XCellR8 Redefining testing

FINAL REPORT: 20ZID01S

In Vitro Assessment of the Skin Irritation Potential of HAND SANITISING GEL FO 28-00046 according to OECD Test Guideline 439 (Reconstructed Human Epidermis Test Method)

Final Report Approval Signatures

	Name	Signature	Date
Approval by Test Facility Management	**Bushra Sim**		26AUG20
Approval by Study Director	**Sarah Bell**		26AUG20

Three annexes are attached to this final report:

Annex 1: Test System QC data sheet
Annex 2: Summary sheet of test conditions and results obtained
Annex 3: XCellR8 GLP compliance certificate

1. Executive Summary

In this study, the skin irritation potential of HAND SANITISING GEL FO 28-00046 was assessed *in vitro* according to OECD Test Guideline 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method.

After 60±1 minute exposure on the surface of the EpiDerm™ reconstructed human epidermis, and a 42 ±4 hour post-exposure incubation time, viability of the tissues was assessed and compared to the negative control.
The percentage of viability obtained was 69.788 % and therefore:

HAND SANITISING GEL FO 28-00046 was classified as Non-Irritant to the skin.

2. Study Director's GLP Statement

I, Sarah Bell, Study Director, certify that the study has been conducted in accordance with:

- The United Kingdom (Good Laboratory Practice Monitoring Authority, Medicines and Healthcare products Regulatory Agency [MHRA]) Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No. 3106 as amended by the Good Laboratory Practice (Codification Amendments etc.) Regulations, 2004, Statutory Instrument 2004, No. 994.

- The OECD Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17 (revised in 1997, Issued January 1998).

This study has been performed under my supervision and the findings provide a true and accurate record of the results obtained.

Study Director: Date: 26 AUG 20

XCellR8 Ltd. Registered in England and Wales. Registered office: Techspace One, Sci-Tech Daresbury, Keckwick Lane, Daresbury, Cheshire, WA4 4AB, UK. Company number 6489519. T: +44 (0)1925 607134. E: info@x-cellr8.com

3. Quality Assurance Statement:

GLP Quality Assurance Statement

Study Number :	**20ZID01S**
Study Title :	***In Vitro* Assessment of the Skin Irritation Potential of HAND SANITISING GEL FO 28-00046 according to OECD Test Guideline 439 (Reconstructed Human Epidermis Test Method)**

This report has been reviewed by Quality Assurance at XCellR8 and is considered to accurately describe the methods and procedures used, and accurately reflect the study raw data.

The following phases of this study conducted at XCellR8 were inspected and findings were reported to the Study Director and the Test Facility Manager:

Study Phase	Dates inspected	Audit Report Issue Date
Study Plan	28 May 2020	28 May 2020
Study Report	10-12 Aug 2020	12 Aug 2020

The following table lists the most recent process/facility inspections considered relevant to this study. These inspections took place in accordance with XCellR8's annual risk-based audit programme and were reported to Study Directors (where relevant) and Test Facility Management.

Audit Name	Dates inspected	Audit Report Issue Date
Assay – Skin Irritation	22 Apr – 13 May 2020	28 May 2020
Test and Reference Item Management	18-19 Mar 2020	02 Apr 2020

Signature: _____ Date: 26 AUG 2020

*On behalf of Quality Assurance**

**Authorised QA Signatories:* *J E Ball MSc, MRQA QA Manager*
T Hand BSc (Hons), MRQA QA Officer

4. Sponsor

Sponsor	ZIDAC LABORATORIES LTD Unit 5, Merlin Park, Airport Service Road, Portsmouth, PO3 5FU Telephone: 07387 018110
Sponsor Contact	Zeljka Putarek zeljka.putarek@zidac.co.uk

5. Test Facility

Test Facility	XCellR8 Ltd. Techspace One, Sci-Tech Daresbury, Keckwick Lane, Daresbury, Cheshire WA4 4AB UK 01925 607134

6. Study Director

Study Director	Sarah Bell Techspace One, Sci-Tech Daresbury, Keckwick Lane, Daresbury, Cheshire WA4 4AB UK 01925 607057

7. Key Personnel

Test Facility Manager	Bushra Sim
Study Scientist(s):	Lottie Roscoe and Josh Fredson
QA Manager:	Jan Ball
Archivist:	Ian Clarke

8. Nature and Purpose of the Study

8.1 Scientific Objective

In vitro assessment of the skin irritation potential of HAND SANITISING GEL FO 28-00046 according to OECD guideline TG439: *In vitro* Skin Irritation: Reconstructed Human Epidermis (RHE) Test Method.

This *in vitro* risk assessment assay predicts the skin irritation potential of a chemical by measurement of its cytotoxic effect on the EpiDerm™ tissue model.

8.2 Technical Parameters

The detailed method followed in this study is described in XCellR8 SOP L0029: "EpiDerm™ *In vitro* Reconstructed Human Epidermis Skin Irritation Test (OECD 439) and is based on the MatTek protocol (MK-24-007-0023, 05/27/20) using the MatTek Corporation EpiDerm™ reconstructed tissue model EPI-200.

9. Test and Reference Items

9.1 Test Item (See Annex 4 for test item Certificate of Analysis)

Supplier	ZIDAC LABORATORIES
Test Item name	HAND SANITISING GEL FO 28-00046
Supplier batch/lot number	21042020
CAS number	Not provided
Purity	Not provided
Receipt Date	11MAY20
Expiry Date	21APR23
Physical state	Viscous clear liquid
Storage Conditions	Room temperature
Administration method	Topical application
Concentration tested	Neat
XCellR8 test item code	ZID001
Study test item code	TA1

9.2 Positive Control

Supplier	MatTek Corporation
Reference Item name	Sodium dodecyl sulphate (SDS)
Lot number	052020LHB
Purity	99.3%
Concentration tested	5%
Administration method	Topical
Solvent	Water
Expiry Date	20MAY21
Storage Conditions	Room Temperature
Study test item code	PC

9.3 Negative Control

Supplier	MatTek Corporation
Reference Item name	Sterile Dulbecco's Phosphate Buffered Saline (DPBS)
Lot number	042820MSA
Concentration tested	Neat
Administration method	Topical
Solvent	Not Applicable
Expiry Date	28APR21
Storage Conditions	Room Temperature
Study test item code	NC

10. Test System

10.1 Description of the test system:

The reconstructed human epidermal model EpiDerm™ (EPI-200-MatTek Corporation) consists of normal human-derived epidermal keratinocytes which have been cultured to form a multi-layered highly differentiated model of the human epidermis. It consists of organised basal, spinous and granular layers and a multi-layered *stratum corneum* containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*.

10.2 Justification for selection of the test system:

Initially, the predictive capacity of the modified EpiDerm™ Skin Irritation Test (SIT) test method using MatTek EpiDerm™ tissue model EPI-200 underwent full prospective validation from 2003-2007. The test method components of this method were used to define the essential test methods components of the original and updated ECVAM Performance Standards (PS).

A modification of the original EpiDerm™ SIT was validated using the original ECVAM PS in 2008. In 2008, ESAC concluded that the Modified EpiDerm™ SIT has sufficient accuracy and reliability for prediction of R38 skin irritating and no-label (non-skin irritating) test substances.

10.3 Characterisation of the test system:

MatTek's EpiDerm™ model has been extensively characterised for multiple parameters including morphology, tissue viability, skin barrier function and sterility (for information see www.mattek.com). QC results for the specific lot of models received (Lot# 30875) were checked in-house for compliance with MatTek acceptance ranges with the following outcome (details in Annex 1):

- Morphology - PASS
- Tissue viability - PASS

- Skin barrier function (ET_{50} value for 1% Triton X-100) where ET_{50} is the time taken for 1% Triton X-100 to reduce the viability of the skin model to 50% relative to the negative control)- PASS
- Sterility testing showed no contamination during long term antibiotic and antimycotic free culture- PASS

10.4 Method of administration and exposure time of test and reference item:

A single topical application of 30 µl of neat test item, negative control (DPBS) or positive control (SDS 5%) to the surface of EpiDerm™ model for 60±1 minutes (25 minutes at room temperature and 35 minutes at 37°C, 5% CO_2, in a humidified environment), followed by 42±4 hours post-treatment incubation, prior to the MTT endpoint; three tissues per condition (n=3).

11. Study Design

Details of materials, reagents and equipment used were recorded in the study data.

11.1 Preliminary test

The test item was first checked for its potential for MTT interference and water coloration. During the preliminary testing, the test was vortexed for 15 seconds at 2,500 rpm using a Stuart vortex mixer. The test item and MTT were mixed with a pipette before incubation.
Viscous test items were checked for compatibility with nylon mesh provided by the MatTek Corporation for use as a spreading support. The mesh was not used in the main test.

11.2 Main Test overview

Day 0: On the day of receipt, EpiDerm™ tissues were pre-incubated overnight at 37°C, 5% CO_2, in a humidified environment.

Day 1: Exposure to, and removal of test and reference items (30µl (liquids) applied topically for 60±1 minutes (25 minutes at room temperature and 35 minutes at 37°C, 5% CO_2, in a humidified environment) (n=3) followed by rinsing steps and a 42±4 hours post-dose incubation at 37°C, 5% CO_2, in a humidified environment).

Day 2: Medium change.

Day 3: MTT viability test, readings at 570 nm without reference filter.

11.3 Study Dates

Study initiation date	01JUN20
Experimental start date (preliminary test)	04JUN20
Experimental end date (main test)	26JUN20
Study completion date	Date Final Report signed by the Study Director

12. Data Analysis

Data Analysis for this study was performed following XCellR8 SOP L0029: "EpiDerm™ *In vitro* Reconstructed Human Epidermis Skin Irritation Test (OECD 439 12DEC17)", using XCellR8 Form F0036C: Data Analysis for EpiDerm™ Skin Irritation Test OECD TG439 (version 7). This is a Microsoft Excel workbook (validated in-house) containing formulae to process the raw data as per OECD TG439/SOP L0029. The final data output is a percentage viability value for EpiDerm™ models exposed to the test item relative to the negative control.

13. Results

(See Annex 2 for Summary of Test Conditions and Results)

Prior to the study, the required compatibility checks (as per SOP L0029) confirmed that the test item did not interfere with MTT and no water colouration was observed.

13.1 Results Summary (Table 1)

Test Item	Percentage of viability (relative to negative control)	Classification Irritant / Non-Irritant
HAND SANITISING GEL FO 28-00046	**69.788%**	**Non-Irritant**

The test item did not reduce the viability to below 50% or below and should be considered as **Non-Irritant** to the skin.

13.2 Data Analysis:

Table 2: Viability measurements after 60±1 minutes of application and 42±4 hours post-incubation of test and reference items and controls.

Condition	Tissue #	Raw data		Blank corrected data		Mean OD	% of Viability
		Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2		
NC	Tissue 1	2.215	2.246	2.113	2.144	2.129	101.357
	Tissue 2	2.146	2.125	2.044	2.023	2.034	96.834
	Tissue 3	2.268	2.212	2.166	2.110	2.138	101.809
PC	Tissue 1	0.208	0.202	0.106	0.100	0.103	4.912
	Tissue 2	0.228	0.245	0.126	0.143	0.135	6.412
	Tissue 3	0.143	0.146	0.041	0.044	0.043	2.032
TA1	Tissue 1	1.479	1.483	1.377	1.381	1.379	65.669
	Tissue 2	1.567	1.596	1.465	1.494	1.480	70.455
	Tissue 3	1.639	1.641	1.537	1.539	1.538	73.240

NC: negative control (DPBS), PC: Positive control (SDS 5%), TA1: Test Item 1.

Table 3: Mean and SD of cell viability measurements and of viability percentages after 60±1 minutes of application and 42±4 hours post-incubation

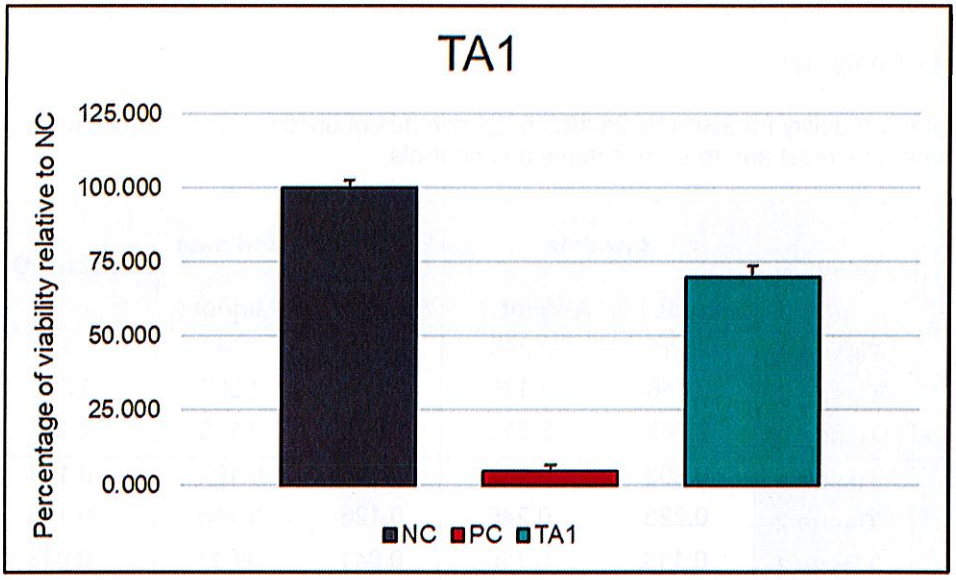
Name	Code	Mean of OD	SD of OD	Mean of viability (%)	SD of viability (%)	CV %	Classification
DPBS	**NC**	2.100	0.058	100.000	2.752	2.752	**Non-Irritant**
SDS 5%	**PC**	0.094	0.047	4.452	2.226	50.006	**Irritant**
Test Item	**TA1**	1.466	0.080	69.788	3.829	5.487	**Non-Irritant**

NC: Negative control (DPBS), PC: Positive control (SDS 5%), TA1: Test Item 1.

Prediction model of irritancy: test items that reduce the viability to 50% or below are Irritant (I), test items with a percentage viability above 50% are considered to be Non-Irritant (NI).

Graph 1: Skin Irritation evaluation following OECD guideline TG439 Reconstructed Human Epidermis Test Method.

Mean of percentage of viability and relative SD are presented here for each condition, relative to NC arbitrarily set to 100%.



NC: Negative control (DPBS), PC: Positive control (SDS 5%), TA1: Test Item 1.

14. Discussion

14.1 Evaluation of the results

Results were checked against the following acceptance criteria:

	Description	Actual values	PASS/FAIL
Acceptance criterion 1	The mean OD_{570} of the **negative control** (treated with DPBS) tissues must be ≥ 0.8 and ≤ 2.8	**2.100**	**PASS**
Acceptance criterion 2	The mean of the **positive control** relative percentage viability must be < 20% of the mean of the negative controls.	**4.452**	**PASS**
Acceptance criterion 3	The **standard deviation** of viability percentages for triplicate skin models in each experimental condition must be ≤18%	**NC: 2.752** **PC: 2.226** **TA1: 3.829**	**PASS**
Acceptance criterion 4	The mean OD of the 6 wells containing extraction solvent alone (blanks) must be ≤ 0.1.	**0.102**	**ACCEPT***

*All acceptance criteria were met except for criterion 4:

Optical Density (OD) values obtained with blanks were higher than 0.1 (0.102) causing a deviation from Acceptance Criterion 4. However, the spectrophotometer was fully validated and had passed all required tests. The OD values for blanks observed in this study are consistent with historical data using this spectrophotometer in the XCellR8 laboratory and meet our current internal acceptance criteria of blank OD values <0.244 (mean + 2SD of XCellR8 historical data, based on blanks obtained during 88 historical runs), therefore this is not considered to be an issue in the interpretation of this study data.

This SOP and guideline deviation was not considered to have affected the integrity or interpretation of the results as no equivocal results were obtained.

14.2 Interpretation of Results following Prediction Model

- A test item is considered an irritant (I) to skin in accordance with UN GHS Category 2 if the skin model viability after exposure and post-treatment incubation is ≤50%.
- A test item may be considered as a non-irritant (NI) if the skin model viability after exposure and post-treatment incubation is >50%.

The percentage of viability obtained with the test item HAND SANITISING GEL FO 28-00046 was **69.788 %,** therefore it was considered as **Non-Irritant** to the skin.

15. Record Retention

Study records will be retained for a minimum of 5 years in the archive at XCellR8, a fire-proof locked filing cabinet accessible only by the XCellR8 Archivist, at the address shown in Section 5.

At the end of this period the Sponsor will be contacted to determine whether the records should be returned, retained or destroyed on their behalf and notified of the financial implication of each of these options.

One copy of the study plan and final report will be held indefinitely by XCellR8.

Records to be retained include:
Ink signed study plan and any amendments; test and reference item formulation records; manually recorded data, authenticated hard copy output from the spectrophotometer; authenticated hard copy of data analysis; the final report and any subsequent report amendment(s) and re-issue(s).

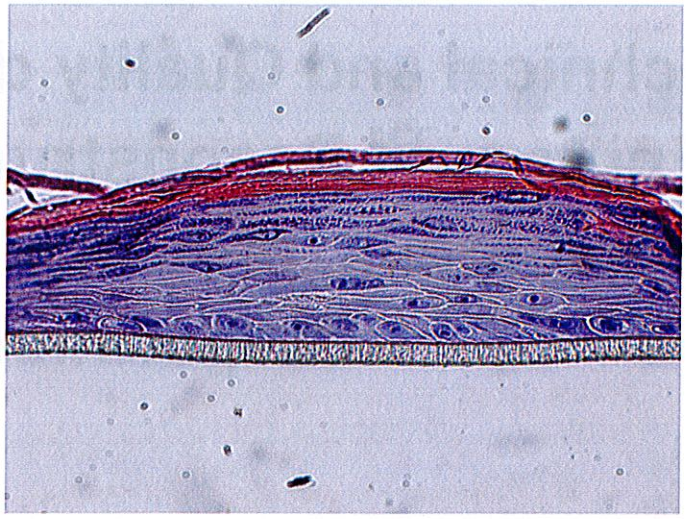
16. Study Report Distribution

Sponsor, Study File

20ZID01S ANNEX 1

Technical and Quality data sheet of EpiDerm™ Reconstructed Human Epidermis, provided by MatTek Corporation

20ZID01S ANNEX 1: Technical and quality data sheet of EpiDerm™ Reconstructed Human Epidermis, provided by MatTek Corporation

Description	**EpiDerm™ (EPI-200) Reconstructed Human Epidermis**
Lot Number	**30875**
Date of Receipt	**23JUN20**
Morphology	**Photo ID# MH1959_400x_1** **EPI-200 Histology**  **Tissue Lot# 30875** **Photo ID# MH1959_400x_1** Histological examination should demonstrate human epidermis-like structure: including multiple layers (at least 4) of viable epithelial cells (basal layer, *stratum spinosum, stratum granulosum*) which are present under multilayered *stratum corneum* QC result = 12 layers are present = **PASS** Tissue thickness should be within the acceptance range of >70 µm and <130 µm. QC result = 100.3 µm = **PASS**
Viability	Optical Density (O.D.) values (the mean and SD of MTT value of 3 tissues exposed to sterile water) should be within the Test Guideline acceptance range of 1.0 – 3.0.

	QC result = 1.815±0.09 = **PASS**
Barrier Function	The ET_{50} of tissues exposed to 100 µL Triton X-100 1%, n=3 should be within the Test Guideline acceptance range of 4.77 hours - 8.72 hours. QC result = 5.04 hrs = **PASS**
Sterility	There should be no evidence of contamination during long term antibiotic and antimycotic free culture. QC result = No contamination reported = **PASS**

20ZID01S ANNEX 2

Summary sheet of test conditions and results obtained

STUDY 20ZID01S - ANNEX 2: Summary sheet of test conditions and results obtained

TEST ITEMS		
Test Item Name HAND SANITISING GEL FO 28-00046	**CAS#** Not provided	
Batch number 21042020	**Physicochemical properties (pH, volatility...)** Not applicable	
Physical state Viscous clear liquid	**Treatment of the test items prior to testing** None	
TEST SYSTEM		
Model EpiDerm™ (EPI-200) Reconstructed Human Epidermis	**Supplier** MatTek Laboratories	
Lot Number 30875	**Keratinocyte Strain** 00267	
INSTRUMENT USED		
Spectrophotometer BMG LabTech FluoStar Optima	**Calibration date** 06MAY20	
SKIN IRRITATION TEST CONDITIONS		
Exposure duration 60±1 minutes	**Amount of test item applied** 30µl	
Wavelength for O.D. measurements (MTT) 570 nm (no reference filter)	**Remarks** None	
TEST ACCEPTANCE CRITERIA		
NC: Mean $O.D._{570nm} \geq 0.8$ and ≤ 2.8	☑ Accept	☐ Reject
PC: Mean viability after 1hour < 20 % of the mean of the negative controls	☑ Accept	☐ Reject
SD of triplicate viability percentages for each condition: ≤18%	☑ Accept	☐ Reject
Blank: Mean $O.D._{570nm}$ (n=3) ≤ 0.1 (See note in Section 14.1)	☑ Accept (<0.244)	☐ Reject

RESULTS

Skin Irritation Classification: n=3, Rounded figures

Name	Mean of OD	SD of OD	Mean of viability (%)	SD %	Prediction (OECD TG439)
NC (DPBS)	2.100	0.058	100.000	2.752	**Non-Irritant**
PC (SDS 5%)	0.094	0.047	4.452	2.226	**Irritant**
TA1	1.466	0.080	69.788	3.829	**Non-Irritant**

Test Item: TA1 (HAND SANITISING GEL FO 28-00046)

TA1 ☐IRRITANT ☑NON IRRITANT **(OECD TG439 classification)**

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20ZID01S ANNEX 3

XCellR8 GLP Compliance Certificate

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Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

XCELLR8 LIMITED
TECHSPACE ONE
SCI-TECH DARESBURY
KECKWICK LANE
DARESBURY
WARRINGTON
WA4 4AB
UNITED KINGDOM

TEST TYPE(S)

Other
in vitro safety testing

DATE OF INSPECTION: 19/06/2019

DATE OF ISSUE: 02/04/2020

An Inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above named test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above named test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

Issued by
Mr Stephen Vinter
Head, UK GLP Monitoring Authority

Medicines & Healthcare products Regulatory Agency

MHRA