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## **A REALITY IN A DEVELOPING COUNTRY- BENIN: DIAGNOSIS OF CANCER WITH THE ACTIVATION OF AUTOMATED IMMUNOHISTOCHEMISTRY**

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### **Abstract**

*Background: In the Benin-West state of Africa, cancer is one of the most frequent causes of death. For this reason from September 2015 at Saint John of God Hospital in Tanguieta – Benin – a Histopathology Laboratory is active with the aim to make diagnosis and address towards adequate therapeutic programs patients affected from cancer and other diseases. The Laboratory has been equipped with a tele pathology system (Cross SS, Dennis T, Start RD. 2002 and Della Mea V. & Beltrami CA. 2000) and from 2018 with an Automatic System for Immunochemistry.*

*Objectives, methods and outcomes: In the last 20 years, in collaboration with the association APOF (Pathologists across the border), diagnostic laboratories have been developed in various African countries: Tanzania - Uganda - Djibouti, etc. Immunohistochemistry has been introduced in these laboratories to formulate a correct and complete diagnosis.*

*In Benin we had a technological breakthrough by introducing an automated immunohistochemistry system. The funds were obtained from the FAI (International Assistance Foundation) of Lugano (Switzerland) and from the UTA – Onlus.*

*The use of validated protocols, the contribution of international experts through telepathology, the training of technical personnel on site, allowed the formulation of correct diagnoses.*

*Conclusion: The development of complete histopathological diagnostic services of automated ICHs, such as that of Benin, is of fundamental importance for the fight against cancer in developing countries. Cancer is fought with research but also with a correct diagnosis.*

### Keywords

Telepathology, Histopathology Laboratory, Immunohistochemistry

## 1. Introduction

In the state of Benin, cancer is a major cause of morbidity and mortality (Jemal A, Bray F, Center MM, Feraly J, Ward E & Forman D -2011 and Shyyan R, Masood S, Badwe RA, Errico KM, Liberman L, Ozmen V. et al. – 2006)

**Table 1:** The explanatory table is taken from a study on the WHO Cooperation Strategy on Benin in the period 2016-2019

Femme	Homme	Enfant
Sein : 32,5 %,	Prostate : 18,2 %	Hémopathies : 38 %
Col de l'utérus : 16,8 %	Hémopathies : 15,4 %	Néphroblastome : 16,9 %
Hémopathies : 6,8 %	Cancer primitif du foie : 13,90 %	Enfants : ND

At the Histopathology Laboratoy of Saint John of God Hospital in Tanguieta – Benin (**Figure 1**), after three years of activity and about 500-600 cases analyzed a diagnostic critical point has emerged.



**Figure 1:** Hospital Entrance and Laboratory Entrance

The different pathologists who study the cases present in Tanguieta have highlighted the need to have available immune histochemical investigations in order to better define the diagnosis formulated.

The case studies Saint John of God Hospital is not only constituted by tumoral pathologies, but we also have inflammatory diseases, diseases generated by various viruses and therefore a diagnostic differentiation allows the application of a correct therapeutic procedure.

The legal impossibility of transferring biological samples, prepared or unprepared, abroad and the urgent need to have diagnoses of the diseases present in the hospital has accelerated the decision by the management to design and activate a histopathology laboratory.

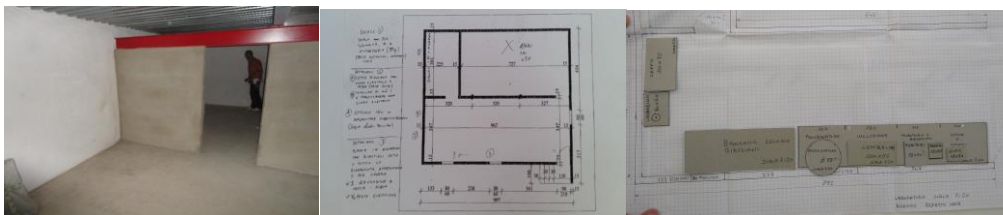
Another problem was that one should not repeat previous experiences, experiences that required the constant presence of an expert and voluntary pathologist.

For this reason, a laboratory has been designed conducted only by trained technicians and equipped with telepathology. (Krupinski EA, Weinstein RS, Bloom KJ & Rozek LS. – 1993 and Weinstein RS, Graham AM, Richter LC, Barker GP, Krupinski EA, Lopez AM, Erps KA, Yagi Y, Gilbertson JR & Bhattacharyya AK. -2009)

Today the network of pathologists connected to the laboratory scanner is about 20 units from different countries of Europe.

### **1.1 Description of the Development of the Project**

In 2014/15 we started the laboratory project starting from a really small and immediate place of any structure. We have studied the layout of the room and planned the arrangement of the necessary equipment on a work plan to be built



**Figure 2:** *Laboratory Rooms and Paper Plans for Design Tools*

However, the development of pathology services in developing countries has to address several problems:

1. funds research
2. design of laboratories suitable for these countries
3. spaces available that are not always suitable
4. on-site procurement problems
5. -staff training

The research of the funds for the realization of the laboratory in this case has been long but fruitful, we have also been careful to divide the design into two phases, the first with the creation of the histopathology laboratory and then the part of automated immunohistochemistry. With reference to the design of laboratories suitable for these countries, we have used local resources by constructing a tiled concrete shelf. (Figure 2)

This was the optimal solution for the available spaces.

As for the supply problems on site, this was solved by purchasing all the equipment and consumables in Italy, and then shipped with a container of the foundation (Table 2).

**Table 2:** *Instrumental Equipment, Personnel*

<b>Resource provider</b>	<b>Equipment</b>	<b>Supplier company</b>	<b>Personnel</b>
FAI	Hood for tissue sampling	Bio-Optica Italia	n. 2-3 Laboratory technicians
	Tissue processor	Bio-Optica Italia	
	Paraffin embedding station	Bio-Optica Italia	
	Microtome	Bio-Optica Italia	
	Water bath	Bio-Optica Italia	
	Slide Stainer	Bio-Optica Italia	
	Slide dryer	Bio-Optica Italia	
	Microscope	Nikon	
	Surgical instruments, consumable material	Bio-Optica Italia	
	Scanner for telepathology	Hamamtsu	
	PC		

After completing the laboratory, we have trained the staff (Figure 3).



**Figure 3:** *Training Of Local Technical Personnel in Histological Techniques and Preparation of Telepathology*

In March 2018 an automatic system for immunochemistry was adopted.

This was possible thanks to donations and the acquisition of an automatic but “easy to use” instrument that was got considering:

- a) Environmental context,
- b) Number of reactions per year,
- c) Antibodies needed to perform diagnosis.

Already during its planning in 2015, the Laboratory had been designed and organized with the future intention to install all necessary instruments for immunochemistry.

### **1.2 Programming and organization of the IHC service**

In 2017 we started to plan the immunochemistry service:

- 1) evaluating cases arrived at the clinical pathology service of the hospital;
- 2) evaluating number and type of antibodies necessary to complete the diagnosis;
- 3) consulting the pathologists that make diagnosis through telepathology;
- 4) Evaluating available spaces in consideration of instruments sizes and connections among them.

### **1.3 Coordination of Equipment**

Then we installed the following equipment: (Figure 4)

- Autostainer 480S – THERMO SCIENTIFIC;
- PC committed to the instrument;
- 2 printers (the first for reagents labeling and the second for slides labeling);
- PT-Module.

instrument and antibodies were purchased with funds from the UTA Onlus organization and the Bio-Optica company.



**Figure 4:** Complete Station Autostainer 480S - Thermo Scientific

In order to install the instrumentation necessary for the execution of immunohistochemistry we reduced the space of the histological instrumentation, subsequently we proceeded to verify the lighting, the wiring of the electrical network, verified the complete functioning of the system (software and hardware) (Giorno R -1984 & Key M. 2009).

Ordered antibodies were checked. Verified the correctness between what was ordered and what was received, together with the technical staff we created a table with the respective dilutions and prepared the analysis protocols.

**Table 3:** *Some Antibodies in Use*

Antibody	Dilution	Company
Estrogen Receptor (ER) 0,5 ml	1:50-1:100	BIOCARE Medical by Bio-Optica - Italy
Progesterone Receptor 1,0 ml - 200 µg YR85	1:50-1:100	BIOCARE Medical by Bio-Optica - Italy
Ki- 67 1,0 ml	1:100 – 1:200	BIOCARE Medical by Bio-Optica - Italy
c- erbB- 2 / HER- 2 / neu 1,0 ml SP3	1:50-1:100	BIOCARE Medical by Bio-Optica - Italy
Keratin, Pan 1,0 ml	1:50-1:100	BIOCARE Medical by Bio-Optica - Italy
CD20 1,0 ml	1:75-1:150	BIOCARE Medical by Bio-Optica - Italy
etc		

It is specified that the optimal dilution depends fundamentally on the thickness of the sections and on the fixation. Therefore the values indicated are those considered optimal but in any case must be verified when the reaction has been evaluated observing the slide under a microscope.

#### 1.4 Training of personnel

Staff training proceeded according the following program: (Figure 5)

- A. Explanation of the instructions for the use of the PT-Module for the preparation of slides to immunochemistry reactions and preparation of the necessary solutions;
- B. Preparation of antibody dilutions;
- C. Explanation of the instructions for the use of the Auto stainer and its programming with insertion of reagents codes, reagents dispensing quantity and type of reactions to perform.

Staff training was carried out by me Dr. T. Zanin



**Figure 5:** *Training of Local Technical Personnel in the Application of Immunohistochemistry*

## **2. Quality Assessment**

Following the guidelines of the Italian Ministry of Health, dell'AIOM (Italian Association of Medical Oncologists), protocols were developed to ensure ongoing quality and reproducibility. Methods used to maintain high quality included: written standard operating procedures, inclusion of positive and negative controls in all staining batches and use the Tele pathology.

To verify the quality of immune histochemical reactions with pathologists, the reactions were performed with antibodies required by the pathologist based on the diagnostic needs of the case under examination.

The quality of immuo histochemical reactions derive from the quality of the histological sections, the technicians after two years of application are able to perform sections of about 2 microns thick, quality that is also reflected through the study of digital images of the scanner in use.

### **2.1 The current immunohistochemistry program: operations and results**

The organization of work in the laboratory is as follows:

- A. arrival of histological samples
- B. registration on the database and numbering of the exam
- C. sampling, processing
- D. inclusion in paraffin and cutting
- E. section coloring and insertion into the scanner
- F. the pathologist takes care of the case, observes the digitized image and requires the antibodies necessary to complete the diagnosis
- G. the technician receives the request on the PC
- H. Cut other sections
- I. starts the necessary immune histochemical protocol

Antigen retrieval for IHC is performed with a PT link machine (Pre Treatment Link, Thermo Scientific by Bio-Optica - Italy). Antigen retrieval is achieved by using high-pH Tris-EDTA (pH 8.0) and a low-pH citrate buffer (pH 6.0). The next step is digestion with Trypsin enzyme for 5 minutes at 37°C -or- for 20 minutes at RT, then proceed with the following protocol:

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher. Primary Antibody: Incubate for 30 minutes at RT. Probe: Incubate for 10 minutes at RT with a secondary probe. Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

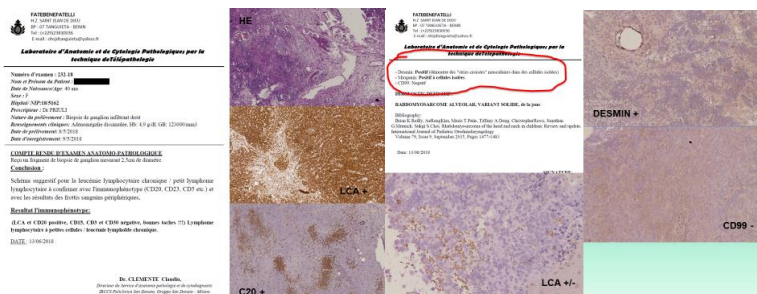


Chromogen: Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Biocare's Warp Red. Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

## 2.2 Technical Note

- 1) With cytokeratin markers, heat retrieval may provide a higher sensitivity assay; whereas, enzyme digestion may produce greater specificity. Users should validate the pretreatment method for their specific application.
- 2) This antibody has been optimized for use with Biocare's MACH 4 Universal HRPPolymer Detection and IntelliPATH Universal HRP Detection Kit. Other Biocare polymer detection kits may be used; however, users must validate incubation times and protocols for their specific application. Use TBS for washing steps.

When the reaction ended, we checked its success at the microscope comparing the slide with a positive and a negative control sample. Once observed the positive result of the immunohistochemistry staining, we scanned the slide so that through telepathology the pathologist requesting the reaction could evaluate the sample and complete the diagnosis. (Figure 6)



**Figure 6:** Complete report of immune histochemical diagnosis - H.E. images and antibody images used

## 2.3 Outcomes

The project started in 2018 so the first significant results can be seen about two years.

We must bear in mind that the number of cases per year is around 600, so a low workload.

## 3. Conclusions

The activation of a complete diagnostic service with integrated morphological histopathological diagnosis and supported by automated immunohistochemistry is certainly a point of reference for cancer patients. The possibility of having a certain and complete diagnosis will give them the certainty of receiving an appropriate anticancer therapy.

However, the activation of these laboratories requires the continuous search for funds to maintain the activity, an activity that, however, will tend to grow.



It is known that when a hospital can offer services, diagnosis and assistance especially in developing countries, it attracts patients from nearby cities and neighboring countries.

Furthermore, at the end of 2018, the Saint John of God Hospital of Tanguieta acquired a TAC, which integrates the modernization of the available tools, offering ever wider and more diversified diagnostic opportunities.

This is the first and only Laboratory of West Africa with complete and automated systems for telepathology and immunochemistry. (Figure 7)



**Figure 7:** *Telepathology Station and Immunohistochemistry Station*

The next step will be the startup of a screening for cervical uterine cancer by Pap Test offered to all women from 20 to 50 years old.

Another study project is to integrate histopathological diagnosis with the application of Molecular Biology

For any further information, please contact in Saint John of God Hospital of Tanguieta ([labanapath\\_tanguieta@yahoo.fr](mailto:labanapath_tanguieta@yahoo.fr))

**Pathologists Make a Diagnosis Can 'Save a Life – To Contact the Laboratory.**

**This project needs your help!!!!!!**



<http://www.tanguieta.org/>

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