Study	Year	Link	Storage conditions	Storage duration for reported outcome	Visualization method	Observation category predominant	Storage effect (An asterisk (*) indicates that the study does not specifically comment on histologic storage effects, but it can be inferred based on their results.)	Effect on morpholog	Effect on morphology gra	Consensus grade	Category of change	Effect on morphology grade notes	Relevant text
Smith 1956	1956	https://pubmed.ncbi	Formol-saline	8 years	Light microscopy after staining for myelin with the Marchi method	Morphologic staining	Intact myelin staining despite extended fixation times, with no reported difference despite the long fixation duration	0	[No independent grade, pilo	0			"It has therefore been shown that the Marchi method is of value in demonstrating degeneration, even after prolonged storage of the tissue in formol-saline."
Hashida 1970	1970	https://pubmed.ncbi	Formalin	9 years	Electron microscopy	Morphologic staining	Claims that morphology was markedly distorted, although unclear if this is a storage effect or due to insufficient initial fixation, and it is also attributed to disease processes	NA	[No independent grade, pilo	NA		There seems to be no clear storage artifact, as one of the better-preserved samples has a relatively longer storage time of 8 years.	
Itoyama 1980	1980	https://pubmed.ncbi	4% formalin	1 year	Light microscopy after immunostaining for myelin basic protein	Biomolecular staining	Reports good staining of myelin sheaths given the correct pretreatment protocol	0	[No independent grade, pilo	0			"When human CNS tissue was stored in unbuffered formalin for more than one year and then embedded in paraffin, we observed good staining of myelin sheaths. But our rat experiments suggest that if these blocks had been immersed for much longer, less intense staining probably would have been observed. If Vibratome sections were cut from blocks of human CNS stored in formalin and were pretreated with ethanol or osmium tetroxide, they contained BP-stained myelin. If pretreatment was omitted, myelin sheaths were not stained." ... "The effects of prolonged immersion in formalin and of pretreatment with osmium tetroxide were also tested in Vibratome sections of brains from 7-day-old rats. If formalin fixation lasted 18 to 24 hours, oligodendroglia and myelin sheaths were well stained by BP antiserum (Fig 4A). When fixation was prolonged to two months, only a few very faintly stained myelin sheaths were observed (Fig 4B) unless the sections had been pretreated with osmium tetroxide densely stained by BP antiserum; the effect on oligodendroglial staining was much less obvious"
Swaab 1982	1982	https://scholar.goo	40% formalin	50 years	Light microscopy after staining for oxytocin	Biomolecular staining	Neurosecretory cells still have some staining for oxytocin, although not "as brilliantly" as samples fixed for shorter amounts of time	1	[No independent grade, pilo	1	Loss of antigenicity		
Swaab 1982	1982	https://scholar.goo	40% formalin	50 years	Light microscopy after staining for vasopressin	Biomolecular staining	Neurosecretory cells still have some staining for vasopressin, although not "as brilliantly" as samples fixed for shorter amounts of time	1	[No independent grade, pilo	1	Loss of antigenicity		
D'Amelio 1983	1983	https://pubmed.ncbi	1.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, 900 mOsm	2 years	Light microscopy following the Golgi-Hortega-Lavilla silver impregnation technique	Morphologic staining	Successful impregnation and histologic visualization of neurons at all levels of the central nervous system	0	[No independent grade, pilo	0			"FIG. 2. Detail of Fig. 1. Pyramidal cell apical dendrite and its bifurcation, showing the clarity of the staining of the spine population" ... "Examples of neurons and neuroglia stained by this method are presented in Figs. 1-5. In general, the technique has been very successful at all levels of the central nervous system with the glutaraldehyde fixative employed. Moreover, it is possible that material fixed in this way might be satisfactorily impregnated even after more than 24 months, which was the longest period in fixative for the samples employed."
Volk 1986	1986	https://pubmed.ncbi	Formalin	0.17 years	Freeze-fracture electron microscopy	Morphologic staining	No effect of storage on membranous cytoplasmic bodies in neurons	0	[No independent grade, pilo	0		Reports storage for "months", so assuming a minimum of 2 months of storage.	"Despite formalin fixation for months, the structures of storage material were well preserved and showed complex and compound MCBs with typical arrangement of lamellae." ... "Our present report shows that even after long periods of formalin fixation, freeze-fracturing of autopsy material is usable and can be performed on storage material such as MCBs"
Rozemuller 1988	1988	https://pubmed.ncbi	Formalin	0.08 years	Light microscopy after immunostaining for several antigens	Biomolecular staining	Decreased or absent staining for several antigens after 1 month of formalin fixation	2	[No independent grade, pilo	2	Loss of antigenicity		"Effect of prolonged fixation and formaldehyde concentration. The influence of prolonged formalin fixation on the immunoperoxidase demonstration of plasma proteins in neurons and glial cells was studied in 12 patients with SDAT (group A). In one-day formalin-fixed tissue, clusters of neuronal and glial cells staining for albumin and IgG were seen in 9 out of 10 cases; in 1-week-fixed tissue these clusters were seen in 8 out of 12 cases and in 1-month-fixed tissue in 7 out of 12 cases. Fixation for a month was associated with a weak or negative staining for fibrinogen, C4c and C3c. Immunolabelling for plasma proteins showed similar results in adjacent tissue blocks fixed in either 10% or 4% formaldehyde. Trypsinization did not influence the immunoperoxidase staining for plasma proteins in formalin-fixed paraffin sections."
Hayashi 1988	1988	https://pubmed.ncbi	Formalin	0.08 years	Light microscopy after immunostaining for bromodeoxyuridine	Biomolecular staining	Longer fixation requires longer protease digestion, which in turn can damage fine histological structures	2	[No independent grade, pilo	1	Loss of antigenicity		"In formalin-fixed materials, BUdR-positive nuclei were barely identifiable (Figure 1B). In formalin-fixed specimens treated with one of the three proteinases, however, nuclei that had incorporated BUdR were readily identified (Figure 1C). Longer fixation times in formalin were associated with less intense staining, and longer incubation with proteinase was required to achieve the same intensity of staining in samples fixed for shorter durations (Table 1)" ... "Finally tissue specimens preserved in formalin can be stained for BUdR even after long storage. The only disadvantage is that prolonged incubation with proteinase occasionally resulted in some destruction of fine histological structures.
Guntern 1989	1989	https://pubmed.ncbi	Formalin	40 years	Light microscopy after immunostaining for neuropeptides	Biomolecular staining	Immunostaining results comparable to freshly fixed brains, after using optimized tissue processing methods	0	[No independent grade, pilo	0			"These treatments enhance the immunolabelling considerably, with results comparable to those obtained with freshly fixed tissues, and facilitate the discrimination between specifically and unspecifically stained structures"
Wilcock 1990	1990	https://pubmed.ncbi	10% formalin	0.5 years	Gallyas silver stain	Morphologic staining	No reported change in staining quality for neurofibrillary tangles and neuritic plaques	0	[No independent grade, pilo	0			"The short-term fixation of tissue, e.g. up to 6 months in formalin does not usually present a problem using any of the three methods tried, e.g. Gallyas, modified Palmgren, or modified Bielschowsky, but once the time lengthens to 6 years or more the demonstration of NFTs and NPs is not so reliable using the first two methods. The modified Bielschowsky method, however, demonstrates well both NFTs and NPs in material stored in formalin or as paraffin blocks for long periods, e.g. 7 years, and also compares favourably with the other methods on freshly processed material and fixed tissue, stored for shorter periods." ... "When using material from AD cases stored in 10% formalin we also found that the modified Bielschowsky method was easier to control than the Gallyas technique, which more readily resulted in overstaining." ... "When using material from AD cases stored in 10% formalin we also found that the modified Bielschowsky method was easier to control than the Gallyas technique, which more readily resulted in overstaining."
Wilcock 1990	1990	https://pubmed.ncbi	10% formalin	0.5 years	Modified Palmgren silver stain	Morphologic staining	No reported change in staining quality	0	[No independent grade, pilo	0			
Wilcock 1990	1990	https://pubmed.ncbi	10% formalin	6 years	Gallyas silver stain	Morphologic staining	Unreliable staining results	1	[No independent grade, pilo	2	Decreased silver staining		
Wilcock 1990	1990	https://pubmed.ncbi	10% formalin	6 years	Modified Palmgren silver stain	Morphologic staining	Unreliable staining results	1	[No independent grade, pilo	2	Decreased silver staining		
Wilcock 1990	1990	https://pubmed.ncbi	10% formalin	7 years	Modified Bielschowsky silver stain	Morphologic staining	No reported change in staining quality	0	[No independent grade, pilo	0			
McQuaid 1990	1990	https://pubmed.ncbi	Formalin	23 years	Light microscopy after immunostaining with measles specific antiserum	Biomolecular staining	Well preserved morphology with the use of the optimal protease, able to detect cellular morphology	0	0	0			"Table 2 summarises the effect of 10 different proteolytic enzymes on serial sections for both ICC and ISH. Positive cells with both techniques had dark red deposits and staining was predominantly cytoplasmic (figs 1 and 2). Positive cells were identified morphologically as neurons and glial cells (predominantly astrocytes)."
Feirabend 1991	1991	https://pubmed.ncbi	10% formalin	10 years	Light microscopy after Nissl staining for cytoarchitecture	Morphologic staining	Reports that staining results are satisfactory depending on the pre-processing procedures*	NA	NA	NA			
Feirabend 1991	1991	https://pubmed.ncbi	10% formalin	10 years	Light microscopy after Klüver-Barrera and Häggqvist stains for myelin architecture	Morphologic staining	Reports that staining results are good depending on the pre-processing procedures*	NA	NA	NA			
Panayotacopoulou 199	1991	https://pubmed.ncbi	Formalin	1 year	Light microscopy after immunostaining for tyrosine hydroxylase	Biomolecular staining	Excellent immunohistochemical staining	0	0	0			"With this procedure we obtained an excellent immunohistochemical staining of human hypothalamic TH-IR neuronal systems. Numerous intensely stained perikarya were revealed on a light background. The majority of the TH-IR neurons were localized within the PVN and SON (Figs. 8c, 2b). Most of the TH-IR cells in these nuclei were large sized and were intensely stained including processes." ... "Our study not only confirms the previously reported finding that in the adult human a large number of PVN and SON magnocellular cells appear to synthesize a catecholamine (Li et al., 1988) but also provides a sensitive method by which these neurons can be visualized in material fixed and stored in formalin for prolonged periods of time. Such material, as far as known, has not been previously used for the demonstration of TH-IR neuronal systems in the human brain."
Ploeger 1993	1993	https://pubmed.ncbi	10% formalin	10 years	Light microscopy after staining with Nissl, Klüver-Barrera, and toluidine blue stains	Morphologic staining	Acidification leads to absent or poor morphological staining, however this can be reversed to *positive* staining by pre-treatment of sections with an alkaline buffer	NA	NA			Unclear to what extent the reversal was back to baseline (so no change) or still a partial change.	"It is possible to reverse the negative effects of acidification by pre-treating paraffin or frozen sections in an alkaline buffer at 55°C. This can be done with microwave irradiation for 1 hour or in a laboratory stove (over 2 hours, without pre-heating the staining solutions). Microwave application is clearly faster than the conventional method in pre-heating with an alkaline buffer. Nevertheless quality differences are not noticed in the stainings, confirming the results of Feirabend et al. (1992)" ... "Acidification in large human brain collections occurs in most fixatives commonly used. Brains are in pathology and anatomy routinely stored in 10% formaline, inducing strong acidification. It would be beneficial to change the storage fluid into neutral buffered formalin 10% or phosphate buffered paraformaldehyde. Brains which have been stored for a long time in acid fixatives have certainly not lost their value for histological staining. Pre-treatment of the sections with an alkaline buffer overcomes the disadvantages of acidification for classical staining."
Ravid 1993	1993	https://pubmed.ncbi	Glutaraldehyde-paraformaldehyde fixative	1 year	Microscopy after staining for several antigens	Biomolecular staining	Preserves the immunoreactivity of vasopressin, oxytocin, and alfa-MSH	0	0	0			"The fixation procedure used and storage of the fixed tissue prior to the application of ICC procedures may affect the staining." ... "Our experience on rat material is that storage in glutaraldehyde-paraformaldehyde fixative preserves immunoreactivity of vasopressin, oxytocin and alfa-MSH for more than a year (Ravid, unpublished results)."
Robards 1993	1993	https://scholar.goo	Modified Karnovsky's fixative (2.5% glutaraldehyde and 2% formaldehyde) with 5 mM calcium chloride	0.08 years	Electron microscopy	Morphologic staining	Prominent appearance of intra- and extracellular "myelin-like" whorls	1	[No independent grade, pilo	1	Myelin-like whorls		"In order to establish the limitations of long-term tissue storage, a study was undertaken specifically to evaluate the effects on ultrastructural morphology of tissue storage at either 4 °C or room temperature (21 °C). Perfusion-fixed liver, kidney, brain and heart samples from rats were stored for 3 h or 1, 6 or 12 months in sodium cacodylate buffered modified Karnovsky's fixative (with 5 mM calcium chloride). The most notable effect of long-term tissue storage was the occurrence of intra- and extracellular 'myelin-like' whorls. Such whorls were observed only rarely in the 3 h samples. After 1 month of storage in fixative, whorls were small and infrequent in liver, kidney and heart, but prominent in brain (Plate 5.1.2). The whorling was most distinctive in all four tissues by 6 months (Plate 5.1.3). At 12 months, the membranous nature of the whorls was less clearly defined, and the whorls were associated with areas of lucency, suggesting lipid leaching. There was no apparent difference in the whorling artifact between specimens held at room temperature versus those held at 4 °C. Other minor changes included a slight loss of glycogen in the liver, and mild clearing of the cytoplasm in the kidney and brain. The preservation of cellular organelles appeared stable with long-term storage."
Robards 1993	1993	https://scholar.goo	Modified Karnovsky's fixative (2.5% glutaraldehyde and 2% formaldehyde) with 5 mM calcium chloride	1 year	Electron microscopy	Morphologic staining	The membranous nature of the whorls is not as well defined, and the whorls are associated with areas of lucency, suggestive of lipid leaching	2	[No independent grade, pilo	2	Myelin-like whorls		
Sillevis Smitt 1993	1993	https://pubmed.ncbi	10% phosphate buffered formaldehyde	2 years	Light microscopy after immunostaining for neurofilament proteins with SMI-31	Biomolecular staining	Immunoreactivity lost	2	2	2	Loss of antigenicity	SMI-31 seems to be the outcome described most clearly of the antibodies used to stain neurofilament proteins, and representative.	"Tissue samples stored in paraffin retained immunoreactivity with SMI-3l for as long as 50 years although the quality of the staining gradually declined. However, no immunoreactivity could be detected in any sample stored in formaldehyde for more than 2 years." ... "Another point underscored by our observations is that, following certain techniques or long periods of fixation, phosphorylated neurofilament could not be demonstrated in parallel fibers, but was obviously present in baskets and white matter axons. Similarly, GFAP could not be demonstrated in Bergmann fibers whereas white matter astrocytes showed abundant staining. Such findings probably reflect differences in level of antigen expression, and that may be another factor involved in the demonstrability of antigens (Trojanowski et al., 1985; Shiurba et al., 1987)"
Sillevis Smitt 1993	1993	https://pubmed.ncbi	10% phosphate buffered formaldehyde	0.5 years	Light microscopy after immunostaining for GFAP with goal of detecting Bergmann fibers	Biomolecular staining	Bergmann fiber reactivity is generally lost after storage for more than 6 months, except for one case with storage for 50 years, but the study does not report the quality of preservation in that case	2	2	2	Loss of antigenicity		"Bergmann fiber reactivity could not be detected in tissue stored in formaldehyde for more than 6 months except in case U-1526 that had been stored for 50 years." ... "Another point underscored by our observations is that, following certain techniques or long periods of fixation, phosphorylated neurofilament could not be demonstrated in parallel fibers, but was obviously present in baskets and white matter axons. Similarly, GFAP could not be demonstrated in Bergmann fibers whereas white matter astrocytes showed abundant staining. Such findings probably reflect differences in level of antigen expression, and that may be another factor involved in the demonstrability of antigens (Trojanowski et al., 1985; Shiurba et al., 1987)"
Sillevis Smitt 1993	1993	https://pubmed.ncbi	10% phosphate buffered formaldehyde	0.125 years	Light microscopy after immunostaining for synatophysin with SY-38	Biomolecular staining	Immunoreactivity lost	2	2	2	Loss of antigenicity		"SY-38 gave strong and evenly distributed staining on tissue sections fixed in Bouin, B5, and Sensofix (Fig. 3a-c). Formaldehyde and paraformaldehyde fixation yielded much weaker and unevenly distributed staining (Fig. 3e). Staining intensity with Sensofix consistently declined after 6 weeks of fixation and could not be detected after 6 months (Fig. 3c, d). After 14 days of formaldehyde fixation, staining intensity declined and was consistently absent after six weeks of fixation".
Wisniewski 1994	1994	https://pubmed.ncbi	10% formalin	58 years	Light microscopy after multiple methods to stain for neurofibrillary tangles	Biomolecular staining	No reported problems with staining for neurofibrillary tangles*	NA	NA				
Evers 1994	1994	https://pubmed.ncbi	4% buffered formaldehyde	0.83 years	Light microscopy after immunostaining for parvalbumin	Biomolecular staining	Good staining after antigen retrieval but not excellent staining as is seen after short fixation periods	1	0	0	Loss of antigenicity		"For PV staining, microwave pretreatment in aluminium chloride solution gave the best results (Fig. 8c). Many cells with nicely stained processes were present in the deep part of layer III and in layers IV, V and VI, which made distinction of cell types of immunopositive neurons possible. In parts of the section, pretreatment in distilled water also led to good results, although they were less homogeneous than the results obtained after pretreatment with the aluminium chloride solution. Pretreatment with thiocyanate or in zinc sulfate solution somewhat improved the staining but did not lead to a good impregnation of dendritic trees. Although a pretreatment in aluminium chloride resulted in good PV staining, it did not reach the excellent quality of staining in all cortical layers that was seen after a short fixation period of 1 week."
Evers 1994	1994	https://pubmed.ncbi	4% buffered formaldehyde	0.83 years	Light microscopy after immunostaining for MAP-2	Biomolecular staining	Staining improved after antigen retrieval but still not as good as short fixation time	1	1	1	Loss of antigenicity		"After microwave pretreatment in distilled water or in aluminium chloride solution the MAP-2 staining largely improved, although no homogeneous staining was observed in the sections (Figs. 2b and 3a). This might be due to wrinkling of sections during microwave pretreatment. Clear pyramidal neurons with their dendrites were observed, mainly in layers V and VI, and in the lower part of III (Fig. 3a). Other types of neurons with dendrites were also visible in layer IV and in layer I (i.e., Cajal-Retzius cells). Many small somata were stained in layer IV. Positive axonal fibers were seen in the white matter. The well-stained parts of these sections can be compared with the results as seen in tissue sections that had been fixed for a short time (+1 week). The staining of neuronal processes was less extensive after zinc sulfate pretreatment and lead thiocyanate pretreatment (Fig. 3b, c). Generally, the frequency of positive somata was also reduced when compared with the aluminium chloride and distilled water pretreatment, but the results were still much better than without microwave pretreatment."
Evers 1994	1994	https://pubmed.ncbi	4% buffered formaldehyde	0.83 years	Light microscopy after immunostaining for MAP-5	Biomolecular staining	Some types of dendrites are not seen even after antigen retrieval	2	2	2	Loss of antigenicity		"MAP-5 staining improved after microwave pretreatment in aluminium chloride or distilled water. After aluminium solution pretreatment parts of basal dendrites were visible in layers V and VI (Fig. 4) which did not show up after pretreatment in distilled water where, in addition to somata, only parts of apical dendrites were observed in layers V and VI. The other pretreatments did not lead to better MAP-5 staining. Although sections showed an improvement when pretreated with aluminium chloride or distilled water, the results cannot be compared with those seen after a short fixation of about 1 week, when MAP-5-positive neurons and dendrites were stained in all cortical layers."
Evers 1994	1994	https://pubmed.ncbi	4% buffered formaldehyde	0.83 years	Light microscopy after immunostaining for calbindin	Biomolecular staining	Partial staining after antigen retrieval, but still not as good as the short fixation time point	1	1	1	Loss of antigenicity		"Only after aluminium chloride/microwave pretreatment could a few neurons with a fine dendritic tree be observed. For the majority of Calb-positive neurons the neuronal processes were partly visible. This was also valid for the neuronal processes in distilled water/microwave-pretreated sections, although to a lesser extent. The other pretreatments showed no improvement, which means that only somata were observed. After pretreatment in the lead thiocyanate solution they were even more indistinct than without pretreatment. On the other hand, in tissue that had been in fixative for a short period, the dendritic processes of the strongly immunopositive neurons in layers II and III were clearly visible, so that it was then possible to distinguish non-pyramidal types".
Evers 1994	1994	https://pubmed.ncbi	4% buffered formaldehyde	0.83 years	Light microscopy after immunostaining for SMI-32	Biomolecular staining	Good staining after antigen retrieval, comparable to that of the short fixation period	0	0	0			"The largest improvement due to pretreatment occurred in the SMI-32 staining. By far the best results were obtained after microwave pretreatment in aluminium chloride solution. Relatively many beautiful layer I (Cajal-Retzius) neurons with dendrites (as compared with Golgi staining) proved to be SMI-32 positive, and pyramidal neurons with branching processes were nicely stained in layers III, V and VI (Fig. 5). In addition, several non-pyramidal neurons were seen in layer II, although this layer's background is dark, as it is in layer VI. The results obtained by this pretreatment were well comparable to the results obtained after a short fixation time of about 1 week."
Eastwood 1994	1994	https://pubmed.ncbi	10% formalin	0.125 years	Light microscopy after immunostaining for synatophysin mRNA	Biomolecular staining	Loss of immunoreactivity	2	2	2	Loss of antigenicity		"A loss of synaptophysin-IR was associated with increasing age (Fig. 4B) and fixation time in formalin (Fig. 7)" ... "Fixation in formalin was also associated with a loss of synaptophysin-IR; our data suggest that this occurs between two and six weeks fixation, agreeing with previous findings."
Evers 1995	1995	https://pubmed.ncbi	4% buffered formaldehyde	1.42 years	Light microscopy after immunostaining for MAP-2	Biomolecular staining	Good staining over the entire section reported after optimized antigen retrieval	0	0	0			Table 1 showing "+ + +, good staining over the entire section"
Evers 1995	1995	https://pubmed.ncbi	4% buffered formaldehyde	1.42 years	Light microscopy after immunostaining for SMI-32	Biomolecular staining	Good staining over the entire section reported after optimized antigen retrieval	0	0	0			Table 1 showing "+ + +, good staining over the entire section"
Gullotta 1996	1996	https://pubmed.ncbi	Formalin	20 years	Electron microscopy	Morphologic staining	Sub-cellular structures able to be identified and distinguished*	NA	NA				"Ultrastructure analysis of the storage inclusions present in macrophages or other cells of LD brains, can also assist in differentiating individual LD genotypes. Laminar, nontubular inclusions (Fig. 1) can be differentiated from the cleft, non-laminar, tubular type (Fig. 2) by demonstrating the periodicity of the lamellae in the former (inset to Fig. 1) and the circular cross-sections through the tubuli in the latter (arrow heads in Fig. 2). We found it to be remarkable that such an analysis was possible using formalin-fixed brain material stored for more than 20 years in the case of Patient A. E."
Evers 1997	1997	https://pubmed.ncbi	4% buffered formaldehyde	4 years	Light microscopy after immunostaining for MAP-2	Biomolecular staining	Optimal immunostaining achieved after optimized antigen retrieval	0	0	0			"MW-pretreated tissue in an antigen retrieval solution pH 8.0 – 10.0 for 15 min at full power (boiling) showed a remarkable increase in immunostaining as compared to the staining results achieved with the standard protocol without pretreatment (Table 2). The best pHs appeared to be 9.0 and 9.5. In these cases immunostainings of all examined antibodies showed both well-stained somata and well-stained dendrites in all cortical layers as well as a very good axonal staining in the case of SMI-312 ".
Evers 1997	1997	https://pubmed.ncbi	4% buffered formaldehyde	4 years	Light microscopy after immunostaining for neurofilament proteins with SMI-32, SMI-311, and SMI-312 antibodies	Biomolecular staining	Optimal immunostaining achieved after optimized antigen retrieval	0	0	0			
Evers 1997	1997	https://pubmed.ncbi	4% buffered formaldehyde	4 years	Light microscopy after immunostaining for calbindin	Biomolecular staining	Optimal immunostaining achieved after optimized antigen retrieval	0	0	0			
Evers 1997	1997	https://pubmed.ncbi	4% buffered formaldehyde	4 years	Light microscopy after immunostaining for parvalbumin	Biomolecular staining	Optimal immunostaining achieved after optimized antigen retrieval	0	0	0			
Evers 1997	1997	https://pubmed.ncbi	4% buffered formaldehyde	4 years	Light microscopy after immunostaining for calretinin	Biomolecular staining	Optimal immunostaining achieved after optimized antigen retrieval	0	0	0			
Evers 1997	1997	https://pubmed.ncbi	4% buffered formaldehyde	8 years	Light microscopy after immunostaining - antigens not specifically described	Biomolecular staining	No longer able to achieve optimal immunostaining after 8 years or more of fixation time	1	1	1	Loss of antigenicity		"However, when fixation times were extremely long (8 years and longer) it was impossible to achieve optimally stained cells."
Dwork 1998	1998	https://pubmed.ncbi	Formalin	51 years	Light microscopy after staining with the fluorescent dye thioflavine S	Biomolecular staining	Detected plaques and tangles with no reported change	0	0	0			"We obtained similar staining of archival and contemporary material with thioflavine S or immunohistochemistry using Ab 39, 4G8, or anti-ubiquitin, provided that immunohistochemistry was preceded by formic acid treatment."

Study	Year	Link	Storage conditions	Storage duration for reported outcome	Visualization method	Observation category predominant	Storage effect (An asterisk (*) indicates that the study does not specifically comment on histologic storage effects, but it can be inferred based on their results.)	Effect on morpholog	Effect on morphology gra	Consensus grade	Category of change	Effect on morphology grade notes	Relevant text
Dwork 1998	1998	https://pubmed.ncbi	Formalin	51 years	Light microscopy after staining for ubiquitin	Biomolecular staining	Detected plaques and tangles with no reported change	0	0	0			
Dwork 1998	1998	https://pubmed.ncbi	Formalin	51 years	Light microscopy after staining with the 4G8 antibody for β-amyloid	Biomolecular staining	Detected plaques with no reported change	0	0	0			
Dwork 1998	1998	https://pubmed.ncbi	Formalin	51 years	Light microscopy after staining with the Ab39 antibody for tau/NFTs	Biomolecular staining	Detected neurofibrillary tangles, including intensely stained neurons	0	0	0			
Dwork 1998	1998	https://pubmed.ncbi	Formalin	9 years	Light microscopy after staining with the Alz50 antibody for tau	Biomolecular staining	Immunoreactivity well preserved	0	0	0			"Alz 50 immunoreactivity, however, while wel preserved after 9 years in formalin, was not preserved after 30 years in formalin, and there were appreciable inconsistencies between Alz 50 and thioflavine S stains after 20 years of formalin fixation. It is conceivable that Alz 50 immunoreactivity could be restored by some other technique, such as microwave treatment or trypsinization, but we have not explored this possibility."
Dwork 1998	1998	https://pubmed.ncbi	Formalin	30 years	Light microscopy after staining with the Alz50 antibody for tau	Biomolecular staining	Immunoreactivity completely absent	2	2	2	Loss of antigenicity		
Sheaffer 1999	1999	https://pubmed.ncbi	Formalin, unclear exact formulation, unclear if ever replaced	53 years	Light microscopy after staining for Verhoeff and Luxol fast blue myelin stains	Morphologic staining	The morphology of individual myelin sheaths is preserved	0	[No independent grade, plic	0			"Verhoeff and Luxol fast blue stains of the resulting sections yielded staining whose quality was unaffected by the duration of fixation. Myelinated and unmyelinated areas were clearly distinguished, and the morphology of individual myelin sheaths was well-preserved. No changes to conventional protocols were required, but it was necessary to carefully monitor the progress of differentiation." ... "LFB stains (Figures 2c, d) showed well-defined myelin sheaths regardless of the fixation interval, but artifactual irregularities were sometimes present in both old and new sections. At low power, MBP immunohistochemistry confirmed the distribution of myelin observed with other methods (Figure 2e). However, at high magnification, the myelin sheaths lacked clear definition, the background was blurred, and there was staining of nuclei and oligodendroglial cytoplasm (Figure 2f). On the other hand, in tissue fixed for less than one year, the myelin sheaths were clearly demarcated, and there was no staining of oligodendroglial cytoplasm (Figure 3). Immunohistochemistry for FAP demonstrated astrocytic morphology clearly, regardless of the fixation interval (Figure 4)."
Sheaffer 1999	1999	https://pubmed.ncbi	Formalin, unclear exact formulation, unclear if ever replaced	53 years	Light microscopy after staining for GFAP	Biomolecular staining	Astrocyte morphology is clearly displayed	0	[No independent grade, plic	0			
Sheaffer 1999	1999	https://pubmed.ncbi	Formalin, unclear exact formulation, unclear if ever replaced	53 years	Light microscopy after staining for myelin basic protein	Biomolecular staining	Fine detail is lost from the myelin sheaths and there is staining of oligodendrocyte cytoplasm and nuclei, which is not typically seen	1	[No independent grade, plic	2	Loss of antigenicity		
Martins 1999	1999	https://pubmed.ncbi	10% formalin	0.21 years	Light microscopy after immunostaining for brain myosin-V	Biomolecular staining	Intact immunostaining	0	[No independent grade, plic	0			"The expression of BM-V IR was consistently and well detected in tissues stored in formalin from 1 week to 2.5 months. Tissues stored in formalin for 8–20 months exhibited a decrease in the expression of BM-V IR, which was not detected in a cerebellum fixed for 28 months."
Martins 1999	1999	https://pubmed.ncbi	10% formalin	1.67 years	Light microscopy after immunostaining for brain myosin-V	Biomolecular staining	Decreased levels of immunostaining	1	[No independent grade, plic	1	Loss of antigenicity		
Martins 1999	1999	https://pubmed.ncbi	10% formalin	2.33 years	Light microscopy after immunostaining for brain myosin-V	Biomolecular staining	Absent immunostaining	2	[No independent grade, plic	2	Loss of antigenicity		
Sarnat 1999	1999	https://pubmed.ncbi	Formalin	0.92 years	Light microscopy after immunostaining for synaptophysin	Biomolecular staining	Expected staining pattern for synaptophysin was lost	2	[No independent grade, plic	2	Loss of antigenicity	Reports many months of storage to up to 6 years. For "many months" assuming the most months than would make sense to report as less than a year (11), for plotting purposes.	"Brain tissue that had been fixed for prolonged periods (many months to years) in formalin lost the expected demonstrable synaptophysin reactivity by ICC and the antigen could not be retrieved by microwave heating for 10 min. Tissue preserved in paraffin over this same period sometimes yielded surprisingly good immunoreactivity, but the quality of ICC of old tissue was not consistent because other tissue blocks yielded poor results."
Heinsen 2000	2000	https://pubmed.ncbi	Gelatin-embedded brain slices in 10% formalin	12 years	Light microscopy after staining with Gallyas silver impregnation	Morphologic staining	No effect on neuronal stainability with Gallyas silver impregnation, but slightly less chromophilic staining in astrocytes and oligodendrocytes	1	[No independent grade, plic	1	Decreased silver staining		"[S]torage of serially sectioned gelatin-embedded slices in formalin had no visible effect on the stainability of neurons. We have recently stained slices that were stored for 12 years in 10% formalin. Only the nuclei of astro- and oligo- dendroglial cells were less chromophilic. A more significant problem is that prolonged storage in 10% formalin caused considerable shrinkage of gelatin-embedded brain slices."
Privat 2000	2000	https://pubmed.ncbi	10% formalin	29 years	Light microscopy after immunostaining for prion protein	Biomolecular staining	Immunostaining is still intact and noted to have marked positivity, the same rating given to samples fixed for only 1 month	0	[No independent grade, plic	0			"Indeed, in all CJD cases except for one (no. 7), and particularly in cases 2 and 3, there was an increased immunolabelling, as compared to the usual immunohistochemical protocol. Interestingly enough, those cases with low or absent immunoreactivity when PK was omitted had been formalin- fixed for a long time (19 and 25 months, respectively). Two other cases (nos. 7 and 8) submitted to very long formalin fixations (29 and 20 years, respectively) were thus studied. The intensity of immunostaining in the cerebral cortex increased significantly after PK proteolysis in one (case 8) but no immunostaining was found whatever the pretreatment in the other (case 7)."
Wu 2002	2002	https://pubmed.ncbi	2% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (PBS) at 4° C	9.5 years	Light microscopy after immunostaining for GFAP	Biomolecular staining	No significant difference in immunolabeling intensity or area when comparing specimens stored for short duration (less than 2 years) to long duration (more than 5 years)	0	[No independent grade, plic	0			"The results for the SD study showed no significant decay of both GFAP immunoreactivity and distribution in long SD (mean 9.3 years) compared to short SD (mean 1.6 years) at all three levels of expression: constitutive, aberrant and total. This suggests that increasing storage duration in fixatives does not lead to significant degradation of GFAP immunoreactivity."
Rosoklija 2003	2003	https://pubmed.ncbi	10% formalin	55 years	Light microscopy after Golgi–Kopsch silver staining	Morphologic staining	Intact neuronal morphology in Golgi–Kopsch impregnation, including dendritic spines, with no qualitative changes in cellular impregnation noted	0	0	0			"However, for tissue with 50 years of fixation, rapid Golgi staining was clearly inadequate, while the Golgi–Kopsch method gave excellent results, comparable to tissue fixed for a few years (Fig. 3). When fixation ranged from 15 months to 20 years, virtually all cases yielded large numbers of apparently well-impregnated neurons. Whether extended fixation produces quantitative changes in extent of impregnation remains to be determined, but qualitative changes are not noted."
Rosoklija 2003	2003	https://pubmed.ncbi	10% formalin	5 years	Light microscopy after rapid Golgi silver staining	Morphologic staining	Fewer impregnated neurons and lower quality results	1	1	1	Decreased silver staining		"Fig. 2. Frontal cortex from human autopsy brain fixed in formalin for 5 years. Impregnations of dendritic trees and spines of individual neurons are similarly extensive with rapid Golgi (A, C) and Golgi–Kopsch (B, D). More neurons are stained with Golgi–Kopsch, and lighter background affords greater clarity."
Larsen 2003	2003	https://pubmed.ncbi	10% formaldehyde	1.17 years	Light microscopy after myelin staining method	Morphologic staining	No reported effect of storage on myelin staining quality.	0	0	0			"The quality of the myelin staining was independent of the different histological procedures used"
Liang 2004	2004	https://pubmed.ncbi	10% neutral buffered formalin	18 years	Light microscopy after staining for VMAT2	Biomolecular staining	No reported differences in immunostaining pattern for VMAT2	0	0	0			"It is interesting that the ISI values for both VMAT2 and TH proteins are similar in the three DA cellular regions across all cases despite coming from brains that were stored for the longest and shortest times in formalin (DA-32 and DA-258, respectively; Tables 1, 2)."
Liang 2004	2004	https://pubmed.ncbi	10% neutral buffered formalin	18 years	Light microscopy after staining for TH	Biomolecular staining	No reported differences in immunostaining pattern for TH	0	0	0			
Lyck 2008	2008	https://pubmed.ncbi	Lillie's phosphate-buffered 4% formaldehyde	0.17 years	Light microscopy after staining for NeuN	Biomolecular staining	Complete disappearance of staining for NeuN	2	2	2	Loss of antigenicity	For this study, the authors report that the some of the samples chosen were not optimally matched and less rigorous. As a result, we focus on the experiments used to test for the effect of storage on four antigens - NeuN, GFAP, CNPase, and CD45 - that were measured with more rigorous approaches such as blinding.	"NeuN was very sensitive to fixation, with complete disappearance of immunohistochemical signal after 2-3 months of storage in 4% Lillies PBFS." ... "With longer time of fixation, staining results varied, raising a demand for a more rigorous test of the effect of fixation time on the staining result than could be achieved with the suboptimally matched samples in tissue array A."
Lyck 2008	2008	https://pubmed.ncbi	0.1% PFA in 0.15 M Sørensens phosphate buffer, pH 7.4, at 4° C	3 years	Light microscopy after staining for NeuN	Biomolecular staining	Deterioration of NeuN immunohistochemical signal, with most of the signal lost	2	1	1	Loss of antigenicity		"[T]he staining signal also deteriorated with storage time in 0.1% PFA at 4C° .. "However, although one specimen maintained a score of 3, most specimens had dropped to a mean score of .1 (four of five specimens: 0–0.6) after 36 months of storage in 0.1% PFA at 4C (Figure 8A)."
Lyck 2008	2008	https://pubmed.ncbi	Lillie's phosphate-buffered 4% formaldehyde	2 years	Light microscopy after staining for CNPase	Biomolecular staining	Deterioration of CNPase signal during fixation, with signal lost at this time point	2	2	2	Loss of antigenicity		
Lyck 2008	2008	https://pubmed.ncbi	0.1% PFA in 0.15 M Sørensens phosphate buffer, pH 7.4, at 4° C	3 years	Light microscopy after staining for CNPase	Biomolecular staining	Decrease in CNPase signal during fixation, but decreased rate of antigen deterioration with these storage conditions	1	1	1	Loss of antigenicity		
Lyck 2008	2008	https://pubmed.ncbi	Lillie's phosphate-buffered 4% formaldehyde	10 years	Light microscopy after staining for CD45	Biomolecular staining	Decrease in CD45 signal during fixation, until signal completely lost	2	2	2	Loss of antigenicity		
Lyck 2008	2008	https://pubmed.ncbi	0.1% PFA in 0.15 M Sørensens phosphate buffer, pH 7.4, at 4° C	3 years	Light microscopy after staining for CD45	Biomolecular staining	No clear decline in antigen signal over time for CD45 with these storage conditions	0	0	0			"The mean score of the staining result for CD45/LCA was negatively correlated to the time stored in 4% Lillies PBFS at room temperature (Figures 7E and 7F). As shown for CNPase, this was independent of the sex of the donor... Regarding PMI, the mean scores were only negatively correlated to time of fixation for specimens with a PMI from 25 to 48 hr... As observed for the GFAP staining, the mean scores for the CD45 staining of specimens from the perfusion- and immersion-fixed brains showed that CD45 was well preserved in this material, even with long-term storage of the specimens in 0.1% PFA at 4C (Figure 8D)"
Lyck 2008	2008	https://pubmed.ncbi	Lillie's phosphate-buffered 4% formaldehyde	10 years	Light microscopy after staining for GFAP	Biomolecular staining	Decrease in GFAP signal during fixation, but signal not completely lost at 10 years	1	1	1	Loss of antigenicity		
Lyck 2008	2008	https://pubmed.ncbi	0.1% PFA in 0.15 M Sørensens phosphate buffer, pH 7.4, at 4° C	3 years	Light microscopy after staining for GFAP	Biomolecular staining	No clear decline in antigen signal over time for GFAP with these storage conditions	0	0	0			"In case of GFAP, the mean scores for the GFAP stainings correlated negatively with storage time in 4% Lillies PBFS at room temperature in specimens from male donors ... but not female donors ... and in specimens with a PMI of 24 hr... Unlike CNPase, the analysis of the specimens from the perfusion- and immersion-fixed brains showed that the GFAP staining was well preserved, even with long-term storage of the specimens in 0.1% PFA at 4C (Figure 8C)."
Aleitu-Paz 2008	2008	https://pubmed.ncbi	Formaldehyde	7 years	Light microscopy after immunostaining for several antigens	Biomolecular staining	Antigen retrieval reportedly restores immunostaining, but the degree to which the tissues may be affected by storage is not specifically reported	NA	NA				"In the present study, we have investigated a new AR method to optimize immunostaining in free-floating human brain sections, showing that, heating formalin-fixed tissue in 0.05% citraconic anhydre solution, pH 7.4, at 95°C for 45 minutes, restores the immunostaining for several antigens."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	14 years	Light microscopy after staining for Aβ with antibody clones 6F/3D, 4G8, and 6E10 with formic acid antigen retrieval	Biomolecular staining	Preserved staining for amyloid beta	0	0	0			"[E]ven after 14 years' fixation, AA-IR aggregates can be detected (i.e. no false-negative results) with all Abs studied when optimal AR methods are used."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	14 years	Light microscopy after staining for Aβ with antibody clone 6E10 without antigen retrieval	Biomolecular staining	Absent staining for amyloid beta	2	2	2	Loss of antigenicity		"When FA AR was used in both tissue types with Clone 6E10, numerous aggregates were seen in most of the cores, but with heat AR, Clone 6E10 displayed only some to moderate IR lesions in the short-term fixed tissue, and no lesions were seen in the long-term fixed tissue."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	14 years	Light microscopy after staining with antibody clone Ubi-1 with heating	Biomolecular staining	Preserved staining with comparable results to long-term fixed tissue	0	0	0			"For Ubq IHC in short-term fixed tissue stored as paraffin blocks, Clone Ubi-1 was more dependent on the AR method used than the polyclonal Ubq-Ab. Clone Ubi-1 clearly required heat AR for optimal staining. It is noteworthy that although the CIs were readily detectable also after FA AR, the extent of Ubq-IR NTs was clearly lower with FA AR than with heat AR. Dwork et al (24) also found that Ubq-IR may be preserved after decades of formalin fixation, and our staining results confirmed this by determining that tissues that had been fixed for a short time were comparable to those that had been fixed for a long time in this regard. Thus, this protein, which is seen in almost all pathological intraneuronal inclusions, can readily be detected on archival tissues that have been fixed for years in formalin."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	14 years	Light microscopy after staining for p62 with antibody clone 3 using autoclave heating in Tris/EDTA	Biomolecular staining	Preserved staining	0	0	0			"The staining results for tissue that had been fixed for short-term or long-term significantly differed (Fig. 3). Substantial numbers of p62-IR CIs and some to moderate numbers of NTs were seen in most of the tissue cores that were fixed for a long time only with Clone 3. The extent of labeled NTs was slightly lower in the long-term than in the short-term fixed tissue specimens, however; this was the case, in particular, after 10 years' fixation. Thus, for p62-IR, of the 16 combinations of Ab and AR methods applied, CIs were seen with all 16 combinations in most tissue cores that had been fixed for a short time, where- as p62-IR CIs were noted with only 11 combinations (5 false- negative) in long-term fixed samples. Results for NTs differed even more extensively, that is, they were seen with only 8 of 16 methodological combinations in the short-term fixed tissue and in only 2 combina- tions (Clone 3 and heating) in long-term fixed tissue."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	14 years	Light microscopy after staining for p62 with most of the other antibodies and antigen retrieval methods tested	Biomolecular staining	Staining of cellular inclusions often decreased, staining of neurites often lost	2	2	2	Loss of antigenicity		
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	0.5 years	Light microscopy after staining for tau using the antibodies against the tau isoforms RD3 and RD4	Biomolecular staining	No immunoreactivity was noted	2	2	2	Loss of antigenicity		"No IR was noted in the samples that were fixed for periods from 6 months to 14 years with Abs RD3 and RD4."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	10 years	Light microscopy after staining for hyperphosphorylated tau following heat antigen retrieval	Biomolecular staining	No significant change in staining for cellular inclusions, but possible decrease in neurite extent, especially with antibody clone AT8	1	1	1	Loss of antigenicity		"Tau-IR CIs, NTs, and NPs were always seen with the Abs against HPtau (Clones AT8, AT180, and AT270), but the results varied using the Abs against the tau isoforms RD3 and RD4 (Fig. 4). Clones AT8, AT180, AT270 labeled numerous CIs in most of the cores regardless of which AR method was used. Clones AT8 and AT270 labeled numerous NTs with all of the AR methods, whereas Clone AT180 benefited from the heat AR method. Only Clones AT8 and AT180 labeled some to moderate NPs. The CIs were seen with both RD3 and RD4 Abs only after heat AR. There were no differences in the staining results of CIs using Clones AT8, AT180, or AT270 in the tissue fixed for short or long durations (Fig. 4). In contrast, the extent of NTs was comparable between short- and long-term fixations only after the heat AR method; however, some decrease of IR NTs might occur after 10 years of fixation, particularly using Clone AT8."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	14 years	Light microscopy after staining for alpha synuclein with clone 42 and antigen retrieval	Biomolecular staining	No significant change in staining for cellular inclusions or neurites	0	0	0			"Immunohistochemistry results for tissue that had been fixed for short and long periods considerably differed (Fig. 5). Substantial numbers of >S-IR CIs and NTs were seen in all tissue cores that were fixed for a long time only with Clone 42."
Pikkarainen 2010	2010	https://pubmed.ncbi	Stored in 10% buffered formalin in 2-L plastic containers at room temperature as 1 cm thick sections	2 years	Light microscopy after staining for alpha synuclein with most antibody clones and antigen retrieval methods	Biomolecular staining	Substantial loss of staining	2	1	1	Loss of antigenicity		"When the tissue had been fixed for more than 2 years, only 5 combinations displayed >S-IR CIs and NTs. In conclusion, of 35 applications, 30 were expected to reveal IR CIs and 28 IR NTs, but only 5 methodological combinations displayed CIs or NTs. Thus, for CIs, 25, and for NTs, 23 methodological combinations yielded false-negative results."
Dall'Oglio 2010	2010	https://pubmed.ncbi	Unbuffered 10% formalin	1.67 years	Light microscopy after staining with the "single-section" Golgi silver impregnation method	Morphologic staining	Preserves neuronal and glial morphology for quantitative studies on the light microscope, with no reported alterations due to storage	0	0	0			"One of the advantages of this method is that it can be applied to formalin-fixed human tissue stored for a long time, as in the routinely fixed anatomical pieces. We developed this technique in brain samples stored for almost 2 years in formalin, but other times can be tested (few months of storage also appeared to provide good results; data not shown)" ... "[N]eurons and glia appear well-impregnated, allowing qualitative and quantitative studies under light microscopy"
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for CD34	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue after antigen recovery methods used	0	0	0			"The combined protocol using formic acid and extended microwave pre-treatments and the CSA system (iv) was able to recover the immunoreactivities of anti-CD34, anti- HLA-DR, anti-CD45, anti-CD68 and anti-Cx43 completely. Using this combined protocol, the recovered immunoreactivities of these antibodies resembled the immunolabeling observed in short-fixed tissue with the exception of anti-HLA-DR, anti-CD45 and anti-CD68 whose recovered immunoreactivities in two control cases (Control 2 and 3) appeared slightly weaker than observed in short-fixed tissue, even though the amount of their immunopositive labeling was similar. We found that the intensity of anti-HLA-DR, anti-CD45 and anti-CD68 recovered immunoreactivities in these two control cases could be improved by using the combined protocol (iv) and doubling the primary antibody concentration (i.e. 1:50 for all three antibodies). The combined protocol (iv) also recovered the majority of anti-caveolin, anti-NeuN and anti-PV immunolabeling, but small areas of weak immunolabeling were still observed. The immunoreactivities of anti-claudin 5 and anti-MRP1 were not recovered using the combined protocol."
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for vWF	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for Claudin5	Biomolecular staining	Immunostaining signal lost	2	2	2	Loss of antigenicity		
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for Caveolin	Biomolecular staining	Immunostaining partially resembles that of short-term fixed tissue, but partially lost	1	1	1	Loss of antigenicity		
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for Pgp with two antibodies	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for BCRP	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for MRP1	Biomolecular staining	Immunostaining signal lost	2	2	2	Loss of antigenicity		
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for NPY	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for MAP2	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue after antigen recovery methods used	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for NeuN	Biomolecular staining	Immunostaining partially resembles that of short-term fixed tissue, but partially lost	1	1	1	Loss of antigenicity		
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for CB	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for PV	Biomolecular staining	Immunostaining partially resembles that of short-term fixed tissue, but partially lost	1	1	1	Loss of antigenicity		

Study	Year	Link	Storage conditions	Storage duration for reported outcome	Visualization method	Observation category predominant	Storage effect (An asterisk (*) indicates that the study does not specifically comment on histologic storage effects, but it can be inferred based on their results.)	Effect on morpholog	Effect on morphology gra	Consensus grade	Category of change	Effect on morphology grade notes	Relevant text
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for HLA-DR	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue after antigen recovery methods used	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for CD45	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue after antigen recovery methods used	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for CD68	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue after antigen recovery methods used	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for GFAP	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for Cx43	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue after antigen recovery methods used	0	0	0			
Liu 2010	2010	https://pubmed.ncbi	10% neutral-buffered formalin at room temperature	10 years	Light microscopy after immunostaining for PCNA	Biomolecular staining	Immunostaining resembles that of short-term fixed tissue	0	0	0			
Dall'Oglio 2010	2010	https://pubmed.ncbi	Unbuffered 10% formalin	1.67 years	Light microscopy after Golgi staining	Morphologic staining	No reported effect of storage on Golgi staining and morphology visualization	0	0	0			"Well-impregnated cells (neurons and glial cells) appear blackstained against a minimal soft yellow background with fewsmall particles of precipitation in the neuropil after osmication(Figs. 1C–F and 2). Following this procedure, the quality of stainwas similar when visually comparing the results obtained along5–11 days of chromation/silver nitrate impregnation. Various kindsof neurons could be observed in the same plane of section withtradialling dendrites. Profusion of thin axons and their collateral processeswere also evident (Fig. 1C and D). In the caudate nucleus,medium-spiny neurons were uniformly impregnated (Fig. 2A andB). They appeared, as in the classical descriptions, with a roundcell body and various primary dendrites that branched and taperedalong the section. Dendritic spines were visualized even withlow magnification. They showed a continuum of different shapesand sizes that could be easily identified, allowing the differentclassifications by shape as thin, stubby, mushroom-like or wida[Fig. 2C)."... "One of the advantages of this method is that it can be applied to formalin-fixed human tissue stored for a long time, as in the routinely fixed anatomical pieces. We developed this technique in brain samples stored for almost 2 years in formalin, but other times can be tested (few months of storage also appeared to provide good results; data not shown)."
Miguel-Hidalgo 2010	2010	https://pubmed.ncbi	Formalin	5.5 years	Light microscopy after cresyl violet staining	Morphologic staining	Macroscopic areas of white discoloration that are randomly distributed and more common with increased storage time. Within these, most cells are morphologically unaltered	0	0	0			"In spite of this difference, the values of glial and neuronal density were not correlated with TF in any of our cohorts (in controls, for glial density r2 = 0.004, p = 0.889; for neuronal density r2 = 0.017, p = 0.755; in alcoholic subjects, for glial density r2 = 0.025, p = 0.571; for neuronal density r2 = 0.037, p = 0.490)."
van Duijn 2011	2011	https://pubmed.ncbi	Stored in sealed plastic bags with a small excess of 10% formalin	42 years	Electron microscopy	Morphologic staining	Macroscopic areas of white discoloration that are randomly distributed and more common with increased storage time. Within these, most cells are morphologically unaltered	0	[No independent grade, pilo	1		Only did histology on the brain samples with longer storage duration, it seems.	
van Duijn 2011	2011	https://pubmed.ncbi	Stored in sealed plastic bags with a small excess of 10% formalin	6 years	Electron microscopy	Morphologic staining	Macroscopic areas of white discoloration that are randomly distributed and more common with increased storage time. Within these, some localized areas have absent neuropil and only membrane remnants	2	[No independent grade, pilo	2	Area of empty neuropil		
van Duijn 2011	2011	https://pubmed.ncbi	Stored in sealed plastic bags with a small excess of 10% formalin	6 years	Light microscopy after staining with H&E and staining for myelin with Kluver's stain	Morphologic staining	Basophilic areas with partial loss of neuropil microstructure and decreased myelin staining, indicating some degree of myelin loss. Cells and vessels are normally distributed in these areas without morphologic alteration	1	[No independent grade, pilo	1	Area of empty neuropil	May be a specific effect on myelin or myelinated axons, since they report that cellular structure is unaltered.	
Mostegl 2011	2011	https://pubmed.ncbi	7% neutral buffered formalin	1.51 years	Light microscopy after staining for DNA with in situ hybridization after using an increased concentration of proteinase K	Biomolecular staining	Decreased ISH signal for DNA, but restored by increasing the concentration of proteinase K treatment, back to the same levels as one day of fixation time	0	[No independent grade, pilo	0			
Martins 2011	2011	https://pubmed.ncbi	4% (w/v) formalin (Merck) buffered with 0.1M sodium phosphate buffer, pH 7.4, at room temperature	0.167 years	Light microscopy after immunostaining for nitric oxide synthase	Biomolecular staining	Following antigen retrieval, no storage effect detected	0	[No independent grade, pilo	0			"Immunostaining after antigen retrieval was maximal in human cerebellar tissue fixed for up to 2months (Table 1), using anti-nNOS (TL) antibody and either 50mM Tris–HCl buffer, pH 9.5 or 10mM sodium citrate buffer, pH 6.0. A moderate staining was achieved when the tissue was fixed for 2–8 months. Fixation of a cerebellar cortex for 20 months or more resulted in an essentially complete loss of neuronal NOS IR (not shown)." ... "A control section of a cerebellar cortex that was formalin-fixed, but cryo-protected and frozen rather than paraffinembedded, and was not subjected to antigen retrieval (Fig. 1B), showed neuronal NOS staining by anti-nNOS (TL) antibody in the same structures that were detected in other cerebellar sections after antigen retrieval in 50mM Tris–HCl buffer, pH 9.5. In contrast, staining of cell bodies and processes in a cerebellar section adjacent to that in Fig. 1A, but without using antigen retrieval, was barely detectable using anti-nNOS (TL)"
Martins 2011	2011	https://pubmed.ncbi	4% (w/v) formalin (Merck) buffered with 0.1M sodium phosphate buffer, pH 7.4, at room temperature	1.67 years	Light microscopy after immunostaining for nitric oxide synthase	Biomolecular staining	Even following antigen retrieval, essentially complete loss of immunostaining	2	[No independent grade, pilo	2	Loss of antigenicity		
Chung 2013	2013	https://pubmed.ncbi	10% formalin at room temperature	6.83 years	3D fluorescence microscopy after staining for neurofilament protein	Biomolecular staining	Effective morphologic visualization and cellular process tracing for neurofilament protein	0	0	0			"We found that CLARITY was suitable for such long-banked human brain, allowing immunohistological visualization and identification of neurons and projections over large volumes (Fig. 5a–g and Supplementary Videos 15, 16, 17). In 0.5-mm-thick blocks of frontal lobe from an autistic patient, stored in formalin for >6 years, we were able to stain for axons with neurofilament protein and myelin basic protein, and trace individual fibres (Fig. 5e and Supplementary Video 15). In addition, by staining for parvalbumin it was possible to visualize the distribution of parvalbumin-positive neurons in the neocortex over large volumes (6.7 × 4.7 × 0.5 mm), and trace individual parvalbumin-labelled processes" ... "82 months in 10% formalin at room temperature" (Also Supplementary Video 15)
Chung 2013	2013	https://pubmed.ncbi	10% formalin at room temperature	6.83 years	3D fluorescence microscopy after staining for myelin basic protein	Biomolecular staining	Effective morphologic visualization and cellular process tracing for myelin basic protein	0	0	0			"Figure 3. Brain tissue with moderate nuclei degradation (25 year storage, the 1970s) H&E 20X magnification. Circled areas highlight nuclear degradation."
Chung 2013	2013	https://pubmed.ncbi	10% formalin at room temperature	6.83 years	3D fluorescence microscopy after staining for parvalbumin	Biomolecular staining	Visualization of the distribution of parvalbumin-positive neurons across long distances	0	0	0			
Liu 2014	2014	https://pubmed.ncbi	10% buffered formalin at 4° C	12 years	Electron microscopy	Morphologic staining	White matter ultrastructure, including myelin sheath thickness and integrity, are not significantly altered due to the long term fixation	0	0	0			"[M]yelin sheath thickness and integrity are not significantly altered due to long term fixation"
Herai 2014	2014	https://pubmed.ncbi	Formalin	21 years	Light microscopy after staining with thionin	Morphologic staining	Cells with pyramidal shaped somas can be visualized and their small RNA content profiled	0	0	0			
Preusse-Prange 2014	2014	https://pubmed.ncbi	10% buffered formalin	0.08 years	Light microscopy after immunostaining for heat shock proteins	Biomolecular staining	Loss of immunoreactivity in a majority of the samples	2	1	1	Loss of antigenicity		"Second, a long-term fixation (>1 month) may also affect immunohistochemical examinations, leading to false-negative results since antigen retrieval may be difficult, as our results show." ... "The results of the immunohistochemical investigation also show that the detectability of the proteins decreases proportional to the fixation duration. When regarding immunohistochemical treated tissue slides, after three months in 58% of samples from cerebrum tissue and cerebellum tissues proteins were no longer detectable. After only 30 min of formalin exposure still 83% of the analyzed cerebrum tissue and 67% of the cerebellum sections showed a strong positive reaction"
Cook 2015	2015	https://www.scirp.o	Formalin	25 years	Light microscopy after hematoxylin and eosin staining	Morphologic staining	Moderate nuclei degeneration	1	1	1	Nuclei degeneration		"Figure 3. Brain tissue with moderate nuclei degradation (25 year storage, the 1970s) H&E 20X magnification. Circled areas highlight nuclear degradation."
Cook 2015	2015	https://www.scirp.o	Formalin	80 years	Light microscopy after hematoxylin and eosin staining	Morphologic staining	Complete nuclei degeneration	2	2	2	Nuclei degeneration		"In general, tissues collected before 1960 showed cell structure degradation with dissolution of some nuclei, washed out cell cytoplasm, and some cell membrane dissolution. These degenerative features were observed to be more severe in the kidney, liver, lung, spleen, and brain than the heart and uterus. ... Figure 4. Brain tissue with complete nuclei degradation (80 year storage, the 1920s) H&E 20X magnification. Nuclear degradation not manually highlighted due to entire representative section being patently affected by nuclear degradation."
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	25 years	3D confocal microscopy after staining for neurofilaments with SMI312	Biomolecular staining	Intact morphological staining for neurons	0	0	0			"Resolved the layered organization of neurons in neocortex and their general morphology" ... "Staining of pan-axonal neurofilaments (SMI312) reveals the general organization of neurons in cortical sections"
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	19 years	3D confocal microscopy after staining for a subset of interneurons with calbindin D28	Biomolecular staining	Intact morphological staining for interneuron population	0	0	0			"Discrete populations of pyramidal neurons including calbindin D28-positive interneurons of layer III were detected, allowing their complex morphology to be visualized" ... "Calbindin expressing interneurons are enriched in layers I/II of the human cortex (male, 88 years old, 19 years of fixation) and show diversified morphologies after 3D reconstruction"
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	15 years	3D confocal microscopy after staining for synapses with VGLUT1 and PSD95	Biomolecular staining	Able to identify individual synapses	0	0	0			"Labeling for the presynaptic protein VGLUT1 and postsynaptic scaffold PSD95 demonstrated the ability of this approach to resolve individual synapses"
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	25 years	3D confocal microscopy after staining for microglia with Iba1	Biomolecular staining	Intact morphological staining for microglia	0	0	0			"Maximum projection of a large field showing 1.95 mm2 (x: 1.95 mm, y 0.9 mm) of tissue with a 30 µm depth labeled for Iba1 (microglia; magenta) and GFAP (astrocytes; green). (e) High magnification image of a GFAP-positive astrocyte 'island' in a cortical section. (f) 3-dimensional reconstructions of cortical protoplasmic GFAP+ astrocytes and Iba1+ microglia (female, 86 years old). (g) GFAP+ astrocytes (green) and Iba1+ microglia (magenta) in cortical layers III/IV show complex interactions in a control brain (female, 86 years old), resolvable by visualizing individual frames of confocal Z-stacks (step-size: 1 µm)."
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	25 years	3D confocal microscopy after staining for astrocytes with GFAP	Biomolecular staining	Intact morphological staining for astrocytes	0	0	0			
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	14 years	3D confocal microscopy after staining for Aβ plaques and tau fibrils with Thiazine red	Morphologic staining	Intact morphological staining for amyloid beta plaques	0	0	0			"Here we provide a broadly accessible method that overcomes several limitations that restrict the amount and type of information recovered from human brain tissue in long-term storage. This approach is attractive because of its independence from complex tissue processing and clearing steps. We demonstrate the robustness of the approach by showing how it simultaneously resolves macro- and microscopic features of brain cells in normal and AD tissues that have been stored up to 25 years. Simultaneous imaging of large territories of human brain (mm2) and 3D analysis of the relationship of different cellular components is a major advantage of this approach. This relies on the ability of the method to deliver low background autofluorescence, excellent antibody penetration, and uniform labeling in thick slices. At the same time, it also affords the ability to resolve subcellular structures in human brain, from cytoskeletal elements in neurons and glia, to pre- and postsynaptic sites. The usefulness of the approach is exemplified by resolving the complex 3D structure of microglia and astrocytes around Aβ plaques in AD."
Bouvier 2016	2016	https://pubmed.ncbi	Formalin	14 years	3D confocal microscopy after staining for phosphorylated tau with PS422 and AT8	Biomolecular staining	Intact staining for paired helical filaments and neurofibrillary tangles	0	0	0			
Phillips 2016	2016	https://pubmed.ncbi	Formalin	7 years	Confocal microscopy for several antigens after tissue clearing	Biomolecular staining	Successful staining in some but not all of the cases, which the authors could not explain and did not attribute to storage effects*	NA	NA	NA		Some of the antibody stainings were unsuccessful, but they do not attribute this to a storage artifact.	"From the seven cases, there were mixed results. There was positive labelling of both NF-H and MBP allowing for the visualisation of myelinated Purkinje cell axons in the granule cell layer of control 1 and Patients 2, 4 and 5 (Fig. 3c). While in the case of control 2, patient 1 and 6 there was no specific staining of any antibody. We are unsure why staining of these tissues was not successful since the length of fixation and post-mortem interval (PMI) of control 2 (fixation: 8 years, PMI: 39 hours), patient 1 (fixation: 6 years, PMI: 21 hours) and 6 (fixation: 2 years, PMI: 76 hours) falls within the range of successful cases (the only exception being control 2 where fixation 1 year over this range) (fixation: 3–7 years, PMI: 21–112 hours). Control 2 demonstrated non-specific staining of the blood vessels and aggregation of the fluorophores in the 488-nm channel. There was some specific staining of both NF-H and MBP in Patient 3, however it was only the larger thicker axons that were labelled."
Harrison 2018	2018	https://pubmed.ncbi	Formalin	2 years	Confocal microscopy of vessels with tomato lectin staining	Morphologic staining	Vasculature visualized at high resolution, including at the capillary level using confocal microscopy, traced to at best 1 nm	0	0	0			"[W]e have demonstrated that this protocol can be successfully applied to tissue volumes that can encompass the whole thickness of the human cerebral cortex in human post-mortem brain samples that have been preserved in formalin for prolonged periods"
Lai 2018	2018	https://pubmed.ncbi	Formalin	30 years	Light microscopy after cresyl violet staining	Morphologic staining	Were able to perform cresyl violet staining for morphologic profiling, but they report that structural preservation was not as good in archival tissue in general	1	1	1	Decreased structural preservation		"Cresyl violet staining is also applicable to prolonged formalin-fixed or FFPE tissues after SDS treatment" ... "It is important to note, however, that although staining was possible, the immunostaining quality, antibody penetration depth and structural preservation were not as good as standard formalin-fixed tissues when treated with the same tissue-clearing and immunostaining procedures."
Lai 2018	2018	https://pubmed.ncbi	Formalin	45 years	Light microscopy after lectin staining	Morphologic staining	Were able to perform 3d blood vessel staining, but they report that structural preservation was not as good in archival tissue in general	1	1	1	Decreased structural preservation		"In this study, a piece of tissue fixed in formalin for 45 years immunostained for zona occludens-1 (ZO-1), was counterstained with DyLight 649-labelled lectin, demonstrating the intricate blood vessel network in the brain" ... "It is important to note, however, that although staining was possible, the immunostaining quality, antibody penetration depth and structural preservation were not as good as standard formalin-fixed tissues when treated with the same tissue-clearing and immunostaining procedures."
Lai 2018	2018	https://pubmed.ncbi	Formalin	50 years	Light microscopy after immunofluorescence with GFAP antibody	Biomolecular staining	Were able to perform GFAP immunostaining, but they report that immunostaining quality, antibody penetration depth and structural preservation were not as good in archival tissue in general	1	1	1	Decreased structural preservation		"Z-stack image of a block of 2 mm-thick cingulate cortex that has been formalin-fixed for 50 years, stained with antibodies against GFAP with an imaging depth of 125.57 µm" ... "It is important to note, however, that although staining was possible, the immunostaining quality, antibody penetration depth and structural preservation were not as good as standard formalin-fixed tissues when treated with the same tissue-clearing and immunostaining procedures."
Quesseveur 2019	2019	https://pubmed.ncbi	Formalin	30 years	Confocal microscopy after staining with different astrocyte markers	Biomolecular staining	No reported effect of storage on successful immunolabeling for astrocytes	0	0	0		This publication is similar to Bouvier 2016, but notes a longer storage time.	
Hildebrand 2019	2019	https://pubmed.ncbi	4% paraformaldehyde in 0.1 M phosphate buffered saline	2.5 years	Fluorescence microscopy following staining with several nonspecific organic dyes	Morphologic staining	Cell body morphology is intact	0	0	0			"This approach has been successful on samples that have been stored in formalin up to 30 years"
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.22 years	Light microscopy after staining for APP	Biomolecular staining	Decreased staining intensity	1	1	1	Loss of antigenicity	Regarding morphology, the authors mention assessing staining intensity in specific cellular compartments (nucleus, cytoplasm, cell membrane), suggesting that morphology is retained in general. The study also notes fixation in 4% buffered formalin, but this is assumed to be a typo.	"MASH is capable of clearing and labeling adult human archival brain samples, even after prolonged storage in formalin (current samples had been fixed for 14 to 30 months)"
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for ATRX	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for MAP2	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.18 years	Light microscopy after staining for NeuN	Biomolecular staining	Decreased staining intensity	1	1	1	Loss of antigenicity		
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for NeuN	Biomolecular staining	Loss of staining	2	2	2	Loss of antigenicity		
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for RbAp48	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for SMI31	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.15 years	Light microscopy after staining for APC	Biomolecular staining	Decreased staining intensity	1	1	1	Loss of antigenicity		
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.13 years	Light microscopy after staining for CD64	Biomolecular staining	Decreased staining intensity	1	1	1	Loss of antigenicity		
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for GFAP	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.15 years	Light microscopy after staining for HLA-DR	Biomolecular staining	Unstable staining intensity	1	1	1	Loss of antigenicity		
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for Iba-1	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for Olig-2	Biomolecular staining	Stable staining intensity	0	0	0			
Lundström 2019	2019	https://pubmed.ncbi	4% buffered formalin	0.26 years	Light microscopy after staining for S100	Biomolecular staining	Stable staining intensity	0	0	0			
Alrafiah 2019	2019	https://pubmed.ncbi	Formalin	20 years	Light microscopy after H&E staining	Morphologic staining	Normal appearance, no reported changes	0	0	0			
Alrafiah 2019	2019	https://pubmed.ncbi	Formalin	20 years	Light microscopy after Luxol fast blue staining	Morphologic staining	Detected myelin, no reported changes	0	0	0			
Alrafiah 2019	2019	https://pubmed.ncbi	Formalin	20 years	Light microscopy after Congo red staining	Morphologic staining	Detected amyloid including amyloid in blood vessels, no reported changes	0	0	0			
Alrafiah 2019	2019	https://pubmed.ncbi	Formalin	20 years	Light microscopy after immunostaining for CD68	Biomolecular staining	Detected microglia, no reported changes	0	0	0			
Alrafiah 2019	2019	https://pubmed.ncbi	Formalin	20 years	Light microscopy after immunostaining for Tenascin-C	Biomolecular staining	Reported positive staining in extracellular matrix and intracytoplasmic	0	0	0			
Alrafiah 2019	2019	https://pubmed.ncbi	Formalin	20 years	Light microscopy after immunostaining for Caspase 3	Biomolecular staining	Detected neurons, no reported changes	0	0	0			
Hildebrand 2020	2020	https://pubmed.ncbi	Formalin [listed in Larsen 2022]	3 years	Light sheet microscopy with DiI staining	Morphologic staining	Good preservation, e.g. spine-like protrusions from dendrites are clearly visible	0	0	0		Also have a different fixation procedure with similar results.	"[B]rains were recovered by calvarian dissection and stored in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS) for 14–30 months"

Study	Year	Link	Storage conditions	Storage duration for reported outcome	Visualization method	Observation category predominant	Storage effect (An asterisk (*) indicates that the study does not specifically comment on histologic storage effects, but it can be inferred based on their results.)	Effect on morpholog	Effect on morphology gra	Consensus grade	Category of change	Effect on morphology grade notes	Relevant text
Costantini 2020	2020	https://www.bioniv	Neutral buffered formalin (pH 7.2-7.4) at room temperature	7 years	Fluorescence microscopy after staining with various antibodies (NeuN, MAP2, etc), to label neurons, interneurons, glia, and vasculature, as well as nuclear stains	Biomolecular staining	Intact imaging of the 3D organization of whole neurons	0	0	0			"In comparison to animal brains, human neural tissues presents high variability of post-mortem fixation conditions and antigens alterations that prevent proper immunostaining recognition. In this work, we combined the SWITCH tissue transformation method with the TDE clearing. The SWITCH technique allows removing lipids from the sample, while maintaining structural integrity, leading to an increase of tissue permeability and a reduction of the tissue refractive index (RI). The TDE clearing method allows homogenizing the RI of the sample with that of the mounting medium to reach the final transparency. The optimized protocol can perform tissue clearing on prolonged formalin-fixed brain samples and homogeneously stain the tissue with small molecules (up to 1 mm in depth) as well as antibodies (up to 500 µm). The compatibility of the protocol with different antibodies is demonstrated by staining neuronal and non-neuronal cells as well as blood vessels with different antibodies. ... The optical sectioning and the high-resolution optical investigation made possible by TPFM, in combination with the tissue clearing technique, allowed imaging the 3D organization of whole neurons without introducing any visual artifacts."
Flor-García 2020	2020	https://pubmed.ncbi	3.7% formalin	0.5 years	Light microscopy after staining for DCX	Biomolecular staining	Abolishes immunostaining signal for DCX	2	2	2	Loss of antigenicity	Very similar data set as Moreno-Jiménez 2019, but more discussion on the storage effects in this article, so this one will supercede that one.	"Note that formalin fixation completely abolished the DCX signal for all the anti-DCX antibodies used and caused the appearance of an unspecific nuclear signal" ... "Staining with the same nine anti-DCX antibodies on samples fixed in 3.7% formalin for 6 months. Note that formalin fixation completely abolished the DCX signal for all the anti-DCX antibodies used and caused the appearance of an unspecific nuclear signal. (ab–ad)"
Flor-García 2020	2020	https://pubmed.ncbi	3.7% formalin	0.5 years	Light microscopy after staining for PSA-NCAM	Biomolecular staining	Abolishes immunostaining signal for PSA-NCAM	2	2	2	Loss of antigenicity		"Staining with an anti–polysialylated-neural cell adhesion molecule (PSA-NCAM) antibody on samples fixed either in 4% freshly prepared PFA for 24 (ae) or 6 (af) h, or in 3.7% formalin for 6 months (ag). PSA-NCAM–specific signal and low background are observed in samples fixed in PFA (ae,af). In contrast, formalin fixation completely abolished the PSA-NCAM signal and caused the appearance of an unspecific nuclear signal"
Flor-García 2020	2020	https://pubmed.ncbi	3.7% formalin	0.5 years	Light microscopy after staining for NeuN	Biomolecular staining	NeuN signal can still be seen, but with a substantially increased background signal	1	1	1	Loss of antigenicity		"Staining with an anti-NeuN antibody on samples that had been fixed either in 4% freshly prepared PFA for 24 (ab) or 6 (ac) h, or in 3.7% formalin for 6 months (ad). Note that the NeuN-specific signal can be observed in the three fixation conditions. However, a substantial increase in the background is observed in samples fixed in formalin"
Chan 2020	2020	https://pubmed.ncbi	Formalin [listed in Larsen 2022]	70 years	Light microscopy after Nissl staining	Morphologic staining	Brains stored for different periods of time have the same neuron and glial cell densities and all cells appear stained with the Nissl stain, without a significant effect of storage time	0	0	0		Effectively the same data set as Larsen 2022. But, there is more discussion on storage effects in this article, so this publication will supercede that one.	"Volume and cell number did not correlate noticeably with age, storage time, and PMI (Fig. 4, PMI data not shown)." ... "From a technical point of view, processing differences (including storage time and PMI) between the two tissue collections is unlikely to create a different number of observable cells in the thionin stain for Nissl substance. All cells appeared stained and we counted both neurons and glial cells, endothelial cells forming only a miniscule uncounted fraction of the total. Thus, our cell number estimates should be robust to this limitation. On the other hand, potential differences in initial tissue processing could cause the observed differences in hippocampal volumes. However, as the observed mean cell densities were virtually identical between groups in most regions (Fig. 3), the volume changes closely matched the robust cell number changes. Therefore, the observed volume changes are most likely of biological origin." ..."As indicated in Fig. 3 and Table 2, we did not detect any significant differences in the numerical density of neurons or glial cells."
Herbin 2021	2021	https://doi.org/10.1	Formalin	92 years	Light microscopy after hematoxylin-eosin and Giemsa staining	Morphologic staining	Fine and cellular features are preserved, with few tissue perforations	0	1	1			"In contrast, the best results were obtained with group F (100% formalin), with even small glial cells visible in the preserved brain tissue. This excellent preservation of fine and even cellular details in the tissue underlines the potential of group F brains for histochemical studies—as long as they are fixed rapidly after the death of the organism"
Larsen 2021	2021	https://pubmed.ncbi	4% formaldehyde in phosphate buffer at neutral pH	21 years	Light microscopy after serial sectioning and staining with toluidine blue	Morphologic staining	Able to identify pyramidal cells, but can't say for sure whether the storage time may have affected some cellular parameters. They didn't describe any obvious storage artifacts, though	NA	NA	NA			
Shan 2022	2022	https://pubmed.ncbi	Formalin	2 years	3D fluorescence microscopy after staining for GFAP	Biomolecular staining	Morphology of astrocytes via GFAP staining appears to be intact*	NA	NA	NA			"Also, even after 2 weeks of storage in AKS solution, most of the fluorescent signals were well preserved, indicating that tissue cleared with AKS was very stable (Additional file 2: Fig. S2)."
Wu 2022	2022	https://pubmed.ncbi	10% neutral buffered formalin	3.33 years	Light microscopy after staining for NeuN	Biomolecular staining	Decreased staining intensity for NeuN, by 19.3%		1	1	Loss of antigenicity		
Wu 2022	2022	https://pubmed.ncbi	10% neutral buffered formalin	3.33 years	Light microscopy after staining for Nissl	Morphologic staining	Decreased staining intensity for Nissl, by 16.6%		1	1	Loss of antigenicity		
Wu 2022	2022	https://pubmed.ncbi	10% neutral buffered formalin	3.33 years	Light microscopy after staining for GFAP	Biomolecular staining	No reported change in staining intensity	0	0	0			
Wu 2022	2022	https://pubmed.ncbi	10% neutral buffered formalin	3.33 years	Light microscopy after staining for IBA-1	Biomolecular staining	No reported change in staining intensity	0	0	0			
Wu 2022	2022	https://pubmed.ncbi	10% neutral buffered formalin	3.33 years	Light microscopy after staining for GAPDH	Biomolecular staining	No reported change in staining intensity	0	0	0			
Wiggermann 2022	2022	https://pubmed.ncbi	1% formalin diluted in phosphate-buffered saline (PBS) at 4° C	14 years	Light microscopy after staining for LFB and PLP	Morphologic staining	No reported difficulties with cellular visualization and identification based on storage time*	NA	NA	NA		The lack of reported collinearity between PLP/LFB-PAS staining metrics and storage time, and their independent contributions to predicting MRI metrics, implies these histological measures do not appear to be substantially associated with storage time based on the data presented.	
Öztürk 2022	2022	https://pubmed.ncbi	10% neutral buffered formalin at room temperature	17 years	Confocal microscopy after staining with the lipophilic dye DiI	Morphologic staining	No reported effect of storage on nerve tracing extent	0	0	0			"Our work showed that a lipophilic dye differentiation of 25.11 ± 9.1 mm and 12.11 ± 8.62 mm of distal and proximal travel on the peripheral nerves of formalin-fixed human fetuses with up to 18-year postmortem delay is feasible. This distance is comparable to those achieved in tissues in which tracing are rapidly processed (with a maximum of 2–3-day postmortem delay) and is meaningful for tracing peripheral nerves"
Insausti 2023	2023	https://pubmed.ncbi	4% paraformaldehyde at 4° C	5 years	Light microscopy and electron microscopy	Morphologic staining	Good histologic results via light microscopy and electron microscopy	0	0	0			"The brain is weighed on a scale and placed in a wide container with a hermetic cover to avoid evaporation and formalin fumes, with the ventral surface up, and abundant gauze at the bottom (Figures 5F–J). 3 L of freshly prepared 4% paraformaldehyde is poured into the container, or enough to cover the brain. On top of the brain, we place additional gauze soaked in 4% paraformaldehyde. The brain is then stored in a cold room (4°C) for postfixation, where it remains intact until processing for study. The time of postfixation is variable, according to the research needs, but we have processed brain tissue up to 5 years after intracarotid perfusion, both at the microscopic and ultrastructural levels with good results (see below)."
Schueth 2023	2023	https://pubmed.ncbi	4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS)	2.5 years	Light-sheet fluorescence microscopy with a morphologic NeuroTrace Nissl stain	Morphologic staining	Morphology is well preserved after tissue processing*	NA	NA				"Subsequently, brain samples were recovered by calvarian dissection and stored in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) for 14–30 months." ... "The morphology of the MASH cleared sample is well preserved after dehydration, delipidation and refractive index (RI) matching, and both grey as well as white matter become highly transparent"
Woelfle 2023	2023	https://pubmed.ncbi	Formalin	18 years	Flouresence microscopy after MAP2 immunostaining	Biomolecular staining	Tissue cleared and imaged with confocal microscopy with reliable MAP2 labeling	0	0	0			Figure 2 - Figure Supplement 1 - "When all six study cases were viewed in a row, the MAP2 antibody provided reliable labeling of its target structures in cleared sections independent of their fixation method and total fixation time, whereas sections without CLARITY-pretreatment showed variable and inconsistent outcomes with sometimes unspecific artifact-rich or even no specific labeling"
Lin 2023	2023	https://www.bioniv	10% formalin	1.12 years	Fluorescence microscopy after staining with GFAP	Biomolecular staining	Loss of antigenicity for astrocyte foot processes	2	1	1	Loss of antigenicity		"The results indicated that three-dimensional (3D) expression patterns of biological markers could be preserved for decades and revealed later by performing MOCAT" ... "The astrocytic foot processes were stained in the brain specimen stored in an FFPE block for 60 days but not in the formalin-preserved brain blocks stored for 1 year and 45 days (Fig. 4a), indicating that FFPE is superior to formalin for antigenicity preservation."