Development of Low Pyrogenic Response and Ultra-low Metal Ion Extractables Zeta Plus™ Filter Medium

Introduction

Cellulose based depth filters are widely used for filtration of biopharmaceutical and blood plasma fraction therapeutics. This 3M Purification Inc. Application Brief describes development of two cellulose based depth filters (the LA and LP grades), employing a specialized grade of cellulose selected to minimize beta glucan extractables. Beta glucan is a naturally occurring component of cellulose, which can cause false positive Limulus Amebocyte Lysate (LAL) test results. In addition, the LA grade of Zeta Plus™ containing a special low beta glucan cellulose, is also constructed with pre-extracted filter aid to minimize metal ion extractables. This unique depth filter construction is ideal for biological fluid filtration where the end product is a parenteral dosage form.

The Process

Cellulose depth filters are used in initial stage blood plasma fractionation to either clarify certain Cohn process fractions or to collect precipitated protein. In biopharmaceutical processing, depth filters are frequently used for media feed filtration, for the initial cell separation process and for downstream purification stages such as chromatography column protection and following ultrafiltration steps to clarify concentrated protein solutions. In both processes, shown below in Figures 1 and 2, specialized beta glucan free and ultra low level metal ion extractable cellulose depth filters can be selected.

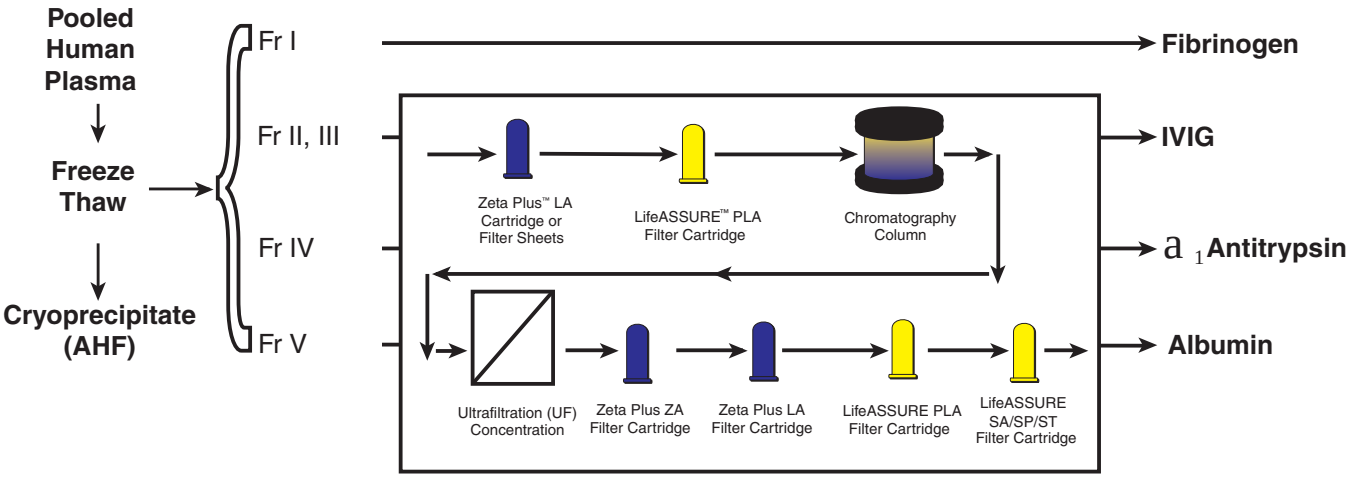

Figure 1 — Blood Plasma Fractionation Process

The Problem

False Positive LAL Test Reaction

It was first noted that the use of cellulose based hollow fiber dialysis filters were associated with positive LAL test results in blood from patients treated with these filters (Petersen, N.J. et al, 1981. Trans. Am. Soc. Artif. Intern. Organs 27: 155-159). Further studies found that the LAL positive reaction was associated with cellulose material extracted from the hollow fiber filters (Pearson, F. C., et al 1984. Applied and Environmental Biology 48:1189-1196). The extracted material was later identified as beta glucan (beta - 1,3). Although beta glucan resulted in a positive LAL test, it was not found to be pyrogenic. Regardless, product having positive LAL test results cannot be released.

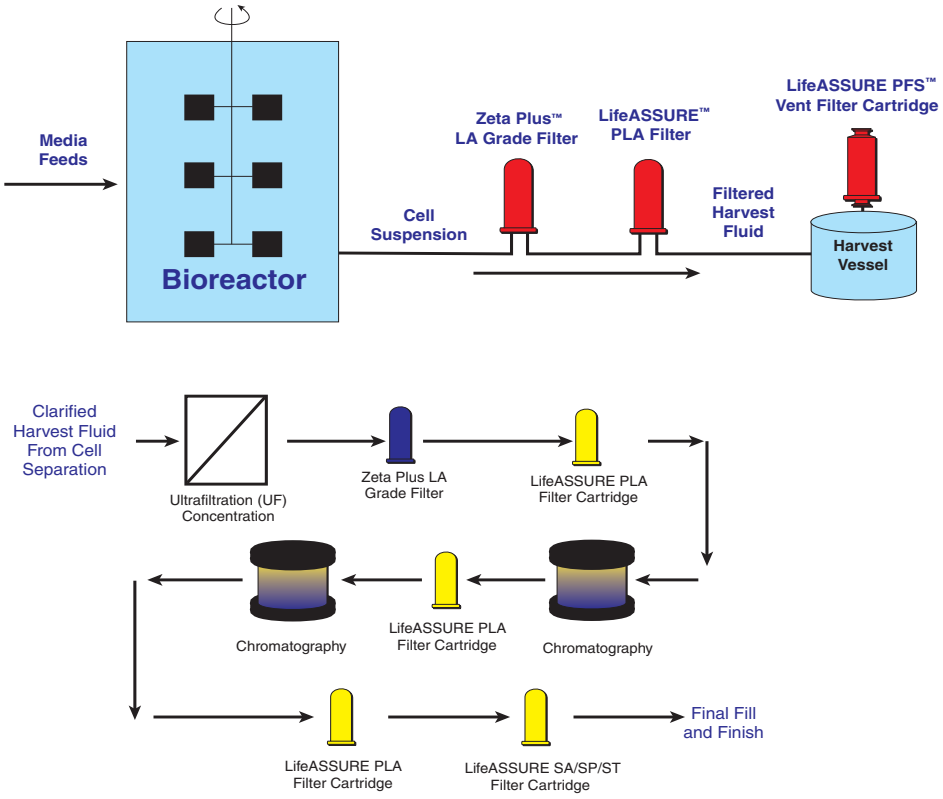

Figure 2 — Biopharmaceutical Processing

In both blood plasma fractionation and biopharmaceutical manufacturing, use of depth filters can contribute extractables to filtered solutions. In the case of cellulose based depth filters, the extractables can be a certain type of beta glucan found to react in the coagulation process of the Limulus Amebocyte Lysate (LAL) test. The particular types of beta glucan found to cause coagulation in the LAL test were those containing varying length glucose polymers linked primarily through beta - 1,3 glycosidic linkages (Morita et al, FEBS Letters 129:318-321, 1981). The reaction of beta glucan causing coagulation in the LAL test is illustrated in Figure 3.

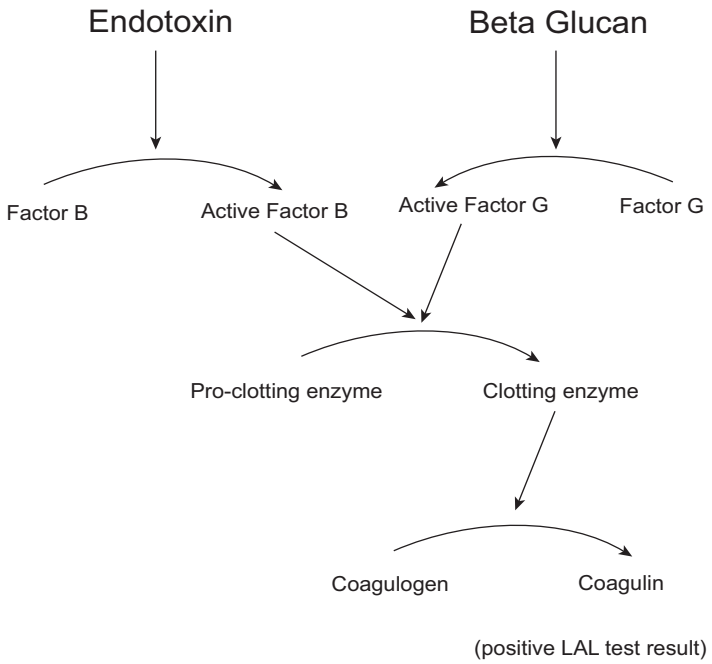

Figure 3 — Limulus Amebocyte Lysate (LAL) Coagulation Cascade

As shown in Figure 3, the presence of beta glucan in filtered product can cause a positive coagulation reaction in the LAL test. It is well-known that lysate (one of the reactants) from some types of endotoxin test kits is sensitive to the presence of beta glucans in samples while lysate from other types of endotoxin test kits is insensitive to the presence of beta glucans in these same samples. This difference in sensitivity to beta glucans sets the stage for the false-positive effect whenever a beta glucan-sensitive type of test kit is being used.

For example, cellulosic filters are typically rinsed prior to use, and post-rinse filtrate or rinsate is sometimes subjected to endotoxin testing. Table 1 contains typical data from such an event. Notice that Kit A (which is sensitive to beta glucans) may well lead to suspected endotoxin problems when one is using non-beta glucan-free cellulosic filter media.

Table 1 — Measured Endotoxin Content of Cellulosic Filter Rinsate (EU/ml)

Test Kit ID	Sensitivity of Kit to Beta glucans	Non LA & LP Grades of Cellulosic Media (Not Beta glucan free)	LA & LP grades of Cellulosic Media (Beta glucan free)
Kit A	Sensitive	0.125 - 0.25	< 0.03
Kit B	Insensitive	< 0.03	< 0.03

If an elevated endotoxin signal is observed under these conditions, a second method (Kit B) and sometimes even a third method (usually one that is more sophisticated and that can directly or indirectly differentiate the beta glucans from endotoxin) are used to determine if beta glucan is the source of a false positive LAL test result.

A further problem of beta glucan extractables is that when present in therapeutic doses and injected into patients, beta glucan detected in the blood can result in a false diagnosis of a bacterial infection.

Metal Ion Extractables

Metal ion extractables, associated with filter aids used in depth filters, can also cause unwanted contamination of parenteral products. Aluminum ions especially have been associated with neurological disease when injected into humans.

The 3M Purification Inc. Solution

Beta Glucan Extractables

As stated, it was determined that certain cellulose containing filters contributed an extractable to filtered fluids resulting in a positive LAL test reaction. Further studies showed that the extracted material, primarily beta - 1,3 glucan, was not by nature pyrogenic, but, that it cross reacts with LAL test reagents resulting in a false positive test result. 3M Purification developed use of a highly purified cellulose that provides an effluent essentially free of beta glucan and is non-reactive as measured by the LAL test.

The highly purified cellulose contains an alpha cellulose content of at least 90% and provides separation media that does not result in false positive LAL tests. It is also noteworthy that this cellulose formulation is produced by the sulphite process. Other types of cellulose are produced using the Kraft process, which employs a caustic leach step known to break down cellulose polymers and, it is believed, to release quantities of beta - 1,3 glucan. The sulphite process is less severe than the Kraft process and may contribute to the noted lack of beta - 1,3 glucan extractables.

Using the highly purified cellulose material described above, 3M Purification Inc. was able to formulate proprietary grades of Zeta Plus™ depth filter media for use in parenteral filtration applications. As will be described, these highly purified cellulose depth filters are also formulated with acid extracted filter aid to produce exceptionally low extractable levels.

Ultra Low Level Metal Ion Extractables

In order to further reduce filter extractables, a process of pre-extracting filter aids used in Zeta Plus filters was developed. Filter aids, typically diatomaceous earth, are mined and thus are often contaminated with metal ions such as aluminum, iron, copper and other metals. Aluminum especially was found to be toxic when present in high amounts in parenteral solutions and infant formula. The method developed by 3M Purification Inc. for use in Zeta Plus ZA and LA grades of filter media to reduce detectable metal ions involves pre-extracting diatomaceous earth with hydrochloric acid. The results of the extraction process for Zeta Plus LA grade filter medium are shown in Table 2.

Table 2 — Effect of Acid Pre-extraction of Zeta Plus™ LA Filter Medium

Metal Ion (ppb)	% of Recommended Rinse Volume		
	33%	67%	100%
Al	23	< 5	< 5
Ca	710	30	12
Cu	47	3	<2
Fe	89	9	4
K	830	43	< 35
Mg	310	12	< 8
Na	3,800	160	75
Zn	28	2	1

The results above represent metal ion extractables, at ppb levels, detected following various rinse volumes. The recommended rinse volume for Zeta Plus filters is 5 liters per square foot of filter area. The percent rinse volume referenced in the table above is based on the recommended rinse volume. In all cases, metal ion extractable concentration is exceptionally low for Zeta Plus LA filter medium.

Conclusion and Summary

This 3M Purification Inc. Application Brief has presented a development with proprietary Zeta Plus depth filter media that incorporates a highly purified form of cellulose which results in effluent that eliminates false positive LAL test results. In other cellulose filters, an extractable identified as beta - 1,3 glucan was identified that can cause a positive LAL test reaction. Use of Zeta Plus LA and LP grades, with highly purified cellulose, will eliminate false positive LAL test results. These filter grades provide an effluent essentially free of beta glucan and is non-reactive as measured by the LAL test.

A second development was described allowing Zeta Plus LA grade filters to provide ultra low levels of metal ion extractables, in addition to minimizing beta glucan levels. The method to accomplish this involves pre-extraction of filter aid used to formulate depth filters. The filter aid, diatomaceous earth, contains associated metals when mined from the earth. A hydrochloric acid pre-extraction process performed prior to formulation, results in a filter medium with substantially reduced metal ion extractables.

Taken together, depth filters with extremely low extractables levels provides users with a choice for critical filtration applications where the end product is a parenteral or extractables must be minimized. The combination of low beta glucan and low metal ion extractables are available in Zeta Plus LA Series filters.

3M Purification Inc.
400 Research Parkway
Meriden, CT 06450, U.S.A.
Tel (800) 243-6894
(203) 237-5541
Fax (203) 630-4530
www.3Mpurification.com

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