CLINICAL PERFORMANCE STUDY REPORT

Performance evaluation of Glyoxal Acid-Free (GAF) used as histological fixative in comparison with Formalin.
An open label, comparative non-inferiority study.

Study dates:	Date of start: 30 January 2020 Date of completion: concluded	**Report date:**	17 February 2022
Clinical Performance Study Protocol (CPSP) identification	ADDAX-GAF-2019	Version: 1.0	19.09.2019
Investigational IVD:	Glyoxal Acid Free Fixative (GAF). It is an innovative reagent that allows optimal tissue fixation at structural and molecular level combined with the absence of toxicity and carcinogenic activity. In Vitro diagnostic medical device. **Indication of Use:** It allows optimal tissue fixation at structural and molecular level for diagnostic purposes (e.g.: breast, prostate, colon, endometrium, and lung).		
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Compliance Statement	The CPSP was performed in accordance with ISO 20916:2019

SIGNATURE PAGE

I have read this report and confirmed that, to the best of my knowledge, it accurately describes the conduct and results of the study.

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1 SUMMARY

Title:	Performance evaluation of Glyoxal Acid-Free (GAF) used as histological fixative in comparison with Formalin. An open label, comparative non-inferiority study.
Introduction:	Glyoxal was proposed in 1943 as a fixative alternative to formalin since it is a simple di-aldehyde. As reported by Harke & Hoeffler glyoxal does not appear to evaporate from solution. Indeed, the reported Henry's law constant of ≤3.38 × 10– 4Pa m3/mol indicates that glyoxal is essentially non-volatile with regard to the aqueous phase. Glyoxal is not classifiable as a human carcinogen, nevertheless its use may cause some adverse reactions such as irritation of skin and eyes. Tumor-promoting activity of glyoxal has been reported in rats subjected to long-term exposure to this agent in drinking water. All these data are providing a clear view that glyoxal has a very low toxicity even though holding a similar reactivity to formaldehyde. Several studies described the effects of glyoxal on tissues and different fixatives based on this reagent were proposed. Nevertheless, some concerns were raised discouraging the use of this fixative as an alternative to formalin. In particular, it has been claimed that glyoxal-fixed tissues show clarity of cellular details, erythrocytes are lysed and microcalcifications are dissolved. In addition, fluorescence in situ hybridization (FISH) analysis led to technically-compromised results and extraction and sequencing nucleic acids proved unsatisfactory. By taking all above into consideration and having observed that commercially available glyoxal is strongly acid, Bussolati and coworkers considered that this peculiar acidity may be responsible for the observed detrimental effect on tissues. Acidification of glyoxal is likely due to its fast oxidation that leads to formation of acids, mainly glyoxilic acid, that is a very strong acid.

	The Glyoxal Acid Free (GAF) Fixative is an innovative reagent that allows optimal tissue fixation at structural and molecular level combined with the absence of toxicity and carcinogenic activity.
Purpose:	The aim of the proposed performance evaluation study is to confirm in a large sample of histological specimens, obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical markers.
Population:	The patients enrolled will not receive any drug or intervention that could modify the clinical outcome. Patient samples will be collected, from surgical specimens of tumors arriving fresh (unfixed) from the Surgical Theatre to the Pathology labs. Samples will be obtained from pathological areas of the following organs: Breast, Colon, Uterus, Prostate and Lung.
Study design	This is an open label, non-inferiority trial, comparing GAF Fixative Vs Formalin as a fixative for histological specimens obtained from surgical biopsies, which are most frequently performed for diagnostic purposes (e.g.: breast, prostate, colon, endometrium, and lung). The study is focused on the immediate preparation of the histological specimens obtained from biopsies performed on surgical samples.
Number of study sites:	3
Statistical method used:	Data from the study will be presented using descriptive statistics. In general, categorical variables will be presented as numbers and percentages, and continuous variables, after evaluation of normality by applying Kolmogorov-Smirnov test, will be presented as mean values, standard deviation (SD), or median value with interquartile range, as appropriate. Surgical biopsies will be included in the study according to the defined groups: A. biopsies fixed in GAF; B. biopsies fixed in PBF.

	All data collected will be tabulated and represented graphically by these two fixative groups.
Results of clinical performance study:	The performance results confirmed the non-inferiority of GAF respect to PBF as highlighted by the previous interim analysis. Therefore, the GAF has the same ability of tissue fixation without toxicity and carcinogenic activity. For the secondary analysis, the performance evaluations for the local laboratories was considered. Also, in this case the results confirmed the non-inferiority of GAF respect to PBF and in terms of median value similar results between fixative groups were observed. The overall mean satisfaction of the local laboratories can be considered positive.
Conclusions:	Confirming the non-inferiority of GAF respect to PBF, the data of the trial highlight the capability of the investigational device to ensure the structural preservation of the: tissue, nuclei, cytoplasm and diagnostic value of the preparations (for the sections of all organs tested). These results are consistent with the rational/justification of the study and confirmed the satisfaction of local laboratories.
Date of study initiation:	30 Jan 2020
Date of study completion:	Site 1: 17/12/2021 Site 2: 16/02/2022 Site 3: 17/02/2022

2 INTRODUCTION

A formaldehyde (FA) solution (Formalin) has been for many years the gold standard for fixation of histological specimens. Nevertheless, the literature contains numerous reports that FA causes some morphological changes, loss of epitopes, or mislocalization of target proteins and that it fixes the samples slowly and incompletely (1-3). On top of this, Environmental Authorities are increasingly concerned for the objective toxicity of this volatile reagent, so that a banning of formalin from 2016 has been proposed in the European Union. This has been stated by the EC Regulation n.605/2014 (4) that modifies the EC Regulation n.1272/2008 (5) defining formalin as a carcinogen (category 1B/2) and mutagen. This regulation should heavily impact on diagnostic pathology methods and procedures, even if so far the main reaction to this significant issue is limited to adoption of protective procedures, designed to prevent excessive exposure of pathology workers to formaldehyde vapours. Many other fixatives have been proposed with the aim to mitigate these problems. Among them, glutaraldehyde seems to be the most frequently used, since it fixes the samples faster and more completely than FA (6). Mixtures of FA and glutaraldehyde result in a more accurate fixation and reduce the lateral mobility of molecules (2), presumably by increasing the level of protein cross-linking. However, this fixative mixture also reduces the efficiency of immunostainings, by blocking the antibody access to epitopes, or by causing particular epitopes to unfold (7). Alcohol-based fixatives, with ice-cold methanol (2), resulted in stable fixation for a subpopulation of cellular structures (such as microtubules), but leaded to poor morphology preservation and to a loss of membranes and cytosolic proteins. Overall, the improvements in fixation induced by glutaraldehyde or methanol do not compensate for their shortcomings, thus in most cases leaving FA as the current fixative of choice. Based on the above assumptions the unmet need still to be addressed is to identify a superior alternative to FA especially since the key artifacts that were substantially negligible with the past conventional microscopy became more critical by the recent progress in super-resolution microscopy (8). In order to find a fixative that maintains high-quality immunostainings while alleviating FA problems, Bussolati G. and co-workers (9) proposed acid-free glyoxal as a substitute of formalin for structural and molecular preservation of tissue samples.

Glyoxal was proposed in 1943 (10) as a fixative alternative to formalin since it is a simple di-aldehyde. As reported by Harke & Hoeffler (11) glyoxal does not appear to evaporate from solution. Indeed, the reported Henry's law constant of ≤3.38 × 10– 4Pa m3/mol (12) indicates that glyoxal is essentially non-volatile with regard to the aqueous phase. Glyoxal is not classifiable as a human carcinogen (13), nevertheless it use may cause some adverse reactions such as irritation of skin and eyes (13). Tumor-promoting activity of glyoxal has been reported in rats subjected to long-term exposure to this agent in drinking water (14). All these data are providing a clear view that glyoxal has a very low toxicity even though holding a similar reactivity to formaldehyde.

Several studies described the effects of glyoxal on tissues (15-17) and different fixatives based on this reagent were proposed. Nevertheless, some concerns were raised discouraging the use of this fixative as an alternative to formalin (18-19). In particular, it has been claimed that glyoxal-fixed tissues show clarity of cellular details, erythrocytes are lysed and microcalcifications are dissolved (20). In addition, fluorescence *in situ* hybridization (FISH) analysis led to technically-compromised results (21,22) and extraction and sequencing nucleic acids proved unsatisfactory (19,21, 23-25).

By taking all above into consideration and having observed that commercially available glyoxal is strongly acid, Bussolati and coworkers (9) considered that this peculiar acidity may be responsible for the observed detrimental effect on tissues. Acidification of glyoxal is likely due to its fast oxidation that leads to formation of acids, mainly glyoxilic acid, that is a very strong acid (26).

The Glyoxal Acid Free (GAF) Fixative is an innovative reagent that allows optimal tissue fixation at structural and molecular level combined with the absence of toxicity and carcinogenic activity.

Therefore, the aim of the currently proposed study is to confirm in a large sample of histological specimens, obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical marker. This is a controlled trial on the values and merits of GAF as

a fixative compared to Formalin on the type of surgical biopsies, which are most frequently performed for diagnostic purposes (e.g.: breast, prostate, colon, endometrium, and lung).

2.1 STUDY RATIONALE

In this study, the objective is to confirm, in a large sample of histological specimens obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical markers.

This is a controlled trial evaluating Glyoxal Acid-Free (GAF) compared to Phosphate buffered Formalin (PBF) as a fixative on the type of biopsies which are most frequently performed for diagnostic purposes (e.g.: breast, prostate, colon, endometrium, and lung).

The study will evaluate the following:

1. **Local Pathologist (each study center)**

   Will answer to the following four questions providing a score according to his/her judgment:

   1) How do you estimate the structural preservation of the tissue
   [possible answers: Valid (1)/ Invalid (0)]
   2) How do you estimate the preservation of the nuclei
   [possible answers: Valid (1)/ Invalid (0)]
   3) How do you estimate the preservation of the cytoplasm
   [possible answers: Valid (1)/ Invalid (0)]
   4) How do you estimate the diagnostic value of these preparations: (this question is focused on the H&E Preparation + the Immunohistochemical preparations).
   [possible answers: Valid (1)/ Invalid (0)]

The answer of each question has a binary score (0 or 1). The total score of the four questions will be collected. The total score obtained by pathologists of each Center will be sent to the CRO and compared with that given by the central pathology reviewer.

In addition, the local reviewers will answer to the following questions:

Do you consider that the preparations obtained on the same case with the two fixatives have the same performance? [possible answers: Yes/No].

Were you satisfied with the use of GAF fixative during the fixation procedure? [score from 1 (not satisfied) to 10 (totally satisfied)]"

2. Central Pathology Reviewer

Will answer to the following four questions providing a score according to his/her judgment:

1) How do you estimate the structural preservation of the tissue
[possible answers: Valid (1)/ Invalid (0)]
2) How do you estimate the preservation of the nuclei
[possible answers: Valid (1)/ Invalid (0)]
3) How do you estimate the preservation of the cytoplasm
[possible answers: Valid (1)/ Invalid (0)]
4) How do you restimate the diagnostic value of these preparations
(this question is focused on the H&E Preparation + the Immunohistochemical preparations) [possible answers: Valid (1)/ Invalid (0)].

The answer of each question has a binary score (0 or 1). The total score of the four questions will be collected.

a) *Evaluation of the results of the relevant pre-clinical testing/assessment carried out*
Not applicable

b) *Evaluation of clinical data that are relevant to the proposed performance evaluation study.*
Not Applicable.

2.2 GUIDELINES

This study will be conducted in conformity with the ethical principles set forth by the Declaration of Helsinki, Good Clinical Practice (GCP) principles according to international standards for clinical performance studies ISO 20916:2019, the laws and regulations of the countries where the study will take place, and indemnity / insurance requirements

2.3 APPROVAL OF THE INDEPENDENT ETHICS COMMITTEE OR INSTITUTIONAL REVIEW BOARD (IRB)

This investigational plan, the informed consent form (if applicable) and any other study related requested documents must be reviewed and approved by the appropriate Ethics Committee where the trial will be conducted and relevant Regulatory Authority, and any additional requirements imposed by the EC and Regulatory Authority shall be followed, if appropriate.

The clinical investigation shall not begin until the required approval/favorable opinion from the EC and Regulatory Authority have been obtained, as appropriate. Changes to the investigational plan that may increase the risk or present new risks to the subject, or that may adversely affect the validity of the trial, must be approved in writing by the Sponsor and by the Ethics Committee.

This performance evaluation study does not require any insurance policy.

3 DESCRIPTION OF THE IVD MEDICAL DEVICE UNDER INVESTIGATION

3.1 DESCRIPTION

The Glyoxal Acid Free (GAF) Fixative is an innovative reagent that allows optimal tissue fixation at structural and molecular level combined with the absence of toxicity and carcinogenic activity. In Vitro diagnostic medical device. IVD medical device composition:

- Glyoxal Acid Free (GAF) Fixative consists of a water solution with a 2% concentration of Glyoxal (Sigma, Milan, Italy) deprived of acid by passage on ion exchange resins (Amberlyst A21, Dow Chemicals, Milan, Italy) in a Phosphate Buffer pH 7,1-7,8 and containing, as a stabilizer, Ethanol <5% , and 5% Glycol (Propylen Glycol, Chim. Strola, Turin, Italy).
- Phosphate buffered Formalin (PBF; comparator): (4% formaldehyde, in 0.1 phosphate buffer pH 7.2-7.4), of the source currently used in the reference laboratory.

3.2 INTENDED USE

In Vitro diagnostic medical device with a mechanical action that is indicated:

• tissue fixation at structural and molecular level for diagnostic purposes (e.g.: breast, prostate, colon, endometrium, and lung).

3.3 CHANGES TO IDV DURING THE CLINICAL INVESTIGATION

No changes to the IVD have been reported.

4 CLINICAL PERFORMANCE STUDY PLAN

4.1 GENERAL

a) Description of the type of performance evaluation study to be performed with rationale for the choice

This is a controlled trial evaluating Glyoxal Acid-Free (GAF) compared to Phosphate buffered Formalin (PBF) as a fixative on the type of biopsies which are most

frequently performed for diagnostic purposes (e.g.: breast, prostate, colon, endometrium, and lung).

The objective of the proposed study is to confirm, in a large sample of histological specimens obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical markers.

b) Description of the measures to be taken to minimize or avoid bias, including randomization and blinding/masking

All primary and secondary outcome measures are objective, reliable, standard and verifiable.

The Trial forecasts a collection overall of 130 cases in duplicate (as 2 different biopsies from each tissue sample will be fixed in PBF and in GAF) with a forecast of 260 H&E preparations and approximately 880 IHC preparations (depending on the requests of the Pathologist in charge of the case). This means that each Centre is expected to collect about 44 cases.

After a collection of 65 cases (about 22 for each Centre) an interim analysis is forecasted.

c) Primary and secondary endpoints, with rationale for their selection and measurement

All primary and secondary outcome measures are objective, reliable, standard and verifiable.

<u>Primary performance Endpoint</u>

Total score calculated in GAF group compared with total score calculated in PBF group on morphological preservation and diagnostic value questions answered by Central Pathology Reviewer

The score is obtained from the answers to 4 questions. The answer to each question has a binary score (0 or 1).

Questions:

1) How do you estimate the structural preservation of the tissue
[possible answers: Valid (1)/ Invalid (0)]
2) How do you estimate the preservation of the nuclei
[possible answers: Valid (1)/ Invalid (0)]
3) How do you estimate the preservation of the cytoplasm
[possible answers: Valid (1)/ Invalid (0)]
4) How do you estimate the diagnostic value of these preparations
(this question is focused on the H&E Preparation + the Immunohistochemical preparations) [possible answers: Valid (1)/ Invalid (0)].

<u>Secondary performance Endpoints</u>

The study secondary hypotheses are to test the differences between the study groups in:

- Total score calculated in GAF group compared with total score calculated in PBF group on morphological preservation and diagnostic value questions answered by local center pathologists;
- To evaluate if score obtained from "Do you consider that the preparations obtained from the same case using two fixative have the same performance?" answered by local centers can be considered as surrogate of total score obtained from 4 answers;
- Descriptive statistical analyses of IHC markers;
- Pathologist's satisfaction evaluated on local centers.

d) Methods and timing for assessing, recording, and analyzing variables:

The study is focused on the immediate preparation of the histological specimens obtained from biopsies performed on surgical samples.

e) Equipment to be used for assessing the performance evaluation study variables and arrangements for monitoring maintenance and calibration

Not applicable.

f) Any procedures for the replacement of subjects

Not applicable.

4.2 IVD MEDICAL DEVICE

a) Description of the exposure to the IVD medical device:

Fixation fluids to be used are:

1. Phosphate buffered Formalin (PBF):

(4% formaldehyde, in 0.1 phosphate buffer pH 7.2-7.4), of the source currently used in the reference laboratory; or

2. Glyoxal Acid-Free Fixative (GAFF):

The GAF fixative consists of a water solution with a 2% concentration of Glyoxal deprived of acid by passage on ion exchange resins in a Phosphate Buffer pH 7,1-7,8 and containing, as a stabilizer, Ethanol <5% , and 5% Glycol (Propylen Glycol, Chim. Strola, Turin, Italy). Phenol Red as an indicator is used to testify the basic pH of the solution.

b) Justification of the choice of comparator

Glyoxal Acid-Free Fixative (GAFF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical markers.

Phosphate buffered Formalin (PBF) represents a golden standard.

c) List of any other medical device or medication to be used during the Performance Evaluation Study Protocol

Not applicable.

d) Number of IVD medical devices to be used, together with a justification

Only the study devices (Glyoxal Acid-Free Fixative (GAFF) or Phosphate buffered Formalin (PBF) will be used in the study, no other devices should be used.

4.3 SUBJECTS

The patients enrolled will not receive any drug or intervention that could modify the clinical outcome. Patient histological specimens for this performance evaluation study will be collected in each participating centre as per local regulation (e.g. from referral biobank, from Surgical Theatre etc.).

- **Total expected duration of the performance evaluation study**

  A total duration of the study of around 6 months is foreseen from the moment of study approval until the data analysis and final results reporting.

- **Number of samples required to be included in the performance evaluation study**

  The Trial forecasts a collection overall of 130 cases in duplicate (as 2 different biopsies from each tissue sample will be fixed in PBF and in GAF) with a forecast of 260 H&E preparations and approximately 880 IHC preparations (depending on the requests of the Pathologist in charge of the case). This means that each Centre is expected to collect 44 cases, about.

  After a collection of 65 cases (about 22 for each Centre) an interim analysis is forecasted.

- **Estimated specimens' collection period**

  Expected date of start: Q4 2019;

Expected date of completion: Q1 2020

4.4 PROCEDURES

a) Description of all the performance evaluation study-related procedures that subjects undergo during the performance evaluation study

The patients enrolled will not receive any drug or intervention that could modify the clinical outcome. Patient histological specimens for this performance evaluation study will be collected in each participating centre as per local regulation (e.g. from referral biobank, from Surgical Theatre etc.).

The following are the procedures for the samples collection and evaluation:

The study is focused on the immediate preparation of the histological specimens obtained from biopsies performed on surgical samples.

1) Collection of Samples

Samples will be collected, from fresh (unfixed) surgical specimens of tumors sent from the Surgical Theatre to the Pathology labs. Samples will be obtained from pathological areas of the following organs: Breast, Colon, Uterus, Prostate and Lung. In each of the 3 Centres, independently and in parallel, samples will be collected using core biopsy needles (gauge between 14 and 18, length 1 cm) or punch devices (size 2-4 mm).
The samples, collected in number of 1 up to 3, will therefore have a volume of 2 up to 4 mm^3 and will immediately be immersed in the fixation fluids.
The collected biopsies will be anonymized with an identification code, composed as follows: a code related to the Centre, a code for type of organ, a code for the samples collected in the Trial and a random code to identify the type of fixative employed (PBF or GAF).

2) Processing

Fixation of the collected specimens in PBF will be processed at room temperature for 6 hours, whereas fixation of the collected specimens in GAF will be processed at room temperature for 3 hours.
Following fixation, the tissue specimens will be collected in casettes, properly labelled (see above) and immersed in Alcohol 80% for a time from 30 min. up to 48 hours to be processed for paraffin embedding using the apparatus of common use in each laboratory (either Leica or Milestone). The processing will involve passages in Alcohol 95%, followed by Absolute Alcohol, Xylene and Paraffin wax.
NB: Specifically passages in additional fixatives, such as Formalin, have to be excluded.

At the end of the embedding process, from the processed biopsies in paraffin blocks 4 micron thick sections will be obtained in the number of 11, using the microtome of common use in the laboratory. One section will be stained in Haematoxylin & Eosin. The other 10 unstained sections, collected in slides and properly marked with the reference number (see above), will be send to the reference Laboratory of Pathological Anatomy and Histology of the University of Turin, Italy Torino for Immunohistochemical staining. All the 11 sections (stained and unstained) will be sent to the centralized laboratory in Turin.
Once the slides have been scanned in Turin, they will be sent back to the Centre of origin, so that the Pathologist in charge of the case will answer to the same questions which will blindly be answered by the Central Reader (see below). The local Laboratory of each of the 3 Centres will retain and archive the H&E and the immunohistochemical preparations as well as the paraffin blocks.

3) Immunohistochemical staining

Central immunohistochemistry will be performed at the Laboratory of Pathological Anatomy and Histology of the University of Turin, Italy, in order to centralize the IHC procedures.
The unstained slides will be processed by de-paraffinization, followed by Antigen Retrieval (AR) procedures optimal for the different antigens, so that some antigens will

not require AR, others AR at 98°C for 1 or for 3 hours, with CC1 or CC2 fluids (Ventana), alternatively with a 0.05% solution of Citraconic Anydride (Sigma) in water buffered at pH 7,4.
The sections will then be processed in Immunohistochemistry using the BenchMark ULTRA Ventana Apparatus.
Immunohistochemical reactions to be regarded as routinary in the management of biopsies of the different organs.

The following markers will be analysed:

Biopsy Organ	**Centre**	**Markers (IHC Markers to be performed in every case)**
Breast (B)	Candiolo (TO)	Ki67, ER, PgR, HER2
Colon (C)	Candiolo (TO) Barcelona	Ki67, EMA, MLH1, CDX2
Lung (L)	Barcelona	Ki67, EMA, MLH1, CK7
Endometrium (E)	Manchester	Ki67, EMA, MLH1, ER
Prostate (P)	Manchester	Ki67, EMA, MLH1, 34βE12

4) Scanning and Presentation

All slides (both a slide stained with H&E and the immunohistochemical preparations of each case) will be scanned using a Hamamatsu apparatus available in the Institute in Turin. The H&E preparation of each case will be scanned at high definition (40x) while the IHC preparations will be scanned at 20x (in order not to overload the process). Each case will be scanned and presented in parallel, the PBF and the GAF fixed preparations, using the code set by the Laboratory of origin (see above). Once scanned, the images

will be loaded on a specialized web based digital pathology platform based on OMERO (planned, produced and operated by CRS4 (Pula, CA - Italy) and then presented on a site defined as GAF Validation Trial.
Access to this site will be permitted only using: Username and Password.
Once the slides will have been scanned in Turin, they will be sent back to the Centre of origin, where they will be stored according to the standard procedures of the Center (together with the paraffin blocks).
The Pathologist in charge of the case will answer to the same questions which will blindly be answered by the Central Reader (see below).

5) Reading

Reading of the slides will be performed by the Central Pathology Reviewer: Prof. Ales Ryska Charles University Hradec Kralove, Czech Republic.
The Central Reviewer will not be informed of the type of fixation employed for each preparation. He will blindly open each case presented in the two variables and answer to the following 4 questions, focused solely on the H&E preparation and to a question focused on the H&E Preparation + the Immunohistochemical preparations. (see Primary & Secondary endpoints)
In addition, the PI of the three Centers will read the slides. The total score obtained by pathologists of each Center will be sent to the CRO and compared with that given by the central pathology reviewer.

6) Sequence of events

The 3 centres will start the collection of samples in close correlation (within short time one from the other). The first 5 cases to be collected will serve as preliminary. A meeting (either a physical meeting or a Skype conference) will be organized in order to exchange opinions and focus on possible problems encountered. At this point the collection of samples/ cases will start. An interim analysis will be performed after a collection of half of the cases.
Each of the 3 Centres is expected to collect samples of the following organs:

the Centre in Candiolo will focus on Breast,
the Centre in Barcellona on Lung and Colon,
the Centre in Manchester on Prostate and Uterus.

b) Description of activities performed by sponsor representatives

No direct activity will be performed by the sponsor's representatives, except monitoring activities.

c) Any known or foreseeable factors that may compromise the outcome of the performance evaluation study or the interpretation of results

The anonymization of the collected biopsies will permit to avoid factors that may compromise the outcome of the performance evaluation study and the interpretation of the results.

4.4 STATISTICAL CONSIDERATIONS

a) Analytical procedures

Data from the study will be presented using descriptive statistics. In general, categorical variables will be presented as numbers and percentages, and continuous variables, after evaluation of normality by applying Kolmogorov-Smirnov test, will be presented as mean values, standard deviation (SD), or median value with interquartile range, as appropriate.

Surgical biopsies will be included in the study according to the defined groups:

A. biopsies fixed in GAF;

B. biopsies fixed in PBF.

All data collected will be tabulated and represented graphically by these two fixative groups.

b) Sample size

Because of primary endpoint of the study is based on the answer of 4 questions formulated ad hoc for the study, no bibliographical references will be considered to calculate the sample size.

For this reason, descriptive statistic of theorical distribution of difference between two final scores obtained from two groups (i.e. interquartile range) will be considered as expected effect in terms of mean difference in final score and his standard deviation.

Sample size estimates are based on one-sided T-test assuming that the actual distribution is normal.

The following assumptions have been made in order to estimate the sample size.

1. Null difference between final scores calculated into each group is considered to demonstrate the non-inferiority of GAF respects to PBF.

2. The 25th percentile of theorical distribution of differences between two final scores is equal to -2 and it is considered as non-inferiority margin.

3. The standard deviation has been calculated as suggested in Conroy R.'s guide. The highest value is maximum of the distribution (it is equal to 4) and the lowest value is the minimum value (it is equal to -4), then the standard deviation has been set on 2 points (27).

4. The significance level (α), i.e. the probability of the study detecting a false positive finding, has been set at 2.5%.

5. The statistical power, i.e. the probability of detecting an effect when the effect really exists, has been set at 80%.

By considering the above-mentioned assumptions, a sample size of 46 biopsies has been estimated.

Planning to randomize a total of 52 biopsies (26 in each fixative group) would allow for a 10% drop-out rate. Sample size estimated above will be considered just for one

organ evaluated, because of surgical biopsies will be collected from 5 different organs (breast, colon, prostate, lung, and endometrium). Then the final sample size will be 260 surgical biopsies (130 in each group). Sample size estimation has been performed using SAS® proc power (SAS software version 9.4 (28)).

c) Study population

All analyses will be performed on all randomized surgical biopsies. In case of some sample will not be analyzable, this will be considered a drop-out and it will be excluded from the analysis.

d) Performance analysis

Primary endpoint

Primary endpoint of the study will be the total score obtained in GAF group compared with total score calculated in PBF group on morphological preservation and diagnostic value questions answered by Central Pathology Reviewer. The score is obtained from the answers to 4 questions. The answer to each question has a binary score (0 or 1).

After evaluation the normality of the distribution of primary outcome, paired t-test or Wilcoxon test for paired data will be performed to assess the difference between samples treated with GAF fixative and samples treated with PBF. If other variables will be evaluated for their effect, an ANOVA model will be estimated. To test non-inferiority of the GAF fixative, the null hypothesis will be: H0: difference between total scores from two groups of fixatives is < -2; if it will be rejected, non-inferiority of the novel fixative will be accepted.

Secondary endpoints

Secondary endpoints of the study and its respective statistical considerations will be:

1. Total score calculated in GAF group compared with total score calculated in PBF group on morphological preservation and diagnostic value questions answered by local center pathologists. This endpoint will be evaluated as primary endpoint;

2. To evaluate if score obtained from "Do you consider that the preparations obtained from the same case using two fixative have the same performance?" answered by local centers can be considered as surrogate of total score obtained from 4 answers. This endpoint will be evaluated as primary endpoint;

3. IHC markers will be analyzed descriptively and results will be presented by two fixative groups;

4. Pathologist's satisfaction evaluated on local centers will be analyzed descriptively and results will be presented by two fixative groups.

e) Safety analysis

Not applicable

f) Interim analysis

After a collection of 65 cases (about 22 for each Centre) an interim analysis is forecasted. At this time point the study will be temporarily put on hold until the interim analysis will be performed and the scientific advisory Board will release its positive evaluation to continue. At that time the study will be restarted up to completion of all the samples required by the protocol.

g) Procedures for reporting any deviation(s) from the original statistical plan

A fully specified Statistical Analysis Plan (SAP) for Clinical Study Report (CSR) will be prepared before the data base lock. The contents of the SAP will include a full and detailed descriptions of the statistical methods for data analysis, as well as a detailed description of the contents of tables, listings and figures. The plan may be reviewed and updated before the start of the statistical analysis, which will start only at the end of data management activities.

h) Specification of subgroups for analysis

Not applicable in this study.

i) Treatment of missing, unused or spurious data, including drop-outs and

withdrawals

Not applicable in this study.

4.5 RISKS AND BENEFITS OF THE IVD MEDICAL DEVICE AND CLINICAL INVESTIGATION

a) Anticipated clinical benefits:

No clinical benefits are expected as the aim of the currently proposed study is to confirm in a large sample of histological specimens, obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical marker

b) Anticipated adverse device effects:

The adverse events listed below may occur:

- Eye contact causes irritation. Symptoms may include: redness, edema, pain and tearing.

- Skin contact may cause moderate irritation. Product contact with the skin causes sensitization (contact dermatitis). The dermatitis originates following an inflammation of the skin, which begins in the skin areas that come into repeated contact with the sensitising agent. The skin lesions may include erythema, edema, papules, vesicles, pustules, scales, fissures and exudative phenomena, which vary according to the stages of the disease and the areas affected. In the acute phase the following prevails: erythema, edema and exudation. In chronic phases the following prevails: scales, dryness, fissuration and thickening of the skin.

- Ingestion may cause health problems, which include pain with burning, nauseous and vomiting.

c) Residual risks associated with the IVD medical device, as identified in the risk analysis

report:

Based on the risk-analysis assessment, it is considered that the device produced and used in compliance with the operating procedures described by ADDAX Biosciences srl, does not present any relevant risks (that are deemed not acceptable) for the study.

d) Risks associated with participation in the performance evaluation study

No risks are expected as the aim of the currently proposed study is to confirm in a large sample of histological specimens, obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical marker

e) Bioptic sampling

Samples will be collected, from surgical specimens of tumors arriving fresh (unfixed) from the Surgical Theatre to the Pathology labs. Samples will be obtained from pathological areas of the following organs: Breast, Colon, Uterus, Prostate and Lung. No specific risks for specimens' collection are foreseen.

f) Possible interactions with concomitant medical treatments

Not applicable.

g) Steps that will be taken to control or mitigate the risks, based on the risk-analysis assessment

Not applicable.

h) Risk-to-benefit rationale.

The performance of Glyoxal Acid-Free (GAF) is given by the fact that represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical marker.

The use of Glyoxal Acid-Free (GAF) should not represent any particular risk. However,

possible side effects shall be considered, like the above mentioned Anticipated adverse device effects to any of the device's ingredient, misuse of the device that might compromise the positive effect of the device when correctly used.

4.6 MONITORING PLAN

The data was recorded in an eCRF. The Investigator entered data and performed corrections as per GCP requirements.

The study monitor (CRA) contacted and visited the investigational site at study initiation, throughout the study and after the study completion to perform the site closure visit. CRA verified the various study records: eCRF, ISF and source data (source data is any information in original records and certified copies of original records on clinical findings, observations or other activities in a study necessary for the reconstruction and evaluation of the study). Source data are contained in source documents – in fully respect of subject's confidentiality - in order to fulfill both the sponsor's responsibility in assuring the proper conduct of the study regarding protocol and GCP adherence and the completeness and accuracy of the data recorded on the eCRF.

The Investigator and/or study team members were expected to be available during the monitoring visits, to answer questions and to provide any missing information. Onsite Monitoring visits were not always possible, due to COVID-19 restrictions. The monitoring visits scheduling was performed as reported below:

	Site 1	**Site 2**	**Site 3**
MONITORING VISITS	**Date**	**Date**	**Date**
Site Initiation Visit (SIV)	30 JAN 2020	25 JUN 2020	19-20 FEB 2020
Monitoring Visit (MV) 1	02 JUL 2021	Not performed	19 OCT 2021

		due to COVID-19 pandemic	
Close-Out Visit (COV)	Planned in Q1 2022	Planned in Q1 2022	Planned in Q1 2022

5 RESULTS

5.1 DISPOSITION OF SLIDES

During the study 200 slides were collected and consequently included in the analysis*.

All the slides were included in accordance with SAP in each analysis as "all analyzed slides".

There are exactly 100 slides collected for each used fixative (GAF and PBF). In particular, 42 (21.0%) slides were collected in Manchester and 68 (34.0%) in Barcelona; the majority of slides were collected in Candiolo (45.0%). (Table 1)

Table 1. Analyzed slides by center and by used fixative

Center		**Slides (N=200)**
Manchester	**N (%)**	42 (21.0)
Barcelona	**N (%)**	68 (34.0)
Candiolo	**N (%)**	90 (45.0)
Fixative		
GAF	**N (%)**	100 (50.0)
PBF	**N (%)**	100 (50.0)

*_**Note**: From each case (100 histological samples), 2 paraffin-embedded tissue blocks were obtained. From the tissue blocks, 5 Slides (1 H&E + 4 IHC) were produced, which were scanned and presented (a total of 1000 slides) for reading by Reviewers._

5.2 PROTOCOL VIOLATIONS

No Protocol violations were recorded during the entire study.

5.3 EFFICACY ANALYSIS

5.3.1 Primary efficacy analysis

The primary endpoint of the study was the evaluation of morphological preservation and diagnostic value questions answered by Central Pathology Reviewer in terms of the total score obtained in GAF group compared with total score calculated in PBF group.

The assumption of normality distribution of total score data is violated (Shapiro-Wilk Test, $P < 0.0001$), so in this analysis, the difference between the two fixative groups was tested using a non-parametric approach.

The mean of total score in GAF fixative group is 3.7 ± 0.5 while in PBF fixative group is 3.9 ± 0.3.
However, in terms of median value we observe similar results between fixative groups, a median value of 4.0 (IQR: 3.5-4.0) with a minimum value of 2.0 and a maximum of 4.0 and a median value of 4.0 (IQR: 4.0-4.0) with a minimum value of 3.0 and a maximum of 4.0 were observed in GAF fixative group and in PBF fixative group, respectively. (Table 2)

Applying Wilcoxon Signed-Rank test to test the non-inferiority (-2.0 of non-inferiority margin), P-value is less than 0.001, therefore the null hypothesis of inferiority can be rejected and the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved.

Moreover, the estimate of the mean difference between fixative groups (GAF and PBF) was -0.20 with a 95% CI from -0.30 to -0.09 and the non-inferiority margin is not included.

The primary efficacy analysis was performed also by organs.

Out of 90 slides collected from breast, the total score mean value for GAF fixative group is 3.7 ± 0.6 and it is 3.9 ± 0.3 for PBF fixative group. As for the overall primary efficacy analysis, the median is 4.0 (IQR: 4.0-4.0) in both fixative groups. In this case the mean difference between fixative groups is -0.2 ± 0.6. (Table 3)

Applying Wilcoxon Signed-Rank test again to test the non-inferiority exclusively in breast slides (-2.0 of non-inferiority margin), P-value is less than 0.001, therefore the null hypothesis

of inferiority can be rejected. Also, the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved in the breast subgroup.

Moreover, the estimate of the mean difference between fixative groups in breast slide (GAF and PBF) was -0.24 with a 95% CI from -0.42 to -0.07 and the non-inferiority margin is not included.

Regarding the 52 slides collected from colon, the total score mean value for GAF fixative group is 3.7 ± 0.5 and 3.9 ± 0.3 for PBF fixative group, similar to the results obtained from breast slides. The median value is 4.0 (IQR: 3.0-4.0) in GAF fixative group and it is 4.0 (IQR: 4.0-4.0) in PBF fixative group. (Table 3)

Applying Wilcoxon Signed-Rank test again to test the non-inferiority, P-value is less than 0.001, therefore the null hypothesis of inferiority can be rejected and also the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved in the subgroup of colon slides.

Moreover, the estimate of the mean difference between fixative groups in colon slide (GAF and PBF) was -0.19 with a 95% CI from -0.39 to 0.01 and the non-inferiority margin is not included.

All 16 slides collected from lung received high score from Central Pathology Reviewer. The total score mean value for GAF fixative group is 3.9 ± 0.4 and it is 4.0 ± 0.0 for PBF fixative group. As for the overall primary efficacy analysis, the median is 4.0 (IQR: 4.0-4.0) in both fixative groups. In this case the mean difference between fixative groups is -0.1 ± 0.4. (Table 3)

Applying Wilcoxon Signed-Rank test again to test the non-inferiority exclusively in lung slides (-2.0 of non-inferiority margin), P-value is less than 0.001, therefore the null hypothesis of inferiority can be rejected. Also, the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved in the lung subgroup.

Moreover, the estimate of the mean difference between fixative groups in lung slide (GAF and PBF) was -0.12 with a 95% CI from -0.42 to -0.17 and the non-inferiority margin is not included.

Regarding the 22 slides collected from endometrium, the total score mean value for GAF fixative group is 3.8 ± 0.4 and 3.9 ± 0.3 for PBF fixative group, similar to the results obtained from breast slides. The median value is 4.0 (IQR: 4.0-4.0) in both fixative groups. (Table 3)

Applying Wilcoxon Signed-Rank test again to test the non-inferiority, P-value is less than 0.001, therefore the null hypothesis of inferiority can be rejected and also the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved in the subgroup of endometrium slides.

Moreover, the estimate of the mean difference between fixative groups in colon slide (GAF and PBF) was -0.09 with a 95% CI from -0.45 to 0.27 and the non-inferiority margin is not included.

For the prostate 20 slides were collected, 10 per fixative group; both received similar score from Central Pathology Reviewer. In particular, total score mean value for GAF fixative group is 3.7 ± 0.5 and 3.9 ± 0.3 for PBF fixative group, similar to the results obtained from breast slides. The median value is 4.0 (IQR: 4.0-4.0) in both fixative groups. (Table 3)

Applying Wilcoxon Signed-Rank test again to test the non-inferiority, P-value is less than 0.001, therefore the null hypothesis of inferiority can be rejected and also the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved in the subgroup of prostate slides.

Moreover, the estimate of the mean difference between fixative groups in colon slide (GAF and PBF) was -0.20 with a 95% CI from -0.65 to 0.25 and the non-inferiority margin is not included.

Table 2. Primary Efficacy Endpoint

Central reviewer Total Score	**Fixative**			
Overall (N=200)	**GAF**	**PBF**	**Difference between fixative**	**P [1]**
N	100	100	100	<0.001
Mean ± SD	3.7 ± 0.5	3.9 ± 0.3	-0.2 ± 0.5	
Median (IQR)	4.0 (3.5-4.0)	4.0 (4.0-4.0)	0.0 (0.0-0.0)	
(Min-Max)	2.0-4.0	3.0-4.0	-2.0-1.0	

SD: Standard Deviation

IQR: Interquartile Range

P[1]: P-value from Wilcoxon Signed-Rank to test non-inferiority of GAF

Notes:

a) Difference between fixative is defined as GAF total score – PBF total score

Table 3. Primary Efficacy Endpoint by organs

Central reviewer Total Score by organ	**Fixative**			
Organ	**GAF**	**PBF**	**Difference between fixative**	**P [1]**
Breast (N=90)				
N	45	45	45	<0.001
Mean ± SD	3.7 ± 0.6	3.9 ± 0.3	-0.2 ± 0.6	
Median (IQR)	4.0 (4.0-4.0)	4.0 (4.0-4.0)	0.0 (0.0-0.0)	

(Min-Max)	2.0-4.0	2.0-4.0	-2.0-1.0	
Colon (N=52)				<0.001
N	26	26	26	
Mean ± SD	3.7 ± 0.5	3.9 ± 0.3	-0.2 ± 0.5	
Median (IQR)	4.0 (3.0-4.0)	4.0 (4.0-4.0)	0.0 (-1.0-0.0)	
(Min-Max)	3.0-4.0	3.0-4.0	-1.0-1.0	
Lung (N=16)				<0.001
N	8	8	8	
Mean ± SD	3.9 ± 0.4	4.0 ± 0.0	-0.1 ± 0.4	
Median (IQR)	4.0 (4.0-4.0)	4.0 (4.0-4.0)	0.0 (0.0-0.0)	
(Min-Max)	3.0-4.0	4.0-4.0	-1.0-0.0	
Endometrium (N=22)				<0.001
N	11	11	11	
Mean ± SD	3.8 ± 0.4	3.9 ± 0.3	-0.1 ± 0.5	
Median (IQR)	4.0 (4.0-4.0)	4.0 (4.0-4.0)	0.0 (0.0-0.0)	
(Min-Max)	3.0-4.0	3.0-4.0	-1.0-1.0	
Prostate (N=20)				<0.001
N	10	10	10	
Mean ± SD	3.7 ± 0.5	3.9 ± 0.3	-0.2 ± 0.6	
Median (IQR)	4.0 (3.0-4.0)	4.0 (4.0-4.0)	0.0 (0.0-0.0)	
(Min-Max)	3.0-4.0	4.0-4.0	-1.0-1.0	

SD: Standard Deviation

IQR: Interquartile Range

P^1: P-value from Wilcoxon Signed-Rank to test non-inferiority of GAF

Notes:

a) Difference between fixative is defined as GAF total score – PBF total score

5.3.2 Analysis of secondary analysis

The first secondary endpoint of the study was the evaluation of morphological preservation and diagnostic value questions answered by local center pathologists in terms of the total score obtained in GAF group compared with total score calculated in PBF group.

The assumption of normality distribution of total score data is violated (Shapiro-Wilk Test, $P < 0.0001$), so in this analysis, the difference between the two fixative groups was tested using a non-parametric approach.

The mean of total score in GAF fixative group is 3.8 ± 0.5 while in PBF fixative group is 4.0 ± 0.1.
However, in terms of median value we observe similar results between fixative groups, a median value of 4.0 (IQR: 4.0-4.0) with a minimum value of 1.0 and a maximum of 4.0 and a median value of 4.0 (IQR: 4.0-4.0) with a minimum value of 3.0 and a maximum of 4.0 were observed in GAF fixative group and in PBF fixative group, respectively. (Table 2)

The only case that scored 1 point was a GAF prostate case analyzed in Manchester (slide "06" regarding prostate). The same case received a score of 3 points from the central reviewer. (Listing 3b, Listing 4)

In general, the scores obtained by the local pathologist are very similar to those received by the central reviewer. Applying Wilcoxon Signed-Rank test to test the non-inferiority (-2.0 of non-inferiority margin), P-value is less than 0.001, therefore the null hypothesis of inferiority can be rejected and the non-inferiority of Glyoxal Acid-Free Fixative to the reference Phosphate buffered Formalin could be considered achieved.

Moreover, the estimate of the mean difference between fixative groups (GAF and PBF) was -0.21 with a 95% CI from -0.31 to -0.11 and the non-inferiority margin is not included.

The second secondary endpoint of the study was the evaluation of Pathologist's satisfaction on local centers. The question concerning satisfaction was only asked for GAF cases and the score went from 1 (not satisfied) to 10 (totally satisfied). The higher mean satisfaction was

recorded in Barcelona (9.8 ± 0.7), with a median of 10 and a minimum of 7 points. (Table 5) The overall mean satisfaction can be considered positive. In particular, overall local center pathologist mean was 9.2 ± 1.1, with a median value of 9.6 points (IQR: 8.7-10.0), a minimum value of 5.4 and a maximum of 10.0. (Table 5)

Regarding the question asked to local centers "Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?", 18 (18.0%) cases were answered negatively, while all the remaining 82 (82.0%) cases were answered "Yes". (Table 5)

The fifth question asked at the centers therefore cannot provide the same detail and accuracy as the score composed of the four questions concerning structural preservation of the tissue, preservation of the nuclei, preservation of the cytoplasm and diagnostic value of these preparations.

Table 4. Total score from four questions evaluated by local reviewers

Local center pathologist Total Score	**Fixative**			
Overall (N=200)	**GAF**	**PBF**	**Difference between fixative**	**P [1]**
N	100	100	100	<0.001
Mean ± SD	3.8 ± 0.5	4.0 ± 0.1	-0.2 ± 0.5	
Median (IQR)	4.0 (3.0-4.0)	4.0 (4.0-4.0)	0.0 (0.0-0.0)	
(Min-Max)	1.0-4.0	3.0-4.0	-3.0-1.0	

SD: Standard Deviation
IQR: Interquartile Range
P[1]: P-value from Wilcoxon Signed-Rank to test non-inferiority of GAF
Notes:

a) Difference between fixative is defined as GAF total score – PBF total score

Table 5. Pathologist satisfaction

Local center pathologist satisfaction	**Study center**				
	Manchester	**Barcelona**	**Candiolo**	**Overall**	**P [1]**
N	21	34	45	100	<0.001
Mean ± SD	8.8 ± 1.6	9.8 ± 0.7	9.0 ± 0.7	9.2 ± 1.1	
Median (IQR)	9.6 (8.3-10.0)	10.0 (10.0-10.0)	9.0 (8.5-9.5)	9.6 (8.7-10.0)	
(Min-Max)	5.4-10.0	7.0-10.0	7.0-10.0	5.4-10.0	
Fifth question					
Yes - N (%)	11 (52.4)	28 (82.4)	43 (95.5)	82 (82.0)	
No - N (%)	10 (47.6)	6 (17.7)	2 (4.5)	18 (18.0)	

SD: Standard Deviation
IQR: Interquartile Range
P[1]: P-value from Wilcoxon Signed-Rank to test non-inferiority of GAF
Notes:

a) Difference between fixative is defined as GAF total score – PBF total score

5.3.3 Analysis of other variables

No analysis of other variables has been planned for this study.

6 DISCUSSION AND OVERALL CONCLUSIONS

6.1 CLINICAL SAFETY AND PERFORMANCE RESULTS

At the end of the study, all primary and secondary endpoints were performed.

In total, 200 slides were considered in the analysis, in particular 100 slides (50%) were performed on GAF and 100 slides (50%) were performed on PBF.

The performance results confirmed the non-inferiority of GAF respect to PBF as highlighted by the previous interim analysis. Therefore, the GAF has the same ability of tissue fixation without toxicity and carcinogenic activity.

In this study, a classic safety evaluation (adverse events, vital signs and physical examination) was not planned, because there was not a direct involvement of subject or patient. However, as mentioned above, Glyoxal was proposed in 1943 (10) as a fixative alternative to formalin since it is a simple di-aldehyde. As reported by Harke & Hoeffler (11) glyoxal does not appear to evaporate from solution. Indeed, the reported Henry's law constant of ≤3.38 × 10– 4Pa m3/mol (12) indicates that glyoxal is essentially non-volatile with regard to the aqueous phase. Glyoxal is not classifiable as a human carcinogen (13), nevertheless it use may cause some adverse reactions such as irritation of skin and eyes (13).

For the secondary analysis, the performance evaluations for the local laboratories was considered. Also, in this case the results confirmed the non-inferiority of GAF respect to PBF and in terms of median value similar results between fixative groups were observed.

The overall mean satisfaction of the local laboratories can be considered positive. The mean value, considering a score scale from 1 (not satisfied) to 10 (totally satisfied), was 9.2 ± 1.1.
The only two insufficient scores were recorded by the Manchester laboratory (5.4 and 5.5 for a prostate and a endometrium slide respectively) even if all the parameter of performance evaluated (structural preservation of the tissue, preservation of the nuclei, preservation of the cytoplasm and diagnostic value) were considered valid.

6.2 ASSESSMENT OF RISKS AND BENEFITS

No clinical benefits are expected as the aim of the currently proposed study is to confirm in a large sample of histological specimens, obtained from different tissues, that an acid-free form of glyoxal (GAF) represents a novel tissue fixative by investigating morphological preservation and diagnostic value to be established on the basis of cellular details and of expression of immunohistochemical marker

As expected, the use of Glyoxal Acid-Free (GAF) seems not represent any particular risk. However, possible side effects were considered, like the above mentioned Anticipated adverse device effects to any of the device's ingredient, misuse of the device that might compromise the positive effect of the device when correctly used.

6.3 CLINICAL RELEVANCE AND IMPORTANCE OF THE RESULTS

Confirming the non-inferiority of GAF respect to PBF, the data of the trial highlight the capability of the investigational device to ensure the structural preservation of the: tissue, nuclei, cytoplasm and diagnostic value of the preparations (for the sections of all organs tested). These results are consistent with the rational/justification of the study and confirmed the satisfaction of local laboratories.

6.4 SPECIFIC BENEFITS OR SPECIAL PRECAUTIONS

As described above, considering the toxicity of formalin, environmental authorities are increasingly concerned for the objective toxicity of this volatile reagent, so that a banning of formalin from 2016 has been proposed in the European union. This has been stated by the ec regulation n.605/2014 (4) that modifies the ec regulation n.1272/2008 (5) defining formalin as a carcinogen (category 1b/2) and mutagen. This regulation should heavily impact on diagnostic pathology methods and procedures, even if so far the main reaction to this significant issue is limited to adoption of protective procedures, designed to prevent excessive exposure of pathology workers to formaldehyde vapors.

At the end of this Clinical Performance Study, the data of this report support GAF could be considered an effective tool as well PBF for the diagnostic procedures for the tumor specimens considered (breast, prostate, colon, endometrium, and lung), but with lower safety concerns than PBF itself.

On the other hand, considering the potential advantages related to the use of GAF, since the fixation time for the tissue specimen was set as 3 h. for GAF, 6 h. for PBF, the results imply that GAF is not only safer, but also faster than PBF, parity of results being considered, potentially speeding up the histological staining procedures.

6.5 IMPLICATIONS FOR FUTURE STUDIES

Even if from literature it is recognized the safety of GAF, on the other hand no data on the safety were collected during this Clinical Performance Study. In future studies, it could be useful to also consider some safety items (e.g., regarding the potential misuse of the IVD) to maintain updated the product vigilance. On the other hand, it could be interesting to expand the plethora of other tumor specimens to be analyzed. In this way, the promising result of this Clinical Performance Study could be verified also in the diagnostic process of other tumors kinds.

6.6 LIMITATIONS OF THE STUDY

A limitation of the study is potentially due to the size of the tissue specimens here investigated, since only small biopsies (and not large specimens were considered. An additional consideration is concerning the questionnaire on the satisfaction of local laboratory, that only allowed binary answers. It would have been useful to know the justification of the negative scores on the performance parameters (structural preservation of the tissue, preservation of the nuclei, preservation of the cytoplasm and diagnostic value).

Also the results obtained with the question: "Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?" can bring some consideration. Eighteen (18.0%) cases were answered negatively. This evaluation was observed both with high and low satisfaction scores. In this case could have been useful to clarify when this discrepancy was due to a considered superiority of the investigational product or not.

The above remarks offer prospects for future focused investigations.

7 LISTING

Listing 1. All analyzed and fixed slides

Slides	Center	Evaluated organ	Fixative
01	Barcelona	Colon	GAF
01	Barcelona	Colon	PBF
01	Barcelona	Lung	GAF
01	Barcelona	Lung	PBF
01	Manchester	Endometrium	GAF
01	Manchester	Endometrium	PBF
02	Candiolo	Breast	GAF
02	Candiolo	Breast	PBF
02	Barcelona	Colon	GAF
02	Barcelona	Colon	PBF
02	Barcelona	Lung	GAF
02	Barcelona	Lung	PBF
02	Manchester	Endometrium	GAF
02	Manchester	Endometrium	PBF
03	Candiolo	Breast	GAF
03	Candiolo	Breast	PBF
03	Barcelona	Colon	GAF
03	Barcelona	Colon	PBF
03	Barcelona	Lung	GAF
03	Barcelona	Lung	PBF
04	Candiolo	Breast	GAF
04	Candiolo	Breast	PBF
04	Barcelona	Colon	GAF
04	Barcelona	Colon	PBF
04	Barcelona	Lung	GAF

Slides	Center	Evaluated organ	Fixative
04	Barcelona	Lung	PBF
04	Manchester	Endometrium	GAF
04	Manchester	Endometrium	PBF
04	Manchester	Prostate	GAF
04	Manchester	Prostate	PBF
05	Barcelona	Colon	GAF
05	Barcelona	Colon	PBF
05	Barcelona	Lung	GAF
05	Barcelona	Lung	PBF
05	Manchester	Endometrium	GAF
05	Manchester	Endometrium	PBF
06	Candiolo	Breast	GAF
06	Candiolo	Breast	PBF
06	Barcelona	Colon	GAF
06	Barcelona	Colon	PBF
06	Barcelona	Lung	GAF
06	Barcelona	Lung	PBF
06	Manchester	Prostate	GAF
06	Manchester	Prostate	PBF
07	Candiolo	Breast	GAF
07	Candiolo	Breast	PBF
07	Barcelona	Colon	GAF
07	Barcelona	Colon	PBF
07	Barcelona	Lung	GAF
07	Barcelona	Lung	PBF
07	Manchester	Endometrium	GAF
07	Manchester	Endometrium	PBF

Slides	Center	Evaluated organ	Fixative
07	Manchester	Prostate	GAF
07	Manchester	Prostate	PBF
08	Candiolo	Breast	GAF
08	Candiolo	Breast	PBF
08	Barcelona	Colon	GAF
08	Barcelona	Colon	PBF
08	Barcelona	Lung	GAF
08	Barcelona	Lung	PBF
08	Manchester	Endometrium	GAF
08	Manchester	Endometrium	PBF
08	Manchester	Prostate	GAF
08	Manchester	Prostate	PBF
09	Candiolo	Breast	GAF
09	Candiolo	Breast	PBF
09	Barcelona	Colon	GAF
09	Barcelona	Colon	PBF
09	Manchester	Endometrium	GAF
09	Manchester	Endometrium	PBF
09	Manchester	Prostate	GAF
09	Manchester	Prostate	PBF
10	Barcelona	Colon	GAF
10	Barcelona	Colon	PBF
10	Manchester	Endometrium	GAF
10	Manchester	Endometrium	PBF
10	Manchester	Prostate	GAF
10	Manchester	Prostate	PBF
11	Candiolo	Breast	GAF

Slides	Center	Evaluated organ	Fixative
11	Candiolo	Breast	PBF
11	Manchester	Endometrium	GAF
11	Manchester	Endometrium	PBF
11	Manchester	Prostate	GAF
11	Manchester	Prostate	PBF
12	Candiolo	Breast	GAF
12	Candiolo	Breast	PBF
12	Barcelona	Colon	GAF
12	Barcelona	Colon	PBF
12	Manchester	Endometrium	GAF
12	Manchester	Endometrium	PBF
12	Manchester	Prostate	GAF
12	Manchester	Prostate	PBF
13	Candiolo	Breast	GAF
13	Candiolo	Breast	PBF
13	Barcelona	Colon	GAF
13	Barcelona	Colon	PBF
13	Manchester	Endometrium	GAF
13	Manchester	Endometrium	PBF
13	Manchester	Prostate	GAF
13	Manchester	Prostate	PBF
14	Candiolo	Breast	GAF
14	Candiolo	Breast	PBF
14	Barcelona	Colon	GAF
14	Barcelona	Colon	PBF
14	Manchester	Prostate	GAF
14	Manchester	Prostate	PBF

Slides	Center	Evaluated organ	Fixative
15	Candiolo	Breast	GAF
15	Candiolo	Breast	PBF
15	Barcelona	Colon	GAF
15	Barcelona	Colon	PBF
16	Candiolo	Breast	GAF
16	Candiolo	Breast	PBF
16	Barcelona	Colon	GAF
16	Barcelona	Colon	PBF
17	Candiolo	Breast	GAF
17	Candiolo	Breast	PBF
17	Barcelona	Colon	GAF
17	Barcelona	Colon	PBF
18	Barcelona	Colon	GAF
18	Barcelona	Colon	PBF
19	Candiolo	Breast	GAF
19	Candiolo	Breast	PBF
19	Barcelona	Colon	GAF
19	Barcelona	Colon	PBF
20	Candiolo	Breast	GAF
20	Candiolo	Breast	PBF
20	Barcelona	Colon	GAF
20	Barcelona	Colon	PBF
21	Candiolo	Breast	GAF
21	Candiolo	Breast	PBF
21	Barcelona	Colon	GAF
21	Barcelona	Colon	PBF
22	Candiolo	Breast	GAF

Slides	Center	Evaluated organ	Fixative
22	Candiolo	Breast	PBF
22	Barcelona	Colon	GAF
22	Barcelona	Colon	PBF
23	Candiolo	Breast	GAF
23	Candiolo	Breast	PBF
23	Barcelona	Colon	GAF
23	Barcelona	Colon	PBF
24	Candiolo	Breast	GAF
24	Candiolo	Breast	PBF
24	Barcelona	Colon	GAF
24	Barcelona	Colon	PBF
25	Candiolo	Breast	GAF
25	Candiolo	Breast	PBF
25	Barcelona	Colon	GAF
25	Barcelona	Colon	PBF
26	Candiolo	Breast	GAF
26	Candiolo	Breast	PBF
26	Barcelona	Colon	GAF
26	Barcelona	Colon	PBF
27	Candiolo	Breast	GAF
27	Candiolo	Breast	PBF
27	Barcelona	Colon	GAF
27	Barcelona	Colon	PBF
28	Candiolo	Breast	GAF
28	Candiolo	Breast	PBF
29	Candiolo	Breast	GAF
29	Candiolo	Breast	PBF

Slides	Center	Evaluated organ	Fixative
30	Candiolo	Breast	GAF
30	Candiolo	Breast	PBF
31	Candiolo	Breast	GAF
31	Candiolo	Breast	PBF
33	Candiolo	Breast	GAF
33	Candiolo	Breast	PBF
34	Candiolo	Breast	GAF
34	Candiolo	Breast	PBF
35	Candiolo	Breast	GAF
35	Candiolo	Breast	PBF
36	Candiolo	Breast	GAF
36	Candiolo	Breast	PBF
37	Candiolo	Breast	GAF
37	Candiolo	Breast	PBF
38	Candiolo	Breast	GAF
38	Candiolo	Breast	PBF
40	Candiolo	Breast	GAF
40	Candiolo	Breast	PBF
41	Candiolo	Breast	GAF
41	Candiolo	Breast	PBF
42	Candiolo	Breast	GAF
42	Candiolo	Breast	PBF
43	Candiolo	Breast	GAF
43	Candiolo	Breast	PBF
44	Candiolo	Breast	GAF
44	Candiolo	Breast	PBF
45	Candiolo	Breast	GAF

Slides	Center	Evaluated organ	Fixative
45	Candiolo	Breast	PBF
46	Candiolo	Breast	GAF
46	Candiolo	Breast	PBF
47	Candiolo	Breast	GAF
47	Candiolo	Breast	PBF
48	Candiolo	Breast	GAF
48	Candiolo	Breast	PBF
50	Candiolo	Breast	GAF
50	Candiolo	Breast	PBF
51	Candiolo	Breast	GAF
51	Candiolo	Breast	PBF
52	Candiolo	Breast	GAF
52	Candiolo	Breast	PBF

Listing 2a. Primary Efficacy Endpoint

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
01	GAF	Colon	Valid	Valid	Valid	Valid
01	PBF	Colon	Valid	Valid	Valid	Valid
01	GAF	Lung	Valid	Valid	Valid	Valid
01	PBF	Lung	Valid	Valid	Valid	Valid
01	GAF	Endometrium	Valid	Valid	Valid	Valid
01	PBF	Endometrium	Valid	Valid	Valid	Valid
02	GAF	Breast	Valid	Valid	Valid	Valid
02	PBF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
02	GAF	Colon	Valid	Valid	Valid	Valid
02	PBF	Colon	Valid	Valid	Valid	Valid
02	GAF	Lung	Valid	Valid	Valid	Valid
02	PBF	Lung	Valid	Valid	Valid	Valid
02	GAF	Endometrium	Valid	Valid	Valid	Valid
02	PBF	Endometrium	Valid	Valid	Valid	Valid
03	GAF	Breast	Valid	Valid	Valid	Valid
03	PBF	Breast	Valid	Valid	Valid	Valid
03	GAF	Colon	Valid	Valid	Valid	Valid
03	PBF	Colon	Valid	Valid	Valid	Valid
03	GAF	Lung	Valid	Valid	Valid	Valid
03	PBF	Lung	Valid	Valid	Valid	Valid
04	GAF	Breast	Valid	Valid	Valid	Valid
04	PBF	Breast	Valid	Valid	Valid	Valid
04	GAF	Colon	Valid	Valid	Valid	Valid
04	PBF	Colon	Valid	Valid	Valid	Valid
04	GAF	Lung	Valid	Valid	Valid	Valid
04	PBF	Lung	Valid	Valid	Valid	Valid
04	GAF	Endometrium	Valid	Valid	Valid	Valid
04	PBF	Endometrium	Valid	Valid	Valid	Valid
04	GAF	Prostate	Valid	Valid	Valid	Valid
04	PBF	Prostate	Valid	Valid	Valid	Valid
05	GAF	Colon	Valid	Valid	Valid	Valid
05	PBF	Colon	Valid	Valid	Valid	Valid
05	GAF	Lung	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
05	PBF	Lung	Valid	Valid	Valid	Valid
05	GAF	Endometrium	Valid	Valid	Valid	Valid
05	PBF	Endometrium	Valid	Valid	Valid	Valid
06	GAF	Breast	Valid	Valid	Valid	Valid
06	PBF	Breast	Valid	Valid	Valid	Valid
06	GAF	Colon	Valid	Valid	Valid	Valid
06	PBF	Colon	Valid	Valid	Valid	Valid
06	GAF	Lung	Valid	Valid	Valid	Valid
06	PBF	Lung	Valid	Valid	Valid	Valid
06	GAF	Prostate	Invalid	Valid	Valid	Valid
06	PBF	Prostate	Valid	Valid	Valid	Valid
07	GAF	Breast	Invalid	Valid	Valid	Valid
07	PBF	Breast	Valid	Valid	Valid	Valid
07	GAF	Colon	Valid	Valid	Valid	Valid
07	PBF	Colon	Valid	Valid	Valid	Valid
07	GAF	Lung	Valid	Valid	Valid	Valid
07	PBF	Lung	Valid	Valid	Valid	Valid
07	GAF	Endometrium	Valid	Valid	Valid	Invalid
07	PBF	Endometrium	Valid	Valid	Valid	Valid
07	GAF	Prostate	Valid	Valid	Valid	Valid
07	PBF	Prostate	Valid	Valid	Valid	Valid
08	GAF	Breast	Invalid	Valid	Valid	Valid
08	PBF	Breast	Valid	Valid	Valid	Valid
08	GAF	Colon	Valid	Valid	Valid	Valid
08	PBF	Colon	Valid	Valid	Valid	Invalid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
08	GAF	Lung	Invalid	Valid	Valid	Valid
08	PBF	Lung	Valid	Valid	Valid	Valid
08	GAF	Endometrium	Valid	Valid	Valid	Valid
08	PBF	Endometrium	Valid	Valid	Valid	Valid
08	GAF	Prostate	Valid	Valid	Valid	Valid
08	PBF	Prostate	Valid	Valid	Valid	Valid
09	GAF	Breast	Invalid	Valid	Valid	Valid
09	PBF	Breast	Valid	Valid	Valid	Valid
09	GAF	Colon	Valid	Valid	Valid	Invalid
09	PBF	Colon	Valid	Valid	Valid	Invalid
09	GAF	Endometrium	Valid	Valid	Valid	Valid
09	PBF	Endometrium	Valid	Valid	Valid	Valid
09	GAF	Prostate	Valid	Valid	Valid	Valid
09	PBF	Prostate	Valid	Valid	Valid	Valid
10	GAF	Colon	Valid	Valid	Valid	Invalid
10	PBF	Colon	Valid	Valid	Valid	Valid
10	GAF	Endometrium	Valid	Valid	Valid	Valid
10	PBF	Endometrium	Valid	Valid	Valid	Valid
10	GAF	Prostate	Valid	Valid	Valid	Valid
10	PBF	Prostate	Valid	Valid	Valid	Valid
11	GAF	Breast	Valid	Valid	Valid	Valid
11	PBF	Breast	Valid	Valid	Valid	Valid
11	GAF	Endometrium	Invalid	Valid	Valid	Valid
11	PBF	Endometrium	Valid	Valid	Valid	Valid
11	GAF	Prostate	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
11	PBF	Prostate	Valid	Valid	Valid	Invalid
12	GAF	Breast	Valid	Valid	Valid	Valid
12	PBF	Breast	Valid	Valid	Valid	Invalid
12	GAF	Colon	Valid	Valid	Valid	Valid
12	PBF	Colon	Valid	Valid	Valid	Valid
12	GAF	Endometrium	Valid	Valid	Valid	Valid
12	PBF	Endometrium	Valid	Valid	Valid	Valid
12	GAF	Prostate	Invalid	Valid	Valid	Valid
12	PBF	Prostate	Valid	Valid	Valid	Valid
13	GAF	Breast	Invalid	Invalid	Valid	Valid
13	PBF	Breast	Valid	Valid	Valid	Valid
13	GAF	Colon	Valid	Valid	Valid	Invalid
13	PBF	Colon	Valid	Valid	Valid	Valid
13	GAF	Endometrium	Valid	Valid	Valid	Valid
13	PBF	Endometrium	Valid	Valid	Valid	Invalid
13	GAF	Prostate	Valid	Valid	Valid	Valid
13	PBF	Prostate	Valid	Valid	Valid	Valid
14	GAF	Breast	Valid	Valid	Valid	Valid
14	PBF	Breast	Valid	Valid	Valid	Valid
14	GAF	Colon	Valid	Valid	Valid	Valid
14	PBF	Colon	Valid	Valid	Valid	Valid
14	GAF	Prostate	Invalid	Valid	Valid	Valid
14	PBF	Prostate	Valid	Valid	Valid	Valid
15	GAF	Breast	Valid	Valid	Valid	Valid
15	PBF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
15	GAF	Colon	Valid	Valid	Valid	Valid
15	PBF	Colon	Valid	Valid	Valid	Valid
16	GAF	Breast	Valid	Valid	Valid	Valid
16	PBF	Breast	Valid	Valid	Valid	Valid
16	GAF	Colon	Valid	Valid	Valid	Valid
16	PBF	Colon	Valid	Valid	Valid	Valid
17	GAF	Breast	Valid	Valid	Valid	Valid
17	PBF	Breast	Valid	Valid	Valid	Valid
17	GAF	Colon	Valid	Valid	Valid	Valid
17	PBF	Colon	Valid	Valid	Valid	Valid
18	GAF	Colon	Valid	Valid	Valid	Valid
18	PBF	Colon	Valid	Valid	Valid	Valid
19	GAF	Breast	Valid	Valid	Valid	Valid
19	PBF	Breast	Valid	Valid	Valid	Valid
19	GAF	Colon	Valid	Valid	Valid	Valid
19	PBF	Colon	Valid	Valid	Valid	Valid
20	GAF	Breast	Valid	Valid	Valid	Valid
20	PBF	Breast	Valid	Valid	Valid	Valid
20	GAF	Colon	Invalid	Valid	Valid	Valid
20	PBF	Colon	Valid	Valid	Valid	Valid
21	GAF	Breast	Valid	Valid	Valid	Invalid
21	PBF	Breast	Valid	Valid	Valid	Valid
21	GAF	Colon	Invalid	Valid	Valid	Valid
21	PBF	Colon	Valid	Valid	Valid	Valid
22	GAF	Breast	Valid	Valid	Valid	Invalid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
22	PBF	Breast	Valid	Valid	Valid	Valid
22	GAF	Colon	Invalid	Valid	Valid	Valid
22	PBF	Colon	Valid	Valid	Valid	Valid
23	GAF	Breast	Valid	Valid	Valid	Valid
23	PBF	Breast	Valid	Valid	Valid	Valid
23	GAF	Colon	Invalid	Valid	Valid	Valid
23	PBF	Colon	Valid	Valid	Valid	Valid
24	GAF	Breast	Invalid	Valid	Valid	Valid
24	PBF	Breast	Valid	Valid	Valid	Valid
24	GAF	Colon	Valid	Valid	Valid	Invalid
24	PBF	Colon	Valid	Valid	Valid	Invalid
25	GAF	Breast	Valid	Valid	Valid	Valid
25	PBF	Breast	Valid	Valid	Valid	Valid
25	GAF	Colon	Valid	Valid	Valid	Valid
25	PBF	Colon	Valid	Valid	Valid	Valid
26	GAF	Breast	Valid	Valid	Valid	Valid
26	PBF	Breast	Valid	Valid	Valid	Valid
26	GAF	Colon	Valid	Valid	Valid	Valid
26	PBF	Colon	Valid	Valid	Valid	Valid
27	GAF	Breast	Valid	Valid	Valid	Valid
27	PBF	Breast	Valid	Valid	Valid	Valid
27	GAF	Colon	Valid	Valid	Valid	Valid
27	PBF	Colon	Valid	Valid	Valid	Valid
28	GAF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
28	PBF	Breast	Valid	Valid	Valid	Valid
29	GAF	Breast	Valid	Valid	Valid	Valid
29	PBF	Breast	Valid	Valid	Valid	Valid
30	GAF	Breast	Valid	Valid	Valid	Valid
30	PBF	Breast	Valid	Valid	Valid	Valid
31	GAF	Breast	Valid	Valid	Valid	Valid
31	PBF	Breast	Valid	Valid	Valid	Valid
33	GAF	Breast	Valid	Valid	Valid	Valid
33	PBF	Breast	Valid	Valid	Valid	Valid
34	GAF	Breast	Invalid	Valid	Valid	Valid
34	PBF	Breast	Invalid	Valid	Valid	Valid
35	GAF	Breast	Valid	Valid	Valid	Valid
35	PBF	Breast	Valid	Valid	Valid	Valid
36	GAF	Breast	Valid	Valid	Valid	Valid
36	PBF	Breast	Valid	Valid	Valid	Valid
37	GAF	Breast	Valid	Valid	Valid	Valid
37	PBF	Breast	Valid	Valid	Valid	Valid
38	GAF	Breast	Valid	Valid	Valid	Valid
38	PBF	Breast	Valid	Valid	Valid	Valid
40	GAF	Breast	Valid	Valid	Valid	Valid
40	PBF	Breast	Valid	Valid	Valid	Valid
41	GAF	Breast	Invalid	Valid	Valid	Invalid
41	PBF	Breast	Valid	Valid	Valid	Valid
42	GAF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
42	PBF	Breast	Valid	Valid	Valid	Valid
43	GAF	Breast	Valid	Valid	Valid	Valid
43	PBF	Breast	Valid	Valid	Valid	Valid
44	GAF	Breast	Valid	Valid	Valid	Valid
44	PBF	Breast	Valid	Valid	Valid	Valid
45	GAF	Breast	Valid	Valid	Valid	Valid
45	PBF	Breast	Valid	Valid	Valid	Valid
46	GAF	Breast	Valid	Valid	Valid	Valid
46	PBF	Breast	Valid	Valid	Valid	Valid
47	GAF	Breast	Invalid	Valid	Valid	Invalid
47	PBF	Breast	Valid	Valid	Valid	Invalid
48	GAF	Breast	Valid	Valid	Valid	Valid
48	PBF	Breast	Valid	Valid	Valid	Valid
50	GAF	Breast	Valid	Valid	Valid	Valid
50	PBF	Breast	Valid	Valid	Valid	Valid
51	GAF	Breast	Valid	Valid	Valid	Invalid
51	PBF	Breast	Valid	Valid	Valid	Valid
52	GAF	Breast	Valid	Valid	Valid	Valid
52	PBF	Breast	Valid	Valid	Valid	Valid

Listing 2b. Primary Efficacy Endpoint

Slides	Fixative	Evaluated organ	Central reviewer Total Score
01	GAF	Colon	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
01	PBF	Colon	4
01	GAF	Lung	4
01	PBF	Lung	4
01	GAF	Endometrium	4
01	PBF	Endometrium	4
02	GAF	Breast	4
02	PBF	Breast	4
02	GAF	Colon	4
02	PBF	Colon	4
02	GAF	Lung	4
02	PBF	Lung	4
02	GAF	Endometrium	4
02	PBF	Endometrium	4
03	GAF	Breast	4
03	PBF	Breast	4
03	GAF	Colon	4
03	PBF	Colon	4
03	GAF	Lung	4
03	PBF	Lung	4
04	GAF	Breast	4
04	PBF	Breast	4
04	GAF	Colon	4
04	PBF	Colon	4
04	GAF	Lung	4
04	PBF	Lung	4
04	GAF	Endometrium	4
04	PBF	Endometrium	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
04	GAF	Prostate	4
04	PBF	Prostate	4
05	GAF	Colon	4
05	PBF	Colon	4
05	GAF	Lung	4
05	PBF	Lung	4
05	GAF	Endometrium	4
05	PBF	Endometrium	4
06	GAF	Breast	4
06	PBF	Breast	4
06	GAF	Colon	4
06	PBF	Colon	4
06	GAF	Lung	4
06	PBF	Lung	4
06	GAF	Prostate	3
06	PBF	Prostate	4
07	GAF	Breast	3
07	PBF	Breast	4
07	GAF	Colon	4
07	PBF	Colon	4
07	GAF	Lung	4
07	PBF	Lung	4
07	GAF	Endometrium	3
07	PBF	Endometrium	4
07	GAF	Prostate	4
07	PBF	Prostate	4
08	GAF	Breast	3

Slides	Fixative	Evaluated organ	Central reviewer Total Score
08	PBF	Breast	4
08	GAF	Colon	4
08	PBF	Colon	3
08	GAF	Lung	3
08	PBF	Lung	4
08	GAF	Endometrium	4
08	PBF	Endometrium	4
08	GAF	Prostate	4
08	PBF	Prostate	4
09	GAF	Breast	3
09	PBF	Breast	4
09	GAF	Colon	3
09	PBF	Colon	3
09	GAF	Endometrium	4
09	PBF	Endometrium	4
09	GAF	Prostate	4
09	PBF	Prostate	4
10	GAF	Colon	3
10	PBF	Colon	4
10	GAF	Endometrium	4
10	PBF	Endometrium	4
10	GAF	Prostate	4
10	PBF	Prostate	4
11	GAF	Breast	4
11	PBF	Breast	4
11	GAF	Endometrium	3
11	PBF	Endometrium	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
11	GAF	Prostate	4
11	PBF	Prostate	3
12	GAF	Breast	4
12	PBF	Breast	3
12	GAF	Colon	4
12	PBF	Colon	4
12	GAF	Endometrium	4
12	PBF	Endometrium	4
12	GAF	Prostate	3
12	PBF	Prostate	4
13	GAF	Breast	2
13	PBF	Breast	4
13	GAF	Colon	3
13	PBF	Colon	4
13	GAF	Endometrium	4
13	PBF	Endometrium	3
13	GAF	Prostate	4
13	PBF	Prostate	4
14	GAF	Breast	4
14	PBF	Breast	4
14	GAF	Colon	4
14	PBF	Colon	4
14	GAF	Prostate	3
14	PBF	Prostate	4
15	GAF	Breast	4
15	PBF	Breast	4
15	GAF	Colon	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
15	PBF	Colon	4
16	GAF	Breast	4
16	PBF	Breast	4
16	GAF	Colon	4
16	PBF	Colon	4
17	GAF	Breast	4
17	PBF	Breast	4
17	GAF	Colon	4
17	PBF	Colon	4
18	GAF	Colon	4
18	PBF	Colon	4
19	GAF	Breast	4
19	PBF	Breast	4
19	GAF	Colon	4
19	PBF	Colon	4
20	GAF	Breast	4
20	PBF	Breast	4
20	GAF	Colon	3
20	PBF	Colon	4
21	GAF	Breast	3
21	PBF	Breast	4
21	GAF	Colon	3
21	PBF	Colon	4
22	GAF	Breast	3
22	PBF	Breast	4
22	GAF	Colon	3
22	PBF	Colon	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
23	GAF	Breast	4
23	PBF	Breast	4
23	GAF	Colon	3
23	PBF	Colon	4
24	GAF	Breast	3
24	PBF	Breast	4
24	GAF	Colon	3
24	PBF	Colon	3
25	GAF	Breast	4
25	PBF	Breast	4
25	GAF	Colon	4
25	PBF	Colon	4
26	GAF	Breast	4
26	PBF	Breast	4
26	GAF	Colon	4
26	PBF	Colon	4
27	GAF	Breast	4
27	PBF	Breast	4
27	GAF	Colon	4
27	PBF	Colon	4
28	GAF	Breast	4
28	PBF	Breast	4
29	GAF	Breast	4
29	PBF	Breast	4
30	GAF	Breast	4
30	PBF	Breast	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
31	GAF	Breast	4
31	PBF	Breast	4
33	GAF	Breast	4
33	PBF	Breast	4
34	GAF	Breast	3
34	PBF	Breast	3
35	GAF	Breast	4
35	PBF	Breast	4
36	GAF	Breast	4
36	PBF	Breast	4
37	GAF	Breast	4
37	PBF	Breast	4
38	GAF	Breast	4
38	PBF	Breast	4
40	GAF	Breast	4
40	PBF	Breast	4
41	GAF	Breast	2
41	PBF	Breast	4
42	GAF	Breast	4
42	PBF	Breast	4
43	GAF	Breast	4
43	PBF	Breast	4
44	GAF	Breast	4
44	PBF	Breast	4
45	GAF	Breast	4
45	PBF	Breast	4

Slides	Fixative	Evaluated organ	Central reviewer Total Score
46	GAF	Breast	4
46	PBF	Breast	4
47	GAF	Breast	2
47	PBF	Breast	3
48	GAF	Breast	4
48	PBF	Breast	4
50	GAF	Breast	4
50	PBF	Breast	4
51	GAF	Breast	3
51	PBF	Breast	4
52	GAF	Breast	4
52	PBF	Breast	4

Listing 3a. Secondary Efficacy Endpoints

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
01	GAF	Colon	Valid	Valid	Valid	Valid
01	PBF	Colon	Valid	Valid	Valid	Valid
01	GAF	Lung	Valid	Valid	Invalid	Valid
01	PBF	Lung	Valid	Valid	Valid	Valid
01	GAF	Endometrium	Valid	Valid	Valid	Valid
01	PBF	Endometrium	Valid	Valid	Valid	Valid
02	GAF	Breast	Valid	Valid	Valid	Valid
02	PBF	Breast	Valid	Valid	Valid	Valid
02	GAF	Colon	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
02	PBF	Colon	Valid	Valid	Valid	Valid
02	GAF	Lung	Valid	Valid	Valid	Valid
02	PBF	Lung	Valid	Valid	Valid	Valid
02	GAF	Endometrium	Valid	Valid	Valid	Valid
02	PBF	Endometrium	Valid	Valid	Valid	Valid
03	GAF	Breast	Valid	Valid	Valid	Valid
03	PBF	Breast	Valid	Valid	Valid	Valid
03	GAF	Colon	Valid	Valid	Valid	Valid
03	PBF	Colon	Valid	Valid	Valid	Valid
03	GAF	Lung	Valid	Valid	Valid	Valid
03	PBF	Lung	Valid	Valid	Valid	Valid
04	GAF	Breast	Valid	Valid	Valid	Valid
04	PBF	Breast	Valid	Valid	Valid	Valid
04	GAF	Colon	Valid	Valid	Valid	Valid
04	PBF	Colon	Valid	Valid	Valid	Valid
04	GAF	Lung	Valid	Valid	Valid	Valid
04	PBF	Lung	Valid	Valid	Valid	Valid
04	GAF	Endometrium	Valid	Valid	Valid	Valid
04	PBF	Endometrium	Valid	Valid	Valid	Valid
04	GAF	Prostate	Valid	Valid	Valid	Valid
04	PBF	Prostate	Valid	Valid	Valid	Valid
05	GAF	Colon	Valid	Valid	Valid	Valid
05	PBF	Colon	Valid	Valid	Valid	Valid
05	GAF	Lung	Valid	Valid	Valid	Valid
05	PBF	Lung	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
05	GAF	Endometrium	Valid	Valid	Valid	Valid
05	PBF	Endometrium	Valid	Valid	Valid	Valid
06	GAF	Breast	Valid	Valid	Valid	Valid
06	PBF	Breast	Valid	Valid	Valid	Valid
06	GAF	Colon	Valid	Valid	Valid	Valid
06	PBF	Colon	Valid	Valid	Valid	Valid
06	GAF	Lung	Valid	Valid	Valid	Valid
06	PBF	Lung	Valid	Valid	Valid	Valid
06	GAF	Prostate	Invalid	Invalid	Invalid	Valid
06	PBF	Prostate	Valid	Valid	Valid	Valid
07	GAF	Breast	Valid	Valid	Valid	Valid
07	PBF	Breast	Valid	Valid	Valid	Valid
07	GAF	Colon	Valid	Valid	Valid	Valid
07	PBF	Colon	Valid	Valid	Valid	Valid
07	GAF	Lung	Valid	Valid	Valid	Valid
07	PBF	Lung	Valid	Valid	Valid	Valid
07	GAF	Endometrium	Valid	Valid	Valid	Invalid
07	PBF	Endometrium	Valid	Valid	Valid	Valid
07	GAF	Prostate	Valid	Invalid	Valid	Valid
07	PBF	Prostate	Valid	Valid	Valid	Valid
08	GAF	Breast	Valid	Valid	Valid	Valid
08	PBF	Breast	Valid	Valid	Valid	Valid
08	GAF	Colon	Valid	Valid	Valid	Valid
08	PBF	Colon	Valid	Valid	Valid	Valid
08	GAF	Lung	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
08	PBF	Lung	Valid	Valid	Valid	Valid
08	GAF	Endometrium	Valid	Valid	Valid	Valid
08	PBF	Endometrium	Valid	Valid	Valid	Valid
08	GAF	Prostate	Valid	Invalid	Valid	Valid
08	PBF	Prostate	Valid	Valid	Valid	Valid
09	GAF	Breast	Valid	Valid	Valid	Valid
09	PBF	Breast	Valid	Valid	Valid	Valid
09	GAF	Colon	Valid	Valid	Valid	Invalid
09	PBF	Colon	Valid	Valid	Valid	Valid
09	GAF	Endometrium	Valid	Valid	Valid	Valid
09	PBF	Endometrium	Valid	Valid	Valid	Valid
09	GAF	Prostate	Valid	Invalid	Valid	Valid
09	PBF	Prostate	Valid	Valid	Valid	Valid
10	GAF	Colon	Valid	Valid	Valid	Valid
10	PBF	Colon	Valid	Valid	Valid	Valid
10	GAF	Endometrium	Valid	Valid	Valid	Valid
10	PBF	Endometrium	Valid	Valid	Valid	Valid
10	GAF	Prostate	Valid	Invalid	Valid	Valid
10	PBF	Prostate	Valid	Valid	Valid	Valid
11	GAF	Breast	Valid	Valid	Valid	Valid
11	PBF	Breast	Valid	Valid	Valid	Valid
11	GAF	Endometrium	Valid	Valid	Valid	Invalid
11	PBF	Endometrium	Valid	Valid	Valid	Valid
11	GAF	Prostate	Valid	Invalid	Valid	Valid
11	PBF	Prostate	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
12	GAF	Breast	Valid	Valid	Valid	Valid
12	PBF	Breast	Valid	Valid	Valid	Valid
12	GAF	Colon	Valid	Valid	Valid	Valid
12	PBF	Colon	Valid	Valid	Valid	Valid
12	GAF	Endometrium	Valid	Valid	Valid	Invalid
12	PBF	Endometrium	Valid	Valid	Valid	Valid
12	GAF	Prostate	Valid	Invalid	Valid	Valid
12	PBF	Prostate	Valid	Valid	Valid	Valid
13	GAF	Breast	Valid	Valid	Valid	Valid
13	PBF	Breast	Valid	Valid	Valid	Valid
13	GAF	Colon	Valid	Valid	Valid	Valid
13	PBF	Colon	Valid	Valid	Valid	Valid
13	GAF	Endometrium	Valid	Valid	Valid	Valid
13	PBF	Endometrium	Valid	Valid	Valid	Valid
13	GAF	Prostate	Valid	Invalid	Valid	Valid
13	PBF	Prostate	Valid	Valid	Valid	Valid
14	GAF	Breast	Valid	Valid	Valid	Valid
14	PBF	Breast	Valid	Valid	Valid	Valid
14	GAF	Colon	Valid	Valid	Valid	Valid
14	PBF	Colon	Valid	Valid	Valid	Valid
14	GAF	Prostate	Valid	Invalid	Valid	Valid
14	PBF	Prostate	Valid	Valid	Valid	Valid
15	GAF	Breast	Valid	Valid	Valid	Valid
15	PBF	Breast	Valid	Valid	Valid	Valid
15	GAF	Colon	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
15	PBF	Colon	Valid	Valid	Valid	Valid
16	GAF	Breast	Valid	Valid	Valid	Valid
16	PBF	Breast	Valid	Valid	Valid	Valid
16	GAF	Colon	Valid	Valid	Valid	Valid
16	PBF	Colon	Valid	Valid	Valid	Valid
17	GAF	Breast	Valid	Valid	Valid	Valid
17	PBF	Breast	Valid	Valid	Valid	Valid
17	GAF	Colon	Valid	Valid	Valid	Valid
17	PBF	Colon	Valid	Valid	Valid	Valid
18	GAF	Colon	Valid	Valid	Invalid	Valid
18	PBF	Colon	Valid	Valid	Valid	Valid
19	GAF	Breast	Valid	Valid	Valid	Valid
19	PBF	Breast	Valid	Valid	Valid	Valid
19	GAF	Colon	Valid	Valid	Invalid	Valid
19	PBF	Colon	Valid	Valid	Valid	Valid
20	GAF	Breast	Valid	Valid	Valid	Valid
20	PBF	Breast	Valid	Valid	Valid	Valid
20	GAF	Colon	Valid	Valid	Invalid	Valid
20	PBF	Colon	Valid	Valid	Valid	Valid
21	GAF	Breast	Valid	Valid	Valid	Valid
21	PBF	Breast	Valid	Valid	Valid	Valid
21	GAF	Colon	Valid	Valid	Invalid	Valid
21	PBF	Colon	Valid	Valid	Valid	Valid
22	GAF	Breast	Valid	Valid	Valid	Valid
22	PBF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
22	GAF	Colon	Valid	Valid	Invalid	Valid
22	PBF	Colon	Valid	Valid	Valid	Valid
23	GAF	Breast	Valid	Valid	Valid	Valid
23	PBF	Breast	Valid	Valid	Valid	Valid
23	GAF	Colon	Valid	Valid	Valid	Valid
23	PBF	Colon	Valid	Valid	Valid	Valid
24	GAF	Breast	Valid	Valid	Valid	Valid
24	PBF	Breast	Valid	Valid	Valid	Valid
24	GAF	Colon	Valid	Valid	Valid	Valid
24	PBF	Colon	Valid	Valid	Valid	Valid
25	GAF	Breast	Valid	Valid	Valid	Valid
25	PBF	Breast	Valid	Valid	Valid	Valid
25	GAF	Colon	Valid	Valid	Invalid	Valid
25	PBF	Colon	Valid	Valid	Valid	Valid
26	GAF	Breast	Valid	Valid	Valid	Valid
26	PBF	Breast	Valid	Valid	Valid	Valid
26	GAF	Colon	Valid	Valid	Valid	Valid
26	PBF	Colon	Valid	Valid	Valid	Valid
27	GAF	Breast	Valid	Valid	Valid	Valid
27	PBF	Breast	Valid	Valid	Valid	Valid
27	GAF	Colon	Valid	Valid	Valid	Valid
27	PBF	Colon	Valid	Valid	Valid	Valid
28	GAF	Breast	Valid	Valid	Valid	Valid
28	PBF	Breast	Valid	Valid	Valid	Valid
29	GAF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
29	PBF	Breast	Valid	Valid	Valid	Valid
30	GAF	Breast	Valid	Valid	Valid	Valid
30	PBF	Breast	Valid	Valid	Valid	Valid
31	GAF	Breast	Valid	Valid	Valid	Valid
31	PBF	Breast	Valid	Valid	Valid	Invalid
33	GAF	Breast	Valid	Valid	Valid	Valid
33	PBF	Breast	Valid	Valid	Valid	Valid
34	GAF	Breast	Valid	Valid	Valid	Valid
34	PBF	Breast	Valid	Valid	Valid	Valid
35	GAF	Breast	Valid	Valid	Valid	Valid
35	PBF	Breast	Valid	Valid	Valid	Valid
36	GAF	Breast	Valid	Valid	Valid	Valid
36	PBF	Breast	Valid	Valid	Valid	Valid
37	GAF	Breast	Valid	Valid	Valid	Valid
37	PBF	Breast	Valid	Valid	Valid	Valid
38	GAF	Breast	Valid	Valid	Valid	Valid
38	PBF	Breast	Valid	Valid	Valid	Valid
40	GAF	Breast	Valid	Valid	Valid	Valid
40	PBF	Breast	Valid	Valid	Valid	Valid
41	GAF	Breast	Valid	Valid	Valid	Valid
41	PBF	Breast	Valid	Valid	Valid	Valid
42	GAF	Breast	Valid	Valid	Valid	Valid
42	PBF	Breast	Valid	Valid	Valid	Valid
43	GAF	Breast	Valid	Valid	Valid	Valid
43	PBF	Breast	Valid	Valid	Valid	Valid

Slides	Fixative	Evaluated organ	How do you regard the structural preservation of the tissue	How do you regard the preservation of the nuclei	How do you regard the preservation of the cytoplasm	How do you regard the diagnostic value of these preparations
44	GAF	Breast	Valid	Valid	Valid	Valid
44	PBF	Breast	Valid	Valid	Valid	Valid
45	GAF	Breast	Valid	Valid	Valid	Valid
45	PBF	Breast	Valid	Valid	Valid	Valid
46	GAF	Breast	Valid	Valid	Valid	Valid
46	PBF	Breast	Valid	Valid	Valid	Valid
47	GAF	Breast	Valid	Valid	Valid	Valid
47	PBF	Breast	Valid	Valid	Valid	Valid
48	GAF	Breast	Valid	Valid	Valid	Valid
48	PBF	Breast	Valid	Valid	Valid	Valid
50	GAF	Breast	Valid	Valid	Valid	Valid
50	PBF	Breast	Valid	Valid	Valid	Valid
51	GAF	Breast	Valid	Valid	Valid	Valid
51	PBF	Breast	Valid	Valid	Valid	Valid
52	GAF	Breast	Valid	Valid	Valid	Valid
52	PBF	Breast	Valid	Valid	Valid	Valid

Listing 3b. Secondary Efficacy Endpoints

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
01	GAF	Barcelona	Colon	No	7.5	4
01	PBF	Barcelona	Colon	.	.	4
01	GAF	Barcelona	Lung	Yes	10	3
01	PBF	Barcelona	Lung	.	.	4
01	GAF	Manchester	Endometrium	Yes	10	4
01	PBF	Manchester	Endometrium	.	.	4
02	GAF	Candiolo	Breast	Yes	10	4
02	PBF	Candiolo	Breast	.	.	4
02	GAF	Barcelona	Colon	Yes	10	4
02	PBF	Barcelona	Colon	.	.	4
02	GAF	Barcelona	Lung	Yes	10	4
02	PBF	Barcelona	Lung	.	.	4
02	GAF	Manchester	Endometrium	Yes	10	4
02	PBF	Manchester	Endometrium	.	.	4
03	GAF	Candiolo	Breast	Yes	8.5	4
03	PBF	Candiolo	Breast	.	.	4
03	GAF	Barcelona	Colon	Yes	10	4
03	PBF	Barcelona	Colon	.	.	4
03	GAF	Barcelona	Lung	Yes	10	4
03	PBF	Barcelona	Lung	.	.	4
04	GAF	Candiolo	Breast	Yes	8	4
04	PBF	Candiolo	Breast	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
04	GAF	Barcelona	Colon	Yes	10	4
04	PBF	Barcelona	Colon	.	.	4
04	GAF	Barcelona	Lung	Yes	8	4
04	PBF	Barcelona	Lung	.	.	4
04	GAF	Manchester	Endometrium	No	6.3	4
04	PBF	Manchester	Endometrium	.	.	4
04	GAF	Manchester	Prostate	Yes	10	4
04	PBF	Manchester	Prostate	.	.	4
05	GAF	Barcelona	Colon	Yes	10	4
05	PBF	Barcelona	Colon	.	.	4
05	GAF	Barcelona	Lung	Yes	10	4
05	PBF	Barcelona	Lung	.	.	4
05	GAF	Manchester	Endometrium	Yes	10	4
05	PBF	Manchester	Endometrium	.	.	4
06	GAF	Candiolo	Breast	Yes	10	4
06	PBF	Candiolo	Breast	.	.	4
06	GAF	Barcelona	Colon	Yes	10	4
06	PBF	Barcelona	Colon	.	.	4
06	GAF	Barcelona	Lung	Yes	10	4
06	PBF	Barcelona	Lung	.	.	4
06	GAF	Manchester	Prostate	No	5.4	1
06	PBF	Manchester	Prostate	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
07	GAF	Candiolo	Breast	No	7	4
07	PBF	Candiolo	Breast	.	.	4
07	GAF	Barcelona	Colon	Yes	10	4
07	PBF	Barcelona	Colon	.	.	4
07	GAF	Barcelona	Lung	Yes	10	4
07	PBF	Barcelona	Lung	.	.	4
07	GAF	Manchester	Endometrium	Yes	10	3
07	PBF	Manchester	Endometrium	.	.	4
07	GAF	Manchester	Prostate	No	6.3	3
07	PBF	Manchester	Prostate	.	.	4
08	GAF	Candiolo	Breast	Yes	8.5	4
08	PBF	Candiolo	Breast	.	.	4
08	GAF	Barcelona	Colon	Yes	10	4
08	PBF	Barcelona	Colon	.	.	4
08	GAF	Barcelona	Lung	Yes	10	4
08	PBF	Barcelona	Lung	.	.	4
08	GAF	Manchester	Endometrium	Yes	10	4
08	PBF	Manchester	Endometrium	.	.	4
08	GAF	Manchester	Prostate	No	7.7	3
08	PBF	Manchester	Prostate	.	.	4
09	GAF	Candiolo	Breast	No	9	4
09	PBF	Candiolo	Breast	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
09	GAF	Barcelona	Colon	No	10	3
09	PBF	Barcelona	Colon	.	.	4
09	GAF	Manchester	Endometrium	Yes	10	4
09	PBF	Manchester	Endometrium	.	.	4
09	GAF	Manchester	Prostate	No	8.6	3
09	PBF	Manchester	Prostate	.	.	4
10	GAF	Barcelona	Colon	Yes	10	4
10	PBF	Barcelona	Colon	.	.	4
10	GAF	Manchester	Endometrium	Yes	10	4
10	PBF	Manchester	Endometrium	.	.	4
10	GAF	Manchester	Prostate	No	8.3	3
10	PBF	Manchester	Prostate	.	.	4
11	GAF	Candiolo	Breast	Yes	10	4
11	PBF	Candiolo	Breast	.	.	4
11	GAF	Manchester	Endometrium	Yes	5.5	3
11	PBF	Manchester	Endometrium	.	.	4
11	GAF	Manchester	Prostate	No	9.6	3
11	PBF	Manchester	Prostate	.	.	4
12	GAF	Candiolo	Breast	Yes	7	4
12	PBF	Candiolo	Breast	.	.	4
12	GAF	Barcelona	Colon	Yes	10	4
12	PBF	Barcelona	Colon	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
12	GAF	Manchester	Endometrium	Yes	8.4	3
12	PBF	Manchester	Endometrium	.	.	4
12	GAF	Manchester	Prostate	No	9.5	3
12	PBF	Manchester	Prostate	.	.	4
13	GAF	Candiolo	Breast	Yes	9	4
13	PBF	Candiolo	Breast	.	.	4
13	GAF	Barcelona	Colon	Yes	10	4
13	PBF	Barcelona	Colon	.	.	4
13	GAF	Manchester	Endometrium	Yes	10	4
13	PBF	Manchester	Endometrium	.	.	4
13	GAF	Manchester	Prostate	No	9.6	3
13	PBF	Manchester	Prostate	.	.	4
14	GAF	Candiolo	Breast	Yes	9	4
14	PBF	Candiolo	Breast	.	.	4
14	GAF	Barcelona	Colon	Yes	10	4
14	PBF	Barcelona	Colon	.	.	4
14	GAF	Manchester	Prostate	No	8.7	3
14	PBF	Manchester	Prostate	.	.	4
15	GAF	Candiolo	Breast	Yes	10	4
15	PBF	Candiolo	Breast	.	.	4
15	GAF	Barcelona	Colon	Yes	10	4
15	PBF	Barcelona	Colon	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
16	GAF	Candiolo	Breast	Yes	9	4
16	PBF	Candiolo	Breast	.	.	4
16	GAF	Barcelona	Colon	Yes	10	4
16	PBF	Barcelona	Colon	.	.	4
17	GAF	Candiolo	Breast	Yes	8.5	4
17	PBF	Candiolo	Breast	.	.	4
17	GAF	Barcelona	Colon	No	7	4
17	PBF	Barcelona	Colon	.	.	4
18	GAF	Barcelona	Colon	No	10	3
18	PBF	Barcelona	Colon	.	.	4
19	GAF	Candiolo	Breast	Yes	8.5	4
19	PBF	Candiolo	Breast	.	.	4
19	GAF	Barcelona	Colon	No	10	3
19	PBF	Barcelona	Colon	.	.	4
20	GAF	Candiolo	Breast	Yes	9	4
20	PBF	Candiolo	Breast	.	.	4
20	GAF	Barcelona	Colon	Yes	10	3
20	PBF	Barcelona	Colon	.	.	4
21	GAF	Candiolo	Breast	Yes	9.5	4
21	PBF	Candiolo	Breast	.	.	4
21	GAF	Barcelona	Colon	Yes	10	3
21	PBF	Barcelona	Colon	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
22	GAF	Candiolo	Breast	Yes	9	4
22	PBF	Candiolo	Breast	.	.	4
22	GAF	Barcelona	Colon	No	10	3
22	PBF	Barcelona	Colon	.	.	4
23	GAF	Candiolo	Breast	Yes	10	4
23	PBF	Candiolo	Breast	.	.	4
23	GAF	Barcelona	Colon	Yes	10	4
23	PBF	Barcelona	Colon	.	.	4
24	GAF	Candiolo	Breast	Yes	9	4
24	PBF	Candiolo	Breast	.	.	4
24	GAF	Barcelona	Colon	Yes	10	4
24	PBF	Barcelona	Colon	.	.	4
25	GAF	Candiolo	Breast	Yes	9	4
25	PBF	Candiolo	Breast	.	.	4
25	GAF	Barcelona	Colon	Yes	10	3
25	PBF	Barcelona	Colon	.	.	4
26	GAF	Candiolo	Breast	Yes	10	4
26	PBF	Candiolo	Breast	.	.	4
26	GAF	Barcelona	Colon	Yes	10	4
26	PBF	Barcelona	Colon	.	.	4
27	GAF	Candiolo	Breast	Yes	9	4
27	PBF	Candiolo	Breast	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
27	GAF	Barcelona	Colon	Yes	10	4
27	PBF	Barcelona	Colon	.	.	4
28	GAF	Candiolo	Breast	Yes	9	4
28	PBF	Candiolo	Breast	.	.	4
29	GAF	Candiolo	Breast	Yes	8.5	4
29	PBF	Candiolo	Breast	.	.	4
30	GAF	Candiolo	Breast	Yes	9.5	4
30	PBF	Candiolo	Breast	.	.	4
31	GAF	Candiolo	Breast	Yes	8.6	4
31	PBF	Candiolo	Breast	.	.	3
33	GAF	Candiolo	Breast	Yes	9	4
33	PBF	Candiolo	Breast	.	.	4
34	GAF	Candiolo	Breast	Yes	9.5	4
34	PBF	Candiolo	Breast	.	.	4
35	GAF	Candiolo	Breast	Yes	8.5	4
35	PBF	Candiolo	Breast	.	.	4
36	GAF	Candiolo	Breast	Yes	9.1	4
36	PBF	Candiolo	Breast	.	.	4
37	GAF	Candiolo	Breast	Yes	9	4
37	PBF	Candiolo	Breast	.	.	4
38	GAF	Candiolo	Breast	Yes	8.1	4
38	PBF	Candiolo	Breast	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
40	GAF	Candiolo	Breast	Yes	9.5	4
40	PBF	Candiolo	Breast	.	.	4
41	GAF	Candiolo	Breast	Yes	8.5	4
41	PBF	Candiolo	Breast	.	.	4
42	GAF	Candiolo	Breast	Yes	7.6	4
42	PBF	Candiolo	Breast	.	.	4
43	GAF	Candiolo	Breast	Yes	9	4
43	PBF	Candiolo	Breast	.	.	4
44	GAF	Candiolo	Breast	Yes	9	4
44	PBF	Candiolo	Breast	.	.	4
45	GAF	Candiolo	Breast	Yes	9	4
45	PBF	Candiolo	Breast	.	.	4
46	GAF	Candiolo	Breast	Yes	8	4
46	PBF	Candiolo	Breast	.	.	4
47	GAF	Candiolo	Breast	Yes	9	4
47	PBF	Candiolo	Breast	.	.	4
48	GAF	Candiolo	Breast	Yes	8.8	4
48	PBF	Candiolo	Breast	.	.	4
50	GAF	Candiolo	Breast	Yes	10	4
50	PBF	Candiolo	Breast	.	.	4
51	GAF	Candiolo	Breast	Yes	10	4
51	PBF	Candiolo	Breast	.	.	4

Slides	Fixative	Center	Evaluated organ	Do you consider that the preparations obtained on the same case with the two fixatives have the same performance?	Were you satisfied with the use of GAF fixative during the fixation procedure?	Local center pathologists Total score
52	GAF	Candiolo	Breast	Yes	10	4
52	PBF	Candiolo	Breast	.	.	4

8 LIST OF ABBREVATIONS AND DEFINITIONS

AE	Adverse Event
ADE	Adverse Device Effect
CA/RA	Competent Authority / Regulatory Authority
CI	Confidence Interval
CPSP	Clinical Performance Study Protocol
CRA	Clinical Research Associate
CRF	Case Report Form
CSR	Clinical Study Report
EC	Ethics Committee
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ISF	Investigator's Site File
ITT	Intention-To-Treat (population)
PI	Principal Investigator
PP	Per Protocol (population)
PVC	Polyvinyl Chloride
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Screening Number
SD	Standard Deviation
SOP	Standard Operating Procedure
UADE	Unanticipated Adverse Device Effect

9 ETHICS

The study was conducted in compliance with the independent Ethics Committee/Institutional Review Board (EC/IRB)'s recommendation, informed consent regulations (written Informed Consent was obtained in response to a fully written and verbal explanation of the nature of the study prior to start any procedure scheduled for the study), Declaration of Helsinki, Good Clinical Practice guidelines, local laws, ISO 20916:2019 and relative Study Protocol. In addition, the study adhered to all applicable local and international laws and regulation.

	Site 1	**Site 2**	**Site 3**
Ethics committee / IRB	22.10.2019/NA	15.10.2019/NA	16.01.2020/NA
Date final protocol approved by EC	19.09.2019_V1	19.09.2019_V1	19.09.2019_V1

10 INVESTIGATORS AND ADMINISTRATIVE STRUCTURE OF STUDY

10.1 PRINCIPAL INVESTIGATOR(S)

As per study design, this is a multicenter study, the centers are below reported including the Coordinating Investigator (*):

Three European Institutions are involved for the sampling:
Istituto per la Ricerca e cura del Cancro (Institute for Cancer Research and Cure, IRCCS of Candiolo (Torino, Italy)). Strada Provinciale 142 km 39,5 - 10060 Candiolo (TO).
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Hospital Universitari Vall d'Hebron; Vall d'Hebron Barcelona Hospital Campus Passeig de la Vall d'Hebron, 119-129 - 08035 Barcelona (Spain)
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The Christie NHS Foundation Trust Wilmslow Road, Manchester, M20 4BX. United Kingdom.
PI: Dr. Pedro Soares de Oliveira consultant in histopathology. Dept. Of pathology. pedro.oliveira@christie.nhs.uk - tel. +44-161-4463275

10.2 EXTERNAL ORGANIZATIONS INVOLVED (CRO, LABORSTORIES, CONSULTANTS)

1MED SA (CRO)
Address: Via Campagna, 13
6982 Agno – CH
Phone: +41 (0)91 605 40 50

1MED supported the Sponsor with the following activities:

- Study Planning, preparation, and project management.

- Study Initiation and Submission.
- Monitoring activities.
- Data management.
- Biostatistics.
- Medical Writing.

Reading of the slides will be performed by the Central Pathology Reviewer: Prof. Ales Ryska Charles University Hradec Kralove, Czech Republic.

10.3 SPONSOR INFORMATION

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12 ANNEXES

12.1 CPSP (INCL. AMENDMENTS)

12.2 IFU

12.3 PRINICIPAL INVESTIGATOR(S)

12.4 EXTERNAL ORGANIZATIONS

12.5 AUDIT CERTIFICATE

No audit performed during the CPS