Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Efficiency of MTT and Trypan Blue Assays for Detection of Viability and Recovery of Different Frozen Cell Lines

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Abstract

THE quantity of healthy cells in a sample is known as cell viability. For all types of cell culture, measuring cell viability is crucial to ensuring a supply of high-quality cell lines through setup, preparation and storage. Although the cryopreservation technique is used in both scientific and clinical research, there are still certain restrictions. At low temperatures, such as −196 °C (i.e., in liquid nitrogen) cells metabolize is almost nothing, which has unavoidable side effects, such as a genetic drift toward biological variations of cell-associated changes in lipids and proteins that could impair cellular activity and structure. Also, cells can be harmed by cryoprotective agents (CPAs), especially when they were administered in high concentrations in conditions where they would typically be used. So, it is important to use a reliable technique to follow up changes affecting cell viability after cryopreservation which were compared with control freshly subcultured cells. In the present study, the MTT assay was used to correlate cell behavior according to the number of healthy and viable cells. Different kinds of cell cultures were cryopreserved with dimethyl sulfoxide (DMSO) at 10% for variable years in a liquid nitrogen tank (-196 °C). Then, different samples were tested for viability using the MTT assay and counted by the trypan blue assay for comparing both results accuracy and confirmed by visual examination in sub-cultured media twenty four hours post-thawing. The MTT assay was easy, fast and showed more accurate results than the trypan blue assay. The visual examination twenty four hours post-subculture indicated that MTT assay can differentiate between healthy and apparently viable cells which have lost their function whereas the trypan blue assay failed to detect healthy cells in some samples.

Keywords: MTT assay, Cell viability, Cryopreservation and trypan blue assay.

Introduction

Viability is the capability of a retained sample to show a specific function, typically enzymatic or metabolic or activity that expressed as a proportion of the same purpose before preservation or by similar fresh untreated samples [1]. In vitro, temperature is a vital environmental element for viability of cell. Incubations with 5% CO_2 and 37°C are habitually used to culture mammalian cells [2]. According to the earlier studies, low temperatures decrease cell proliferation and have effect on embryonic development [3]. While slight heat stress accelerates growth and increases the rate of cell proliferation [4]. In addition, prolonged contact to subzero temperatures has the potential to significantly diminish the mammalian cells or embryos viability [5]. Cryopreservation is a procedure that keeps cells, tissues and organelles or any other biological agents by very low temperatures to get frozen. The living cells reactions to ice construction are practically and theoretically useful as formation of ice crystal, osmotic shock, and membrane injury through freezing and thawing will cause death of cell and viable tissues or cells which have great potential for research using in addition to medicinal applications cannot be stored for an extended period of time with simple cooling or freezing (6). With the use of cryoprotective agents (CPAs) and control tools temperature, efficacious cryopreservation of cells

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(Received 04/01/2024, accepted 25/02/2024)
DOI: 10.21608/EJVS.2024.260687.1764

or tissues and their clinical implementations will require explanation the chemical and physical features that occur in thawing and freezing cells cycles [6]. Among CPAs, DMSO (Dimethyl sulfoxide) that was initially developed in 1866 and has been extensively utilized for mammalian cells cryopreservation in culture. DMSO has numerous advantages such as somewhat cytotoxicity and low cost. It can reduce content of electrolyte at any given temperature in the remaining or unfrozen solution around the cell. Besides, it can diminish rate of survival and induction of cell differentiation by methylation of DNA and histone amendment. Nevertheless, these detrimental effects of DMSO on cryopreservation make it more challenging to apply for clinical aspects [7]. Numerous forms of tests can be employed to determine the number of viable cells. These assays relies on cells various functions comprising permeability of cell membrane, enzyme activity, adherence of cell, production of adenosine triphosphate (ATP), activity of nucleotide and uptake production of co-enzyme [8]. Among viability assays that highlight conversion of substrate to chromogenic product by living cells is MTT assay [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide]. MTT test is a simple colorimetric assay of cell survival and proliferation which was established by [9] and modified by [10] for measuring human lung cancer cell lines chemosensitivity. The assay is based on conversion of MTT by living cells into formazan crystals, which displays mitochondrial function [11]. The mechanism of action of MTT assay mainly relies on reduction of tetrazolium salt to insoluble formazan dye by dehydrogenase enzyme present in the viable cells at 37 °C. Then, dissolving of insoluble formazan salt by addition of DMSO as solubilizing agent followed by initiation of mitochondrial reductase enzyme which converts water-soluble yellow dye MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazo-liumbromide] to an insoluble purple formazan. After that, formazan is solubilized and using a spectroscopic multiplate reader, colored product concentration is determined quantitatively by optical density at 570 nm [11]. Minor alterations in metabolic activity can induce great variations in MTT permitting detection of cell stress in the absence of direct cell death upon contact to a toxic agent. The test has been optimized for non-adherent or adherent cells grown in multiple wells. Moreover, it can detect up to 10^6 cells per well in standard 96 well plate including 500-10,000 cells per well indicating the sensitivity of test for detection of viable cells [12]. The colored product intensity is directly correlated to viable cell numbers existing in cell culture because the ability of dead cells was lost to decrease salts of tetrazolium to formazan colored products while active metabolism of viable cells can convert MTT to formazan purple colored product at 570nm of absorbance [13]. Also, MTT assay was constructed for rapid assessment of viability and cell proliferation (14). The drawbacks of viability metabolic based assays and MTT in estimation of cell proliferation comprising interference of polyphenols or phytochemicals with succinate dehydrogenase activity or intervention directly with MTT assay [15].

The trypan blue stain assay was originally established in 1975 to estimate viable cell count and it is still utilized for detection of alterations in viable cell induced by toxins or drugs as confirmatory test [16]. The assay was used to determine the viable cell numbers in cell suspensions [17]. This assay based on negatively charged molecule and living integral cell membranes can exclude trypan blue stain whereas dead cells can be stained with trypan blue stain. Viable cells have characteristic clear cytoplasm while dead cells have blue cytoplasm and suspension of cells examined under light microscope [18]. Therefore, the current study was conducted to compare between MTT and trypan blue assays for estimation of viable and unviable different cell cultures along periods of different times.

Material and Methods

Ethical approval

- Institutional Animal Care and Use Committee at Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt and Animal Health Research Institute, Mycoplasma Department, Dokki, Giza, Egypt acknowledge the research manuscript, which has been reviewed under our research authority and is deemed in compliance with bioethical standards in good faith.

Study period and location

- The study was conducted in June 2022 at Central Laboratory for Evaluation of Veterinary Biologics (CLEVB),

(ARC), Cairo, Egypt and Veterinary Serum and Vaccine Research Institute, Newcastle Department (VSVRI), (ARC), Cairo, Egypt.

Cell culture types

Madin-Darby canine kidney (MDCK), Baby Hamster Kidney Fibroblast (BHK), Rabbit kidney (RK), African Green Monkey Kidney Cell (VERO), Madin-Darby bovine kidney (MDBK) were obtained from reference strain bank, the Central Lab. For Evaluation of Veterinary Biologics. The period of cryopreservation and number of samples were shown in **(Table-1).**

MEM (Minimum ***essential media), trypsin, DMSO and trypan blue 0.4%.***

Positive control cells: the positive control cells were composed of three different cell lines including freshly obtained BHK, MDBK and VERO cells. These cells were supplied from VACSERA, Agouza, Giza in 2023 and were used to compare the sheet growth cells and viability of various tested cell lines samples under inverted microscope.

TABLE 1. Date of cryopreservation for different types of cell lines and number of samples used in the study.

Number of samples	Date of cryopreservation	Cell culture type
1	2022	MDCK
2	2015	MDCK
3	2013	MDCK
4	2011	MDCK
5	2018	BHK
6	2015	BHK
7	2012	BHK
8	2012	BHK
9	2011	BHK
10	2020	RK
11	2019	RK
12	2018	RK
13	2022	VERO
14	2020	VERO
15	2019	VERO
16	2012	VERO
17	2022	MDBK
18	2021	MDBK
19	2019	MDBK
20	2012	MDBK
21	2023	Positive control

Preparation of MTT solution

Preparation of MTT solution was carried out by MTT dissolving in Dulbecco's phosphate-buffered saline (DPBS) (5 mg/ml) at pH 7.4. Then, using 0.2-µm filter the solution was filtered, sterilized and transferred to light protected sterile container. The dissolving solution was prepared with 40% (v/v) DMF dimethylformamide comprising 2% (v/v) glacial acetic acid under fume hood ventilation. Then, 16% (W/V) sodium dodecyl sulfate (SDS) was added to solution with pH optimization at 4.7. SDS precipitation is inhibited by storage of solubilization solution at room temperature otherwise heating at 37◦C is implemented for re-solubilization to inhibit precipitation. MTT solution should be guarded from light and reserved at -20 °C till analysis or kept at 4 °C for using at once [19].

Methodology

In 96 well plates, 100 µl /well of cell suspension were added with or without the tested compounds and incubated at 37 °C with 5% CO_2. Then, 10 µl of MTT solution was added to each well with 0.45 mg/ml as final concentration and incubated for one to four hours at 37°C. After that, 100 µl of solubilization solution were used for formazan crystals dissolving and multiplate reader was used to measure absorbance at 570 nm [19].

Calculation

- The next equation was used for calculation of cell viability percent:

Viability% = mean OD sample / mean OD blank ×100 [19].

Preparation of trypan blue stain assay

Trypan blue stain assay composed of 0.4% trypan blue stain, phosphate-buffered saline (PBS) or serum-free medium. Trypan blue stain should be reserved in dark place and clarified following long storage. Serum free medium should be used as trypan blue stain combined with serum protein leading to imprecise outcomes [20].

Methodology

The tested suspension of cell is centrifuged at 100 × g for five minutes then; resuspension of pellet was applied in 1-ml PBS or serum free media with decant the supernatant. After that, one part of cell suspension was mixed with nine parts of trypan blue stain and the mix was kept for three minutes at room temperature. Counting of cells should be implemented within three to five minutes with trypan blue to avoid cell death and viability counts reduction upon prolonged incubation period. Then, transfer a drop of mixture to haemocytomemter on binocular microscope stage. Counting of unviable stained cells and viable unstained cells in

haemocytomemter was implemented to all four sets of sixteen corners squares [20].

Calculation

- The whole number of viable cells per milliliter of aliquot is calculated by multiplying the viable cell whole number by ten as trypan blue dilution factor.
- The sum number of cells per milliliter of aliquot is calculated by addition of the number of nonviable cells and viable cells.
- The next equation was used to estimate the viable cells percent:
- %Viable cells = Total number of viable cells per milliliter of aliquot / Total number of cells per milliliter of aliquot × 100 [20].

Results

Results of using MTT assay on cell viability percent of different cell lines and visual examination of cell culture (Table-2)

The optical density of MTT assay of 1, 2, 3 and 4 samples (representing MDCK cell culture that were previously preserved on (2022, 2015, 2013 and 2011) were 0.748, 0.895, 0.810, and 0.905, respectively and the viability percent was 69.5%, 83%, 75%, and 85% correspondingly.

The visual inspection under inverted microscope for the sample 1 (MDCK preserved in 2022) revealed overgrowth sheet (cells seemed viable but not expanded) and this might be due to contamination. While, the samples 2, 3, and 4 demonstrated a healthy confluent sheet. The optical density of MTT assay of 5, 6, 7, 8, and 9 samples (representing BHK cell culture preserved on (2018, 2015, 2012, 2012 and 2011) were 1.223, 0.778, 0.754, 1.056, and 0.918 respectively and viability percent was 100%, 72%, 70%, 92%, and 86%.

The visual examination under inverted microscope revealed healthy sheet showing overgrowth for samples 5 and 8, and a healthy weak sheet for samples 6, 7, and 9. The optical density of MTT assay of samples 10, 11, and 12 representing RK cell culture preserved (2020, 2019, and 2018) were 0.630, 0.589 and 0.696 respectively and viability percent was 58%, 53%, and 64% correspondingly. The visual inspection under inverted microscope for samples illustrated healthy and weak sheet. The optical density of MTT assay of samples 13, 14, 15, and 16 representing VERO cell culture preserved (2022, 2020, 2019 and 2012) were 0.653, 0.726, 0.851, and 0.7 and the viability percentage of was 60%, 67%, 80%, and 64%.The visual examination under inverted microscope for samples showed viable seemed cells for sample 13 but not expanded while healthy weak sheet for samples 14 and 16 and confluent healthy sheet for sample 15. The optical density of MTT assay of samples 17, 18, 19, and 20 representing MDBK cell culture preserved (2022, 2021, 2019 and 2012) were 0.637, 1.167, 0.698, and 1.005 and viability percent was 100%, 94%, and 64%.

The visual examination under inverted microscope for samples revealed weak sheet with a lot of dead cells for samples 17 and 19 and a confluent healthy sheet for samples 18 and 20. The optical density of MTT assay of control sample was 1.26, viability percentage was 100% and the visual examination under inverted microscope was a confluent healthy sheet.

Results of using trypan blue assay on cell viability percent of different cell lines using hamocytometer (Table-3)

The viability percent for trypan blue assay of samples 1, 2, 3, and 4 representing MDCK cell culture previously preserved (2022, 2015, 2013 and 2011) was 96%, 94.8%, 96.8%, and 98%, respectively. The viability percent for trypan blue assay of samples 5, 6, 7, 8, and 9 demonstrating BHK cell culture preserved (2018, 2015, 2012, 2012 and 2011) was 98%, 96.2%, 88.2%, 99%, and 94.7% correspondingly. The viability percent for trypan blue assay of samples 10, 11, and 12 displaying RK cell culture preserved (2020, 2019, and 2018) was 81.8%, 81.2%, and 5% respectively. Samples 13, 14, 15, and 16 illustrating VERO cell culture reserved (2022, 2020, 2019 and 2012) had viability percent of 93%, 73%, 96%, and 76%.

Samples 17, 18, 19, and 20 showing MDBK cell culture kept (2022, 2021, 2019 and 2012) had viability percent of 80%, 98%, 79%, and 96%. The percentage of viability was 100% in the positive control cells.

TABLE 2. Results of MTT assay for different cell culture, viability percentage and visual reading of cell culture.

Number of samples(S)	Average readings of three samples	Viability %	Visual reading of cell culture sheet 24 hour post test
1	0.748	69.5%	over growth sheet (Cells seemed viable but not expanded)
2	0.895	83%	Confluent healthy sheet
3	0.810	75%	Confluent healthy sheet
4	0.905	85%	Healthy sheet showed overgrowth
5	1.223	100%	Healthy sheet showed overgrowth
6	0.778	72%	Healthy weak sheet
7	0.754	70%	Healthy Weak sheet
8	1.056	92%	healthy sheet showed overgrowth
9	0.918	86%	Healthy weak sheet
10	0.630	58%	Healthy weak sheet
11	0.589	53%	Healthy weak sheet
12	0.696	64%	Healthy weak sheet
13	0.653	60%	Cells seemed viable but not expanded
14	0.726	67%	Healthy weak sheet
15	0.851	80%	Confluent healthy sheet
16	0.7	64%	Healthy weak sheet
17	0.637	58%	Weak sheet with a lot of dead cells
18	1.167	100%	Confluent healthy sheet
19	1.005	94%	Confluent healthy sheet
20	0.698	64%	Weak sheet with a lot of dead cells
21	1.240	100%	Confluent healthy sheet

S1: MDCK (2022) S2: MDCK (2015) S3: MDCK (2013) S4: MDCK (2011) S5: BHK (2018) S6: BHK (2015) S7: BHK (2012) S8: BHK (2012) S9: BHK (2011) S10: RK (2020) S11: RK (2019) S12: RK (2018) S13: VERO (2022) S14: VERO (2020) S15: VERO (2019) S16: VERO (2012) S17: MDBK (2022) S18: MDBK (2021) S19: MDBK (2019) S20: MDBK (2012) S21: positive control cells.

TABLE.3 Trypan blue stain assay of different cell culture using hemocytometer and viability%

Number of samples (S)	Average count of live cells for 4 squres /ml	Average count of dead cells for 4 squres /ml	Sum of live and dead cells	Viability %
1	**155×10^5**	**5×10^5**	**160×10^5**	**96.8%**
2	**146×10^5**	**8×10^5**	**154×10^5**	**94.8%**
3	**123×10^5**	**4×10^5**	**127×10^5**	**96.8%**
4	**152×10^5**	**3×10^5**	**155×10^5**	**98%**
5	190×10^5	3×10^5	193×10^5	98%
6	52×10^5	2×10^5	54×10^5	96.2%
7	60×10^5	8×10^5	68×10^5	88.2%
8	186×10^5	2×10^5	188×10^5	99%
9	80×10^5	5×10^5	85×10^5	94.7%
10	**36×10^5**	**8×10^5**	**44×10^5**	**81.8%**
11	**18×10^5**	**5×10^5**	**23×10^5**	**81.2%**
12	**76×10^5**	**17×10^5**	**93×10^5**	**91.5%**
13	77×10^5	5×10^5	82×10^5	93%
14	44×10^5	16×10^5	60×10^5	73%
15	82×10^5	3×10^5	85×10^5	96%
16	48×10^5	15×10^5	63×10^5	76%
17	32×10^5	8×10^5	40×10^5	80%
18	200×10^5	4×10^5	204×10^5	98%
19	41×10^5	11×10^5	52×10^5	79%
20	52×10^5	2×10^5	54×10^5	96%
21	200×10^5	2×10^5	202×10^5	100%

S1: MDCK (2022) S2: MDCK (2015) S3: MDCK (2013) S4: MDCK (2011) S5: BHK (2018) S6: BHK (2015) S7: BHK (2012) S8: BHK (2012) S9: BHK (2011) S10: RK (2020) S11: RK (2019) S12: RK (2018) S13: VERO (2022) S14: VERO (2020) S15: VERO (2019) S16: VERO (2012) S17: MDBK (2022) S18: MDBK (2021) S19: MDBK (2019) S20: MDBK (2012) S21: positive control cells.

Discussion

The mammalian cell viability in a lab mainly depends on temperature optimization as one of the most fundamental factors. Quantity and function of live cells are significantly decreased after three days of short-term preservation at 40°C. [21]. However, short-term processing before cryopreservation at 4°C can progress recovery of cell in comparison with cells kept at room temperature [22].

Earlier reports displayed that the percent of carbon dioxide and oxygen within the storage vessel can affect viability of cell in addition to mammalian cells were capable of sustaining a high level of viability and recovery after being transported for 36 hours at environmental temperature [23].

Various cell culture systems are commonly used not only to prepare and evaluate reference animal vaccinal strains but also, serological tests. BHK-21 cells were used to prepare FMD SAT2/2012 vaccinal strain and then used in neutralization test of virus and ELISA [24]. Vero cell adapted tissue culture vaccine was used for propagation and development of Rift valley fever vaccine [25] Also, MDBK cell line was used in lumpy skin disease virus titration [26]. Cryopreservatives applications must have low toxic effect and capable of entering cells for induction of biological effects by raising solutes concentrations causing growth ice inhibition.

Hence, cryoprotectants administration, their removal as well as processes of freezing and thawing are significantly influenced by osmosis and diffusion. Cryoprotectants like 10% DMSO were used for slow freezing and thawing that involves keeping cell-containing vials at -80°C overnight before transferring to liquid nitrogen tank [27]. Cryopreservative vapor-phase liquid nitrogen reduces cross contamination danger particularly related to buildup pressure. So, storage of cells at -80 °C should only be used for short-term transport or storage because cells kept at -80°C will miss their viability through devitrification [28]. The ability of cryopreserved cells to redevelop is extremely variable after thawing. Constant recovery is achieved by conserving a steady rise in temperature during thawing that enhances viability of cell. Stability and growth kinetics of cell can be affected by thawing into a new cultural medium and/or matrix with other cultures. For instance, when cells are thawed from feeder-containing situations to feeder-free ones, the outcomes can often be unexpected. So, monitoring cell conditions for prolonged time is crucial to assess their recovery after thawing as post thawing assessment resulting in imprecise findings of cell integrity [28].

Non-viable cells appeared in culture due to improper storage, uncorrected thawing or thawing media resulting in damage of cells so frozen cells should be appropriately thawing and diluted slightly before plating in pre warmed growth medium. Also, excessive dilution, improper handling of cells and storage of DEMSO or glycerol in light of liquid freezing resulting in conversion of glycerol to acrolein which is toxic to cells causing deaths of cells [29]. After cell removal from cryopreservation, DMSO should be decanted from media after gentle centrifugation and thawing. Also, cell subculture in specific media is required to adapt the environment as they may lose ability to attach [29]. Numerous factors attributed to slow cell growing such as presence of unsuitable growth medium, multiple passages and confluency past growing and avoiding such matters the recommended pre-warmed growing medium should be used, fewer passages of healthy cells are suggested and passaging of mammalian cells should be applied before confluence during the log-phase. Also, spoor quality serum, contamination of culture and reagents are involved in slow cell growing [30]. Counting of cells is fundamental procedures for quality control to characterize viable or non-viable cells. Trypan blue assay is commonly used tool for cell counting, this assay is rapid, simple, inexpensive and require small number of cells depending on integrity of membrane. However, it has disadvantages such as inaccuracy, time consuming, cell cytotoxicity effect, entry of debris to cell, resulting in false positive outcomes. Also, it is only suitable for suspensions of monolayer cells that required trypsinization and consequently this accordant with findings of this study [31].Similarly, errors count may occur due to wrong dilution, poor cell dispersion, contamination of counting chambers, presence of air bubbles and reading variations between users that cannot differentiate between healthy cells and viable cells that have lost their function, and these agreed with the results (Table 2 and 3) for samples 1 and 13 that showing seemed viable cells and not healthy as reported by [32]. To overcome the shortcomings associated with trypan blue stain, automated cell counters were standardized to recognize specific structures and reduce the possibility of false negative and positive. In this respect MTT assay was used in this study to estimate cell viability and proliferation using different lines of cell culture preserved at different periods of time (Table-1). In agreement with study, colorimetric MTT assay was used as popular test for detection of metabolic viable cell cultures owing to its accuracy, sensitivity and applicability to both adherent cell and cell suspensions [14]. Also, MTT assay was used for detection of human normal liver (THLE2) cell viability [33]. On the contrary, MTT assay is not water soluble, require extra formazan dilution

step and interfere with cell culture media. Likewise, it is affected by PH, ion cell concentration, enzymatic regulation and cell cycle alterations [13]. Opposite to this study, using MTT and trypan blue assays revealed the same outcomes for HepG2 cell line viability under diverse conditions (assay time and cell density) [34].

Collectively, this research supports using of MTT assay in detection of cell viability and progression than trypan blue assay following cryopreservation.

Conclusion

It is critical to progress and apply quantitative procedures for evaluating tissue viability after cryopreservation of banked cell culture samples. Because homogeneous results were attained under all examination conditions, the MTT test was suggested as the main approach in tissue culture bank for measuring viability. Moreover, viability percent of MTT outcomes correlate with microscopical examinations for sub-cultured cells inspected twenty four hours post-thawing. Obvious rules for the handling and appropriate quality assurance processes should be provided for the banking of cell culture to overcome dangers and viability damage. This study recommends preserving different tissue cultures in adequate nutrient medium with a cryoprotective agent like DMSO to inhibit crystal formation through rapid proper thawing consequently, high viability and quality cell culture can be certified for challenging task.

Conflict of interest

Authors declared no conflict of interest

Funding statement

This work did not receive any fund

Acknowledgments

The materials used in this study were supplied by the Central Laboratory for Evaluation of Veterinary Biologics and the Veterinary Serum and Vaccine Research Institute, Egypt. The authors are thankful to all the technical staff of the Microbiology and Virology Department of the Central Laboratory for Evaluation of Veterinary Biologics for their contribution in the practical part of this study.

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كفاءة فحوصات MTT و trypan الزرقاء للكشف عن حيوية واستعادة خلايا الزرع النسيجى المختلفة المجمدة

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تُعرف كمية الخلايا السليمة في العينة بصلاحية الخلية. بالنسبة لجميع أنواع زراعة الخلايا، يعد قياس صلاحية الخلية أمرًا بالغ الأهمية لضمان توفير خطوط الخلايا عالية الجودة من خلال الإعداد والإعداد والتخزين. على الرغم من استخدام تقنية الحفظ بالتبريد في كل من الأبحاث العلمية والسريرية، إلا أنه لا تزال هناك بعض القيود. عند درجات الحرارة المنخفضة، مثل -196 درجة مئوية (أي في النيتروجين السائل)، لا تقوم الخلايا باستقلاب أي شيء تقريبًا، الأمر الذي له آثار جانبية لا يمكن تجنبها، مثل الانجراف الوراثي نحو الاختلافات البيولوجية للتغيرات المرتبطة بالخلية في الدهون والبروتينات والتي يمكن أن تضعف النشاط الخلوي. والهيكل. أيضًا، يمكن أن تتضرر الخلايا بسبب عوامل الحماية من البرد (**CPAs**)، خاصة عند تناولها بتركيزات عالية في الظروف التي تُستخدم فيها عادةً. لذلك، من المهم استخدام تقنية موثوقة لمتابعة التغييرات التي تؤثر على بقاء الخلية بعد الحفظ بالتبريد والتي تمت مقارنتها بالخلايا الضابطة المزروعة حديثًا. في هذه الدراسة، تم استخدام اختبار **MTT** لربط سلوك الخلية وفقًا لعدد الخلايا السليمة والقابلة للحياة. تم حفظ أنواع مختلفة من مزارع الخلايا بالتبريد باستخدام ثنائي ميثيل سلفوكسيد (**DMSO**) بنسبة 10% لسنوات مختلفة في خزان النيتروجين السائل (-196 درجة مئوية). بعد ذلك، تم اختبار عينات مختلفة للتأكد من صلاحيتها باستخدام اختبار **MTT** وتم حسابها بواسطة اختبار التريبان الأزرق لمقارنة دقة النتائج وتأكيدها عن طريق الفحص البصري في الوسائط المزروعة فرعيًا بعد أربع وعشرين ساعة من الذوبان. كان اختبار **MTT** سهلاً وسريعًا وأظهر نتائج أكثر دقة من اختبار التريبان الأزرق. أشار الفحص البصري بعد أربع وعشرين ساعة من الزراعة الفرعية إلى أن اختبار **MTT** يمكن أن يفرق بين الخلايا السليمة والقابلة للحياة على ما يبدو والتي فقدت وظيفتها في حين فشل اختبار التريبان الأزرق في اكتشاف الخلايا السليمة في بعض العينات.

الكلمات الدالة : اختبار MTT ، حيوية الخلية، والحفظ بالتبريد، اختبار التريبان الأزرق