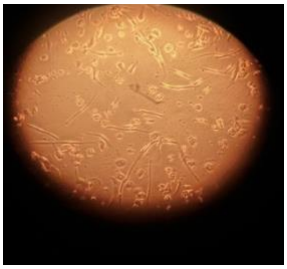
L2 Bio White Paper on Stem Cell Banking Process and Quality

Stem cell quality is a crucial factor in stem cell research and applications and is the highest priority of L2 Bio. Ensuring the quality of stem cells is essential for their safe and effective use in various fields, including regenerative medicine and drug discovery. Several studies have addressed the issue of stem cell quality and have proposed different approaches to assess and improve it. L2 Bio works with the top professionals in the industry and uses state of the art FDA and GMP compliant processes to ensure the production of better and safer Stem Cells for their immediate use and for years to come. L2 Bio cells are a safer option to the millions of people who seek out Stem Cell treatments in countries that lack the rigorous standard of the United States FDA.

To ensure better quality of adipose derived mesenchymal stem cells (AD-MSCs) during the expansion or culture process, we incorporate specific measures into their standard operating procedures (SOPs). These measures aim to optimize culture conditions, enhance cell proliferation, maintain cell characteristics, and minimize the risk of contamination. By implementing these strategies, L2 Bio can improve the quality and consistency of MSCs for various applications in regenerative medicine and research.

Figure 1- Example of a typical Stem Cell grown in culture by l2 Bio: This is a good-looking, or beautiful stem cell because it is round and has a large, prominent nucleus. It also has a clear cytoplasm.



The choice of culture medium and supplements is crucial for maintaining MSC quality during expansion. It is common for laboratories to use media formulations that contain essential nutrients, vitamins, and supplements to support cell growth and maintain cell characteristics. The L2 Bio process and all affiliated CMO Lab relationships do not use fetal bovine serum (FBS) or any such media that does not normally exist in the human body. Much of our process is proprietary and considered intellectual property and cannot be shared here in its exact formulation. However, what we can talk about is the basic media formulations used to supplement the culture process.

The use of FBS raises concerns about potential contamination and batch-to-batch variability. To address these issues, we choose to use serum-free or xeno-free media formulations, which eliminate the risk of contamination and ensure consistency in MSC culture. The inclusion of serum-free or xeno-free media in SOPs can contribute to better MSC quality and reduce the risk of introducing unknown factors into the culture system.

Another area where we are more advanced is Cryopreservation. There are common methods known to preserve stem cells for future use. Xie et al. (2022). It is important not to damage cells during the Cryopreservation process. During Cryopreservation we have implemented specific quality control parameters to be assessed during the process such as an even slower freeze time, not utilizing vials over 2 ml in volume, and while not exceeding 5-6 million MSCs per ml. This special Cryopreservation process helps ensure maximum viability better ensuring the highest percentage of live, viable. Manufacturing, clinical-grade stem cell. This study Xie et al. (2022) emphasizes the significance of preventing cryodamage ensuring the quality of cryopreserved stem cells. However, we have made a tremendous advancement in Cryopreservation utilizing a DMSO-free special "sugar water" allowing the L2 Bio stem cell product to be stored in a -20c environment losing only 1% of its thawed viability compared to a vial of stem cells being stored in a minus 85 degree centigrade environment. This not only contributes to the convenience of the administering physician but it means that a doctor does not have to spend thousands of dollars on a minus 85 centigrade cryo-freezer. If a doctor does not have a minus 85 degree centigrade cryo-freezer and a patient has to cancel their treatment date, the patients MSCs can be stored in a common refrigerator freezer compartment for up to 30 days.

Figure 2-The L2 Bio MSC has virtually the same Viability when thawed when stored in a -20c vs a -80c environment for 30 days with only a 1% difference. When a margin of error of 3-6% is factored into the equation there is basically no difference with a consistent 86.5-94% cell viability based on storage method.

L2 Bio Cell Viability Validation Report

	AUTHORED BY:	DOCUMENT APPROVED BY:
Name:	Nebiyu Arega	Roma Borkar
Signature:		
Position:	Lab Technician	Scientific Officer
Date:	08/24/2022	08/24/2022

OBJECTIVE

The objective of this report is to document the cell count of adipose autograft (AA) in different temperature condition (-20°C and -80°C for 1 month) and compare it with the original storage condition (liquid nitrogen).

VALIDATION PROCESS

Six AA vials each stored at -196 °C and -80 °C and five AA vials stored at -20 °C were used for the validation. Each AA vials were placed into mylar bag and heat sealed. Each Mylar bag was then transferred to bubble wrap pouch and placed in different conditions as described below:

Sr. No	Location	Temperature	No. of Vials	Duration
1.	Liquid nitrogen (Original source)	-196°C	6	N/A
3.	Deep Freezer	-80°C	6	1 Month
4.	Freezer	-20°C	5	1 Month

Cells were thawed for 5 minutes post each timepoint and cell count/viability was obtained using MUSE cell analyzer.

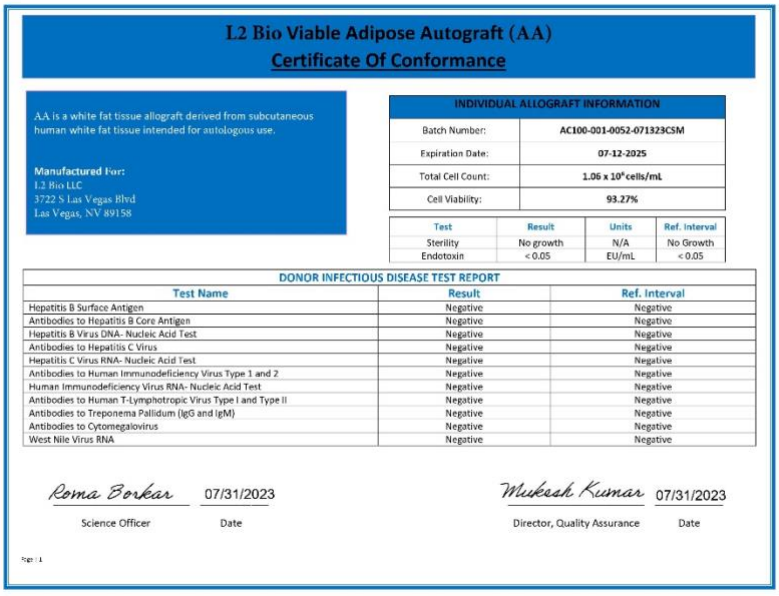
Test Performed By:	Nebiyu Arega	Date: 8/24/2022
Reviewed By:	Roma Borkar	Date: 08/24/2022

CONCLUSIONS:

- The change in viable cell count between sample stored at -196 degrees (liquid nitrogen) and -80 degrees freezer was 12.357 %.
- The change in viable cell count between sample stored at -196 degrees (liquid nitrogen) and -20 degrees freezer was 13.308 %.

Our SOPs implement strict quality control measures to ensure the purity and identity of MSCs during the expansion process. These measures include regular monitoring of cell morphology, growth rate, and surface marker expression. Flow cytometry analysis is commonly used through the process to assess the expression of MSC-specific markers. We can't disclose some of the specific markers as this is part of "secret sauce" and we believe why L2 Bio's stem cells are so healthy. We monitor our cells to ensure that they contain certain markers that are considered normal and healthy, and we make sure they are in abundance. By regularly monitoring these parameters, L2 Bio and our partners can be ensured that the expanded cells maintain their MSC characteristics and are free from contamination or phenotypic changes.

Figure 3- Attached is one example of the long list of SOPs of L2 Bio's internal extensive infectious disease testing requirements not required by the FDA for Autologous Stem Cells but part of L2's commitment to safety, and excellence.



L2 Bio Viable Adipose Autograft (AA)
Certificate Of Conformance

AA is a white fat tissue allograft derived from subcutaneous human white fat tissue intended for autologous use.

Manufactured For:
L2 Bio LLC
3722 S Las Vegas Blvd
Las Vegas, NV 89158

INDIVIDUAL ALLOGRAFT INFORMATION

Batch Number:	AC100-001-0052-071323CSM
Expiration Date:	07-12-2025
Total Cell Count:	1.06 x 10⁶ cells/mL
Cell Viability:	93.27%

Test	Result	Units	Ref. Interval
Sterility	No growth	N/A	No Growth
Endotoxin	< 0.05	EU/mL	< 0.05

DONOR INFECTIOUS DISEASE TEST REPORT

Test Name	Result	Ref. Interval
Hepatitis B Surface Antigen	Negative	Negative
Antibodies to Hepatitis B Core Antigen	Negative	Negative
Hepatitis B Virus DNA- Nucleic Acid Test	Negative	Negative
Antibodies to Hepatitis C Virus	Negative	Negative
Hepatitis C Virus RNA- Nucleic Acid Test	Negative	Negative
Antibodies to Human Immunodeficiency Virus Type 1 and 2	Negative	Negative
Human Immunodeficiency Virus RNA- Nucleic Acid Test	Negative	Negative
Antibodies to Human T-Lymphotropic Virus Type I and Type II	Negative	Negative
Antibodies to Treponema Pallidum (IgG and IgM)	Negative	Negative
Antibodies to Cytomegalovirus	Negative	Negative
West Nile Virus RNA	Negative	Negative

Roma Borkar 07/31/2023
Science Officer Date

Mukesh Kumar 07/31/2023
Director, Quality Assurance Date

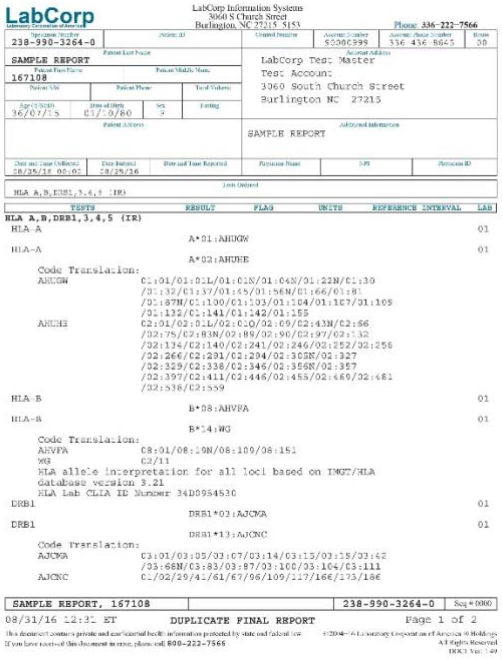
To minimize the risk of contamination, we also incorporate aseptic techniques and stringent cleaning procedures into their SOPs. This includes working in a clean room under laminar flow hood when sterility is essential, using sterile equipment and reagents, and regularly disinfecting the culture area. Additionally, we implement unannounced random spot and regular testing for mycoplasma contamination to ensure the integrity of the MSC cultures. These measures help to maintain a controlled and sterile environment, reducing the risk of introducing contaminants that could compromise the quality of the MSCs.

L2 Bio offers an HLA Type test option to match stem cells grown from L2 Bio donors to blood relatives. This is important because HLA compatibility is essential for successful stem cell transplantation. L2 Bio's HLA Type test can be used to identify blood relatives who are a good match for stem cell transplantation, even if they are not in the same location as the patient.

Stem cells grown from L2 Bio donors can be used for current or future treatments in blood relatives to address the same conditions that are being studied in clinical trials

today in the USA, or for treatments overseas for where such medical tourism is legal. HLA typed stem cells also can be used for safer research to companies engaged in FDA Approved Clinical Trials in the USA. This means that patients may have access to stem cell therapy for their condition, even if it is not yet available in their country or if they are not eligible for a clinical trial.

Here are some examples of how L2 Bio's HLA Type testing and stem cell therapy can be used to benefit families:



LabCorp

LabCorp Information Systems
3060 S Church Street
Burlington, NC 27215 5153
Phone: 336-222-7566

238-990-3264-0

SAMPLE REPORT

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LabCorp Test Master
Test Account
3060 South Church Street
Burlington NC 27215

SAMPLE REPORT

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HLA A,B,DRB1,3,4,5 (IR)

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
HLA A,B,DRB1,3,4,5 (IR)					
HLA-A	A*01:AHUGW				01
HLA-A	A*02:AHUBE				01

Code Translation:
AHUGW C1:01/01:01L/01:01N/01:04N/01:22N/01:30
/01:32/01:37/01:45/01:56N/01:66/01:81
/01:87N/01:100/01:103/01:104/01:107/01:108
/01:132/01:141/01:142/01:155
AHUBE C2:01/02:01L/02:CQQ/02:69/02:43N/02:66
/02:75/02:83N/02:89/02:90/02:97/02:132
/02:134/02:140/02:241/02:246/02:252/02:256
/02:266/02:291/02:294/02:305N/02:327
/02:329/02:338/02:346/02:356N/02:357
/02:397/02:411/02:446/02:455/02:469/02:481
/02:538/02:559

HLA-B	B*08:AHVPA				01
HLA-B	B*14:WG				01

Code Translation:
AHVPA C8:01/08:19N/08:109/08:151
WG C2/11
HLA allele interpretation for all loci based on IMGT/HLA database version 3.21
HLA Lab CLIA ID Number 34D0954530

DRB1	DRB1*03:AJCMA				01
DRB1	DRB1*13:AJCNC				01

Code Translation:
AJCMA C3:01/03:05/03:07/03:14/03:15/03:15/03:42
/03:68N/03:83/03:87/03:100/03:104/03:111
AJCNC C1/02/29/41/61/67/98/109/127/166/173/186

SAMPLE REPORT, 167108 | 238-990-3264-0 | Seq # 0060

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- A mother or father may be able to protect their children's future by having their HLA type tested and by storing stem cells as a first-degree relative donor. If one of their children develops a condition that can be treated with stem cell therapy, the family will already have a matched donor ready.
- A son or daughter may be able to offer stem cells to their parents, or other matched relatives with little to no chance of rejection. This could be a lifesaver for patients with conditions such as leukemia, lymphoma, and sickle cell anemia.

L2 Bio is committed to making stem cell therapy more accessible and affordable for everyone. By offering HLA Type testing and stem cell storage, L2 Bio is helping families to protect their loved ones and give them hope for the future.

All laboratories add various measures to their standard operating procedures (SOPs) to ensure better quality of MSCs during the expansion or culture process, but not all labs are created equal. We at L2 Bio are constantly looking at our SOPs and new ways to improve and optimizing culture conditions, using serum-free or xeno-free media formulations, implementing strict quality control measures, and maintaining aseptic techniques. By following these SOPs, we can enhance MSC proliferation, maintain cell characteristics, minimize the risk of contamination, and improve the overall quality and consistency of MSCs for regenerative medicine and research applications.

References:

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