Frequently asked questions on the use of GAF®:

1. How is GAF stored?

GAF® Fixative (Glyoxal Acid-Free) is stable (pinkish colour, pH ≥ 7) for 24 (twenty-four) months when stored between 2°C and 8 °C. Before use, keep the product at room temperature to allow the dissolution of any crystals formed due to excessively low temperatures, and perform the fixation with the product at room temperature.
The device can be stored at room temperature (5 °C - 25 °C) for at least 90 (ninety) days.
Do not use it in case of acidification (colour change to yellow).
PLEASE NOTE: Tissues with a naturally acidic pH, such as gastric tissue, pose no concern. When introduced into GAF®, the solution may turn yellow due to the acidic nature of the tissue. However, this color change does not impact the performance of the product. The crucial factor is that GAF® remains pink prior to tissue introduction, indicating its effective preservation and activity at the time of use. The subsequent color changes after tissue introduction are inconsequential.

2. Does GAF need a cold chain transportation?

No need of cold chain transportation.
GAF® can be shipped at environmental temperature, then stored 4-8°C once at the delivery place, for long term conservation.

3. Do tissues fixed in GAF need a special processor? What changes in routine?

Basically nothing. The timing of fixation is the same. The fixation mechanism of GAF is cross-linking as for formalin.
Tissues fixed in GAF can be processed safely in the usual processors currently used in laboratories for formalin (VENTANA; LEICA; AGILENT-DACO; LUNAPHORE).
The tank from which the cases start the process is irrelevant whether it is filled with 70-80% alcohol or formalin (the tissues placed inside the processor are already fixed and do not undergo any detrimental process due to immersion in an additional fixative).
The passage into the formalin tank of the processor therefore has no impact, it is not influential, you can obviously skip it, but if you prefer to keep it, that does not change.
The operations of processing, embedding, cutting, staining etc. remain the same.

4. Immunohistochemical staining has the same protocols?

In general, most IHC protocols applied to formalin-fixed tissues are applicable, without modification, to GAF-fixed tissues.
We therefore recommend adopting the protocol currently in use. In fact, the staining is subject to the subjective preferences of the pathologist in terms of intensity, minor adjustments/optimisation according to specific preferences may possibly be useful.
Protocols for the most widely used antibodies (tested on 'Ventana Benchmark Ultra') for GAF-fixed tissues are published on the ADDAX Biosciences website, as mentioned above, which are substantially similar to those for formalin-fixed tissues.

5. Samples left in fixative for 1 month are preserved as in formalin?

Yes the retention of overfixed tissue for days or weeks is the same as with formalin. Slightly better for GAF due to the absence of acids. In addition, the tissue will be less hardened and greying.
We recommend keeping a ratio 20:1 in volume as per formalin.

6. Tissue blocks fixed in GAF after 10 years are still usable for diagnosis or any other purpose?

The first tissue blocks fixed in GAF are dated 2014, therefore more than 10 years old. In 2024, a check was carried out in which the same diagnostic investigations carried out initially (EE; special histochemical and immunohistochemical staining) were repeated on these blocks in parallel with the formalin-fixed cases, the results of which were absolutely comparable to those obtained initially for both fixatives.
GAF-fixed tissues will be absolutely usable for any revision of diagnosis or any other purpose that may be necessary.
It is also worth remembering that tissues fixed in GAF retain longer DNA fragments on average than those extracted from formalin-fixed tissues, so the retention of the same Nucleic Acids over time, particularly with regard to DNA, could also be advantageous compared to that guaranteed by formalin.

7. Why do previous commercial fixatives based on glyoxal not allow diagnostic accuracy?

Previous attempts to replace formalin with glyoxal-based fixatives have all had very little uptake: Greenfix (DIAPATH), Glyofixx (SHANDON), Safefix (FISHER), Histofix (ITW). Etc.

All of them share a common feature, namely, the presence of the acid component of glyoxal (oxalic acid / glyoxylic acid / formic acid / etc etc), which produces known cell structure-damaging effects on nuclei, cytoplasm and membranes, (such as lysis of haematoses / microcalcifications dissolution / failure of IHC and FISH).

8. Why do previous commercial glyoxal-based fixatives not allow the blocks to be reused after months?

In addition to the already known detrimental effects (such as lysis of haemocytes/dissolution of microcalcifications/failure of IHC and FISH), they also damage tissue following fixation and inclusion in paraffin (this is due to the penetration of this acid component into the tissue, which obviously has effects that become more and more pronounced as time passes).

9. Clone sensitiveness

GAF® is not specifically clone-dependent exactly like formalin is, as the fixation mechanism is identical: linking is made through the creation of methylene bridges with proteins.

During antigen retrieval process bridges broke and proteins released are detected from antibodies clones.

Typically used clones in human and vet diagnostics have been used for GAF validations. As per formalin, obviously, new or non-standard clones must be tested.