Landelijk protocol indicaties ex-situ machine perfusie donorlevers

Versie 1.0 (datum 15-04-2021)

Versie 1.2 (datum 01-06-2021)

Versie 2.0 (datum 27-09-2021)

Universitair Medisch Centrum Groningen

Erasmus MC
Universitair Medisch Centrum Rotterdam

L U
M C
Leids Universitair
Medisch Centrum

Auteur en contactpersoon:
Prof.dr. Robert J. Porte, chirurg
Sectie HPB Chirurgie en Levertransplantatie
Afdeling Chirurgie
Universitair Medisch Centrum Groningen
Postbus 30.001
9700 RB Groningen

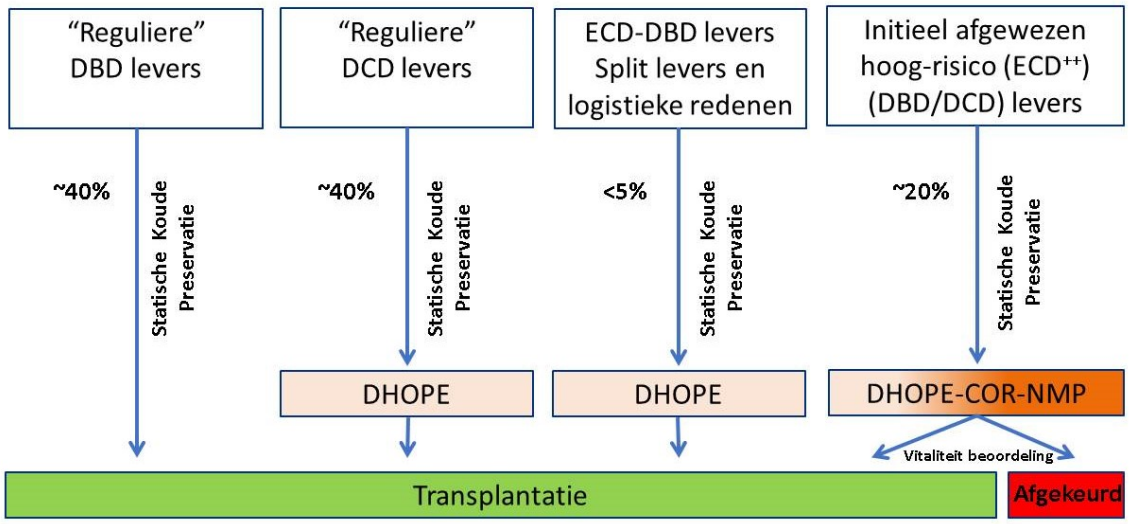
Tel. 050-3612896
E-mail: r.j.porte@umcg.nl

Inhoudsopgave

1. Achtergrond

Per 1 januari 2022 vallen *ex situ* machine perfusies van donorlevers voor transplantatie onder vergoede zorg. In dit protocol worden de indicaties en werkwijze beschreven voor machinale perfusie van donorlevers in Nederland.

Bij *ex situ* machine perfusie van donorlevers worden twee vormen onderscheiden: hypotherme geoxygeneerde machine perfusie (HOPE of DHOPE) en normotherme machine perfusie (NMP). Deze twee technieken hebben ieder hun eigen indicatiegebied, doch zorgen er beide voor dat er meer levertransplantaties mogelijk zijn. (D)HOPE heeft als belangrijkste doel om ischemie-reperfusie schade gerelateerde complicatie na levertransplantaties te vermijden en retransplantaties te voorkomen. NMP heeft als belangrijkste doel om hoog-risico levers, die niet zonder meer geschikt zijn voor transplantatie, eerst te testen om zo levers te selecteren die anders niet getransplanteerd zouden zijn of een hoog risico gehad zouden hebben op vroegtijdig transplantaat falen. In Nederland wordt op dit moment zowel DHOPE als NMP uitsluitend eind-ischemisch toegepast, dat wil zeggen na een periode van koude statische preservatie tijdens transport van het donor-ziekenhuis naar het transplantatie-ziekenhuis (Figuur 1).



Figuur 1. Grafische weergave van de indicaties voor de verschillende vormen van ex situ machinale leverpreservatie in Nederland.

Naast *ex situ* machine perfusie wordt in Nederland in onderzoeksverband ook *in situ* machine perfusie toegepast bij DCD-donoren ouder dan 60 jaar. Deze vorm van machine perfusie wordt normotherme regionale perfusie (NRP) genoemd en valt buiten de context van dit protocol voor *ex situ* machine perfusie.

In dit protocol is beschreven welke vorm van machine perfusie geïndiceerd is voor welk type donorlever. Dit protocol is opgesteld door de drie levertransplantatiecentra op basis van het huidige wetenschappelijke bewijs. Omdat de ontwikkeling van de machinale orgaanpreservatietechnieken snel verloopt kunnen de indicaties voor machine perfusie de komen de jaren veranderen.

2. Hypotherme geoxygeneerde machine perfusie (HOPE of DHOPE)

Voor eind-ischemische DHOPE bestaan in Nederland de volgende indicaties:

- Alle donorlevers afkomstig van een DCD (*donation after circulatory death*) donor. Hierbij dient DHOPE gedurende tenminste 2 uur voorafgaand aan de transplantatie te worden uitgevoerd (1).
- Split lever procedures waarbij een postmortale lever wordt gebruikt voor twee ontvangers (meestal in twee verschillende ziekenhuizen) (2).
- Levers afkomstig van *extended criteria donor* (ECD) hersendode donoren (*donation after brain death* of DBD) (3). Hierbij gaat het om een kleine groep DBD levers met een verhoogd risico op door ischemie-reperfusie-schade geïnduceerde complicaties en/of vroeg transplantaatfalen, waarbij geen indicatie bestaat om de functie eerst te testen middels NMP. Te denken valt hierbij aan DBD levers met forse (>30%) steatose. Indien een dergelijke levers door alle drie centra is afgekeurd om medische redenen bestaat een indicatie voor NMP (zie hier onder).
- Voor “reguliere” levers afkomstig van DBD-donoren bestaat momenteel geen standaard indicatie voor (D)HOPE. De resultaten van de HOPE-trial welke eind 2021 worden verwacht zouden dit kunnen veranderen (4).

De taakverdeling en het werkprotocol voor het uitvoeren van DHOPE zijn beschreven in Appendix I en II.

3. Normotherme machine perfusie (DHOPE-COR-NMP)

Eind-ischemische NMP wordt in Nederland toegepast bij hoog-risico levers (van zowel DBD als DCD-donoren) welke om medische redenen door alle drie centra zijn afgezegd voor een reguliere transplantatie. Hieronder vallen onder andere levers van DCD-donoren ouder dan 60 jaar. Om het risico van ischemie-reperfusie schade tijdens de NMP-procedure te verminderen wordt NMP voorafgegaan door een korte periode van DHOPE en wordt de overgang van koude naar warme perfusie geleidelijk uitgevoerd middels *controlled oxygenated rewarming* (COR). Samen is dit bekend als het DHOPE-COR-NMP-protocol (5).

De taakverdeling en het Werkprotocol voor het uitvoeren van DHOPE-COR-NMP zijn beschreven in Appendix I en III.

4. Nader te bepalen indicaties

Naast de bovengenoemde bewezen zinvolle indicaties voor machine perfusie van donorlevers worden momenteel andere toepassingsvormen van ex situ machine perfusie onderzocht in studieverband.

Het gaat hierbij om de toepassingen met een logistiek en/of medisch voordeel:

- Verlengde DHOPE om nachtelijke transplantaties te voorkomen en de transplantatie tijdens dag-uren te kunnen uitvoeren (DHOPE-PRO trial in Groningen) (6).

- NMP om logistieke redenen, zoals een gelijktijdig lever- en pancreasaanbod, waarbij eerst de pancreastransplantatie zal worden uitgevoerd (lever NMP implementatie-onderzoek in Leiden) (7).

Literatuur

1. van Rijn, et al. Hypothermic machine perfusion in liver transplantation – A randomized trial. N Engl J Med 2021; 384:1391-1401.

2. Thorne, et al. Ex situ dual hypothermic oxygenated machine perfusion for human split liver transplantation. Transpl Direct 2021;7:e666.

3. Czigany, et al. Hypothermic oxygenated machine perfusion (HOPE) reduces early allograft injury and improves post-transplant outcomes in extended criteria donation (ECD) liver transplantation from donation after brain death (DBD): Results from a multicenter randomized controlled trial (HOPE ECD-DBD). Ann Surg 2021, doi: 10.1097/SLA.0000000000005110. (Online ahead of print)

4. Dutkowski, et al. HOPE-Trial. Clinicaltrials.gov NCT01317342 (www.clinicaltrials.gov).

5. van Leeuwen, et al. Transplantation of high-risk donor livers after ex situ resuscitation and assessment using combined hypo- and normothermic machine perfusion: A Prospective clinical trial. Ann Surg 2019; 270:906-914.

6. Brüggenwirth, et al. Prolonged dual hypothermic oxygenated machine preservation of human liver grafts: a feasibility and safety trial (DHOPE-PRO Trial). Netherlands Trial Register No. NL8740 (www.trialregister.nl/trial/8740).

7. Huurman, Alwayn, unpublished data (available upon request via: I.P.J.Alwayn@lumc.nl).

Addendum I – Algemene Logistiek Machinale Lever Perfusie Procedures

In dit addendum wordt beschreven wat het begin en einde van een lever machine perfusie procedure is. Daarbij wordt onderscheid gemaakt tussen hypotherme en normotherme machine perfusie. Hypotherme machine perfusies zijn procedures die relatief kort duren en nagenoeg altijd leiden tot een transplantabele lever. Normotherme procedures daarentegen leiden niet altijd tot een transplantatie, omdat de lever tijdens een dergelijke procedure kan worden afgekeurd voor transplantatie. Van elke procedure wordt door de orgaanperfusionist/transplantatie-coördinator (OPTC) een administratie bijgehouden en na afloop een verslag gemaakt.

Hypotherme geoxygeneerde machine perfusie (HOPE of DHOPE)

(D)HOPE wordt uitgevoerd bij geselecteerde donorlevers (zie hier boven) waarbij geen twijfel bestaat over de transplantabiliteit van het orgaan. Doel is het reduceren van complicaties (en bijkomende kosten) na levertransplantatie.

- Wanneer een donorlever, die in aanmerking komt voor (D)HOPE, is geaccepteerd voor transplantatie wordt de dienstdoende OPTC hiervan telefonisch op de hoogte gesteld. Hierbij worden relevante donorgegevens en de verwachte aankomsttijd van het orgaan besproken.
- De OPTC zorgt er voor dat het perfusieapparaat opgebouwd gereed is wanneer de lever in het transplantatiecentrum arriveert. Nadat de chirurg het orgaan heeft geïnspecteerd en voorbereid voor aansluiting op de perfusiemachine (de zogenaamde backtable procedure) worden de vaatcannules ingebracht. Met assistentie van de OPTC sluit de chirurg het orgaan aan op de perfusiemachine. De OPTC is verantwoordelijk voor het bedienen van de perfusiemachine en de monitoring van de verschillende perfusieparameters tijdens de gehele procedure. De OPTC controleert op vaste tijdstippen de oxygenatie van de perfusievloeistof middels bloedgasanalyses.
- (D)HOPE wordt verricht voor minimaal 2 uur. Definitief einde van de (D)HOPE procedure hangt af van de voortgang van de hepatectomie bij de ontvangende patiënt. Zolang de nieuwe lever nog niet geïmplanteerd kan worden bij de patiënt blijft deze op de perfusiemachine aangesloten.

- In uitzonderlijke gevallen (<5%) kan het zijn dat een lever tijdens (D)HOPE wordt afgekeurd voor transplantatie. Bijvoorbeeld wanneer er door ernstige leververvetting onvoldoende perfusie van de lever mogelijk is. In dat geval wordt de procedure beëindigd en de lever bij Eurotransplant afgemeld als niet transplantabel.
- Na het beëindigen van een succesvolle (D)HOPE procedure wordt de lever steriel vervoert naar de operatiekamer waar de transplantatie plaats vindt. De OPTC is verantwoordelijk voor het afbouwen van de perfusiemachine, het opruimen van de perfusieruimte en de administratie. Van elke procedure wordt door de OPTC een verslag gemaakt.

Normotherme machine perfusie (NMP)

NMP wordt uitgevoerd bij organen waarbij de transplantabiliteit onduidelijk is en waarbij eerst een functionele beoordeling dient plaats te vinden tijdens ex situ machine perfusie. Doel is het vergroten van het aantal donorlevers wat succesvol getransplanteerd kan worden.

- Zodra een lever, die in aanmerking komt voor NMP, beschikbaar komt wordt de dienstdoende OPTC hierover geïnformeerd door de dienstdoende chirurg. Net als bij (D)HOPE worden relevante donorgegevens en de verwachte aankomsttijd van het orgaan besproken.
- Wanneer het om een DCD donor (‘*donation after circulatory death’*) gaat wordt de OPTC op de hoogte gehouden van de relevante parameters tijdens de agonale fase van de donor. Indien een potentiële DCD donor binnen 2 uur na abstineren niet is overleden wordt de donatieprocedure beëindigd. Aan de OPTC wordt doorgegeven dat de NMP procedure niet doorgaat. Dit wordt door de OPTC vastgelegd.
- Wanneer de DCD procedure wel doorgang vindt wordt de OPTC geïnformeerd over de definitieve aankomsttijd van de lever in het transplantatiecentrum.
- De OPTC zorgt er voor dat het perfusieapparaat opgebouwd en gereed is wanneer de lever in het transplantatiecentrum arriveert. Nadat de chirurg het orgaan heeft geïnspecteerd en voorbereid voor aansluiting op de perfusiemachine (de zogenaamde backtable procedure) worden de vaatcannules ingebracht. Met assistentie van de OPTC sluit de chirurg het orgaan aan op de perfusiemachine. De OPTC is verantwoordelijk voor het bedienen van de perfusiemachine en de monitoring van de verschillende perfusieparameters tijdens de gehele procedure.
- Om ischemie-reperfusieschade tijdens de start van NMP te voorkomen wordt NMP voorafgegaan door een korte periode van DHOPE en wordt tussen DHOPE en NMP het orgaan gecontroleerd opgewarmd (‘*controlled oxygenated rewarming’* of COR).

Dit wordt het DHOPE-COR-NMP protocol genoemd. De DHOPE en COR fases duren ieder 1 uur. Het wisselen van perfusievloeistof tussen de DHOPE fase en de COR-NMP fase duurt gemiddeld 20 minuten en is de verantwoordelijkheid van de OPTC. De chirurg is verantwoordelijk voor het ontkoppelen en weer aansluiten van de lever bij het wisselen van de perfusievloeistof. Tijdens de gehele DHOPE-COR-NMP procedure is de OPTC verantwoordelijk voor de monitoring van de perfusie. Na 2,5 uur NMP wordt beoordeeld of het orgaan voldoet aan de vitaliteitscriteria (Tabel 2). Definitieve beoordeling van de transplantabiliteit van de lever is de verantwoordelijkheid van de chirurg aan de hand van de vitaliteitscriteria en perfusiekarakteristieken. Indien een lever niet aan deze criteria voldoet wordt de NMP procedure beëindigd. De lever wordt bij Eurotransplant afgemeld als “niet transplantabel”. De patiënt wordt geïnformeerd en zo mogelijk weer naar huis ontslagen.

- Indien een orgaan na 2,5 uur NMP wel als “transplantabel” wordt beoordeeld wordt de transplantatieprocedure bij de ontvangende patiënt opgestart. De donorlever blijft aangesloten op de perfusiemachine zolang deze nog niet bij de ontvanger kan worden geïmplanteerd. De OPTC blijft gedurende die tijd verantwoordelijk voor de monitoring van het orgaan op de perfusiemachine.
- Na het beëindigen van de NMP procedure wordt de lever steriel vervoert naar de operatiekamer waar de transplantatie plaats vindt.
- De OPTC is verantwoordelijk voor het afbouwen van de perfusiemachine, het opruimen van de perfusieruimte en de administratie. Van elke NMP procedure wordt door de OPTC een verslag gemaakt, waarbij wordt vermeld of de lever wel of niet is getransplanteerd.

Tabel 2. Hepatobiliaire vitaliteitscriteria 2,5 uur na start lever NMP

Hepatocellular	Cholangiocellular
Lactate clearance	Bile pH > 7.45
Perfusate pH stabilization	Delta pH
Bile production	Delta bicarbonate
	Delta glucose

Addendum II – Werkprotocol DHOPE

Het volgende werkprotocol wordt gebruikt in het UMC Groningen en dient als voorbeeld voor de andere centra. Ieder centrum zal zijn eigen werkprotocollen dienen te maken.

DHOPE Protocol

Dual Hypothermic Oxygenated Perfusion

Protocol for organ perfusionists

Important agreements

Since the DHOPE-DCD randomized controlled trial has been finished, all DCD livers that are accepted for regular transplantation will be perfused hypothermically. Potentially, this can also be performed for DBD livers if there is a clinical benefit (e.g. expected long hepatectomy in a retransplant patient).

Responsibilities

The surgeon is always responsible for handling the liver. The organ perfusionists are responsible for following the protocol and handling the machine. The liver team students are responsible for the biopsies and samples taken during the procedure.

Histology and experiment number

- All indication DHOPE livers that are not part of the trial will be part of experiment number: 349
- A histology number will be assigned to each liver
- Stickers and a log form will be located in a yellow envelope in the liver team room at the COL.

Photos

Photos taken during the procedure will be uploaded to the COL drive within 24h and deleted from mobile devices.

Inclusion/exclusion criteria and informed consent

As DHOPE is currently used as clinical care for patients, no informed consent is required. However, several exclusion criteria do apply:

- donor HIV positive
- donor HCV positive
(- donor HBV positive)

When a DCD liver is accepted for DHOPE

- The surgeon calls the on-call perfusionist about the planned procedure
- On-call perfusionists arranges a team.
- On-call perfusionist informs the on-call liver student and instructs them to bring the research cases, dry ice and liquid nitrogen. The perfusionists also provides an estimate of when the liver biopsies are taken.
- The surgeon calls or a WhatsApp group is made with further information about the ETA of the liver
- Arrive at OPR-unit approximately 45 minutes before the ETA of the liver

Preparations at the OPR-unit

- Go through the OPR-unit checklist for arrival
- Check to make sure that the blood gas analyzer is working
- Check to make sure that there are at least 8 bags of CarnaMedica PumpProtect in the fridge. Check the expiration date
- Check to make sure that there are at least 8 packs of ice in the freezer
- Call the scrub nurses for preparing the back table: 45837. Make sure that they prepare the backtable at least 15 minutes before ETA of the liver.
- Retrieve the liver scale from the 3rd floor
- Retrieve a disposable from the sterile storage unit
- Write down the date, donor number, histology number, experiment number and lot number of the disposable set on the log form.
- Collect ice from the ice machine in the OPR cool box and place it next to the Liver Assist

Installing the machine

- Plug in the power cord and earthing system of the Liver Assist.
- Wear sterile gloves and check all caps and connections of the disposable for loose connections.
- Tighten them, but not too tight
- Place the disposable in the machine in the correct manner
- Attach sensors
- Attach water bath tubing to the oxygenators in the correct manner. The red rotor should be at the bottom of the oxygenator.

Oxygenation

- Attach the oxygen and air supply to the gas mixer and make sure they are plugged into the wall
- Attach the gas mixer with tubing to the Y piece (provided with the disposable) to both oxygenators.
- Make sure everything is properly connected
- Check to make sure you hear and feel the gas flow

Oxygenation settings

FiO_2: 100%
Gas flow: 1L/min

Priming of the machine

- Wear unsterile gloves
- Check expiration date of the disposable
- Close the connections of the infusion systems
- Attach 3 bags of 1L CarnaMedica PumpProtect to the infusion pole. (Watch out, these bags are fragile)
- Attach the arterial and portal infusion system to the bags
- Fill the system with 3L CarnaMedica PumpProtect by opening the connections. De-air the de-airing chamber first.
- Guard the bags while the perfusion solution passively enters the system
- When bags are empty, close all connections and attach a new bag if required
- Otherwise, close all connections and leave empty bags attached for sterility and de-airing later on.
- During the whole procedure, count the amount of used CarnaMedica bags on the corresponding log form

- Start the pump units of the Liver Assist. First portal followed by arterial.
- Go through the menu

- When you arrive at the step *priming:*
    - Increase flow and remove air bubbles starting at the reservoir and following the stream
    - Use a syringe or the back of a clamp to tap out and remove air bubbles.
    - De-air the oxygenator and bubble trap by holding them upside down. Be careful not to disconnect the filling lines. Never tap the bubble trap to hard!
    - Use the infusion system to remove bubbles at the top of the oxygenator.
    - Use a syringe to remove air from the bubble trap, sample ports, and pressure lines
    - Check for air bubbles under the flow sensor

- Continue going through the menu
- When you arrive at the step *pressure zeroing*:
    - Check to make sure reservoir is placed correctly in the bowl
    - Remove the bottom cap of both of the pressure sensors
    - Turn transducer valves as mentioned on the screen op display by turning them vertical and 'stop' is facing downward.
    - Press enter to start zeroing
    - The measured pressures should be between -3 and +3. If out of range, check pressure lines again and repeat pressure zeroing by restarting the pump units.
- When the screen says *Turn transducer valve*:
    - Turn transducer valves back to a horizontal position
- Screw the white cap back on the pressure sensors
- Set the machine to the correct settings:

Portal pressure	**4 mm Hg**
Arterial pressure	**25 mm Hg**
Temperature	**<12 degrees Celsius (Cooling)**

- Make sure that the rotors of the water bath are moving in a fast pace. If not, squeeze tubing on one side to remove air.
- Add ice to the water bath
- If needed, remove water by opening the valve in the tubing and catching this in a white bucket
- This helps the cooling process and should be repeated throughout the procedure to keep temperatures low.
- Stop when the menu says *Press to start perfusion*

Last check and sampling for timepoint -1

- Wear unsterile gloves
- Remove dead volume in sample port with a syringe
- Take 18,5 mL of perfusate:
    - Store 10 mL in a 15 mL falcon tube on ice as **T-1**
    - Store 4 mL in a EDTA tube (pink)
    - Store 4,5 mL in a heparin tube (mint green)
- Take 1 mL of perfusate with a blood gas syringe, place cap, remover air, and analyze directly.
- Check O_2 level, to make sure the oxygen supply is sufficient: > 106 kPa
- Place a lab order using the MDN of the liver with the correct analysis (see table) and print stickers
- Add stickers to the EDTA and heparin tube and send to the clinical chemistry lab (with urgency) using pneumatic transport (buizenpost).
- *If pneumatic transport does not work, call the emergency courier to deliver it to the clinical chemistry lab (nr. 12345).*

During the procedure additional laboratory analysis are taken. These are currently mainly for research, but might prove usable for clinical application.

Medium: "*bijzonder vocht*"

Leukocyten	Natrium	Eiwit totaal
Leukocyten differentiatie	Kalium (potassium)	LDH
Hb	Chloride	ASAT
Ht	Kreatinine	ALAT
MCV	Ureum	Alkalische fosfatase
Trombocyten	Calcium	Gamma-GT
Fibrinogeen	Fosfaat	Bilirubine totaal
CRP	Magnesium	Bilirubine direct
Osmolariteit	Albumine	Triglyceride

During back table

- Write down timepoints on the log form
- Please remember to explain to the surgeons the option of performing an arterial reconstruction during portal vein-only HOPE, to reduce cold ischemic periods.
- Provide the portal and aortic cannulas to the back table
- Provide infusion system to the back table
- Make sure the liver team student is ready to accept the liver and bile duct biopsies.
- Make sure the scale is on a hard surface and turned on
- Note the weight on the log form
- Get 1 L CarnaMedica ready for the flush, by placing it in the cool box next to the machine

Flush

- Wear unsterile gloves
- Accept perfusion system with connections from the back table
- Close all connections
- Hang 1 bag of CarnaMedica on the infusion pole when the surgeon is ready for flush
- If necessary (judgment surgeon), a second liter of CarnaMedica can be used for flush. This depends on whether the flush out is clean. Please write down on the log form whether the flush out was either 'clean', 'mildly bloody' or 'severely bloody'.
- De-air the de-airing chamber by opening the connections
- De-air the infusion system in communication with the surgeon
- Note the time of start flush on the log form
- When the surgeon says yes, start flush. Don't put pressure on the bag during portal flush. Mild pressure can be used for the arterial flush
    - The surgeon collects the first 20 mL of flush with a sterile syringe. Store this in a 15 mL as **T0** on ice.
- Flush at least 1L with Carnamedica, but naturally more if the effluent is not clear yet after 1L. At least 0.75L through portal vein, and at least 0.25L through hepatic artery. The more the better!

Placing of the cannulas

The opening of the portal cannula to the right

Position of the liver

The gallbladder towards the front of the machine, dorsal side upwards.

Connecting the liver to the Liver Assist

- When the back-table procedure is finished and the machine has reached the desired temperature (8-9 °C), the liver is ready to be connected to the Liver Assist.
- In communication with the surgeon, start the portal perfusion. Start at 4 mmHg aiming for a flow around 100ml/min +/- 20%. Adjust accordingly limiting to 6 mmHg max. Focus more on flow aim instead of pressure.
- If flow is <100mL/min at 4mmHg, follow the following steps:

- Check the portal vein and cannula for air bubbles. Follow the portal vein until the bifurcation! If air is discovered, disconnect the cannula from the machine. Turn down the pressure to 2-3mmHg, and maintain an adequate flow for connecting the liver (150-300ml/min).
- Cut the net until the liver descends a little bit. Preferably cut the part of the net on the cranial side of the liver, as the flow will then go downwards.
- If there is still no flow, cut the entire net away and let the liver float.
- If there is still no flow, disconnect the portal cannula from the machine and shut down the portal device. The artery will continue to perfuse, and provide oxygen to the graft. Rapidly start up the portal device again, and turn up the priming speed. De-air only the pressure line again. Go to pressure zeroing, and ask the surgeon to keep the portal vein cannula in the reservoir completely horizontal. Then reconnect.
- If this does not work, call Robert, or Vincent for troubleshooting or cancel the procedure.

- In communication with the surgeon, start the arterial perfusion. Communicate the flows and pressures to the surgeon.
- Take a picture of the liver without the lid

Throughout the procedure we aim for a portal flow of +/- 150 mL/min and 1/3 (+/- 50 mL/min) of the portal flow as arterial flow. Always keep the pressure as low as possible to prevent endothelial injury. The lower the pressures, the better.

Toolkit:
The surgeon packs the toolkit in kidney organ bags

- 1st bag: Toolkit with UW-CS solution
- 2nd bag: NaCl 0,9%
- 3rd bag: no fluids

Place toolkit in the original Styrofoam organ box on ice.

During the perfusion procedure

- Cover back table to keep sterile
- One perfusionist must always be with the liver.
- Make sure that a sterile bowl with CarnaMedica is ready in case the machine fails.
- Have a bag of sterile ice ready in the OPR.
- Write down the perfusion parameters accordingly on the log form
- Collect perfusate samples according to the log form.
- Collects samples for clinical chemistry analysis at the given time points as mentioned above in the protocol. Discuss further with clinical chemists – price, relevance etc
- Copy the donor forms and place the original back in the Styrofoam box of the liver.
- Check cool rotors, temperature, ad ice if needed, and check oxygen flow.

Towards the end of the perfusion procedure

In general, the procedure is two hours. However, this depends on the surgery. Communicate with the surgeons in OR 19 about the exact timeline.

Before ending the perfusion procedure

- Cool down the centrifuge to 4 degrees Celsius
- Make sure the liver team student is ready to accept the liver and bile duct biopsy.
- Make sure the scale is on a hard surface and turned on
- Take last perfusate samples and note the perfusion parameters
- Make sure a sterile bowl with ice and Carnamedica is ready. This will be done by the surgeon (in a sterile setting).

- Make sure a second empty bowl is ready for weighing the liver, and that the scale is covered with a sterile sheet
- Put some extra ice in the machine. The cooler the liver is prior to disconnecting, the less ischemia.

Ending DHOPE

- Take a picture of the liver without the lid
- Ask the surgeon to take the biopsies
- The surgeon will disconnect the cannulas and at the same time the machine must be stopped
- Note these time points on the log form
- The surgeon will weigh the liver. Note the weight on the log form
- Discus whether the surgeon wants to bag the liver or transport the liver in the bowl.

For transportation in the bowl:

- Make sure the cart is covered with a sterile sheet
- Place the Styrofoam box with the original donor forms, toolkit, and the cultivation (kweek) on the bottom of the cart.
- The surgeon will place the bowl on the cart and cover the bowl
- Assist the surgeon to OR 19

For transportation in organ bag and Styrofoam box:

The surgeon packs the liver in liver organ bags together with a second sterile person:

- 1st bag: Liver with Carnamedica solution
- 2nd bag: ice cold NaCl 0,9%
- 3rd bag: no fluids
- Place liver in the original Styrofoam organ box on ice. Make sure the toolkit, donor forms and the cultivation is also in the box.
- Assist the surgeon to OR 19

Cleaning of the OPR

- Place perfusate samples in the centrifuge and centrifuge at:
  - 4 degrees Celsius
  - 3220 RCF
  - 15 minutes
- Place log form and copy of the donor forms back in the yellow envelope.
- Count the gauzes with two people
- Discard everything from the back-table except surgical tools and white tube/probe (sonde) in the blue basket!!
- DO NOT discard the disposable after DHOPE. It will be used for animal experiments, contact one of the researchers or store it at the COL.
- Empty water bath and refill with demi water
- Mop the floor
- Disinfect all surfaces including the machine
- Call cleaning crew: 44903
- Call OR assistants for collecting the surgical tools: 45837
- Write the OR report in EPIC
- Fill in the OPR checklist
- Place perfusate samples back on ice and hand them over to the liver student or place in the freezer

Addendum III – Werkprotocol DHOPE-COR-NMP

Het volgende werkprotocol wordt gebruikt in het UMC Groningen en dient als voorbeeld voor de andere centra. Ieder centrum zal zijn eigen werkprotocollen dienen te maken.

DHOPE-COR-NMP

Protocol

Important agreements

Responsibilities
The surgeon is always responsible for handling the liver. The organ perfusionists are responsible for the perfusion procedure and handling the machine. The researcher (or in case there is no researcher present, the responsible organ perfusionist) are responsible for the biopsies and samples taken during the NMP procedure.

Histology and experiment number
- All livers are part of experiment number: 347
- A histology number will be assigned to each liver.
- New stickers and a log form are located in the OPR binder located in the OPR.

Photos
Photos taken during the procedure will be uploaded to the COL drive within 24h and deleted from mobile devices.

In- and exclusion criteria

This is checked by the organ perfusionist in consultation with the on call HPB surgeon

Inclusion criteria

Organ recipient
- Adult (≥ 18 years old)
- Verbal informed consent provided (check waiting list email from secretariaat OLT)

Donor grafts
- Donors with a bodyweight of ≥40 kg
- Donors within the Netherlands → if not the case discuss these cases with Robert or Vincent

Exclusion criteria

Organ recipient
- Participation in alternative studies that can influence this study
- Cognitive disabilities which make it impossible for the recipient to understand the study.
- On the waiting list due to liver failure or primary non function (previous transplant or retransplant) → discuss these cases with Robert or Vincent
- Recipient tested positive for HIV → discuss these cases with Robert or Vincent

Donor grafts

- Donor positive for HIV, Hepatitis B or C → "it is safe to perfuse these organs using the Liver Assist. The water bath (and the rest of the machine) must be cleaned thoroughly afterwards" → discuss these cases with Robert or Vincent
- Split of partial liver grafts
- Domino donor livers

Accepting an ECD liver

Preliminary acceptance

Tasks of the surgeon
- Checks inclusion and exclusion criteria
- Discusses patients with the hepatologist.
- Informs scrub nurses about the NMP procedure (45837)
    - 2 scrub nurses are present during the back table procedure.
- Informs the organ perfusionists
- Orders RBC's

Organ perfusionist:
- In charge of gathering a team. Everyone contacts the OP on call for joining the procedure.
- Informs/assigns a researcher
- Notifies the NMP WhatsApp group.
- Is contacted by the transplant coordinator or surgeon over the NMP offer.
- Checks:
    - Name patient (recipient) and MDN-number patient
    - Donor EuroTransplant (ET) -number
    - Start time donor procedure
    - Who is the on call HPB surgeon
    - Checks to make sure the RBCs are ordered (3 packets) on the patient file of the organ not the recipient! (Always O neg, unless donor is O pos, then O pos)
    - Asks the transplant coordinator to inform the donor transplant coordinator about the retrieval according to perfusion standards and about the liver whatsapp group.
    - Checks inclusion and exclusion criteria in EPIC and in the donor data

<u>Graft explantation criteria</u>
- Liver: explantation with segment of 5 cm supratruncal cilinder/circular aorta instead of Carrel patch of celiac trunk
- Cystic duct: ligation, no cholecystectomy
- Extrahepatic bile duct: flushing with UW & preservation of long trajectory
- Portal vein: flushing with minimally 2L UW & preservation of long trajectory
- Very rapid hepatectomy

The hepatologist (MDL-arts):
Responsible for contact with potential recipient.

Definite acceptance of the liver

When the macroscopy is approved:
- The transplant coordinator calls the OP with the results and the ETA or the OP can extract this information from the whatsapp group.
- The surgeon calls the scrub nurses

Organ perfusionist:
- Schedules 1 hour for preparations at the OPR. (1,5 hours when also going to the COL).

- Check to make sure the RBCs are ordered
- Make a note in EPD with the blood group of the liver donor
- Check to make sure that there is enough CarnaMedica (8-10 bags) and a goodiebag available.
- Check to make sure that the scrub nurses are up to date and that they prepare the back table approximately 15 minutes before the ETA of the liver.

Gathering research necessities from the lab

The following items are necessary for taking samples during the perfusion procedure. Collect them all from the lab and place them on the liver team cart in communication with the liver team student.

1. LIVERTEAM-suitcase
2. NMP-suitcase (filled)
3. Pocket balance
4. Dry ice ("kostenplaats" 75600)
5. 2 containers of liquid nitrogen

Preparations at the OPR-unit

Organ perfusionist:

- Go through the OPR-unit checklist for arrival
- Check to make sure that the blood gas analyser is working
- Check to make sure that the following items are in the OPR fridge/freezer and check the expiration date:
  - 8 bags of CarnaMedica PumpProtect
  - Albumin
  - Goodiebag (medication)
  - 8 packs of ice (Frozen saline) → take 2 out of the fridge immediately.
  - 8 bags of UW – cold storage
  - Cold NaCl 0.9%, minimal 3L
- Call the scrub nurse for preparing the back table: 45837. Make sure that the backtable is prepared at least 15 minutes before ETA of the liver.
- Retrieve the liver scale from the 3rd floor
- Retrieve a Liver Assist disposable set from the sterile storage unit
- Retrieve 3 L of sterile water and 0,5 L Gelofusine from the storage unit
- Retrieve 1 45 cm extension tube from the storage unit and connect it to the portal side for medication supplementation
- Retrieve 5 vacuum disposable pots
- Write down the date, donor number, histology number, experiment number and lot number of the disposable set on the log form.
- Collect ice from the ice machine in the OPR cool box and place it next to the Liver Assist
- All medication will be added via the portal side.

Installing the machine

- Plug in the power cord and earthing system of the Liver Assist.
- Wear sterile gloves and check all caps and connections of the disposable for loose connections. Tighten them, but not too tight.
- Add an extra line (extension tube of 4-5 cm) and three-way valve to the portal side for administration of medication with sterile gloves.
- Place the disposable in the machine in the correct manner
- Attach sensors

- Attach water bath tubing to the oxygenators in the correct manner. The red rotor should be at the bottom of the oxygenator.

Oxygenation

- Attach the oxygen and air supply to the gas mixer and make sure they are plugged into the wall
- Attach the gas mixer with tubing to the Y piece (provided with the disposable) to both oxygenators.
- Make sure everything is properly connected
- Check to make sure you hear and feel the gas flow

Oxygenation settings

DHOPE
FiO_2: 100%
Gas flow: 1L/min

Priming of the machine for DHOPE

- Wear unsterile gloves
- Check all caps and connections of the disposable again (before filling the circuit)
- Close the connections of the infusion systems
- Note log number of CarnaMedica PumpProtect and check the expiration date
- Attach 3 bags of 1L CarnaMedica PumpProtect to the infusion pole. (Watch out, these bags are fragile)
- Attach the arterial and portal infusion system to the bags
- Fill the system with 3L CarnaMedica PumpProtect by opening the connections of the filling lines. De-air the de-airing chamber in the filling lines first.
- Guard the bags while the perfusion solution passively enters the system
- When bags are empty, close all connections and attach a new bag if required
- Otherwise, close all connections and leave empty bags attached for sterility and de-airing later on.

- Start the pump units of the Liver Assist. First portal followed by arterial.
- Go through the menu
- When you arrive at the step *priming:*
  - Increase flow (900 ml/min max) and remove air bubbles starting at the reservoir and following the stream
  - Use a syringe or the back of a clamp to tap out and remove air bubbles.
  - De-air the oxygenator and bubble trap by holding them upside down. Be careful not to disconnect the filling lines. Never tap the bubble trap to hard!
  - Use a syringe to remove air from the bubble trap and sample ports.
  - De-air the pressure lines using a syringe and check if there are no air bubbles in the pressure lines and sensors.
  - Check for air bubbles under the flow sensor

- Continue going through the menu
- When you arrive at the step *pressure zeroing*:
  - Check to make sure reservoir is placed correctly in the bowl
  - Turn transducer valves as mentioned on the screen op display by turning them vertical and ‘stop’ is facing downward.
  - Remove the side cap of both of the pressure sensors
  - Press ‘OK’ to start zeroing

    - The measured pressures should be between -3 and +3. If out of range, check pressure lines, pull the blue strap once again and repeat pressure zeroing by restarting the pump units.
- When the screen says *Turn transducer valve*:
    - Screw the white cap back on the pressure sensors
    - Turn transducer valves back to a horizontal position, pointing towards the side cap
- Set the machine to the correct settings:

DHOPE	
Portal pressure	**≤4 mm Hg**
Arterial pressure	**≤25 mm Hg**
Temperature	**<12 °C (Cooling) → strive during HMP: 10-12°C**

- Make sure that the rotors of the water bath are moving in a fast pace. If not, squeeze tubing on one side to remove air.
- Add ice to the water bath
- If needed, remove water by opening the valve in the tubing and catching this in a white bucket
- This helps the cooling process and should be repeated throughout the procedure to keep temperatures low.
- Stop when the menu says *Press to start perfusion*

Last check and sampling for timepoint -1

- Wear unsterile gloves
- Remove dead volume in sample port with a syringe
- Take 10 mL of perfusate:
- Take 1 mL of perfusate with a blood gas syringe, place cap, remover air, and analyse directly.
- Check O_2 level, to make sure the oxygen supply is sufficient: > 106 kPa

During back table before DHOPE

Researcher/ liver team student (under supervision of the OP):

- Write down time points on the log form and white board
- Note the amount of used gauzes on the white board
- Copy the donor form and place the original back into the Styrofoam box,
- Code the filters, cassettes and eppendorfs
- Weigh the bile eppendorfs (MO- eppendorfs)
- Prepare everything for the biopsies on a fibre mat:
    - Forceps
    - Knife
    - Petri dish
    - Container with liquid nitrogen
    - Jar of formalin
    - Gloves
    - The coded filters and cassettes
- You will receive a sample of the preservation solution. Store this in the Styrofoam box of the liver
- Take liver biopsies and store these accordingly

Organ perfusionist:

- Please remember to explain to the surgeons the option of performing an arterial reconstruction during portal vein-only HOPE, to reduce cold ischemic periods.
- Provide the portal and aortic cannulas to the back table

- Provide infusion system to the back table
- Make sure the researcher is ready to accept the liver and bile duct biopsies.
- Make sure the scale is on a hard surface and turned on
- Note the weight on the log form. (The weight will be used for calculating the desired flow of 110% of the weight during NMP).
- Get **2** L CarnaMedica ready for the flush, by placing it in the cool box next to the machine.
- Mark all the used CarnaMedica bags on the corresponding log form (during the whole procedure)
- Get R studio ready if there is extra time. Create a new CSV and PDF.

Flush

- Wear unsterile gloves
- Accept perfusion system with connections from the back table
- Close all connections
- Hang 1 bag of CarnaMedica on the infusion pole when the surgeon is ready for flush
- If necessary (judgment of the surgeon), additional CarnaMedica can be used for flush. This depends on whether the flush out is clean. Please note on the log form whether the flush out was either 'clean', 'mildly bloody' or 'severely bloody'. There is no maximum flush, flush until the flush out is clear.
- De-air the de-airing chamber by opening the connections and tilting the de-airing chamber.
- De-air the infusion system in communication with the surgeon
- When the surgeon is ready, start flush. Don't put pressure on the bag during portal flush. Mild pressure can be used for the arterial flush
    - Ask the surgeon to collect the first 20 mL of flush with a sterile syringe. Use this for a bloodsmear and for collecting possible thrombi in the Tissue-Tek biopsy bag (see sample appendix).
- Note the time of start flush on the log form
- Flush at least 0,75 L through the portal vein, but please flush as much as possible until effluent is clear
- Flush at least 0,25 L through the hepatic artery
- **NOTE**: Flushing after SCS: 1 L (minimal) with CarnaMedica Flushing between DHOPE/COR: 2L NaCl 0.9%. Flushing after NMP: 2L UW. Ratios are the same for all flushing moments.

Placing of the cannulas
The opening of the portal cannula to the right

Position of the liver
The gallbladder towards the front of the machine, dorsal side upwards.

Phase 1: DHOPE

- When the back-table procedure is finished and the machine has reached the desired temperature (7-9 °C), the liver is ready to be connected to the Liver Assist.
- In communication with the surgeon, start the portal perfusion. Start at 4 mmHg aiming for a flow around 150ml/min +/- 20%. Adjust accordingly limiting to 5 mmHg max, temporarily to 6 mmHg. Focus more on flow aim instead of pressure.
- If flow is <100mL/min at 4mmHg, follow the following steps:
    - Check the portal vein and cannula for air bubbles. Follow the portal vein until the bifurcation! If air is discovered, disconnect the cannula from the machine. Turn down the pressure to 2-3mmHg, and maintain an adequate flow for connecting the liver (150-300ml/min).
    - Cut the net until the liver descends a little bit. Preferably cut the part of the net on the cranial side of the liver, as the flow will then go downwards.
    - If there is still no flow, cut the entire net away and let the liver float.
    - If there is still no flow, disconnect the portal cannula from the machine and shut down the portal device. The artery will continue to perfuse, and provide oxygen to the graft. Rapidly start up the portal device again, and turn up the priming speed. De-air only the pressure line again. Go to pressure zeroing, and ask the surgeon to keep the portal vein cannula in the reservoir completely horizontal. Then reconnect.
    - If this does not work, call Robert, Vincent or a collegue for troubleshooting or cancel the procedure.
- In communication with the surgeon, start the arterial perfusion. Communicate the flows and pressures to the surgeon.
- Take a picture of the liver without the lid

Throughout DHOPE we aim for a portal flow of +- 150-200mL/min and 1/3 (+- 50 mL/min) of the portal flow as arterial flow. Always keep the pressure as low as possible to prevent endothelial injury. The lower the pressures, the better.

<u>Toolkit:</u>
The surgeon packs the toolkit in kidney organ bags

- 1st bag: Toolkit with UW-CS solution
- 2nd bag: NaCl 0,9%
- 3rd bag: no fluids

Place toolkit in the original Styrofoam organ box on ice.

During DHOPE

- Cover back table to keep sterile
- One perfusionist must always be with the liver
- Make sure that a sterile bowl with UW CS is ready in case the machine fails
- Write down the perfusion parameters accordingly on the log form
- Collect perfusate samples according to the log form. Take 10 mL of perfusate and store this in a 9 mL citrate tube on ice
- Check cool rotors, temperature, ad ice if needed, and check oxygen flow

Prepare for NMP

- Collect RBC's from the fridge
- Collect 1 bag of albumin, 1 goodiebag and 1 bag of gelofusine
- Get 3 bottles of sterile water ready
- Get 2 bags of cold NaCl 0.9% ready in cool box for flush
- Have a bag of sterile ice ready in the OPR.
- Get 2 big vacuum bags and hoses and from the storage unit
- Retrieve 20mL sodium bicarbonate 8.4% in a sterile syringe
- Retrieve 10mL (1 flacon) calcium gluconate in a sterile syringe
- Retrieve 10 mL of NaCl 5% in a sterile syringe
- Assemble the vacuum system
- Get the bile drain (8Fr) ready
- Get the 75 cm cava drain with a three way valve ready. Make sure the **LL male** side is cut off the extension tube and the 3-way valve is connected to the **LL female** side
- Get another bucket of water for the flow sensors ready
- Place RBCs outside of the fridge so that they can adjust to room temperature.

Stop DHOPE, and switch to COR-NMP

The surgeon will disconnect the liver from the Liver Assist and place it in ice cold UW CS or CarnaMedica with sterile ice.

- Stop the pumps, but **do not switch off** the machine
- Place flow sensors in water buckets
- Change the machine settings:

Switch:
Temperature: 20°C
FiO_2: 21%
Air flow: op 200mL/min

The surgeon will empty the circuit in a sterile manner with the vacuum pump and will eventually connect the tubes to the portal and arterial cannulas.

- Turn down the vacuum pump to its lowest setting. This is for better preservation of the oxygenators
- Continue to empty the circuit while the surgeon focuses on the back table
- When all the tubing is empty, add 3L of sterile water into the reservoir in a sterile manner
- Disconnect the pump heads and hold them up so the that the circuit empties completely
- Empty the vacuum disposables when full

The surgeon will cannulate the bile duct with a 8Fr probe. Make sure the tip is cut off. A flush of the bile duct might be desired. The probe will be attached with stitches. Furthermore the vena cava will be cannulated with a 14 cm probe, a three way valve and an extra 150 cm probe. Make sure the **LL male** side is cut off the extension tube and the 3-way valve is connected to the **LL female** side

Refilling the system

- When the whole system is empty, refill as followed:

<u>Portal side</u>

1. Goodiebag
2. ½ pack of Gelofusine

<u>Arterial side</u>

1. Albumin
2. ½ pack of Gelofusine

- Add to the reservoir:
  - 3x Packets RBCs with bag a jets
  - 20 mL sodium bicarbonate 8,4%
  - 10 mL Calcium gluconate

For a faster switch of perfusate it can also be desired to bag a jet the Goodiebag, Albumin, Gelofusine, and all packets of RBCs and not use the infusion lines at all .

- Ask the surgeon to place the lid back on the reservoir
- Turn off the machine and turn it back on. Place flow sensors back on the appropriate tubing as soon as all tubes are filled with perfusate and only 'smaller' bubbles are left in the circuit
- De-air the system again as mentioned before. Don't use flows that are too high as it will generate more bubbles in the circuit. Fill pressure and sample lines with perfusate. Zero the pressure sensors again. Make sure to remove the cap and turn the valve. Set the machine settings to:

Start COR	
Portal pressure	**5 mm Hg**
Arterial pressure	**25 mm Hg**
Temperature	**20°C**

Last check and sampling for time point T-baseline

- Wear unsterile gloves
- Remove dead volume in sample port with a syringe
- Take 10 mL of perfusate and store this in a 9 mL citrate tube on room temperature as **T-Baseline**
- Take 1 mL of perfusate with a blood gas syringe, place cap, remover air, and analyse directly.
- Check blood gas values before proceeding with the back table flush
- As the back table flush often takes 5-10 minutes, take another blood gas directly before connection of the liver, with the sole intention of checking the CO2/pH.

Back table flush

- Wear unsterile gloves
- Close all connections
- Remove CarnaMedica bags from infusion system and attach the bags of NaCl 0,9% that were previously stored in the cool box
- De-air the de-airing chamber by opening the connections
- De-air the infusion system in communication with the surgeon
- When the surgeon says yes, start flush. Don't put pressure on the bag during portal flush. Mild pressure can be used for the arterial flush.
- Note the time of start flush on the log form
- Use 1,5 L through the portal vein
- Use 0,5 L trough the artery
- ***NOTE**: Flushing after SCS: 1 L (minimally) with UW. Flushing between DHOPE/COR: 2L NaCl 0.9%. Flushing after NMP: 2L UW. Ratios are the same for all flushing moments.*

Phase 2: COR

- Place a new sterile sheet on the Liver assist
- In communication with the surgeon, start the portal perfusion. **The desired flow is about 300 ml/min**
- In communication with the surgeon, start the arterial perfusion. Communicate the flows and pressures to the surgeon. (Desired flow arterial, 1/3 of portal flow?, although the arterial flow usually takes a bit longer to rise in comparison to the portal venous)
- Take a picture of the liver without the lid
- Cover back table to keep sterile
- Use the COR log form to increase temperature and pressures and observe the desired flow throughout the hour.
- Prepare syringes for all the relevant medications for NMP, and **sticker the syringes immediately. Double check medication before administering**

Temperature	**Gradual rewarm to minimize ischemia/reperfusion injury**
Pressure and flow	Pressures are increased with an increase in compliance of the vascular bed of the liver. Increase pressure after the increase in temperature. If the flow does not lag behind, do not increase pressure. See desired flows on the COR log form (**MAXIMUM 110% of the liver weight, preferably around 90-100%**). Make sure that the flows and thus pressures are not too high. The flows are leading, not the pressures.
Internal environment, supplementation and oxygenation	During COR and NMP we will correct the values of electrolytes and pH by means of supplementation and gas flow. During COR, an imbalance between the acid/base environment will occur towards the end of rewarming. It is important to keep the pH low during the COR, as low pH protects against IRI. Be very careful with ventilation as the liver does not produce a lot of CO_2 yet so everything you hyperventilate, will not come back. Keep your base excess between 0 and -13, and administer NaBic 8,4% if it drops below -13.

Phase 3: NMP

- Always have 2 people with the machine in case it fails
- Always have sterile ice and cold UW-CS ready in case the machine fails
- Make sure that a sterile bowl with CarnaMedica or UW-CS is ready in case the machine fails.
- Get a pair of sterile gloves ready in case the machine fails
- Write down the perfusion parameters accordingly on the log form and in EPD
- Collect perfusate samples according to the log form.
- Take 10 mL of perfusate and store this in a 9 mL citrate tube on room temperature.
- Collect bile samples for analyses and blood gas analyses and note everything on the bile log form.
- Note all blood gas values in R Studio
- Check temperature, and check oxygen flow
- Collect samples for clinical chemistry as followed:
    - Store 4 mL in a EDTA tube (pink)
    - Store 4,5 mL in a heparin tube (mint green)
    - Store 4 mL in a citrate tube (blue)
    - Place a lab order using the MDN of the liver with the correct analysis (see table; orderset 'OPR Lever Lab' plus 'IPF' minus ATPP and PT) and print stickers
    - Add stickers to the EDTA and heparin tube and send to the clinical chemistry lab (with urgency) using pneumatic transport (buizenpost).
    - *If pneumatic transport does not work, call the emergency courier to deliver it to the clinical chemistry lab (nr. 12345).*

Leukocyten	**Natrium**	**Eiwit totaal**	**IPF**
Machine differentiatie	Kalium (potassium)	LDH	Osmolariteit
Hb	Chloride	ASAT	Albumine
Ht	Kreatinine	ALAT	Triglyceride
MCV	Ureum	Alkalische fosfatase	CRP
Trombocyten	Calcium	Gamma-GT	Magnesium
Fibrinogeen	Fosfaat	Bilirubine totaal	Bilirubine direct

Oxygenation	**Based on venous pO_2 and arterial pCO_2**
Internal environment	During NMP we will correct the values of electrolytes and pH by means of supplementation and gas flow.
Supplementation	Administer all medication via the portal sample line. Be aware! There is a high pressure on this line so be careful. Use a luer lock syringe. o Add 0,2 mL (1000 units) of heparin every hour of NMP (see log form). o Note all administrated medication on the log form.

Total volume of perfusate: 2200 mL, volume used for calculation: 2000 mL					Medication: Storage unit near OR 19	
Medium			**Normal-values**	**Clinical relevance**	**Medication**	**Notes**
Medication	**Anti-coagulation**	**Heparin**		Start NMP: 0,2 mL (1000 units). Then every hour: 0,2 mL.	5000 IE/ml	
Arterial perfusate	**Metabolites**	**Lactate (cLac)**	<1,8 mmol/L after 150 min of NMP	Develops during anaerobe metabolism. A functioning liver clears lactate. Rising lactate levels correlate with liver dysfunction.		
		Glucose (cGluc)	>4,0 mmol/L	Decreases when a liver functions well due to metabolism. When the decrease is mild, the metabolism could be weak. Values can rise due to cellular leakage caused by IRI. Not the most important value to monitor.	Glucose 50% 2,775 mmol/mL	Strive 8-12mmol/L glucose. Use only 2-4 mL per correction.
	Electrolytes	**Sodium (cNa^+)**	135–145 mmol/L	Extracellular electrolyte Rises with a good metabolism due to the sodium/potassium channels. (Na out of the cell, K into the cell)	NaCl 5% 0,86 mmol/mL	Only administer if no NaBic is given, to increase sodium to 135mmol/L. Do not correct before T10.
		Potassium (cK^+)	3,5-5,0 mmol/L	Intracellular electrolyte. electrolyte Decreases with a good metabolism due to the sodium/potassium channels. (Na out of the cell, K into the cell)	KCl 74,6 mg/ml 1 mmol/mL	Only correct for hypokalemia. Do not over correct!
		Chloride (cCl^-)	>90 mmol/L	The Cystic fibrosis transmembrane conductance regulator (CFTR) channel facilitates transmembral chloride flow toward the bile. Anion exchange protein 2 (AE2) is a membrane transport protein that exchanges Cl^- for HCO_3^- in the bile. Alkali bile is dependent on Cl^- values.	NaCl 5% 0,86 mmol/mL	
		Calcium (cCa^{2+})	≥0,70 mmol/L	Ca^{2+} is an important cofactor for bile production.	Calcium-gluconate 0,225 mmol/mL	Add 10 mL at the start of COR, afterwards usually another 10 mL when the liver is warm. Add per 5 mL.
	Blood gas	**pH**	COR: <7.30 NMP: 7,35–7,45	Twee factors influence the pH: 1) Metabolism: more acidic during anaerobe metabolism due to lactate levels. At the start of NMP there is a pH drop due to lactate. There could be a shortage of HCO_3^-. A mild acidic environment could be protective against IRI. 2) Gas exchange: CO_2 is acidic, a higher airflow results in a higher clearance of CO_2.	$NaHCO_3$ 8.4% 1 mmol/ml	
		pCO_2	4.5-6.0 kPa	An acidic product of metabolic activity and a substrate of $HCO3^-$. You can get rid of it by increasing the gas flow.	↓gas flow = ↓pH ↑gas flow =↑pH	Be really careful with your airflow during COR, since there is almost no CO2 production. Only turn up the gasflow if the liver starts producing CO2. Maintain a stable pCO2 during the perfusion, adjust when necessary.
		pO_2	During COR & NMP: 10–13 kPa	Needed for aerobic metabolism. Hypoxia is toxic due to reactive oxygen species.	Gas flow (ml/min) FiO_2 (%)	Do not let the go below 10kPa. Increase FiO_2 with 1 to 2 %.
		sO2	≥ 90%	Percentage with O2 saturated haemoglobin	Gas flow (ml/min) FiO_2 (%)	No indication. Focus on venous fiO_2 and kPO_2
		Bicarbonate ($cHCO_3^-$(p)c)	>20 mmol/L	An alkaline substrate for correction of the pH. Physiological increase during NMP.	$NaHCO_3$ 8.4% 1 mmol/ml	The liver will produce HCO3 while clearing lactate. Therefore, do not over correct during COR.
Medium			**Normal-values**	**Clinical relevance**	**Medication**	**Notes**

Arterial perfusate	**Blood gas**	**Arterial Base excess (AABEc)**	+3.0 to -3.0	Metabolic component of the acid-base imbalance Not correctable with gas flow. If there is a drastic drop during the first 150 min of nmp, bad sign for the viability.	$NaHCO_3$ 8.4% 1 mmol/ml	Think about administrating 10 mL $NaHCO_3$ when ABEc <-13 during COR
Venous perfusate	**Bloedgas**	**fiO_2**	≥ 55%	Percentage with O_2 saturated haemoglobin.	Gas flow (ml/min) FiO2 (%)	Focus on the venous fiO2 en kPO2. Make sure the arterial kPO2 stays between 10-13 kPa
Bile	**Macroscopic**	**Production**		**<u>Macroscopy is less important than biochemistry.</u>** Metabolic activity. Dependent of arterial chloride and pH. Sticky and stringy bile is a sign of bile duct viability.		See arterial corrections pH and chloride
		Viscosity	Sticky			
	Metabolites	**Glucose**	*n.v.t.*	Glucose is reabsorbed in the bile duct. When levels are higher than 30, it's not reabsorbable. High glucose levels mean moderate bile duct viability.		Absolute value is irrelevant. See Δ and ratio Glucose
	Blood gas	**pH**	~7,45 See Δ pH	Alkali bile protects the bile duct against bile stones. pH is a sensitive marker for biliary tract viability. A lower bile pH means moderate bile duct viability. pH is dependent on both perfusate chloride and **HCO_3^-**.		See arterial corrections pH and chloride
		Bicarbonate (HCO_3^-)	~ ≥ 18 mmol/L Zie Δ HCO3-	See pH.	See arterial pH and chloride	Absolute values are irrelevant. See Δ HCO3-
	Oxymetrics	**Bilirubin**	*n.v.t.*	Bile pigment. When absent, there is no bile duct? production, or the bile duct cannula is blocked.		
	calculations	**Δ Glucose**	< -5	Measure for reabsorbed glucose, a bigger delta means more bil educt viability.		Gal minus perfusate
		Ratio glucose	<0.8			Gal/perfusate
		Δ pH	>0.1	Measure AE2-pump activity. Bile duct viability when there is an alkaline environment.	See arterial pH and chloride	Gal minus perfusate
		Δ HCO_3^-	>5	Measure AE2-pump activity. Bile duct viability when there is an alkaline environment. Says more than ΔpH.	See arterial pH and chloride	Gal minus perfusate

Phase 4: Assessment of the liver

After 2,5 hours of NMP, the liver and bile duct function is assessed as followed. Always compare the values of the liver to the graphs in R studio.

Liver function	
o **Bile production**	**≥ 10 grams, of which ≥ 4 gram in the last hour**
o **Lactate**	**<2.0 mmol/L**
o **pH of arterial perfusate**	**7.35 – 7.45**
Bile duct function	
o **Bile pH**	>7.45
Other indications (Not scientifically validated yet, not to be used for definite assessment, follow colour scheme)	
o **Δ Glucose (bile – perfusate)**	< -5
o **Ratio Glucose (bile/perfusate)**	<0.8
o **Δ pH (bile – perfusate)**	>0.1
o **Δ HCO^-_3 (bile – perfusate)**	>5

When the liver is approved for transplantation:

- Continue protocol and NMP
- Especially now it is important to have two people with the liver at all times
- Check if the emergency sterile ice is still frozen, otherwise replace and put the defrosted ice back into the freezer, have UW-CS 2L ready in the OPR.
- Get a pair of sterile gloves ready
- Communicate NMP time with the surgeon. This depends on the hepatectomy time.

When the liver is declined for transplantation:

- The surgeon will call the transplant coordinator. The TC will inform EuroTransplant.
- When EuroTransplant declines the liver for transplantation, check with all HPB-researchers for potential experiments, if none: call on-call slice lab phone: +31 6 45185434
- Collect biopsies in the same manner as before DHOPE while liver is still on the pump
- Flush liver with cold UW-CS after stop perfusion

Phase 5: Stop perfusion

Before ending the perfusion procedure

- Make sure the researcher is ready to accept the liver and bile duct biopsy.
- Make sure the scale is on a hard surface and turned on
- Take last perfusate samples and note the perfusion parameters
- Make sure a sterile bowl with ice and UW-CS is ready. This will be done by the surgeon (in a sterile setting).
- Make sure a second empty bowl is ready for weighing the liver, and that the scale is covered with a sterile sheet
- Discuss with the surgeon that the bile drain will be kept *in situ* for bile analysis after reperfusion.

Ending NMP

- Take a picture of the liver without the lid
- Ask the surgeon to take the biopsies
- The surgeon will disconnect the cannulas and at the same time the machine must be stopped
- Note these time points on the log form
- The surgeon will weigh the liver **after flush**. Note the weight on the log form

Flush

- Wear unsterile gloves
- Close all connections
- Attach new bags of UW- CS to the infusion system
- De-air the de-airing chamber by opening the connections, do this **before** stop of NMP.
- De-air the infusion system in communication with the surgeon, do this **before** stop of NMP.
- When the surgeon says yes, start flush. Don't put pressure on the bag during portal flush. Mild pressure can be used for the arterial flush.
- Note the time of start flush on the log form
- Flush 1,5 L through the **portal vein**
- Flush 0,5 L trough the **artery**
- **NOTE**: Flushing after SCS: 1 L (minimally) with UW. Flushing between DHOPE/COR: 2L NaCl 0.9%. Flushing after NMP: 2L UW. Ratios are the same for all flushing moments.

Transportation of the liver

- Discus whether the surgeon wants to bag the liver or transport the liver in the bowl.

For transportation in the bowl:

- Make sure the cart is covered with a sterile sheet
- Place the Styrofoam box with the original donor forms, toolkit, and the cultivation (kweek) on the bottom of the cart.
- The surgeon will place the bowl on the cart and cover the bowl
- Assist the surgeon to OR 19

For transportation in organ bag and Styrofoam box:

The surgeon packs the liver in liver organ bags together with a second sterile person:

- 1st bag: Liver with UW-CS solution
- 2nd bag: ice cold NaCl 0,9%
- 3rd bag: no fluids
- Place liver in the original Styrofoam organ box on ice. Make sure the toolkit, donor forms and the cultivation is also in the box.
- Assist the surgeon to OR 19

Cleaning

- Place log form and copy of the donor forms in a yellow envelope.
- Count the gauzes with two people
- Discard everything from the back-table except surgical tools and white tube/probe (sonde) in the blue basket!!
- discard the disposable
- Empty water bath and refill with demi water
- Mop the floor
- Disinfect all surfaces including the machine
- Call cleaning crew: 44903
- Call the scrub nurse for collecting the surgical tools: 45837
- Write the OR report in EPIC
- Fill in the OPR checklist

Researcher tasks

- Place perfusate samples in the centrifuge and centrifuge at:
  - 4 degrees Celsius
  - 3220 RCF
  - 15 minutes
- Store al samples at the COL in -80 and note location in the OR report.
- Go to OR 19 for sample collection
- Take a bile sample after graft reperfusion and analyse it with the blood gas analyser, using the same EPIC number. Take the sample after arterial reperfusion.