

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor:

Steven Clyde PETTIT

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CELL CULTURE MEDIA CONTAINING
COMBINATIONS OF PROTEINS

Docket No.: 50091-CNT

Customer No.: 23589

Group Art Unit No.: 1656

Confirmation No.: 8304

Examiner: Paul J. HOLLAND

Mail Stop Amendments
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R. 1.132

I, Lorne Jordan, attest and declare as follows:

1. I have a Ph.D. in Biochemistry and Molecular Biophysics from Kansas State University, a Master of Science degree from the University of Oklahoma in Chemistry and Biochemistry, and an undergraduate degree in Biology and Chemistry from Bowling Green State University. I am currently a Scientist, Analytical Biochemistry, at Ventria Bioscience, the assignee of the above-referenced application.

2. I understand that the Examiner has cited WO 2007/002762 (Deeter et al.) against the above-referenced application. I have been asked to provide this declaration to explain the information described in this reference as understood by those skilled in this field.

3. The basic premise asserted by the Examiner on page 5 of the Action is that “the human serum albumin taught by Deeter et al. would inherently lack any endotoxin.” This statement is incorrect. In fact, in the process carried out by Deeter et al. endotoxin levels were observed in the final product. However, these values were not reported in the patent application at that time. As noted by the Examiner endotoxin is found in Gram-negative bacteria which are ubiquitous organisms. However, it is not correct to assume that because the Deeter et al. system is plant-based, it would be free of endotoxin contamination. When albumin is extracted from recombinant systems, it is exposed to and binds endotoxin. Endotoxin is a well-known

Wuhan Healthgen Biotechnology Corp. v. Ventria Bioscience Inc.

contaminant of recombinant protein systems. Due to its strong amphiphilic nature, albumin has an uncharacteristically strong interaction with endotoxin (compared to other plasma proteins). This has resulted in the unusually challenging development of a specific and non-obvious purification strategy for achieving recombinant albumin with low endotoxin levels, which was one of the aims of the current invention.

4. The current application actually contains data directly comparing the “old” approach described in Deeter et al. with the current invention. The Examiner’s attention is directed to Example 5 (“Purification and characterization of recombinant serum human albumin (rHSA)) in the current Application, also designated as Process “B000”. Notably, the protocol in Example 5 is identical to the protocol described in Example 7 of Deeter et al. (paragraphs [0116]-[0117]). Further, the Examiner’s attention is directed to Table E3 on page 104 of the current Application. For ease of reference, the table is copied below. This table shows the endotoxin levels detected in albumin extracted and purified using the old “B000” Deeter et al. process.

Table E3 Cellastim - Old Process B000								
Production Date	Batch Number	Grams product	EU/mg Dry Material	Detergent (ppm)	Viability (number of viable cells / ml / 10 ⁵)			
					1mg/ml	2 mg/ml	5 mg / ml	10 /mg ml
2/27/08	B202	70.6	25.3	815.3	15	7.9	4.1	0.9
2/27/08	B203	58.8	23.1	267.7	15.8	12.4	6.5	4.1
2/28/08	B204	59.9	29.2	589.2	16.3	11.1	5.2	2.9
2/28/08	B205	63	64.3	133.4	14	11.6	6.9	3.8
3/3/08	B206	41.1	35.1	1182.2	14.9	2.3	0.0	0.0
3/3/08	B207	94.2	28.7	388	16.5	12.7	7.6	4
3/4/08	B208	24.5	>80	467.2	15.3	11.4	7.1	2.7
3/4/08	B209	66.1	91.8	406.4	16.7	15.2	6.9	4.1
3/4/08	B210	87.3	67.8	76.4	17.5	19.1	15.4	8.5
3/11/08	B217	62.09	4.4	127.3	16.7	18.7	13.2	6.6
Averages			44.97	445.31	15.87	12.24	7.29	3.76

As can be seen, significant levels of endotoxin are present in albumin produced using the approach of Deeter et al. Further, this data demonstrates that the endotoxin levels in Deeter et al. were not inherently low or not present, as asserted in the Office Action. Moreover, in the case of the B000 protocol, cell viability is seen to decrease, dose-dependently, from 16 (0.5 mg/mL) to 4 (10 mg/mL).

5. The ability to achieve recombinant albumin with such low endotoxin levels was unexpected. Conceptually, a specific endotoxin-removal step was suggested and utilized with the goal of using detergent to remove endotoxin from protein. A process was developed to leverage the amphiphilic nature of detergent. Detergent and endotoxin interact with one another in an amphiphilic manner to form a more thermodynamically stable phase. Thus, conceptually, detergent was explored to bind and “extract” endotoxin from protein, despite the strong endotoxin-albumin interaction.

However, although detergent was found to successfully separate the endotoxin from the protein of interest, the detergent itself is a contaminant of the end product with detrimental effects on cells in culture. Therefore, the detergent-endotoxin complex must be subsequently removed from the solution. To accomplish this, we developed a protocol to absorb the detergent-endotoxin complex from the solution onto a hydrophobic detergent-binding resin. As a result of the process, protein without endotoxin flows through the chromatography resin/column and detergent-endotoxin complex is bound to the resin and separated from the protein.

6. Following the above concepts, the initial process B000, was designed (Example 5). However, this approach still resulted in high endotoxin levels (44.97 EU/mg average), and also had high detergent levels (445.31 ppm average), which was detrimental to cell viability in a dose-dependent manner, as noted.

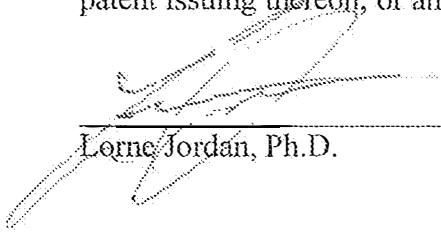
7. Thus, a more advanced process, B0000C, was designed using Tr-X-100 detergent and SDR (Pall) hydrophobic resin, as described in the Application. This new approach unexpectedly resulted in endotoxin reduction by 125-fold and detergent reduction by 129-fold compared to the Deeter et al. process B000. Data for the B0000C process are shown in Table E4, copied below for ease of reference.

Table E4								
Cellastim - New Process B0000C								
Production Date	Batch Number	Grams product	EU/mg Dry Material	Detergent (ppm)	Viability (number of viable cells / ml / 10 ⁵)			
					0.5mg/ml	2 mg/ml	5 mg / ml	10 /mg ml
2/2/2009	B0032C	209.4	0.35	3.1	13.1	15.7	13.4	11.2
2/2/2009	B0033C	271.4	0.26	7.9	18.4	17.4	15.1	14.3
2/10/2009	B0041C	247.1	0.18	5.4	15.4	17.2	14.1	12.1
4/27/2009	B0118C	617.4	0.11	Less than 1	15.7	15.4	19.2	16.8
5/14/2009	B0138C	610.6	0.48	Less than 1	12.5	15.8	15.1	14.6
6/9/2009	B0158C	598.0	0.13	3.9	13.7	17.1	13.2	11
6/15/2009	B0162C	618.6	0.20	2.0	15.5	17.5	17.2	14.7
7/28/2009	B0196C	507.0	0.36	3.9	17.4	17.3	18.8	14.8
8/26/2009	B0219C	851.4	0.86	8.2	17.9	16.3	16.6	13.7
8/31/2009	B0220C	897.8	0.93	Less than 1	13.5	16	15.5	15.1
9/9/2009	B0227C	929.9	0.13	Less than 1	11.1	14.4	12.5	11.4
Averages			0.36	3.45	14.92	16.37	15.52	13.6

As can be seen, the improved process achieves an *endotoxin average of 0.36 EU/mg* and *detergent levels of 3.45 ppm*. Cell viability was also substantially increased by 3x, even at high protein concentration 10 mg/mL indicating the absence of toxicity. Importantly, there is little variation on viability across protein concentration levels.

8. In summary, a comparison of the performance of the original B000 process (Deeter et al.) and the more advanced B0000C process (Currently invention) across several different batches of albumin demonstrates that the original B000 process produced recombinant albumin with high endotoxin content and high detergent content, whereas the advanced B0000C process produced recombinant albumin with significantly lower endotoxin content (less than 1 EU/mg) and detergent. Moreover, a comparison of the performance of the improved B0000C process with low endotoxin also improved cell culture performance as measured by cell viability per ml, especially at higher levels of albumin inclusion in cell culture media.

I hereby declare that all statements made herein of my own knowledge are true and that statements made herein 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, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this Declaration is directed.



Lorne Jordan, Ph.D.

11-17-19

Date