PREDICTIVEBIOTECH™
A Predictive Technology Group Company

WHARTON'S JELLY BIRTH TISSUE PROCESSING: MAINTAINING CHARACTERISTICS OF SOURCE TISSUE WHILE MAXIMIZING CONSISTENCY, QUALITY, AND SAFETY

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INTRODUCTION

Research on the therapeutic potential of perinatal birth tissue in a clinical setting has increased exponentially as clinicians, scientists, researchers, and manufacturers continue to innovate and utilize this tissue with patients for its regenerative properties. In order to effectively measure outcomes, several factors must be considered: 1) Birth tissue is dynamic and varies widely from one donation to the next; 2) Standardization (reduction of variations from each lot in birth tissue) of a final product is critical to clinical utility. Without proper processing techniques, it is not possible to address variations and ensure product quality, product safety and product consistency.

Predictive Biotech's Wharton's Jelly human cell and tissue product (HCT/P), WJ Flow™ (Wharton's Jelly Flow), is derived from donated, full-term birth tissue. Equipped with state-of-the-art analytical equipment to ensure the safety and viability of its allografts, Predictive Biotech's laboratory and proprietary processes maintain the tissue's biological components, yielding a normalized dose. WJ Flow is primarily comprised of epithelial cells, endothelial cells, perivascular cells, mesenchymal stromal cells (MSCs), cytokines, growth factors, exosomes, and scaffolding proteins.

BACKGROUND

Wharton's Jelly

The umbilical cord is a structure that connects the placenta to the developing fetus, serving as a nutrient conduit. At term, in humans, the umbilical cord is typically 40–60 cm long, with a diameter of 1–2 cm. The structure is made of a single layer of amniotic epithelium that encloses a mucoid connective tissue through which three vessels – a vein and two arteries – carry oxygenated and deoxygenated blood between the placenta and fetus, respectively. The three vessels are supported within a layer of connective tissue known as Wharton's Jelly.

Function of Wharton's Jelly

Wharton's Jelly is classified as connective tissue. It is a gelatinous substance within the umbilical cord largely made up of mucopolysaccharides (hyaluronic acid and chondroitin sulfate). As a mucoid tissue, the Wharton's Jelly protects and insulates umbilical blood vessels. Extracellular matrix (ECM) components of Wharton's Jelly are similar to those of articular cartilage.[1] The ECM is primarily comprised of collagen, which organizes over 50% of the defatted dry weight of tissue. Wharton's Jelly also has large amounts of glycosaminoglycans, especially hyaluronic acid, forming about 70% of total glycosaminoglycans content.[2] The hyaluronic acid component found in Wharton's Jelly is reported to be higher in molecular weight, 1100± 200 Kda.[3] The high percentage of hyaluronic acid characterizes Wharton's Jelly as a strongly hydrated and viscous tissue, suitable as a natural hydrogel-type biomaterial for providing cushioning and lubrication.[4]

Wharton Jelly Flow (WJ Flow™) Product Description

WJ Flow is a minimally manipulated human tissue allograft made from the Wharton's Jelly layer of the umbilical cord. WJ Flow is processed to preserve the structural integrity of Wharton's Jelly and meets the definition of minimally manipulated as described in the Food and Drug Administration's (FDA) published "Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use", for structural tissue (2017). The processing of WJ Flow does not alter the original tissue's function as a mucoid connective tissue, providing physical support and cushioning.

WJ Flow is where science meets nature. Predictive Biotech has invested years into developing and optimizing tissue processing techniques to increase the viability of the tissue contents and increase the value of the cytokine and growth factor profiles. WJ Flow is the purest form of Wharton's Jelly and is the accomplishment of meticulous planning and testing in our laboratory. WJ Flow's composition has been analyzed using flow cytometry, an in-depth human cytokine array, an enzyme-linked immunosorbent assay (ELISA) array, and an extracellular matrix characterization.

For regulatory purposes, WJ Flow is:

- minimally manipulated
- not combined with other articles, exception of a cryogenic preservative (DMSO)
- not dependent on metabolic activity of living cells for product's primary function
- designated as an HCT/P under CFR 1271 Section 361

CELLULAR CHARACTERIZATION

Cells are classified by the type of work they do within the environment in which they are found. Visually, cells can be classified either by: 1) the cell's shape, or 2) surface markers which are identical across cells that serve the same function. However, in most situations, it is difficult to classify cells by normal visual means due to the microscopic size of cells and the vast number of cell subtypes.

Predictive Biotech aliquots approximately 1.5 million nucleated cells in each 1 ml unit of Wharton's Jelly Flow (after manufacturing). Flow cytometry allows for the rapid identification, separation, and characterization of various cell subtypes by virtue of size and morphology from a fluid heterogeneous mixture. This process uses antibodies to fluorescently label individual cells in a sample. Flow cytometry is used to analyze each product lot to determine cell count and cell viability. With this data, Predictive Biotech normalizes WJ Flow to provide approximately 1.5 million nucleated cells per 1 ml of product. This product is cryopreserved at -196 degrees Celsius to maintain cell viability.

WJ Flow is aliquoted to contain approximately 1.2 million (+/- 150k) viable MSCs per 1 ml of allograft material after being thawed (from cryopreservation) and ready for administration by provider. The native tissue MSCs identified in WJ Flow are characterized using CD90, CD146, CD45, CD31, and CD326.

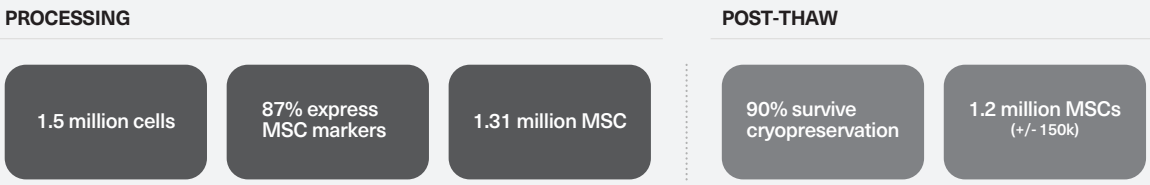

Figure 1: Predictive Biotech's standard operating procedure directs flow cytometry be used on each processed umbilical cord to standardize units of product to 1.0 million MSCs per 1 ml WJ Flow MSCs are characterized using markers: CD90, CD146, CD45, CD 31 and CD326.

Over the past two years, Predictive Biotech has worked to improve upon its Wharton's Jelly products. One objective has been to minimize variability that can occur in the products due to variability in donor tissues. Predictive Biotech has reduced the variability in cell counts that can occur during processing (Pre-freeze) or upon thawing of the products (Post-thaw). Figure 2 demonstrates this reduction in variability between WJ Flow and CoreCyte as seen in the size of the error bars for each sample. The error bars for CoreCyte are much larger and represent about a 30% variance while the bars for WJ Flow represent only about a 10% difference.

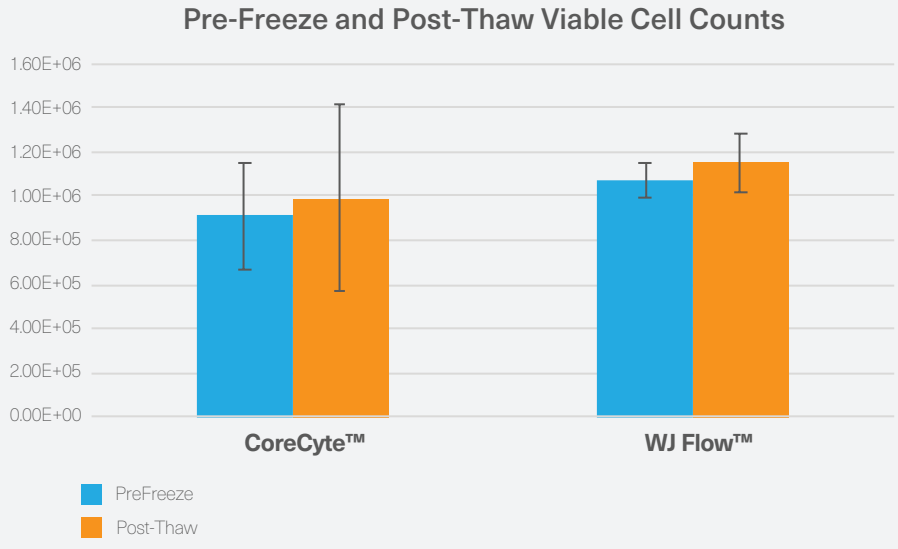

Figure 2: Pre-Freeze and Post-Thaw Viable Cell Counts (n = 11 for each sample). WJ Flow has less variability between product lots both after processing and after thawing.

With reduced variability between samples, the next measure of the product was to ensure that the cellular content of the product was not changed between these products. Figure 3 shows the cellular characterization of CoreCyte compared to WJ Flow. No statistical difference was found between any of the extracellular markers measured (not shown: CD45, CD31, and CD326).

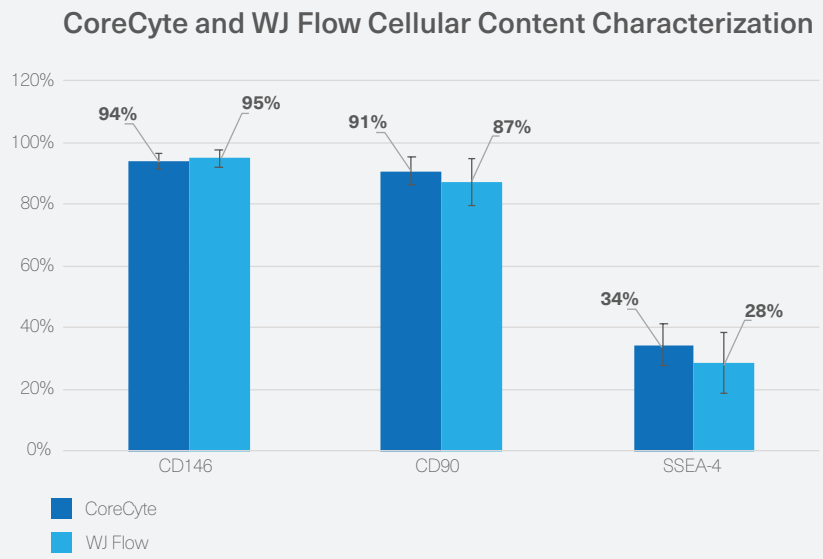

Figure 3: CoreCyte and WJ Flow Cellular Content Characterization (n = 11 for each sample). CD146 is a marker for pericytes, a cell that is thought to be a "precursor" to mesenchymal stromal cells. CD90 identifies mesenchymal stromal cells, while SSEA-4 is a measure of pluripotency (ability to grow, divide, and differentiate into other cell types in a laboratory setting). No statistical difference was found between these products (Student's t-test).

CYTOKINES

Cytokines are a broad group of small cellular proteins involved in biological function. Birth tissue contains a rich and diverse population of cytokines, underscoring the dynamic nature of the tissue. These often-overlooked proteins are critical tissue components that must be accounted for and maintained in the manufacturing process of donated tissue. Predictive Biotech products were analyzed by RayBiotech in a Quantibody® analysis, an array-based multiplex ELISA system for simultaneous quantitative measurement of various cytokines, growth factors, proteases, soluble receptors and other proteins (**Figure 4**).

Description	WJ Flow (avg. pg/ml)
2B4	10.1
Activin A	5.6
ADAM9	167.9
Adiponectin	117.0
Adipsin	8,221.7
AFP	3,061.1
AgRP	158.8
Albumin	2,256.4
ALCAM	561.4
AMICA	48.3
ANG-1	102.6
ANG-2	91.5
Angiogenin	1,268.5
Angiostatin	8,146.1
Angiotensinogen	3,198.6
ANGPTL4	199.9
APRIL	6,217.9
Axl	62.9
B2M	1,060.4
B7-H1	98.2
B7-H3	78.2
BCAM	142.7
BCMA	24.6
BDNF	1.2
bFGF	187.6
bIG-H3	21,904.8
BMP-2	14,766.5
BMP-9	28.4
CA125	1,664.8
CA15-3	26.1
CA19-9	2,097.2
Cadherin-11	10.3
Cathepsin B	407.6
Cathepsin L	114.9
Cathepsin S	132.7
CD14	3,349.7
CD163	11,132.8
CD200	380.9
CD40	30.3
CD40L	13.0
CD97	3,544.8
CD99	413.5
CEA	216.3
CEACAM-1	37.3
Chemerin	4,890.1
CHI3L1	2,005.2
Ck beta 8-1	793.7
Clusterin	10,635.7
Cripto-1	16.5
CRP	334.1

Description	WJ Flow (avg. pg/ml)
CRTAM	158.0
CXCL14	324.7
CXCL16	333.4
Cystatin B	1,085.4
Cystatin C	4,708.4
Cystatin E M	364.4
DAN	187.9
Decorin	1,009.3
DKK-1	173.6
Dkk-3	7,203.5
DLL1	103.1
DPPIV	14,850.6
DR6	128.5
E-Cadherin	497.0
EG-VEGF	30.4
EGF R	21.5
EMMPRIN	121.4
ENA-78	10.2
Endoglin	7.1
EpCAM	4.7
ErbB2	7.9
FAP	513.6
Fas	16.0
FAS L	14.1
Fcg RIIBC	63.9
Ferritin	132,550.1
Fetuin A	843,467.3
FGF-19	309.5
FLRG	7,576.3
Flt-3L	4.8
Follistatin	659.5
Follistatin-like 1	119.2
Furin	16,070.3
G-CSF	25.8
Galectin-1	3,906.8
Galectin-3	431.1
Galectin-7	2,202.1
Galectin-9	346.2
GDF-15	24.9
gp130	951.6
Granulysin	545.5
GROa	852.1
HAI-2	51.4
HCC-1	360.3
HCC-4	3.1
hCGb	123.8
HGF	150.1
HGF R	173.0
I-309	2.7
I-TAC	32.1

Description	WJ Flow (avg. pg/ml)
ICAM-1	1,707.6
ICAM-2	563.8
ICAM-3	60.6
IGF-1R	1,042.2
IGF-2	457.4
IGF-2R	421.8
IGFBP-1	2,811.8
IGFBP-2	4,434.7
IGFBP-3	17,626.2
IGFBP-4	3,603.3
IGFBP-6	6,261.6
IL-1 F7	833.3
IL-1 R3	127.3
IL-1 R4	154.6
IL-10 Rb	9.5
IL-13 R1	295.9
IL-13 R2	288.4
IL-16	3.8
IL-17B	597.4
IL-17F	2.8
IL-1ra	27.7
IL-2 Ra	34.1
IL-2 Rb	276.2
IL-2 Rg	184.4
IL-23	242.6
IL-24	131.7
IL-3	233.5
IL-32 alpha	14.3
IL-33	58.3
IP-10	39.0
Kallikrein 14	15.0
L-Selectin	369.4
L1CAM-2	998.9
LAP(TGFb1)	47.5
Layilin	65.2
LDL R	27.1
Legumain	56.8
Leptin	145.3
Lipocalin-2	294.1
LYVE-1	614.3
MBL	65.9
MCP-1	33.5
MCP-2	5.8
MCP-3	2.3
MCP-4	5.7
MCSF	10.6
MCSF R	792.0
MDC	4.4
Midkine	2,237.5
MIF	1,080.9

Figure 4: Representative list of 150 of the 228 present in the cytokine array characterization in WJ Flow, as analyzed by RayBiotech (average pg/ml, n = 5).

Predictive Biotech has developed an in-house assay based on the same technology as that used by Ray Biotech. In this assay, a much smaller set of cytokines is measured. These cytokines were chosen based on biological function and expression levels in the hope that this test could eventually become a Quality Control measure to ensure that each vial contained pre-specified levels of these cytokines. Improvements to the manufacturing process used in WJ Flow result in increased cytokine levels within the product, a finding similar to that seen with the data obtained by Ray Biotech (**Figure 5)**.

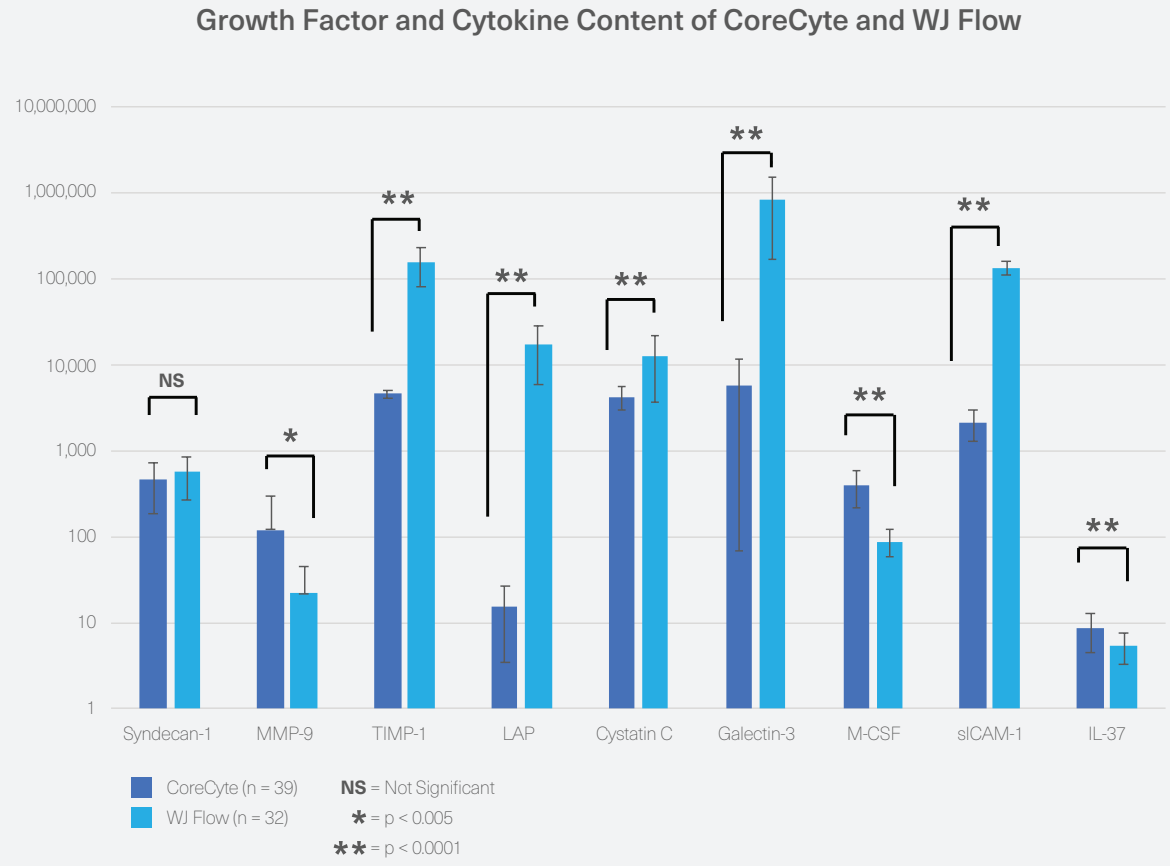

Figure 5: Predictive Biotech cytokine assay of CoreCyte and WJ Flow. Student's t-test was used to determine statistical significance between cytokine levels in products.

EXOSOMES

Predictive Biotech quantified the exosome presence of its products as a part of the product's tissue profile using a standard ELISA (**Figure 6**). Post-thaw analysis confirmed that WJ Flow contains naturally occurring exosomes from birth tissue and quantified the average value to be approximately 6.3 billion exosomes per 1 ml of product. Exosomes are membrane-bound extracellular vesicles that are present in all bodily fluids under both normal and pathophysiological conditions.[5] Exosomes are produced in the endosomal compartment of most eukaryotic cells. An ELISA is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.

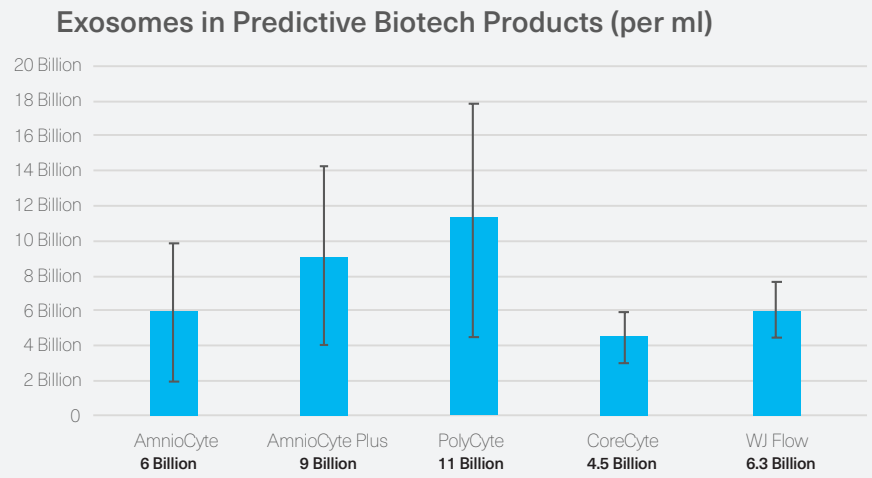

Figure 6: Quantitative analysis of exosomes in Predictive Biotech's products was performed using an ExoQuick-TC Ultra kit to isolate the exosomes and then was quantified using the ExoELISA-ULTRA Complete Kit. Quantification was accomplished using a spectrophotometric plate reader (Thermo MultiSkan FC) at 450 nm (n ≥ 4 for each sample).

EXTRACELLULAR MATRIX

The extracellular matrix is composed of tissue components, such as collagen and glycoproteins, that not only provide structural support, but also supplement important functions. Extracellular components in WJ Flow samples were validated by R&D Biotechne (**Figure 7**).

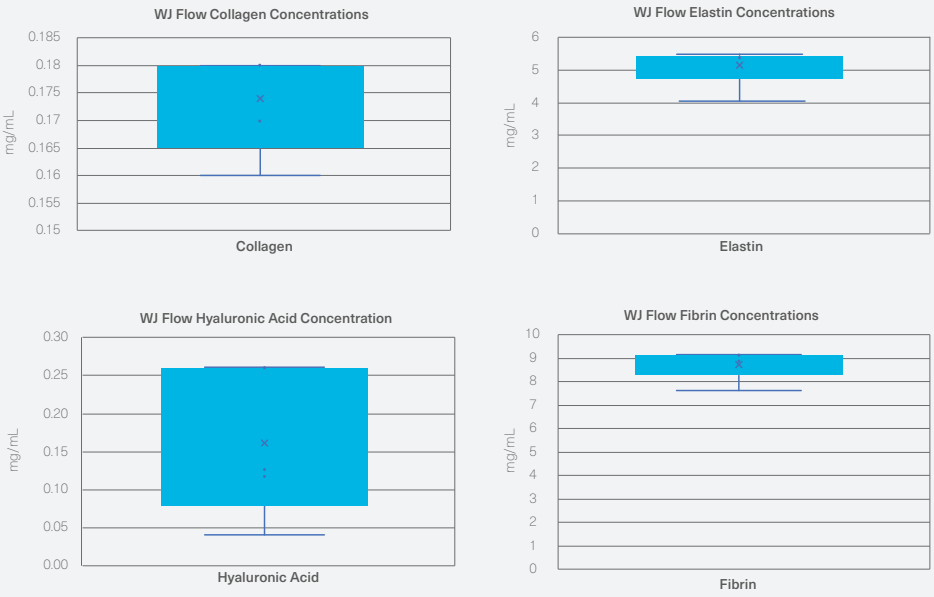

Figure 7: Quantitative analysis of extracellular matrix found in WJ Flow, as analyzed by R&D Biotechne (n = 10 for each measurement).

PRODUCT SAFETY

WJ Flow is manufactured under Current Good Manufacturing Practice (cGMP), Current Good Tissue Practice (cGTP) and is ISO 13485 accredited. WJ Flow is produced from birth tissues after normal, full-term pregnancies. Each donor is carefully screened, with comprehensive medical and social histories of donors collected for review by a medical doctor. All tissue is procured, processed and tested in accordance with FDA requirements to minimize potential risks of disease transmission to recipients. Infectious disease testing is performed at a certified laboratory in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493.

PRODUCT SAFETY PROCESS

Birth tissue is shipped to Predictive Biotech's lab within 24 hours of birth. Upon arrival, Predictive Biotech's quality assurance team performs inspections to verify that tissue viability has been properly maintained during transport. All tissue is processed in Predictive Biotech's ISO 7 cleanroom under ISO 5 biological safety cabinets. Once produced, three (3) samples of each lot of WJ Flow are sent for safety testing. All remaining product is placed into cryogenic quarantine (release pending third-party validation and medical director approval). Two (2) of the samples are sent to a third-party laboratory for serology and bacterial endotoxin testing. The third sample is screened in-house for particulates using a Horizon Halo system for subvisible particle analysis in order to meet USP 790 standards.

14 Day Microbial Screening

Sample ID	Product Description	Result	Status
S-012820-05826	C20023007	No Growth, Day 14	Final

Method Description

14-day microbial screening in accordance with USP <71>, 21CFR610.12.

Microbial screening looks for bacterial, fungal and/or mold growth. If growth present in the sample, the entire lot will be discarded, and the growth will be characterized to understand the type of contamination.

Bacterial Endotoxin Test

Sample ID	Result Units	Result	Status
S-012820-05826	EU/ml	0.0718	Final

Method Description

Endotoxin testing in accordance with USP <85>, ANSI / AAMI ST72:2011.

Endotoxins are bacterial structural components that are released when such a cell is lysed. These components are toxic if administered to humans and animals, causing a pyrogenic response (rise in body temperature). All samples must have an EU/ml of less than 3.0.

Particulate Test

Halo System - Background membrane imaging

Clean Brightfield | Failed Brightfield | Clean Simi View | Failed Simi View

Method Description

Particulate testing in accordance with USP <787>, USP <788>, USP <790>.

Figure 8: Safety tests (microbial screening, bacterial endotoxin, and particulate) are performed on every lot of WJ Flow. Sample results.

Predictive Biotech's medical director reviews donor's medical records and blood test, and deems the donor eligible or ineligible to donate tissue.

DONOR ELIGIBILITY SCREEN

Test Description	Standards	Status
HIV-1/HIV-2 Antibody	Non-Reactive	Final
HIV-1/HIV-2 NAT	Non-Reactive	Final
Hepatitis B Surface and Core Antibody	Non-Reactive	Final
Hepatitis B NAT	Non-Reactive	Final
Hepatitis C Antibody	Non-Reactive	Final
Hepatitis C NAT	Non-Reactive	Final
Syphilis	Non-Reactive	Final
West Nile Virus	Non-Reactive	Final
Cytomegalovirus IgM	Not Detected	Final
Microbial Screening – Bacterial	No Growth (USP 71)	Final
Microbial Screening – Fungal	No Growth (USP 71)	Final
Bacterial Endotoxin (BET)	≤3 EU/ml	Final
Particle Contaminant	USP 787, 788, 790	Final

Method Description

Infectious disease testing is performed at a certified laboratory in accordance with the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and 42 CFR part 493.

Figure 9: Donor eligibility screening panel.

Should tissue fail any safety test (microbial screening, endotoxin, and particulate) or the donor eligibility requirements, the entire lot of WJ Flow will fail quarantine and be discarded.

Once third-party analysis is received and approved, Predictive Biotech's quality assurance team conducts a final review of the entire process. If review is approved, the individual lot of product will be released from quarantine. WJ Flow is shipped overnight on dry ice to keep product at appropriate temperature to maintain product viability.

CONCLUSION

WJ Flow is processed at Predictive Biotech's state-of-the-art laboratory, where each step is monitored carefully for quality assurance. WJ Flow is primarily comprised of epithelial cells, endothelial cells, perivascular cells, mesenchymal stromal cells (MSCs), cytokines, growth factors, exosomes, and scaffolding proteins as validated by third-party labs and internal measures.

Our investment in research, processes, equipment, facilities, quality assurance, and third-party testing has provided Predictive Biotech the ability to develop the safest and most consistent Wharton's Jelly derived allografts available in the market. With over 140,000 allografts shipped, Predictive Biotech's processing, safety, and normalization standards will position WJ Flow as a market leading product.

Disclaimer

Our Human Cell and Tissue Products (HCT/Ps) are not FDA approved or licensed for the prevention, treatment, diagnosis, mitigation and/or cure of any disease or condition, including COVID-19.

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