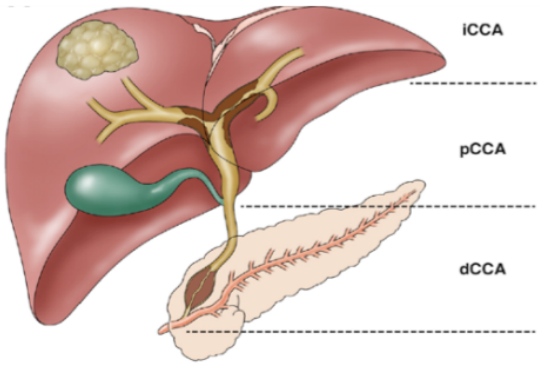
European Network for the Study of Cholangiocarcinoma (ENS-CCA)

International Registry on
Cholangiocarcinoma (CCA)



European CCA Registry

Sample Processing Protocol

Founders: European Network for the Study of Cholangiocarcinoma (ENS-CCA)

SAMPLE PROCESSING PROTOCOL

- **Serum**
  1- Collect blood in serum tube
  2- Keep upright 30 min at Room Temperature
  3- Centrifuge at 1,500g, 20 minutes, at Room Temperature
  4- Aliquote the serum in volumes of 1 ml

- **Plasma**
  1- Collect blood in EDTA tube
  2- Centrifuge at 1,500g, 20 minutes, at Room Temperature
  3- Aliquote the plasma in volumes of 1 ml
  3.1- Collect gDNA with **Flexigen DNA Kit** (Automated or Manually)

- **Bile**
  1- Collect bile
  2- Centrifuge at 1,500g, 20 minutes, at Room Temperature
  3- Aiquote the bile in volumes of 1 ml

- **Urine**
  1- Collect the urine
  2- Aliquote 3 ml of urine in volumes of 1 ml
  2.1- Centrifuge the remainder urine at 3,000g, 5 minutes, at Room Temperature
  2.2- Aliquote the urine in volumes of 1 ml

- **Stools**
  1- Collect stools
  2- Freeze at -80ºC

- **Saliva**
  1- Collect saliva with **Oragen DNA kit (Genotek)**
  2- Follow the protocol

- **Tumor tissue and surrounding tissue**

  - **Frozen**
    1- Cut a piece of non-tumoral and tumoral tissue
    2- Put them of eppendorf tubes and storage them at -80ºC

  - **Frozen on OCT**
    1- Cut a piece of non-tumoral and tumoral tissue
    2- Put each piece in a cryomold with OCT
    3- Immerse the cryomolds into isopentane at -80ºC
    4- Keep the cryomolds for 5-10 minutes in isopentane
    5- Leave it drying and storage at -80ºC

6- If there is enough tissue, put also a piece of non-tumoral and tumoral tissue in a eppendorf
7- Immerse the eppendorfs into isopentane at -80ºC
8- Storage at -80ºC

- **Embedded in paraffin**
  1- Cut a piece of non-tumoral and tumoral tissue
  2- Put each piece in a cassette
  3- Immerse the cassettes in formaldehyde 4%
  4- Keep in the formaldehyde until the next day
  5- Introduce the cassettes in Ethanol 96% during 1h 30 minutes (x2) and then introduce them in Ethanol 96% for 1h
  6- Put the cassettes in Ethanol 100% for 1h 30 minutes (x2)
  7- Place the cassettes in xilol for 1 h (x3)
  8- Soak up the cassettes in paraffin at 55-60ºC for 2 h (x2)
  9- Put the paraffined cassettes on a “cold plate” to form the block
  10- Cut and make the slides