the glucose polymer solution.

In a third aspect, there is provided a method for manufacturing a dialysis solution, the method comprising: providing a glucose polymer; performing a bioburden test on the glucose polymer in an acidic medium and at an elevated temperature to detect contaminating *Alicyclobacillus acidocaldarius*; testing the glucose polymer with a reagent derived from silkworm larvae plasma, wherein the reagent is reactive with any peptidoglycan in the glucose polymer to determine a peptidoglycan concentration; and using the glucose polymer to prepare the dialysis solution if the peptidoglycan concentration is determined to be about 10 ng/mL or less.

In a fourth aspect, there is provided a method of testing a glucose polymer for peptidoglycan contamination, the method comprising: providing a glucose polymer; performing a bioburden test on the glucose polymer in an acidic medium and at an elevated temperature to detect *Alicyclobacillus acidocaldarius*, which is a source of peptidoglycan; and testing the glucose polymer with a reagent derived from silkworm larvae plasma, wherein the reagent is reactive with any peptidoglycan in the glucose polymer to determine a peptidoglycan concentration in the glucose polymer.

SUMMARY OF THE INVENTION

The present disclosure~~invention~~ generally relates to the detection of gram positive microbial contaminants. In particular, the present disclosure~~invention~~ relates to methods and compositions that employ the detection of peptidoglycan in peritoneal dialysis solutions. The inventors have surprisingly discovered a novel cause of aseptic peritonitis--aseptic peritonitis associated with gram positive microbial contamination of a dialysis solution. Peptidoglycan is a major component of a gram positive bacterial cell wall and thus can serve as a marker for gram positive bacteria. Thus, testing for peptidoglycans can be utilized to effectively prevent peritonitis in patients that use the peritoneal dialysis solutions, such as peritoneal dialysis solutions that contain a glucose polymer including an icodextrin and the like.

Icodextrin is derived from corn starch, a natural product. It is well known that products of natural origin are contaminated with a wide variety of micro-organisms. The inventors have found that some natural products, such as corn starch, contain an acidophilic thermophilic bacteria, such as *Alicyclobacillus acidocaldarius*. The later organism is ubiquitous in the food industry, particularly in acidic beverages. It is the alicyclobacillus that produces guaiacol, which is a causative substance for an 'off' flavour orange juice. See, for example, Matsubara H, Goto K, Matsumara T, et al. Alicyclobacillus acidiphilus sp. Nov., a novel thermo-acidophilic, omega-alicyclic fatty acid-containing bacterium isolated from acidic beverages. Int J Syst Evol Microbiol 2002; 52:1681-5.

Peritoneal dialysis solutions and parenteral solutions in general have not been recognized to have contaminated by this organism or its degradation products. This is mainly because the current testing procedure for microbial contamination of peritoneal

dialysis solutions and parenteral solutions in general are not capable of detecting this organism or its degradation products.

To this end, in an embodiment, the present disclosure~~invention~~ provides a method for manufacturing a peritoneal dialysis solution. The method includes providing a glucose polymer-based solution; adding a reagent to the glucose polymer-based solution wherein the reagent is capable of reacting with a peptidoglycan; determining an amount of the peptidoglycan using the reagent; and using the glucose polymer-based solution to provide the peritoneal dialysis solution if it is determined that a sufficiently low level of the peptidoglycan is present.

In an embodiment, the reaction initiates a serine protease cascade.

In an embodiment, the serine protease cascade includes a prophenol oxidase cascade.

In an embodiment, the reagent is derived from a silkworm larvae plasma.

In an embodiment, the amount of peptidoglycan is further determined by a colorimetric measurement in response to the reaction between the peptidoglycan and the reagent.

In an embodiment, the sufficiently low level of the peptidoglycan is about 10 ng/mL or less.

In an embodiment, the glucose polymer-based solution includes an icodextrin.

In an embodiment, the reagent is added to the icodextrin in a raw material form that is used to make the glucose polymer-based solution.

In another embodiment, the present disclosure~~invention~~ provides a method of providing peritoneal dialysis to a patient. The method includes preparing a peritoneal dialysis solution utilizing a reagent to ensure that the peritoneal dialysis solution has a sufficiently low level of a peptidoglycan so as to prevent peritonitis in the patient; and providing the peritoneal dialysis solution to the patient.

In an embodiment, the peritoneal dialysis solution includes an icodextrin-based solution.

In an embodiment, the peritoneal dialysis includes an automated peritoneal dialysis, a continuous ambulatory peritoneal dialysis and the like.

In an embodiment, the patient is monitored for peritonitis during peritoneal dialysis. For example, a dialysis effluent can be collected from the patient to determine an IL-6 response that correlates to an incidence of peritonitis.

In an embodiment, the reagent is used to determine if the amount of the peptidoglycan exceeds about 10 ng/mL in the peritoneal dialysis solution prior to use during peritoneal dialysis.

In yet another embodiment, the present disclosure~~invention~~ provides a method of testing a peritoneal dialysis solution for an amount of a peptidoglycan that exceeds a level sufficient to cause peritonitis. The method includes adding a reagent to the peritoneal dialysis solution wherein the reagent is capable of reacting with the peptidoglycan to initiate a serine protease cascade; and determining the amount of the peptidoglycan. For example, the icodextrin-based solution can be tested to determine if the amount of peptidoglycan exceeds about 10 ng/mL.

In still yet another embodiment, the present disclosure~~invention~~ provides an icodextrin composition that includes a reagent capable of reacting with a peptidoglycan. .

In an embodiment, a level of peptidoglycan can be established above which the product would produce sterile peritonitis.

In an embodiment, use of affinity columns with resins that bind peptidoglycan can be used to minimize the contamination of peptidoglycan in glucose polymers.

An advantage of the present disclosure~~invention~~ is to provide improved peritoneal dialysis solutions.

Another advantage of the present disclosure~~invention~~ is to provide improved methods for manufacturing and using peritoneal dialysis solutions that employ a detection protocol to determine the presence of peptidoglycan in the peritoneal dialysis solution.

Yet another advantage of the present disclosure~~invention~~ is to provide improved testing procedures that can be employed to prevent peritonitis in patients that receive peritoneal dialysis therapy.

Yet still another advantage of the present disclosure~~invention~~ is to provide an improved icodextrin composition that utilizes a detection procedure in the manufacture thereof to determine the presence of peptidoglycan.

Additional features and advantages of the present disclosure~~invention~~ are described in, and will be apparent from, the following Detailed Description of the Invention and the figures.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates a correlation between IL-6 response in a PBMC assay and peptidoglycan concentration in icodextrin. IL-6 response was measured in freshly isolated monocytes from healthy volunteers. Each symbol and line represent data from a single donor.

Figure 2 illustrates an effect of peptidoglycan on the infiltration of neutrophils in the peritoneal fluid of rats. Peritoneal fluid was collected six hours after single injection (35 mL/kg) of icodextrin containing peptidoglycan.

Figure 3 illustrates an effect of peptidoglycan on TNF-α in the peritoneal fluid of rats.

Figure 4 illustrates an effect of peptidoglycan on IL-6 in the peritoneal fluid of rats.

Figure 5 illustrates frequency (%) of aseptic peritonitis reported with use of icodextrin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention generally relates to the detection of gram positive organisms and fragments thereof. In particular, the present invention relates to methods and compositions that employ the detection of peptidoglycan in peritoneal dialysis solutions. The inventors have surprisingly discovered a novel cause of aseptic peritonitis--aseptic peritonitis associated with gram positive microbial contamination of a dialysis solution. Peptidoglycan is a major component of a gram positive bacterial cell wall and thus can serve as a marker for gram positive bacteria. In this regard, testing for peptidoglycans can be utilized to effectively prevent peritonitis in patients that use the peritoneal dialysis solutions, such as peritoneal dialysis solutions that contain a glucose polymer including an icodextrin and the like.

Aseptic peritonitis associated with icodextrin-based peritoneal dialysis solutions is believed to be the largest adverse event reported for a peritoneal dialysis solution due to a contaminant of microbial origin. Based on the experimental