

SHIPPING STUDY TO EVALUATE THE PERFORMANCE OF THE LabCyte EPI-MODEL 24 TISSUES FOR USE IN THE SKIN IRRITATION TEST (OECD TG 439) AFTER LONG-HAUL AIRFREIGHT



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ABSTRACT

It is recommended that an evaluation of the impact of shipping of Reconstructed human Epidermis (RhE) tissues be conducted especially after long-haul airfreight shipments. The OECD Test Guideline 439 (OECD TG 439), *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method, recommends that users do so by verifying the barrier properties of the tissues after receipt. In this study, LabCyte EPI-MODEL 24 tissues were received in the USA after an overnight shipment from Japan and were tested to evaluate their performance after shipment using several endpoints. First, the viability of untreated tissues incubated overnight in culture medium was assessed using the vital dye MTT and expressed as Optical Density (OD₅₇₀₋₆₅₀) values. The calculated OD values were 1.2 (J-TEC) and 0.99 (IIVS) and within the range established by the manufacturer (0.8 - 2.5). The barrier function was further evaluated after the tissues were exposed to the assay negative control (Phosphate Buffered Saline - PBS) and to four concentrations of the positive control, Sodium Lauryl Sulfate (SLS) (1.0, 2.0, 3.0 and 4.0 mg/mL). The calculated IC₅₀ values were 2.7 mg/mL (J-TEC) and 3.4 mg/mL (IIVS), respectively, and within the established historical range (1.4 - 4.0 mg/mL). The analysis of the results generated by the two labs (J-TEC and IIVS) demonstrated that the tissue lot met the acceptance criteria developed by the tissue manufacturer under conditions of shipping stress. Finally, the histological analysis of untreated fixed tissues identified all tissue layers and supported the conclusion that the tissue model was acceptable for use in subsequent studies after long-haul airfreight shipment. Such shipping studies are critical to gaining confidence in the tissues' performance when used for research, industrial, and regulatory testing purposes.

MATERIALS & METHODS

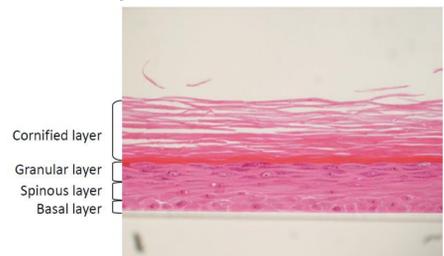
Test System – LabCyte EPI-MODEL 24



Packaging of LabCyte EPI-MODEL 24

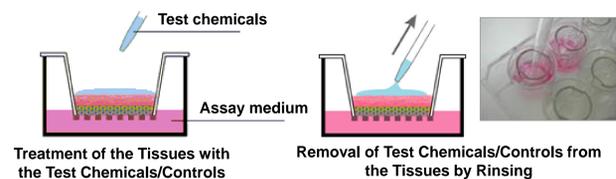


LabCyte EPI-MODEL 24 Tissues in Culture



Hematoxylin-Eosin Staining of LabCyte EPI-MODEL 24 Tissues

Standard Testing Protocol for the Skin Irritation Test (SIT – OECD 439) using the LabCyte EPI-MODEL 24 Tissues



Tissue Viability Assay Using the Vital Dye MTT



Extraction of MTT into Isopropanol → Reading of Optical Density Values and Conversion into % Tissue Viability



Cell Viability Assessment

Standard Protocol

Step	Time/Conditions
Tissues were pre-incubated in 500 μ L of assay medium	16 to 29 hours, 37°C, 5% CO ₂ , 95% humidity
The MTT media was warmed up	30 minutes at 37°C (water bath)
A set of three tissues was incubated in 500 μ L of MTT	3 hours
The tissues were transferred in tubes containing 200 μ L isopropanol	At least 1 hour
A volume of 150 μ L of extracted MTT was transferred into a 96-well plate and the absorbance was read	Absorbance reading at 570 _{nm} and corrected by the reading at 650 _{nm}

Results

Acceptable range established by the manufacturer (J-TEC) OD ₅₇₀₋₆₅₀ : 0.8 – 2.5	
Japan Laboratory (Manufacturer): J-TEC	USA Laboratory (Testing): IIVS
1.2	0.99



Barrier Function Assessment

Standard Protocol

Step	Time/Conditions
Tissues (3/treatment group) were pre-incubated in 500 μ L of assay medium	16 to 29 hours, 37°C, 5% CO ₂ , 95% humidity
Tissues were transferred into fresh assay media (500 μ L) and treated with 25 μ L of: Negative Control: Phosphate Buffered Saline – PBS Positive Control: Sodium Lauryl Sulfate – SLS (1.0 mg/mL; 2.0 mg/mL; 3.0 mg/mL and 4.0 mg/mL, respectively)	18 hours, 37°C, 5% CO ₂ , 95% humidity
The treatments were removed by aspiration and the tissues rinsed	10 times with 500 μ L of PBS
The tissues were incubated in 500 μ L of MTT	3 hours
The tissues were transferred in tubes containing 300 μ L isopropanol	At least 1 hour
A volume of 200 μ L of extracted MTT was transferred into a 96-well plate and the absorbance was read	Absorbance reading at 570 _{nm} and corrected by the reading at 650 _{nm}

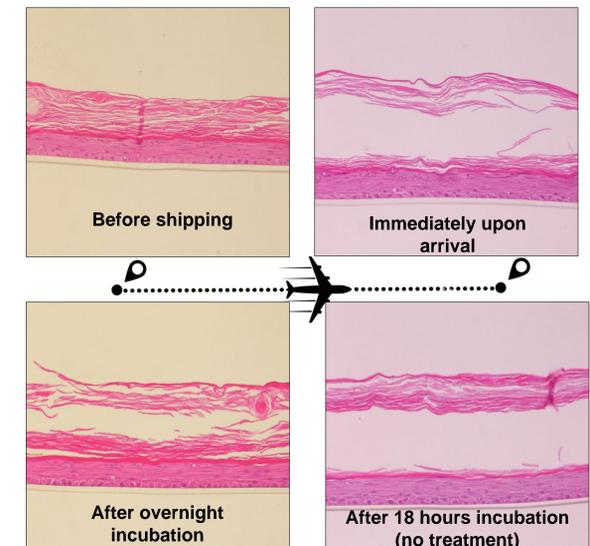
Results

Acceptable range established by the manufacturer (J-TEC) IC ₅₀ : 1.4 mg/mL – 4.0 mg/mL	
Japan Laboratory (Manufacturer): J-TEC	USA Laboratory (Testing): IIVS
2.7	3.4

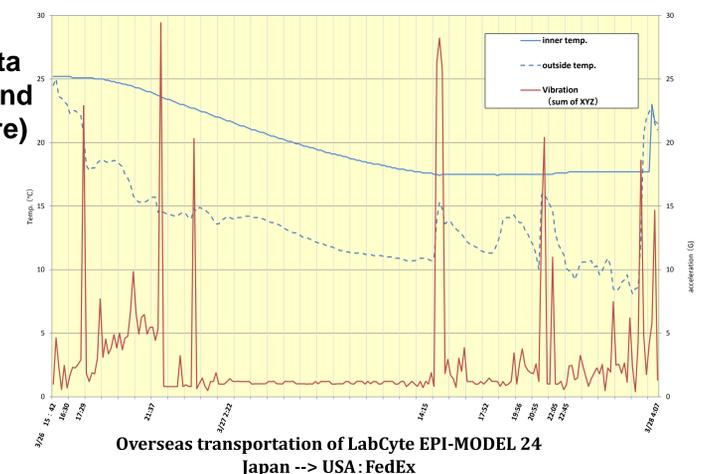


RESULTS

Histological Analysis (Hematoxylin-Eosin staining, x200)



Logger Data (Vibration and Temperature)



CONCLUSIONS

1. The cell viability and barrier function assessment demonstrated that the LabCyte EPI-MODEL 24 tissue lot met the acceptance criteria developed by the tissue manufacturer under conditions of shipping stress.
2. The histological analysis of untreated fixed tissues identified all tissue layers and supported the conclusion that the tissue model was acceptable for use in subsequent studies after long-haul airfreight shipment. The separation of the *stratum corneum* could represent an artifact of histology samples preparation (sectioning).
3. The logger data (temperature and vibration) analyzed by the tissue manufacturer (shipper) showed that the parameters were relatively constant during shipping and were not anticipated to have adversely impacted the tissues' performance while used by the Testing Laboratory.
4. Such shipping studies are critical to gaining confidence in the tissues' performance when used for research, industrial, and regulatory testing purposes.

REFERENCES

OECD. (2015). OECD GUIDELINE FOR THE TESTING OF CHEMICALS (OECD TG 439). *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. LabCyte EPI-MODEL Three-dimensional Cultured Human Epidermis Model Manual. Available at: http://www.jp-te.co.jp/english/business/LabCyte/LabCyte_EPI-MODEL_Instruction_Manual_Mar2017.pdf. Characteristics of the LabCyte model: <http://www.jp-te.co.jp/english/business/LabCyte/LabCyte.html>.