Prolonging fixation time of an alternative fixative to formalin for dermatological samples using standard laboratory protocols

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ABSTRACT

Aims Though formalin remains to be the gold standard fixative in pathology departments, analytical challenges persist for nucleic acid evaluations. In our laboratory, formalin fixation of skin samples in particular impairs diagnostic accuracy and demands repetition of biopsies and analytical procedures. PAXgene Tissue Systems may be an alternative; however, according to manufacturer specifications it only allows fixation for 48 hours before having to add a stabiliser. This may be a challenge in laboratories, which are closed in weekends and bank holidays. Our aim was to validate this alternative fixative for dermatological samples with prolonged fixation times using standard laboratory protocols developed for formalin-fixed specimens. We compared the results with gold standard formalin fixation.

Methods Skin specimens were formalin or PAXgene fixed for either 2 hours, 24 hours, 3 days or 7 days, paraffin-embedded, analysed and scored by observers.

Results Generally, formalin outperformed PAXgene fixation in H&E stains and fluorescence in situ hybridisation (FISH), but both seem usable for diagnostics. Time of PAXgene fixation did not have an impact on alcian blue-Van Gieson (ABVG), H&E (p=0.48), nor immunohistochemistry (p=0.74). There was a tendency towards best PAXgene performance at 24 hours of fixation for FISH, and for DNA integrity analysis 24 hours or 3 days.

Conclusions Prolonging PAXgene fixation time to 3 days before adding stabiliser does not seem to have major impact on performance of general diagnostic analysis, but our preliminary results show optimisation of internal protocols are needed. PAXgene is an expensive alternative and may be confined to some dermatological samples.

INTRODUCTION

Formalin is relatively inexpensive, produces long-lasting and 'easy to store' formalin-fixed paraffin-embedded (FFPE) blocks.[1–3] However, the cross-linking mechanism that allows formalin to fixate can represent problems for molecular analysis, which in recent years are becoming more common in pathology departments. Not only does cross-linking occur with surrounding histones, but formaldehyde may react directly with nucleotides. This blocks nucleic acid retrieval, degrade DNA or may even alter the DNA sequence through introduction of DNA artefacts.[4–8]

In recent studies, the PAXgene Tissue System (PAXgene) seem to outperform other non-formalin-based fixatives regarding nucleic acid preservation, but also microscopic morphological evaluations seem to be comparable to FFPE blocks.[8–19] PAXgene is formalin-free and amenable to be processed for paraffin embedding.[20] Being an alcohol-based solution, it denatures and coagulates tissue proteins, changing their molecular structure to render them insoluble without establishing cross-links. The PAXgene fixative is supposed to be less harmful to health than formalin,[21] despite not being totally devoid of chemical hazards. However, a potential concern preventing PAXgene from replacing formalin is cost constraints. Also, tPAXgene only allows fixation for 48 hours before it is necessary to add a stabiliser (according to the manufacturer), which may be a practical problem with specimens arriving just before weekends or bank holidays. Implementation of new fixation standards requires flexibility in fixation time due to practicalities in a high-throughput laboratory.

At our Department of Pathology, Rigshospitalet (Copenhagen, Denmark), small dermatological or head/neck tissue biopsies sent from practitioners outside the hospital have often proven to be particularly challenging. The DNA is often fragmented and the overall yield is to sparse for subsequent molecular analysis. For example clonality analyses, which require DNA fragments >300 bp, may have inconclusive reports and result in repeated biopsies at the inconvenience of patients and personnel, hence being time and resource consuming. To our knowledge, a focus on dermatological samples and prolonged PAXgene fixation times has not previously been studied with concomitant evaluation of histomorphology, antigenicity and biomolecules.

Therefore, this study aimed to evaluate the effects of different fixation times regarding histochemical stains, immunohistochemistry (IHC), fluorescence in situ hybridisation (FISH) performance and DNA yield and quality. To achieve this, skin tissues from the breast were PAXgene-fixed for up to 7 days before adding the required stabiliser. The tissues were then paraffin-embedded, processed and analysed according to our department's routine standard laboratory procedures and protocols. Tonsil samples were also fixed in PAXgene to compare with a different type of tissue. PAXgene results were compared with gold standard formalin and scored by observers.

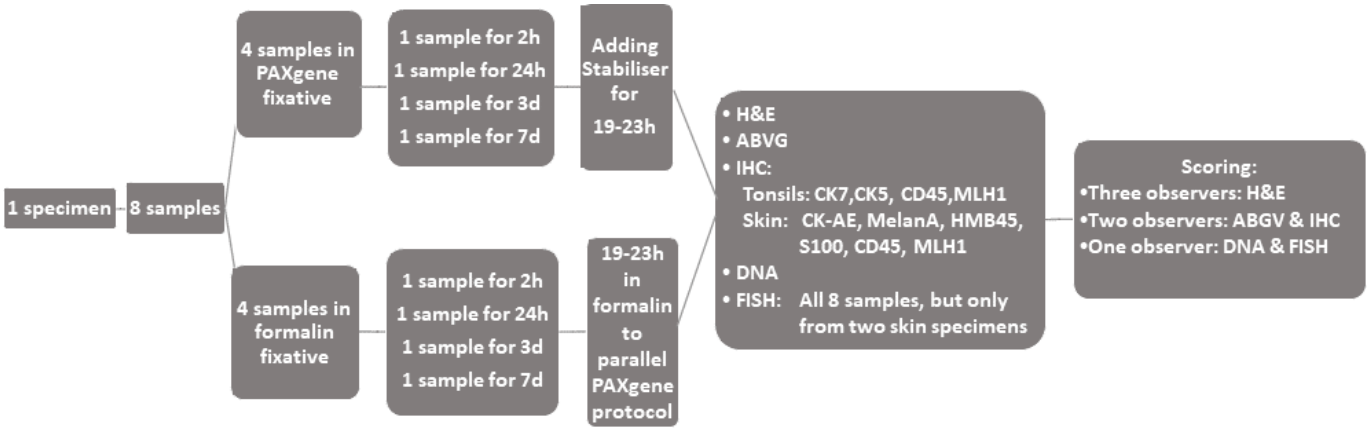

Figure 1 Present study included three tonsil and three skin specimens in total and the flowchart shows the workflow: from one specimen and tissue fixation to scoring of the various analysis. In this study, we compared the PAXgene Tissue System (PreAnalytiX, Switzerland) with the gold standard fixative formalin. PAXgene is a two-reagent fixative system in which tissues are fixed in a solution containing methanol and acetic acid (PAXgene Tissue FIX). This is followed by stabilisation in an ethanol solution (PAXgene Tissue STABILIZER). The manufacturer recommend fixation between 2 and 24 hours (preferably 3 hours minimum) for small tissue samples up to 4×15×15 mm before adding stabiliser. For larger tissue samples up to 20×20×20, the recommendation is 6–48 hours, but preferably 8–24 hours. In the product circular, it is noted that longer fixation periods may lead to degradation of biomolecules. Yet, this was what we tested in present study. Immune markers: leucocyte common antigen CD45; specific cytokeratin markers CK-AE, CK5, CK7; human melanoma black HMB45; melanoma antigen MelanA; neural marker S100; and MutL homolog 1 colon marker MLH1; DNA, DNA acid integrity analysis. ABVG, Alcian Blue/Van Gieson stain; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry.

MATERIALS AND METHODS

See figure 1 for the workflow performed between September and December 2017.

Tissue specimens

Three unfixed skin specimens from fresh mastectomies were randomly collected on two consecutive days from the routine gross examination at the Department of Pathology, Rigshospitalet, Copenhagen, Denmark. The skin specimens were placed in fixative within 4 hours after the mastectomy.

To observe PAXgene on a different type of tissue than skin, we included three fresh-frozen tonsil specimens of random adult patients, which were collected from Gentofte Hospital (Gentofte, Denmark). The tonsils were all removed due to acute tonsillitis. After removal, the tonsils were sent to our lab, sectioned into pieces of approximately 5 mm in thickness and fast frozen in dry ice, all within 30–45 min; then stored at −80°C, until defrosted at room temperature and placed in fixative.

The eligibility criteria established was the absence of carcinogenicity in the tissue.

The study was performed at Department of Pathology, Rigshospitalet, Copenhagen, Denmark and approved by the institutional review board.

We only used donors registered with consent for anonymised use of collected tissue at the 'Danish Registry for Use of Tissue' (Vævsanvendelsesregisteret) administrated by the 'Danish Health Data Protection Agency' (Sundhedsdatastyrelsen). Hence no further permissions were necessary according to the Danish Health Act.[22]

Fixation and paraffin embedding

The three skin specimens and three tonsils were each divided into eight small samples (approximately 3–5 mm each edge). Paired samples were fixed in either PAXgene fixation (PreAnalytiX GmbH, Switzerland) or 10% neutral buffered formalin (CellPath, UK) for either 2 hours, 24 hours, 3 days or 7 days at room temperature (see figure 1).

After PAXgene fixation, the Tissue FIX container reagent was replaced by PAXgene Stabiliser diluted in ethanol 99% according to the manufacturer's specifications. The PAXgene-fixed samples remained in the Stabiliser between 19 and 23 hours at room temperature. The formalin-fixed samples remained in the formalin fixative in parallel. This way, a total of 48 samples were prepared for paraffin embedding.

After the fixation protocol, all samples were processed on a PELORIS II Premium Tissue Processing System (Leica Biosystems, Germany) and, to avoid tampering by formalin, all samples fixed in PAXgene skipped the first step containing formalin, going directly to the steps with ethanol 85%, isopropyl alcohol and paraffin in the processing procedure. All samples were embedded manually using an HistoStar Embedding Workstation (Thermo Fisher Scientific, USA), creating a total of 48 blocks, that is, 8 blocks from each patient specimen (see figure 1).

Microtomy

With a 450 sliding microtome, Thermo Microm HM (Thermo Fisher Scientific, USA), sections of 1–1.5 µm were sliced and transferred to glass slides.

For DNA analysis, four 10 µm sections from the same sample were aseptically placed in a sterile microtube (Eppendorf, Germany). The microtome was cleaned with alcohol 70% and *NucleoClean Decontamination Solution* (Merck Millipore, Germany) between samples to prevent DNA contamination. The tubes were stored at 4°C for a maximum of 2 weeks before DNA extraction and analysis.

H&E and ABGV stains

Both tonsil and skin samples were processed with the department's routine haematoxylin and eosin (H&E) stain protocol with *Dako CoverStainer* (Agilent, USA). The 48 samples were also evaluated after Alcian Blue van Gieson (ABVG) stain processed on a *Tissue-Tek Prisma* (Sakura, USA) slide stainer, following the standard protocol of the department.

Immunohistochemistry

The antibodies evaluated targeted CK5 (Clone D5/16, Roche, Switzerland) and CK7 (Clone SP52 Roche, Switzerland) for all tonsil samples, CK-AE (Clone AE1/AE3, Agilent, USA), MelanA (Clone A103 Red detection, Agilent, USA), HMB45 (Clone HMB45, Agilent, USA) and S100 (Clone poly, Agilent, USA) for all skin samples, and CD45 (Clone 2B11&PD7/26, Roche, Switzerland) and MLH1 (Clone ES05, Agilent, USA) for both tissues. The immunostains were performed with BenchMark ULTRA (Ventana, USA) according to standard protocols.

The MLH1 immunomarker was included because loss of expression seems to be related to inadequate fixation.[23]

FISH

The FISH assays were performed on two skin specimens. The PAXgene-fixed paraffin-embedded (PAXFPE) tissues (fixed for 2 hours, 24 hours, 3 and 7 days) were compared only with their 24 hours FFPE tissue match. Three probes FUS, SS18 and HER2 (Zytovision, Germany) were applied to the slides and followed the departments laboratory routine procedures for FISH using *HYBrite* Dako hybridiser (Agilent, USA) and stained with 4′,6-diamidino-2-phenylindole (DAPI), (Zytovision, Germany).

DNA yield and quality

For both tonsil and skin specimens, DNA was extracted using *Maxwell RSC DNA FFPE Kit* (Promega, USA) and performing the lysis with proteinase K. DNA yields and qualities were assessed by spectrophotometry using *NanoDrop 2000* (ThermoScientific, USA) and the concentrations were adjusted to a maximum of 50 µg/µL to proceed to the PCR reaction for the size evaluation. DNA fragment size evaluation was done by preparing a mastermix with a primer able to amplify amplicons with sizes 100 bp, 200 bp, 300 bp, 400 bp and 600 bp, RedEx enzyme and deionised water.[24] PCR was performed with DNA from each sample, a negative control (water) and a positive clonal control DNA (IVS 0029, Invivoscribe) and performed on a *S1000 Thermal Cycler* (Bio-Rad, USA). The obtained size distribution for each sample was visualised using *QIAxcel Advanced* and *QIAxcel ScreenGel V.1.5.0* software (QIAGEN, Germany).

Scoring systems

H&E, ABVG and IHC stains

The scores for the morphology and quality of stains were given based on an arbitrary scale between 0 (unacceptable), 1 (poor quality but acceptable) and 2 (good quality) using a *BX53* microscope (Olympus, Japan). See details below for each stain.

H&E

Two medical laboratory scientists and one pathologist blinded evaluated the quality. The scoring table was divided into four specific criteria: nuclei, cytoplasm, other layers (eg, connective tissue) and artefacts. Each of these criteria was given a score, which included evaluation of nuclear and cytoplasmic details, disruption of the membranes, loss of components or changes in the cell's proportions as well as artefacts such as staining, shrinkage or sectioning artefacts. The staining quality was also assessed under these criteria, and was based on intensity, colour and specificity of the stain. Hence, the criteria evaluation resulted in an overall assessment score used for data processing.

ABVG

The stain was blindly evaluated by two experienced medical laboratory scientists and based on two criteria: scoring the specificity (unspecific stain, staining artefacts) and intensity/contrast of the stain. These scores were incorporated into an overall assessment score, which was used for data processing.

IHC

The IHC slides were evaluated blinded by the same two experienced medical laboratory scientists. The scoring tables were based on three criteria: the signal intensity, signal sensitivity and unspecific signalling (background noise), each given a score, which was incorporated into an overall assessment used for data processing.

FISH

Evaluation of FISH slides were performed by one experienced medical laboratory scientist, considering the integrity and intensity of the probe signals and the presence of background signalling, when counting the signals as done routinely. The score was given on an arbitrary scale of 0 (unsuitable), 1 (suitable for a preliminary counting but requires repetition for confirmation), 2 (sufficient for counting, but with small artefacts) and 3 (allows clear and unambiguous evaluation). The presence of artefacts, such as over digestion of the nuclei, autofluorescence and other features were also noted. The evaluation was performed with software LAS core (Leica, Germany) and *Leica DM6000B/DFC7000T/CTR6000* fluorescence microscope (Leica, Germany).

DNA

The obtained amplicon size distribution from the electrophoresis was evaluated blindly by one experienced molecular biologist with regards to the intensity and size of the largest fragment visualised. For the evaluation of DNA integrity: score 0 was given to bands below 400 bp being unsuitable for DNA analysis; score 1e to poorly visible bands of 400 bp; score 2 to well visible bands of 400 bp; score 3 to poorly visible bands of 600 bp; and score 4 to well visible bands of 600 bp corresponding to good preservation of DNA.

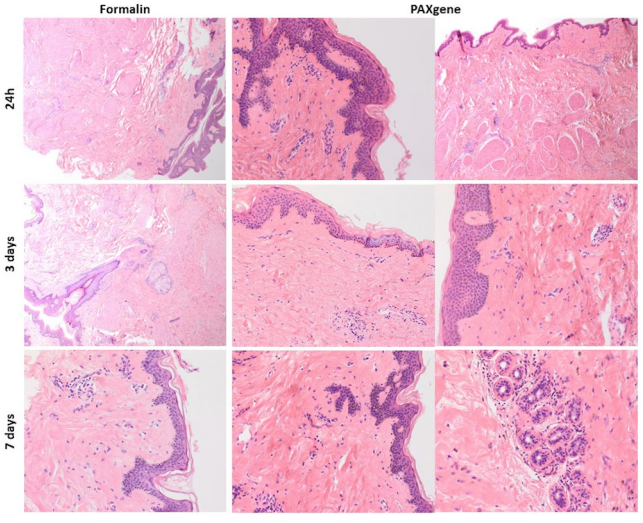

Figure 2 H&E stain of formalin and PAXgene-fixed skin samples for 24 hours, 3 days and 7 days. Overall morphology preservation in PAXgene-fixed tissues were acceptable in comparison to formalin-fixed tissues with the central cores of tissue preserved. Like for other alcoholic fixations, a more intense and eosinophilic stain was observed in the PAXgene-fixed samples.

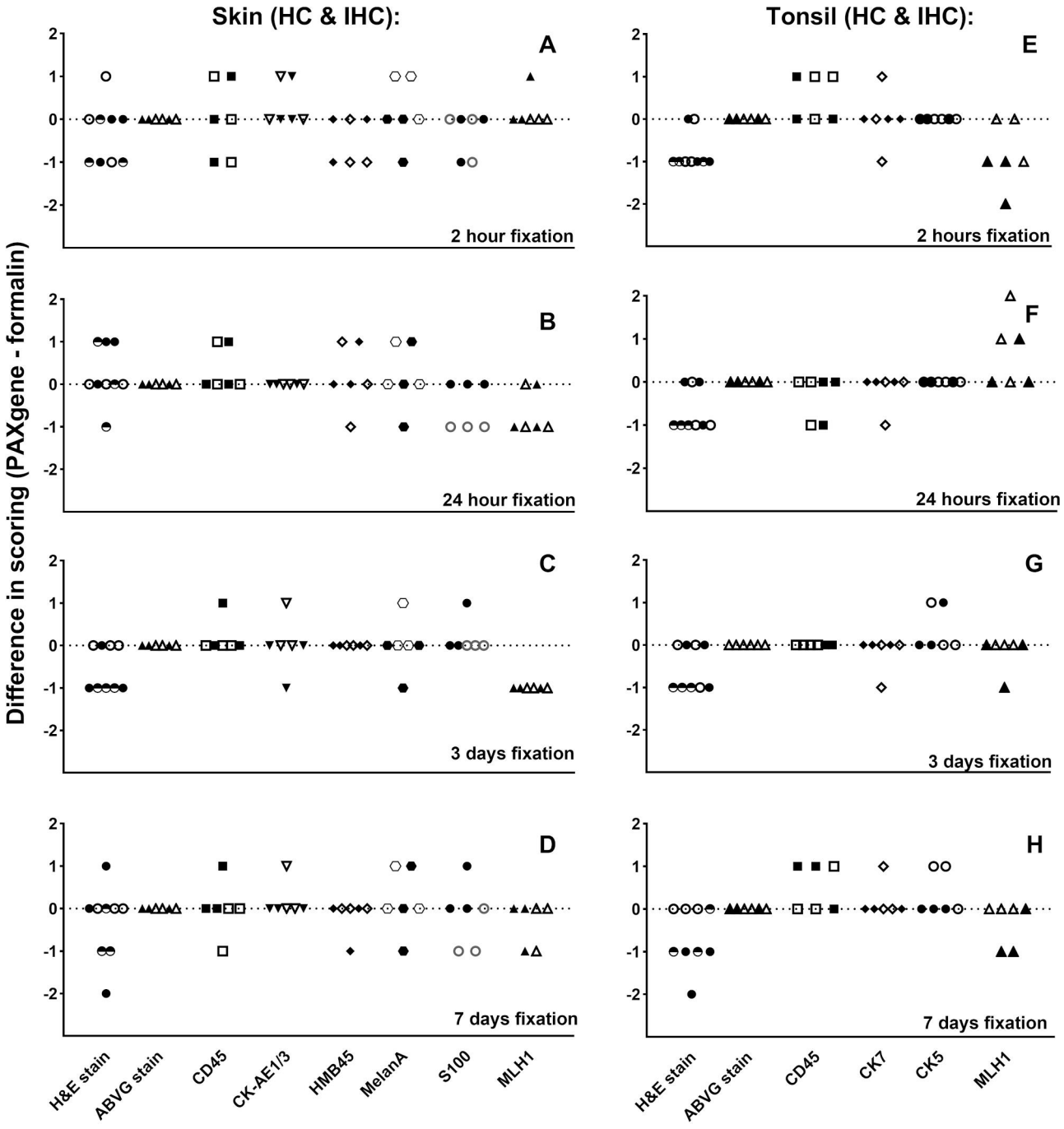

Figure 3 Three skin and three tonsil specimens fixed at four time lengths with PAXgene and formalin fixation. One symbol illustrates fixation differences from the same paraffin-embedded block. Positive score: PAXgene outperforms formalin; negative score: formalin outperforms PAXgene; null score: fixation types perform equally. For the immunostains the evaluation of the slides focused on the intensity, specificity and background marking. There was no apparent relationship between the time of fixation and a variation on the preservation of antigenicity on both formalin and PAXgene-fixed samples. Open symbols, observer 1; filled black symbols, observer 2; half-black symbols observer 3 (H&E only). Immune markers: leucocyte common antigen CD45; specific cytokeratin markers CK-AE, CK5, CK7; Human Melanoma Black HMB45; melanoma antigen MelanA; neural marker S100; and MutL homolog 1 colon marker MLH1. ABVG, Alcian Blue/Van Gieson stain; HC, histochemistry; IHC, immunohistochemistry.

Data analysis

The categorical data were evaluated by Fisher's exact test, alpha level set to 0.05. Also, χ^2 test of goodness-of-fit was applied to evaluate the effect of fixation time on H&E and IHC scores. The GraphPad Prism V.7 software was applied. The figures in the Result section show the difference in scoring, that is, by subtraction: PAXgene score minus the formalin score (positive score: PAXgene outperforms formalin; negative score: formalin outperforms PAXgene; null score: fixation types perform equally).

RESULTS

H&E stain

Examples of H&E stains of formalin and PAXgene-fixed skin samples at 24 hours, 3 days and 7 days are shown in figure 2.

Table 1 Number of scores where PAXgene fixation were superior, inferior or similar to formalin fixation for three skin and three tonsil specimens

Type of analysis and fixation time	Number of scores (n)		
	PAXgene superior	PAX=formalin	Formalin superior
H&E*			
2 hours	1	6	11
24 hours	3	8	7
3 days	–	8	10
7 days	1	9	8
ABVG†			
2 hours	–	12	–
24 hours	–	12	–
3 days	–	12	–
7 days	–	12	–
IHC‡			
2 hours	11	36	13
24 hours	9	39	12
3 days	6	44	10
7 days	11	40	9
FISH§			
2 hours	–	–	6
24 hours	–	4	2
3 days	–	–	6
7 days	–	1	5

*Three observers, six specimens, four time periods=72 scores.
†Two observers, six specimens, four time periods=48 scores.
‡Three specimens with six antibodies+three specimens with four antibodies, two observers, four time periods=240 scores.
§One observer, two skin specimens, four time periods, three probes=24 scores.
ABVG, Alcian Blue/Van Gieson stain; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry.

H&E evaluation of the three skin and three tonsil specimens was performed by three observers, and scoring variation was 12.6% for slides with FFPE tissue and 18.1% for slides with PAXFPE tissue. The pathologist tended to score PAXFPE tissue slides lower (figure 3: H&E stain: observer 3, half black circles).

Artefacts seemed a little more pronounced in the PAXFPE tonsil samples than in the PAXFPE skin samples, and the pathologist also tended to score the tonsil samples lower. This difference may be because the tonsil samples were frozen prior to fixation procedures.

Figure 3 and table 1 shows in the H&E panel for both tonsil and skin specimens, FFPE and PAXFPE tissue slides scored similar in 43% of the cases, and in 50% of the scores FFPE outperformed PAXFPE tissue slides. PAXFPE tissues only scored better than FFPE in 7% of the cases.

There was a tendency towards PAXFPE tissue performed best after a 24 hours fixation (figure 3, panel B and table 1), however, the χ^2 test showed no significant differences among the groups (p=0.48).

The central core of the tissue samples looked well preserved and means that PAXgene fixative was able to fix all the way through the tissue and maintain the tissue structures sufficiently (only two sample pairs scored −2 according to figure 3D,H. In those two pairs, PAXFPE slides scored 0 and FFPE scored 2 by the same observer).

ABVG stain

No discrepancies were seen between samples fixed in formalin or PAXgene (all slides scored 0 according to figure 3, table 1). The ABVG scoring was made by two observers and all slides scored 2 points independent of fixation type or fixation time.

Bearing in mind that the morphological changes were not part of the evaluation, the focus was the staining intensity, contrast and presence of artefacts.

Immunohistochemistry

For the specific markers it was also difficult to conclude that one type of fixation was superior to the other, with all the scores lying between −1 and +1. This was also confirmed by the statistical tests:

Figure 3 shows that IHC stains for FFPE and PAXFPE tissue got the same score in 68.3% of the paired cases. FFPE was superior to PAXFPE tissue in 18% of the scored slides, and PAXFPE was superior to FFPE in 15% of the slides. There was no difference between FFPE and PAXFPE tissues was confirmed by Fischer's exact test (p>0.999 for tonsil samples and p=0.4 for skin samples). We tested if length of fixation time had an impact on quality of outcome by comparing four groups: 2 hours, 24 hours, 3 days and 7 days; and three groups for outcome: PAXFPE superior to FFPE, FFPE=PAXFPE, FFPE superior to PAXFPE. The χ^2 test showed no significant differences among the groups (p=0.74), see table 1.

The scoring for all IHC stains were made by two observers. The variation between observers in overall formalin scoring were 10% (minimum variation was for CD45 (1.7%) and maximum for MelanA (31.4%)) and for the PAXFPE tissue slides the interobserver variation was 8.4% (minimum variation was for MLH1 (0%) and maximum for S100 (31.3%)).

FISH

The FISH evaluation was performed by one observer. According to figure 4 and table 1, the FFPE tissue scores outperformed PAXFPE tissue independent of fixation time, except for the 24 hours PAXFPE tissue which seemed to perform similar to FFPE tissue (no statistical calculations could however be done due to lack of enough data).

The 2 hours and 7 days produced weaker signals and more intense background noise; but some signals were still possible to evaluate using single filters on the microscope.

DNA yield and quality

The difference in scoring for the DNA fragment size distribution from the electrophoresis are shown in figures 5 and 6 . For 37.5% of the samples, PAXFPE and FFPE tissues obtained the same score (score 0) and for the remaining samples, 62.5%, PAXFPE tissue obtained a higher score (score ≥1). This was particularly evident for the skin samples, where the Fisher's exact test showed PAXFPE samples to score higher than FFPE tissue samples (p=0.005), when scores 0 to 2 were pooled and score 3 and 4 were pooled for the two fixatives tested, to allow statistical testing. This difference was not found for the tonsil samples (p>0.999). For both skin and tonsil (see table 2), 24 hours to 3 days seemed to give the best result for PAXFPE samples, whereas the quality of DNA was affected after 7 days of fixation for PAXFPE samples (no statistical calculations because of too few data).

DISCUSSION

Extracting DNA from skin samples fixed with formalin can be problematic with regard to subsequent molecular analysis and repeated biopsies are therefore often needed which is inconvenient and costly. PAXgene may be an alternative fixation method,

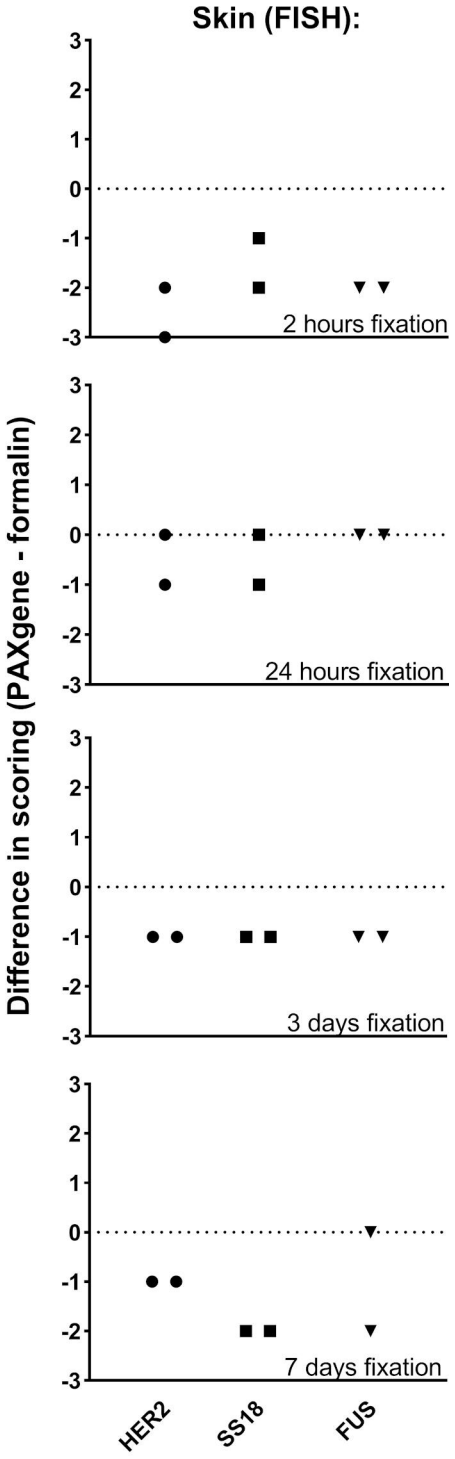

Figure 4 FISH analysis of two skin specimens fixed at four different time lengths with PAXgene. Compared with 24 hours formalin fixations, which all had the maximum score 3 (used as reference). The scores were given by one observer. Our study was low in sample number; however, optimisation is needed. FISH is performed manually, which may explain some of the variability in results. Each symbol corresponds to a specific probe: HER2; SS18, FUS RNA binding protein. FISH, fluorescence in situ hybridisation; FUS, Fused in Sarcoma; HER2, Human epidermal growth factor receptor 2; SS18, Synovial sarcoma.

but the requirement to add a stabiliser after 24–48 hours of fixation may be problematic in some laboratories. Therefore, we evaluated the impact of prolonged PAXgene fixation times for dermatological assessments using standard laboratory protocols developed for formalin-fixed specimens.

H&E and ABVG stains

The central core of PAXgene-fixed tissue looked well preserved with the evidence of chromatin detail and nucleoli. Even though all samples were evaluated blindly, a trained eye would be able to distinguish between slides fixed in formalin or PAXgene. This is due to light shrinkage in nuclei and muscle fibres on PAXFPE slides, which became more evident with longer fixation (3 and 7 days). Also, the eosin stain was more intense for PAXFPE slides resulting in a higher degree of contrast (figure 2). These features are known fixation artefact associated with alcohol-based fixation[3 25] and have also been reported in other studies.[7 11] However, this could hinder some of the fine details of the tissue's components[26] and lead to the attribution of lower scores regarding morphological evaluation and perhaps the reason why the pathologist (compared with the two medical laboratory scientists) consequently scores PAXFPE tissues lower than FFPE apart from a score at 24 hours. All slides from all time periods seemed usable for diagnostics, despite there being discrepancy between the evaluators.

Two medical laboratory scientists evaluated the ABGV stains and found no difference between fixatives, also regarding time periods of fixation. However, morphology was not evaluated. Similar to other studies, PAXFPE samples were increased in contrast.[9 11 19]

Immunohistochemistry

In this study, time of fixation did not have a significant impact on the results. Apart from a very low number of samples, one must bear in mind that no changes were made in the standard protocols for the immunostains made on PAXFPE tissue, meaning that heat-induced antigen retrieval (HIER) was performed on both types of tissue. Omitting antigen retrieval for PAXFPE tissues might be considered an option for further investigations, since this alcoholic fixative does not produce cross-links between the antigens.[4]

FISH

The FISH standard protocol needs optimisation if applying PAXgene fixation partly due to an increase in red background, which appeared resolved with repetition of stringent washes. Perhaps the alcoholic properties were responsible for these background results, as this feature was also reported by Oberauner-Wappis *et al*.[17]

DNA yield and quality

From the tonsil specimens, longer periods of PAXgene fixation produced 'thicker' bands in electrophoresis than their formalin-fixed counterpart (figure 6)—perhaps DNA was more amenable with only few formalin cross-links or less inhibition even though the same amount of DNA was added to each PCR reaction. However, both formalin and PAXgene fixation consistently obtained DNA with 600 bp, the ideal for molecular analysis, hence there was no advantage in using PAXgene fixation for tonsil samples.

In contrast, skin samples yielded more heterogeneous DNA quality for both fixatives. DNA extracted from FFPE samples amplified fragments longer than 400 bp with some inexistent, unusable or with a low amount that compromised band visualisation. The DNA from PAXFPE samples resulted in fragments of at least 400 bp, the minimum required to perform subsequent

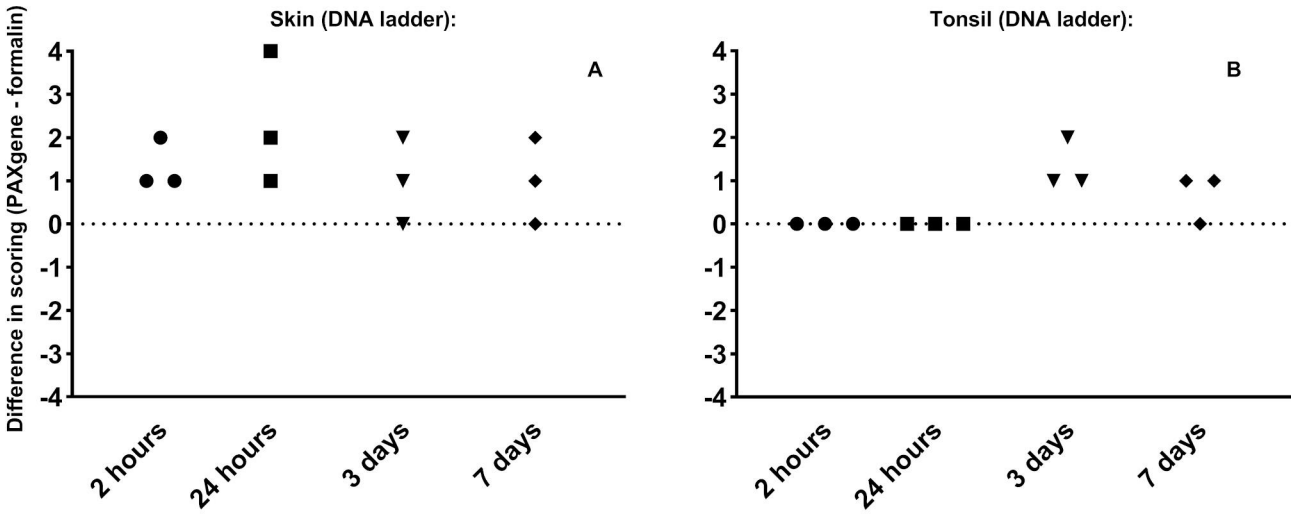

Figure 5 Three skin and three tonsil specimens were fixed at four different time lengths with PAXgene and formalin fixation. DNA extraction, PCR amplification of different sized amplicons and electrophoresis were performed. Differences in amplicons obtained for different samples due to differences in DNA integrity are shown (amplicon size distribution). Each symbol corresponds to a specific fixation time length.

molecular analysis such as clonality analysis. This is similar to previous reports on PAXgene and formalin.[5 11 13–15]

DNA extracted with the best quality was derived from fixations of 24 hours and 3 days for PAXgene. This is not fully concordant with the manufacturers' recommendations, and it would be interesting to study these preliminary data further.

Limitations

Only few samples were included in our study and data should be regarded as an indicative preliminary study.

Pathologists are trained in diagnostic details, but the majority of our histological stains were evaluated by medical laboratory scientists.

Part of our aim was to apply our standard routine protocols developed for formalin-fixed specimens and with no optimisation for PAXgene (including DNA extraction methods), however, this is also a limitation and PAXgene may be able to perform better than described in this study.

Our tonsil tissue was frozen prior to fixation procedures, which may pose a bias in our morphology results compared with the skin samples, which were not frozen prior to fixation. We included tonsil tissue to serve as 'control tissue' given that formalin fixation has not previously been considered as problematic for tonsil DNA yield as for skin and other tissue types. However, two different types of tissue that react differently to fixatives will not be directly comparable.

CONCLUSIONS

For dermatological tissues, a substitute for formalin needs to adequately preserve histomorphology, antigenicity and biomolecules—also if fixation time is prolonged during weekends and bank holidays. This preliminary study shows that the less health hazardous PAXgene may be such a substitute, but protocols need optimisation to match individual laboratory customs. PAXgene may prevent the need to repeat molecular analyses due to suboptimal DNA input and repetition of fortuneless dermatological

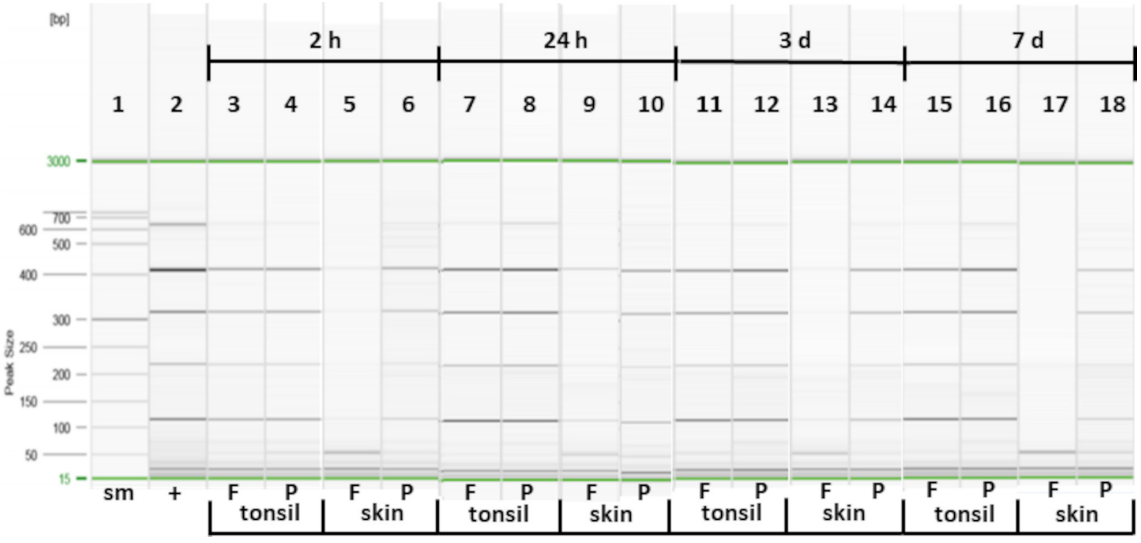

Figure 6 Electrophoresis results of amplifiable PCR amplicons from skin and tonsil samples fixed with formalin (F) and PAXgene (P) at four different time intervals (2 hours, 24 hours, 3 days and 7 days). Sample 1 (sm): size marker and sample 2 (+): positive control DNA.

Table 2 DNA size evaluation from electrophoresis.

		Formalin					PAXgene				
Number of samples		**0**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
Skin	2 hours	–	2	1	–	–	–	–	1	2	–
	24 hours	1	1	1	–	–	–	–	–	2	1
	3 days	–	–	3	–	–	–	–	1	1	1
	7 days	2	–	1	–	–	–	1	2	–	–
Tonsil	2 hours	–	–	–	3	–	–	–	–	3	–
	24 hours	–	–	–	–	4	–	–	–	–	4
	3 days	–	–	1	2	–	–	–	–	–	4
	7 days	–	–	–	3	–	–	–	–	1	2

The distribution of the number of DNA integrity scores after fixation of skin and tonsil samples in formalin or PAXgene at different time lengths. DNA visible band scores: score 4 is the most optimal score, whereas score 0 is unsuitable for DNA analysis.

biopsies from the same patient. However, PAXgene may only be a realistic alternative for a confined set of samples, like skin, as it is a very expensive alternative to formalin fixation.

Take home messages

- PAXgene fixation outperformed formalin for skin, but not tonsil samples in DNA integrity analysis.
- Formalin fixation outperformed PAXgene in H&E and fluorescence in situ hybridisation (FISH) analysis, but PAXgene seem usable for diagnostics.
- No difference between fixatives was observed between the fixatives in Alcian Blue/Van Gieson stain and immunohistochemistry analysis.
- PAXgene fixation up to three days before adding stabiliser may be feasible after optimisation to individual laboratory customs. PAXgene may be an alternative for a confined set of samples, like repeated dermatological biopsies, due to high costs of this fixative.

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