

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

HUGEL, INC.

Petitioner,

v.

MEDYTOX INC.

Patent Owner.

Case No. IPR2023-00604

Patent No. 11,331,598

**EXPERT DECLARATION OF RONALD C. BATES, PH.D. IN
SUPPORT OF PETITION FOR *INTER PARTES* REVIEW**

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1. My name is Ronald C. Bates. I have been retained by counsel for Hugel, Inc. (“Petitioner”) to provide my opinions regarding the patentability of claims 1-9 of U.S. Patent No. 11,331,598 (EX1001, “the ’598 patent”), which I understand is assigned to Medytox Inc. (“Patent Owner”). I understand that Petitioner is petitioning for *inter partes* review of the ’598 patent, and is requesting cancellation of claims 1-9. My opinions in this expert declaration support Petitioner’s request for *inter partes* review of the ’598 patent and the cancellation of claims 1-9.

I. QUALIFICATION AND BACKGROUND

A. Education and Experience

2. I completed my undergraduate work from Rensselaer Polytechnic Institute in 1993. I obtained my Ph.D. in Chemical and Biochemical Engineering from the University of Maryland in August 1999.

3. Upon obtaining my Ph.D. I joined Pfizer, Inc. as Research Scientist, and later Senior Research Scientist. At Pfizer, I supervised two research groups responsible for purification process method development, process scale-up, and delivery of bulk drug substance for discovery, development, and manufacturing using separation techniques including high pressure liquid chromatography (HPLC) with various stationary phases (chiral, silica, reversed phase, cyano), medium

pressure chromatography (silica, hydrophobic), filtration, precipitation, crystallization, and liquid-liquid extraction.

4. I left Pfizer in 2003 to join Bristol-Myers Squibb as Manager, and later, senior manager. At Bristol-Myers Squibb, I managed the process development group responsible for development and improvement of recovery and purification processes for late-stage biological moieties, including antibodies. I also provided technical guidance and support to manufacturing operations and manufacturing deviation investigations and led a cross-functional team including manufacturing, cell culture, recovery, and downstream development and technical services to identify root cause of harvest operation issue. I also authored and reviewed several CMC Sections of IND, BLA, sBLA and MAA and Downstream Process Validation reports.

5. I joined Allergan in 2007 as Senior Manager, and later director. At Allergan, I led the Biologics Development team for pre-clinical/Phase I biological moieties. I developed and implemented high throughput screening for cell line selection, resin screening, process optimization, and process characterization. I also led technical transfer, process characterization, and process validation teams for next generation process including cell bank selection, manufacture, and stability monitoring, DOE range finding studies for inoculation, fermentation, harvest and

downstream steps, impurity challenge studies, process control strategy, stability strategy.

6. I returned to Bristol-Myers Squibb in 2013 as Director where I managed the Drug Substance Manufacturing Sciences & Technology group responsible for commercial support of biological moieties including Pharmacovigilance (PV)/ Process Performance Qualification (PPQ), regulatory filings, audit support, campaign monitoring, manufacturing support, raw material and consumable strategies and implementation, deviation remediation, technical transfer, continuous improvement, and next generation process development and characterization at several internal and external manufacturing sites.

7. In 2020, I joined Immunovant as Vice President, CMC. In this position, I am responsible for Chemistry Manufacturing and Control (CMC) and Supply Chain activities to rapidly develop and commercialize biopharmaceutical assets in support of Immunovant's strategic objectives.

8. Over the course of my career, I have published over 50 publications in peer reviewed scientific journals, including papers relating to chromatographic purification of proteins, chaired dozens of conference sessions and roundtable discussions, and am a member of the Editorial Board of the Biotechnology Journal. I am also an inventor on a number of patents related to botulinum toxin purification.

9. I have included a copy of my *curriculum vitae* setting forth in more detail my education, training and experience that inform this declaration.

B. Bases for Opinions and Materials Considered

10. In addition to my education, knowledge of the relevant published art, training, and experience, in forming the opinions I provide in this declaration, I have also considered the exhibits in the exhibit list at the end of this declaration.

C. Scope of Work

11. I have been retained by Petitioner as an expert in this matter to provide my various opinions regarding the '598 patent. I receive compensation for my services. No part of my compensation is dependent upon my opinions given or the outcome of this proceeding. I do not have any current or past affiliation with Medytox, or the named inventors on the '598 patent.

II. LEGAL STANDARDS

12. For rendering my opinions in this declaration, I understand that it requires applying various legal principles. As I am not an attorney, I have been informed about various legal principles that govern my analysis. I have used my understanding of those principles in forming my opinions. I summarize my understanding of those legal principles as follows:

13. **Burden of Proof.** I understand that Petitioner bears the burden of proving unpatentability in this proceeding by a preponderance of the evidence. I am

informed that this preponderance of the evidence standard means that Petitioner must show that unpatentability is more probable than not.

14. **Claim Construction.** I have also been told that when I review and consider the claims, the claim term(s) should be analyzed under their ordinary and customary meaning as understood from the perspective of one of ordinary skill in the art at the time of the invention, taking into account the claim language itself, specification, and prosecution history pertaining to the patent, as well as relevant extrinsic evidence. I have applied this standard in formulating my opinions and set forth my understanding of the scope of particular claim terms discussed below.

15. **Obviousness.** I have been asked to consider the question of obviousness/non-obviousness. Again, I am told that this analysis must be from the perspective of the person of ordinary skill in the art, and whether such person would consider any differences between the prior art and what is claimed to have been obvious. To make this assessment, I have been informed that the concept of patent obviousness involves four factual inquiries: the scope and content of the prior art; the differences between the claimed invention and the prior art; the level of ordinary skill in the art; and so-called secondary considerations of non-obviousness.

16. I have further been instructed that one must consider what the person of ordinary skill in the art knew, and what the art taught; suggested; or motivated the person of ordinary skill in the art to further pursue; and to differentiate between steps

that were routinely done (such as in response to known problems, steps, or obstacles), and those which, for example, may have represented a different way of solving existing or known problems. Further I have been informed that one cannot use the challenged patent itself (here, the '598 patent) as a guide from which to select prior art elements, or otherwise engage in hindsight.

17. When there is some recognized reason to solve a problem, and there are a finite number of identified, predictable, and known solutions, a person of ordinary skill in the art has good reason to pursue the known options within his or her technical grasp. I understand that if such an approach leads to the expected success, it is likely not the product of innovation but of ordinary skill and common sense. In addition, when a patent simply arranges old elements with each performing its known function and yields no more than what one would expect from such an arrangement, the combination is obvious.

18. I understand that before reaching any final conclusion on obviousness, the obviousness analysis requires consideration of objective indicia of non-obviousness, if offered. These must be considered to ensure that, for example, there were not some unanticipated problems, obstacles, or hurdles that may seem easy to overcome in hindsight, but which were not readily overcome prior to the relevant invention date of the patents/claims at issue here. I understand that these objective indicia are also known as “secondary considerations of non-obviousness,” and may

include long-felt but unmet need and unexpected results, among others. I also understand, however, that any offered evidence of secondary considerations of non-obviousness must be comparable with the scope of the challenged claims. However, I understand that secondary considerations will not overcome a strong showing of obviousness.

19. I have been informed that where the prior art discloses ranges that overlap the ranges recited in the claims, the burden shifts to the applicant or patentee to come forward with evidence of teaching away, unexpected results, criticality, or other pertinent objective indicia indicating that the overlapping range would not have been obvious.

III. PERSON OF ORDINARY SKILL IN THE ART

20. As I mentioned above, I have been informed by counsel that my analysis is to be conducted from the perspective of a POSA at the time of the invention. I also understand that the person of ordinary skill in the art is assumed to know, understand, and be familiar with all of the relevant prior art, and that such person is not an automaton, but rather a person of ordinary creativity.

21. I have also been informed by counsel that in defining a person of ordinary skill in the art, the following factors may be considered: (1) the educational level of the inventor(s); (2) the type of problems encountered in the art; (3) prior art

solutions to those problems; (4) rapidity with which innovations are made; and (5) sophistication of the technology and educational level of active workers in the field.

22. After considering the above-mentioned factors, it is my opinion that a POSA to whom the '598 patent is directed would have been an individual with at least a graduate degree, such as a masters or Ph.D., in a relevant discipline such as biochemistry, process chemistry, protein chemistry, chemical engineering and/or biochemical engineering, among others, with at least four years of experience in those areas after receiving the graduate degree.

23. While my level of education and experience exceeds that of a POSA in some respects, I have performed my analysis and formed my opinions from the perspective of a POSA as defined herein.

IV. THE '598 PATENT

24. I have read the '598 patent, which is entitled "Method of Isolating Botulinum Toxin from Botulinum Toxin-Containing Solution." EX1001 at Cover. I am very familiar with the state of the art at the time the priority date of the '598 patent, which I have been asked to assume is October 4, 2016.

25. I have reviewed the '598 patent claims, the specification, and the prosecution history. I understand that Petitioner is challenging claims 1-9. I have reproduced those claims below:

Claim No.	Claim Language
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Independent Claim 1	A method of isolating a botulinum toxin type A macro complex from a botulinum toxin-containing solution, the method comprising: culturing <i>Clostridium botulinum</i> in an animal product-free medium to obtain a culture; removing <i>Clostridium botulinum</i> cells from the culture to obtain a botulinum toxin-containing solution; adding an acid to the botulinum toxin-containing solution to precipitate the botulinum toxin and obtain a precipitate; dissolving the precipitate in a medium to obtain a solution; filtering the solution to obtain a botulinum toxin-containing solution; and (a) contacting the botulinum toxin-containing solution with an anion exchange chromatography medium at a pH lower than an isoelectric point (PI) of botulinum toxin; contacting a flow-through portion of the botulinum toxin-containing solution, which is not bound to the anion exchange chromatography medium, with a cation exchange chromatography medium at a pH lower than the PI of botulinum toxin; and separating the botulinum toxin type A macro complex from the cation exchange chromatography medium; or (b) contacting the botulinum toxin-containing solution with a cation exchange chromatography medium at a pH lower than a PI of botulinum toxin; separating botulinum toxin from the cation exchange chromatography medium; contacting a solution containing the botulinum toxin separated from the cation exchange chromatography medium with an anion exchange chromatography medium at a pH lower than the PI of botulinum toxin; and
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	separating the botulinum toxin type A macro complex from a flow-through portion of the solution containing the botulinum toxin, which is not bound to the anion exchange chromatography medium.
Dependent Claim 2	The method of claim 1, further comprising adjusting a pH of the botulinum toxin-containing solution to a pH lower than the PI of botulinum toxin, before the contacting of the botulinum toxin-containing solution with the anion exchange chromatography medium at the pH lower than the PI of botulinum toxin in (a); adjusting a pH of the flow-through portion of the botulinum toxin-containing solution that is not bound to the anion exchange chromatography medium, before the contacting of the flow-through portion of the botulinum toxin-containing solution with the cation exchange chromatography medium at the pH lower than the PI of botulinum toxin in (a); adjusting a pH of the botulinum toxin-containing solution, before the contacting of the botulinum toxin-containing solution with the cation exchange chromatography medium at the pH lower than the PI of botulinum toxin in (b); or adjusting a pH of the solution containing the botulinum toxin separated from the cation exchange chromatography medium, before the contacting of the solution containing the botulinum toxin separated from the cation exchange chromatography medium with the anion exchange chromatography medium at the pH lower than the PI of botulinum toxin in (b).
Dependent Claim 3	The method of claim 2, wherein the adjusting comprises mixing the botulinum toxin-containing solution, the flow-through portion of the botulinum toxin-containing solution, or the solution containing the botulinum toxin with a buffer having a pH lower than the PI of botulinum toxin.
Dependent Claim 4	The method of claim 3, wherein the buffer is a phosphate buffer, a citrate buffer, or an acetate buffer.

Dependent Claim 5	The method of claim 3, wherein the buffer has a pH of 3.5 to 6.0.
Dependent Claim 6	The method of claim 3, wherein the buffer comprises a 10 mM to 100 mM phosphate buffer, a 10 mM to 100 mM citrate buffer, or a 10 mM to 100 mM acetate buffer.
Dependent Claim 7	The method of claim 1, wherein the anion exchange chromatography medium comprises DEAE or Q anion exchange resin.
Dependent Claim 8	The method of claim 1, wherein the cation exchange chromatography medium comprises CM, S, or SP cation exchange resin.
Dependent Claim 9	The method of claim 1, wherein the pH lower than the PI of botulinum toxin is pH 3.5 to 6.0.

26. The patent specification includes a single example entitled “Culture of *Clostridium botulinum* and Purification of *Clostridium botulinum* Toxin.” EX1001 at 7:17 – 10:16. In that example, “a large amount of *Clostridium botulinum* type A toxin was expressed outside cells by culturing *Clostridium botulinum* in an animal product-free medium.” *Id.* at 7:23-25. Following culturing, *Clostridium botulinum* was purified from the culture medium by (1) acid purification, (2) concentration and filtration, (3) anion exchange chromatography, (4) concentration and filtration, and (5) cation exchange chromatography. *Id.* at 8:14 – 10:16.

27. During the prosecution of the ’598 patent, I understand the Examiner rejected the claims as obvious over Kim ’741, and that in response to the rejection, Patent Owner submitted a “Declaration signed by a co-inventor, Jung Hyun SONG”

which compared “the relative efficiency of botulinum toxin recovery between AEX [anion exchange chromatography] using a pH lower than the PI of botulinum toxin and an AEX using pH higher than the PI of botulinum toxin.” EX1006, 7-22-2021 Response at 9. “In the experiments, pH 5.5 and pH 6.5 were selected as a pH lower than the PI of botulinum toxin and a pH higher than the PI of botulinum toxin, respectively.” *Id.*

28. Patent Owner pointed to Figure 1 of the Declaration which “shows a chromatogram for flow through solution and eluted solution from the AEX using pH 5.5.” *Id.* “As shown in FIG. 1, the botulinum toxin containing sample was detected immediately after loading the sample.” *Id.* Patent Owner explained that “[t]his indicates that the botulinum toxin is contained in the flow through solution,” meaning that it did not bind to the anion exchange resin. *Id.* “On the contrary, no significant peaks were (*sic*) appeared in the eluted solution with buffer B (pH 6.5).”

29. Patent Owner next pointed to Figure 3 of the Declaration which “shows a chromatogram for a flow through solution and eluted solution from the AEX using pH 6.5.” *Id.* “As shown in FIG. 3, the botulinum toxin containing sample was detected at about 50 ml retention time and after starting the elution with buffer B after loading the sample.” *Id.* at 9-10. According to Patent Owner, “[t]his indicates that the botulinum toxin is not contained in the flow through solution, but contained

in the eluted solution with buffer B,” meaning that the botulinum toxin bound to the anion exchange resin had to be eluted with elution buffer. *Id.* at 10.

30. Patent Owner stated that “[t]hese results from using pH 5.5 and pH 6.5 show that 19S can be purified from the flow through solution when pH 5.5 is used but at pH 6.5, 19S was not present in the flow through solution but present in the eluted solution.” *Id.* According to Patent Owner, the “unexpected benefit of the claimed invention” is that “when pH 5.5 is used, 19S can be simply purified from the flow through solution with high purity and there is no need to add an additional eluting step.” *Id.*

31. The Examiner allowed the claims, finding that that the inventor declaration submitted during prosecution “was persuasive to overcome the 35 USC 103 rejection of claim 1 as being unpatentable over KIM.” EX1006, 1-21-2022 Notice of Allowance at 3. Specifically, the Examiner stated:

KIM discloses a broad pH range of 3-8 and provides only embodiments where the pH levels are above the pI of botulinum toxin. As argued by Applicant in the reply filed 20 December 2021, one of ordinary skill in the art would not have been motivated to select a pH lower than the pI of botulinum toxin from the pH range of 3-8 disclosed by KIM because KIM does not disclose or provide any reason that the anion exchange or cation exchange processes can be performed at such a lower pH level. Applicant has also provided a declaration by co-inventor SONG (22 July 2021) comparing the relative efficiency of botulinum toxin

recovery from an anion exchange process between using a pH lower than the pI of botulinum toxin and a pH higher than the pI of botulinum toxin, thereby showing the criticality of such a claimed limitation. This showing of criticality was persuasive to overcome the 35 USC 103 rejection of claim 1 as being unpatentable over KIM.

Id., 1-21-2022 Notice of Allowance at 3.

32. As explained below, it is my opinion that the Examiner erred in finding that the declaration showed the criticality of the pH claim limitation.

V. BACKGROUND OF THE STATE OF THE ART

33. The claims of the '598 patent relate to a method of isolating a botulinum toxin type A macro complex comprising routine protein purification steps, including culturing *Clostridium botulinum* in a suitable media, followed by, *inter alia*, centrifugation, acid precipitation, filtration, and ion exchange chromatography. By October 4, 2016, at least the following information was known about botulinum toxin and purifying botulinum toxin:

A. Botulinum Toxin

34. Botulinum toxin was known to be divided into seven types of A through G according to their serological characteristics. I note the claims at issue here are directed to isolating botulinum toxin type A.

35. Each toxin was known to include an about 150 kDa toxin protein, and to be composed of a complex with a variety of non-toxic proteins bound thereto. An

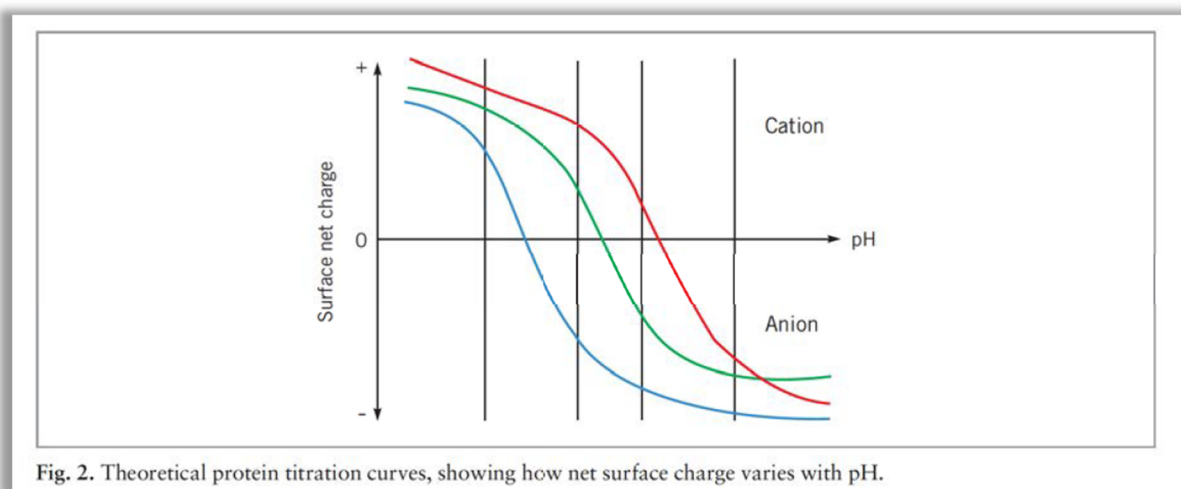
intermediate complex (300 kDa) was known to be composed of a toxin protein and a non-toxic non-hemagglutinin (HA) protein, and large/macro complexes of 500 kDa and 900 kDa, each having a structure in which the intermediate complex is bound to hemagglutinin. EX1010. I note the claims at issue are directed to isolating the botulinum toxin type A macro complex.

B. Purification of Botulinum Toxin from *Clostridium Botulinum*

36. Several methods for purifying botulinum toxin from *Clostridium botulinum* have been published. *See, e.g.*, EX1010-1015. The process usually starts with culturing *Clostridium botulinum* in a suitable media, followed by conventional protein purification steps to separate the toxin from cellular components, such as, acid precipitation, depth filtration (DF), microfiltration (MF), ultrafiltration (UF), sterile filtration, membrane chromatography (MC) and/or centrifugation, to provide a solution containing botulinum toxin. *See also* EX1016 at 153, Appendix 1 (“Sample Preparation”). This solution comprises botulinum toxin and various impurities, and therefore, additional purification steps, such as chromatography, must be performed on the solution so as to obtain a purified botulinum toxin composition.

37. One example of chromatography that can be used to purify botulinum toxin is ion exchange chromatography (“IEX”). Generally, IEX separates proteins (and other biological molecules such as peptides, nucleic acid, or amino acids) on

the basis of surface charge. EX1016 at 9, 13. IEX has been widely used for separation of biomolecules since the 1960s. *Id.* at 9. Each protein has an isoelectric charge. The overall isoelectric charge and the surface charge of a protein will vary with the surrounding pH. A protein present at its isoelectric point (pI) has an equal number of positive and negative charges, and therefore contains a net charge of zero. *Id.* at 13. At its isoelectric point, a protein will still interact with charge matrices because the protein may still contain charged amino acids. *Id.* In general, a protein at a pH above its pI binds stronger to a positively charged separation matrix (*i.e.*, an anion exchanger) because its overall surface charge is negative; but it will bind less strongly to (and flow through) a negatively charged separation matrix. Conversely, a protein at a pH below its pI will bind stronger to a negatively charged separation matrix (*i.e.*, a cation exchanger) because its overall surface charge is positive; but it will bind less strongly to (and flow through) a positively charged separation matrix. *Id.* The relationship between pI and pH is illustrated in the graph below:



EX1016 at 13 (where each line represents a different protein). As can be seen from the graph, each of these proteins has a different pI (e.g., where each line crosses the horizontal line representing a surface charge of 0).

38. Interactions between the surface charges of proteins and an oppositely-charged IEX matrix can be modulated (*e.g.*, by adjusting the pH of a solution) to favor either binding of proteins to the separation matrix, electrostatic repulsion of the proteins from the separation matrix thus preventing binding, or eluting of bound proteins from the separation matrix, thereby achieving separation of proteins from other contaminants or impurities having a different charge. EX1016 at 13.

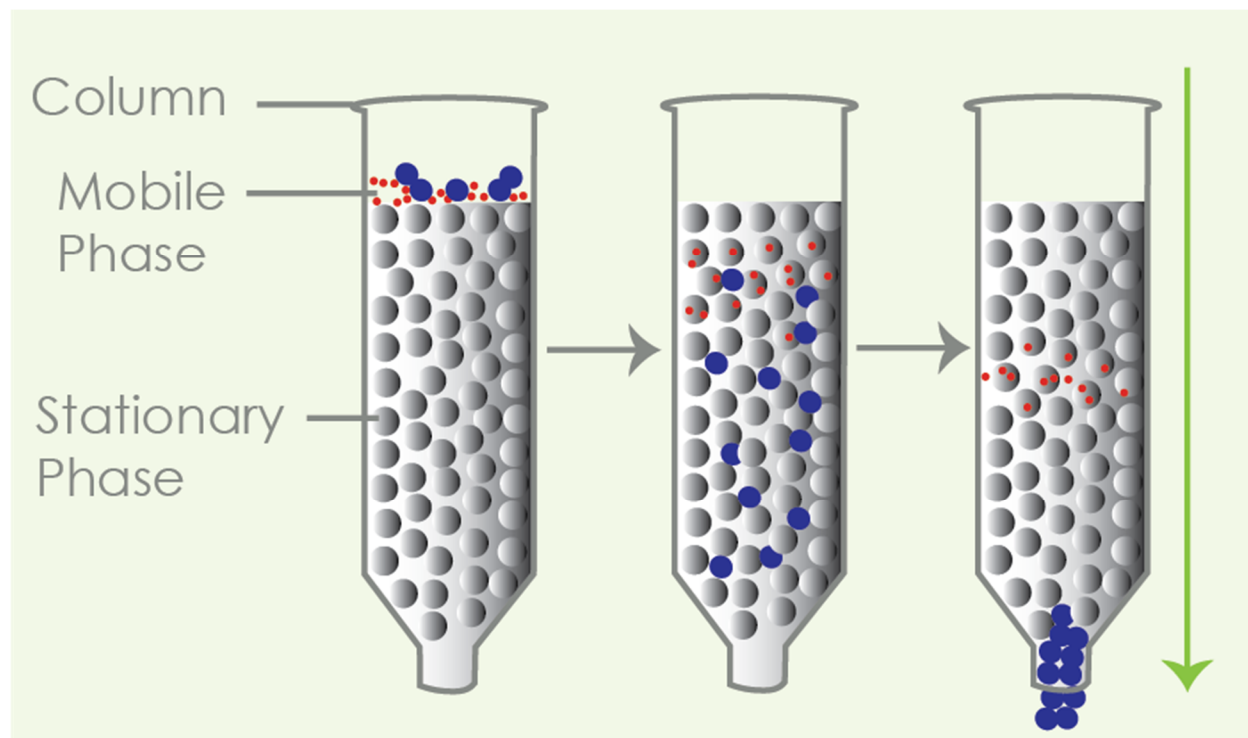
39. IEX conducted under conditions that favor binding of the protein of interest to the separation matrix is typically referred to as “binding mode,” whereas IEX conducted under conditions that favor electrostatic repulsion of the protein of interest from the separation matrix is typically called “flow-through mode.”

40. Ionic strength is another variable that needs to be controlled when conducting ion exchange chromatography. *Id.* at 14. Ionic strength refers to the concentration of ionic charge in solution containing the protein. For example, when conducting IEX in binding mode, protein samples are typically applied to separation matrices at low ionic strength (normally at $<0.05\text{M}$) to maximize the interactions between the protein of interest and the matrix.

41. A POSA as of 2016 understood that the particular pH and ionic strengths of a protein solution during IEX were important to achieve effective protein binding and/or separation. As noted above, the surrounding pH of proteins can impact their surface charges, which will in turn affect their ability to associate with the IEX separation matrix. Similarly, a POSA would have known that a high ionic strength of a protein solution could hinder the interaction of the protein of interest with the IEX separation matrix. Further, it was also known that proteins often begin to dissociate from an IEX separation matrix about 0.5 pH units from their pIs at an ionic strength of about 0.1M. EX1016 at 37. Accordingly, a POSA would have been very familiar with optimizing pH and ionic strength of a protein solution such that the solution is applied to the separation matrix under conditions suitable for the protein to interact with (or flow through or elute from) the separation matrix, while the stability and bioactivity of the protein of interest is maintained during the performance of the separation.

42. Ion exchange chromatography is typically conducted in columns, where the ion exchange resin is packed in columns and the sample containing the biomolecule of interest is applied and allowed to flow into the column containing the resin. Biomolecules that interact with the resin remain in the column while those that do not interact with the resin flow through. An elution buffer can then be applied to the column to release the biomolecules that were bound to the resin, allowing

them to flow through the column. A simple schematic is shown below (where the “mobile phase” contains the biomolecule of interest, and the “stationary phase” is the ion exchange resin):



VI. CLAIM CONSTRUCTION

43. In my opinion, a person of ordinary skill in the art would reach at least the following conclusions regarding the claim language discussed below.

A. Botulinum Toxin Type A Macro Complex

44. Independent claim 1 states “[a] method of isolating a botulinum toxin type A macro complex.” According to the specification, “botulinum toxin type A macro complex, *i.e.*, **900 kDa**, may be efficiently isolated from the botulinum toxin-containing solution.” *Id.* at 6:56-60. Thus, the term “botulinum toxin type A macro

complex” should be construed to include *a botulinum toxin type A of 900 kDa*. See also EX1006, 1-21-2022 Notice of Allowance at 2 (under “Claim Interpretation” the Examiner noting that “[a]s noted in paragraph 6 of the Specification, a botulinum toxin macro complex is 900 kDa and includes 150 kDa of botulinum toxin (A-G) and a complex of a variety of non-toxic proteins.”).

B. A pH Lower than the PI of Botulinum Toxin

45. Independent claim 1 recites conducting anion and cation exchange chromatography “at a pH lower than the PI of botulinum toxin.” According to the specification, “[a]n isoelectric point of botulinum toxin A may be about 6.06. Therefore, the ‘pH lower than the isoelectric point’ may differ depending on the type of the toxin to be isolated. For example, in the case of type A toxin, the pH may be 6.06 or lower ...” EX1001 at 4:16-23. Thus, the term pH lower than the PI of botulinum toxin would be understood by a POSA reading the ’598 patent to mean *a pH lower than 6.06*. See also EX1006, 1-21-2022 Notice of Allowance at 2 (under “Claim Interpretation” the Examiner noting that “[a]s noted in paragraph 21 of the Specification, the isoelectric point (pI) of botulinum toxin Type A is about 6.06.”).¹

¹ A POSA would have been able to calculate the isoelectric point of botulinum toxin type A based on its amino acid sequence (*see, e.g.*, EX1027), for example by using Expasy bioinformatics suite, https://web.expasy.org/compute_pi/, which was known

VII. DETAILED OPINIONS

A. The Asserted Prior Art²

1. Jung

46. Based on the cover of Jung (U.S. Patent No. 8,993,268, EX1002), entitled “Method of producing *Clostridium botulinum* toxin using media containing plant-derived components and flexible closed container,” I see that it issued on March 31, 2015. EX1002 at Cover. This date is before October 4, 2016, the assumed effective filing date of the ’598 patent. I understand that Jung is therefore prior art to the ’598 patent.

47. Based on the cover of Jung, I also see that four of the inventors overlap with the inventors of the ’598 patent. I also note that Medytox is the assignee of both Jung and the ’598 patent.

prior to 2016 and was “provided as a service to the life science community” and “free for academic users” as noted on pages 3784-3785, and “computes the theoretical isoelectric point (pI).” EX1026.

² Throughout this declaration, I refer to certain references that are not explicitly enumerated in Ground 1 and 2. These references are being used to show the common knowledge in the art at the relevant time.

48. Jung discloses “a method of producing *Clostridium botulinum* toxin by using a media containing plant-derived components.” EX1002 at Abstract. Jung states that:

[T]he inventors of the present application found that a large amount of *Clostridium botulinum* toxin was expressed outside the cell by using a composition of plant derived components, and *Clostridium botulinum* toxin could be effectively produced ... In addition, the inventors of the present application found that *Clostridium botulinum* toxin could be easily and very simply purified from the culture from which the cell was removed.

Id. at 2:46-55. The Jung process producing *Clostridium botulinum* toxin by using a media containing plant-derived components includes the following steps:

In operation (a), the culturing may be performed by introducing a culture media in a closed container including an inlet and an outlet and comprised of a flexible material, inoculating the culture media with *Clostridium botulinum*, and culturing the cell. ... [T]he cell is removed from the obtained culture to obtain the portion including *Clostridium botulinum* toxin expressed outside the cell, and the toxin is isolated from the portion (operation (b)). ... [O]peration (b) may include: removing the cell from the culture, adding an acid to the obtained culture to precipitate *Clostridium botulinum* toxin, and then isolating the precipitation (operation (b-1)); dissolving the isolated precipitation in a liquid medium and ultra-filtrating the resulting solution (operation (b-2)); and performing anion exchange chromatography on the ultra-filtered solution, i.e., the retentate.

Id. at 3:63 – 4:48.

49. Example 3 of Jung is entitled “Isolation of *Clostridium botulinum* Type A Toxin from a Supernatant of Culture Using a Media Containing Plant-Derived Components.” *Id.* at 11:24-27. The Example includes culturing *Clostridium botulinum* cells, removing the cells from the culture, precipitating the botulinum toxin with acid, dissolving the resulting precipitate, filtering the resulting solution, and applying the filtered solution to anion exchange chromatography at pH 5.5. *Id.* at 11:27-39.

50. Specifically, in Example 3, “*Clostridium botulinum* was cultured using [animal protein free medium] and *Clostridium botulinum* type A toxin was isolated from a supernatant from which the cell was removed.” *Id.* at 11:27-30. “0.1 N sulfuric acid was added to a bag including 20 L of a culture Supernatant to adjust the pH of the resulting Solution to be in the range of 3.0 to 4.5, and as a result, toxin protein was precipitated.” *Id.* at 11:33-37. “[T]he resulting solution was filtered through a sterilized disposable filtration system.” *Id.* at 11:38-40. “25 mM citrate buffer (pH 5.5) was added to the collected precipitation to re-dissolve toxin protein existing in the precipitation.” *Id.* at 11:51:54. “Then, DEAE anion exchange chromatography and membrane chromatography were performed on the sample not in contact with the outside.” *Id.* at 12: 9-11.

After the sample was loaded on the system, the *Clostridium botulinum* type A toxin protein in the sample was not bound to a column while other impurities [*sic*] were bound to the column, and thus a fraction flowing out of the system was collected. As a result, impurities were removed with no contact with the outside, and *Clostridium botulinum* type A toxin protein with high purity was collected. In addition, the yield of *Clostridium botulinum* type A toxin purified in the media containing plant-derived components was about 5-10 times higher than the yield of the toxin purified in the animal-derived medium used as a control.

Id. at 12:12-22.

2. Kim '741

51. Based on the cover, I see that Kim '741 (U.S. Patent Publication No. 2018/0251741, EX1004), entitled "Method for producing botulinum toxin," claims priority to Korean Patent Application No. 10-2015-0168196, filed on November 30, 2015. EX1004 at Cover.³ Kim '741's priority application pre-dates October 4, 2016,

³ I understand that Petitioner has provided a certified translation of the Kim '741 priority application KR10-2015-0168196 (EX1008) as well as the original document in its native Korean language. EX1019. I have reviewed the certified translation of KR10-2015-0168196 and confirm that it contains substantially the same disclosure as Kim '741 in all material respect.

the assumed effective filing date of the '598 patent. I understand that Kim '741 is therefore prior art to the '598 patent.

52. Kim '741 discloses “a method for producing a botulinum toxin by an animal product-free (APF) process.” EX1004 ¶ [0001]; EX1008 ¶ [28]. “The botulinum toxin purified according to the production method of the present invention may have a form of 7S or 19S (molecular weight: 150-900 kDa).” EX1004 ¶ [0046]; EX1008 ¶ [48]. Kim generally discloses culturing *Clostridium botulinum* cells, adding an acid to the culture to precipitate the botulinum toxin, dissolving the precipitate, clarifying the resulting solution, subjecting the clarified solution to anion exchange chromatography and cation exchange chromatography.

53. Specifically, the process of Kim '741 includes:

- (a) treating a culture of a botulinum toxin-producing strain with acid to form a botulinum toxin-containing precipitate;
- (b) adding a buffer to the botulinum toxin-containing precipitate of step (a), and then clarifying by one or more method selected from the group consisting of depth filtration (DF), microfiltration (MF), ultrafiltration (UF), sterile filtration, membrane chromatography (MC) and centrifugation;
- (c) subjecting the botulinum toxin-containing solution of step (b) to UF diafiltration, ammonium sulfate precipitation or hydrochloric acid precipitation, and then diluting a retentate resulting from the UF diafiltration in a buffer, or dissolving a precipitate resulting from the

ammonium sulfate precipitation or hydrochloric acid precipitate in a buffer; and

(d) subjecting the retentate dilution, ammonium sulfate precipitate solution or hydrochloric acid precipitate solution of step (c) to anion-exchange chromatography (AEX), thereby purifying the botulinum toxin.

EX1004 ¶¶ [0042] – [0045]; EX1008 ¶¶ [42] – [45].

54. Kim '741 discloses that “the present invention provides a method for producing a botulinum toxin, further comprising, after step (d), the steps of: (e) adjusting the pH of the botulinum toxin-containing anion exchange chromatography fraction upward; (f) purifying the pH-adjusted anion exchange chromatography fraction resulting from step (e) by anion exchange chromatography; and (h) purifying the diluted botulinum toxin resulting from step (f) using cation exchange chromatography (CEX)” EX1004 ¶¶ [0077] – [0080]; EX1008 ¶¶ [78] – [84]. Kim '741 discloses that “the method may further comprise, between step (f) and step (h), step (g) of adding a buffer to the purified anion exchange chromatography fraction to dilute the botulinum toxin.” EX1004 ¶ [0081].

55. According to Kim '741, “[t]he anion-exchange chromatography in step (d) may be performed at a pH of 2-9, preferably 3-8, and a conductivity of 2-40 mS/cm, preferably 3-30 mS/cm. In step (d), botulinum toxin may be collected as a botulinum toxin-containing fraction (flow-through mode) of a flow-through (FT)

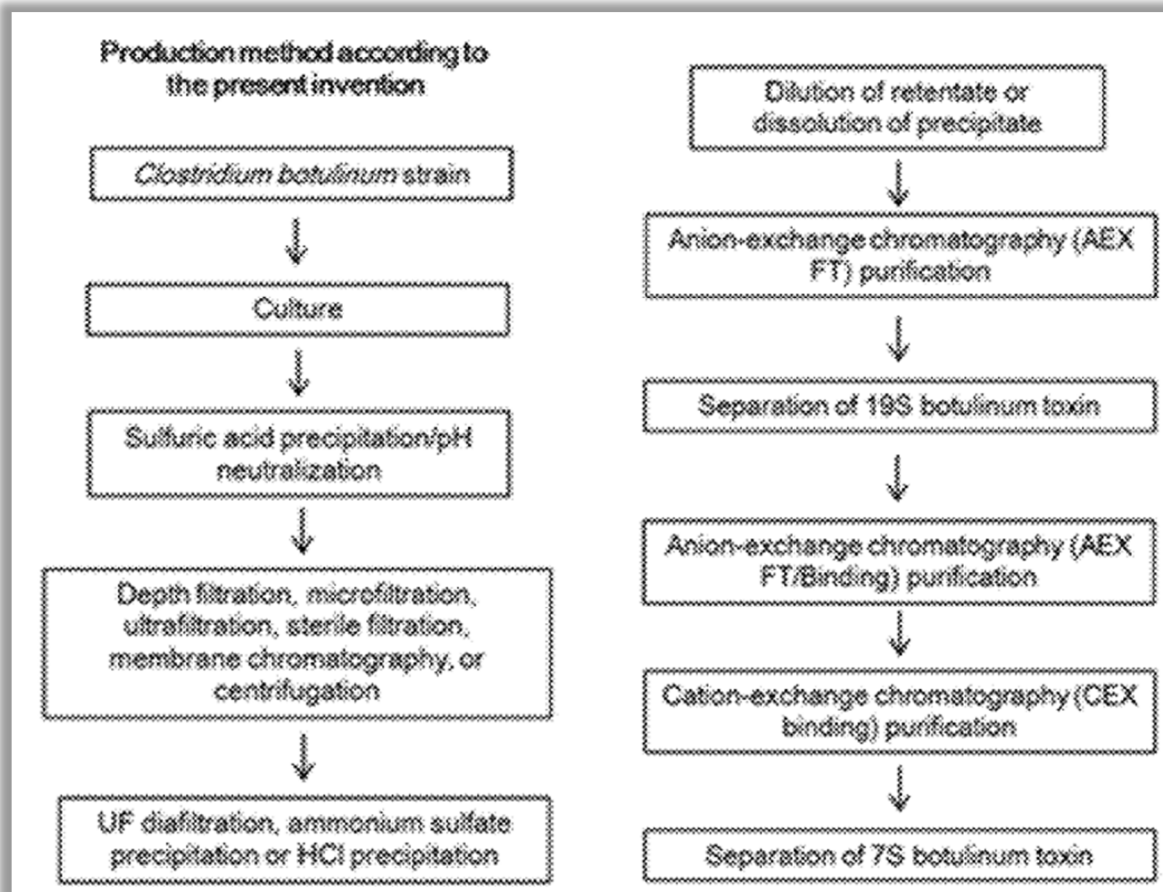
fraction eluted from anion-exchange chromatography or as a fraction containing the botulinum toxin bound to anion exchange chromatography resin.” EX1004 ¶ [0072]; EX1008 ¶ [74]. Kim ’741 explains that:

The term “FT (flow-through)”, “flow-through process” or “flow-through purification”, as used interchangeably herein, means a separation process in which at least one target molecule (e.g., botulinum toxin) contained in a biopharmaceutical formulation together with one or more impurities passes through a material and one or more impurities generally bind to the material and the target molecule generally does not bind to the material (that is, flows through the materials).

EX1004 ¶ [0072]; EX1008 ¶ [75].

56. According to Kim ’741, “the cation-exchange chromatography in step (h) may be performed at a pH of 2-9, preferably 3-8 [and] the botulinum toxin may be collected as a fraction containing the botulinum toxin bound to the cation-exchange chromatography resin.” EX1004 ¶ [0087]; EX1008 ¶ [93].

57. Kim ’741 summarizes the disclosed process as follows:



EX1004 at Figure 1; EX1008 at Figure 1.

3. Kim '565

58. Based on the cover of Kim '565 (International Patent Publication WO2016/175565, EX1023), entitled "Medium composition for preparing botulinum toxin," I see that it was filed on April 28, 2016. EX1023 at Cover. April 28, 2016 pre-dates October 4, 2016, the assumed effective filing date of the '598 patent. I understand that Kim '565 is therefore prior art to the '598 patent.

59. Kim '565 "provides a medium composition for culture of *Clostridium botulinum*, the medium composition comprising: at least one plant-derived peptone

selected from the group consisting of a garden pea hydrolysate, a cotton seed hydrolysate and a wheat gluten hydrolysate.” EX1023 ¶ [21]. Kim ’565 states that:

In the present invention, it was attempted to prepare a medium that further increases the growth rate of *Clostridium botulinum* compared to a medium that is in current use (original medium) and have no risk of infection with TSE or the like. Thus, an animal protein-free (APF) medium containing plant-derived peptones were used, and the growth of a bacterium in the APF medium was examined. As a result, the APF medium showed an increased growth rate of the bacterium compared to a medium that is in current use. Thus, if the APF medium is used, a high concentration of botulinum toxin can be produced by culturing a bacterium in a safe manner under TSE-free conditions.

EX1023 ¶ [37]. According to Kim ’565:

[T]he term “medium that is in current use or original medium” means a medium comprising casein hydrolysate, yeast extract and thioglycollate medium, which are animal-derived medium components. The term “APF medium (animal protein-free medium)” means a medium that contains no animal-derived protein and that contains plant-derived peptones, minerals and glucose.

Id. ¶ [38].

4. Ton

60. Based on the cover of Ton (U.S. Patent No. 8,932,827, EX1003), entitled “Process and system for obtaining botulinum neurotoxin,” I see that Ton

issued January 13, 2015, which is before October 4, 2016, the assumed effective filing date of the '598 patent. I understand that Ton is therefore prior art to the '598 patent.

61. Ton discloses “a substantially APF chromatographic process for obtaining a biologically active botulinum neurotoxin.” EX1003 at 9:33-35. The process of Ton comprises the steps of:

(a) providing a substantially APF fermentation medium; (b) fermenting *Clostridium botulinum* bacteria in the fermentation medium, and; (c) recovering the biologically active botulinum neurotoxin from the fermentation medium by contacting the fermentation medium with an anion exchange chromatography media followed by contacting an eluent from the anion exchange chromatography medium with a cation exchange chromatography media, to thereby obtain the biologically active botulinum neurotoxin from the substantially APF chromatographic process.

Id. at 9:36-45.

62. In a specific example, Ton discloses conducting anion exchange chromatography at pH 6.5 and eluting the botulinum toxin with “50 mM sodium acetate, pH 4.8.” *Id.* at 40:8-18. Ton discloses diluting the eluate pool with 1 column volume of 50 mM sodium acetate, pH 4.8, and then applying the eluate pool to a cation exchange chromatography column. *Id.* at 40:19-26. Specially:

The cation exchange column (e.g. Poros® 20HS) was equilibrated with a minimum of 5 CVs of equilibration buffer (50 mM sodium acetate, pH 4.8). After equilibration, the diluted eluate pool from the anion exchange column was loaded onto the cation exchange column. ... Elution of bulk toxin complex from the cation exchange column was carried out utilizing elution buffer (e.g., 250 mM sodium chloride in 50 mM sodium acetate, pH 4.8).

Id. at 40:26-47.

B. Ground 1: Claims 1-9 Would Have Been Obvious to a POSA in 2016 in view of Jung in Combination with Ton

63. I was asked to review the Challenged Claims of the '598 patent and compare them to the disclosures of Jung and Ton. It is my opinion that the Challenged Claims would have been obvious to a POSA in view of Jung in combination Ton.

1. Independent Claim 1

a) “A method of isolating a botulinum toxin type A macro complex from a botulinum toxin-containing solution”

64. Jung discloses this limitation. For example, the Jung specification discloses “*a method of producing Clostridium botulinum toxin* by using a media containing plant-derived components.” EX1002 at Abstract. Jung states that “[t]he *Clostridium botulinum* toxin may be one selected from the group consisting of botulinum toxin A, B, C, D, E, F, and G, and *preferably, botulinum toxin A.*” *Id.* at 6:36-38. Jung explains that “[e]ach toxin has about 150 kDa of toxin protein, and

naturally comprises a complex with a variety of non-toxic proteins bound thereto. An intermediate complex (300 kDa) comprises toxin protein and non-toxic non-hemagglutinin protein, and a large complex (500 kDa) and *a macro complex (900 kDa).*” *Id.* at 1:43-49. Moreover, in Jung, “the *toxin existing in the culture* from which the cell is removed is isolated.” EX1002 at 4:37-38. According to the ’598 patent, “the botulinum toxin-containing solution may be a *culture of Clostridium botulinum.*” EX1001 at 3:28-30. Thus, “the culture from which the cells are removed” in Jung is a “botulinum toxin-containing solution” according to the ’598 patent.

b) “culturing *Clostridium botulinum* in an animal product-free medium to obtain a culture”

65. Jung discloses this limitation. For example, the Jung specification discloses that “*Clostridium botulinum was cultured* using the medium 5 of Example 1 or 2.” EX1002 at 11:28-29. Medium 5 of Example 1 or 2 is *animal product-free.* *Id.* at Table 1. Moreover, the “culturing” step described in Jung is essentially the same as that disclosed in the ’598 patent:

Jung Example 3	’598 Patent Example
“ <i>Clostridium botulinum was cultured</i> using the medium 5 of Example 1 or 2.” EX1002 at 11:28-29. “The culturing conditions and time and the cell removal process were the same as those in Example 1.” <i>Id.</i> at 11:29-32.	“In this Example, a large amount of <i>Clostridium botulinum</i> type A toxin was expressed outside cells by <i>culturing Clostridium botulinum</i> in an animal product-free medium.” EX1001 at 7:23-25.

Medium 5: phytone peptone 1%; <i>yeast extract 1%; glucose 0.5%.</i> <i>Id.</i> at Table 1. In Example 1, “200 ml of each of the media of Table 1 and the control media was filtered through a sterilized single use microfiltration system ... so as to be injected into Cultibag™ ... <i>Then, each media was inoculated with 1ml of a seed inoculum including Clostridium botulinum type A with a concentration of 10⁶-10⁷ cells/ml through a sample injection port.</i> Cultibag™ is a disposable bioreactor that may use rocking motion in order to cause mixing.” <i>Id.</i> at 8:56 – 9:2.	“The used medium was a medium containing 4% <i>yeast extract and 0.5% glucose.</i>” <i>Id.</i> at 7:26-27.⁴ “200 ml of the medium thus prepared was filtered through a sterile single use microfiltration system ... and injected into Cellbag™ ... <i>Then, the medium was inoculated with 1 ml of a seed culture of Clostridium botulinum at a density of 10⁶ cells/ml – 10⁷ cells/ml through a sample injection port.</i> Cellbag™ is a disposable bioreactor that uses rocking motion in order to cause mixing under low shear stress.” <i>Id.</i> at 7:31-39.
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EX1022.

c) **“removing *Clostridium botulinum* cells from the culture to obtain a botulinum toxin-containing solution”**

66. Jung discloses this limitation. For example, the Jung specification discloses that “[i]n an embodiment, operation (b) may include ***removing the cells from the culture***, adding an acid to obtain a precipitate.” EX1002 at 6:45-47.

⁴ The '598 patent also discloses that “[t]he medium containing plant-derived components may include phytone peptone, and preferably, phytone peptone, yeast extract, and glucose.” EX1001 at 3:43-45.

Moreover, the “removing *Clostridium botulinum* cells” step described in Jung is essentially the same as that disclosed in the ’598 patent:

Jung Example 3	’598 Patent Example
“After the culturing was terminated, a sterile connector QDC connector (manufactured by Satorious) was connected to the sample port of the bag ... and <i>the culture was filtered through depthfilter and the microfilter to remove the cell.</i> ” EX1002 at 9:36-46.	“After the culturing was terminated, a QDC connector (manufactured by Satorious) which is a sterile connector was connected to the sample port of the bag... and <i>the culture was filtered therethrough to remove the cells.</i> ” EX1001 at 8:1-9.

EX1022.

- d) “adding an acid to the botulinum toxin-containing solution to precipitate the botulinum toxin and obtain a precipitate”

67. Jung discloses this limitation. For example, the Jung specification discloses that “0.1 N ***sulfuric acid was added*** to a bag including 20 L of a culture supernatant to adjust the pH of the resulting solution to be in the range of 3.0 to 4.5, and as a result, ***toxin protein was precipitated.***” EX1002 at 11:33-36. Moreover, the “acid precipitation” step described in Jung is essentially the same as that disclosed in the ’598 patent:

Jung Example 3	’598 Patent Example
“0.1 N <i>sulfuric acid was added</i> to the bag including 20 L of a culture supernatant to adjust the pH of the resulting solution to be in the range of 3.0 to 4.5, and as a result, <i>toxin</i>	“1 N <i>sulfuric acid was added</i> to a bag containing 20 L of the culture supernatant obtained as above to adjust pH from 3.0 to 4.5, and as a

<i>protein was precipitated.</i> ” EX1002 at 11:33-36.	result, <i>toxin protein was precipitated.</i> ” EX1001 at 8:19-21.
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EX1022.

e) **“dissolving the precipitate in a medium to obtain a solution”**

68. Jung discloses this limitation. For example, the Jung specification discloses that “10 L of a 25 mM citrate buffer (pH 5.5) was added to the collected precipitation to *re-dissolve toxin protein existing in the precipitation.*” EX1002 at 11:51-53. Moreover, the “dissolving” step described in Jung is essentially the same as that disclosed in the ’598 patent:

Jung Example 3	’598 Patent Example
“10 L of a 25 mM citrate buffer (pH 5.5) was added to the collected precipitation to <i>re-dissolve toxin protein</i> existing the precipitation.” EX1002 at 11:51-53.	“10 L of citrate buffer (25 mM, pH 5.5) was added to the collected precipitate to <i>redissolve toxin protein</i> existing in the precipitate.” EX1001 at 8:37-39.

EX1022.

f) **“filtering the solution to obtain a botulinum toxin-containing solution”**

69. Jung discloses this limitation. For example, the Jung specification discloses that “sterilized disposable filtration system (0.2 um, ULTA Cap HC, GE, USA) was connected to the bag including the redissolved toxin protein and *filtration was performed on the toxin protein.*” EX1002 at 11:54-57. Moreover, the

“filtering” step described in Jung is essentially the same as that disclosed in the ’598 patent:

Jung Example 3	’598 Patent Example
<i>“[F]iltration was performed on the toxin protein through the system not in contact with the outside. As a result, the supernatant was collected in a sterilized disposable bag and insoluble impurities were removed... and a buffer exchange was performed on the enriched resultant by using a 25 mM citrate buffer (pH 5.5).” EX1002 at 11:57 – 12:1.</i>	<i>“[F]iltration was performed without being in contact with the outside. As a result, the supernatant was collected in a sterile disposable bag and insoluble impurities were removed.” EX1001 at 8:43-46.</i>

EX1022.

- g) **“contacting the botulinum toxin-containing solution with an anion exchange chromatography medium at a pH lower than an isoelectric point (PI) of botulinum toxin”⁵**

70. Jung discloses this limitation. For example, the Jung specification discloses a buffer exchange step following the filtration step “using a 25 mM citrate buffer (**pH 5.5**).” EX1002 at 11:65 – 12:1 (“Then, the sample was filtered with no contact with the outside to be enriched, and a **buffer exchange** was performed on

⁵ Claim 1 of the ’598 patent recites an element (b) as an alternative to element (a). I understand that a claim element reciting alternatives is disclosed if the prior art discloses one of the alternatives.

the enriched resultant by using a **25 mM citrate buffer (pH 5.5).**”). Jung then discloses applying the buffer-exchanged toxin containing solution to a DEAE anion exchange chromatography resin. *Id.* at 12:9-12. While Jung does not expressly disclose equilibrating the anion exchange resin prior to sample application, a POSA would understand that this step was done because equilibration is an essential step in any ion exchange chromatography protocol. As explained in GE Healthcare’s 2002 handbook on Ion Exchange Chromatography, “[t]he first stage is **equilibration** in which the ion exchanger is brought to a starting state, in terms of pH and ionic strength, which allows the binding of the desired solute molecules.” EX1030 at 12; *id.* at 83 (“[I]t is **essential** that the exchanger is brought to **equilibrium with start buffer before sample application.**”); *id.* at 85 (“The ionic composition [of the sample] **should be the same** as that of the starting buffer.”). Thus, a POSA, reading Jung, would have understood that the column in Jung was first equilibrated with **citrate buffer pH 5.5**, e.g., the buffer containing the toxin sample (which a POSA would also call the “start buffer” or “load buffer”).⁶ A POSA would therefore have

⁶ Indeed, as taught by “Instructions 56-1191-00 AF” by GE Healthcare (which include instructions for GE’s DEAE anion exchange resin, the same resin used by Jung), “[t]o **equilibrate**, pump approximately 100 ml of **start buffer** through the column [until] the pH and/or conductivity of the effluent is the same as the start

understood that the anion exchange chromatography step in Jung was performed at *pH 5.5* since the buffer containing the toxin prior to conducting anion exchange chromatography was *buffer exchanged with a citrate buffer pH 5.5* and the column was *equilibrated with citrate buffer pH 5.5*. A pH of 5.5 is “lower than an isoelectric point (PI) of botulinum toxin” as that term is properly construed, *e.g.*, a pH lower than 6.06. Moreover, the “anion exchange chromatography” step described in Jung is essentially the same as that disclosed in the ’598 patent:

Jung Example 3	'598 Patent Example
“Then, the sample was filtered with no contact with the outside to be enriched, and a <i>buffer exchange</i> was performed on the enriched resultant by using <i>a 25 mM citrate buffer (pH 5.5)</i>. ... <i>Then, DEAE anion exchange chromatography and membrane chromatography were performed</i> ... The disposable chromatography system was pre-washed. After the sample was loaded on the system, the <i>Clostridium botulinum type A toxin protein in the sample was not bound to a column</i> while other	<i>“DEAE anion exchange chromatography was performed ...</i> In detail, the citrate buffer (25 mM pH 5.5) was passed through a column packed with a DEAE anion exchange resin to equilibrate the column, and the toxin-containing <i>citrate buffer (25 mM pH 5.5)</i> was passed through the column. In this process, <i>Clostridium botulinum type A toxin was not adsorbed to the anion exchange resin</i>, and most impurities were adsorbed thereto and removed.” EX1001 at 8:60 – 9:2.

buffer.” EX1028 at 6. Although my opinion is that a POSA would have known that Jung equilibrated the DEAE column with start buffer prior to sample application, it would have been routine for a POSA to consult the GE manuals (EX1028 and EX1030) for guidance.

impurities [<i>sic</i>] were bound to the column, and <i>thus a fraction flowing out of the system was collected.</i> EX1002 at 12:11:65 – 12:16.	<i>“Next, a fraction containing Clostridium botulinum type A toxin was collected from the flow-through solutions</i> which were not bound to the anion exchange resin during the anion exchange chromatography.” <i>Id.</i> at 9:5-8.
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EX1022.

- h) **“contacting a flow-through portion of the botulinum toxin-containing solution, which is not bound to the anion exchange chromatography medium, with a cation exchange chromatography medium at a pH lower than the PI of botulinum toxin”**

71. Jung discloses the collection of the flow-through portion of the botulinum toxin-containing solution, which is not bound to the anion exchange chromatography medium. For example, the Jung specification discloses that “[a]fter the sample was loaded on the system [containing the anion exchange chromatography column], ***the Clostridium botulinum type A toxin protein in the sample was not bound to a column*** while other impurities [*sic*] were bound to the column, ***and thus a fraction flowing out of the system was collected.***” EX1002 at 12:12-19. In other words, the anion exchange chromatography step disclosed in Jung is operated in flow-through mode.

72. Jung does not disclose applying the flow-through fraction of the anion exchange chromatography step to a cation exchange chromatography column. However, by 2016, a POSA would have been motivated to modify Jung by adding a

cation exchange chromatography binding step downstream of Jung's flow-through anion exchange chromatography step in view of Ton, with a reasonable expectation of success, as shown below.

73. First, Ton would have motivated a POSA to add a cation exchange chromatography step downstream of Jung's flow-through anion exchange chromatography step. Ton discloses "**high purity**, highly potent **botulinum neurotoxins** obtainable by rapid, smaller scaled, commercially useful, high yield, **animal protein free, chromatographic systems and processes.**" EX1003 at 6:60-64. According to Ton, "[a]n important aspect of our invention is use of an anion exchange (capture) media chromatography ***followed by use of cation exchange (polishing) media chromatography*** to purify botulinum neurotoxin from an APF fermentation medium in which *Clostridium botulinum* bacterium have been fermented." *Id.* at 24:33-38. Ton states "that use of anion exchange ***followed by use of cation exchange chromatography*** media provides an effective and rapid method for obtaining **high purity, high yield botulinum neurotoxin.**" *Id.* at 24:38-41. Therefore, Ton discloses combining anion exchange chromatography with cation exchange chromatography for the purification of botulinum toxin from *Clostridium botulinum*.

74. In a specific example, Ton discloses that “[f]ollowing the run-through of the anion exchange column, *cation exchange chromatography operation was undertaken.*” *Id.* at 40:25-26. Specifically:

Following the run-through of the anion exchange column, cation exchange chromatography operation was undertaken. The cation exchange column (e.g. Poros® 20HS) was *equilibrated* with a minimum of 5 CVs of equilibration buffer (50 mM sodium acetate, *pH 4.8*). *After equilibration, the diluted eluate pool from the anion exchange column was loaded onto the cation exchange column.*

Id. at 40:8-31. As disclosed in Ton, the eluate pool of the anion exchange chromatography of Ton is at a *pH of 4.8*. EX1003 at 40:8-24. A pH 4.8 is “a pH lower than the PI of botulinum toxin” as that term is properly construed, e.g., a pH lower than 6.06. Therefore, Ton discloses conducting cation exchange chromatography at “a pH lower than the PI of botulinum toxin.”

75. A POSA would have been motivated to conduct Ton’s cation exchange chromatography step downstream of Jung’s flow-through anion exchange chromatography step, particularly in light the express motivation provided by Ton “that use of *anion exchange followed by use of cation exchange chromatography* media provides an effective and rapid method for obtaining *high purity, high yield botulinum neurotoxin.*” EX1003 at 24:38-41; *also see id.* at 24:47-49 (“*Another important aspect of our invention is that it results in high purity* botulinum

neurotoxin”). In my opinion, motivation would have also come from a POSA’s common knowledge that it is generally accepted that more than one chromatography step is needed to achieve a high level of purity.

76. Indeed, as I explained in a book chapter published in 2006:

In certain instances, the buffer conditions can be manipulated to allow either the product to flow through the column unretained and the impurities to be bound to the absorber, or the product to be bound and the impurities to be unretained. This is especially useful to remove nucleic acids (which are negatively charged) from a basic protein on an anion-exchanger column. Care must be taken when using chromatography resins to bind impurities since not all impurities are likely to bind to the resin. *If used early in the purification process, flow-through columns are extremely productive, but it is generally accepted that a binding step (i.e., where the desired solute is bound to a resin) is needed downstream of a flow-through chromatographic step.*

EX1009 at 461⁷; see also EX1024 at 484-487 (discussing monoclonal antibody processes that typically use 3-columns: Protein A capture chromatography, followed

⁷ I note that flow-through chromatography can be productive when conducted either upstream or downstream of a binding step. The point I was trying to emphasize in this Chapter is that to ensure adequate impurity removal, a purification process

by bind/elute cation exchange, and either bind/elute anion or flow-through anion exchange chromatography); EX1025 at 305-308 (discussing Protein A capture chromatography, cation exchange chromatography and anion exchange chromatography (flow-through mode) and stating that “[t]hese three steps together comprise a process that, while meeting stringent purity and throughput restrictions still produces a high yield of antibody.”) Thus, in my opinion, a POSA, seeking to develop a method for purifying botulinum toxin type A with high purity (which I note is the alleged “industrial applicability” of the ’598 patent (EX1001 at 10:20-22)), would have been motivated to modify Jung by adding a further chromatography step, such as the binding cation exchange chromatography step disclosed in Ton.

77. A POSA would have had a reasonable expectation of success in making such a combination. For example, Ton teaches “that use of anion exchange followed by use of cation exchange chromatography media provides an effective and rapid method for obtaining *high purity, high yield botulinum neurotoxin.*” EX1003 at 24:38-41. Moreover, as I explained in my book chapter, flow-through chromatography in combination with by a binding chromatography step can be

should contain at least one binding step, in addition to a flow through step, if such a step is included in the process.

“extremely productive.” EX1009 at 461. And a POSA would have known to select the pH of a protein solution so that the desired protein will either bind or flow through the separation matrix during ion exchange chromatography. Therefore, adding a binding cation exchange chromatography step downstream of Jung’s flow through anion exchange chromatography step would have been routine to a POSA in 2016.

78. I understand that Patent Owner may argue that Ton “teaches away” from flow-through anion exchange chromatography (because it discloses anion exchange chromatography in binding mode), and as such, a POSA would not have combined Ton with Jung. Having reviewed Ton, however, I have not seen any disclosure in Ton that criticizes, discredits, or otherwise discourages flow-through anion exchange chromatography. Moreover, as noted, Ton discloses *eluting* the botulinum toxin from the anion exchange resin with an elution buffer having a *pH of 4.8* (indicating that the botulinum toxin *does not bind* to the anion exchange resin at pH 4.8). Thus, a POSA reading Ton, would have understood that flow-through anion exchange chromatography *can be achieved* at “a pH lower than the PI of botulinum toxin.” Thus, a POSA would not have been discouraged from attempting flow through anion exchange chromatography on a botulinum toxin containing solution from reading Ton.

i) “separating the botulinum toxin type A macro complex from the cation exchange chromatography medium”

79. Ton discloses this limitation. For example, the Ton specification discloses that “[e]lution of bulk toxin complex from the cation exchange column was carried out utilizing elution buffer.” EX1003 at 40:45-46; *also see id.* at 27:25-27 (“Unless otherwise set forth in the examples “toxin” or “botulinum toxin” means a botulinum toxin *type A complex with a molecular weight of about 900 kDa.*”)

* * *

80. In view of the disclosures described above, it would have been obvious to a POSA to perform the claimed method in view of Jung and Ton. EX1021 ¶¶64-82. As an initial matter, Jung and Ton are related to the same field of endeavor—botulinum toxin purification—as the ’598 patent. EX1001, Title; EX1002, Title; EX1003, Title. Furthermore, the methods of Jung and Ton would have been reasonably pertinent to the problems facing the named inventors, *e.g.*, how to improve the purification of botulinum toxin from *Clostridium botulinum*. EX1001, 2:50-53; EX1002, 2:45-56; EX1003, 6:52-56. In particular, Jung expressly discloses all of the limitations of claim 1, except for the cation exchange chromatography step. However, this limitation is disclosed in Ton, and the POSA would have been motivated, with a reasonable expectation of success, to modify Jung by adding a cation exchange chromatography step, particularly given Ton’s express teaching that combining anion exchange and cation exchange “provides an effective and rapid

method for obtaining high purity, high yield botulinum neurotoxin.” EX1003 at 24:38-41. And a POSA would have had a reasonable expectation of success in doing so in view of Ton’s successful purification method of combining anion and cation exchange chromatography to purify botulinum toxin.

81. Moreover, as noted, claim 1 recites alternative elements (a) and (b), where element (b) recites conducting cation exchange chromatography first and anion exchange chromatography second. EX1001 at Claim 1. A POSA would understand that the order of the ion exchange chromatography steps is somewhat arbitrary and a matter of simple design choice that a POSA can easily implement. A POSA would understand that for obtaining high purity, high yield botulinum toxin, what really matters is the performance of *multiple chromatography steps*. Thus, in my opinion, the order of the ion exchange chromatography steps recited in claim 1 is immaterial to the alleged invention, and, regardless of the order, the claims unpatentable over Jung and Ton.

82. This is supported by the ’598 patent itself. For instance, the specification of the ’598 patent lacks any working example where cation exchange is conducted first, and anion exchange is conducted second. EX1001 at 8:56 – 10:5 (example limited to anion exchange followed by cation exchange). Indeed, in describing alternative element (b), the ’598 patent explains:

The contacting of the botulinum toxin-containing solution with the cation exchange chromatography medium at a pH lower than PI and the separating of the botulinum toxin from the cation exchange chromatography medium **are the same as described above, except that the botulinum toxin-containing solution is used instead of “the solution which is not bound to the medium”**. Further, “the contacting of the solution containing the toxin which is separated from the medium, with the anion exchange chromatography medium at a pH lower than PI, and the isolating of the botulinum toxin from the solution which is not bound to the medium” **are the same as described above, except that “the solution containing toxin which is separated from the medium” is used instead of “the botulinum toxin-containing solution”**.

Id. at 6:31-44. In my opinion, this is an admission by the inventors that the order of anion exchange and cation exchange steps is immaterial and can easily be switched because all the POSA needs to do is (i) apply the “botulinum-toxin containing solution” obtained from the *Clostridium botulinum* culture to a cation exchange, and (ii) apply the “solution containing toxin which is separated from the [cation exchange] medium” to the anion exchange. Other than that, the steps “***are the same as described above***” for a method where anion exchange is conducted first, and cation exchange is conducted second.

2. Dependent Claim 2

83. Claim 2 depends on claim 1 and recites four alternatives, only one of which is recited below:⁸

adjusting a pH of the botulinum toxin-containing solution to a pH lower than the PI of botulinum toxin, before the contacting of the botulinum toxin-containing solution with the anion exchange chromatography medium at the pH lower than the PI of botulinum toxin in (a)

84. Jung discloses this limitation. For example, the Jung specification discloses that the botulinum toxin-containing solution “was filtered with no contact with the outside to be enriched, and a *buffer exchange* was performed on the enriched resultant by using a 25 mM citrate buffer (*pH 5.5*).” EX1002 at 11:65 –

⁸ I understand that a claim element reciting alternatives is disclosed if the prior art discloses one alternative. Regardless, the step of “adjusting” the pH of a solution prior to conducting any ion exchange chromatography step is a matter of routine practice to those skilled in the art of protein purification. *See, e.g.*, EX1028 at 7 (“[T]he sample may be transferred to start buffer by dialysis or by *buffer exchange* ... ”); EX1002 at 12:8-10 (“enrichment and *buffer exchange* were completed. Then, DEAE anion exchange chromatography and membrane chromatography were performed on the sample.”); EX1001 at 9:13-14 (*buffer exchange* was performed prior to anion and cation exchange chromatography).

12:1. A pH of 5.5 is “pH lower than the PI of botulinum toxin” as that term is properly construed, e.g., pH lower than 6.06. Following the buffer exchange step, Jung teaches that “*DEAE anion exchange chromatography* and membrane chromatography were performed on the sample not in contact with the outside.” EX1002 at 12:9-11. Therefore, Jung discloses “adjusting a pH of the botulinum toxin-containing solution to a pH lower than the PI of botulinum toxin,” e.g., by buffer exchanging the solution with a citrate buffer pH 5.5, “before the contacting of the botulinum toxin-containing solution with the anion exchange chromatography medium at the pH lower than the PI of botulinum toxin in (a).”

3. Dependent Claim 3

85. Dependent claim 3 depends on claim 2 and recites that “the adjusting comprises mixing the botulinum toxin-containing solution, the flow-through portion of the botulinum toxin-containing solution, or the solution containing the botulinum toxin with a buffer having a pH lower than the PI of botulinum toxin.”

86. Jung discloses this limitation. For example, the Jung specification discloses buffer exchanging the botulinum toxin-containing solution with a *citrate buffer pH 5.5* prior to performing the anion exchange chromatography step. EX1002 at 11:65 – 12:11. A pH of 5.5 is lower than the PI of botulinum toxin” as that term is properly construed, e.g., pH lower than 6.06. Therefore, Jung discloses “mixing the botulinum toxin-containing solution, the flow-through portion of the

botulinum toxin-containing solution, or the solution containing the botulinum toxin with a buffer having a pH lower than the PI of botulinum toxin.”

4. Dependent Claim 4

87. Dependent claim 4 depends on claim 3 and recites that “the buffer is a phosphate buffer, a citrate buffer, or an acetate buffer.”

88. Jung discloses this limitation. For example, the Jung specification discloses buffer exchanging the botulinum toxin-containing solution with a ***citrate buffer*** pH 5.5 prior to performing the anion exchange chromatography step. EX1002 at 11:65 – 12:11.

5. Dependent Claim 5

89. Dependent claim 5 depends on claim 3 and recites that “the buffer has a pH of 3.5 to 6.0.”

90. Jung discloses this limitation. For example, the Jung specification discloses buffer exchanging the botulinum toxin-containing solution with a ***citrate buffer pH 5.5*** prior to performing the anion exchange chromatography step. EX1002 at 11:65 – 12:11.

6. Dependent Claim 6

91. Dependent claim 6 depends on claim 3 and recites that “the buffer comprises a 10 mM to 100 mM phosphate buffer, a 10 mM to 100 mM citrate buffer, or a 10 mM to 100 mM acetate buffer.”

92. Jung discloses this limitation. For example, the Jung specification discloses buffer exchanging the botulinum toxin-containing solution with a **25 mM citrate buffer** pH 5.5 prior to performing the anion exchange chromatography step. EX1002 at 11:65 – 12:11.

7. Dependent Claim 7

93. Dependent claim 7 depends on claim 1 and recites that “the anion exchange chromatography medium comprises DEAE or Q anion exchange resin.”

94. Jung discloses this limitation. For example, the Jung specification discloses performing **DEAE anion exchange chromatography**. EX1002 at 12:4-16. Jung also discloses “Q Sepharose” and “Q sephadex.” *Id.* at 4:62-65.

8. Dependent Claim 8

95. Dependent claim 8 depends on claim 1 and recites that “the cation exchange chromatography medium comprises CM, S, or SP cation exchange resin.”

96. Ton discloses conducting cation exchange chromatography using a **POROS® 20HS**. EX1003 at 40:25-29 (“The cation exchange column can be a POROS® 20HS cation exchange resin from Applied Biosystems”). A POSA would have known that POROS® 20HS is a sulfopropyl (“SP”) cation exchange resin. EX1020.

9. Dependent Claim 9

97. Dependent claim 9 depends on claim 1 and recites that “the pH lower than the PI of botulinum toxin is pH 3.5 to 6.0.”

98. Jung discloses conducting anion exchange chromatography at *pH 5.5*. EX1002 at 11:65 – 12:16. Ton discloses conducting cation exchange chromatography at *pH 4.8*. EX1002 at 40:25-32.

C. Ground 2: Claims 1-9 Would Have Been Obvious to a POSA in 2016 in view of Kim '741 in Combination with Kim '565 and Jung

99. I was asked to review the Challenged Claims of the '598 patent and compare them to the disclosures of Kim '741, Kim '565 and Jung. It is my opinion that the Challenged Claims would have been obvious to a POSA in view of Kim '741, Kim '565 and Jung.

1. Independent Claim 1

a) “A method of isolating a botulinum toxin type A macro complex from a botulinum toxin-containing solution”

100. Kim '741 discloses this limitation. For example, the Kim '741 specification teaches “a more efficient and safe *method for producing botulinum toxin* ...” EX1004 ¶ [0023]; EX1008 ¶ [28]. “The *botulinum toxin purified according to the production method* of the present invention may have a form of 7S or 19S (*molecular weight: 150-900 kDa*).” EX1004 ¶ [0046]; EX1008 ¶ [48]. “[T]he term ‘botulinum toxin’ as used herein is meant to include *botulinum toxin*

serotypes A, B, C, D, E, F and G ...” EX1004 ¶ [0048]; EX1008 ¶ [50]. Moreover, in Kim ’741, the botulinum toxin is purified from a “***culture of a botulinum toxin-producing strain.***” EX1004 ¶ [0026]; EX1008 ¶ [31]. According to the ’598 patent, “the botulinum toxin-containing solution may be a ***culture of Clostridium botulinum.***” EX1001 at 3:28-30. Thus, “culture of a botulinum toxin-producing strain” in Kim ’741 is a “botulinum toxin-containing solution” according to the ’598 patent.

b) “culturing *Clostridium botulinum* in an animal product-free medium to obtain a culture”

101. Kim ’741 discloses culturing *Clostridium botulinum* in a “conventional medium.” EX1004 ¶ [0051]; EX1008 ¶ [53]. According to Kim ’741, “a medium for culture of *Clostridium botulinum* strain may include a casein hydrolysate, a yeast extract, glucose and the like.” EX1004 ¶ [0052]; EX1008 ¶ [54]. Casein hydrolysate is a protein derived from cow milk. Thus, Kim ’741 does not disclose “culturing *Clostridium botulinum* in an animal product-free medium to obtain a culture.” However, in my opinion, a POSA would have been motivated to substitute the animal product-containing medium of Kim ’741 with the animal product-free medium of Kim ’565, and the POSA would have done so with a reasonable expectation of success.

102. As stated in Kim ’565, “Transmissible spongiform encephalopathy (TSE) is known as a neurodegenerative disorder causing serious degeneration of

neurons.” EX1023 ¶ [12]. Kim ’565 explains that “if an animal abnormal prion known as an agent that causes transmissible spongiform encephalopathy is contained in the animal components due to contamination, it poses problems in a process for producing botulinum toxin.” *Id.* ¶ [11]; *also see id.* ¶ [15] (“if a pharmaceutical composition is prepared by drug substance produced using animal-derived components, it can subject the patient to a potential risk of receiving various pathogens or infectious agents.”). The object of Kim ’565’s invention was therefore “to provide a medium composition comprising plant-derived peptones having no risk of transmissible spongiform encephalopathy (TSE) infection.” *Id.* ¶ [19].

103. Specifically:

In the present invention, it was attempted to prepare a medium that further increases the growth rate of *Clostridium botulinum* compared to a medium that is in current use (original medium) and have no risk of infection with TSE or the like. ***Thus, an animal protein-free (APF) medium containing plant-derived peptones were used***, and the growth of a bacterium in the APF medium was examined. As a result, the ***APF medium showed an increased growth rate of the bacterium compared to a medium that is in current use***. Thus, if the APF medium is used, a high concentration of botulinum toxin can be produced by culturing a bacterium in a safe manner under TSE-free conditions.

Id. ¶ [37]; *also see id.* ¶ [38] (“the term ‘medium that is in current use or original medium’ means medium comprising casein hydrolysate ...”). Thus, a POSA in

2016 would have been motivated to use a culture medium that does not include casein hydrolysate in order to remove the risk TSE.⁹

104. A POSA would have had a reasonable expectation that using a culture medium disclosed in Kim '565 would be successful. For example, Kim '565 states that:

In an example of the present invention, in order to produce botulinum toxin by culturing *Clostridium botulinum* under transmissible spongiform encephalopathy (TSE)-free conditions, an APF medium comprising TSE-free plant-derived peptone was prepared and compared with a medium that is in current use (containing an animal component). As a result, it could be seen that an ***optimal medium composition for culturing Clostridium botulinum*** is one comprising a plant-derived peptone, ... ***and the optimal growth of the bacterium in this medium was found.***

Id. ¶ [39]. According to Kim '565, “the APF medium showed an ***increased growth rate*** of the bacterium compared to a medium that is in current use.” *Id.* ¶ [37]. Thus,

⁹ Although Kim '741 uses casein in the culture medium, the overall goal of Kim '741 is consistent with that of Kim '565, which is to provide “a method of producing botulinum toxin by an ***animal product-free (APF) process*** ... to solve safety problems such as transmissible spongiform encephalopathy infections caused by such animal-derived components worldwide.” EX1004 ¶ [0021].

in my opinion, a POSA would have had a reasonable expectation of success in substituting the culture medium of Kim '741 with the *animal product-free culture medium* of Kim '565.

c) **“removing *Clostridium botulinum* cells from the culture to obtain a botulinum toxin-containing solution”**

105. Kim '741 does not disclose removing the cells from the culture prior to adding an acid to precipitate the toxin. However, this cell removal step (which as I note below, the '598 patent itself admits is not necessary) would have been obvious in view of Jung. The method of Jung includes “(a) culturing *Clostridium botulinum* in a media containing plant-derived components to express *Clostridium botulinum* toxin outside a cell, and (b) removing the cell from the obtained culture to obtain a portion including *Clostridium botulinum* toxin expressed outside the cell and isolating the *Clostridium botulinum* toxin from the portion.” *Id.*, 2:67-3:6. According to Jung, “removing the cell may be performed using at least one method selected from the group consisting of depth filtration and microfiltration.” *Id.*, 4:49-5. A POSA, seeking to develop a method for purifying botulinum toxin from *Clostridium botulinum* would have been motivated by Jung, with a reasonable expectation of success, to remove the cells from the culture prior to adding an acid to precipitate the toxin because, as disclosed in Jung, “the inventors of the present application found that a large amount of *Clostridium botulinum* toxin was *expressed*

outside the cell by using a composition of plant-derived components, and ... *Clostridium botulinum* toxin could be easily and very simply purified from the culture from which the cell was removed.” EX1002, 2:46-56. In other words, a POSA at the relevant time wanting to obtain a pure botulinum toxin preparation “easily and very simply” would have followed Jung’s teaching of removing cells prior to adding acid to precipitate the toxin. Moreover, cell removal is a routine step in the art of protein purification. *E.g.*, EX1024 at 481 (“The first unit operation in a downstream process is the removal of cells and cell debris from the culture broth and clarification of the cell culture supernatant that contains the antibody product.”); EX1031 at 2329 (“The first step of downstream operations in monoclonal antibody (mAb) manufacturing is the removal of cells and cellular debris from cell culture via centrifugation and depth filtration, prior to capture chromatography”); EX1025 at 302 (“[mAb] recovery includes harvest, which is removal of cells and cell debris by tangential flow filtration or centrifugation (van Reis *et al.*, 1991), chromatography for antibody purification, and formulation by tangential flow filtration.”). Therefore, removing the cells prior to adding an acid to precipitate the botulinum toxin would have been routine and obvious to a POSA in 2016.

d) **“adding an acid to the botulinum toxin-containing solution to precipitate the botulinum toxin and obtain a precipitate”**

106. Kim ’741 discloses this limitation. For example, the Kim ’741 specification discloses “(a) treating a culture of botulinum toxin-producing strain with acid to form a botulinum toxin-containing precipitate.” EX1004 ¶ [0042]; EX1008 ¶ [42]. I note that the ’598 patent states that “the botulinum toxin-containing solution may be a culture of *Clostridium botulinum* or a solution derived therefrom.” EX1001 at 3:27-30. A POSA would therefore understand that the acid may be added *directly to the culture of Clostridium botulinum* (without first “removing the cells”) to precipitate the botulinum toxin. Moreover, the ’598 patent states that “after the cells produce the toxin therein, the cells excrete the toxin spontaneously, and the *cells containing the toxin expressed therein are also lysed* ...” *Id.* at 3:37-41. Thus, Patent Owner’s argument during prosecution to distinguish Kim ’741 (EX1006, 12-20-2021 Response at 7), namely, that “[i]n the claimed method, there is no need to remove or treat nucleic acids from bacteria cells since *Clostridium botulinum* cells from the culture are removed,” lacks merit since the ’598 patent states that the *cells are lysed upon expression of the toxin*, which necessarily means that nucleic acids are *released in the culture medium upon lysis of the cells*. And challenged claim 1 recites acid precipitation and filtration steps, so Patent Owner’s argument that “there is no need to include the step of ‘performing

one process selected from among UF diafiltration, ammonium sulfate precipitation and hydrochloric acid precipitation” as purportedly distinguishing claim 1 from Kim ’741 makes little sense. *Id.* at 8.

e) “dissolving the precipitate in a medium to obtain a solution”

107. Kim ’741 discloses this limitation. For example, the Kim ’741 specification discloses “(b) adding a buffer to the botulinum toxin-containing precipitate of step (a).” EX1004 ¶ [0043]; *also see id.* ¶ [0067] (“step (b) comprises a step of *dissolving the toxin precipitate* resulting from step (a) by addition of phosphate buffer”); EX1008 ¶ [44], [69].

f) “filtering the solution to obtain a botulinum toxin-containing solution”

108. Kim ’741 discloses this limitation. For example, the Kim ’741 specification discloses “clarifying” the solution in (b) “by one or more method selected from the group consisting of *depth filtration* (DF), *microfiltration* (MF), *ultrafiltration* (UF), *sterile filtration*, membrane chromatography (MC) and centrifugation.” EX1004 ¶ [0043]; EX1008 ¶ [44].

- g) “contacting the botulinum toxin-containing solution with an anion exchange chromatography medium at a pH lower than an isoelectric point (PI) of botulinum toxin”¹⁰

109. Kim ’741 discloses this limitation. For example, the Kim ’741 specification discloses subjecting the botulinum toxin solution to *anion exchange chromatography* at a pH of 2-9, *preferably 3-8*. EX1004 ¶ [0072]. As disclosed in Kim ’741:

The anion-exchange chromatography in step (d) may be performed at a pH of 2-9, preferably 3-8, and a conductivity of 2-40 mS/cm, preferably 3-30 mS/cm. In step (d), botulinum toxin may be collected as a botulinum toxin-containing fraction (flow-through mode) of a flow-through (FT) fraction eluted from anion-exchange chromatography or as a fraction containing the botulinum toxin bound to anion exchange chromatography resin. The finally purified botulinum toxin may have a form of 7S or 19S.

Id.; also see *id.* ¶ [0085]; EX1008 ¶¶ [74], [77].

110. Thus, Kim ’741 discloses performing anion exchange chromatography at pH ranges that *overlap* with “a pH lower than an isoelectric point (PI) of botulinum

¹⁰ Claim 1 of the ’598 patent recites an element (b) as an alternative to element (a).

I understand that a claim element reciting alternatives is disclosed if the prior art discloses one of the alternatives.

toxin,” *i.e.*, **a pH lower than 6.06**. I have not seen any evidence of teaching away, unexpected results, criticality, or other pertinent objective indicia indicating that the overlapping range would not have been obvious over Kim ’741.

111. As noted above, Patent Owner submitted an inventor declaration during prosecution of the ’598 patent to attempt to demonstrate the alleged criticality of the claimed pH range. Specifically, the declaration described an experiment comparing anion exchange chromatography conducted at a pH of 5.5 and 6.5. According to Patent Owner, results showing that “19S can be purified from the flow through solution when pH 5.5 is used but at pH 6.5, 19S was not present in the flow through solution ... is an unexpected benefit of the claimed invention.” EX1006, 7-22-2021 Response at 10. In a later response, Patent Owner stated that “[t]he experiments and results provided in the SONG Declaration show the criticality of the use of ‘a pH lower than the pI of botulinum toxin.’” *Id.*, 12-20-2021 Response at 13.

112. But there is ***nothing special or unexpected*** about that observation. Jung, Medytox’s patent having the same lead named inventor, Hyun-Ho Jung discussed in Ground 1, disclosed a method of purifying botulinum toxin type A comprising the steps of (1) culturing *Clostridium botulinum* in an animal product-free medium, (2) removing the cells from the culture, (3) adding an acid to precipitate the botulinum toxin from the culture medium, (4) dissolving the precipitated toxin with a buffer, (5) filtering the solution, and (6) ***applying the***

solution to an anion exchange chromatography medium at a pH of 5.5. EX1002 at 11:20 – 12:16. As disclosed in Jung, “the *Clostridium botulinum type A toxin protein in the sample was not bound to a column* while other impurities [*sic*] were bound to the column, and thus a fraction flowing out of the system was collected.” *Id.* at 12:13-16. Thus, *the experiment and results described in the inventor declaration submitted during prosecution were already disclosed in Jung.* The table below shows a side by side of Jung Example 3 compared with the Inventor Declaration submitted during prosecution.

Jung Example 3	Inventor Declaration (’598 Patent PH)
“<i>Clostridium botulinum</i> was cultured using the medium 5 of Example 1 or 2.” EX1002 at 11:28-29. “The culturing conditions and time and the cell removal process were the same as those in Example 1.” <i>Id.</i> at 11:29-32. Medium 5: phytone peptone 1%; yeast extract 1%; glucose 0.5%. <i>Id.</i> at Table 1. In Example 1, “200 ml of each of the media of Table 1 and the control media was filtered through a sterilized single use microfiltration system ... so as to be injected into Cultibag™ ... Then, each media was inoculated with 1ml of a seed inoculum including Clostridium botulinum type A with a concentration of 10⁶-10⁷ cells/ml through a sample injection port. Cultibag™ is a disposable	“In this experiment, a large amount of <i>Clostridium botulinum</i> type A toxin was expressed outside cells by culturing Clostridium botulinum in an animal product-free medium.” EX1006, 7-22-2021 Declaration at 1. “The used medium was a medium containing 4% yeast extract and 0.5% glucose.” <i>Id.</i> “200 ml of the medium thus prepared was filtered through a sterile single use microfiltration system ... and injected into Cellbag™ ... Then, the medium was inoculated with 1 ml of a seed culture of Clostridium botulinum at a density of 10⁶ cells/ml-10⁷ cells/ml through a sample injection port. Cellbag™ is a disposable bioreactor that uses rocking motion in order to

bioreactor that may use rocking motion in order to cause mixing.” <i>Id.</i> at 8:56 – 9:2.	cause mixing under low shear stress.” <i>Id.</i> at 2.
“After the culturing was terminated, a sterile connector QDC connector (manufactured by Satorious) was connected to the sample port of the bag ... and <i>the culture was filtered through depthfilter and the microfilter to remove the cell.</i> ” <i>Id.</i> at 9:36-46.	“After the culturing was terminated, a sterile connector QDC connector (manufactured by Satorious) which is a sterile connector was connected to the sample port of the bag ... and <i>the culture was filtered therethrough to remove the cells.</i> ” <i>Id.</i>
“ <i>[S]ulfuric acid was added</i> to the bag including 20 L of a culture supernatant to adjust the pH of the resulting solution to be in the range of 3.0 to 4.5, and as a result, <i>toxin protein was precipitated.</i> ” <i>Id.</i> at 11:33-36.	“ <i>[S]ulfuric acid was added</i> to a bag containing 20 L of the culture supernatant obtained as above to adjust pH from 3.0 to 4.5, and as a result, <i>toxin protein was precipitated.</i> ” <i>Id.</i> at 3.
“ <i>10 L of a 25 mM citrate buffer (pH 5.5)</i> was added to the collected precipitation to <i>re-dissolve toxin protein</i> existing the precipitation.” <i>Id.</i> at 11:51-53.	“ <i>10 L of citrate buffer (25 mM, pH 5.5)</i> was added to the collected precipitate to <i>redissolve toxin protein</i> existing in the precipitate.” <i>Id.</i>
“ <i>[F]iltration was performed on the toxin protein</i> through the system not in contact with the outside. As a result, the <i>supernatant was collected</i> in a sterilized disposable bag and insoluble impurities were removed... and a buffer exchange was performed on the enriched resultant by using a <i>25 mM citrate buffer (pH 5.5).</i> ” <i>Id.</i> at 11:57 – 12:1.	“ <i>[F]iltration was performed</i> without being in contact with the outside. As a result, the <i>supernatant was collected</i> in a sterile disposable bag and insoluble impurities were removed.” <i>Id.</i>
“Then, DEAE <i>anion exchange chromatography</i> and membrane chromatography <i>were performed</i> ... After the sample was loaded on the system, the <i>Clostridium botulinum</i>	“ <i>Q anion exchange chromatography was performed</i> [and] <i>[t]he flow through solution was analyzed.</i> ” <i>Id.</i> “As shown in FIG. 1, the botulinum toxin containing sample was detected

<i>type A toxin protein in the sample was not bound to a column</i> while other impurities [<i>sic</i>] were bound to the column, and <i>thus a fraction flowing out of the system was collected.</i>” <i>Id.</i> at 12:4-16.	immediately after loading the sample ... This indicates that the <i>botulinum toxin is contained in the flow through solution.</i>” <i>Id.</i>
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See also EX1022.

113. Moreover, as I explain above, ion exchange chromatography is a chromatographic process that separates molecules, such as proteins, on the basis of surface charges. Each protein has an overall surface isoelectric charge as well as specific charged regions. The net surface charge as well as the number and magnitude of specific charged regions of a protein will vary with pH. The pH at which a protein has a net charge of zero (*e.g.*, the same number of positively and negatively charged amino acids) is called the isoelectric point (pI). A protein at a pH below its pI will carry a positive surface charge and is less likely to interact with a positively charged separation matrix (anion exchanger). Rather, it is more likely to flow through the matrix. Conversely, a protein at a pH above its pI, *i.e.*, a pH at which the protein carries a negative surface charge, is more likely to interact with and remain bound to a positively charged matrix. This was a widely accepted concept. See, *e.g.*, EX1030 at 71 (“Below its isoelectric point a protein has a net positive charge and can therefore adsorb to cation exchangers. Above its pI the protein has a net negative charge and can be adsorbed to anion exchangers.”);

EX1007 at 218 (“The pI of bovine hemoglobin (Hb) is 6.9. Theoretically, if pH is selected between 6.1 and 6.8 [e.g., below the pI of bovine hemoglobin], ***Hb would flow through an anion exchange column due to its positive charge***, while albumin and CA [which have pIs of 4.9 and 6.0, respectively] would be retained due to their negative changes.”)

114. Applying these well-known ion exchange chromatography principles here, a POSA ***would not have been surprised*** that anion exchange chromatography conducted at “a pH lower than the pI of botulinum toxin” (e.g., pH of 5.5) results in the toxin flowing through the matrix (*i.e.*, the fraction that does not bind to the anion exchange matrix). To the contrary, a POSA would have ***expected*** the toxin to flow through at pH 5.5.

115. Additionally, I note that in Kim ’741’s Example 2-3-1, entitled “TQ Purification (Anion Exchange Chromatography ***Flow-Through*** (AEX FT)),” anion exchange chromatography was performed at a ***pH of 6.4-6.6***, and “it was shown that the ***botulinum toxin was not adsorbed onto the TQ resin***, and most major impurities were removed by adsorption, and thus a botulinum toxin could be produced with high purity.” EX1004 ¶¶ [0102] – [0108]. “In addition, the resulting botulinum toxin had a ***form 19S (molecular weight: about 900 kDa)***.” *Id.* ¶ [0108]. In other words, Kim ’741 discloses conducting anion exchange chromatography in ***flow-through mode at a pH higher than the pI of botulinum toxin***. In my opinion, this

demonstrates that pH is *not critical* to the claimed invention since flow-through anion exchange chromatography can be conducted at pH *outside* the claimed range of “a pH lower than an isoelectric point (PI) of botulinum toxin.”

116. Moreover, as I explain above, a POSA would have been very familiar with optimizing pH of a protein solution such that the solution is applied to the separation matrix under conditions (pH and ionic strength) suitable for the protein to interact with (or flow through) the separation matrix. pH is easy to modulate in the context of ion exchange chromatography, and it would have routine for a POSA to optimize the pH and observe whether varying the pH affected the binding of the toxin to the matrix. This is routine experimentation in the field, and purification using ion exchange would be optimized using pH adjustment to determine a pH lower than the PI of botulinum toxin to permit flow through for anion exchange and capture by cation exchange chromatography.

117. Finally, I disagree with Patent Owner’s argument during prosecution that Kim ’741 teaches away from “a pH lower than the PI of botulinum toxin.” EX1006, 7-22-2021 Response at 7-8. To the contrary, Kim ’741 expressly teaches that both anion and cation exchange chromatography *can be conducted* at a pH of 3-8, which includes “a pH lower than the PI of botulinum toxin.” Moreover, I have seen no disclosure in Kim ’741 that criticizes or discredits the use of “a pH lower than the PI of botulinum toxin.”

118. Therefore, Kim '741 discloses “contacting the botulinum toxin-containing solution with an anion exchange chromatography medium at a pH lower than an isoelectric point (PI) of botulinum toxin.”

- h) **“contacting a flow-through portion of the botulinum toxin-containing solution, which is not bound to the anion exchange chromatography medium, with a cation exchange chromatography medium at a pH lower than the PI of botulinum toxin”**

119. Kim '741 states that following the anion exchange chromatography “[i]n step (d), botulinum toxin may be collected as a botulinum toxin-containing fraction (*flow-through mode*) of a *flow-through (FT) fraction* eluted from anion-exchange chromatography.” EX1004 ¶ [0072]. As disclosed in Kim '741:

The term “FT (*flow-through*)”, “flow-through process” or “flow-through purification”, as used interchangeably herein, means a separation process in which at least one target molecule (e.g., botulinum toxin) contained in a biopharmaceutical formulation together with one or more impurities passes through a material and one or more impurities generally bind to the material and *the target molecule generally does not bind to the material (that is, flows through the materials)*.

Id. ¶ [0073]; *id.* ¶ [0085]; EX1008 ¶¶ [74] – [75].

120. Kim '741 discloses that a further “*cation-exchange chromatography in step (h) may be performed at a pH of 2-9, preferably 3-8.*” *Id.* ¶ [0087]; EX1008 ¶ [93]. Thus, Kim '741 discloses performing cation exchange chromatography at “a

pH lower than an isoelectric point (PI) of botulinum toxin,” *i.e., a pH lower than 6.06.*¹¹

121. Therefore, Kim ’741 discloses “contacting a flow-through portion of the botulinum toxin-containing solution, which is not bound to the anion exchange chromatography medium, with a cation exchange chromatography medium at a pH lower than the PI of botulinum toxin.”

i) “separating the botulinum toxin type A macro complex from the cation exchange chromatography medium”

122. Kim ’741 discloses this limitation. For example, the Kim ’741 specification discloses that “[i]n the cation-exchange chromatography of step (h), the botulinum toxin *may be collected as a fraction containing the botulinum toxin bound to the cation-exchange chromatography resin.*” EX1004 ¶ [0087]. Therefore, Kim ’741 discloses “separating the botulinum toxin type A macro complex from the cation exchange chromatography medium.”¹²

¹¹ Based on my review of the file history, Patent Owner did not present any evidence of alleged criticality during prosecution for the pH range of the cation exchange chromatography step.

¹² While Kim ’741 discloses that the final purified botulinum toxin is a form of 7S (molecular weight of about 150 kDa), a POSA would understand that this is because Kim ’741’s working examples disclosed conducting cation exchange

* * *

123. In view of the above, it would have been obvious to a POSA to perform the claimed method of Kim '741 as modified by Kim '565 and Jung. As an initial matter, Kim '741, Kim '565 and Jung are related to the same field of endeavor—botulinum toxin purification—as the '598 patent. EX1001, Title; EX1004, Title; EX1023, Title; EX1002, Title. Furthermore, the methods of Kim '741, Kim '565 and Jung would have been reasonably pertinent to the problems facing the named inventors, for example, how to improve the purification of botulinum toxin from *Clostridium botulinum*. EX1001, 2:50-53; EX1002, 2:45-56; EX1004 ¶[0023]; EX1023 ¶[19]. In particular, Kim '741 expressly teaches most of the steps of claim

chromatography at a pH of 6.9-7.1. EX1004 ¶ [0122]. A POSA would understand that at a pH of 6.9-7.1, the toxin complex breaks down into the individual components (e.g., hemagglutinin – HA – and non-toxic non-hemagglutinin – NTNHA – and the 150 kDa toxic component). But at a pH below the pI of botulinum toxin, which Kim '741 expressly states *can be used for cation exchange chromatography*, a POSA would have expected botulinum toxin type A to be present as a complex, e.g., 900 kDa. In my opinion, this knowledge would be yet an additional motivation for the POSA to conduct anion and cation exchange chromatography at a lower pH, e.g., below the pI of botulinum toxin.

1 of the '741 patent, including the steps of culturing *Clostridium botulinum*, adding an acid to the culture to precipitate the botulinum toxin, dissolving the precipitate, filtering the solution, anion exchange chromatography in flow-through mode, and cation exchange chromatography in bind-elute mode. In my opinion, these steps were merely routine protein purification steps with which a POSA as of 2016 would have been very familiar. While Kim '741 does not disclose removing *Clostridium botulinum* cells from the culture prior to adding an acid to precipitate botulinum toxin, such a step would have been obvious to a POSA in view of Jung, which expressly discloses that step and states that that “*Clostridium botulinum* toxin could be easily and very simply purified from the culture from which the cell was removed.” EX1002, 2:46-56. Thus, a POSA at the relevant time wanting to obtain a pure botulinum toxin preparation “easily and very simply” would have followed Jung’s teaching of removing cells prior to adding acid to precipitate the toxin. Moreover, a POSA also would have been motivated to culture *Clostridium botulinum* in an animal protein-free medium as disclosed in Kim '565, and the POSA would have done so with a reasonable expectation of success as discussed above.

124. With respect to the limitation of conducting anion and cation exchange chromatography “at a pH lower than an isoelectric point (PI) of botulinum toxin,” Kim '741 disclose pH ranges that overlap with the claimed range, which I understand creates a presumption of obviousness. And as I explained above, Patent Owner’s

evidence presented during prosecution do not overcome that presumption because a POSA would have been familiar with well-known ion exchange chromatography principles and would have expected botulinum toxin to flow through an anion exchange column at a pH lower than the pI of botulinum toxin. Moreover, pH is easy to modulate in the context of ion exchange chromatography, and it would have been routine for a POSA to optimize the pH and observe whether varying the pH affected the binding of the toxin to the matrix. This is routine experimentation in the field, and purification using column exchange would be optimized using pH adjustment to determine a pH lower than the PI of botulinum toxin to permit flow through for anion exchange and capture by cation exchange chromatography.

2. Dependent Claim 2

125. Claim 2 depends on claim 1 and recites four alternatives, only one of which is recited below:¹³

adjusting a pH of the botulinum toxin-containing solution to a pH lower than the PI of botulinum toxin, before the contacting of the botulinum toxin-containing solution with the anion exchange chromatography medium at the pH lower than the PI of botulinum toxin in (a)

¹³ I understand that a claim element reciting alternatives is disclosed if the prior art discloses one alternative.

126. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses a “dilution or dissolution of the botulinum toxin in step (c) compris[ing] a step of adding phosphate buffer. ... [t]he pH of the phosphate buffer is preferably about 4.0-8.5, and a base may be added to *adjust the final pH to 4.5-8, preferably 5.5-7.5, more preferably 6-7.*” EX1004 ¶ [0068]; EX1008 ¶ [70]. The dilution or dissolution in step (c) precedes the anion exchange chromatography step (d). I understand that the overlap in pH range creates a presumption of obviousness and as explained above, there is nothing critical about the claimed pH range.

3. Dependent Claim 3

127. Dependent claim 3 depends on claim 2 and recites that “the adjusting comprises mixing the botulinum toxin-containing solution, the flow-through portion of the botulinum toxin-containing solution, or the solution containing the botulinum toxin with a buffer having a pH lower than the PI of botulinum toxin.”

128. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses a “dilution or dissolution of the botulinum toxin in step (c) compris[ing] a step of adding phosphate buffer. ... [t]he pH of the phosphate buffer is preferably about 4.0-8.5, and a base may be added to *adjust the final pH to 4.5-8, preferably 5.5-7.5, more preferably 6-7.*” EX1004 ¶ [0068]; EX1008 ¶ [70]. I understand that the overlap in pH range creates a presumption of obviousness and as explained above, there is nothing critical about the claimed pH range.

4. Dependent Claim 4

129. Dependent claim 4 depends on claim 3 and recites that “the buffer is a phosphate buffer, a citrate buffer, or an acetate buffer.”

130. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses “dilution or dissolution of the botulinum toxin in step (c) compris[ing] a step of adding *phosphate buffer*.” EX1004 ¶ [0068]; EX1008 ¶ [70].

5. Dependent Claim 5

131. Dependent claim 5 depends on claim 3 and recites that “the buffer has a pH of 3.5 to 6.0.”

132. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses “dilution or dissolution of the botulinum toxin in step (c) compris[ing] a step of adding phosphate buffer. *The pH* of the phosphate buffer is *preferably about 4.0-8.5*.” EX1004 ¶ [0068]; EX1008 ¶ [70]. I understand that the overlap in pH range creates a presumption of obviousness and as explained above, there is nothing critical about the claimed pH range.

6. Dependent Claim 6

133. Dependent claim 6 depends on claim 3 and recites that “the buffer comprises a 10 mM to 100 mM phosphate buffer, a 10 mM to 100 mM citrate buffer, or a 10 mM to 100 mM acetate buffer.”

134. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses a *phosphate buffer* having a concentration of “**15-70 mM, preferably about 20-60 mM.**” EX1004 ¶ [0075]; EX1008 ¶ [77]. I understand that the overlap in concentration range creates a presumption of obviousness and I am not aware that there is anything critical about the claimed concentration range.

7. Dependent Claim 7

135. Dependent claim 7 depends on claim 1 and recites that “the anion exchange chromatography medium comprises DEAE or Q anion exchange resin.”

136. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses that “the resin that is used in the anion-exchange chromatography process includes *diethylaminoethyl (DEAE)*, quaternary aminoethyl (QAE) and *quaternary amine (Q)* groups.” EX1004 ¶ [0088]; EX1008 ¶ [94].

8. Dependent Claim 8

137. Dependent claim 8 depends on claim 1 and recites that “the cation exchange chromatography medium comprises CM, S, or SP cation exchange resin.”

138. Kim '741 discloses this limitation. For example, the Kim '741 specification discloses that “the resin that is used in the cation-exchange chromatography process preferably includes *carboxymethyl (CM)*, sulfoethyl (SE),

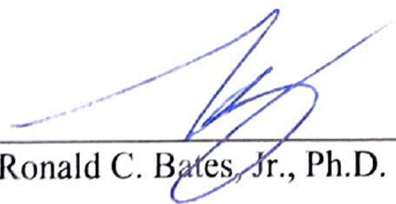
sulfopropyl (SP), phosphate (P), and *sulfonate (S)*.” EX1004 ¶ [0089]; EX1008 [95].

9. Dependent Claim 9

139. Dependent claim 9 depends on claim 1 and recites that “the pH lower than the PI of botulinum toxin is pH 3.5 to 6.0.”

140. Kim ’741 discloses this limitation. For example, the Kim ’741 specification discloses that the “anion-exchange chromatography in step (d) may be performed at a *pH of 2-9, preferably 3-8*” and that “the cation-exchange chromatography in step (h) may be performed at a *pH of 2-9, preferably 3-8*.” EX1004 ¶¶ [0072], [0087]; EX1008 ¶¶ [74], [93]. I understand that the overlap in pH range creates a presumption of obviousness, and as explained above, there is nothing critical about the claimed pH range.

141. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.



Ronald C. Bates, Jr., Ph.D.

Date: February 21, 2023

EXHIBIT LIST

Exhibit	Description
1001	U.S. Patent No. 11,331,598 (“the ’598 Patent”)
1002	U.S. Patent No. 8,993,268 (“Jung”)
1003	U.S. Patent No. 8,932,827 (“Ton”)
1004	U.S. Patent Publication No. 2018/0251741 (“Kim ’741”)
1005	U.S. Patent Publication No. 2015/0337281 (“Kim ’281”)
1006	’598 Patent Prosecution History
1007	Lu, Xiuling et al. <i>Purification of hemoglobin by ion exchange chromatography in flow-through mode with PEG as an escort</i> . Artificial cells, blood substitutes, and biotechnology 32.2 (2004): 209-227 (“Lu”)
1008	Certified English Translation of Korean Patent Application No. KR10-2015-0168196
1009	Ozturk and Hu, eds. <i>Cell culture technology for pharmaceutical and cell-based therapies</i> . CRC press, 2005, Chapter 13 (Ronald C. Bates) (“Bates Chapter”).
1010	Sugiyama. <i>Clostridium botulinum neurotoxin</i> . Microbiological reviews 44.3 (1980): 419-448.
1011	Park et al. <i>Binding of Clostridium botulinum type B toxin to rat brain synaptosome</i> . FEMS microbiology letters 72.3 (1990): 243-247.
1012	Montecucco and Schiavo. <i>Tetanus and botulism neurotoxins: a new group of zinc proteases</i> . Trends in biochemical sciences 18.9 (1993): 324-327.
1013	Poulain et al. <i>Neurotransmitter release is blocked intracellularly by botulinum neurotoxin, and this requires uptake of both toxin polypeptides by a process mediated by the larger chain</i> .

Exhibit	Description
	Proceedings of the National Academy of Sciences 85.11 (1988): 4090-4094.
1014	Schantz and Johnson. <i>Properties and use of botulinum toxin and other microbial neurotoxins in medicine</i> . Microbiological reviews 56.1 (1992): 80-99.
1015	U.S. Patent No. 6,558,926
1016	Amersham Biosciences, Ion Exchange Chromatography & Chromatofocusing: Principle and Methods, No. 11-0004-21, Amersham Biosciences Limited (GE Healthcare) (Ed. AA) (2004) (“GE Handbook”)
1017	Reserved
1018	<i>Curriculum vitae</i> of Ronald C. Bates, Ph.D.
1019	Korean Patent Application No. KR10-2015-0168196
1020	POROS™ HS Analytical Columns for Cation Exchange Chromatography Technical Sheet
1021	Expert Declaration of Dr. Ronald Bates in Support of Petition for Post-Grant Review of U.S. Patent No. 11,331,598 (“Bates Declaration”)
1022	Table Comparing Jung Example 3, ’598 Patent Example, and Inventor Declaration Submitted During Prosecution of the ’598 Patent
1023	International Patent Application Publication No. WO2016175565 (“Kim ’565”)
1024	Liu et al. <i>Recovery and purification process development for monoclonal antibody production</i> . MAbs. Vol. 2. No. 5. Taylor & Francis, 2010.
1025	Fahrner et al. <i>Industrial purification of pharmaceutical antibodies: development, operation, and validation of chromatography</i>

Exhibit	Description
	<i>processes</i> . Biotechnology and Genetic Engineering Reviews 18.1 (2001): 301-327.
1026	Gasteiger et al. <i>ExPASy: the proteomics server for in-depth protein knowledge and analysis</i> . Nucleic acids research 31.13 (2003): 3784-3788.
1027	Binz et al. <i>The complete sequence of botulinum neurotoxin type A and comparison with other clostridial neurotoxins</i> . Journal of Biological Chemistry 265.16 (1990): 9153-9158.
1028	GE Healthcare Ion Exchange Instructions 56-1191-00 AF (2004)
1029	GE Healthcare BPG Column Data File 18-1116-23 AD
1030	Amersham Biosciences, Ion Exchange Chromatography: Principle and Methods, No. 18-1114-21, Amersham Biosciences Limited (GE Healthcare) (Ed. AB) (2002)
1031	Kang et al. Development of a novel and efficient cell culture flocculation process using a stimulus responsive polymer to streamline antibody purification processes. Biotechnology and Bioengineering 110.11 (2013): 2928-2937.