SC1-BHC-07-2019
Regenerative medicine: from new insights to new applications

ORGANTRANS

Controlled Organoids transplantation as enabler for regenerative medicine translation

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Duration: 36 months

= Deliverable D1.2 =

Protocol for the large-scale production of liver organoids

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PU	Public	x
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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Executive Summary

In order to obtain clinically sufficient numbers of LGR5-positive stem cells in the form of organoids, there is a need to generate Standard Operating Procedure (SOP) to upscale the expansion of organoids in spinner flasks. We established a method for the expansion of large quantities of human liver organoids in spinner flasks. Due to improved oxygenation in the spinner flasks, organoids rapidly proliferated and reached an average 40-fold cell expansion after 2 weeks, compared with 6-fold expansion in static cultures. This highly efficient method for culturing large numbers of organoids of organoids, paves the way for the application of organoids for tissue engineering and liver transplantation. The deliverable 1.2 has yielded one manuscript in the prestigious journal Hepatology and a SOP (Appendix I) has been generated which ensures future GMP compliance.

Table of Contents

1. Introduction

The current deliverable D1.2 is part of Work Package 1 (WP1). The overall aim of WP1, Stem cell source and requirements, is to create standardized liver spheroids with hepatic function and tissue architecture. In order to achieve this, we will isolate six individual adult stem cell cultures from liver tissue (male and female), differentiate them towards the hepatocyte- and cholangiocyte-lineage, combine the organoid-derived hepatocytes and cholangiocytes with supporting cells including bone-marrow derived Multipotent Stromal Cells (BM-MSCs) and endothelial cells, create standardized spheroids using the Sphericalplates 5D from KUG, assess function and tissue architecture of the multicellular spheroids and scale-up the culture of adult stem cells from the liver cultured as organoids. For the upscaling we needed a standardised method that allowed large scale organoid expansion. For this we used spinner flasks (Figure 1) which provide a continuous agitation to the organoid culture hence providing an improved oxygenation and distribution of nutrients and waste products. Upscaling cultures using stirred bioreactors or spinner flasks is an essential step in the upscaling of cell cultures needed for ATMP development. The current developed technology has been turned into a Standard Operating Procedure (SOP) that would allow future GMP compliancy.

FIG 1. Example of commercially available Corning spinner flasks (125 ml) in a culture stove. Spinner flasks are inoculated with 2.5 million cells in a 25 ml starting volume (expansion media) with 10% (v/v) Matrigel. Every other day media is added, and cells are cultured for a duration of two weeks. Rotation speed of the spinner flasks is 80 rpm.

2. Results and Discussion

To establish a suspension culture for efficient organoid expansion, we inoculated spinner flasks with 10^5 human liver organoid cells per millilitre of organoid EM (Huch et al. 2015) supplemented with 10% (vol/vol) Matrigel, cultured the cells for 2 weeks, and compared the proliferation with that of cells conventionally cultured in Matrigel droplets (static controls; 10^5 cells/100 µL Matrigel). Light microscopy showed that the single cells grew out to form organoids within the first 4 days of culture in both static controls and spinner flasks (Fig. 2A). From culture day 7 onward, the diameter of the organoids in the spinner flasks increased and the outer cell layers appeared thicker compared with those in static controls. This was confirmed in histological sections, where spinner flask organoids often consisted of a multilayer of cells, whereas organoids in static controls consisted of a single epithelial layer (Fig. 2C). On day 14, organoid sizes were heterogeneous, with those in the spinner flasks clearly larger compared with those in static controls. The biggest organoids reached a diameter of about 5 mm in the spinner flasks and 1 mm in the static controls (Fig. 2A). To quantify the cells, we harvested an aliquot from the spinner flasks every 2 to 3 days, and trypsinised and counted the single cells. Expansion rates varied between donors, but were significantly increased in spinner flasks compared with static controls for all five donors that were analysed in independent experiments (Fig. 2B). The average cell expansion after 2 weeks was 43-fold in spinner flasks and 6-fold in static controls (Fig. 2B). Relative cell numbers for each donor are provided in Supporting Table S1. Immunohistochemical staining for Ki67 confirmed that almost every cell in the spinner flasks was proliferative (Fig. 2C), and quantitative RT-PCRs showed comparable expression of the stem cell marker LGR5 in spinner flasks and static controls. We next analysed whether the stem cell phenotype and the high proliferation of the organoids were sustainable over several weeks, to obtain billions of cells as required for transplantations or tissue engineering. We inoculated four independent spinner flasks with single cells from four different donors and passaged the organoids every 2 weeks. After passaging, one new spinner flask per donor was inoculated with organoid fragments equal to a density of 10^5 cells/mL and expanded in EM. Quantitative RT-PCR showed that the expression of LGR5 in spinner flasks samples remained stable over the course of 6 weeks (data not shown). For quantification of the cells, an aliquot from the spinner flasks was trypsinised and single cells were counted every week. We observed an exponential cell expansion during the 6 weeks of culture for organoids from all four donors. The calculation of the theoretical number of cells after 6 weeks of culture revealed a total amount of cells ranging between 5.1×10^{10} and 2.7×10^{12}, depending on the donor (Fig. 2D). Therefore, with a starting number of 2.5 million cells, our method allowed us to produce sufficient numbers of cells to restore 17%-900% of the liver mass of an adult person in only 6 weeks. To assess what caused the high proliferation rate in the spinner flasks, we performed mRNA sequencing on organoids from three different donors that were cultured in spinner flasks or in Matrigel droplets for 14 days. We selected genes that were down-regulated or up-regulated more than 4-fold in the spinner flasks compared with their respective static controls, and obtained a list of 69 annotated down-regulated genes and 62 annotated up-regulated genes that were overlapping for organoids from all three donors. Gene ontology analysis of those genes revealed that three major pathways were differentially regulated in spinner flask organoids compared with static controls: cell cycle genes (n = 19) were significantly up-regulated; and genes related to cell adhesion (n = 17) and genes related to hypoxia (n = 14) were significantly down-regulated in the spinner flask organoids (Fig. 2E), suggesting that the increased expansion rate in the spinner flasks may be associated with an improved oxygen supply.

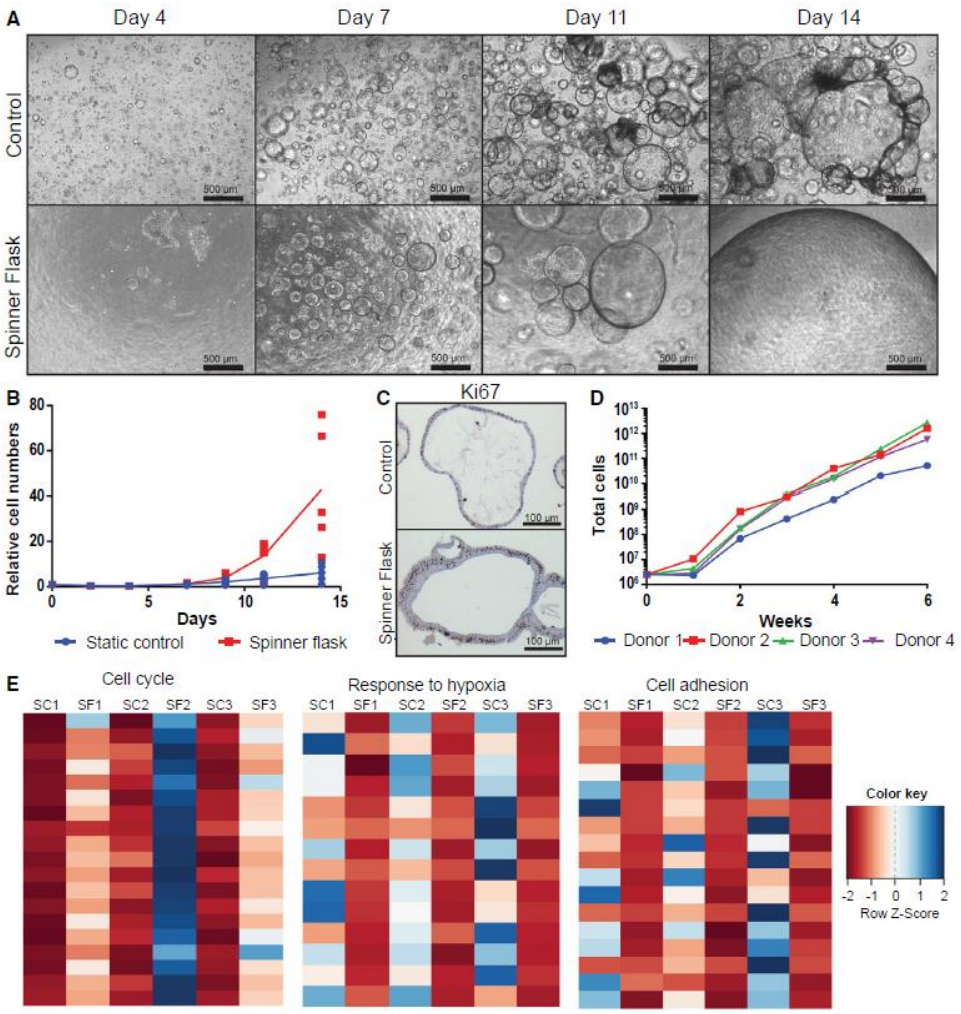

FIG. 2. Enhanced liver organoid expansion in spinner flasks. Spinner flasks were inoculated with 2.5 × 106 single organoid cells (105 cells/mL) at day 0 and cultured in human organoid EM supplemented with the Rho kinase inhibitor Y27632 and 10% vol/vol Matrigel; 4-5 different donors were analyzed in independent experiments. As controls, single cells were seeded in Matrigel droplets (10^5 cells/100 µL Matrigel) and cultured in EM supplemented with Y27632. (A) Light microscopy images of organoids grown from single cells in spinner flasks or in static control. (B) In vitro growth curves. An aliquot of cells was counted every 2-3 days, and cell numbers relative to day 0 were calculated. Each red dot represents a different donor. Blue dots represent the corresponding donors cultured in static control. Lines represent the mean of five different donors. (C) Ki67 stainings confirmed that organoids were highly proliferative at day 14 after seeding in spinner flasks and controls. (D) Long-term in vitro growth curves. Organoids from four different donors were cultured for 6 weeks in spinner flasks. Cultures were split every 2 weeks, and new spinner flasks were inoculated with organoid fragments corresponding to 2.5 × 10^6 cells. An aliquot of cells was counted every week, and theoretical total cell numbers were calculated. (E) mRNA sequencing data from organoids from three different donors that were cultured in spinner flasks or in static control for 14 days. Heatmaps show the three major pathways that were differentially regulated in spinner flask organoids compared with static controls. Abbreviations: SC, static control; SF, spinner flask.

In this study, we describe a robust spinner flask–based method for large-scale culture of human LGR5- positive adult stem cell–derived liver organoids. This method allows high proliferation levels, repopulation potential, and maturation toward cholangiocyte-like and hepatocyte-like cells. These cells do not induce tumour formation and can be serially passaged and maintained for at least 6 weeks (Fig. 1A-D). This greatly improves upon current techniques for the expansion of large numbers of liver stem cells, and therefore approaches to liver regeneration.

Reference:

Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, Ellis E, van Wenum M, Fuchs SA, de Ligt J, van de Wetering M, Sasaki N, Boers SJ, Kemperman H, de Jonge J, Ijzermans JN, Nieuwenhuis EE, Hoekstra R, Strom S, Vries RR, van der Laan LJ, Cuppen E, Clevers H. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015 Jan 15;160(1-2):299-312. doi: 10.1016/j.cell.2014.11.050. Epub 2014 Dec 18.

3. Conclusions

We have developed a method for highly efficient and safe expansion of LGR5-positive liver stem cells, reaching an average expansion of 40-fold in two weeks of spinner flask culture compared to a 6-fold expansion in static conditions. With this procedure, published and written down as an SOP (see Appendix I), we can expand 2.5 million cells (inoculation) into, on average, 160 billion cells in six weeks. As an average human liver holds approximately 150 billion cells, this is far more than sufficient for clinical application.

4. Degree of Progress

The Task 1.2: Cell procurement and large-scale production of adult stem cells (UU, KUG), was successful for the large-scale production of organoids (D1.2). The second part of the task, differentiation, is ongoing (part of D1.1).

5. Dissemination Level

The deliverable is public, the procedure was published in the journal Hepatology as part of the ORGANTRANS project:

Schneeberger K, Sánchez-Romero N, Ye S, et al. Large-Scale Production of LGR5-Positive Bipotential Human Liver Stem Cells. Hepatology. 2019;10.1002/hep.31037. doi:10.1002/hep.31037.

6. Appendix I: Standard Operating Procedures

OrganTrans Process for organoids transplantation	**Code:** **Version:** **Page:** **Valid from:** **Valid until:**

INTSRUCTION – CORNING® DISPOSABLE SPINNER FLASK SYSTEM USED FOR ORGANOID UPSCALING

DOCUMENT IDENTIFICATION:

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Bart Spee	26-06-2020
Associate Professor	Date / Signature
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Karolina Pal-Kutas	26-06-2020
Quality Management	Date / Signature
Document authorized by:	
FULL NAME	
Head of Quality Management	Date / Signature
Status of document:	**New document**
Change history:	
Previous version:	**Modifications**
n.a.	**n.a.**
Processing data:	

OrganTrans Process for organoids transplantation	**Code:** **Version:** **Page:** **Valid from:** **Valid until:**

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DOCUMENT IDENTIFICATION:

Place of production:		☐ **Room ID:** BSL 1 E 5.17		
Name of Authorised staff:			**Signature:**	
Name of Controller*:			**Signature:**	
Start of processing:	**Date:**		**Time:**	
End of processing:	**Date:**		**Time:**	

The Controller controlled the steps marked with „"

Preparations:

used equipment:	**used:**	**Signature:**
Aspiration pump		
BioRad Cell Counter	☐ Bench BSL1 E 5.17	
Calculator	☐ Bench BSL1 E 5.17	
Eppendorf Centrifuge 5810R	☐ Bench BSL1 E 5.17	
Corning disposable spinner flask	☐ Culture stove BSL1 E 5.16	
Corning stirrer plate	☐ Bench BSL1 E 5.16	
Corning stirrer plate controler	☐ Bench BSL1 E 5.16	
Freezer	☐ Bench BSL1 E 5.16	
Fridge	☐ Bench BSL1 E 5.16	
Heating block	☐ Bench BSL1 E 5.16	
Incubator	☐ Bench BSL1 E 5.16	
Micropipette 2 – 20µL	☐ Bench BSL1 E 5.16	
Micropipette 100 – 1000µL	☐ Bench BSL1 E 5.16	
Pipetboy	☐ Bench BSL1 E 5.16	
Stir plate	☐ Culture stove BSL1 E 5.16	
used basic material:	**Internal lot no.:**	**Signature:**
Advanced DMEM/F12	☐	
Ethanol 70%	☐	
TrypLE Express Enzyme (1X) no phenol red	☐	
used consumables:	**Internal lot no.:**	**Signature:**
Falcon Centrifuge tube 50mL	☐	
Falcon Centrifuge tube 15 mL	☐	
Micropipette tip 2 – 20µL	☐	
Micropipette tip 100 – 1000µL	☐	
Protein LoBind Tube 5mL	☐	
Serological pipette 5mL	☐	
Serological pipette 10mL	☐	
used intermediate:	**Internal lot no.:**	**Signature:**

OrganTrans Process for organoids transplantation	**Code:** **Version:** **Page:** **Valid from:** **Valid until:**

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n.a.	n.a.	n.a.

Operating state/preventive measures:	**Signature:**
All required equipment is in operation state according to instruction.	
The work area has been disinfected according to instruction. All required basic materials and consumables are locked in cleanroom according to instruction *SOP xxxx*.	
All required basic materials and consumables are within the shelf life. Media and solutions are clear.	
The safety precautions have been applied according to instruction.	

Labelling:

Product name:	Spinner Flask Corning – 125 ml	
Internal no:	/	(dd.mmm.yyyy)

☐ Labelling according to SOP

Requirements for label (example):	**Used label:**
Container label: Batch no.: /dd.mmm.yyyy[†] Volume: mL Expiry: dd.mmm.yyyy[‡] [‡]Expiry: Expiry of original product	Container label: Storage box label:

Processing:

1) General description of the Corning® disposable spinner flask system (125 ml)

a) The Corning® disposable spinner flask system comes ready-to-use with paddle and integrated magnet, eliminating the need for time-consuming assembly or cleaning and reassembly

b) Molded from virgin polystyrene and gamma-irradiated, each spinner flask system assures a clean and sterile unit that is nonpyrogenic. No more concerns with detergent residues or contamination.

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c) Made of USP Class VI and ISO 10993-18 polystyrene, the vessel is comparable to conventional glass spinner flasks for growth of suspension cell lines and any attachment-dependent cultures using microcarrier beads

d) The paddle size and height is optimized for each vessel size. A unique integrated magnet provides a smooth, even rotation at required speeds on any laboratory slow speed stirrer. Heat build-up in the vessel is reduced by means of a specially designed flange that raises the vessel off the stir-plate surface. This flared base also provides extra stability and support.

e) Corning 125 mL disposable spinner flask is approximately 63.5 mm diameter, with a 70 mm screw cap and it has two approximately 18.8 mm long angled sidearms with approximately 32 mm screw caps. The flask is sterile and disposable. (See picture Figure 1.)

Figure 1. Example of an 125 mL Corning Spinner flask.

2) Instruction to usage of the Spinner Flask

a) Sterilize a low speed stir plate appropriate for use in CO_2 incubator by spraying with 70% ethanol
b) Place the low speed stir plate on the bottom shelf of a standard tissue culture incubator
c) Make sure the shelves above are positioned with enough room to fit the spinner flask on the stir plate comfortably
d) Move the controller cable to the side so it can exit the incubator without hanging in front of tissue culture dishes
e) Hold it on place with a piece of tape on the outside of the incubator and check that the incubator doors can still close securely
f) Connect the power plug of the stirring plate controller to a wall outlet
g) Set the desired rotational speed by pressing the + or - buttons on the controller until the desired speed is shown in the display (= 85 rpm)

Section 2 completed: ☐ yes ☐ no Signature:

3) Trypsinisation of organoids from standard static culture

a) Continuation of "Organoid Static Culture" (SOP organoid culture and media)

a) Add 2 ml of cold advanced DMEM/F12 (4 °C) to each well for a total of 5 wells of a 12-well culture plate using a 10 mL serological pipette

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b) Resupend the organoids in cold advanced DMEM/F12 and pipette the media containing cells up and down using the 10 mL serological pipette

c) Divide the cell suspension over 2 15ml Falcon tubes and fill the tubes up until 15ml with cold advanced DMEM/F12

d) Spin down at 400 g for 5 minutes at 4 °C in the centrifuge

e) Take off supernatant using a 10 mL serological pipette

f) Add 1 ml TrypLE Express to each of the two cell pellets using an aspiration pipette and a 1000µl micropipette tip, using the same micropipette tip, transfer the cell pellet and Tryple Express mixture to a 5 ml Protein LoBind Tube and incubate in heat block at 37°C for 30 minutes

Start of incubation (hh:mm): ……., End of incubation (hh:mm): …….,
Total incubation time: ……min

g) Mechanically fragment every 5 minutes using an aspiration pipette and a 1000µl micropipette tip to break down the organoids until 90-95% is single cell (check with a little drop under the microscope when no cell clumps are visible)

h) Add 4 mL of cold advanced DMEM/F12 to each LoBind tube using an aspiration pipette and a 1000µl micropipette tip to the trypsinised sample and mix through inverting the LoBind Tube

i) Spin down at 400 g for 5 minutes at 4 °C in the centrifuge

j) Take off supernatant using a 5 mL serological pipette

k) Add 2 mL of advanced DMEM/F12 to each of the LoBind tube using a 5 mL serological pipette and pool both cell suspensions into one of the tubes (total volume = 4 ml)

Section 3 completed: ☐ yes ☐ no Signature:

4) IPC – Cell count

b) Take the LoBind Tube containing the cell suspension and bring it to the BioRad cell counter (see SOP about BioRad cell counter)

c) Take 10 µl of the cell suspension and mix with 10 µl Trypan Blue in an Eppendorf vial using an aspiration pipette and a 20µl micropipette tip

d) Add 10 µl of the cell suspension/ Trypan Blue mixture to the BioRad cell counter chip using an aspiration pipette and a 20µl micropipette tip and place the chip in the Biorad cell counter machine (the machine will start counting automatically), see also Figure 2

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Load the sample onto a slide. | Insert the slide into the TC20 cell counter; counting automatically begins. | Obtain a total cell count (without trypan blue) or total and live cell counts (with trypan blue) in 30 seconds.

Figure 2. Loading of the sample in the BioRad cell counter chip using an aspiration pipette and a 20µl micropipette tip at the end. Inserting the chip into the cell counter. View the results on the screen.

e) Repeat the measurement one more time

f) Write in table 1 below the sample ID with batch number, actual passage (P0 or P1), date (as an example: P008spez10xx_P0_yyyymmdd)

g) Check the presence of cells and the number of viable cells on the screen of the BioRad cell counter and note it in table 1. Average the two measurements with a calculator.

h) Calculate the total amount of cells in the cell suspension with the average cell count (cells/ml) according to step 5.

Table 1: IPC cell count

Parameter	Acceptance criteria	Further procedure
Sample ID:		
Viability of cells: **1: ….** **2: ….** **Average:**	☐ < 50% cell viability	SOP Deviation → disposal according to SOP …., new organoid culture according to SOP ….
	☐ ≥ 50% cell viability	Continue with step 8)
Number of viable cells: **1: ….** **2: ….** **Average:**	☐ <0.625 million cells per mL	SOP Deviation → disposal according to SOP …., new organoid culture according to SOP ….
	☐ ≥0.625 million cells	Continue with step 6)

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	per mL	

Section 4 completed: ☐ yes ☐ no Signature:

5) Seeding density calculation

a) Calculate the dilution you need for seeding spinner flasks based on the number of cells you counted in "Cell count" (step 5-Table 1):

$$Dilution = \frac{counted\ viable\ cells\ (million)\ per\ mL}{0.1\ million\ cells\ per\ mL}$$

Setpoint: dilution of 6,25 fold

Finding: >6,25 fold ☐ corresponds ☐ does not correspond

b) Calculate the amount of cell suspension in mL you have to seed per spinner flask:

$$\boldsymbol{x} = amount\ of\ cell\ suspension = \frac{25'000}{\text{dilution}}$$

$\boldsymbol{x} =$ …. mL/ spinner flask

Section 5 completed: ☐ yes ☐ no Signature:

6) Seeding spinner flask culture (day 0)

a) Add the calculated amount of cell suspension (step 6/b) to each spinner flask using a 5 mL serological pipette, changing to a new, sterile pipette for each flask

b) Add culture media (SOP organoid culture and media) up to a total volume of 22.5 mL using a 25 mL serological pipette

c) Add 2.5 ml cold Matrigel

d) Close the spinner flasks, distribute the content over the spinner flask by gently swirling the flasks and place the flasks into the incubator at 36-38°C in vertical position on the stirrer plate.

e) Incubate for 14 days

Section 6 completed: ☐ yes ☐ no Signature:

7) Further procedure (day 2, 4, 6, 8, 10, 12)

a) After 2-3 days (according to Table 2) take the flasks out from incubator and add the appropriate amount of culture media and liquid Matrigel (see SOP organoid culture and media)

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Table 2: Media addition

Day	Date	Media addition	Volume of Culture media (with 10% v/v Matrigel)
	Start (dd.mmm.yyyy): .....,		
2	Date (dd.mmm.yyyy): .....,	☐ Yes	12.5 mL
4	Date (dd.mmm.yyyy): .....,	☐ Yes	12.5 mL
7	Date (dd.mmm.yyyy): .....,	☐ Yes	25 mL
9	Date (dd.mmm.yyyy): .....,	☐ Yes	25 mL
11	Date (dd.mmm.yyyy): .....,	☐ Yes	25 mL
	End (dd.mmm.yyyy): .....,		

Section 7 completed: ☐ yes ☐ no Signature:

8. IPC – Cell count

a) Take the spinner flask out of the incubator, quickly take one mL out of the spinner flask using aspiration pipette and a 1000µl micropipette tip (do not allow the cells to settle), add it to a 15ml Falcon tube and fill the tube up until 15ml with cold advanced DMEM/F12

b) Spin down at 400 g for 5 minutes at 4 °C in the centrifuge

c) Take off supernatant using a 10 mL serological pipette

d) Add 1 ml TrypLE Express to the cell pellet using an aspiration pipette and a 1000µl micropipette tip and transfer to a 5 ml Protein LoBind Tube and incubate in heat block at 37°C for 30 minutes

e) Mechanically fragment every 5 minutes using an aspiration pipette and a 1000µl micropipette tip to break down the organoids until 90-95% is single cell (check with a little drop under the microscope when no cell clumps are visible)

f) Add 4 mL of cold advanced DMEM/F12 to the LoBind tube using an aspiration pipette and a 1000µl micropipette tip to the trypsinised sample and mix through inverting the LoBind Tube

g) Spin down at 400 g for 5 minutes at 4 °C in the centrifuge

h) Take off supernatant using a 5 mL serological pipette

i) Add 1 mL of advanced DMEM/F12 to each the LoBind tube using a 5 mL serological pipette and thoroughly resuspend the cells

j) Take the LoBind Tube containing the cell suspension and bring it to the BioRad cell counter (see SOP about BioRad cell counter)

k) Take 10 µl of the cell suspension and mix with 10 µl Trypan Blue in an Eppendorf vial using an aspiration pipette and a 20µl micropipette tip

l) Add 10 µl of the cell suspension/ Trypan Blue mixture to the BioRad cell counter chip using an aspiration pipette and a 20µl micropipette tip and place the chip in the Biorad cell counter machine (the machine will start counting automatically), see also Figure 3

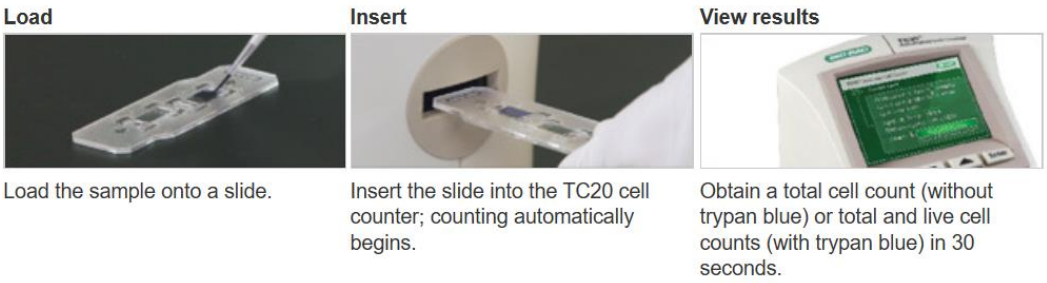

Figure 3. Loading of the sample in the BioRad cell counter chip using an aspiration pipette and a 20µl micropipette tip at the end. Inserting the chip into the cell counter. View the results on the screen.

m) Repeat the measurement one more time

n) Write in table 2 the sample ID with batch number, actual passage (P0 or P1), date (as an example: P008spez10xx_P0_yyyymmdd)

o) Check the presence of cells and the number of viable cells on the screen of the BioRad cell counter and note it in table 2. Average the two measurements with a calculator.

p) Calculate the total amount of cells in the spinner flask with the average cell count (cells/ml)

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Table 2: IPC cell count

Parameter	Acceptance criteria	Further procedure
Sample ID:		
Viability of cells: **1: ….** **2: ….** **Average:**	☐ < 50% cell viability	SOP Deviation → disposal according to SOP …., new organoid culture according to SOP ….
	☐ ≥ 50% cell viability	Continue with step 8)
Number of viable cells: **1: ….** **2: ….** **Average:**	☐ <0.48 million cells	SOP Deviation → disposal according to SOP …., new organoid culture according to SOP ….
	☐ ≥0.48 million cells	Continue with step 10)

Section 9 completed: ☐ yes ☐ no Signature:

8) Total amount of cells per spinner flask:

$$x = amount\ of\ cells\ (\text{million})\ \text{per mL} * 125$$

$x =$ …. ′million cells / spinner flask

9) Further processing

a) Take the spinner flask from the culture stove

b) Proceed with SOP processing organoids for spheroid formation

Storage	**Signature:**
☐ Product stored at ……in ……	

Shelf life	**Signature:**

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DOCUMENT IDENTIFICATION:

Product expiry date:		

Clean up	**Signature:**
All used consumables have been disposed according to instruction. The work area has been cleaned and disinfected according to instruction.	

Conformation of processing:

processing according to instruction: Authorised staff:	Date / Signature	
processing according to instruction: Controller:	Date / Signature	
Deviation/NoE according SOP:	☐ yes ☐ no	No.:
Note: Attachments: Attachment ....., filled ....., on .....(date), ... pages		

Check and evaluation:

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The following material has been released:	(internal number/date)
The organoid cell suspension is: ☐ **released** ☐ **conditionally released** ➔ Please indicate the conditions in the note field below ☐ **rejected** ➔ Please indicate the justification in the note field below	
Note: The organoid cell suspension can be released when a minimum yield of 60 million cells has been measured per spinner flask. A conditional release is allowed with a yield between 40-60 million cells. A yield below 40 million cells will be rejected.	
Date:	Signature Department: