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COMPARATIVE IMMUNOHISTOCHEMICAL AND HISTOCHEMICAL ANALYSIS IN HISTOLOGICAL SPECIMENS PROCESSED WITH OTTIX AND WITH TRADITIONAL METHOD

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1 – PRELIMINARY REMARKS

OTTIX is a product able to replace alcohol and xylene and/or toluene in processing and staining of cytohistological preparation. OTTIX is less harmfulness than solvents used nowadays in histological laboratories.
Being a product of recent introduction on the market, data concerning reliability of OTTIX in diagnostic and research methods are quite limited. [1]

2 – INTRODUTION AND ISSUE OF THE WORK

To obtain an histological specimen embedding in paraffin with classical method, it is custom to process it as follows:

1. **Dehydration:** through passage in an increasing series of alcohol 70°-95°-100° (in general with a stay of some hours in each of them)
2. **Stay in paraffin solvent** xylene or toluene
3. **Impregnation:** a sufficient long step in melted paraffin till complete impregnation
4. **Embedding:** it consists in packaging of the block

Issue of these passages is to transform tissues, which have a different solidity and elasticity, in an homogenous mass, whose parts will have, during cutting, a quite homogenous behaviour. [2]

Elements used in these phases of the process are::

Ethyl alcohol 70°-95°-100°, which, apart from inflammability, doesn't present particular aspects concerning harmfulness for operators , and xylene.

Commercial **xylene** is compounded by a mixture of the following compounds in a variable percentage according to manufacturer [3] [4]:

- Xylene >50%
- Ethilbenzene till 25%
- Toluene < 0,5%
- Benzene till 100 ppm.

A new product able to replace alcohol and solvent in processing of histological preparation has been put on the market recently. The product is *Ottix by Diapath srl.*

Ottix is compounded by:

- Alkyl C_5-C_{12}
- Aliphatic alcohol
- Other non-toxic compounds

This new product has features of lower ranger and harmfulness than alcohol and solvents used nowadays in histological laboratories.

Being a new product, there are limited data concerning reliability in diagnostic and research methods which use specimen embedded in paraffin.

The issue of this work is to evaluate results of Eosin Haematoxylin staining (routine staining), of immunohistochemical and histochemical reactions on 100 histological specimens obtained by processing with Ottix. They will be compared with results obtained by processing with routine techniques with alcohol and xylene

3 – MATERIALS AND METHODS

100 consecutive specimens of various histopathological case histories have been prepared, without interfering in fulfilment of current diagnostic activity, where it was possible to prepare *similar preparations mirror in double*:

- 1 specimen processed with Ottix in blue biocassette
- 1 specimen processed with routine technique in fuchsia biocassette

In the following pages there is the list of 100 cases with different anatomic seats, pathologies and diagnosis.

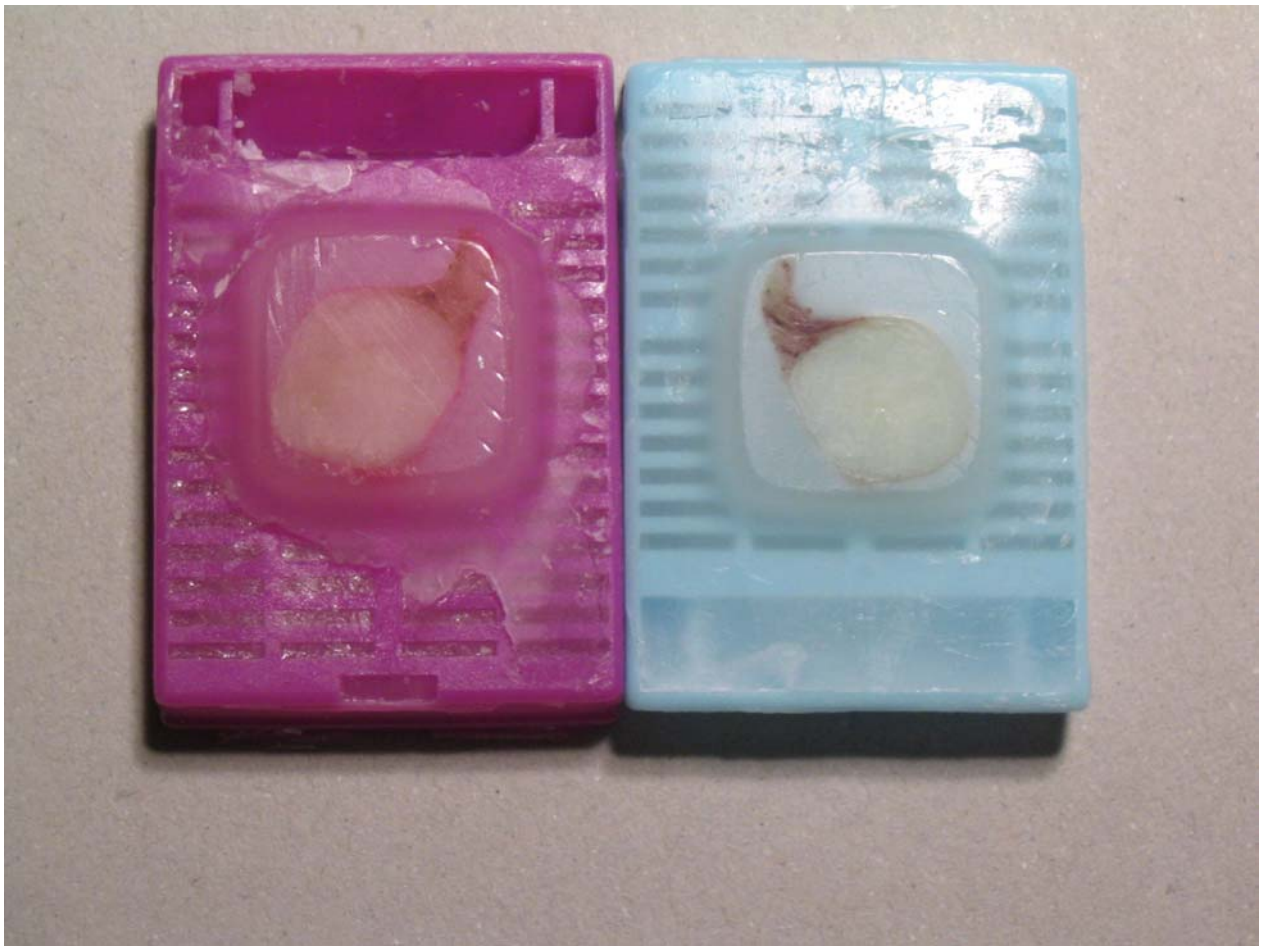

Photo 1 – Used biocassette

	ANATOMICA SEAT	*DIAGNOSIS*
1	Cholecyst	Phlogosis
2	Prostate	Aden carcinoma
3	Lymph node	Lymphoma
4	Skin	Spine cellular carcinoma
5	Heel skin	Naevus
6	Lipoma	Lipoma
7	Portio and cervical duct	Normal
8	Scalp skin	Seborrheic keratosis
9	Appendix	Inflammation
10	Gengiva neoformation	Spine cellular carcinoma
11	Placenta	Infarct
12	Uterus	Prolapse of the uterus
13	Mamma	Mastopathy
14	Mamma	Carcinoma
15	Skin	Spine cellular carcinoma
16	Mamma	Fibro adenoma
17	Uterus	Myoma
18	Mamma	Mastopathy
19	Jejunum	Normal
20	Jejunum	Lymphoma
21	Seminal bladder	Normal
22	Sinus	Sinus pilonidalis
23	Appendix	Inflammation
24	Ovular material	Abortion
25	Placenta	Intrauterine death
26	Spleen	Normal spleen
27	Pancreas	Aden carcinoma
28	Appendix	Phlogosis
29	Cervical duct	Myoma
30	Prepuce	Phimosis
31	Skin	Pilonidale cyst
32	Mamma	Fibro adenoma
33	Salpinx	Clot
34	Encephalon	Atypical meningioma
35	Colon	Normal
36	Peritoneum	Endometrisys
37	Thyroid	Normal
38	Stomach	Normal
39	Duodenum	Normal
40	Pancreas	Aden carcinoma
41	Ileum	Leukemia lymphoma t
42	Ileum	Intestinal lymphoma t
43	Skin	Seborrheic keratosis
44	Skin	Seborrheic keratosis
45	Cholecyst	Phlogosis
46	Testicle	Normal
47	Mamma	Mastopathy
48	Tonsil	Tonsillitis
49	Skin	Fibrohistiocitoma
50	Skin	Thrichilemmale cyst

	ANATOMICA SEAT	*DIAGNOSIS*
51	Subcutaneiously	Lymphoma
52	Parotid	Pleomorphic adenoma
53	Parotid	Normal
54	Ovary	Normal
55	Uterus	Leiomioma
56	Stomach	Aden carcinoma
57	Stomach	Normal
58	Limphonode	Methexis of aden carcinoma
59	Femoral head	Necrosis
60	Ovary	Cyst
61	Vulva	Cyst
62	Maxillar sinus	Polyposis
63	Cervical duct	Normal
64	Uterus	Myoma
65	Ovary	Normal
66	Colon	Aden carcinoma
67	Colon	Normal
68	Menimix	Meningioma
69	Subcutaneously	Lipoma
70	Uterus	Abortion
71	Uterus	Leiomioma
72	Mamma	Normal
73	Colon	Perforated diverticulitis
74	Appendix	Appendicitis
75	Stomach	Normal
76	Embryonic material	Abortion
77	Appendix	Appendicitis
78	Uterus	Fibroleiomioma
79	Omentum	Normal
80	Uterus	Myoma
81	Thyroid	Goitre
82	Spleen	Normal
83	Uterus	Mullerianic tumour
84	Skin	Basalioma
85	Vescica	Papillary tumor
86	Menimix	Meningioma
87	Lymphonode	Methexis of adenocarcinoma
88	Colon	Colic adenocarcinoma
89	Skin	Methesis of melanoma
90	Parotid	Adenoma
91	Liver	Hepatocarcinoma
92	Skin	Seborrheic keratosis
93	Subtaneously	Bursitis
94	Lymphonode	Lymphome with t cell
95	Uterus	Prolapse
96	Uterus	Myoma
97	Ovary	Follicolinic cyst
98	Tube	Paratubaric cyst
99	Ovary	Normal
100	Uterus	Leiomioma

3.1- CASES PROCESSED WITH CLASSICAL METHOD

An automatic vacuum-sealed processor "Pathcenter" by Shandon was used for processing of histological examinations of experimentation with classical method, which used alcohol/xylene.

The used processing protocol, which needs the use of 14 reagent positions and lasts 14 hours and 45 minutes, is the following:

PROCESSOR PATHCENTER SCHEDULE				
PROGRESSIVE	***REAGENT***	***TEMPERATURE [°C]***	**DURATION** [minutes]	**VACUUM**
1	ALCOHOL 70	35	60	YES
2	ALCOHOL 95	35	45	YES
3	ALCOHOL 95	35	45	YES
4	ALCOHOL 95	35	60	YES
5	ALCOHOL ASS	35	75	YES
6	ALCOHOL ASS	35	75	YES
7	ALCOHOL ASS	35	75	YES
8	ALCOHOL ASS	35	75	YES
9	XYLENE	room temperature	90	YES
10	XYLENE	35	90	YES
11	PARAFFIN A	60	30	YES + PRESSION
12	PARAFFIN B	60	45	YES
13	PARAFFIN C	60	60	YES
14	PARAFFIN D	60	60	YES

3.2- CASES PROCESSED WITH OTTIX

An automatic vacuum-sealed processor "Hypercenter" by Shandon was used for processing histological examinations of Ottix experimentation.

The used processing protocol, given by Diapath, needs the use of 7 reagent positions and lasts 14 hours and 10 minutes.

Before processing cassettes , there is a wash step in Ottix Shaper to remove the excess of formalin. Passages in alcohol solutions with different graduations and the following ones in xylene are replaced by passages in Ottix. Ottix allows dehydration and clarification with an unique component. By this way it is possible to process with fewer passages.

PROCESSOR HYPERCENTER SCHEDULE				
PROGRESSIVE	***REAGENT***	***TEMPERATURE [°C]***	***DURATION [minutes]***	***VACUUM***
1	OTTIX SHAPER	RT	10	NO
2	OTTIX	35	60	SI
3	OTTIX	35	180	SI
4	OTTIX	35	180	SI
5	OTTIX	35	180	SI
6	PARAFFIN	60	90	SI
7	PARAFFIN	60	150	SI

3.3- PROCESSING, PARAFFIN EMBEDDING, CUTTING, STAININGS AND EVALUATION OF RESULTS

All specimens processed with classical method and with Ottix are embedded in paraffin and cut.

Some first sections were cut and stained with routine ***Eosin Hematoxil*** stain, and a first evaluation is done concerning :

- processing
- cutting
- morphology
- chromatism

with principles of evaluation :

- inferior
- equivalent
- superior

Other sections for ***histochemical*** detections followed and were evaluated according to:

- brilliance
- chromatic fidelity

always with principles of evaluation :

- inferior
- equivalent
- superior

Other sections for ***immunhoistochemical*** detections followed evaluated according to:

- antigenic conservation
- intensity of signal
- bottom of staining

always with principles of evaluation :

- inferior
- equivalent
- superior

3.4- CUTTING AND STAINING WITH EOSIN EMATOXILIN

All specimens processed with classical method and Ottix were cut with sliding microtome (Microm HM400) or rotative automatic (LKB) and stained with automatic stainer (Varistain Gemini) with routine Eosin Ematoxilyn staining.

3.5- HISTOCHEMICAL RESEARCHES

Staining done are routine ones:

- **Argentafin reticular fibre** to remark reticular fibres
- **PAS** for polysaccharides and mucus. [5]
- **Alcian Blu with pH 2,5** for mucus, for mucus, hyaluronic acid , sialic and solpho mucin. [5]

Here follows three staining processes used for experimentation:

Argentafin Reticular fibres

1. Dewax sections and bring them to distilled water with a decreasing series of alcohol
2. Put on section 5 drops of reactive A (Potassium Permanganate Solution) and 5 drops of reactive B (Acid activating tampon) , Wait 5 minutes
3. Wash in distilled water
4. Put on section 10 drops of reactive C (Solution of oxalic acids) and wait 3 minutes
5. Wash in distilled water
6. Put on section 10 drops of reactive D (Solution of ammoniacal iron) and wait 3 minutes
7. Wash in distilled water
8. Put on section 10 drops of reactive E (Ammoniacal solution) and wait 3 minutes
9. Wash in distilled water
10. Put on section 10 drops of reactive F (formaldehyde Solution) and wait 5 minutes
11. Wash in 2 changes of distilled water
12. Put on section 12 drops of reactive G (Sodium hyposulphite Solution) and wait 5 minutes.
13. Wash in water for 5 minutes
14. Dehydrate, clarify and mount

PAS

1. Dewax sections and bring them to distilled water with a decreasing series of alcohol
2. Treat sections with periodic acid 1% for 10 minutes (1 g of periodic acid in 10 ml of distillate water)
3. Wash in 3 changes of distilled water
4. Treat sections with *Schiff Reactive* at room temperature for 10 minutes
5. Wash in distilled water
6. Stain nuclei with Mayer haematoxylin for 1 minute
7. Dehydrate, clarify and mount

ALCIAN BLUE pH 2,5

1. Dewax sections and bring them to distilled water with a decreasing series of alcohol
2. Mordant with *acetic acid 3%* for 3 minutes (add to 3 ml of concentrated acetic acid 100 ml of distilled water)
3. Deep slides in Alcian bleu 1% acetic acid pH 2,5 for 30 minutes (1 g of Alcian bleu 8GX. in 100 ml of acetic acid 3% . Filter and add some thymol crystal. Ad just pH solution to 2,5: if it is higher, add some drops of acetic acid 3%, if it is lower, add some drops of sodium Hydroxide 1%)
4. Wash in current water for 10 minutes
5. Wash in distilled water
6. Stain nucleus with *Nuclear Fast Red* for 5 minutes
7. Wash in current water for 1 minute
8. Dehydrate, clarify and mount

3.6- IMMUNOHISTOCHEMICAL RESEARCHES

Immunohistochemical reactions are done either manually or automatically with immunostainer using different protocols of antigenic unmasking (water bath with citrate tampon pH6/7 or TET or with proteolytic enzymes as pronase or trypsin) and with many revelation systems (Avidin-Biotin, Envision TN, Picture Plus, Powervision™, detection sistems). [6]

Here follows the list of tested antibodies:

Antigens	Clone	Working diluition	Company	Host	Antigenic unmasking
Estrogen	6F11	1/150	Novo Castra	Mono Mouse	Citrate tamp.pH 7
Progesteron	Pgr 636	1/3	DAKO	Mono Mouse	Citrate tamp.pH 7
p53	DO-7	1/3	DAKO	Mono Mouse	Citrate tamp.pH 7
BCL2	124	1/3	DAKO	Mono Mouse	Citrate tamp.pH 7
EGFR	3IG7	5/100	DBA	Mono Mouse	pronase
CerbB2	CB11	1/250	Novo Castra	Mono Mouse	no
CyclinA	6E6	4/100	Novo Castra	Mono Mouse	Citrate tamp.pH 7
CyclinD1	DCS-6	1:100	Neo Markers	Mono Mouse	TET
CyclinD3 Ab-1	DCS-22	2/100	Neo Markers	Mono Mouse	Citrate tamp.pH 6
p16	JC8	1/200	Neo Markers	Mono Mouse	Citrate tamp.pH 7
p21	EA10Waf1	2/100	Calbio Chem	Mono Mouse	Citrate tamp.pH 7
p27	Kip1mAb	1/250	Transduction	Mono Mouse	Citrate tamp.pH 7
Ki-67	Mib 1	1/100	Dako	Mono Mouse	Citrate tamp.pH 7
Calretinin P.	poli SW	1/4000	Swant	Poli Rabbit	Citrate tamp.pH 7
cyticheratina. 34b132	34b12	20/100	Dako	Mono Mouse	Citrate tamp.pH 7
CromograninA	LK2H10	½	Biogenex	Mono Mouse	Citrate tamp.pH 6
Actin 1A4	1A4	1/3	Dako	Mono Mouse	Citrate tamp.pH 7
Desmin	D33	½	Dako	Mono Mouse	Citrate tamp.pH 7
Vimentin	V9	1/100	Dako	Mono Mouse	Citrate tamp.pH 7
CD1a	010	15/100	Immunot.	Mono Mouse	Citrate tamp.pH 7
CD3	F7.2.38	1/3	Dako	Poli Rabbit	Citrate tamp.pH 7
CD5	4c7	1:50	Novo Castra	Mono Mouse	Citrate tamp.pH 7
CD8	cd8/144b	1/3	Dako	Mono Mouse	Citrate tamp.pH 7
CD15	C3D-1	2/150	Dako	Mono Mouse	pronase
CD20	L26	1/10	Dako	Mono Mouse	Citrate tamp.pH 7
CD21	1F8	4/100	Dako	Mono Mouse	pronase
CD30	MP-1	1/100	Novo Castra	Mono Mouse	Citrate tamp.pH 7
CD45 Lca	PD7/26	½	Dako	Mono Mouse	Citrate tamp.pH 6
CD68 mac.	KP1	½	Dako	Mono Mouse	pronase
Kappa	Poli Dako	1/2000	Dako	Poli Rabbit	pronase
Lamda	Poli Dako	1/2000	Dako	Poli Rabbit	pronase
S100	Poli Dako	1/2000	Dako	Poli Rabbit	no
Anti melanoma	HMB45	½	Dako	Mono Mouse	pronase
PSA	ER-PR8	5/300	Dako	Mono Mouse	no
Sinaptofisina	Snp88	1/100	Biogenex	Mono Mouse	Citrate tamp.pH 6
CD34	QBEnd-10	1/50	Dako	Mono Mouse	Citrate tamp.pH 7
Neurofilaments	2F11	2/100	Dako	Mono Mouse	Citrate tamp.pH 6
GFAP	6F-2	1/100	Dako	Mono Mouse	Citrate tamp.pH 7
RB	3H9	1/150	MBL	Mono Mouse	Citrate tamp.pH 6
CD4	4B12	1/100	Novo Castra	Mono Mouse	Citrate tamp.pH 7
CDX2	CDX2-88	1/200	Biogenex	Mono Mouse	Citrate tamp.pH 7
p63 Ab1	4A4	1/200	Neo Markers	Mono Mouse	Citrate tamp.pH 7
Cytokeratin 20	IT-Ks20.8	No dil.	Biogenex	Mono Mouse	pronase

CD138	B-B4	1/100	Oxford	Mono Mouse	Citrate tamp.pH 7
CD10	56C6	5/300	Novo Castra	Mono Mouse	Citrate tamp.pH 7
Bcl6	PG-B6p	1/25	Dako	Mono Mouse	TET
CD79a/mb-1/B-cell	HM47/A9	1/100	Neo Markers	Mono Mouse	Citrate tamp.pH 7
Cytokeratina 7	OV-TL12/30	1/3	Biogenex	Mono Mouse	pronase
Tyrosinase	T311	1/200	Santa Cruz	Mono Mouse	Citrate tamp.pH 7
CD117 c-kit	c-kit	1/100	Dako	Poli Rabbit	Citrate tamp.pH 7
p14 ARF	14PO2	1/100	Biogenex	Mono Mouse	Citrate tamp.pH 7
TTF1(Tyroid-Tr)	SPT 24	1/100	Novo Castra	Mono Mouse	Citrate tamp.pH 7
Cytokeratina MNF116	MNF116	1/50	Dako	Mono Mouse	pronase
PLAP Ab3	H7	1/100	Neo markers	Mono Mouse	no
MART-1	lot.799P007	1/100	Neo Markers	Mono Mouse	Citrate tamp.pH 6

Here follows procedure of immunohistyochemical staining on fixed and embedded in paraffin material.

IMMUNOHISTICHEMICAL STAINING

Common part to all revelation systems used :

1. Dewax sections and bring them to distilled water with a decreasing series of alcohol
2. Endogenous *Inhibition of peroxidase* by deeping slides for 10 minutes in a aqueous solution of hydrogen peroxide at 3%
3. Wash slides with distilled water
4. *Antigen unmasking,* differentiated according to different fixation and antigen to investigate:
    - Treatment in water bath: deep slides in a basin containing Citrate Tampon pH6 or 7. Basin has to be placed in water bath containing distilled water. Once temperature of 95° is reached by the buffer, slides are treated for 40 minutes. At the end of the treatment, basin has to be took out from Bain Marie and let cool at room temperature for about 15-20 minutes
    - Enzymatic treatment: Cover sections with Pronase 0,05%(working solution) for 15 minutes at room temperature in humid chamber
    - Alternatively to Pronase, if requested we can use ready to use Tripsina (melting one pill in 1 ml of deionised water) for a time changeable from 3 to 5 minutes at room temperature
    - Treatment with TET: in 500 ml of distilled water melt 1.2 g of TRIS and 0.12 g of EDTA, bring pH to 9 finally add 2.5 microliters of Tween 20
5. Wash 2-3 times in *Tbs tampon with Tween 20* for about 1 minute
6 Delimit section with Pap Pen.

At this point depending on used revelation system, staining is different :

- **<u>BIOTIN SYSTEM WITH IMMUNOSTAINER</u>** requires the use of own auxiliary product :
    7. Incubation with *Blocking Serume* (ready to use solution) at room temperature for 5 minutes
    8. Incubation with *Primary Antibody*. The primary antibody ha to be diluted in a ready to use diluent for primary antibodies. Incubation time is 45 minutes at room temperature
    9. Wash 2-3 times in Tbs with Tween 20 for about 1 minute
    10. Incubation with *Secondary Antibody biotinilate*. Proceed to incubation with secondary antibody (ready to use) with an incubation time of 15 minutes at room temperature
    11. Wash 2-3 times in Tbs with Tween 20 for about 1 minute
    12. Incubation with *Tertiary Antibody Streptoavidinabiotin* . Proceed to incubation with tertiary antibody (ready to use) with an incubation time of 15 minutes at room temperature
    13. Wash 2-3 times in Tbs with Tween 20 for about 1 minute
    14. Development in *Chromogenous compound* . Incubate in Diamino Benzidin (ready to use DAB) for 10 minutes at room temperature
    15. Wash for 2-3 changes in distilled water
    16. Counterstain with Haematoxylin of Mayer for 3-5 minutes
    17. Differentiate in running water
    18. Dehydrate, clarify and mount

- **ENVISION SYSTEM, POWERVISION TM SYSTEM and PICTURE PLUS SYSTEM**

7. Incubation with *Primary Antibody*. The primary antibody ha to be diluted in a ready to use diluent for primary antibodies. Incubation time is 60 minutes at room temperature
8. Wash 2-3 times in Tbs with Tween 20 for about 1 minute
9. Incubation with *Secondary Antibody coniugated with peroxidase.* Proceed to incubation with secondary antibody (ready to use) with an incubation time of 30 minutes at room temperature.
10. Wash 2-3 times in Tbs with Tween 20 for about 1 minute
11. Development in *Chromogenous compound* . Incubate in Diamino Benzidin (ready to use DAB) for 10 minutes at room temperature
12. Wash for 2-3 changes in distilled water
13. Countersstain with Haematoxylin of Mayer for 3-5 minutes
14. Differentiate in running water
15. Dehydrate, clarify and mount

4 – RESULTS OFCOMPARATION

4.1- PROCESSING:

For processing the following parameters had been evaluated:

- Reagent consumption and consequent reduction of disposal cost
- Processing time
- Practicality

Reagent consumption: Ottix allows to save about 30% of reagent thanks to the smaller number of passages. So there is a further saving on reagent disposal and on their nature which contains substances less harmfulness than Xylene

Processing time: processing times are equivalent. Necessary total time for specimens preparation is unchangeable.

Practicality: the reduced number of reagents, in terms of maintenance and change of processor reagents, makes the practicality of Ottix method superior to classical method.

4.2- MICROTOME CUT PHASES:

During cutting of histological examinations the following parameters had been evaluated:

- Consistency
- Thickness of section
- Completeness of section

Consistency: hardness of specimen embedded in paraffin during cutting is resulted substantially equivalent for all treated specimens except for those ones whose anatomic seat correspond to thyroid. In this case specimens treated with Ottix resulted slightly harder.

Thickness of section: thickness of cur sections is resulted perfectly equivalent for specimens treated either with classical method or with Ottix .

Completeness of section: completeness results to be equivalent apart from rare coarctation phenomenon either with classical method or with Ottix. The reason of coarctations is due to a non proportional fixation.

4.3- STAINING WITH EOSIN HEMATOXYLIN

Staining with Eosin Haematoxylin has been done to evaluate the following parameters:

- Morphology
- Chromatism

Morphology: morphological quality of specimens processed with Ottix is resulted equivalent to that one with classical method.

Chromatism: also in this case there is an equivalence.

4.4- HISTOCHEMICAL STAININGS

Histochemical surveys had been evaluated in terms of:

- Brilliance
- Chromatic fidelity

Brilliance: results of comparison are equivalent.

Chromatic fidelity: results of comparison are equivalent

4.5- IMMUNOHISTOCHEMICAL STAININGS

Immunohistochemical surveys had been evaluated in terms of:

- Antigenic conservation
- Intensity of signal
- Bottom of staining

Antigenic conservation: results of comparison are equivalent

Intensity of signal: in some Ottix specimens immunohistochemical reactions show a more intense signal for Chromogranin, Granzyme and Estrogenous Receptors. In the remaining specimens, reactivity in term of intensity is equivalent.

Bottom of staining: any big differences are shown between bottom of Ottix and traditional specimens.

Additional notes:

All used systems of antigenic unmasking have shown good results in specimens treated with Ottix.

All different systems of relevation have shown good results in specimens treated with Ottix.

4.6- SUMMARIZING SCHEDULE

PHASE	*EVALUATED PARAMETER*	*RESULTS WITH OTTIX*
PROCESSNG	· Reagent Consumption	Lower consumption(Superior)
	· Processing time	Equivalent
	· Practicality	Superior
MICROTOME CUT PHASE	· Consistency	Equivalent (1)
	·Thickness of section	Equivalent
	· Completeness of section	Equivalent
STAINING WITH EOSIN HEMATOXYLIN	· Morphology	Equivalent
	· Chromatism	Equivalent
HISTOCHEMICAL STAININGS	· Brilliance	Equivalent
	· Chromatic fidelity	Equivalent
IMMUNOHISTOCHEMICAL STAININGS	· Antigenic conservation	Equivalent
	· Intensity of signal	Equivalent/Superior
	· Bottom of staining	Equivalent

(1) Thyroid specimens resulted slightly harder to cut

5 – REMARKS ON HARMFULNESS

Aromatic compounds or aromatic hydrocarbon, like xylene, are organic substances which have similar benzene chemical properties (it is the easier term of this chemical series) . Peculiar features of these substances are due to their particular molecular structure. It is composed by a plane molecule, perfectly symmetric, where six atoms of carbon lying on the same plane placed on vertexes of a regular hexagon, linked with an angle of 120°. This particular kind of structure has a high stability of molecule and distinguishes the toxicity of product derived from reaction of substitution on hexagonal ring (it is very important as these substances are used diffusely).

From the toxicological point of view, aromatic hydrocarbons have similar features concerning acute exposition, acting as neurotoxics. Different pathological pictures exist after chronic exposition: benzene is very myelotoxic , while the homologues haven't activity. Toxicity of these solvents is quite known because they can penetrate in organism either by respiratory tracts or cutaneous and digestive, In working activity the first one is prevalent, while digestive tract is involved in general in case of accident [7] [8]

Ottix has lower contents of :

- Benzene < 10 ppm (a quantity order inferior to that on of xylene, which on commercial formulation is <=100 ppm)
- Toluene < 10 ppm
- Xylene < 10 ppm.

Total supply of aromatics is certified not lower than limit of10 ppm.

CLASSIFICATION ACCORDING TO 67/548/CEE DIRECTIVE:	
OTTIX	XYLENE
SYMBOL : F+, Xn RISK PHRASES R : - R11 – Easily Inflammable - R65 – Harmful. It can cause damages in case of ingestion - R36/38-Irritant for eyes and skin. RISK PHRASES S: - S7 – Keep container well closed. - S16 – Keep far from fire and sparks Don't smoke	SYMBOL : F+,Xn RISK PHRASES R : - R11 - Easily Inflammable - R20/21 – harmful for inhalation and contact with skin - R38- Irritant for skin. RISK PHRASES S: - S7 - Keep container well closed. - S16 - Keep far from fire and sparks Don't smoke - S25 – Avoid contact with eyes

6 – CONCLUSIONS

Ottix allows in *processing* to save about 30% of reagents thanks to the inferior number of steps, with a consequent saving on their disposal.

During *cutting* to microtome, specimens are equivalent with both methods .

Morphological preservation and chromatism marked with *Eosin Hematolin* gave equivalent results for processed specimens with both methods.

Brilliance and chromatic fidelity of *histochemical* stainings , tested with Alcian blue pH2,5, PAS stainings and Argentafin reticular fibre, gave equivalent results.

Antigenic preservation and the possibility of *immunohistochemical* techniques application was equivalent in both methods and in some cases, in Ottix specimens, reactions showed a more intense signal.

Finally it is important to consider that Ottix has features of lower *danger and harmfulness* than Xylene used nowadays in histopathological laboratories as it substitutes Xylene (aromatic hydrocarbon) with other organic compounds (alkyls) less harmful.[9]

	XYLENE	**OTTIX**
ADVANTAGES	- Known product - Good quality of products	- Less harmful - Good quality of products - No aromatics - Bigger practicity - Fewer consumptions
DISADVANTAGES	- Bad odour - High harmfulness - Cancerogenous suspicion - Classified as dangerous waste - It causes dermatitis and penetrates skin	- Few experimented - Classified as dangerous waste - More expensive

7- BIBLIOGRAPHY

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4. MERCK **- Scheda di sicurezza Xilene del 20/09/2002**
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6. Eugenio Leonardo **– Diagnostica Immunoistochimica in Istopatologia**
7. Pasquale Iovinella , Paolino Petillo **- La metodica HPLC per la valutazione del rischio di esposizione a Sostanze Aromatiche (Xilene) in Laboratorio -** ASL CE/2 Aversa
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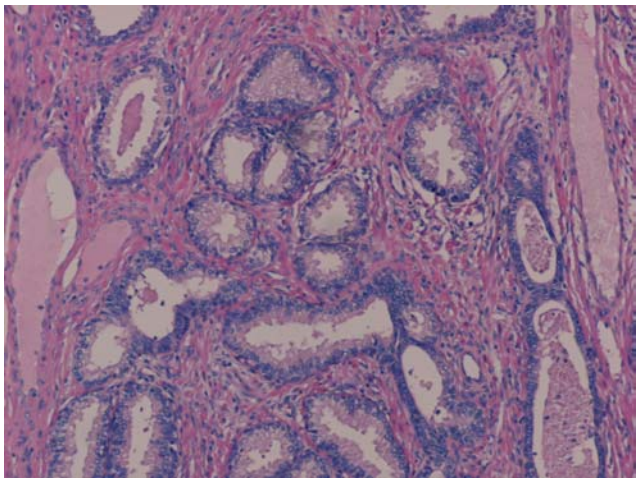
10. Stanley S. Raphael **- -Tecniche di laboratorio di Lynch -** Piccin
11. Leong/Cooper/Leong **- Manual of diagnostic antibodies for immunohistology -** GMM

8.1 - Eosin Hematoxylin images appendix



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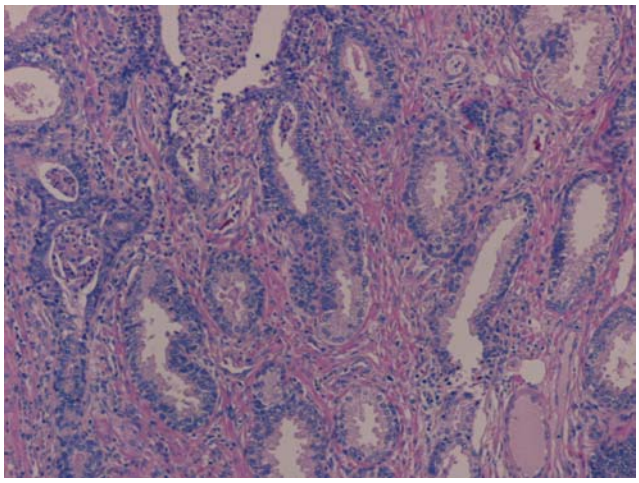

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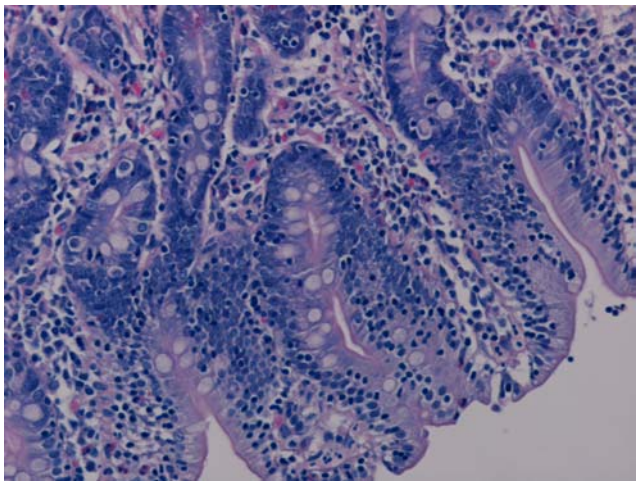

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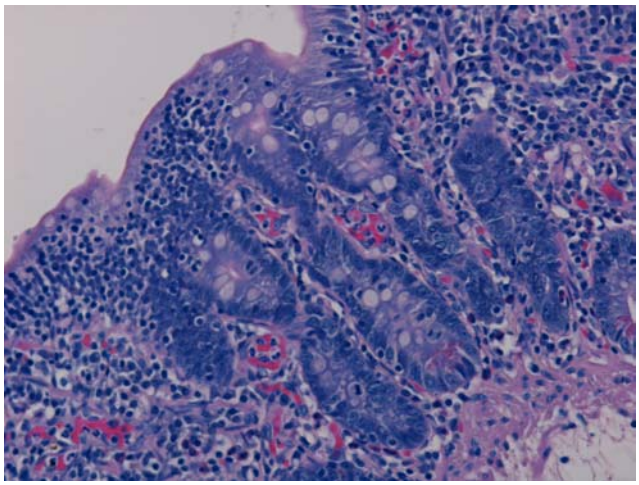

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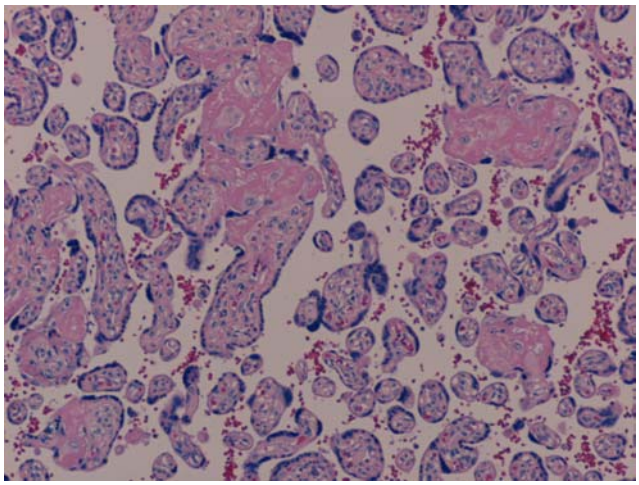

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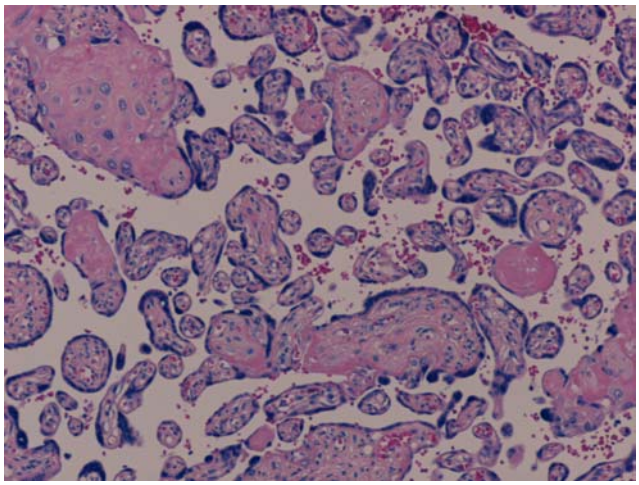
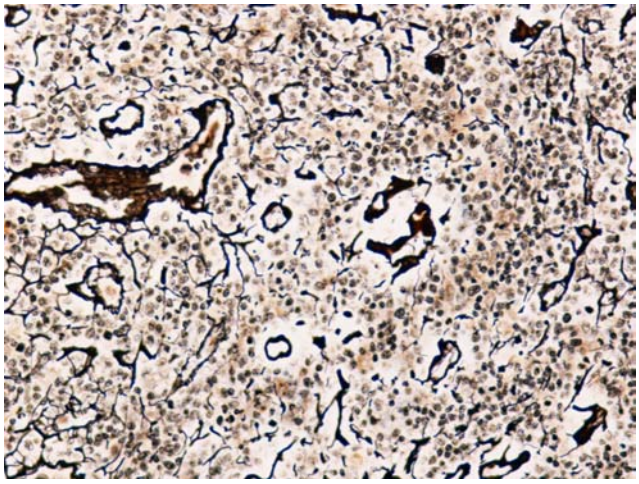


- EOSIN HEMATOXYLIN CASE N°11 (Placenta) OTTIX PROCESSING

8.2 - Histochemistry images appendix



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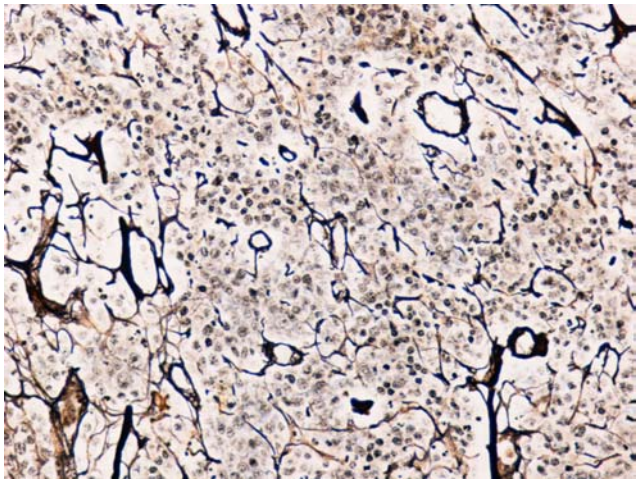

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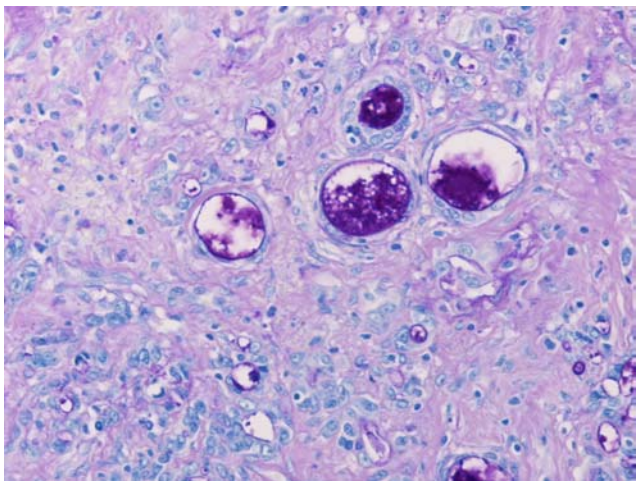

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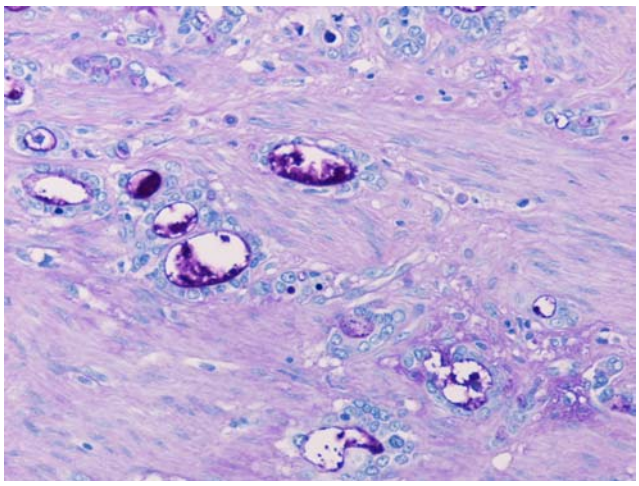

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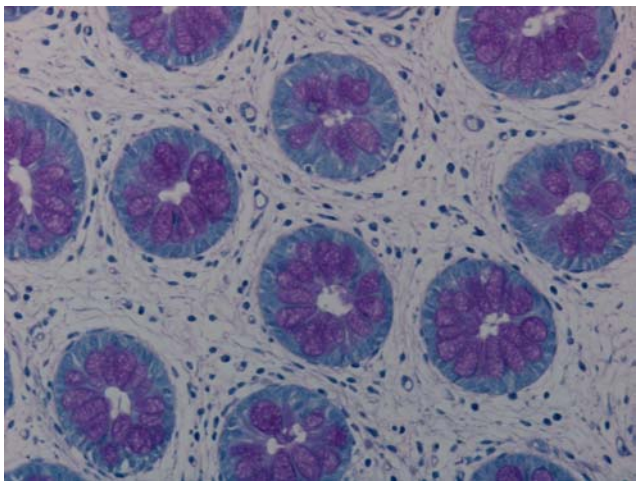

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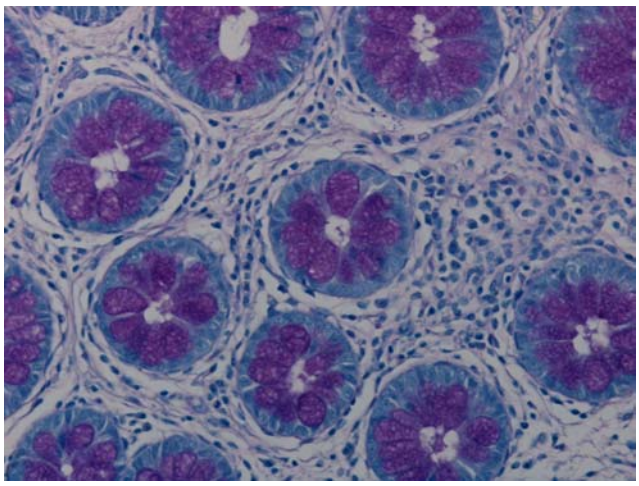

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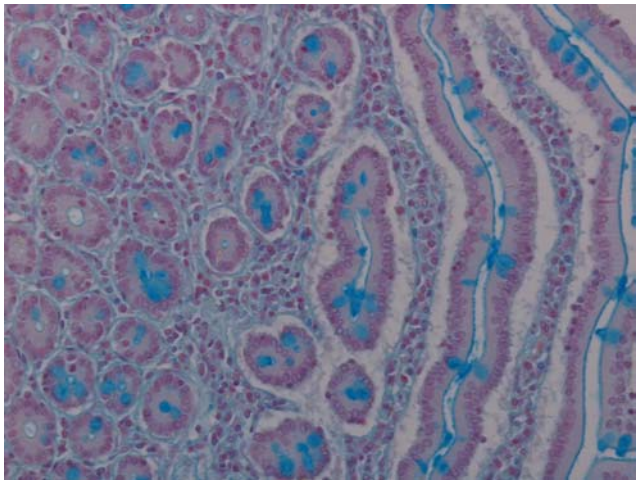
8.2 - Histochemistry images appendix



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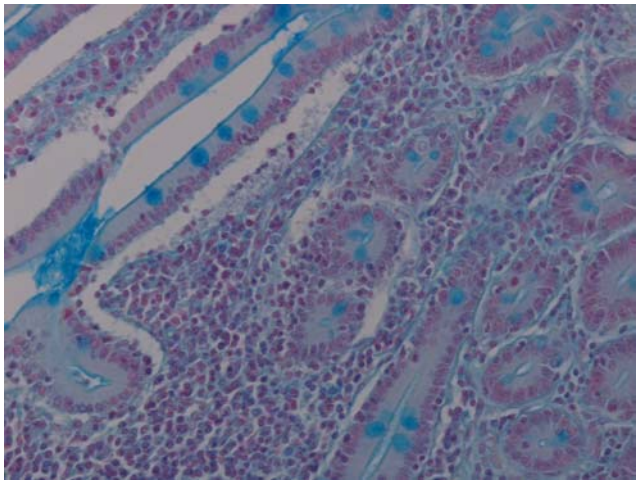

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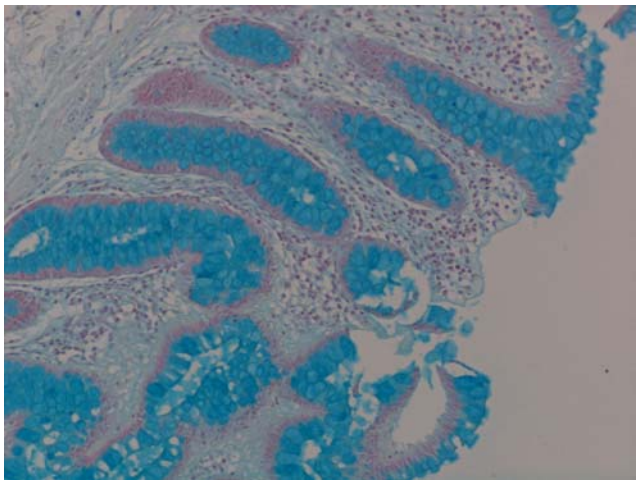

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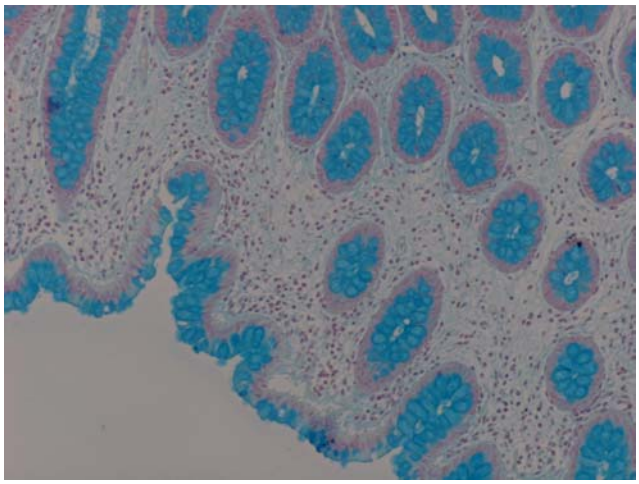

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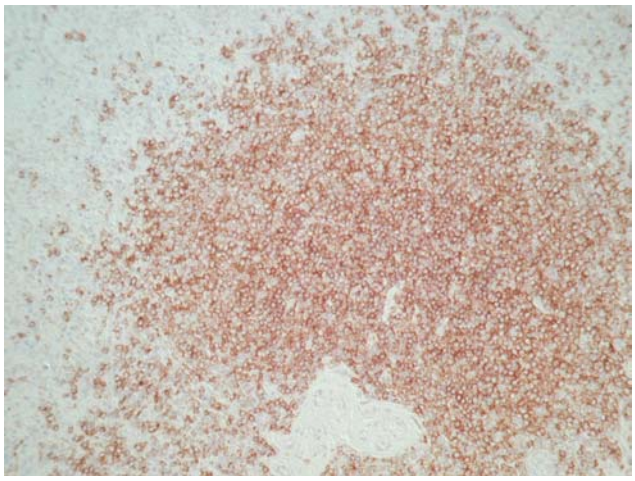
8.3 - Immunohistochemistry images appendix



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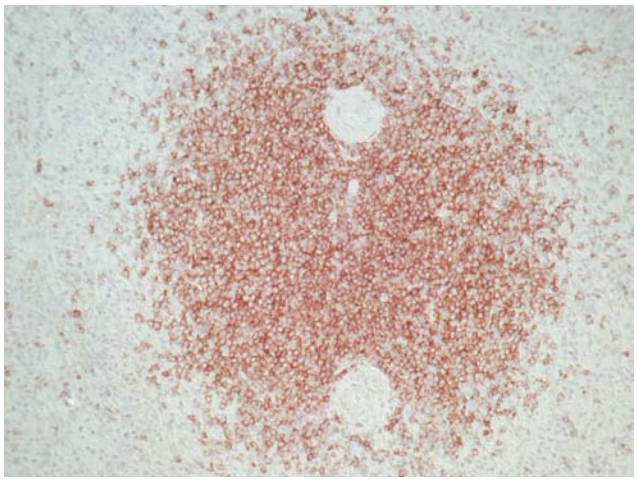

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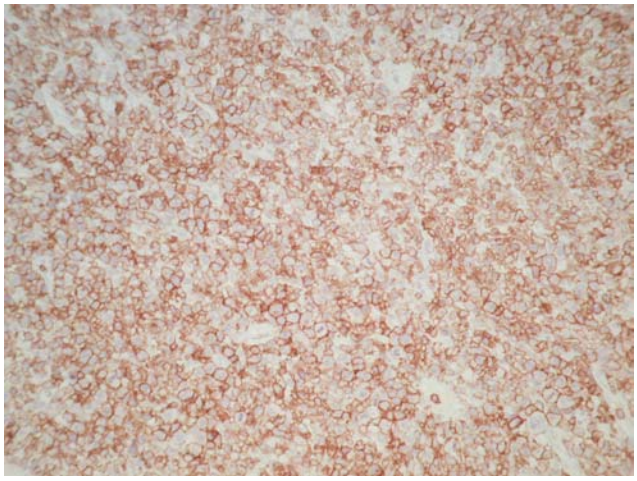

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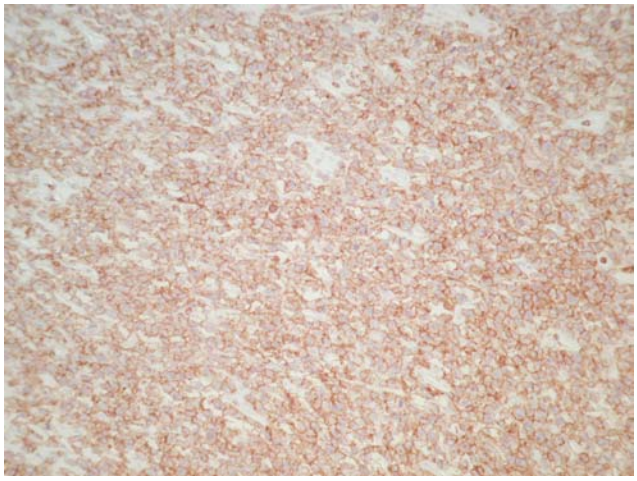

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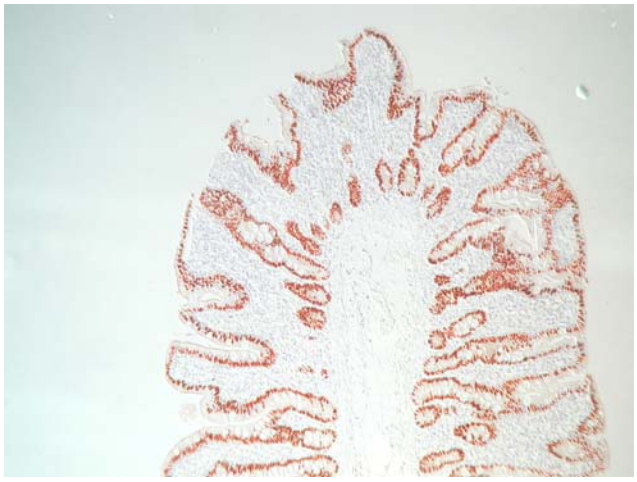

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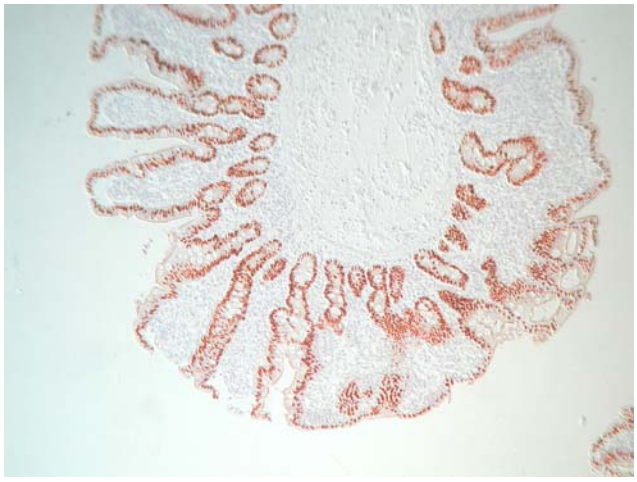

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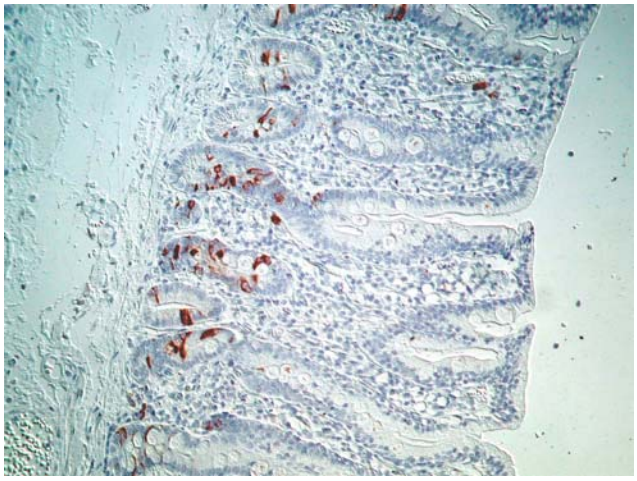
8.3 - Immunohistochemistry images appendix



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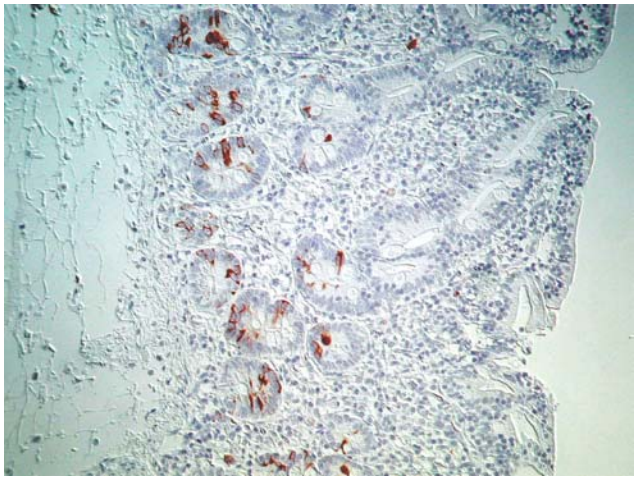

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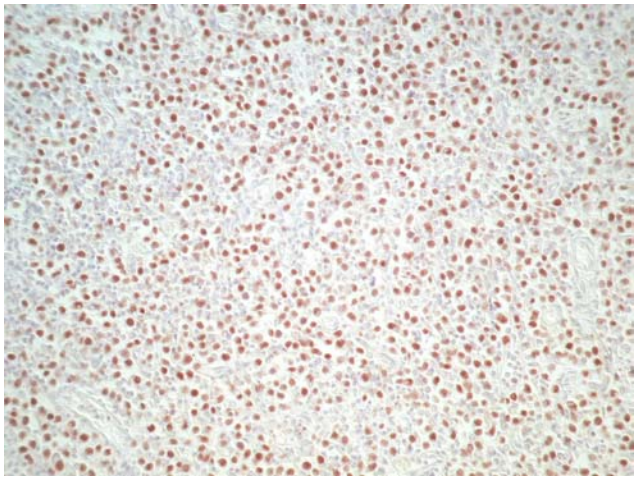

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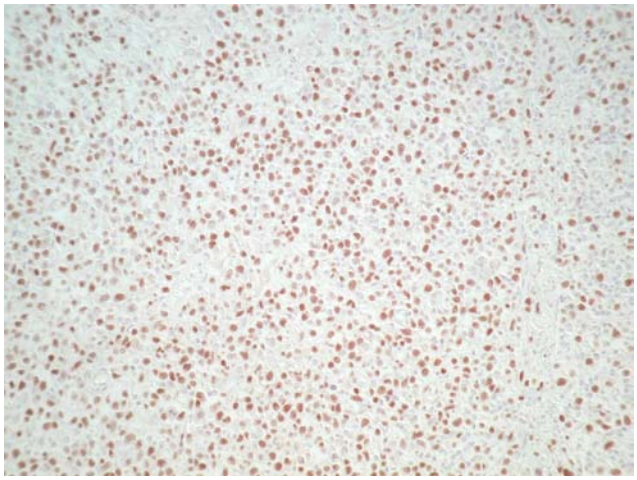

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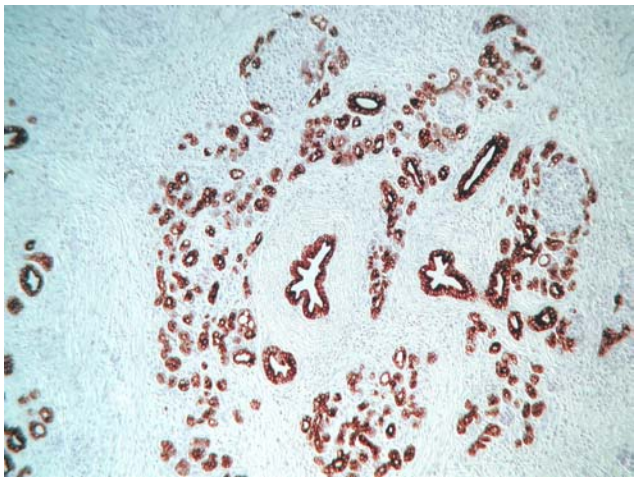

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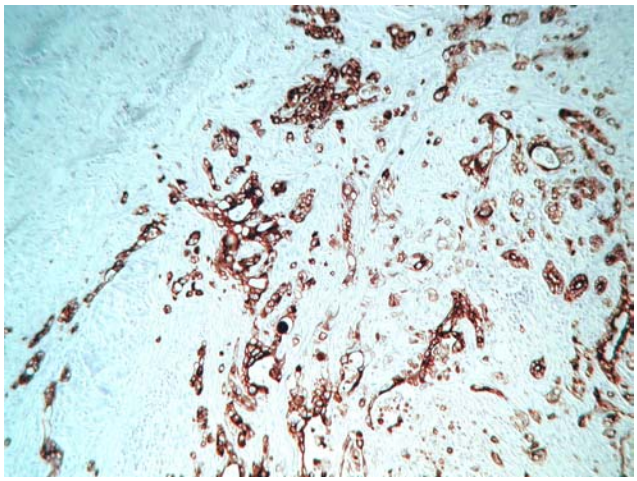

-Ck7 CASE N°40 (Pancreas)
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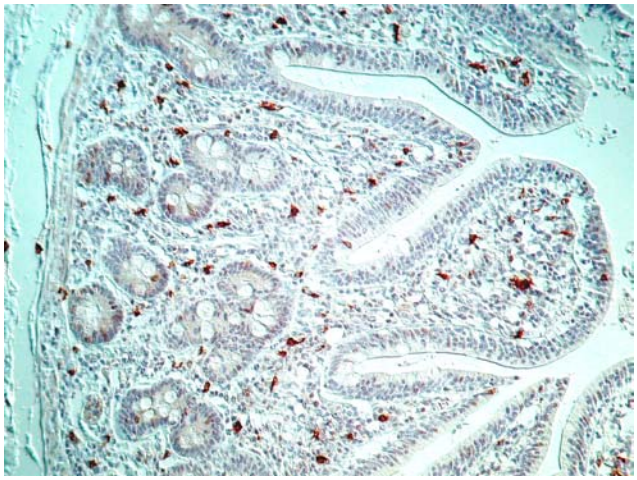
8.3 - Immunohistochemistry images appendix



-CD117 C-KIT CASE N°19 (Jejunum)
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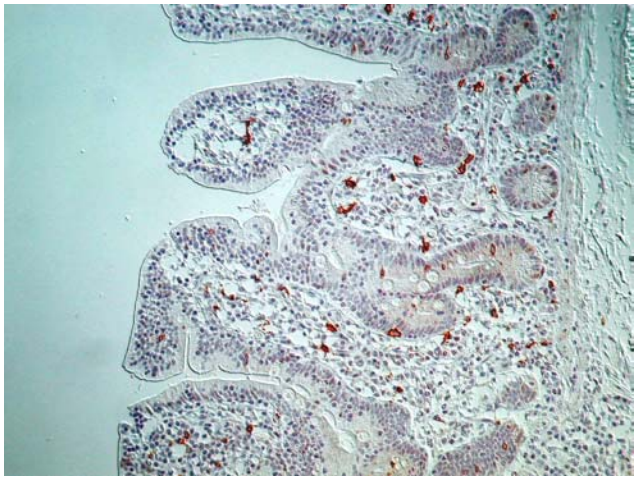

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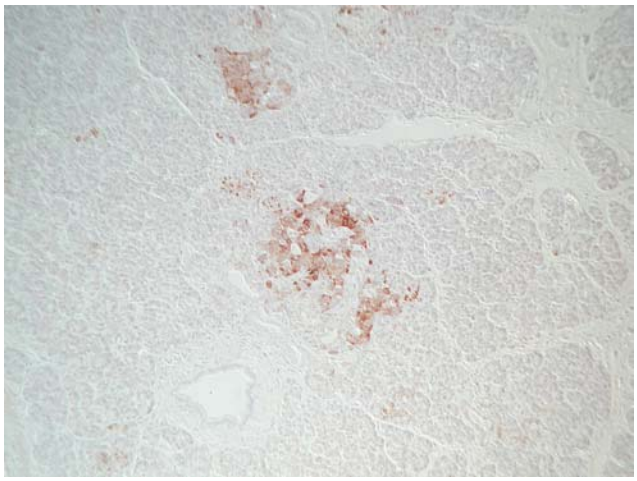

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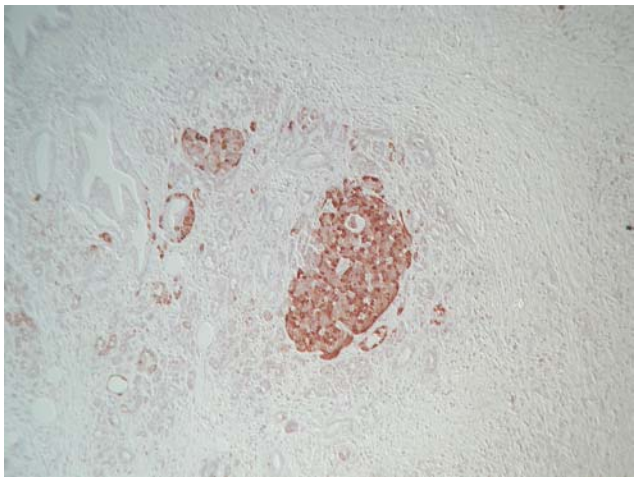

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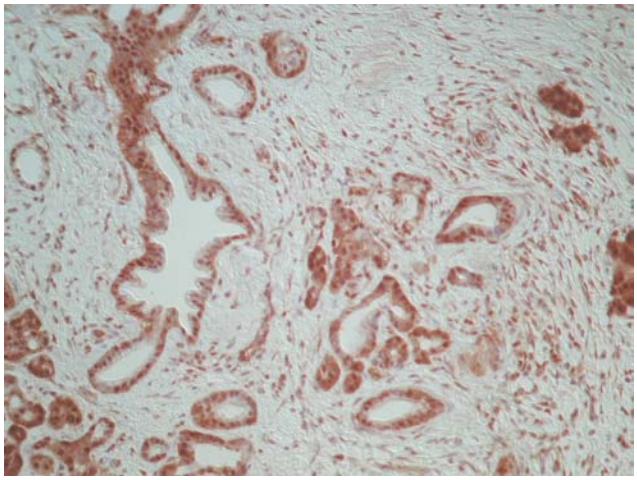

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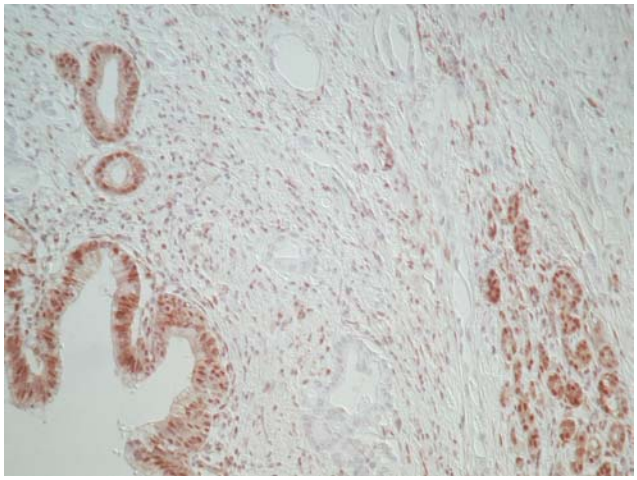

-DPC4 CASE N° 40 (Pancreas)
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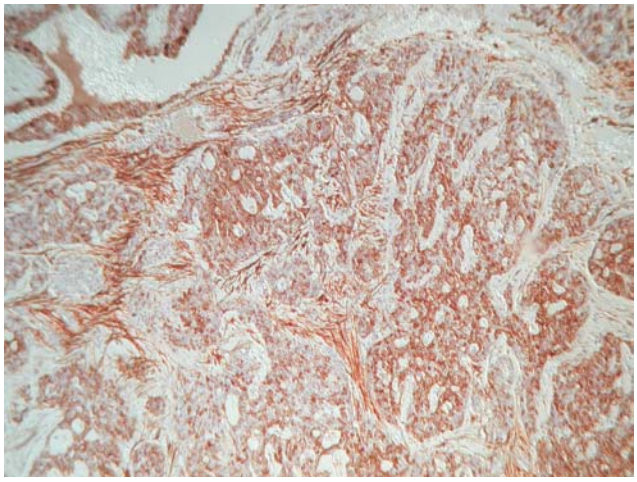
8.3 - Immunohistochemistry images appendix



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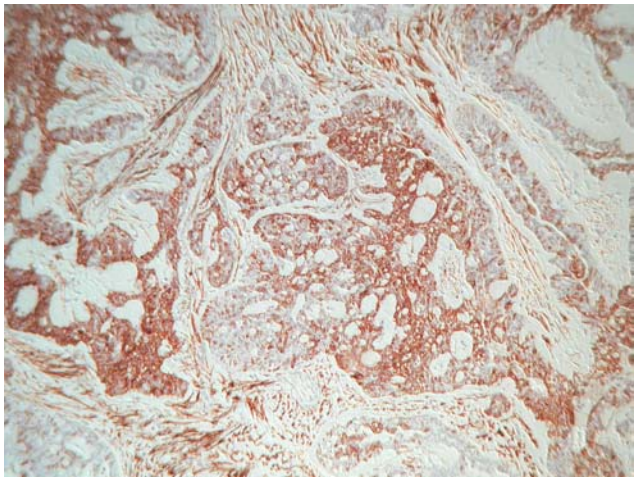

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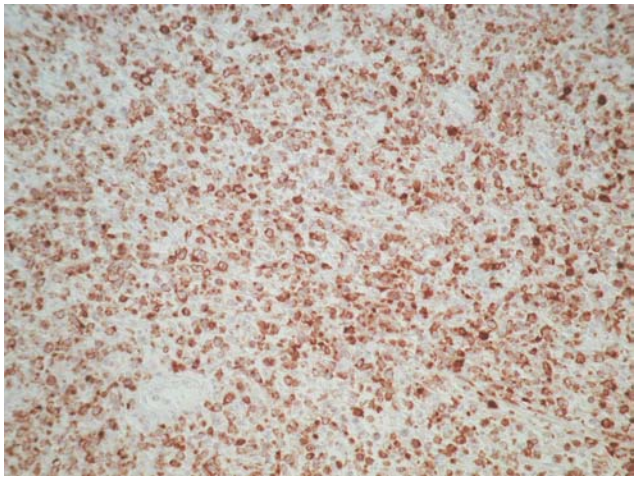

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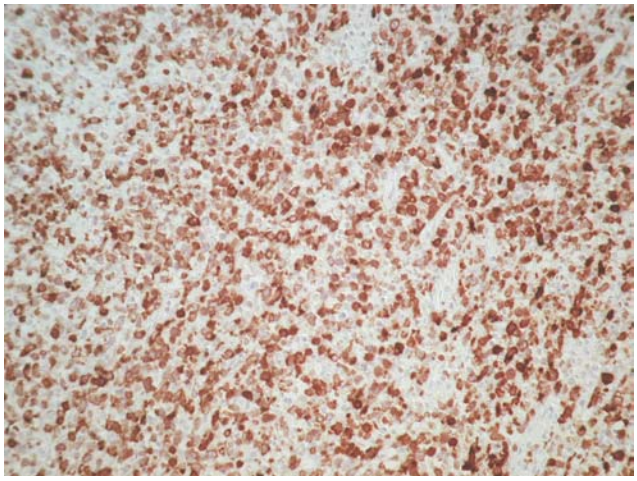

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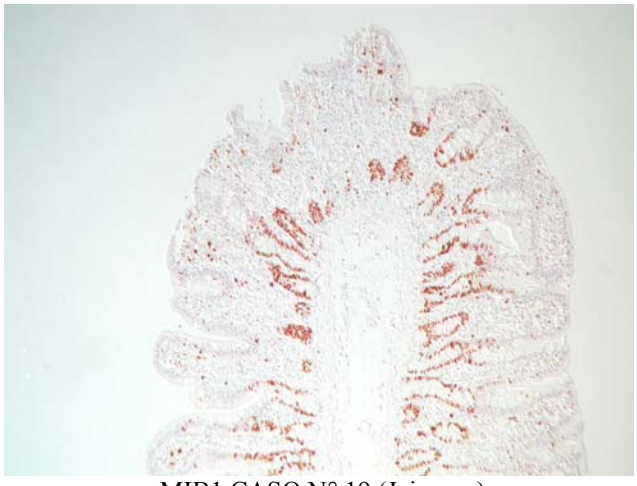

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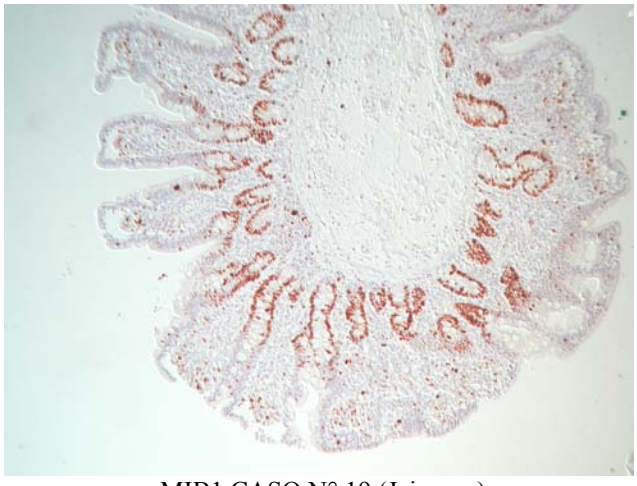

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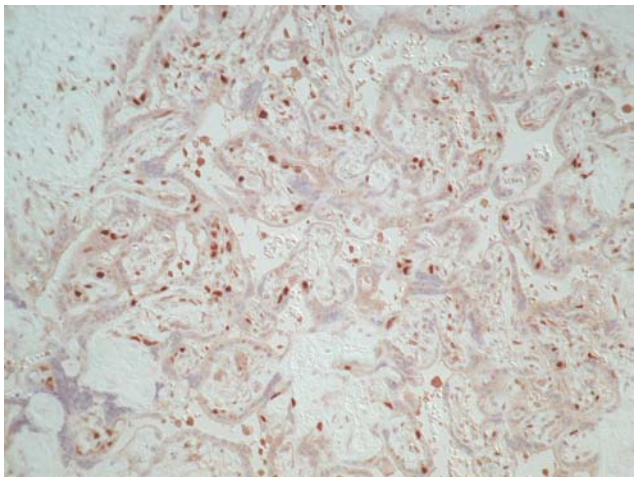
8.3 - Immunohistochemistry images appendix



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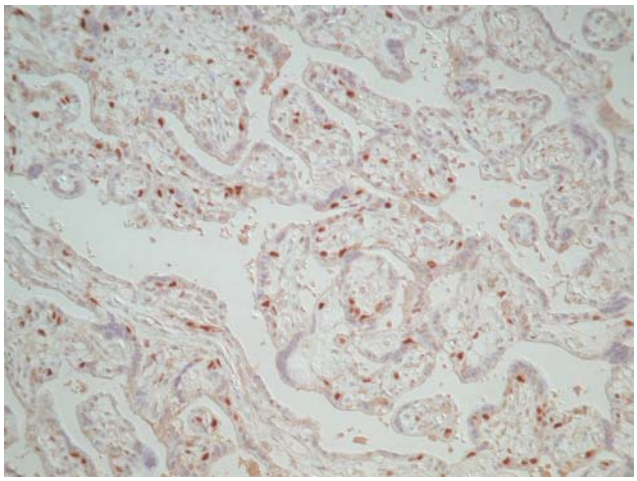

-P57 CASE N° 25 (Placenta)
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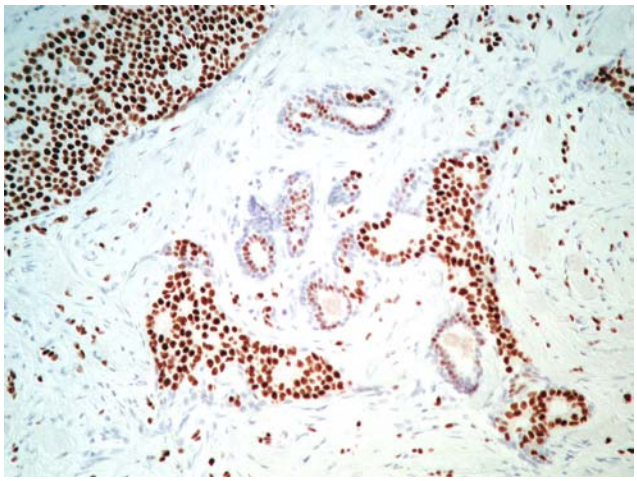

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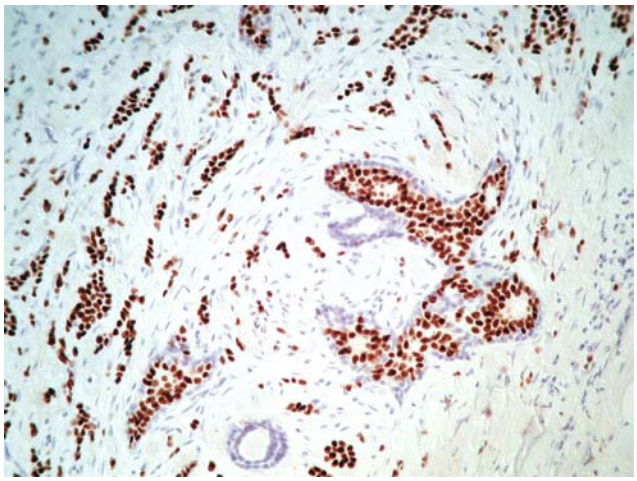

-RE CASO N° 14 (Breast)
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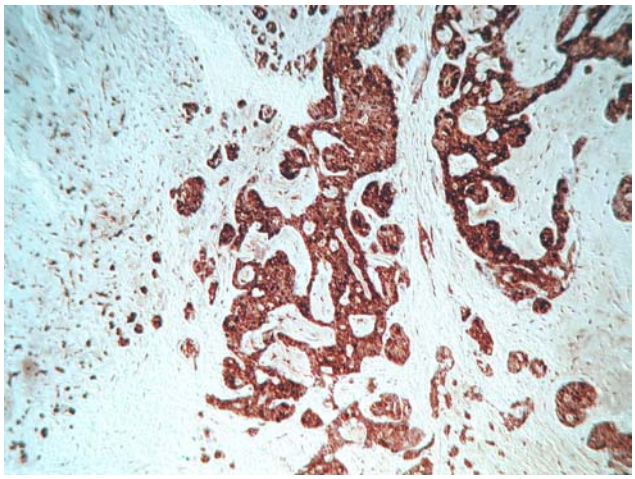

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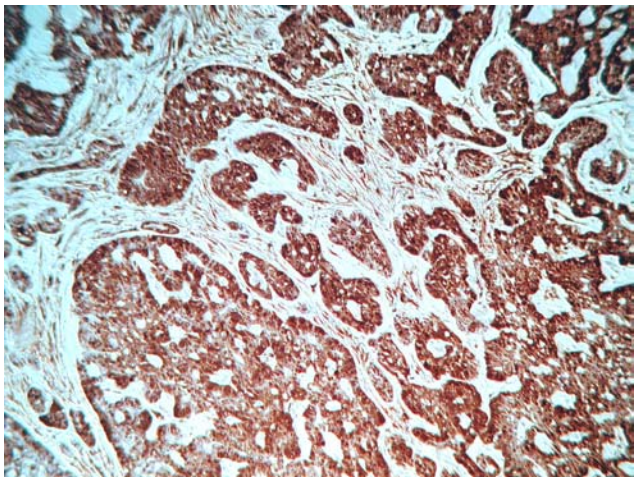

-S100 CASE N° 53 (Parotid)
OTTIX PROCESSING

9- THANKS

I would like to thank the pathological anatomy chief technician of "San Martino" Hospital of Belluno Mr. Enzo Meggiolaro and the lab manager Dr. Ettore Macrì for their collaboration.