ELSEVIER

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat





Cluster analyses of contemporary embalming protocols in central European anatomy institutions: A collaborative effort to minimize chemical exposure

Sara Mohebimoushaei ^a, Veronica Antipova ^a, Uta Biedermann ^b, Beate Brand-Saberi ^c, Lars Bräuer ^d, Svenja Caspers ^e, Sara Doll ^f, Maren Engelhardt ^g, Timm J. Filler ^e, Martin Gericke ^h, Yakup Gümüsalan ⁱ, Stefanie Gundlach ^j, Gregor U. Hayn-Leichsenring ^b, Stephan Heermann ^{i,k}, Laura Hiepe ^l, Bernhard Hirt ^m, Lena Hirtler ⁿ, Christoph Kulisch ^o, Marko Konschake ^p, Stefanie Kürten ^q, Tobias Lange ^{b,r}, Mario Lorenz ^{a,s,t}, Christina Nassenstein ^u, Peter H. Neckel ^m, Ute Nimtschke ^v, Friedrich Paulsen ^d, Andreas Prescher ^w, Michael L. Pretterklieber ^a, Andreas Schmiedl ^x, Sven Schumann ^{y,z}, Volker Spindler ^{r,aa}, Gundula Schulze-Tanzil ^{ab}, Thomas Tschernig ^{ac}, Axel Unverzagt ^{ad}, Ursula Valentiner ^r, Ivan Varga ^{ae}, Thilo Wedel ^j, Janet Weigner ^{af}, Wolfgang J. Weninger ⁿ, Jürgen Westermann ^{ag}, Monika Wimmer-Roell ^g, Jens Waschke ^{ad}, Niels Hammer ^{a,ah,ai}, ^{*}

- a Division of Macroscopic and Clinical Anatomy, Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria
- ^b Institute of Anatomy I, University Hospital Jena, Jena, Germany
- ^c Department of Anatomy and Molecular Embryology, Institute of Anatomy, Bochum, Germany
- ^d Institute of Functional and Clinical Anatomy, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany
- ^e Institute for Anatomy I, Medical Faculty & University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
- f Department of Anatomy and Cell Biology, Heidelberg University, Heidelberg, Germany
- g Department of Anatomy, Johannes Kepler University, Linz, Austria
- ^h Institute of Anatomy, Leipzig University, Germany
- ¹ Institute for Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, Freiburg, Germany
- ^j Institute of Anatomy, Christian-Albrechts-Universität Kiel, Kiel, Germany
- ^k Fakultät III Health, Medical & Life Sciences, Furtwangen University, Germany
- ¹ Institute for Anatomy, Rostock University Medical Center, Rostock, Germany
- m Institute of Clinical Anatomy and Cell Analysis, University of Tübingen, Tübingen, Germany
- ⁿ Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria
- o Institute of Functional Anatomy, Charité-Universitätsmedizin Berlin, Berlin, Germany
- P Department of Anatomy, Histology and Embryology, Institute of Clinical and Functional Anatomy, Medical University of Innsbruck (MUI), Innsbruck, Austria
- ^q Institute of Neuroanatomy, Faculty of Medicine, University of Bonn and University Hospital Bonn, Bonn, Germany
- ^r Institute of Anatomy and Experimental Morphology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
- s Department of Orthopedic and Trauma Surgery, University of Leipzig, Leipzig, Germany
- ^t Chemnitz University of Technology, Professorship for Production Systems and Processes, Chemnitz, Germany
- ^u Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany
- v Institute of Anatomy, Technical University Carl Gustav Carus Dresden, Dresden, Germany
- [™] MOCA, Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany
- x Institute of Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany
- y Institute of Anatomy, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany
- ^z Institute of Anatomy, Brandenburg Medical School, Neuruppin, Germany
- aa Department of Biomedicine, University of Basel, Basel, Switzerland
- ab Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg and Salzburg, General Hospital Nuremberg, Nuremberg, Germany
- ac Institute of Anatomy and Cell Biology, Saarland University, Homburg, Saar, Germany
- ad Chair of Vegetative Anatomy, Institute of Anatomy, Faculty of Medicine, LMU Munich, Munich, Germany
- ^{ae} Institute of Histology and Embryology, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia
- ^{af} Institute of Veterinary Anatomy, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
- ag Institute of Anatomy, University of Lübeck, Lübeck, Germany

E-mail addresses: niels.hammer@medunigraz.at, nlshammer@googlemail.com (N. Hammer).

https://doi.org/10.1016/j.aanat.2025.152403

Received 22 November 2024; Received in revised form 23 February 2025; Accepted 11 March 2025

Available online 19 March 2025

0940-9602/© 2025 The Author(s). Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

^{*} Correspondence to: Division of Macroscopic and Clinical Anatomy, Gottfried Schatz Research Center, Medical University of Graz, Auenbruggerplatz 25, Graz, Austria.

- ^{ah} Department of Orthopedic and Trauma Surgery, University of Leipzig, Leipzig, Germany
- ai Division of Biomechatronics, Fraunhofer Institute for Machine Tools and Forming Technology Dresden, Dresden, Germany

ARTICLE INFO

Keywords: Cluster analysis Conservation Elbow method Embalming Fixation Formaldehyde

ABSTRACT

Background: Anatomical embalming forms a mainstay in the provision of teaching materials based on human post-mortem tissues. As a consequence of regulations worldwide to minimize the use of formaldehyde due to its harmful nature, effective measures must be taken to minimize its application in the anatomy laboratory. This study aimed to identify commonalities among embalming protocols used in Austrian, German and Swiss anatomies to reduce formaldehyde usage and provide a foundation for joint accreditation.

Methods: Survey data on all procedures, technologies and chemicals related to embalming were collected from 28 participating institutions and analyzed using an automated clustering approach. Special attention was given to chemicals, explosion-proof facilities, and air ventilation technology. After conducting principal component analyses, the data was evaluated using an unsupervised K-means learning algorithm.

Results: Six distinct clusters of common chemical usage were identified, namely (1) Thiel embalming with explosion-proof facilities, (2) potassium- and sodium salt-based embalming with downdraft ventilation, (3) ethanol/glycerin/glutaraldehyde/sodium alkyl ether sulphate-based embalming, (4) ethanol-based embalming, (5) formaldehyde-based embalming, and (6) ethanol/phenoxyethanol/alkyl (C12–16) dimethylbenzyl ammoniumchloride-based embalming. All clusters included the use of formaldehyde to varying extent. These clusters demonstrated high stability for fixation, combined fixation and conservation, as well as the incorporation of explosion-proof facilities and air ventilation technology. Moreover, a number of institutions used more than one similar protocol, so-called 'Superclusters', with Clusters (4) and (1) or Clusters (5) and (1) combined being the most common observation.

Conclusion: These findings provide a basis for optimizing, streamlining and sharing embalming protocols among central European anatomy institutions.

1. Introduction

Gross anatomy research and study have largely been facilitated by the introduction of organic embalming chemicals, notably the application of formaldehyde as a fixative by Ferdinand Blum (Benerini Gatta et al., 2012; Blum, 1893, 1894; Fox et al., 1985; Musiał et al., 2016; Puchtler and Meloan, 1985). Formaldehyde and other chemicals effectively prevent tissues from decomposing and bacterial contamination, thereby allowing for an extended usability of the tissues (Balta et al., 2019; Eisma and Wilkinson, 2014; Ramesh et al., 2017; Thavarajah et al., 2012). A broad variety of different protocols have been established for fixation and conservation in anatomy, which accommodate the visual and haptic properties of tissues needed for undergraduate or postgraduate study (Hammer, 2022; Hammer et al., 2015; Hammer et al., 2012; Hayashi et al., 2014; Rakuša and Kocbek Šaherl, 2022; Thiel, 1992, 2002; Tutsch, 1975; Zanini et al., 2012). Due to its unique features, formaldehyde is one of only two chemicals listed as embalming agents in the European regulations on the use of biocidal substances for fixation purposes, and therefore forms a common component among most of the existing embalming protocols (Durongphan et al., 2023; Eisma et al., 2013; Kerner et al., 2023; Pfeil et al., 2020; Waschke et al., 2019).

While embalming chemicals undoubtedly serve a purpose in preserving post-mortem tissues (Brenner, 2014; Coleman and Kogan, 1998; Crosado et al., 2020), large-scale studies on formaldehyde have provided substantial evidence of its carcinogenic effects (Albert et al., 1982; Hauptmann et al., 2003, 2004; Kang et al., 2021; Lam et al., 2021; Swenberg et al., 1980; Swenberg et al., 2013). Consequently, the use of such chemicals has been continuously restricted (Bruinen de Bruin et al., 2022; Kerner et al., 2023; Scheepers et al., 2018; Waschke et al., 2019). This development aligns with workplace safety regulations, as it helps protect those in anatomy exposed to peak levels of formaldehyde and other chemicals, especially prosection staff (Eickmann and Thullner, 2017; Hauptmann et al., 2004, 2009; Pflaumbaum et al., 2019; Tupper and Garg, 2024). However, these restrictions also limit anatomists' ability to customize post-mortem tissues to their needs.

A recent initiative by the Anatomische Gesellschaft (German Anatomical Society), the "Working Group on the Reduction of Formaldehyde Exposure", aimed to establish measures to minimize chemical exposures to anatomy staff and students in a joint effort between the German-speaking institutions (Waschke et al., 2019). In an effort to gather information on the collective use of chemicals, protocols, and infrastructure, our group conducted a large-scale 537-item survey among German-speaking departments in Austria, Germany, and Switzerland (Kerner et al., 2023). It was found that embalming protocols and facilities varied among institutions. Table-borne air ventilations were closely linked to the use of formaldehyde, while ethanol was closely linked to downdraft- and floor-borne ventilation technology (Kerner et al., 2023; Russ and Hammer, 2025). Although this information helped outlining the nature and link between chemicals and building infrastructure, understanding potential shared protocols between anatomy institutions was lacking.

Shared embalming protocols not only have the advantage of sharing knowledge on the utility of modifications and their limitations, but may also offer a sound basis for accreditation purposes between institutions in the near future. Such shared protocols could help maximize workplace safety in line with biocidal substances accreditation purposes, while also reducing the high cost of such accreditation.

This study aimed to determine commonalities among embalming protocols in Germany, Austria, and Switzerland. Cluster analyses were conducted to prioritize the mixtures of the most common embalming chemicals combined with relevant infrastructure frameworks.

It was hypothesized that certain clusters, i.e., groups of similar protocols, exist among the various institutions, and that these clusters can be further characterized by commonalities among facility infrastructure.

2. Material and methods

2.1. Survey conceptualization and distribution

The survey data used for this evaluation have been obtained as part of a larger study on all German-speaking anatomy departments (Kerner et al., 2023). In brief, the methods and related procedures pertaining to the embalming of human bodies have been surveyed from anatomies in the DACH region including Germany, Austria, and Switzerland. Participating institutions included the members of the 'Working Group on the Reduction of Formaldehyde Exposure' of the Anatomische Gesellschaft (German Anatomical Society). The survey comprised 537

items, related to the procedures prior to the arrival of the body in anatomy, facility infrastructure including air ventilation techniques and chemicals used for each of the steps. The survey questions can be found in Supplement Documents 1 and 2.

The survey's response rate was 58 % (29/50) institutions. Seven of the fifty anatomy departments had no active body donation system at the time of the survey, fourteen institutions declined participating or did not respond to at least two reminders inviting them to participate, as outlined elsewhere (Kerner et al., 2023). Data was only processed if the respective institutions had their own active body donation system and/or actively used human bodies for their anatomy training. Data from 28/50 institutions (56 %) were processed as part of this given study. More information on the nature of the datasets can be found in Kerner et al. (2023).

2.2. Data retrieval

Records and features retrieved from the database were defined primarily for all chemicals related to the embalming procedure. Each record presented a fixation/conservation protocol, identified by city name, and if more than one protocol was used in a city, it was considered as a separate record and indicated with a number as a suffix to make a difference among protocols of a city; each feature represents a different chemical, ventilation, and explosion-proof facility. Fixation was defined as the (initial) process of exposing the post-mortem tissues to chemicals in an effort to interrupt autolysis and microbial contamination resulting in the degradation of the tissues (Hammer et al., 2015, 2012). Primarily, fixation was conducted via injection and submersion of the bodies. Conservation was defined as the embalming following fixation in an effort to maintain the state of fixation. For 'fixation only', a total of 37 records or protocols with 37 features were used, and 37 records with 39 features were included for 'combined fixation and conservation'. For 'fixation, ventilation type, and explosion-proof facilities', 37 records with 42 features were included, as well as 37 records with 44 features for 'combined fixation and conservation, ventilation type, explosion-proof facilities'. Explosion-proof facilities have been defined as specialized infrastructures designed to safely handle, process, and store potentially explosive chemicals involved in tissue procedures,

incorporating resistant materials, ventilation, containment, and fire suppression systems. Such facilities comply with ATEX standards in the EU and Switzerland, alongside other local regulations.

2.3. Principal component analysis and data transformation

In the next step, all the data points were visualized using principal component analysis (PCA) before proceeding with the clustering algorithms. PCA is a powerful technique within data analysis and machine learning, employed to decrease the dimensionality of a dataset while preserving as much of the original variability as possible. It is utilized to extract a compact set of low-dimensional features from an extensive array of variables, a process known as dimensionality reduction, or to visualize data with higher dimensions (Jolliffe, 2002).

In the given case, PCA has been utilized as a preprocessing step for dimensionality reduction before applying clustering algorithms, i.e., to identify the principal components as linear combinations of the original features and to reduce noise. These components capture the directions in the data where the variability is maximal. The original data was then transformed into a new set of coordinates based on these components. Following this, the transformed data was used as an input for visualization of the dataset. Fig. 1 summarizes the findings obtained from PCA for combined fixation and conservation, as well as ventilation type and explosion-proof facilities, Supplementary Figure 1 for fixation embalming only.

2.4. Data clustering

For data clustering purposes, K-means, a hierarchical approach, or Density-Based Spatial Clustering of Applications with Noise (DBSCAN) can be applied to identify groups or patterns within the transformed data. Following the clustering, the results can be interpreted in the reduced-dimensional space. Visualization of the clusters may be more straightforward in the reduced space, especially if the original data had a high dimensionality.

The K-means algorithm is an unsupervised learning algorithm that partitions a dataset into K clusters (groups), based on similarity. The algorithm aims to minimize variance within each cluster, making the

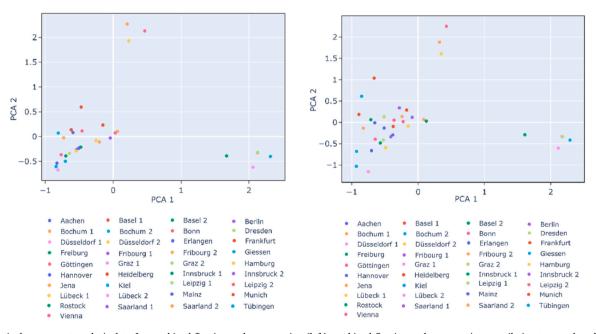


Fig. 1. Principal component analysis data for combined fixation and conservation (left) combined fixation and conservation, ventilation type and explosion-proof facilities (right). PCA 1 represents the linear combination of original variables with the maximum possible variance and PCA 2 captures the next highest variance in the data. If more than one protocol was used in an institution, this was considered a separate record and indicated with a number as a suffix to differentiate among protocols of this given institution. Due to the overlapping of some of the protocols, not all of them are visible in the figure.

data points within a cluster as similar as possible. The K-means algorithm is well suited to capturing the data structure especially if clusters have a spherical-like shape. K-means attempts to construct a spherical shape around a centroid. K-means comprises the following sub-steps: (1) initialize 'K' and centroid values, (2) assign data points to the closest clusters by calculating the Euclidean distance, (3) recompute the clusters' centroid values by calculating the average of data points, and (4) a repetition of sub-steps 2 and 3 until all the clusters are stable.

Euclidean distance
$$(d) = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

An optimal K-value is defined as having a minimum variance within each cluster; with data points having the most similarity within clusters and the most difference between clusters. Subsequently, the 'Elbow method' has been used to choose the optimal K-value. The Elbow method identifies the point at which the within-cluster sum of squares (WCSS) begins to decrease at a slower rate, thus creating an elbow shape in the plot. The point at which this slowdown occurs is considered a good estimate of the optimal number of clusters (Han, 2006).

3. Results

3.1. Clustering based on the Elbow method offers similar results for fixation only and for combined fixation and conservation

The Elbow method was used for each data set based on the previously mentioned features to define the optimal number of clusters. The Elbow method offers K=4 as optimal number of clusters for the dataset on fixation embalming only. More detailed information on the K=4 clusters for fixation-only is outlined in Supplementary Table 1 and Supplementary Figures 2, 3. With combined fixation and conservation embalming, the optimal K-value was reached with five clusters (K=5). A graphical summary representing these findings based on the Elbow approach is shown in Fig. 2.

3.2. Clustering of combined fixation and conservation and related chemicals

For the combined fixation and conservation embalming (K = 5),

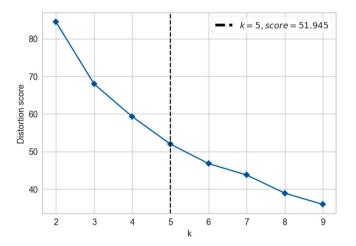


Fig. 2. Optimal K-values obtained from Elbow analyses for combined fixation and conservation embalming (K = 5). The X-axis (k) represents the number of clusters, and the Y-axis (distortion score) represents the within-cluster sum of squares (WCSS), indicating cluster compactness. The plot shows that the distortion score decreases as clusters increase. The "Elbow" point, marked by the dashed vertical line at K=5 with a score of 51.945, indicates that adding more clusters beyond this point does not significantly improve compactness. Thus, K=5 is the optimal number of clusters for this dataset according to the Elbow method.

Cluster 1 entailed six protocols from six institutions, Cluster 2 three protocols from three institutions, Cluster 3 four protocols from four institutions, Cluster 4 fourteen protocols from 14 institutions and Cluster 5 ten protocols from nine institutions (Table 1). Four institutions grouped in Cluster 4 reported not to have installed specific explosion-proof installations in their chemical storage or prosection area at the time of the survey. A detailed overview of the geographic distribution is outlined in Fig. 3. All five clusters included the use of formaldehyde.

3.3. Embalming protocol superclusters

Considering the number of different clusters based on fixation and conservation protocols, a series of anatomies used multiple protocols similar to other institutions. To this end, the secondary grouping of multiple clusters resulted in two so-called superclusters. A total of 5 institutions were found using two different combinations of protocols as outlined in Fig. 3.

3.4. Clustering of combined fixation and conservation, ventilation type and explosion-proof facilities

The Elbow method offers K = 6 as the optimal number of clusters for the protocols including combined fixation and conservation embalming, ventilation type and explosion-proof facilities (Fig. 4). With K = 6, the six clusters outlined below have been retrieved, all involving the use of formaldehyde. Cluster 1 was defined by the commonly used Thiel protocol and explosion-proof facilities. It included six protocols from six institutions (Table 2; Fig. 5). Cluster 2 entailed three protocols from three institutions and was defined by the chemical compositions outlined below and ventilation infrastructure (Table 2). Downdraft ventilation delivers fresh air from above the room occupants and embalmed tissues and draws it down past them to an extraction point, e.g., at the level of the tissues or slightly below near floor level. This ensures that the room occupants breathe fresh air and fumes from the embalming chemicals are carried away from the room occupants. Cluster 3 included four protocols from four institutions. Ten protocols from 10 institutions grouped to Cluster 4. The protocols in Cluster 4 were primarily defined by formaldehyde and ethanol. Eleven protocols from ten institutions grouped in Cluster 5 were defined by formaldehyde, thus forming the largest cluster. Cluster 6 included three protocols from three institutions, defined by the chemical compositions outlined in Table 2. The institutions grouped in this cluster reported not to have installed specific explosion-proof installations in their chemical storage or prosection area at the time of the survey. Of note, the clusters were further defined by sub-variables not given in the table with the primary chemicals. More detailed information on fixation embalming, ventilation type and explosion-proof installation clustering is outlined in Supplementary Figures 4, 5, and Supplementary Table 2.

3.5. Combined fixation and conservation, ventilation type and explosion-proof facilities superclusters

Based on the combined characteristics resulting in the clusters from combined fixation and conservation embalming, air ventilation type and explosion-proof facilities, it was found that a series of anatomies fell within the same combination as others. Two superclusters were identified. A total of 5 institutions grouped in two superclusters of 2 and 3 institutions outlined in Fig. 5.

Upon further evaluation, it was found that three of the five or six clusters remained highly stable regarding the chemicals involved (Clusters 1–3), whereas only the remaining Clusters 4, 5 and 6 changed regarding their chemical composition. This finding was irrespective of the K-value targeted, the isolated evaluation of 'fixation only' or 'combined fixation and conservation' embalming as well as the additional inclusion of parameters such as air ventilation technology and explosion-proof facilities.

Table 1 Fixation and conservation clusters (K = 5). The table shows common denominators of all protocols contained in the individual clusters.

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde
Boric acid	Potassium nitrate	Glycerin	Ethanol	
Chlorocresol	Potassium sulphate	Ethanol		
Potassium nitrate	Sodium bicarbonate	Glutaraldehyde		
Ammonium nitrate	Sodium sulphate	Sodium alkyl ether sulphate		
Sodium sulfide	Sodium chloride	-		

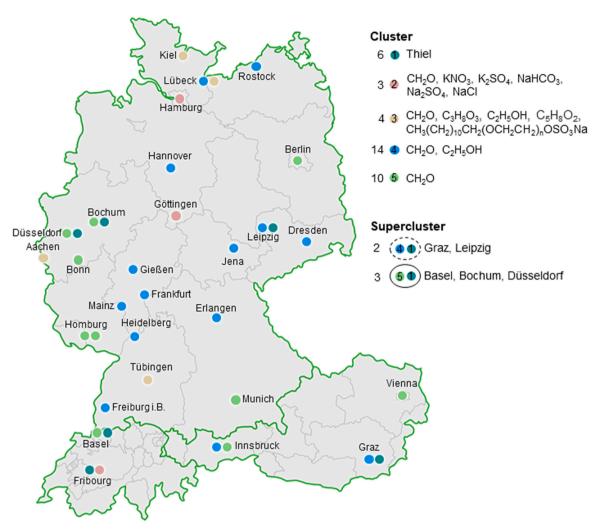


Fig. 3. Cluster analyses based on K = 5 for fixation and conservation embalming.

4. Discussion

4.1. Automated cluster analyses offer a reliable approach to group embalming technology based on similarity and difference; but yield limitations in usability

Clustering helps compare groups of data based on similarities and differences within datasets (Kafieh and Mehridehnavi, 2013; Rodriguez et al., 2019). In a previous manual clustering approach, data on embalming chemicals and related infrastructure from the Central European (DACH) anatomies have been analyzed (Kerner et al., 2023). This previous method operated under the assumption that formaldehyde, ethanol and 'other chemicals' constituted three primary embalming groups, with embalming features primarily depending on chemical and infrastructural characteristics deduced from the institutions' specific information. It was discovered that each institution

possessed a highly variable set of data entitled 'fingerprints'. However, this manual clustering approach faced several limitations, including complex interrelationships among predefined fixation, chemicals safety, ambient conditions, and air exchange, making it difficult to interpret the dependencies between the different features from an end-user perspective.

In contrast to the manual method utilized above, an automated approach using the K-means clustering with Elbow analyses offer well-established statistical tools to group a large series of data points based on their features (Ball and Hall, 1967; Zhou and Gao, 2014). K-means offers access to an unsupervised learning algorithm, mitigating to overcome the issue of user bias and the limitations of empirical knowledge (Liao et al., 2016; Qarmiche et al., 2023). This type of automated clustering has rendered helpful in identifying patterns which otherwise might have remained obscure to the blunt eye. Moreover, applying PCA before the clustering facilitated dealing with the

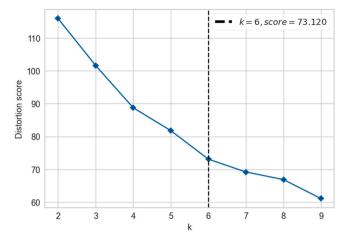


Fig. 4. Distortion scores for K=6 based on the Elbow method for fixation and conservation embalming, ventilation technology and explosion-proof facility.

high-dimensional data, as it helped overcome the challenges related to dimensionality, to reduce data noise and to focus on the most informative aspects of the data.

4.2. Clusters on chemicals remain consistent for a majority of compositions

For fixation embalming only, a different K-value optimum (K=4) was observed when compared to combined fixation and conservation embalming (K=5) using the Elbow method (Fig. 2; Supplementary Figure 2). In line with these findings, by including 'ventilation type' and 'explosion-proof infrastructure' as additional variables for 'fixation' embalming, the optimal K of Elbow method remained the same as K for 'fixation' embalming (K=4). The nature of Clusters 1, 2 and 3 remained consistent concerning the chemical composition. Only the remaining one (K=4) two (K=5) or three (K=6) clusters included as a common the following chemicals: formaldehyde only (K=4); formaldehyde with ethanol or formaldehyde only and formaldehyde with ethanol, phenoxyethanol, alkyl (K=4) dimethylbenzyl ammoniumchloride' (K=6); Table 2; Supplementary Table 2).

The findings on the consistency of chemical protocols appear remarkable, as they underpin the common understanding of the needs for optimal tissue properties and tissue durability, likely, to achieve certain teaching and learning objectives in undergraduate and professional education (Antipova et al., 2023; Balta et al., 2015; Crosado et al., 2020; Estai and Bunt, 2016; Kaliappan et al., 2023). Such consistency may indicate that the given embalming protocols have been trialed following their publication or adopted between institutions, likely as part of the staff mobility common in the DACH countries (Brenner, 2014; Kerner et al., 2023; Waschke et al., 2019). However, the extent of the publications is limited (Kerner et al., 2023; Waschke et al., 2019). In consequence, in spite of the broad diversity observed among all institutions surveyed, this outcome on a number of institutions falling within similar clusters offers hope for establishing joint embalming

protocols as part of future certification processes to address health and safety concerns for those exposed to the chemicals; and to meet contemporary and future regulations set by the European Commission (Bruinen de Bruin et al., 2022; Scheepers et al., 2018) and on a Federal State level (Pflaumbaum et al., 2019; Pipke and Wilmes, 2022).

To determine an optimal clustering approach as done in the given study, it is crucial to also examine closely the difference between the K = 4 versus K = 5 clusters, i.e., the difference between 'fixation only' embalming versus 'combined fixation and conservation' embalming protocols. The Elbow method reaches an optimal K for 'fixation only' and 'combined fixation and conservation' embalming at K=4 and K= 5, respectively (Fig. 2; Supplementary Figure 2). In line with these numerical findings, Clusters 4 and 5 offered more detailed information on the commonalities among the chemicals at K = 5. Moreover, the results achieved for 'fixation only' offered outcomes similar to 'combined fixation and conservation' embalming but included fewer features from institutions, and in consequence only a subselection but an encompassing picture. The same pattern can be observed when comparing K = 5 with K = 6 for 'combined fixation and conservation' versus 'combined fixation and conservation, ventilation type and explosion-proof facilities'.

To this end, the interpretation summarized below will primarily focus on K=6, i.e., 'combined fixation and conservation embalming, ventilation type and explosion-proof facilities'. The additional data from 'fixation only' and 'fixation, ventilation type and explosion-proof facilities' are additionally presented to offer the reader an unbiased view of the nature of the data. One common for all clusters included the use of formaldehyde. In consequence, our hypothesis that groups of similar protocols referred to as 'clusters' exist among the DACH anatomy institutions can therefore be approved.

4.3. Cluster 1: Thiel embalming and explosion-proof facilities

The first cluster consisted of chemicals commonly associated with Thiel embalming (Thiel, 1992, 2002). Interestingly, a secondary finding was that facilities using this protocol also shared the feature 'explosion-proof facilities'. This finding may in part be related to the properties of the chemicals involved, e.g., ammonium nitrate powder. However, Cluster 1 also coincides with Clusters 4 and 5 where ethanol and formaldehyde are used. As Thiel embalming is exclusively utilized for postgraduate purposes with the exception of its use in dental medical education in Graz, making its application limited (Antipova et al., 2023; Niedermair et al., 2023).

4.4. Cluster 2: potassium and sodium salts and downdraft ventilation and cluster 3: ethanol, glycerin, glutaraldehyde, sodium alkyl ether sulphate

The application of the protocols underlying Clusters 2 and 3 likewise appears to be limited. This may in part be due to the multitude of the embalming protocols with six and five chemical components, which may complicate the application of this type of embalming for broader use. Downdraft ventilation formed a second feature of Cluster 2. Cluster 2 has only been used in two institutions at the western part of Germany and in Fribourg (Switzerland), Cluster 3 in the northwestern part of Germany and in Tübingen. Its relation to the specifics of embalming

Table 2 Clusters on fixation and conservation, ventilation type and explosion-proof installation (K = 6).

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Explosion-proof	Downdraft ventilation	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde
Formaldehyde	Formaldehyde	Glycerin	Ethanol		Ethanol
Boric acid	Potassium nitrate	Ethanol			Phenoxyethanol
Chlorocresol	Potassium sulphate	Glutaraldehyde			Alkyl (C12-16) dimethylbenzyl ammoniumchloride
Potassium nitrate	Sodium bicarbonate	Sodium alkyl ether sulphate			
Ammonium nitrate	Sodium sulphate				
Sodium sulfide	Sodium chloride				

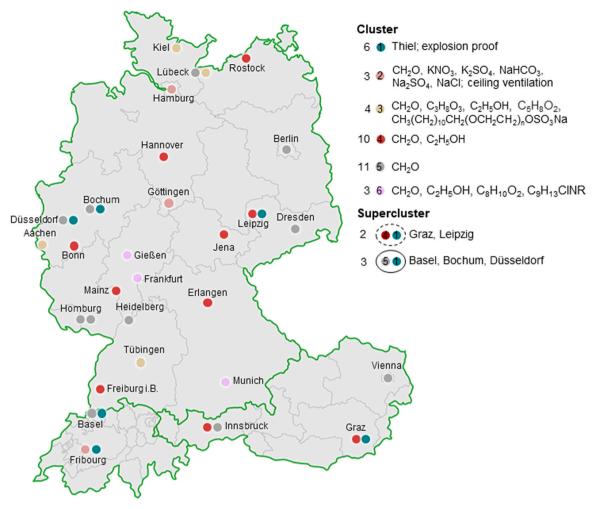


Fig. 5. Cluster analyses based on K = 6 for combined fixation and conservation embalming, ventilation type and explosion-proof facilities.

remains unelucidated at this point, but it may be hypothesized that this type of air ventilation had been considered state-of-the-art at the time of the installation. Future research should involve institutions beyond the DACH region to extend ideas and concepts for the reduction of formal-dehyde on a more international level.

4.5. Cluster 4: ethanol

This cluster is the second most frequently observed among the six clusters. Its use is spread homogeneously among institutions without any indication on regionality. The absence of explosion-proof installations in some institutions could be attributed to the differing quantities of the chemicals used, as well as potential variations in regulations between prefectures, countries, or EU regulations.

4.6. Cluster 5: formaldehyde

This cluster formed the largest one and it involved predominantly formaldehyde forming the principal component. Those institutions in Cluster 5 were also spread homogeneously amongst the DACH region.

4.7. Cluster 6: ethanol, phenoxyethanol, alkyl (C12-16) dimethylbenzyl ammoniumchloride

Three institutions were found to be allocated to Cluster 6 where 'ventilation type and explosion-proof facilities' were included to chemicals compositions as additional features on combined fixation and

conservation embalming.

4.8. Superclusters as a secondary grouping approach to share multiple clusters with related protocols

The concept of superclusters is based on the observation that a number of institutions jointly fell within more than one cluster, thereby enhancing the commonalities these institutions had regarding their infrastructure. Of the institutions falling within the superclusters, the combined Clusters 4 and 1 and the combined Clusters 5 and 1 were observed for a total of five institutions (Figs. 3, 5). For 'fixation only' and 'fixation, ventilation type and explosion-proof facilities', only one supercluster was observed, which combined Clusters 4 and 1 and included all institutions of the two previously mentioned superclusters (Supplementary Figures 3, 5), demonstrating the consistency of the findings even on a supercluster level.

5. Conclusions

This given work sets the basis for future joint efforts to optimize embalming protocols, and to identify approaches to minimize the use of formaldehyde, and to optimize measures to remove its exposure. Six clusters were identified based on chemicals, ventilation technology and explosion-proof facilities. All of the institutions involved in data retrieval were allocated to one or more of the clusters, and five institutions to superclusters comprising two clusters each. While the given cluster-based approach helped identify common groups based on

similarities between institutions and protocols, a next step could involve these institutions sharing in more detail knowledge, protocols, and potentially exchanging tissues for further evaluation. This collaborative effort could aim to optimize joint protocols while preserving the specific embalming techniques unique to each institution. Such an approach may be a necessary step toward certifying joint protocols in compliance with national and international standards, such as biocidal product regulations.

5.1. Limitations

Contrary to supervised learning, automated clustering analysis does not possess a solid evaluation metric that can be used to evaluate the outcomes of different clustering algorithms. Of further interest, the K-means requires k as input, and is not trained based on all data, i.e., there is no one correct or incorrect answer regarding the number of clusters to solve the research question. As done in this case, additional manual steps and evaluations may be needed to contextualize the data and fulfill the given objective. Furthermore, owing the number of survey respondents, this automated clustering approach is limited to those institutions providing their data. It would be desirable if future analyses would include a more encompassing and detailed dataset from an even larger cohort of anatomy institutions.

Ethical statement

This study does not involve human tissues; therefore, no ethical statement is required.

CRediT authorship contribution statement

Mohebimoushaei Sara: Writing - original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. Antipova Veronica: Writing - review & editing, Writing - original draft, Investigation, Data curation. Biedermann Uta: Writing - review & editing, Writing - original draft, Investigation, Data curation. Brand-Saberi Beate: Writing - review & editing, Writing - original draft, Investigation, Data curation. Bräuer Lars: Writing – review & editing, Writing – original draft, Investigation, Data curation. Caspers Svenja: Writing review & editing, Writing – original draft, Investigation, Data curation. Doll Sara: Writing - review & editing, Writing - original draft, Investigation, Data curation. Engelhardt Maren: Writing – review & editing, Writing - original draft, Investigation, Data curation. Filler Timm J.: Writing - review & editing, Writing - original draft, Investigation, Data curation. Gericke Martin: Writing - review & editing, Writing - original draft, Investigation, Data curation. Gümüsalan Yakup: Writing - review & editing, Writing - original draft, Investigation, Data curation. Gundlach Stefanie: Writing – review & editing, Writing – original draft, Funding acquisition, Data curation. Hayn-Leichsenring Gregor U.: Writing - review & editing, Writing - original draft, Investigation, Data curation. Heermann Stephan: Writing - review & editing, Writing original draft, Investigation, Data curation. Hiepe Laura: Writing - review & editing, Writing - original draft, Investigation, Data curation. Hirt Bernhard: Writing - review & editing, Writing - original draft, Investigation, Data curation. Hirtler Lena: Writing - review & editing, Writing - original draft, Funding acquisition, Data curation. Kulisch Christoph: Writing – review & editing, Writing – original draft, Funding acquisition, Data curation. Konschake Marko: Writing - review & editing, Writing - original draft, Funding acquisition, Data curation. Kürten Stefanie: Writing – review & editing, Writing – original draft, Investigation, Data curation. Lange Tobias: Writing – review & editing, Writing - original draft, Investigation, Data curation. Lorenz Mario: Writing – review & editing, Writing – original draft, Funding acquisition, Data curation. Nassenstein Christina: Writing - review & editing, Writing - original draft, Funding acquisition, Data curation. Neckel Peter H.: Writing - review & editing, Writing - original draft,

Investigation, Data curation. Nimtschke Ute: Writing - review & editing, Writing - original draft, Investigation, Data curation. Paulsen Friedrich: Writing - review & editing, Writing - original draft, Investigation, Data curation. Prescher Andreas: Writing - review & editing, Writing - original draft, Funding acquisition, Data curation. Pretterklieber Michael L.: Writing - review & editing, Writing - original draft, Investigation, Data curation. Schmiedl Andreas: Writing - review & editing, Writing – original draft, Investigation, Data curation. Schumann Sven: Writing - review & editing, Writing - original draft, Investigation, Data curation. Spindler Volker: Writing - review & editing, Writing - original draft, Investigation, Data curation. Schulze-Tanzil Gundula: Writing - review & editing, Writing - original draft, Investigation, Data curation. Tschernig Thomas: Writing – review & editing, Writing - original draft, Investigation, Data curation. Unverzagt Axel: Writing - review & editing, Writing - original draft, Investigation, Data curation. Valentiner Ursula: Writing – review & editing, Writing - original draft, Investigation, Data curation. Varga Ivan: Writing - review & editing, Writing - original draft, Investigation, Data curation. Wedel Thilo: Writing - review & editing, Writing - original draft, Investigation, Data curation. Weigner Janet: Writing – review & editing, Writing – original draft, Investigation, Data curation. Weninger Wolfgang J.: Writing - review & editing, Writing - original draft, Investigation, Data curation. Westermann Jürgen: Writing – review & editing, Writing - original draft, Investigation, Data curation. Wimmer-Roell Monika: Writing - review & editing, Writing - original draft, Investigation, Data curation. Waschke Jens: Writing – review & editing, Writing - original draft, Validation, Investigation, Data curation. Hammer Niels: Writing - review & editing, Writing - original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors have no conflict of interest related to the given study.

Acknowledgements

The authors would like to thank Ingo Bechmann (Leipzig, Germany), Luis Filgueira (Fribourg, Switzerland), Helga Fritsch (Innsbruck, Austria), Estifanos Ghebremedhin (Frankfurt a. Main, Germany), Ingrid Hettwer-Steeger (Göttingen, Germany), Markus Kipp (Rostock, Germany), Rudolf Leube (Aachen, Germany), Christian Mühlfeld (Hannover, Germany), Magdalena Müller-Gerbl (Basel, Switzerland), Mirko Schmitt (Dresden, Germany), Katja Schmidt (Leipzig, Germany), Stefanie Schliwa (Bonn, Germany), Claudia Schneider (Bochum, Germany), Christof Schomerus (Frankfurt a. Main, Germany), Johannes Streicher (Krems an der Donau, Austria), Imke Weyers (Lübeck, Germany), Sabine Wittschonnke (Düsseldorf, Germany) for their valuable input and contribution on their anatomy departments. Alexander Kerner and Vivian Leliebre Lara helped in obtaining the data, Sandra Maria Pietras and Andreas Bauer provided their support in creating the underlying illustrations (all Graz, Austria). Proofreading the paper has been conducted using https://www.perplexity.ai.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aanat.2025.152403.

References

Albert, R.E., Sellakumar, A.R., Laskin, S., Kuschner, M., Nelson, N., Snyder, C.A., 1982. Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in the rat. J. Natl. Cancer Inst. 68, 597–603.

Antipova, V., Niedermair, J.F., Siwetz, M., Fellner, F.A., Löffler, S., Manhal, S., Ondruschka, B., Pietras, S.M., Poilliot, A.J., Pretterklieber, M.L., Wree, A., Hammer, N., 2023. Undergraduate medical student perceptions and learning

- outcomes related to anatomy training using Thiel- and ethanol-glycerin-embalmed tissues. Anat. Sci. Educ. 16, 1144–1157.
- Ball, G.H., Hall, D.J., 1967. A clustering technique for summarizing multivariate data. Behav. Sci. 12, 153–155.
- Balta, J.Y., Cryan, J.F., O'Mahony, S.M., 2019. The antimicrobial capacity of embalming solutions: a comparative study. J. Appl. Microbiol 126, 764–770.
- Balta, J.Y., Lamb, C., Soames, R.W., 2015. A pilot study comparing the use of Thiel- and formalin-embalmed cadavers in the teaching of human anatomy. Anat. Sci. Educ. 8, 86–91
- Benerini Gatta, L., Cadei, M., Balzarini, P., Castriciano, S., Paroni, R., Verzeletti, A., Cortellini, V., De Ferrari, F., Grigolato, P., 2012. Application of alternative fixatives to formalin in diagnostic pathology. Eur. J. Histochem 56, e12.
- Blum, F., 1893. Der Formaldehyd als Hartungsmittel. Z. Wiss. Mikros 314-315.
- Blum, F., 1894. Notiz über die Anwendung des Formaldehyds (Formol) als Härtungs-und Konservierungsmittel. Anat. Anz. 9, 229–231.
- Brenner, E., 2014. Human body preservation old and new techniques. J. Anat. 224, 316–344.
- Bruinen de Bruin, Y., Franco, A., Ahrens, A., Morris, A., Verhagen, H., Kephalopoulos, S., Dulio, V., Slobodnik, J., Sijm, D.T., Vermeire, T., 2022. Enhancing the use of exposure science across EU chemical policies as part of the European Exposure Science Strategy 2020–2030. J. Expo. Sci. Environ. Epidemiol. 32, 513–525.
- Coleman, R., Kogan, I., 1998. An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching. J. Anat. 192 (Pt 3), 443–446.
- Crosado, B., Löffler, S., Ondruschka, B., Zhang, M., Zwirner, J., Hammer, N., 2020. Phenoxyethanol-based embalming for anatomy teaching: an 18 years' experience with crosado embalming at the university of Otago in New Zealand. Anat. Sci. Educ. 13, 778–793.
- Durongphan, A., Rungruang, J., Nitimanee, E., Panichareon, B., 2023. The effects of enhanced formaldehyde clearance in a gross anatomy laboratory by floor plan redesign and dissection table adjustment. Environ. Sci. Pollut. Res. 30, 1–8.
- Eickmann, U., Thullner, I., 2017. Tätigkeiten mit Formaldehyd im Gesundheitsdienst: was sind angemessene Schutzmaßnahmen? Zent. F. üR. Arb., Arb. und Ergon. 67.
- Eisma, R., Lamb, C., Soames, R.W., 2013. From formalin to Thiel embalming: what changes? One anatomy department's experiences. Clin. Anat. 26, 564–571.
- Eisma, R., Wilkinson, T., 2014. From "silent teachers" to models. PLoS Biol. 12, e1001971.
- Estai, M., Bunt, S., 2016. Best teaching practices in anatomy education: a critical review.

 Ann. Anat. 208. 151–157.
- Fox, C.H., Johnson, F.B., Whiting, J., Roller, P.P., 1985. Formaldehyde fixation.
 J. Histochem. Cytochem 33, 845–853.
- Hammer, N., 2022. Thirty years of Thiel embalming—a systematic review on its utility in medical research. Clin. Anat. 35, 987–997.
- Hammer, N., Löffler, S., Bechmann, I., Steinke, H., Hädrich, C., Feja, C., 2015. Comparison of modified Thiel embalming and ethanol-glycerin fixation in an anatomy environment: potentials and limitations of two complementary techniques. Anat. Sci. Educ. 8, 74–85.
- Hammer, N., Löffler, S., Feja, C., Sandrock, M., Schmidt, W., Bechmann, I., Steinke, H., 2012. Ethanol-glycerin fixation with thymol conservation: a potential alternative to formaldehyde and phenol embalming. Anat. Sci. Educ. 5, 225–233.
- Han, Ja.M.K., 2006. Data mining: concepts and techniques. Morgan Kaufmann series in data management systems.
- Hauptmann, M., Lubin, J.H., Stewart, P.A., Hayes, R.B., Blair, A., 2003. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J. Natl. Cancer Inst. 95, 1615–1623.
- Hauptmann, M., Lubin, J.H., Stewart, P.A., Hayes, R.B., Blair, A., 2004. Mortality from solid cancers among workers in formaldehyde industries. Am. J. Epidemiol. 159, 1117–1130.
- Hauptmann, M., Stewart, P.A., Lubin, J.H., Beane Freeman, L.E., Hornung, R.W., Herrick, R.F., Hoover, R.N., Fraumeni Jr., J.F., Blair, A., Hayes, R.B., 2009. Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. J. Natl. Cancer Inst. 101, 1696–1708.
- Hayashi, S., Homma, H., Naito, M., Oda, J., Nishiyama, T., Kawamoto, A., Kawata, S., Sato, N., Fukuhara, T., Taguchi, H., Mashiko, K., Azuhata, T., Ito, M., Kawai, K., Suzuki, T., Nishizawa, Y., Araki, J., Matsuno, N., Shirai, T., Qu, N., Hatayama, N., Hirai, S., Fukui, H., Ohseto, K., Yukioka, T., Itoh, M., 2014. Saturated salt solution method: a useful cadaver embalming for surgical skills training. Medicine 93, e196.
- Jolliffe, I.T., 2002. Principal Component Analysis.
- Kafieh, R., Mehridehnavi, A., 2013. A comprehensive comparison of different clustering methods for reliability analysis of microarray data. J. Med Signals Sens 3, 22–30.
- Kaliappan, A., Motwani, R., Gupta, T., Chandrupatla, M., 2023. Innovative cadaver preservation techniques: a systematic review. Maedica 18, 127–135.
- Kang, D.S., Kim, H.S., Jung, J.-H., Lee, C.M., Ahn, Y.-S., Seo, Y.R., 2021. Formaldehyde exposure and leukemia risk: a comprehensive review and network-based toxicogenomic approach. Genes Environ. 43.
- Kerner, A.M., Biedermann, U., Bräuer, L., Caspers, S., Doll, S., Engelhardt, M., Filler, T.J., Ghebremedhin, E., Gundlach, S., Hayn-Leichsenring, G.U., Heermann, S., Hettwer-Steeger, I., Hiepe, L., Hirt, B., Hirtler, L., Hörmann, R., Kulisch, C., Lange, T., Leube, R., Meuser, A.H., Müller-Gerbl, M., Nassenstein, C., Neckel, P.H., Nimtschke, U., Paulsen, F., Prescher, A., Pretterklieber, M., Schliwa, S., Schmidt, K., Schmiedl, A., Schomerus, C., Schulze-Tanzil, G., Schumacher, U., Schumann, S.,

- Spindler, V., Streicher, J., Tschernig, T., Unverzagt, A., Valentiner, U., Viebahn, C., Wedel, T., Weigner, J., Weninger, W.J., Westermann, J., Weyers, I., Waschke, J., Hammer, N., 2023. The chemicals between us-First results of the cluster analyses on anatomy embalming procedures in the German-speaking countries. Anat. Sci. Educ. 16, 814–829.
- Lam, J., Koustas, E., Sutton, P., Padula, A.M., Cabana, M.D., Vesterinen, H., Griffiths, C., Dickie, M., Daniels, N., Whitaker, E., Woodruff, T.J., 2021. Exposure to formaldehyde and asthma outcomes: a systematic review, meta-analysis, and economic assessment. PLoS One 16, e0248258.
- Liao, M., Li, Y., Kianifard, F., Obi, E., Arcona, S., 2016. Cluster analysis and its application to healthcare claims data: a study of end-stage renal disease patients who initiated hemodialysis. BMC Nephrol. 17, 25.
- Musiał, A., Gryglewski, R.W., Kielczewski, S., Loukas, M., Wajda, J., 2016. Formalin use in anatomical and histological science in the 19th and 20th centuries. Folia Med. Cracov 56, 31–40.
- Niedermair, J.F., Antipova, V., Manhal, S., Siwetz, M., Wimmer-Röll, M., Hammer, N., Fellner, F.A., 2023. On the added benefit of virtual anatomy for dissection-based skills. Anat. Sci. Educ. 16, 439–451.
- Pfeil, S., Hieke, H., Brohmann, P., Wimmer, M., 2020. Low cost and effective reduction of formaldehyde in gross anatomy: long throw nozzles and formaldehyde destruction using InfuTrace™. Environ. Sci. Pollut. Res. 27, 45189–45208.
- Pflaumbaum, W., von Hahn, N., Kolk, A., Liedtke, M., Schelle, F., Kaulbars, U., von der Heyden, T., Peters, S., Pelzer, J., Schmidt, V., 2019. Grenzwerteliste 2019: Sicherheit und Gesundheitsschutz am Arbeitsplatz. DGUV/IFA.
- Pipke, R., Wilmes, A., 2022. Risikokommunikation zum Schutz vor Gefahrstoffen am Arbeitsplatz. Bundesgesundheitsblatt-Gesundh. -Gesundh. 65, 589–598.
- Puchtler, H., Meloan, S.N., 1985. On the chemistry of formaldehyde fixation and its effects on immunohistochemical reactions. Histochemistry 82, 201–204.
- Qarmiche, N., El Kinany, K., Otmani, N., El Rhazi, K., Chaoui, N.E.H., 2023. Cluster analysis of dietary patterns associated with colorectal cancer derived from a Moroccan case-control study. BMJ Health Care Inf. 30.
- Rakuša, M., Kocbek Šaherl, L., 2022. Thiel embalming method used for anatomy dissection as an educational tool in teaching human anatomy, in research, and in training in comparison of different methods for long term preservation. Folia Morphol. (Praha).
- Ramesh, G., Katiyar, A., Sujatha, R., Raj, A., Gupta, B., Kumar, A., 2017. Detection of microorganisms on formalin-fixed and stored pathology tissues: a microbiological study. J. Oral. Maxillofac. Pathol. 21, 64–69.
- Rodriguez, M.Z., Comin, C.H., Casanova, D., Bruno, O.M., Amancio, D.R., Costa, L.D.F., Rodrigues, F.A., 2019. Clustering algorithms: a comparative approach. PLoS One 14, e0210236
- Russ, J., Hammer, N., 2025. Departing from standard practices: Strategic application of value engineering in the anatomy laboratory to enhance formaldehyde extraction using high-impact, low-cost, and low-maintenance solutions. Anat Sci Educ. 18 (3), 241–253. https://doi.org/10.1002/ase.2553. Epub 2025 Jan 20. PMID: 39831516.
- Scheepers, P.T.J., Graumans, M.H.F., Beckmann, G., van Dael, M., Anzion, R.B.M., Melissen, M., Pinckaers, N., van Wel, L., de Werdt, L.M.A., Gelsing, V., van Linge, A., 2018. Changes in work practices for safe use of formaldehyde in a university-based anatomy teaching and research facility. Int. J. Environ. Res. Public Health 15.
- Swenberg, J.A., Kerns, W.D., Mitchell, R.I., Gralla, E.J., Pavkov, K.L., 1980. Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. Cancer Res 40, 3398–3402.
- Swenberg, J.A., Moeller, B.C., Lu, K., Rager, J.E., Fry, R.C., Starr, T.B., 2013.
 Formaldehyde carcinogenicity research: 30 years and counting for mode of action, epidemiology, and cancer risk assessment. Toxicol. Pathol. 41, 181–189.
- Thavarajah, R., Mudimbaimannar, V.K., Elizabeth, J., Rao, U.K., Ranganathan, K., 2012. Chemical and physical basics of routine formaldehyde fixation. J. Oral. Maxillofac. Pathol. 16, 400–405.
- Thiel, W., 1992. The preservation of the whole corpse with natural color. Ann. Anat. 174, 185-195.
- Thiel, W., 2002. Supplement to the conservation of an entire cadaver according to W. Thiel. Ann. Anat. 184, 267–269.
- Tupper, C., Garg, R., 2024. OSHA Formaldehyde Safety, StatPearls. StatPearls Publishing Copyright © 2024. StatPearls Publishing LLC, Treasure Island (FL).
- Tutsch, H., 1975. Eine geruchsfreie, gut konservierende Injektionslösung für Kursleichen. Anat. Anz. 138, 126–128.
- Waschke, J., Bergmann, M., Bräuer, L., Brenner, E., Buchhorn, A., Deutsch, A., Dokter, M., Egu, D.T., Ergün, S., Fassnacht, U., Fietz, D., Gundlach, S., Heermann, S., Hirt, B., Kugelmann, D., Müller-Gerbl, M., Neiss, W., Nimtschke, U., Plendl, J., Pretterklieber, M., Redies, C., Scaal, M., Schmidt, M.H.H., Schmiedl, A., Schnittler, H.J., Schomerus, C., Sebestény, T., Spittau, B., Steiniger, B., Tschernig, T., Unverzagt, A., Viebahn, C., Voigt, E., Weigner, J., Weyers, I., Winkelmann, A., Winkler, M., Paulsen, F., 2019. Recommendations of the working group of the anatomische gesellschaft on reduction of formaldehyde exposure in anatomical curricula and institutes. Ann. Anat. 221, 179–185.
- Zanini, C., Gerbaudo, E., Ercole, E., Vendramin, A., Forni, M., 2012. Evaluation of two commercial and three home-made fixatives for the substitution of formalin: a formaldehyde-free laboratory is possible. Environ. Health 11, 59.
- Zhou, H., Gao, J., 2014. Automatic optimization algorithm of clusters number based on maximum distance. Adv. Mater. Res. 951, 231–234.