RNA PRESERVATION IN FFPE TISSUES REQUIRES PRE-ANALYTICAL STANDARDIZED PROCESSING

BACKGROUND: Modalities of handling of surgical specimens need standardization in order to guarantee optimal preservation structure, protein antigenicity and nucleic acid sequences. For the transfer of Surgical specimens to Pathology laboratories, two types of modalities are currently practiced worldwide, according to local conditions and habits: A) Tissues are transferred fresh, B Tissues are immersed in Formalin containers. Preservation of RNA for Gene Expression profiling is notoriously poor in Formalin Fixed Paraffin Embedded (FFPE) tissues, thus hampering extended gene sequencing in routinely processed histo-pathological tissues.

a) Tissues left fresh

No Fixation (material available for banking) Temperature: Room Temperature (about 20°C) In some realities, tissues (free in a vessel or in a bag) are transferred to the grossing room. Time interval: relatively short (according to local conditions) Time in C before grossing: variable from a few minutes up to several hours. Optimal: 30 minutes

b) Tissues immersed in formalin in large boxes, then transferred (within hours) to the Pathology labs.

Advantages: -No drying -Fixation already starts at the periphery (penetration = 1 mm./hour) -Anti-septic

Drawbacks (large specimens):

- Degradation continues in deep areas
- Tissue banking is hampered
- Formalin containing vessels heavy to carry
- Spilling of formalin may occur
- Fumes dispersed while grossing
- Nurses refuse to handle this "carcinogen" in surgical theatre (and without hoods)
- Tissue forgotten by the surgeon because
- "already safe in formalin"

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Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin

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DESIGN: Our hypothesis was that RNA degradation in surgical biopsies is caused by RNAse activation before and during formalin fixation and that low temperature (4°C) could inhibit this activity and prevent degradation. In our Hospital (a large, pavillon hospital in Piedmont, Italy) the traditional habit of transferring specimens from the surgical theatre to the Pathology laboratory while immersed in large boxes filled with formalin was substituted by Under-Vacuum sealing (UVS) using the Tissue- Safe[©] apparatus (Milestone, Soresole, Italy). Sealed specimens were kept at 4°C for 1-72 h. until transfer. Grossing, fixation in Phosphate-buffered Formalin (PBF) and paraffin embedding followed. Selected specimens were processed by a 24 h. fixation in PBF at controlled temperature.

Project: "....Towards a Formalin-free Hospital" "Molinette" Hospital, Turin

1162 Beds; >54.000 yearly admissions; > 40.000 histopathological exams. (2008)

--- "Small" = < 2 cm Ø Formalin(pre-filled vials) Biopsies 25% — "Large" surgical specimens U.V. bags



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ICC evaluation of therapeutic/prognostic paremeters in a continuos series (N=375) of breast cancers Years 6/05-6/07 vs. 7/07-7/09.

c) Tissues preserved under vacuum

Merits:

- No more formalin in surgical theatre (except for small specimens, where pre-filled tubes are employed)
- No spilling
- No fumes
- No drying of tissues
- Colours preserved
- Lack of insulating air around tissues allows fast cooling
- Tissues (bags) light and easy to carry
- Structure (RNA, Antigens) preserved up to days
- Banking (selective) allowed
- Demonstrating of operated tissues is convincing for students and surgeons



RIN Rat Liver U.V.

RNA Integrity Index (RIN) is heavily dependent from storing conditions. Optimal values are still obtained after 72 hours, in UVS, cooled specimens

RESULTS: UVS is well accepted by both Surgery and Pathology staff and this procedure, now in use since 2 years, assured proper histological and immuno-histochemical quality as well as RNA preservation (RIN values above 7). Fixation in PBF at controlled conditions improved RNA preservation and gene expression analysis of paraffin-embedded tisues.

Breast ca	ncers	process	ed by USV and cooling (4°C		
histo ID	gan	DIN	histo ID	gan	DIN
1285/09/3	gap 1h	9	6054/09/3	gap 6h	8.6
1385/09/3	60h	7.9	6056/09/3	24h	7.3
1394/09/3	60h	7,9	6058/09/3	24h	7.9
1599/09/3/B	60h	7,4	6226/09/3	2h	6.9
1271/09/3	1h	7,8	6263/09/3	5h	7,8
1498/09/3	5h	7,5	6368/09/3	72h	7,6
1469/09/3	5h	7,9	6369/09/3	72h	8
1422/09/3	2h	8,7	6422/09/3	24h	7,1
1267/09/3	5h	8,2	6520/09/3	24h	7,7
1698/09/3/m	24h	8,7	6552/09/3	24h	7,1
1783/09/3	60h	7,9	6753/09/3	24h	7,8
1/93/09/3	60h	7,6	6849/09/3	72h 24h	7,8
1844/09/3	111 115	8,7 8,5	0907/09/3III 7004/00/3	2411 6b	ð,/ 8 3
1904/09/3	111 24h	8, <i>3</i> 7 3	7004/09/3 273/09/Domen	$\frac{011}{2h}$	8,5
1992/09/3	2411 5h	7,3	7129/09/3	211 6h	8.1
1995/09/3	72h	8.1	7249/09/3	2h	9.1
2032/09/3	7h	7,5	7250/09/3	4h	9,6
2163/09/3	60h	8,1	7362/09/3	6h	9,4
2241/09/3	1h	8,9	7405/09/3	6h	7,9
2238/09/3	6h	8,4	7489/09/3	5h	9,1
2237/09/3	5h	8	7514/09/3	24h	8
2574/09/3	6h	7,9	7613/09/3	6h	7,3
2621/09/3	24h	7,6	7781/09/3	8h	7,9
2622/09/3	24h	6,8	7782/09/3	6h	7,7
2/12/09/3	/2h 20h	/,4	/805/09/3	5h	/ 7.0
2003/09/3	20n 72h	7,8 6.4	7880/09/3	5n 24b	7,9
2745/09/3	7211 24h	0,4	8153/09/3	2411 7h	8,0 7
2817/09/3m	24h	7,2	8215/09/3	71 72h	9
3216/09/03	1h	7	8413/09/3	72h	6.5
3365/09/3	7h	8,1	8542/09/3	5h	7,2
3368/09/03	7h	7,7	8623/09/3	5h	7,6
3400/09/3	24h	8,7	8687/09/3	7h	8,6
3673/09/3	1h	9,6	9000/09/3	24h	7,4
3657/09/3	72h	7,5	9112/09/3	24h	7,8
3/01/09/3	24h		9182/09/3	4h	8,1
3/64/09/3	48h 72h	6,6 7.9	9183/09/3	/h 491-	6,9
3888/09/3	72fi 72h	/,0	9248/09/3	4811 24h	8 8 7
3850/09/3	7211 70h	0,2 7 Δ	9324/093	2411 5h	8,7 7 9
3993/09/3	70h 72h	7,4	9461/09/3	7h	7,5
4014/09/3	2h	7,6	9585/09/3	24h	8.6
4073/09/3	6h	9,1	9687/09/3	4h	7,1
4075/09/3	5h	7,9	108/10/3	5h	7,7
4122/09/3	20h	7,3	143/10/3	5h	8
4221/09/3	70h	7,4	305/10/3	2h	7,2
4211/09/3	70h	6,2	435/10/3	5h	8,2
4275/09/3	7h	9,4	506/10/3	6h	8,6
4298/09/3	24h	6,6	651/10/3	5h 21	8,5
44/1/09/3	48n 20h	8,0	650/10/3	3n 22h	0,8 6 8
4555/09/5	5011 72h	0 6 2	049/10/3	2311 72h	0,8
4617/09/3	72n	8.9	1267/10/3	7211 7h	8,0 7 8
4668/09/3	24h	8.6	1316/10/3	5h	7,3
4831/09/3	5h	8,3	1453/10/3	24h	6,5
4937/09/3	72h	8	1677/10/3	6h	9
5038/09/3	24h	8,2	1775/10/3	7h	9,2
5118/09/3	72h	6,2	1944/10/3	7h	8
5357/09/3	24h	7,3	1945/10/3	4h	7,4
5525/09/3	5h	7,8	2033/10/3	5h	9,1
5598/09/3	1h	7,9	2036/10/3	24h	7,4
5661/09/3	7h	7,1	2096/10/3	24h	9,5
5/04/09/5 5877/00/2	on 5h	0,2 7 <i>A</i>	2082/10/3	SN	0,0

One hundred twenty nine consecutive cases of breast cancers, stored in UVS at 4°C for a time between 1 and 72 hours (mean 23 h), before grossing. A specimen (punch biopsy) was taken, immersed in RNA later[©] and sent for Gene Expression Analysis (GEA). Evaluation of RNA values proved that in all cases the material was fit for GEA analysis (RIN value mean 7.9)

CONCLUSIONS: Standardization of pre-analytical processing is mandatory for a proper preservation and processing of surgical biopsies. Their transfer in UVS assured standard quality for structural, antigenic and nucleic acids analysis.