Iceland Liechtenstein Norway grants

Exchange in Cluj-Napoca

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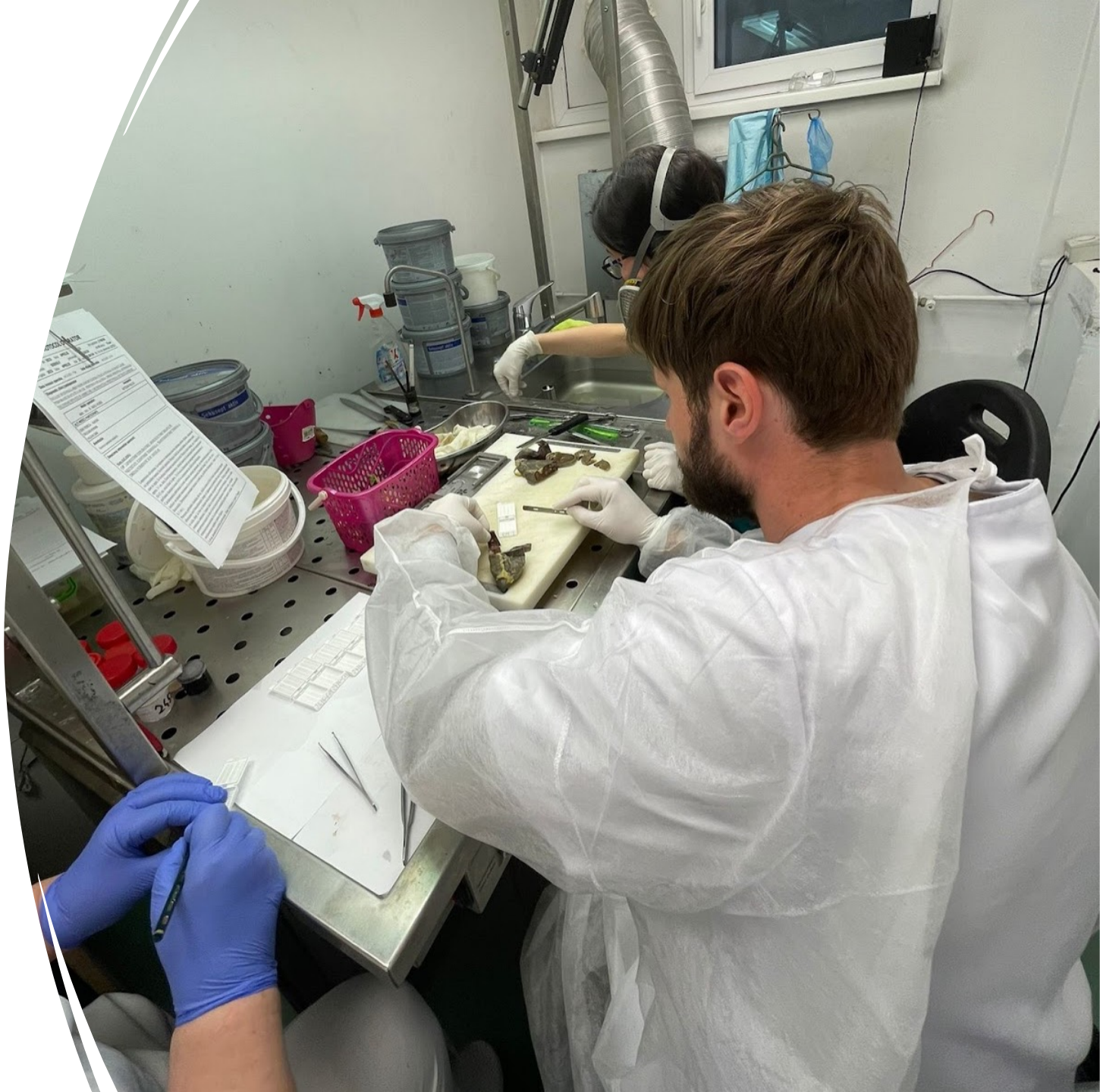
NORWAY

First week (25.04.2023)

1.The tissue it is taken from the **autopsy**
•the resected part (a part from each end, the tumor and the ganglions (e.g. from the piece of colon) - cut and put in boxes
•
from the pieces of the organ, pieces of tissue are taken that look suspicious - they are cut enough to fit in the boxes
•the tissue put in boxes is kept in formalin
Samples: Riad 1/2/3 - gallbladder
Riad 4/5/6/7 - autopsy
2.The tissue samples in the boxes go to a device that creates a vacuum for their **preparation for waxing.**
Steps:
1. Dehydration - water comes out and is replaced by ethanol (approx. 1h)
2. Clarification - Xylene (3 baths/ approx. 1h)
3. Paraffin - approx. 1 hour
*everything is done under vacuum, because for large and thick sections it takes approx. 1h for the solutions to penetrate the test about 1-2 mm) => they will stay on schedule overnight



- **3 Parafining**
- The paraffin blocks are taken from the machine and taken to the paraffin machine
- There are aluminum boxes of different sizes in the device
- Put hot paraffin in the aluminum box and then the tissue from the plastic box
- Move the box to a cooler place to fix the tissue to the bottom (add the box over for support and then move to the ice).

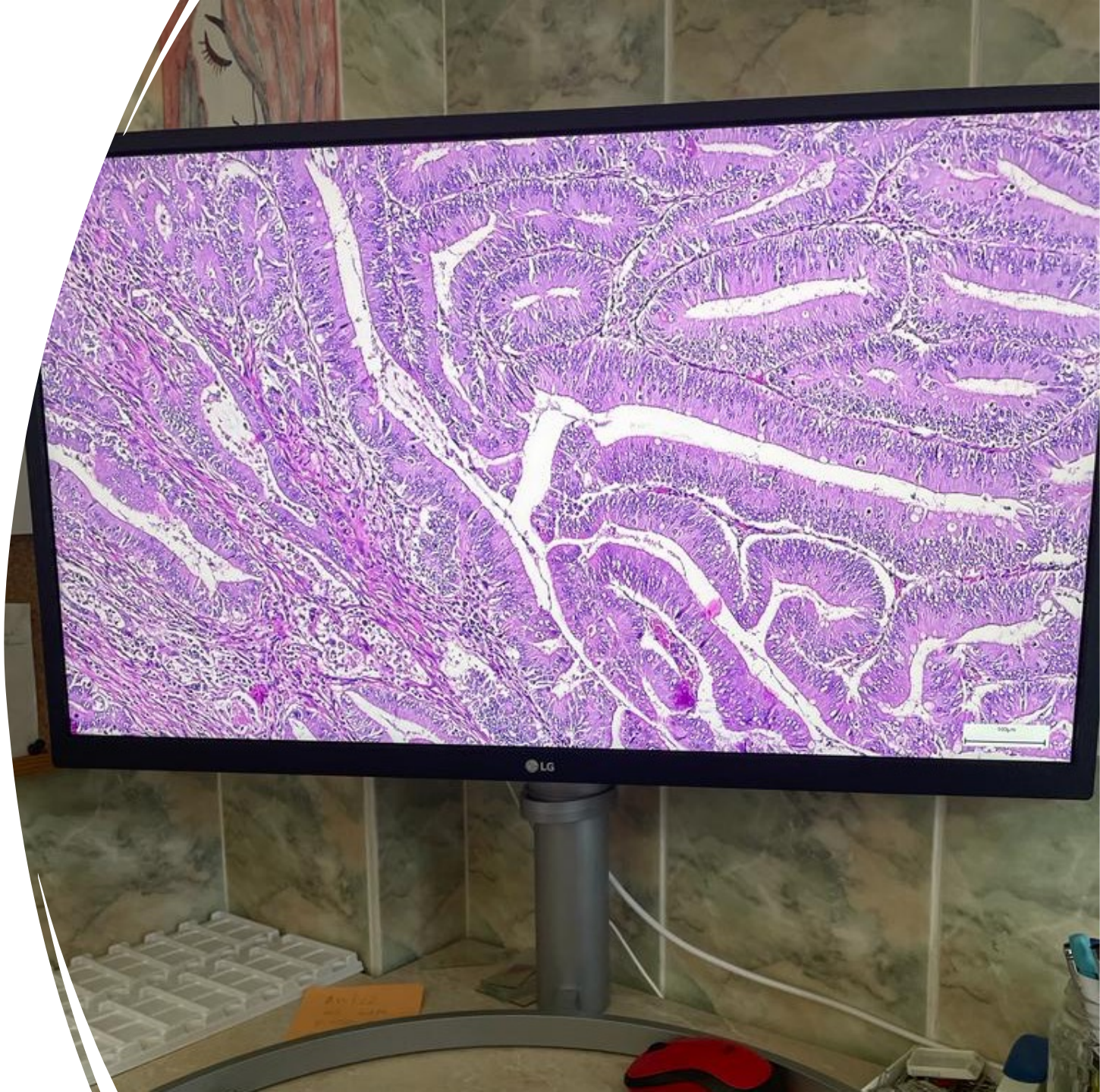
4.Cutting

- With the help of the microtome - with a thickness of 3 or 2 microns
- They are placed in a water bath (43C) and then on the slide (about 4 sections)

5. Standard coloring of the slides

- The samples are placed in the Leica Autostain XL device
- . Hematoxylin - nuclei staining
- Eosin - cytoplasm staining
- Balm
- he lamella on top
- Manual staining:

1. Hematoxilin - 5 min
2. Washing - 5 min
3. Eozin - 3 min
4. Rinsing - 1 min
5. Alcool - max 1min



Second week (Immunohistochemistry)

1. Slides preparation (2 section on one slide for economy)
2. The paraffin blocks are cut and placed on slides and then in thermoblock overnight
   - ***Ventana machine**
   - 1. Cupru DAB
   - 2. DAB = peroxydase (removes impurities)
   - 3. Chromogene DAB
   - 4. DAB inhibitor
   - 5. HRP Multimer - post primeri
   - 6. Reagent
   - 7. Hematoxilin
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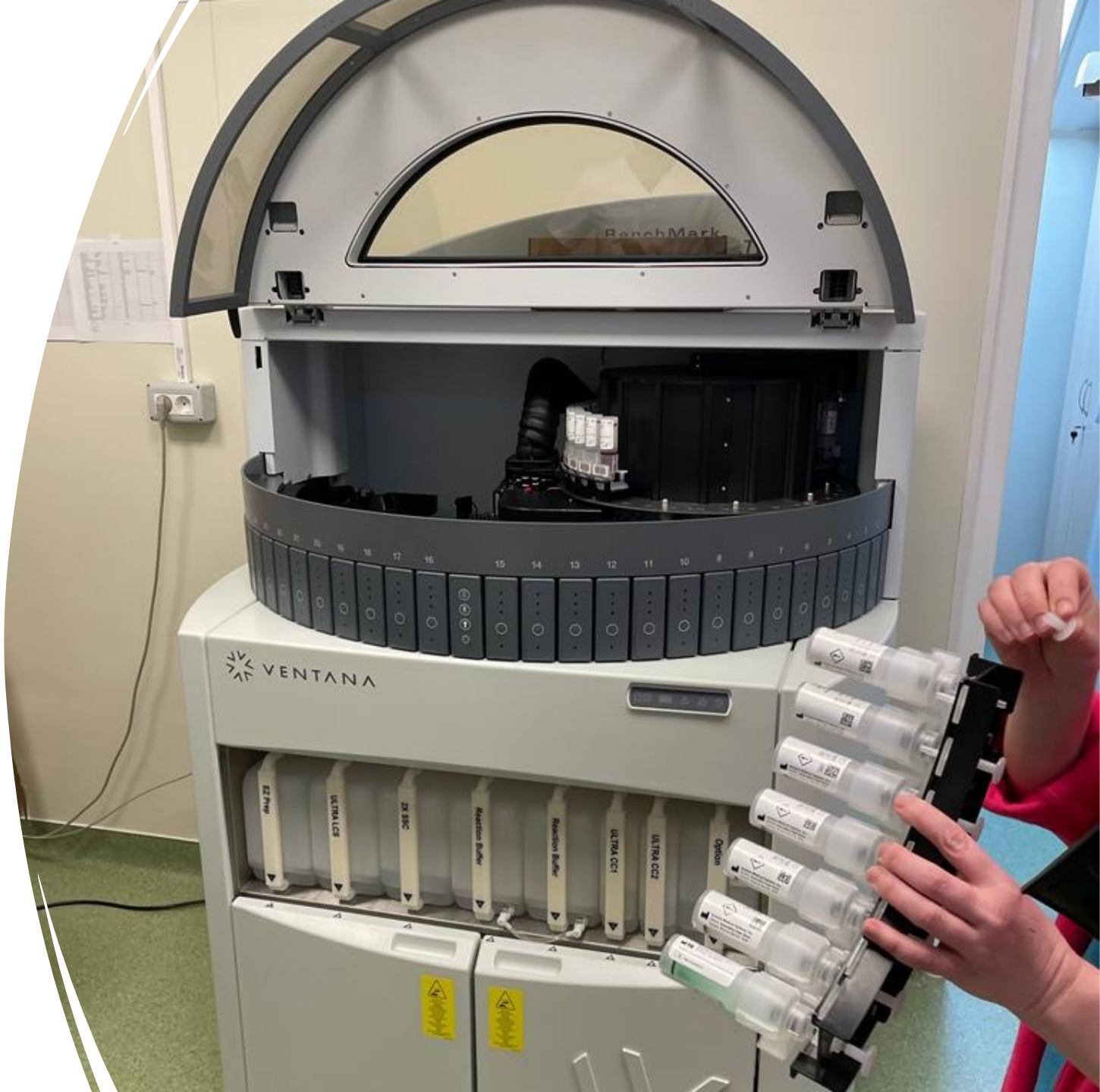

Solutions:
- EZ prep. - deparafination
- Ultra LCS - is oil that covers slides to avoid evaporation of the solutions
- 2X SSS?
- Reaction Buffer - for washing
- Ultra CC1 and 2 - pH 6 and 9

- !!! total decontamination is done once every 3 months -

1. Select antibodies (max 30 slides)
2. Open the programme and add antibodies, name the slides and print.
3. Place the slides in the machine
4. Place antibodies in the machine
5. Go (2h 30 min)

- **Manually**

1. Persoxidase block - blocking 5 min
2. Boiling (enzimatic) - reveal epitop inside - 30 min
3. First antibody - 1 hour RT
4. Second antibody - post primary - 2 minutes
5. DAB (amio benzidine) = chromogene -> for coloration reaction - 5 min * 2
6. We want to see the tissue, so we use hematoxilin (not just brown spots) - 3-5 min
7. Wash -> alcool -> xylene -> mounting

- *Melanomas - special pigments
- **Leica Bond Max.**
- MLM, MSH = microsatellites
    1. The labels are printed
    2. The labes are added to the slides
    3. The slides are placed in the machine -> scan.
    4. Go

Thanks to you for an amazing stay

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