

**EUROPE
BIOBANK
WEEK**

SEPTEMBER 13-16, 2016
VIENNA - AUSTRIA

**Martina Loibner: "Preservation of Tissues at Room Temperature for
Molecular Analysis"**



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Europe Biobank Week

Preservation of Tissues at Room Temperature for Molecular Analysis

Martina Loibner

15/09/2016

Preservatives for tissues and other biological specimens have to block all chemical processes to:

- 1) Preserve morphology**
- 2) Allow retrieval of antigens**
- 3) Snap-shot current biomolecular status**
- 4) Inactivate pathogens**

= 10 % formalin solution containing 3,7 % or 4 % formaldehyde by mass or volume, respectively, buffered to pH 6,8 to pH 7,2.

100% Formalin = saturated formaldehyde solution containing a mass fraction of 37% (corresponding to a volume fraction of 40%) formaldehyde.

CEN/TS 16827-1:2015

**Molecular in vitro diagnostic examinations -
Specifications for pre-examination processes for FFPE tissue.**

Origin: Blum F. Der Formaldehyd als Härtungsmittel. Z wiss Mikrosk **1893**;10:314-5.

Mechanism of action:

- Tissues are penetrated by hydrated formaldehyde (= methylene glycol) and formaldehyde.
- Formation of cross-links between and within proteins and nucleic acids.
- Fragmentation of nucleic acids

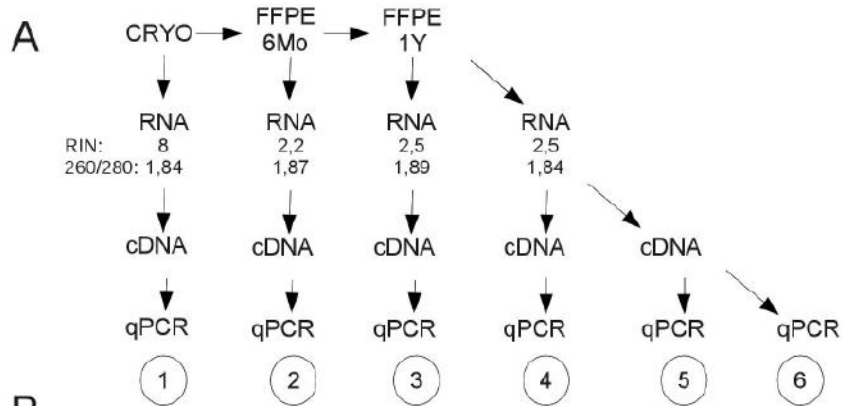
Advantages

Utility
Costs
Good morphologic preservation
Routinely trained persons available

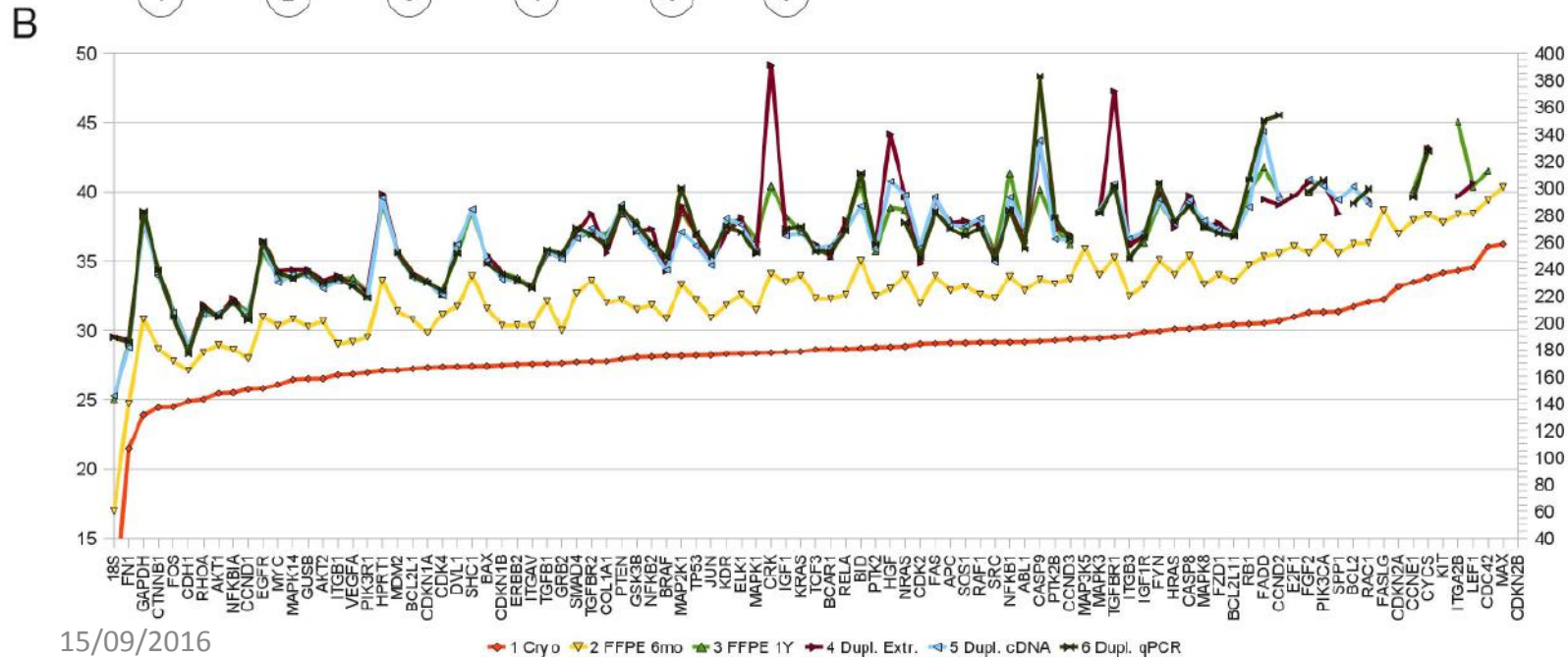
Disadvantages

Biohazard
Carcinogen
Limits quality of nucleic acids and proteins
Less usable for downstream molecular diagnostics

Fixation and storage introduces major gene-to-gene variations in qRT-PCR.

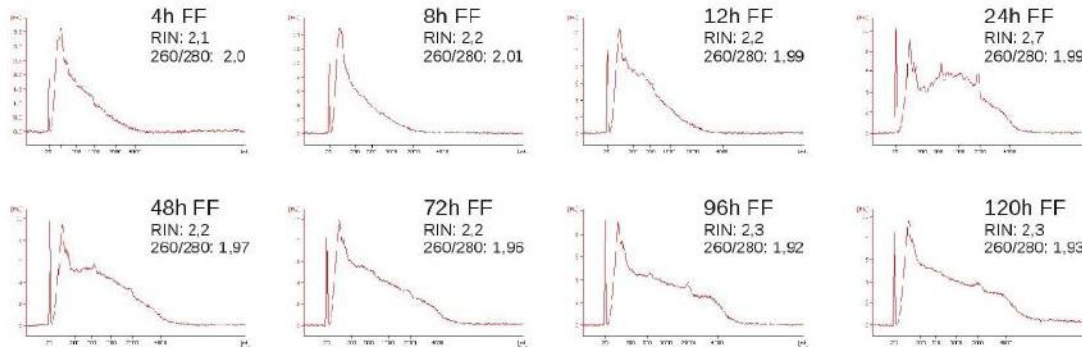


Kashofer et al. Quality Control of RNA Preservation and Extraction from Paraffin-Embedded Tissue: Implications for RT-PCR and Microarray Analysis. PLoS One. 2013; 8(7): e70714.



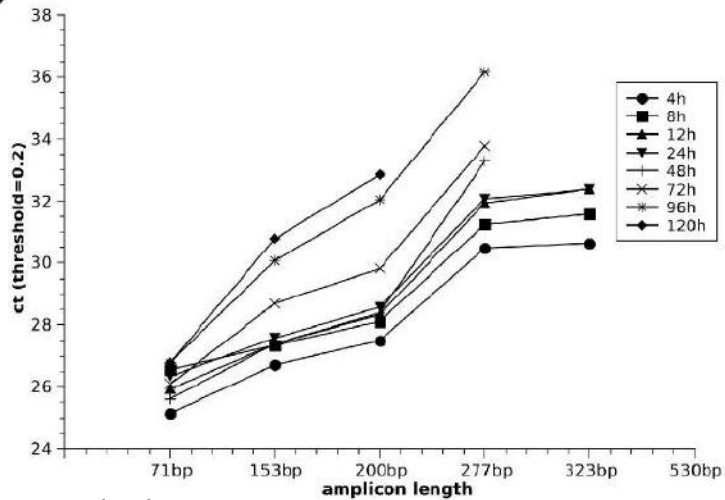
Formalin fixation impairs RNA integrity in a time dependent manner.

A



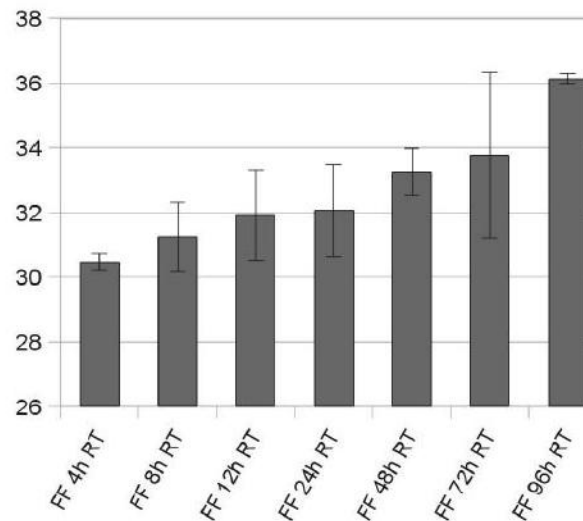
Kashofer et al. 2013

B



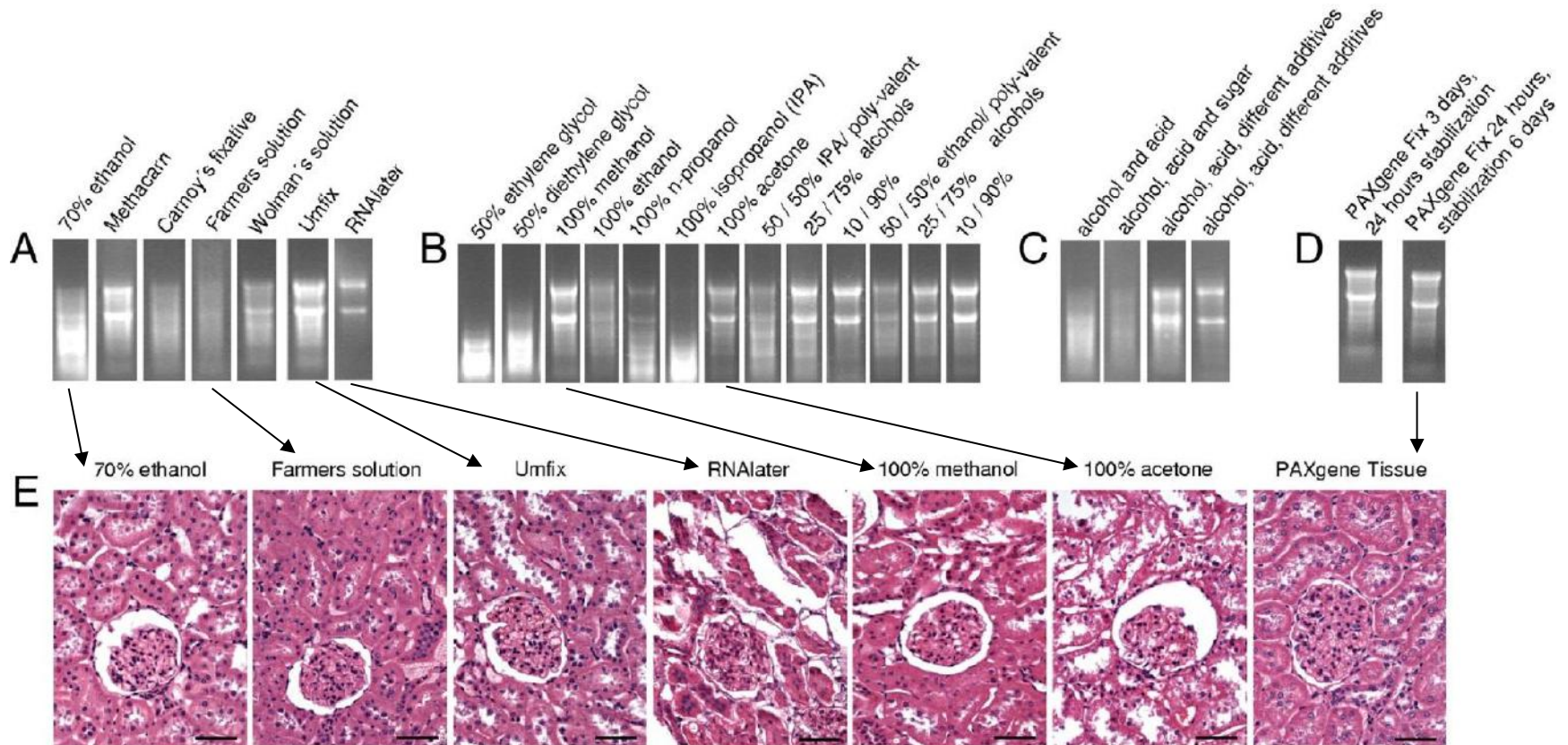
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C



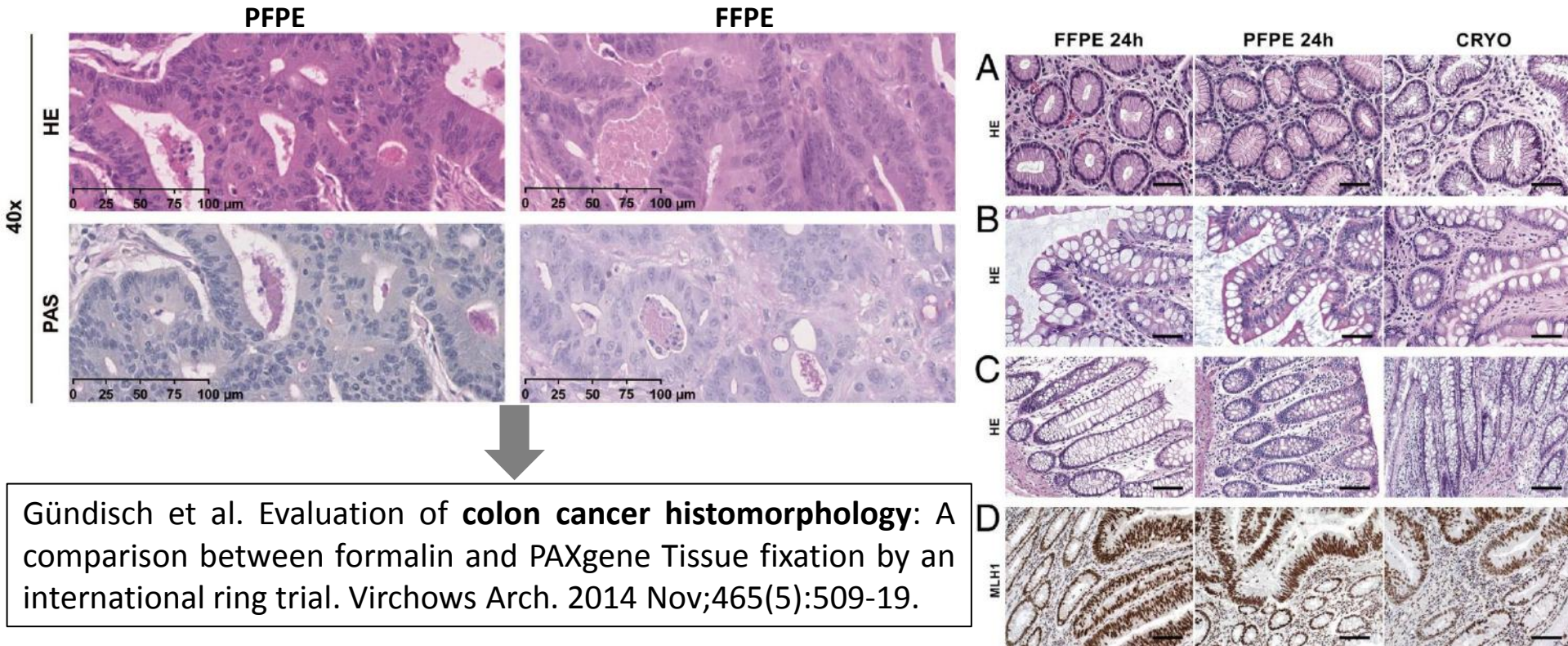
Variety of alternative tissue fixatives (commercially available):

- **PAXgene tissue (PreAnalytix, Qiagen)**
- **RCL2 (Alphelys, France)**
- **RNAlater (Invitrogen, UK)**
- **zink-based Z7**
- **Allprotect (Qiagen, Germany)**
- **FineFIX (Milestone, Italy)**
- **HOPE (DCS, Germany)**
- **Streck Tissue Fixative (Streck, US)**
- **UMFix (Sakura Finetec, US)**
- **Etc...**



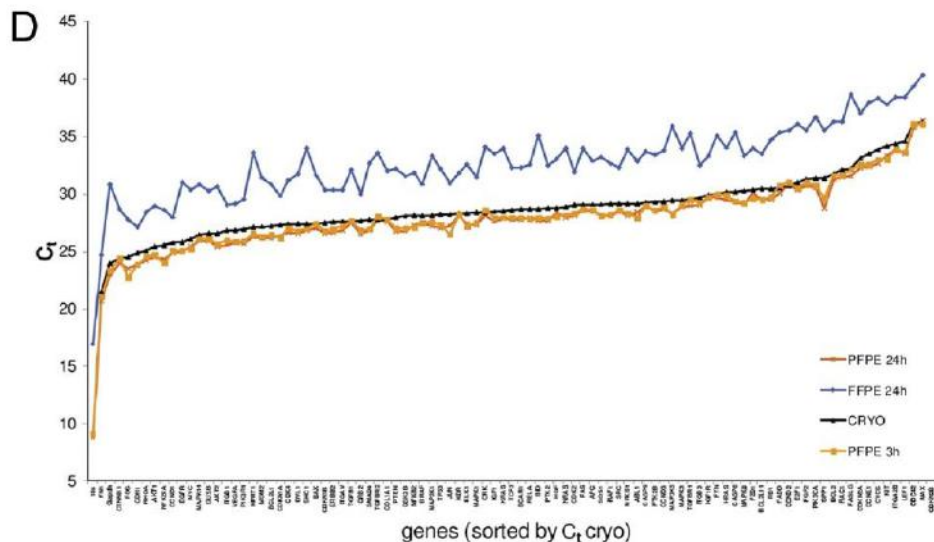
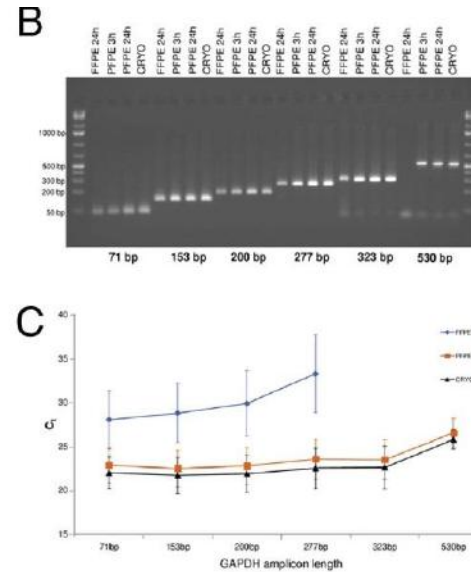
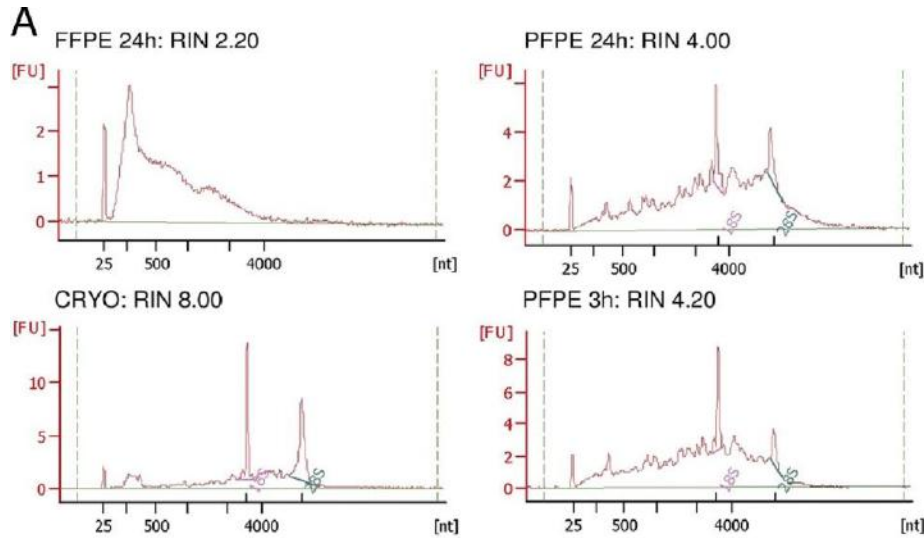
Viertler et al. A New Technology for Stabilization of Biomolecules in Tissues for Combined Histological and Molecular Analyses. *J Mol Diagnostics*, Volume 14, Issue 5, 458-466 (2012).

www.spidia.eu Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics (FP7-HEALTH-2007-1.2.5).

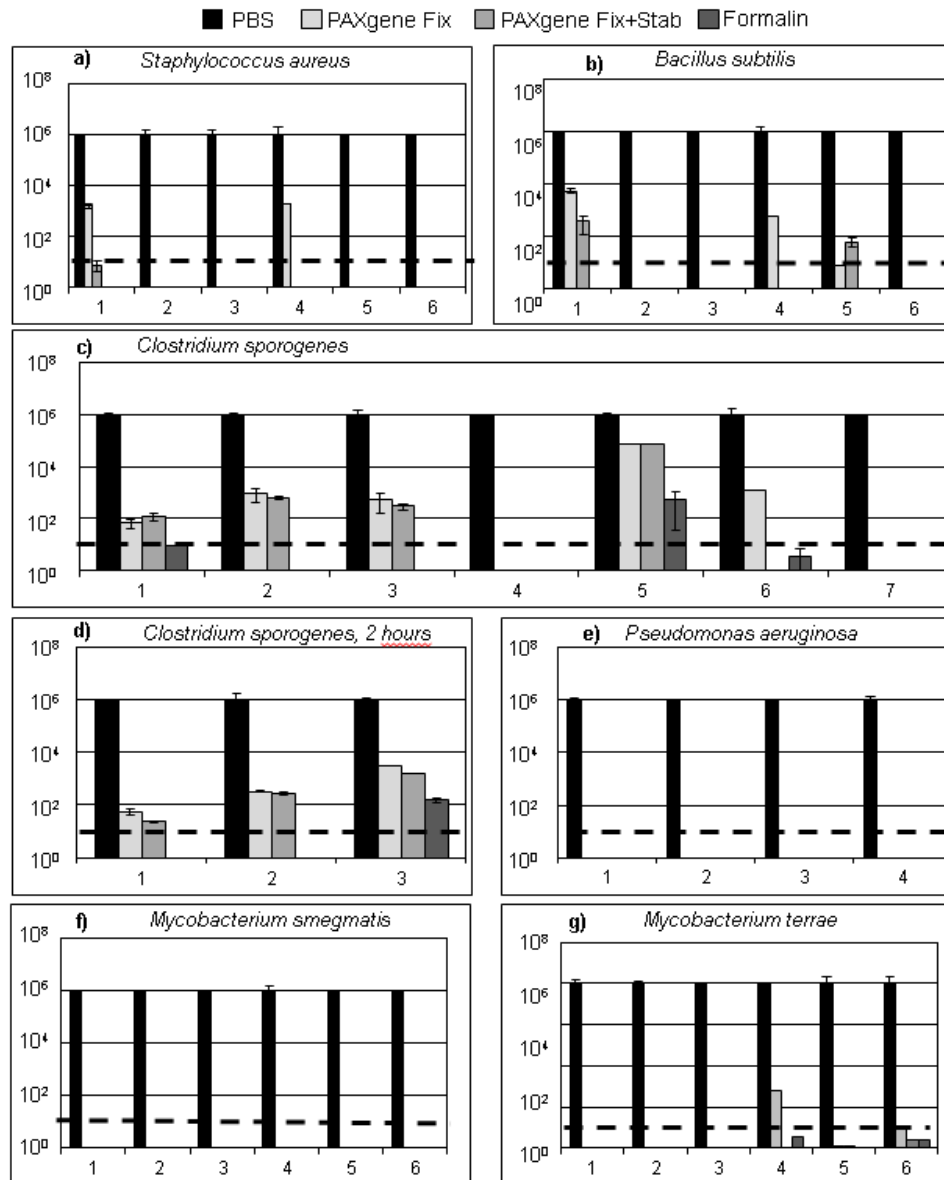


Gündisch et al. Evaluation of **colon cancer histomorphology**: A comparison between formalin and PAXgene Tissue fixation by an international ring trial. Virchows Arch. 2014 Nov;465(5):509-19.

Viertler et al. 2012. **H&E staining of tissue from human stomach** (antrum) (A), ileum (B), and (C) rectum, D: Immunohistochemical analysis of **MLH1**, a typical screening marker routinely used for hereditary colon cancer. Original magnification: 400 (A and B); 200 (C and D). Scale bars: 50 μm (A and B); 100 μm (C and D).

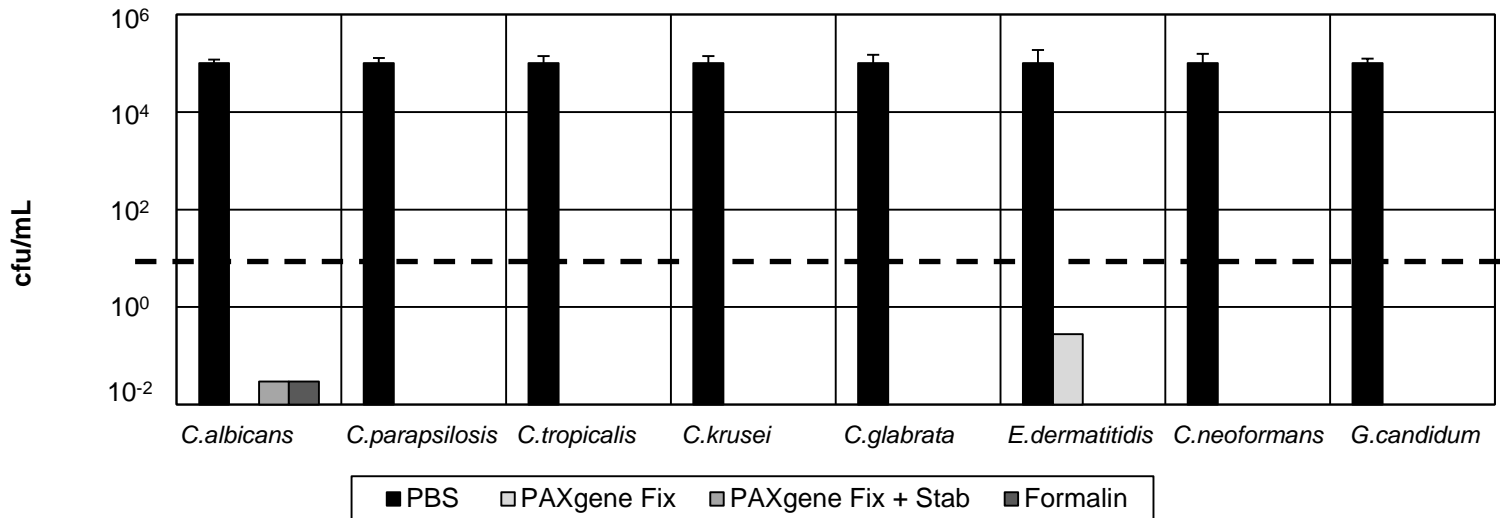


Viertler et al. (2012).
RNA preservation and gene expression.
A: RNA integrity
B: Gel electrophoresis of qRT-PCR products for different amplicon lengths of human GAPDH amplified from human nonmalignant liver tissue.
C: Summary of CT values for more than 800 qRT-PCR assays, based on different amplicon lengths of GAPDH (71 to 530 bp).
D: Gene expression analysis of 92 cancer genes.



Loibner et al.
Pathogen Inactivating Properties and Increased Sensitivity in Molecular Diagnostics by PAXgene, a Novel Non-Crosslinking Tissue Fixative. March, 2016, PLoS ONE 11(3): e0151383. doi: 10.1371/journal.pone.0151383

Reduction of Yeasts



Reduction of Mould Fungi	Strains + experiments n =	Mean dilution of PBS growth controls	cfu/ml after PAXgene Fix	cfu/ml after PAXgene Fix + Stabilizer	cfu/ml after formalin fixation
<i>Aspergillus flavus</i>	6	10 ⁻¹²	0.8	0.0	0.1
<i>Aspergillus fumigatus</i>	6	10 ⁻¹²	8.0	0.0	4.0
<i>Aspergillus niger</i>	3	10 ⁻¹²	2.0	0.3	0.0
<i>Aspergillus terreus</i>	6	10 ⁻¹²	8.0	0.0	0.0
<i>Penicillium chrysogenum</i>	6	10 ⁻¹²	3.1	0.3	6.8
<i>Absidia corymbifera</i>	6	10 ⁻¹²	0.0	0.0	0.0
<i>Rhizopus oryzae</i>	6	10 ⁻¹²	0.0	0.1	0.0
<i>Cunninghamella bertholletiae</i>	6	10 ⁻¹²	0.0	0.0	3.0
<i>Rhizomucor pusillus</i>	2	10 ⁻¹²	0.0	0.0	0.0
<i>Alternaria alternata</i>	2	10 ⁻¹²	0.0	0.0	0.0
<i>Scedosporium apiospermum</i>	6	10 ⁻¹²	0.0	0.0	0.0
<i>Fusarium solani</i>	6	10 ⁻¹²	0.0	0.0	0.0
<i>Paecilomyces lilacinus</i>	4	10 ⁻¹²	0.0	0.0	0.0
<i>Scopulariopsis brevicaulis</i>	6	10 ⁻¹²	0.0	0.0	0.0

CMV inactivation and reinfection of MRC-5 cells. Time course and IHC

15 min

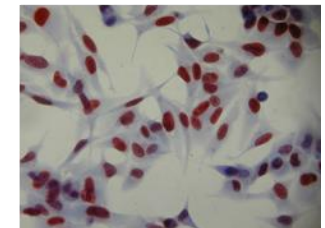
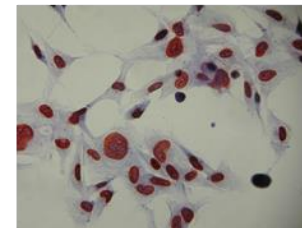
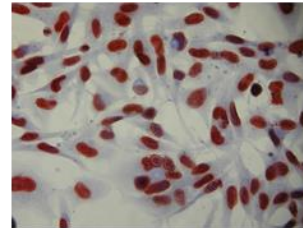
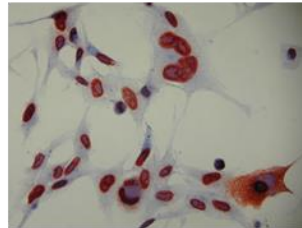
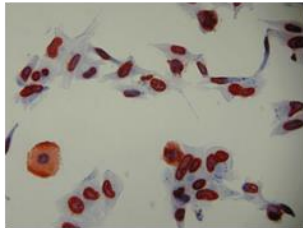
1 h

3 h

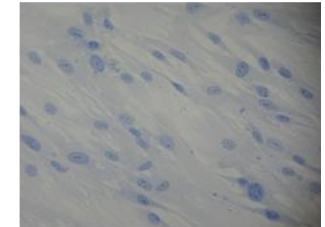
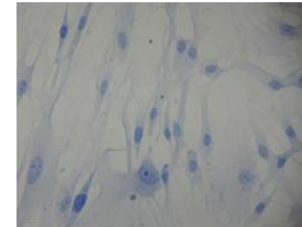
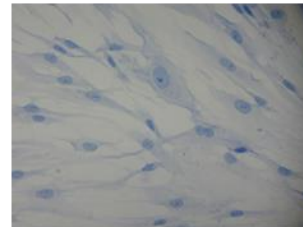
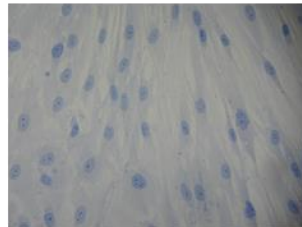
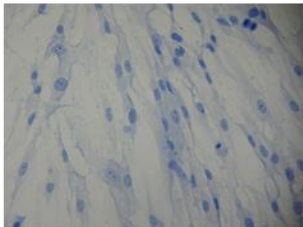
6 h

24 h

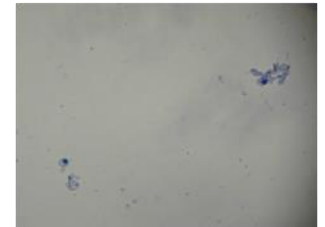
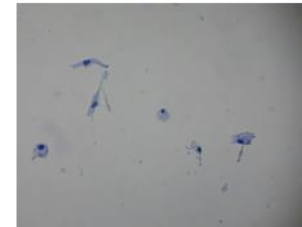
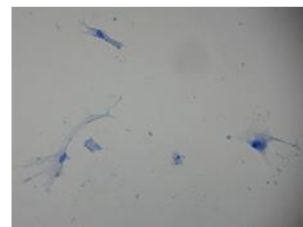
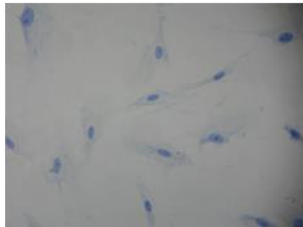
PBS



PAXgene



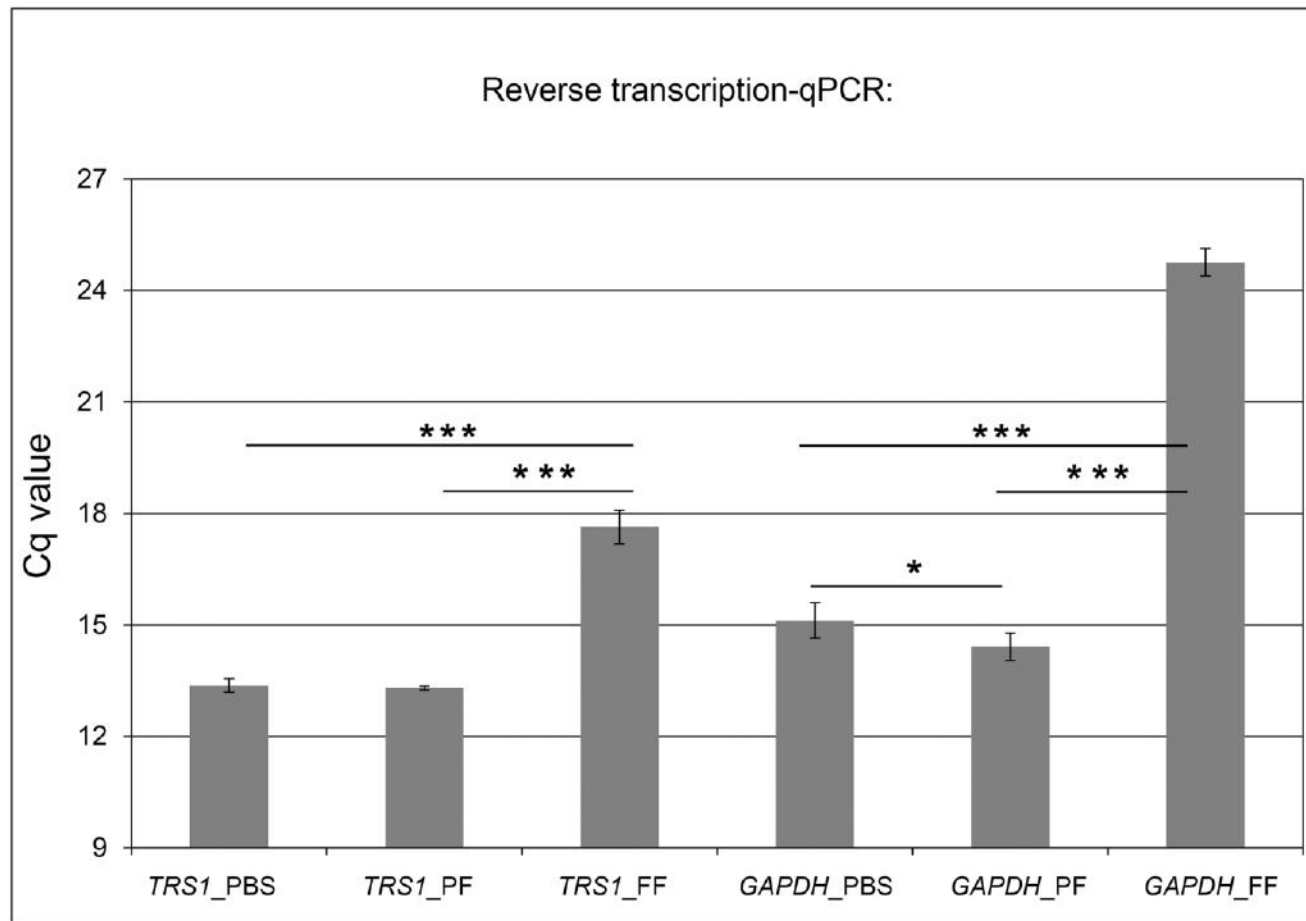
Formalin



Published in: Kap M, Aron G, **Loibner M**, Hausleitner A, Siaulyte G, Zatloukal K, et al. Inactivation of influenza virus, adenovirus and cytomegalovirus with PAXgene tissue fixative and formalin. Biopres Biobank 2013;11(4):229-34.

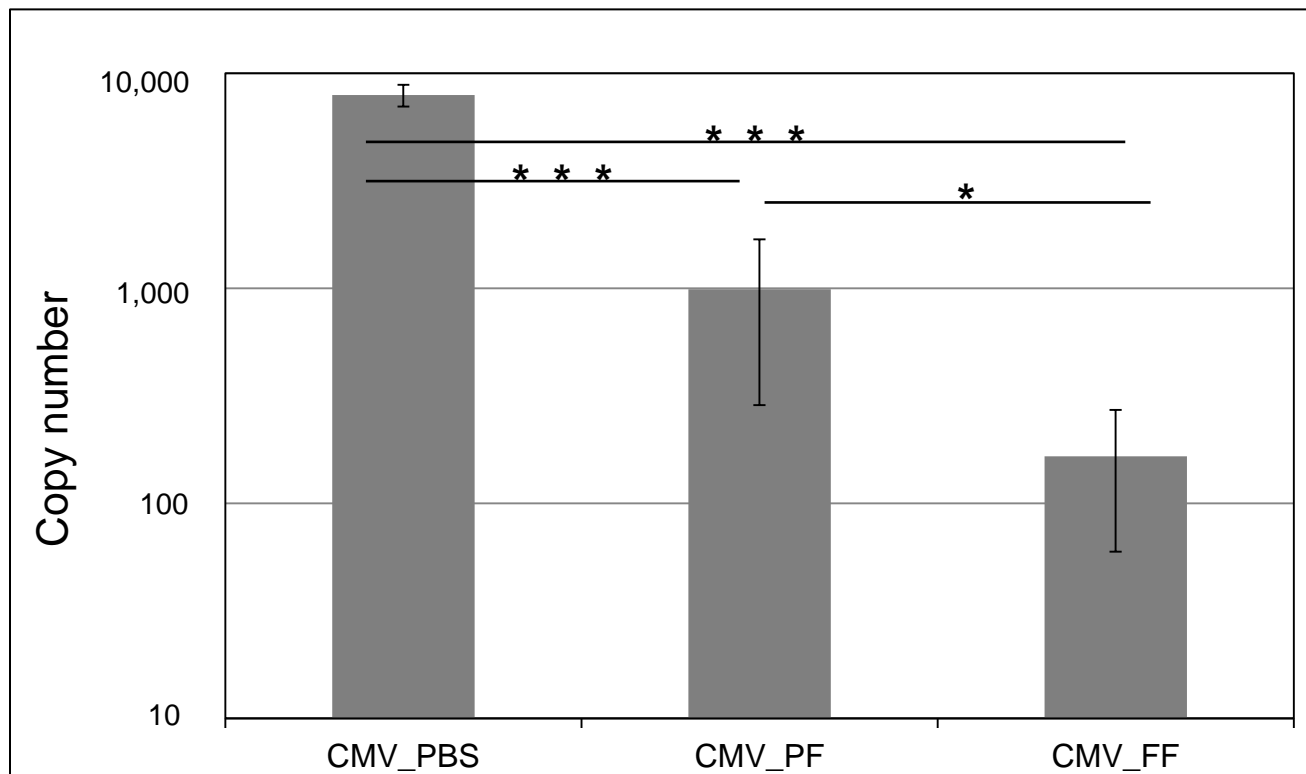
Reverse transcription qPCR: comparison of Cq values of PAXgene and formalin-fixed CMV samples. Low Cq values indicate early detection.

Statistical significance $p < 0.0001$ (***) or $p < 0.03$ (*). Loibner et al. 2016.



Quantitative real-time PCR, comparison of detected CMV copy numbers of PAXgene and formalin-fixed samples. CMV-DNA copy numbers detected by IVD-approved artus CMV RG PCR Kit (Qiagen) show a significant difference between PAXgene (CMV_PF) and formalin fixed CMV (CMV_FF) infected samples compared to unfixed CMV samples (CMV_PBS).

Statistical significance $p < 0.0001$ (***) or $p < 0.05$ (*). Loibner et al. 2016.



Fixative	Morphology	Immuno-histochemical Staining	Pathogen Inactivation	RIN	qPCR Applicability	Sequencing Applicability	Western Blot	FISH
Formalin	+++	+++	+++	-	-	+	+	+++
PAXgene Tissue	+++	++	++	++	+++	+++	+++	+++
RCL2	+	+	?	-	-	?	?	++
RNAlater	-	-	?	+	?	?	?	?
Zink-based Z7	+++	++	?	-	-	?	?	++
Allprotect	-	-	?	+++	+++	+++	+++	-
FineFIX	+	+	?	+	+	?	?	?
HOPE	+/-	+	?	+	+/-	?	+	?
UMFix	+/-	+	?	+	++	++	+	?

- PAXgene allows a variety of molecular applications
- No cooling chain necessary
- Field sampling is applicable
- Long term storage provides reliable data

Thank you for your attention!

Thank you to the teams

**at the Institute of Pathology,
Medical University Graz, Austria**

Univ. Prof. Dr. Kurt Zatloukal
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Daniela Pabst
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Dr. Karl Kashofer

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Dr. Tomasz Krenz