Insert BA Categorisation (Hazard Group 1 or 2/ or GMO Class 1):
HG1

Loughborough University

Health & Safety Unit Use Only	
Ref No:	
Department Use Only	
Ref No:	CBE/BRA/095

RISK ASSESSMENT OF WORK WITH BIOLOGICAL AGENTS

Please note the following before completing this form:

1. University Health and Safety Policy requires that risk assessment of all work with biological agents (BAs) must be carried out in advance of work commencing. A key requirement of The Control of Substances Hazardous to Health Regulations (COSHH) is to assess the risks associated with any work activity involving the use of biological materials may contain biological agents.
2. YOU SHOULD COMPLETE ALL OF PART A, THE APPROPRIATE SECTIONS OF PART B, AND ALL OF PART C. WHERE HAZARD GROUP 2 BIOLOGICAL MATERIAL IS INTENDED TO BE USED THE RISK ASSESSMENT MUST BE REVIEWED BY THE DEPT/SCHOOL BIOLOGICAL SAFETY ADVISOR AND EXPLICIT APPROVAL IS ALSO REQUIRED FROM THE UNIVERSITY BIOLOGICAL SAFETY OFFICER. THIS FORM SHOULD BE SUBMITTED TO THE HEALTH, SAFETY & ENVIRONMENT UNIT FOR REVIEW VIA YOUR DEPARTMENTAL BIOLOGICAL SAFETY ADVISOR.
3. It is the responsibility of the Principal Investigator/Supervisor to ensure compliance to these requirements and that this risk assessment remains valid.
4. This risk assessment form **IS NOT** for assessing the risks associated with **Genetically Modified Organism activities**.

Date Submitted:	10/12/2014	Date Approved:	11/12/2014
Version Number:	1.0	Supersedes *(insert version number if applicable)*	N/A

PART A: Please provide the following general information:

School/Department			
Wolfson School of Mechanical and Manufacturing Engineering / Healthcare Engineering			
Title of Project			
Evaluation of mechanical dispensing of Vavelta (Clinical quality human Dermal Fibroblasts)			
Project Reference Number:	N/A		
Person responsible for this work (*Principle Investigator*)			
Name:	Dr Alex Lyness	Position:	Enterprise Fellow in Delivery of Cell Therapies.
Department:	Healthcare Engineering	University School:	Wolfson School of Mechanical and Manufacturing Engineering
Person conducting this assessment			
Name:	Mark McCall	Position:	Enterprise Fellow
Department:	Healthcare Engineering	Date Risk Assessment Undertaken:	10/12/2014
Proposed Project Start Date:	11/12/2014	Proposed Project End Date:	30/01/2015

Review History: *required at least once a year or immediately following any significant change to the project. Significant revisions must be detailed on a revision form. The person responsible must ensure that this RA remains valid.*

	Review 1	Review 2	Review 3	Review 4	Review 5
Due Date					
Date Conducted					

A1 PROJECT SUMMARY

A1.1 Scientific Goals of the Project.

This provides a useful background for the reviewer and reader. It need only be brief and should provide an overview of the scientific goals.

To evaluate the accuracy of a Loughborough University designed cell delivery device in the consistent administration of human dermal fibroblasts (hDF) in suspension.

A1.2 Description of the Experimental Procedures

Describe laboratory procedures to be used and highlight any non-standard laboratory operations. This may need cross reference to supporting documentation i.e. protocols.

1. Receipt of up to six sealed vials of hDF in suspension to the CBE facility.
2. Transfer of the cells from these transport vials to the cell delivery device.
3. Automated dispensing of the hDF from the device into an open beaker sitting on the mass balance in the chemical fume hood in H34 (Analytical Suite, CBE) or a mass balance in a biological safety cabinet (H23).
4. Measurements of the dispensed volume/weight from the cell delivery device
5. Disposal of biological material.

A full description of the protocol is addressed in the "Performance Verification Protocol for CTCDV01 Device", D000183-NC2014013 Version 1.0 dated 11 Dec 2014 (attached).

PART B: Please provide information in one or more of the following sections, as appropriate. Only sections which you complete should be submitted:

Section 1: *micro-organisms (prions, viruses, bacteria, fungi, parasites in ACDP category 2 and pathogens controlled by the Department for the Environment, Food and Rural Affairs).* ***[Work with ACDP category 3 and 4 pathogens is not currently permitted in the University.]***

Section 2: *cell cultures, tissues, blood, body fluids or excreta*

Section 3: *plants and plant material*

Section 4: *animals and animal tissues*

SECTION 2: CELL CULTURES, TISSUES, BLOOD, BODY FLUIDS OR EXCRETA

B2.1 HAZARD & RISK IDENTIFICATION : NATURE OF CELLS, TISSUES OR BODY FLUIDS

*This information gives an indication of the **potential** harm that the biological material may cause*

B2.1.1 List all cells or tissues to be used. *For cells indicate if primary, continuous or finite.*

Indicate in the adjacent box if Not Relevant (N/R)			
Cell or tissue type and ID	Organ Source	Species	From where will it be obtained?
Human neonatal fibroblasts	Foreskin	Human	Intercytex

B2.1.2 List all blood, body fluids or excreta to be used

Indicate in the adjacent box if Not Relevant (N/R)		N/R
Material type	Species	From where will it be obtained?

B2.1.3 Has any material listed in section B2.1.1 been genetically modified in any way?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If Yes, complete Genetically Modified Organisms (GMO) Risk Assessment Form	

B2.1.4 Will material be screened for infectious agents? (if from a cell culture collection answer B2.1.6 instead)

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	Yes
Cells have come from Intercytex. The working bank was screened for contamination and mycoplasma. This certificate is attached to FSOP008.1 for the Quality Managers approval. There is also a brief commentary from the Intercytex Operations Manager that the vials sent to Loughborough University came from the tested working bank. These vials contained cells expanded and stored in Intercytex GLP laboratories under their quality system to prevent contamination (Letter from Joan Benson dated 11 Dec 2014). Hence we can assume that the risk of them being contaminated by infection agents is minimal.	

B2.1.5 Will any clinical history (if relevant) be provided with this material?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes give details:	
If yes, will a policy of rejection of samples from diseased patients be adopted?	
If yes, how will the information be disseminated in the course of the project?	

B2.1.6 If obtained from a cell culture collection, is safety information provided?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	N/R
If Yes, summarise here:	

B2.1.7 Has any of the material listed in section B2.1.1 been identified in the list of cross-contaminated or misidentified cell lines, available on HPA website
(http://www.hpacultures.org.uk/media/E50/3B/Cell_Line_Cross_Contaminations_v6_0.pdf

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If Yes, provide details of the route of provenance back to the originator of the cell line, together with a Certificate of Analysis; identifying the methods used to qualify the cell type.	

B2.2 RISK TO HUMANS

B2.2.1 What is the likelihood of infection of this material? Indicate as None, Low Risk, Medium Risk, High Risk, Known Infected*

Cell type and ID	Risk Category	Justification for Selection
Fibroblasts	None	
		If none proceed to section B2.2.4

see The Managing the risks in laboratories and healthcare premises – available at
http://www.hse.gov.uk/biosafety/biologagents.pdf

B2.2.2 If low, medium or high risk (section B2.2.1), name and classify the Biological Agents this material could be infected with. List the biological agents and indicate the ACDP hazard group classification*

Name of Agent	Classification

see The Approved List of Biological Agents – available on the Health & Safety website or
http://www.hse.gov.uk/pubns/misc208.pdf.

B2.2.3 Describe the route(s) of infection (in humans) for these adventitious agents (place a 'X' in the relevant box)

Percutaneous	Mucocutaneous	Inhalation	Ingestion	N/R
Details:				

B2.2.4 Are there any other biological hazards (other than adventitious infectious risk) associated with the materials e.g. aggressive tumourogenic cell lines

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	N/R
If Yes, describe:	

B2.3 HUMANS AT INCREASED RISK OF INFECTION

B2.3.1 Do any of the agents listed in section 2.1 present an overt risk to humans at increased risk (including immunocompromised workers, pregnant workers, breast feeding mothers)?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, Occupational Health must be consulted:	

B2.4. PROPAGATION OR CONCENTRATION OF ADVENTITIOUS AGENTS

B2.4.1 Will any culturing of this material take place?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	
If yes, identify the cells and the conditions these will grow:	

B2.4.2 If culturing, will CD4+ cells be present. Describe what cells and for how long these cultures will be allowed to grow

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.4.3 If culturing, what is the maximum volume of culture grown?

Indicate in the adjacent box if Not Relevant (N/R)	

B2.4.4 Will the cells be manipulated in any way that could result in a concentration of any adventitious biological agent present?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain:	

B2.5 WORKING WITH MATERIAL DONATED BY YOURSELF OR COLLEAGUES :

Workers **MUST NEVER** culture, deliberately transform or modify their own cells or cells from their co-workers or workers otherwise associated with the experimental work. *NOTE: This presents a particular hazard since any self-inoculation injury could have potentially serious consequences as cells would essentially circumvent the normal protection of the immune system.*

B2.5.1 Will any cells be donated by persons working in or has access to the lab?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, explain what precautions are to be taken to prevent that person being exposed to the cells:	
If yes, where will this material be collected:	
If yes, provide justification for not using a safer source:	
If yes, how will confidentiality be assured:	

If yes, has Ethics Committee approval been obtained:

B2.6 ENVIRONMENTAL CONSIDERATIONS:

B2.6.1 Are any of the agents capable of causing disease or other harm in animals, fish or plants?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.6.2 Will there be any other environmental risks?

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, describe:	

B2.7 OTHER HAZARDS

B2.7.1 Are there any other hazards associated with this work? For example, hazardous chemicals (especially carcinogens, mutagens, substances toxic to reproduction, cytotoxins), cryogenic gases, ionising radiation.

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	No
If yes, identify these:	
If yes, have these been risk assessed and any necessary approval obtained? n/r	

B2.7.2 Are there any conditions associated with the hazards described in B2.7.1 that require special attention in Section C of this risk assessment? For example, material incompatibilities with disinfectants such as Virkon or hazardous product decomposition associated with high temperatures (ie autoclaving).

Indicate in the adjacent box as: Yes, No or Not Relevant (N/R)	
If yes, provide details and ensure that appropriate control measures are addressed in Section C:	

PART C: CONTROL MEASURES

C1. CONTROL MEASURES

The risk of exposure must be prevented or adequately controlled to minimise the chance of harm arising. COSHH Regulations require minimum containment measures for laboratories handling organisms from the different ACDP hazard groups (http://www.hse.gov.uk/pubns/misc208.pdf)
The hazard group number typically indicates the level of containment (includes physical measures & working practices) that must be used for its handling).

C1.1 Preventing Exposure

C1.1.1 Substitution with a Safer Alternative

Is substitution with a safer alternative practical, by for example, replacement of a clinical strain or pathogen with one that is lab adapted? Provide reasons for your answer:

No. These are clinical grade cells and safe for work in the labs.

C1.1.2 Isolation/Segregation

(i) Is/Are the laboratory(s) to be used for this work to be shared with other workers not directly involved in this activity?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, provide details: The CBE is a shared facility but operates at HG2 level containment which is above and beyond the required control level for this cell type.	
(ii) Is access to the laboratory(s) to be used for this work restricted?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, provide details: Access is controlled by the lab manager.	

C1.2 Controlling Exposure

C1.2.1 Are sharps (needles, blades, scissors, forceps, glass or capillary tubes) to be used at any stage during this activity?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, list the sharps:	
If yes, justify there use – is there an alternative?	
If yes, describe there use and disposal:	
If yes, describe any additional precautions employed to reduce risk:	

C1.2.2 Containment and Ventilation

(i) Is the use of BSC required for the protection of the worker i.e. do the work procedures generate aerosols or splashes that pose a risk to workers?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, specify the type(s) and when they will be used:	
(ii) Are there any requirements for room ventilation e.g. negative pressure, temperature control?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	Yes
If yes, specify: Yes to maintain air balance for optimal performance of BSCs.	

C1.2.3 Transport and Storage within the laboratory

How and where are materials to be stored? Fridge or Cold Store.
How will this material be transported within the laboratory e.g. between BSC and incubator? Detail the containment measures which will be used to prevent or contain accidental splashes or spills. With 1 level of containment at all times (Flask or vial)

C1.2.4 Local transport out of the laboratory

How will this material be transported on-site (e.g. research material between labs on campus or movement of waste containing viable agents e.g. to a remote autoclave? Detail the containment measures which will be used to prevent or contain accidental splashes or spills. Will not be transported out of the CBE. Spills will be dealt with in accordance to SOP038

C1.2.5 Shipment of Biological Material

Will this material be shipped elsewhere in the UK or abroad?						
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)						No
If yes, give details to support compliance to the relevant regulation (e.g. category of material, correct packaging instruction):						
Description of material to be shipped (*indicate in available boxes*). Is this:						
Category A		UN2814		UN2900		*Packaging instruction 602 or 620 must be followed*
Or?						
Category B		UN3373				*Packaging instruction 650 must be followed*
Or?						
Non-hazardous	X					*Should be packaged to protect sample*

C1.2.6 Receipt of material

If material will be received from other sites or organisations, what precautions are being taken to ensure that the material is shipped correctly? Although not hazardous samples will be protected with 2 levels of containment with temperature control (Wet ice cool packs)

C1.2.7 Centrifugation

(i) If material is to be centrifuged will sealed buckets and rotors be used?
Indicate in the adjacent box as No, Yes or Not Relevant (N/R) — N/R
(ii) Where will these rotors/buckets be opened?
(iii)Describe the procedures in place to deal with leaks and spillages in the centrifuge

C1.2.8 Incubators

If incubators are to be used, what type of incubator (e.g. shaking, static) is used and describe procedures to prevent and contain spillages. No incubation required

C1.2.9 Disinfection

Specify the type and concentration of disinfectants to be used: IMS (70% CBE/COSHH/108) and Virkon (1%CBE/COSHH/39) will be used
Have these disinfectants been validated for use with the recipient biological material? Indicate in the adjacent box as No, Yes or Not Relevant (N/R) — Yes
If yes, describe the procedure: For biologics belonging to HGO 1 and 2 it is normally sufficient to rely on the manufacturer's data providing the recommended concentrations and exposure time. Hence 1% Virkon is used per manufacturer's instructions and according to local Code of Practice and SOP006- "*Selection and Use of Virkon Disinfectant*" Independent studies have reported that 1% Virkon completely destroys a wide spectrum of organisms within a contact time of 10mins

C1.2.10 Personal Protective Equipment (PPE)

(i) What type of lab coats will be worn and where will they be stored? *Howie* type lab coats will be worn at all times within the CBE laboratories. They are stored in the dedicated first change area. Guidance on appropriate use of PPE will be taken from CBE SOP037 "*Use of Personal Protective Equipment*"
(ii) What type of gloves will be worn and where will they be stored? • *Depends on circumstance:* • Disposable latex powder free gloves for general use will be worn at all times when in the CBE facility, as directed by SOP037 "*Use of Personal Protective Equipment*" Autoclave gloves, stored near the autoclave will be worn at all times when operating the autoclave as directed by SOP025 "*Use and Maintenance of Systec VX-95 autoclave*"

(iii) Describe any other PPE to be used:

Full length aprons to be used when using the autoclave

SOP025 "*Use and Maintenance of Systec VX-95 Autoclave CBE045*"

Safety goggles may be required in accordance of specific SOPs.

C1.2.11 Hygiene Measures

Describe the hygiene facilities available and where they are located

Eye wash stations and hand washing facilities are located in the change room of each laboratory; other hand basins are situated directly inside the analytical laboratory and in the main change area as entering and exiting the facility

C1.2.12 Vaccination

Are effective vaccines available against any of the agents listed in Section1, 2, 3, or 4 of Part B?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe:	

C1.2.13 Waste Treatment before Disposal

How must waste to be treated before disposal and how has it been validated as being effective?

Type of Waste	Treatment before disposal	Validation of this treatment
Liquid waste	Virkon Decontamination according to SOP003 "Disposal of Biological Waste"	According to manufacturer's instructions, see section C2.1.9
Solid waste	Autoclave Decontamination according to SOP003 *"Disposal of Biological Waste"*	Treatment Cycle is validated according to SOP024 "Maintenance of Systec VX-95 Autoclave CBE044". Annual validation is conducted by an external contractor

C1.2.14 Autoclave sterilisation

If waste is treated by autoclave sterilisation then this section must be completed. If this section is not relevant then hatch the box

Type of Waste	Composition of waste	Autoclave cycle (temp, cycle time)	Treatment monitor
Liquid waste	N/R	N/R	N/R
Solid waste	Cell Culture Consumables	Minimum 121°C for 15 minutes (under clinical vacuum) CYCLE#4	Designated Autoclave tape monitors
Location of autoclave	Servicing	Location of back-up autoclave	Designated area for

	details		storage of unsterilised waste
CBE- Autoclave Room	Annual	CBE/045- In autoclave room H31	Second Change.

C1.2.15 Liquid Waste Disposal

How will liquid waste be disposed of?
To the drain?
As solid waste? No
Other? Liquid waste will be aspirated into aspirator bottle containing Virkon Disinfectant solution and the contaminated flask will be autoclaved. Refer to SOP003 "*Disposal of Biological Waste*"

C1.2.16 Solid Waste Disposal

Describe the waste category and disposal route. *(For guidance refer* to http://www.environment-agency.gov.uk)

Colour Code	Categorisation	*Hatch relevant box(es)*	Disposal Method
Yellow	Sharps (not contaminated with cytotoxic/cytostatic material)		Yellow Sharps bin>autoclave sterilisation if known or potentially infected >clinical waste disposal (incineration)
Purple/Yellow Special case, contact DSO	Sharps (contaminated with cytotoxic/cytostatic material)		Purple/Yellow lidded Sharps bin>clinical waste disposal (incineration @ 1000C)
Yellow	Human body parts, organs, including blood bags and blood preserves and excreta (unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal)		Yellow rigid one way sealed tissue bins>clinical waste disposal (incineration)
Yellow	Animal body carcasses or recognisable parts ((unless identified as medium or high risk or known infected in Section 2.2.1 of this RA in which case they must be pre-treated before disposal		Yellow rigid one way sealed tissue bins > clinical waste disposal (incineration)
Special Case – Contact DSO	Potentially or known infected lab wastes (including sharps) of HG2, GM Class 2, DEFRA Cat 2 or higher, that have not been pre-treated before leaving the site.		This is not a route of preference and is subject to special requirements
Orange	Infected or potentially infected lab wastes that have been pre treated before leaving the site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > orange clinical waste bags > clinical waste disposal (incineration)

Yellow	Infected or potentially infected animal or human body parts, organs or excreta that have been pre treated before leaving site	X	Disinfection or sterilisation (as identified in C1.2.14) in the laboratory suite > yellow one way sealed tissue bins > clinical waste disposal (incineration)

C1.2.17 Work with Animals or Vectors (if none proceed to Section C1.2.18)

(i) Are animals or vectors to be infected with any of these biological agents?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe the procedure and describe where this aspect of the work will be conducted:	
(ii) Is shedding of infectious materials by the infected animals possible or expected?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If yes, describe the routes of shedding, risk periods for such shedding and the additional precautions required to control exposure:	
(iii) Who will perform the inoculations of animals/vectors? What training have they received?	
Indicate in the adjacent box if Not Relevant (N/R)	N/R
Provide details of the training required:	

C1.2.18 Bioreactor/Fermenters (if none proceed to Section C1.2.19)

Will a bioreactor/fermenter be used to culture a biological agent?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No
If yes, describe the size, and type of the bioreactor/fermenter.	
(ii) Are any supplementary containment measures required, for example, the use of a BSC or spill tray.	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	~~Yes~~ N/R
If yes, describe: Spill tray to be used in BSC during initial tissue explant as a precautionary method to minimise opportunity for adventitious agents to reach the surface floor. A Ruano 11 Dec 2014	

C1.2.19 Other Control Measures Required?

Initial pre expansion (P0-P1) will be carried out in the presence of anti-bacterial, fungal and mycotic agents.

A Ruano 11 Dec 2014

C1.3 Emergency Procedures

C1.3.1 Describe the procedures in place for dealing with spillages *(specify disinfectants and any special containment for large volumes)*

Within the BSC:

Several SOPs outline protocol to be followed in the event of a spill.

- SOP006- Selection and Use of Virkon disinfectant
- SOP009- Use and Maintenance of Herasafe KS Class II BSC
- SOP104- Use and Maintenance of HERASAFE KS Class II re-circulating BSCs
- SOP038- Biological Spill Response

Labelled spill kits are located in the CBE unit and signs are posted throughout the CBE unit to enable workers to locate the nearest biological (and chemical) spill kit and also to advise on spill response and reporting procedures.

Within the laboratory but outside the control measure e.g. BSC, spill tray

SOP038- Biological Spill Response

Outside the laboratory e.g. during transport

Cells in this instance are not to leave the confines of the CBE laboratory space without appropriate revision of the risk assessment.

Describe the procedures in place for an accidental exposure (if necessary describe different procedures for different types of exposure e.g. eye splash or percutaneous inoculation)

The CBE code of practice and SOP 038 "biological spill response" detail the response procedure. Additionally there are supplementary information posters in the CBE lab space advising users of what to do. Eye wash stations are located in change areas and first aid kit is located outside the laboratory.

A list of qualified first aiders and contact details are posted in the labs.

The departmental safety officer Bob temple must be notified of any accident that occurs within the lab and the incident logged in the accident and near miss record.

C2 ASSIGNMENT OF CONTAINMENT LEVEL

The laboratory Containment Level is directly related to each of the 4 Hazard Groups; organisms categorised as HG1 (lowest hazard rating) should normally be handled in CL1 facilities (minimum level of containment), and likewise up to HG4 (highest hazard rating) in CL4 facilities (maximum level of containment). Where the identity or presence of a biological agent is not known the following rules apply: a) where uncertainty exists over the presence of pathogenic biological agent – minimum of CL2; b) where the presence of a pathogenic biological agent is known or suspected – minimum of Containment Level appropriate to the agent, where the assessment is inconclusive but where the activity might involve serious risk – minimum CL3

C2.1. What containment level is required for this work? (see COSHH Schedule 3, Part II for a list of criteria)

Containment level 1 is required for work with the indicated cell type (assessed hazard group 1). However, all procedures will be carried out under containment level 2 (CL2). This is to safeguard quality of work and the work of other users.

C2.2. Describe extra controls or derogation from certain controls

No

C3 FACILITIES

C3.1 Where will this work take place?

Room(s)	Building	Campus	Person in Control of area
H23 & H34 Cell Culture Suites	Centre for Biological Engineering	Holywell Park	C.J. Hewitt (Biological Safety Officer) R.Temple (Department Safety Officer) K.Sikand/C.Kavanagh (Laboratory Manager)

C4 PERSONNEL

C4.1 Names of Personnel involved in the Project

Surname	Initials	University ID	Position
Heathman	T	B120328	PhD Student
Lyness	A. M.	5017250	Enterprise Fellow

C4.2 Information, Instruction and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity. Instruction should include the 'Local Rules' or 'Local Codes of Practice' which focus on the working instructions to be followed by all persons involved in the work activity to control or prevent exposure to hazardous biological agent(s). These should be written and readily available to all workers working at Containment Level 2. A formal record of training should be kept for all individuals working at Containment Level 2.

In order to obtain access to the CBE lab space users must gain prior authorisation. Authorisation is granted on the basis of the user satisfying minimum criteria for entry set by the managing oversight. The criteria includes the user under taking some practical training sessions, reviewing the CBE CoP, submission of appropriate risk assessment documentation and review by the departmental safety officer.

Once authorisation has been approved it is the responsibilities of the user to identify and embark on specific training needs. SOPs and risk assessments relevant to project equipment and/or procedures can be used as training aids. Training files are live documents and must be continually updated to record all training acquired.

C4.3 Relevant Experience/Training:

Surname	Experience/Training
Heathman	4 years experience in cell culture. Experience and Training recorded in the training folder
Lyness	No training in cell culture. Will be observing work done by Heathman.

C4.4 Other people who may be at risk from the activity e.g. cleaners, maintenance workers or other workers in shared laboratory

Details:

None

C5 OCCUPATIONAL HEALTH

C5.1 Vaccination

Is an effective vaccination available for any of the pathogens associated with this work? Advice can be obtained from the Occupational Health Adviser (OHA) if required. All workers involved with handling unscreened blood, blood products and other tissues are recommended to have Hepatitis B immunization

C5.2 Health Surveillance

Is health surveillance required? (Health surveillance is typically applied if working with a hazardous substance that: a) produces an identifiable disease or adverse health effect that can be related to exposure; b) there is a reasonable likelihood that the disease or effect may occur under the conditions of work, and c) there are valid techniques for detecting indications of the disease or effect).

C6. NOTIFICATIONS: Human Tissue Act

C6.1.1 Relevant material covered by the Human Tissue Act

Are any of the cells, tissues or fluids to be used covered by the Human Tissue Act?	
Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	No

C6.1.2 Does This Work Have Ethical Approval? If Yes, Provide Details

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)			N/R
Approval number:			
Date obtained:		Ethics committee name:	

C6.1.3 Are other registrations/notifications required for this work? For example HSE notification under COSHH, Home Office notification under anti-terrorism, crime and security act etc

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R
If Yes, give details:	

7. LICENSING REQUIREMENTS FOR ANIMAL PRODUCTS

C7.1.1 Are there any licensing requirements for this work?

Indicate in the adjacent box as No, Yes or Not Relevant (N/R)	N/R

The regulations covering the import of animal products (including tissue cultures, tissues, body fluids or fractions thereof) are in a state of flux. Current procedures to be followed:

- If you wish to import any animal products that you know are not infected with an animal pathogen, or have good reason to expect that they are not infected with an animal pathogen, from within or outside of the EC you must apply for a Research Sample Licence using the Defra form IAPPO1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/iapppo1.htm

- If you wish to import such an animal product but it is known or suspected of being infected with an animal pathogen then you must use DEFRA form IM137. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/inttrade/im137.htm

- If you wish to import an animal pathogen listed under the Specified Animal Pathogens Order then you must use DEFRA form PATH1. Follow this link to download the form http://www.defra.gov.uk/corporate/docs/forms/ahealth/path1.htm

In all cases the instructions for their submission is stated on the forms themselves.

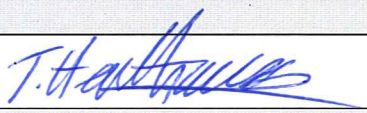
ALL APPLICATIONS SHOULD BE REVIEWED BY THE DEPARTMENTAL SAFETY OFFICER AND THE UNIVERSITY BIOLOGICAL SAFETY OFFICER BEFORE SUBMISSION.

8. DECLARATION

*The declaration must be signed **before** submitting this assessment to the Departmental Safety Officer and University Biological Safety Officer*

I, the undersigned:

- confirm that all information contained in this assessment is correct and up to date
- will ensure that **suitable and sufficient instruction, information and supervision** is provided for all individuals working on the activity
- will ensure that no work will be carried out until this **assessment has been completed and approved** and that all necessary control measures are in place
- that all information contained in this assessment must remain correct and up to date (the assessment should be **reviewed once a year** and whenever any **significant changes** to the work activity occur)
- will re-submit the assessment for approval if any significant changes occur

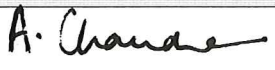
Name: Person conducting assessment	**Signature:**	**Date:**
Alex Lyness		11/12/14
Name(s): All named persons involved in the project *(add additional rows below, as required)*	**Signature:**	**Date:**
Tom Heathman		11/12/14
Name: Principal Investigator/Supervisor/Line Manager	**Signature:**	**Date:**
David Williams		

9.APPROVAL

For work involving **Hazard Group 1** biological agents: Review and approval is required by authorised and designated members of CBE staff before the work begins

For work with **Hazard Group 2** biological agents: Explicit approval is required from the Departmental Biological Safety Advisor and the University Biological Safety Officer before work begins.

If the biological agent has been **Genetically Modified** this form, (approved by the relevant authority, as above) should be submitted with the GMO risk assessment to the Departmental Biological Safety Advisor and both forms forwarded to the LU GM Safety Committee for final approval.

Name: Authorised CBE Personnel (please indicate position)	**Signature**	**Date**
A. Chandra Research Associate		11 Dec 2014
Name: Departmental Biological Safety Advisor	**Signature**	**Date**
Name: University Biological Safety Officer (or Deputy)	**Signature**	**Date**

Intercytex Ltd

Intercytex Ltd
Core Technology Facility
46 Grafton Street
Manchester
M13 9NT
United Kingdom

T +44 (0)1 606 7204

This statement certifies that the ICX-RHY cells provided to Alex Lyness of Loughborough University were manufactured to GLP grade in an Intercytex laboratory and according to Intercytex SOPs.

Date: 11-DEC-2014

Joan Benson
Operations Manager

QC Syringeability Test

Appendix 1 **QC Syringeability Test**

Batch Number: INTRODUCER STUDY **Expiry:** 20 DEC 14

Date of syringeability test: 09 DEC 14

- Mix the vial thoroughly by inverting several times
- Fit an 18 gauge needle to the syringe
- Draw up a 1.0mL volume of ICX-RHY/ICX-RHY-013 into the syringe
- Remove the 18 gauge needle (safely) and place into a sharps bin
- Fit a 30 gauge needle to the syringe
- Expel the 1.0mL volume back into the vial
- Dispose of the needle and syringe into the sharps bin

Acceptance Criterion	Pass / Fail and Initial
1.0mL product can be drawn up through the 18 gauge needle and expelled back out through the 30 gauge needle with relative ease	PASS JE

If the sample being tested fails the above criterion initiate an OOS investigation in accordance with QSOP0050 'Out Of Specification Test Results'.

Performed by: _____ **Date:** 09 DEC 14

Checked by: _____ **Date:** 09 DEC 14

Review and Release of Master and Working Human Dermal Fibroblast Cell Banks

Appendix 2

QUALITY ASSURANCE CHECKLIST FOR HDF WORKING CELL BANK

Working Cell Bank Batch Number: B/2013/066

Passage No.: 6 **No. Vials:** 197

Document for Review	Report No.	Result	Sign	Date
Batch Manufacturing record	N/A	complete	Savanne	11 NOV 13
Deviations (Detail Below)	See below	N/A	Savanne	11 NOV 13
Sterility Testing (First and Last)	0012782/1	PASS	Savanne	11 NOV 13
Bacteriostasis and Fungistasis	0012120/1	PASS	Savanne	11 NOV 13
Mycoplasma	8292135	No species were detected	Savanne	11 NOV 13
In vitro adventitious viral screen (14 day)	8292135	PASS	Savanne	11 NOV 13
Cell Morphology	100% Fibroblasts	meet Specification	Savanne	11 NOV 13
Identity Test (RAPD)	8292135	PASS	Savanne	11 NOV 13
Viable cell count	23.0×10^6	meets Specification	Savanne	11 NOV 13
Population doublings	3.2	meets specification	Savanne	11 NOV 13
Vial labelling	N/A	Satisfactory	Savanne	11 NOV 13

DEVIATION DETAILS			
Ref. No.	Comments	Sign	Date
Dev/2013/017	Deviation complete see attached	Savanne	11 NOV 13

N/A

Review and Release of Master and Working Human Dermal Fibroblast Cell Banks

COMMENTS:

Data above completed by QA (SO), with full review of batch documentation by Head of operations (JC) and CEO (PK).

11 NOV 13

Batch documentation reviewed and found to be satisfactory

PAUL IGGLE		11/11/13
Print	Sign	Date

Review and Release of Master and Working Human Dermal Fibroblast Cell Banks

Appendix 5

CONFIDENTIAL

HUMAN DERMAL FIBROBLAST WORKING CELL BANK RELEASE CERTIFICATION

BATCH NUMBER: B/2013/066 **NUMBER OF UNITS RELEASED:** 197

OTHER APPLICABLE REFERENCE: B/2013/097 (Test article BMC)

DONOR ID: N/A

This document certifies that this batch has been manufactured, tested and approved in accordance with regulatory requirements, Intercytex procedures and policies, and all applicable cGMPs.

Signed: Paul Lyn Date: 11/11/13
~~Regulatory Affairs Manager~~ CEO
SO 11 NOV 13

Signed: N/A * Date: .....
Head of Operations

Signed: Date: 11 NOV 13
Quality Assurance

See next page (POF) SO 12 Mar 13

Signed: ..... Date: .....
Qualified Person

* Signature not required. SOP to be updated
Paul Lyn 11/11/13

Review and Release of Master and Working Human Dermal Fibroblast Cell Banks

Appendix 5

CONFIDENTIAL

HUMAN DERMAL FIBROBLAST WORKING CELL BANK RELEASE CERTIFICATION

BATCH NUMBER: B/2013/066 **NUMBER OF UNITS RELEASED:** 197

OTHER APPLICABLE REFERENCE: B/2013/097 (TEST ARTICLE BMC)

DONOR ID: N/A

This document certifies that this batch has been manufactured, tested and approved in accordance with regulatory requirements, Intercytex procedures and policies, and all applicable cGMPs.

Signed: Paul Lyn Date: 11/11/13
Regulatory Affairs Manager CCB
SO 11 NOV 13

Signed: W A * Date:
Head of Operations

Signed: Date: 11 NOV 13
Quality Assurance

Signed: Date: 12 NOV 2013 *
Qualified Person

* Batch review completed concurrently with release of ICX-RHY/ICX-RHY-013 batch B/2013/074 but not signed physically until 12 NOV 2013 due to printing issues.
12 NOV 2013.

* Signature not required. SOP to be updated
Paul Lyn 11/11/13

PERFORMANCE VERIFICATION PROTOCOL

Protocol Number:	D00183-NC2014013
Project:	ICX-3D-00183
Protocol Version	Version 1.0 FINAL
Date:	

Study Start Date:	11th December 2014
Experimental Start Date:	11th December 2014
Experimental End Date:	12th December 2014
Study Monitor:	N/A
Experimental Lead:	Alex Lyness

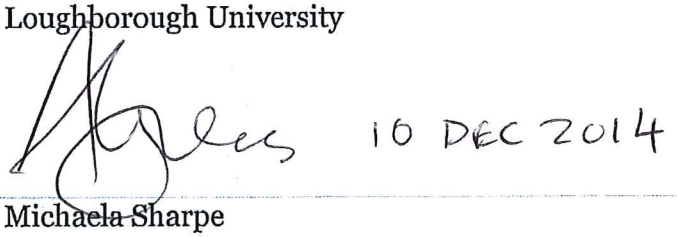
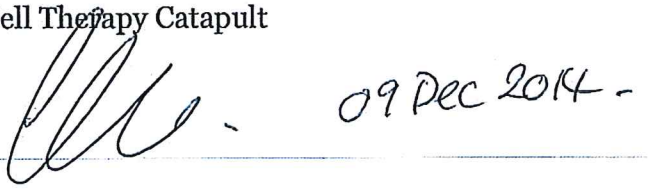
Study Location: Address:	H34 Analytical Suite Centre of Biological Engineering Hollywell Park Loughborough University Leicestershire LE11 3TU
Study Monitor: Job Title: Organisation: Signature:	N/A
Experimental Lead: Job Title: Organisation: Signature:	Alex Lyness External Consultant Loughborough University  10 DEC 2014
Approved by: Job Title: Organisation: Signature:	Michaela Sharpe Head of Non-Clinical Safety Cell Therapy Catapult  09 Dec 2014

Table of Contents

1 Introduction

This study is designed to better understand the parameters required for an automated injectable device to deliver a cell therapy product (in this instance Vavelta®; Intercytex Ltd).

Previous investigations into the performance of the CTC prototype delivery device have determined the optimal delivery parameters into ex vivo skin [1], these have then been shown to deliver to the correct depth at a non-clinical trial [2]. The accuracy and the consistency of the expected dose volume (26µl) has been verified in vitro also [3]. It is important to note that all of these studies so far have been performed using to either water or phosphate buffer solution (PBS) that has been dyed blue. This is due to ease of handling and avoiding the biological hazards of working with cells.

There has been one study, the cell viability study [4], that has been performed using fibroblasts at CTC to assess viability of the cells post-ejection in vitro. This study showed that the cell viability was not significantly affected by simulated delivery from a syringe, through microbore tubing and 27 G needle. However, one observation made by study monitor was that occasionally the pressure required to depress the plunger of the syringe required more force than expected. This is likely due to the high viscosity of the cell suspension as well as to the formation of cell aggregates that had to be forced through the system.

2 Experimental Aim

The purpose of this study is to determine whether the properties of Vavelta effect the performance of the CTCDV01 device and whether the device is consistently capable of delivering the minimum required dose of 26µl +/1 as that has been specified [5].

3 Experimental Objectives

The study will closely repeat the method used in the previous Syringe Dose Test [3]. The accuracy and the consistency of the dose volume (26µl) will be verified using GLP grade Vavelta ejected through a 27 G needle. Water will be used as the control.

The experiment will also investigate the effect of leaving the device stationary for an elongated period (50 mins) and whether the cells settle and this has an adverse effect on the dose volume accuracy; i.e. do the cells clump and cause issues with the dosing? Counting the cells of the first and last ejections from a syringe that has been motionless will also establish the homogeneity of the dosing over time.

Needles with larger bore size (lower gauge) will be used to see whether this effects and could mitigate any dose performance issues that may arise with a 27 G needle.

4 Equipment and Materials

4.1 Equipment

The test will be conducted using the CTC initial prototype (CTCDV01) programmed with firmware version CAH-0108-BD-vB. The settings to be used are injection speed = 60 mm/s, dwell time = 1 s and dose size = 26 µl. The dose speed will initially be set to 390 µl/s but may be altered during the study. Any changes to dose speed will be recorded.

The CTCDV01 will be attached to a clamp and aimed to dispense directly into a container placed on the balance. This set up will be equivalent to what was achieved during the dosing study carried out by EGT shown in Figure 4-1 [3].

Figure 4-1 Experimental set up from Syringe Dose Test (CAH-0112-TR-vB).

The volume of liquid dispensed will be determined by measuring the weight on a Denver Instruments APX-100 Precision Balance (Readability: 0.1mg, Max Capacity: 100g, Linearity: 0.2mg).[1]

The viability and presence of cells of the samples will be measured using a NucleoCounter NC-3000® (Chemometec). Mean cell diameter of the analysed population is also provided by each automated cell count, which will be used in combination with cell viability to demonstrate stability of Vavelta throughout the delivery process.

4.2 Consumables

For the previous tests, the syringe was filled with de-ionized water giving a nominal density of 1g/ml therefore for all measurements in this test 1g = 1ml (0.001g = 1µl). The nominal density of Vavelta that is to be used is unknown.

The other consumables to be used are:

- Terumo 2.5ml Luer Lock Syringe

[1] The balance used is an order of magnitude more sensitive than that used in the Syringe Dose Test [3].

- Terumo 27, 25, 22 G ¾" Hypodermic Needles
- 75mm Microbore Extension Tubing (Initial Production Batch - Non-Sterile)

5 Protocol

The following work is to be carried out in the H34 Analytical Suite at the Centre of Biological Engineering, Hollywell Park, Loughborough University.

5.1 Study Design

The study is divided into the following two experiments, to be performed one after the other using two 1ml vials of Vavelta:

1. The first experiment will measure the precision of which the device dispenses Vavelta product at 26µl both immediately after a WCS 50 minute period [2].
2. The second experiment will investigate whether increasing the needle bore size (reducing the gauge) effects the dose volume delivered.

Once both experiments are complete repeats will be performed using water will act as the control.

5.2 Methods

The materials and equipment will be set up as follows;

5.2.1 Vavelta Preparation

The Vavelta will be shipped in 1ml vials from Intercytex Ltd. and received to the CBE facility and handled according to the correct Biological Risk Assessment [6]. The Vavelta will be stored at 2-8°C until time of use. When required the vials will be warmed in a water bath to 20°C (room temperature) as established by Prof. McGrath who developed the clinical protocol for a recently reported clinical trial [8].

5.2.2 System Loading Procedure

To load the syringe, the contents of a 1ml vial of Vavelta (or water) will be drawn up into a 2-mL syringe using a 19 G sterile 'filling' needle. The syringe will then be attached to the microbore tubing and the narrower 27 G 'delivery' needle [3] (the sub-assembly)

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[2] This is a worst-case scenario as Vavelta must be used within 1 hour of warming to room temperature.

[3] Previously, in two different clinical trials in the UK and Australia, 21 G and 25 G needles have been used [8], [9]. The instructions for Vavelta produced by Intercytex indicate a 29-30 G needle should be used [10].

5.2.3 Device Set Up

This sub-assembly will then be placed onto the CTCDV01 and the system will be primed. The device will then be placed on a clamp stand pointing down into a weighing vessel placed on the mass balance.

5.2.4 Dosing Volume Measuring Method

1. The CTCDV01 device will be loaded and set up as explained in the Loading Procedure and Device Set Up sections.
2. Tare (zero) the balance.
3. The device will then be triggered to eject liquid with the lowest dose volume setting (26µl) into the weighing vessel on the mass balance.
4. After each ejection the value will be recorded and the mass balance will be tared (zeroed) [7].
5. Steps 2 and 3 will be repeated for as long as is specified.
6. The results will be recorded manually and then transferred into Microsoft Excel for further analysis. A QC of data transfer will be performed.
7. The collected ejectate at the end of the experiment will be disposed of according to the Biological Risk Assessment [6].

5.2.5 Cell Counting Method

The dose volume will be measured and the cumulative fluid (130µl) will be transferred to the NucleoCounter. It is necessary to use cumulative dosing as the sample sizes are too small on their own. Only the beginning and end of the experiment are to be counted. The protocol is as follows:

1. The cell suspension is mixed to obtain a homogenous suspension. The cell sample is drawn by inserting the tip of the Via1-Cassette™ into the cell suspension and pressing the piston.
2. The loaded Via1-Cassette™ is immediately placed on the tray of the NucleoCounter® NC-3000.
3. "Viability and Cell Count Assay" and sample unit Via1-Cassette™ are selected and the sample is run.

After approximately 1 minute the viability (in percent) and the concentrations (cells/mL) of all cells and non-viable cells are displayed in the result box. Moreover, a rough estimate of the cell diameter (in µm) and information about cell aggregation are also provided. The data will be recorded for 5 samples.

5.3 Experiments

5.3.1 Experiment 1 – Investigating the effects Vavelta has on dosing accuracy

The experiment will be carried out accordance to the methods already outlined.

1. Firstly, 5 repeats will be performed. The dose volume will be measured and the cumulative fluid (130µl) will be transferred to the NucleoCounter in order to establish cell count and viability at the start of the experiment.
2. Then 15 further repeats will be performed and dose volume measured.
3. Then the device will be left for 50 minutes to allow for the WCS cell settling.
4. Then 15 further repeats will be performed and dose volume measured.
5. Finally, 5 more repeats will be performed. The dose volume will be measured and the cumulative fluid (130µl) will be transferred to the NucleoCounter in order to establish cell count and viability at the end of the experiment.

A total of 40 injections (1.04ml) with Vavelta will be performed. For the control using water, the time delay is not required and only 20 repeats are to be performed.

5.3.2 Experiment 2 – Investigating the effect of needle gauge

The experiment will be carried out accordance to the methods already outlined.

1. The system will first be filled and a 25 G needle (260µm bore size) will be used in place of a 27 G (210µm bore size) for 20 repeats.
2. The system will remain primed and the needle switched for a 22 G (413µm bore size) for 20 repeats.

A total of 40 injections (1.04ml) with Vavelta will be performed. For the control tests with water, 20 repeats are to be performed for each of the needles used.

5.4 Planned Test Summary

Exp.	Needle	Material	No.	Cumulative Volume (µl)	Cell Count Volume (µl)	Comment
1	27 G	ICX Vial 1	5	130	130	Cell Count (Moving)
	27 G	ICX Vial 1	15	520	-	Flushed through
	27 G	ICX Vial 1	15	910	-	50 min delay
	27 G	ICX Vial 1	5	1040	130	Cell Count (Settled?)
	27 G	Water	20	N/A	-	Control
2	25 G	ICX Vial 2	20	520	-	Increase needle bore 1
	22 G	ICX Vial 2	20	1040	-	Increase needle bore 2
	25 G	Water	20	N/A	-	Control 1
	22 G	Water	20	N/A	-	Control 2

6 References

[1] G. Leoni, "Intercytex Ex Vivo Pilot Study Report (D00183-NC2014006)," 2014.

[2] J. Cartwright, "Pathology Draft Report 1 (D00183-NC2014003)," 2014.

[3] D. Warwick, "Syringe Dose Test Report (CAH-0112-TR-vB)," 2014.

[4] G. Leoni, "Cell Viability Study Protocol (D00183-NC2014002)," 2014.

[5] "Acceptance Test Criteria – CTC Development Contract - Schedule 5," 2014.

[6] "Biological Risk Assessment CBE/BRA/072," 2014.

[7] CBE, "Use and Maintenance of Mettler Toledo balances (SOP136)," 2012.

[8] G. Petrof, M. Martinez-Queipo, J. E. Mellerio, P. Kemp, and J. a McGrath, "Fibroblast cell therapy enhances initial healing in recessive dystrophic epidermolysis bullosa wounds: results of a randomized, vehicle-controlled trial.," *Br. J. Dermatol.*, vol. 169, no. 5, pp. 1025–33, Nov. 2013.

[9] S. S. Venugopal, W. Yan, J. W. Frew, H. I. Cohn, L. M. Rhodes, K. Tran, W. Melbourne, J. a Nelson, M. Sturm, J. Fogarty, M. P. Marinkovich, S. Igawa, A. Ishida-Yamamoto, and D. F. Murrell, "A phase II randomized vehicle-controlled trial of intradermal allogeneic fibroblasts for recessive dystrophic epidermolysis bullosa.," *J. Am. Acad. Dermatol.*, vol. 69, no. 6, pp. 898–908.e7, Dec. 2013.

[10] "Vavelta Instructions - Information for the Healthcare Professional supplied with Product," 2013.