

Qualitative Evaluation of Modified Polyvinyl Alcohol Gel Used as A Preservative Medium Compared to Traditional Modified Kaiserling III Solution in Pathology Museum

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 05 Dec 2023	Pathology and anatomy museums preserve body tissues and organs traditionally in modified Kaiserling III solution and display in transparent glass jars, which serve as valuable teaching materials for students. Other techniques tried in preserving specimens are embedding in molten wax, embedding in epoxy resin, and plastination techniques. Modified Carbopol-940 gel has been tried recently as a preservative mounting medium in pathology museum. However, the use of modified Polyvinyl alcohol (PVA) gel as a preservative medium is not mentioned in the literature. Gel preservative medium can prevent leakage, toxic vapors, and irritation of eyes and skin. Aims: Qualitative evaluation of modified Polyvinyl alcohol gel as a preservative medium for specimens in pathology museums compared to modified Kaiserling III solution over one year. Objectives: 1) To qualitatively evaluate physical integrity of mounted tissue specimens stored in modified Kaiserling III solution and modified Polyvinyl alcohol gel for one year. 2) To qualitatively evaluate physical, chemical and microbiological parameters of modified Kaiserling III solution and modified Polyvinyl alcohol gel for one year. Materials and methods: In this qualitative cross-sectional study, six different organs, two sets each, were preserved in jars containing modified Kaiserling III solution and modified Polyvinyl alcohol gel. The media were evaluated for stability by physical tests, chemical test, and microbiological test, before and after the study. The specimens were assessed for physical disintegration by observing for tissue shredding, and evaluated for histopathological appearances, before and after the study. Results showed mild variability in stability of the preservative media but good tissue integrity of the specimens over a period of one year.
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1. Introduction

Normally, body tissue specimens are preserved in museums, immersed in solution containing formalin reagent. This prevents tissue disintegration, increases tissue hardening and prevents contamination by micro-organisms. In 1897⁽¹⁾, Kaiserling developed a buffered solution which contained 400 ml of 40 % formalin, 30 g potassium nitrate, 60 g potassium acetate and 2000 ml tap water. This Kaiserling I solution, was used to fix the tissue for a period of up to two weeks. However, there was a loss of color contrast. He discovered that immersion in 80 % ethyl alcohol, Kaiserling II solution, restored the original color of the tissue. The specimen was then mounted in glass jars, containing a solution of 500 ml. glycerin, 200 ml of 1 % arsenious acid, potassium acetate 250 g and Thymol 2.5 g. The specimen mounted in this solution and sealed in glass jars could be stored for many years. This solution was named Kaiserling III solution. This solution was further modified and developed at Westminster Medical School ⁽¹⁾, and contained glycerin 30 %, Sodium acetate 10 %, and formalin 0.5 %, with pH adjusted to 8.0.

Usage of gel medium has a few advantages ⁽²⁾. The gel en-sheathes the specimen, preventing leakage of contents on tilting the jar. The specimen will not get dislodged, and sloshing of the liquid medium will be prevented during rough handling and transport. Being jelly like, it will not leak through fine cracks in the glass jar; consequently, the examiner's hand, body or clothes will not be soiled. The gel itself is odorless, nontoxic, noninflammable and nonreactive to the skin, nose or eyes. Leaching of

pigments from the tissue like bilirubin or blood will be localized due to the absence of conduction or convection currents of the fluid particles.

Gelatin embedding ⁽¹⁾ with arsenious acid-gelatin was carried out, however gelatin tends to yellow with age and undergoes liquefaction. Embedding in solid plastic blocks is possible for hard tissues, certain insects and plants but difficult for normal pathological specimens. Cadaveric organs can be kept as dry specimens, for teaching and mounting in museum, by a new wax embedding technique ^(3,4). Plastination was invented by Gunther Von Hagens ^(5,6) in 1977, wherein tissue was impregnated with synthetic resin. Insects have been embedded in resins ⁽⁷⁾. Carbopol-940 gel has been modified and used as preservative medium for storing specimens in pathology museum ⁽²⁾. Literature on use of Polyvinyl alcohol (PVA) gel for preservation of tissue specimen is not available.

2. Materials And Methods

The specimens for mounting were selected purposively to include common identifiable lesions in different organs viz. spleen infarct, lipoma, gallbladder with chronic inflammation, kidney with chronic pyelonephritis, ovary with serous cyst and uterus with fibroid ⁽²⁾. The organs were well fixed in 10% formalin. Unfixed and specimens undergoing autolysis were not included as also unidentifiable lesions.

The specimens were tied by string using needle and fixed on glass plates. Acrylic plates are avoided as they are light and would float up in the polyvinyl alcohol medium, so mounting would be difficult. Appropriate two sets of similar organs were mounted in six jars, each containing modified Kaiserling III solution in one set and modified Polyvinyl alcohol (PVA) gel in another set. Before sealing the mounted jars, the media and tissue specimens were subjected to various physical, chemical and histopathological tests and the results were recorded as pre study data. The mounted specimens were sealed by glass lids on the glass jars using a sealant (M-seal, Pidilite Industries, India) and stored for one year in room temperature. After a period of one year, the lids were reopened and the media and the specimens were again subjected to retesting, as done previously. The results were recorded as post study data.

The modified Kaiserling III solution was prepared as follows ⁽¹⁾:

100 g sodium acetate was dissolved in 500 ml of distilled water. To this was added 5.0 ml of 40% formalin solution. Next 300 ml of glycerin was added to the mixture and the solution was made up to 1000 ml by distilled water. This was filtered and used as mounting medium.

The Polyvinyl alcohol gel was prepared as follows:

In a microwave proof bowl, 400 ml distilled water was taken, to which was added 42 g Polyvinyl alcohol powder (Manufacturer: Loba Chemie Pvt. Ltd., India). The powder was allowed to soak in the water overnight. Next day, the mixture was heated in microwave for 30 seconds two times and cooled. The bowl was taken out and solution stirred to dissolve the powder. This was repeated two times again for 30 seconds each, and stirred to dissolve the powder. The mixture was again heated twice, 30 seconds each and stirred and allowed to cool for some minutes. After total microwaving time of 3 minutes, the powder finally dissolved in water. Boiling of the solution was avoided. Two ml of 40% formalin was added to the cooled solution.

Next, a 4% borate solution was prepared by dissolving 20 g di-Sodium tetra borate decahydrate (Emplura®, Merck, India), in 500 ml of distilled water. The solution was stored in 200 ml plastic dropper bottles.

The borate solution was gradually added drop wise to the hot PVA mixture while constantly stirring the mixture. The mixture turned into a viscous, transparent gel on addition of borate solution. The bubbles formed in the medium cleared on standing after a few hours.

The Kaiserling III solution, Polyvinyl alcohol gel and the tissue specimens were subjected to various physical tests, chemical test, microbiological test and histopathological tests as follows:

Color ⁽²⁾: The media were checked for changes in color visually. Sometimes, due to ageing, the media may become pale yellowish. The tissue specimen may color the medium due to hemorrhage, bile or blood pigment in to light red, brown, or brownish black color.

Turbidity ⁽²⁾: The media may develop turbidity. The turbidity can be observed semi quantitatively by placing a sheet of paper with letters typed on it, behind the transparent jar. If the typed letters are read properly, with slight smokiness of medium it is called mild turbidity (+), If there is cloudiness of the medium with haziness of the typed letters it is called moderate turbidity (++). If there is marked

turbidity and the typed letters are not visible, with opaque medium, then it is called severe turbidity (+++).

The pH⁽²⁾: This was measured using paper pH strips which showed a variation in colors from pink (acidic pH) to light greenish (neutral pH) to dark blue (alkaline pH).

Specific gravity⁽²⁾: This test was recorded using Hand held ERMA Labart Professional Salinity Refractometer with dual scale salinity tester 0-100PPT and 1.000-1.070 specific gravity (Amazon.in, India). The instrument is calibrated with distilled water. A drop of the water was placed on the clean surface of the objective and covered by the attached lid. By viewing through the eyepiece directed towards a light source, the reading of specific gravity was confirmed as 1.000. Subsequently, drops of Kaiserling III solution and Polyvinyl alcohol gel were put on the viewing surface and their specific gravity estimated.

Growth of organisms⁽²⁾: The media were tested for organisms by gram staining. A loop full of the medium was taken and smeared on the clean slide. It was heat fixed on a flame. The smear was laid on a horizontal slide rack and covered with gram stain for one minute. Gram's iodine was poured over the smear to act as a mordant for a minute. The smear was washed and few drops of acetone alcohol were poured over it for de-colorization. The smear was counterstained with safranin stain for 30 seconds. The smear was washed, air dried and observed under oil immersion objective lens for presence of gram positive blue stained bacteria or gram-negative orange-stained bacteria. Fungal organisms can also be confirmed by the stain.

Specimen color⁽²⁾: The tissue may become purple to brown to black in color due to putrefaction. The tissue may show color changes due to presence of blood in congestion and hemorrhage. Also presence of bilirubin and breakdown of blood may impart a golden brown to greenish pigmentation.

Specimen histopathology⁽²⁾: Tissue slices, measuring 3-5 mms thick, are taken from the specimens and subjected to tissue processing. It is passed through beakers of ascending grades of alcohol 70 %, 80%, 90%, 100%, 100% for an hour each to dehydrate the tissue. Then it passes through two cycles of xylene reagent of an hour each, followed by two cycles of one hour each in molten paraffin wax and finally is embedded in paraffin block. The tissue paraffin block is sectioned by microtome (Leica Microsystems, India) and 3-4-micron thick sections are taken on a slide, fixed and stained by Haematoxylin and eosin stain. The prepared slide is observed under 100x magnification and cellular characteristics photographically recorded at 400x magnification. Disintegrated tissue shows loss of architecture, absence of nuclear staining, and fading of the stains with absence of blue and pink color differentiation. Well preserved tissue will show sharply stained blue nucleus, and pink cytoplasm and matrix, with regular tissue architecture.

3. Results and Discussion

The mounted spleen, lipoma, gallbladder, kidney, ovarian cyst and uterus fibroid specimens in the two different media, both pre study and post study, showed mild variability in the stability of the media but good integrity in the morphology and histology of the tissue.

The specimen jars of gallbladder (Table 3.0, Figure 3.1, 3.2, 3.3, & 3.4), Kidney (Table 4.0, Figure 4.1, 4.2, 4.3, & 4.4) and uterus fibroid (Table 6.0, Figures 6.1, 6.2, 6.3, & 6.4) displayed excellent features of gross preservation of tissue and stability of the mounting media, Kaiserling III solution and Polyvinyl alcohol gel. The specimen of spleen (Table 1, Figure 1.4) in polyvinyl alcohol, post study, showed dark discoloration due to blood leaching into the medium with haziness of the medium, and moderate turbidity (++). The specimen of Lipoma (Table 2.0, Figure 2.4) in polyvinyl alcohol gel, post study, showed haziness on the surface mainly and diffuse smokiness with blurring of the typed writing seen through the medium i.e. moderate to severe turbidity (++/+++). In all the above cases, no organism was detected by gram staining of the media samples. The specimen of ovarian cyst showed mild haziness with mild turbidity (+) of the polyvinyl alcohol medium (Table 5.0, Figure 5.3) pre study, and severe haziness with turbidity (+++) of the post study polyvinyl alcohol medium (Table 5.0, Figure 5.4). The post study sample of polyvinyl alcohol medium of ovarian cyst (Table 5.0, Figure 5.4) showed growth of gram positive bacilli (Figure 7.1 and 7.2)

Physical disintegration of the tissues was absent. The histopathological appearance showed well stained blue nuclei of the cells of various tissues in a background of pink eosinophilic matrix, which was indicative of good preservation of all the tissues viz. spleen (Table 1.0, Figures 1.5 to 1.8), lipoma (Table 2.0, Figures 2.5 to 2.8), gallbladder (Table 3.0, Figures 3.5 to 3.8), kidney (Table 4.0, Figures 4.5 to 4.8), ovarian cyst (Table 5.0, Figures 5.5 to 5.8) and fibroid uterus (Table 6.0, Figures

6.5 to 6.8) pre and post study of a period of one year, in both the media, modified Kaiserling III solution and modified Polyvinyl alcohol gel.

Discussion:

The various parameters assessed for the media are color, turbidity, specific gravity, pH, gram staining of bacterial and fungal organisms, and for the tissue organs were color, gross disintegration and histological changes seen in tissue disintegration.

Color of medium: The color of the Kaiserling III solution and Polyvinyl alcohol gel was colorless. If the tissue specimens were hemorrhagic or bilious the media would be colored blackish, brownish, yellowish or greenish because of leaching of the pigment into the media. Similarly contamination with pigment producing bacteria or fungi would turn the media blackish, brownish or greenish. When the pigment leaching occurs in liquid media, it would diffusely spread throughout the media, whereas in gel media it would remain localized due to absence of conduction or convection of the fluid particles. In our case, the slice of the spleen organ (Table 1.0, Figure 1.4) showed large dilated vascular spaces on the cut surface engorged with blood. During extrication of the specimen to take tissue bits for histopathology examination, the rubbery gel was peeled off the specimen. Squeezing and compression of the specimen, during extrication, released blood and pigment into the medium, resulting in the dark color and turbidity. The medium didn't show growth of any organism, and tested negative for gram stain of organisms.

Turbidity of medium: The media may show turbidity due to impurities in the medium and faulty polymerization during preparation, presence of contaminating micro-organisms like fungi and bacteria or disintegration of the tissue following autolysis. Separation of the shredded tissue particulate matter within the media would cause turbidity and opacity. Fat particulate matter would float on the surface of the liquid and gel media. The specimen of Lipoma (Table 2.0, Figure 2.4) in Polyvinyl alcohol gel, post study, shows haziness of medium more on the surface suggestive of fat globules released by the sliced lipoma tissue, during manipulation of the specimen. Since the covering fascia of the lipoma was stripped off and sliced, the loose fat globules were dislodged, rising to the surface. The specimen of spleen shows dark discoloration with leaching out of hemoglobin pigment, as well as shedding of blood clots and formalin fixed red blood cells which have resulted in mild to moderate turbidity. In all the above cases, no organism was detected by gram staining of the media samples. The ovarian cyst specimen medium shows opacity due to growth of micro-organisms causing severe turbidity and inability to read the typewritten letters in the background. This specimen showed growth of gram-positive bacilli resulting in turbidity (Table 5.0, Figure 5.4). The mild turbidity observed in the post study specimen of kidney in polyvinyl alcohol media (Table 4.0, Figure 4.4) may be caused by dehydration and loss of water content of the medium, resulting in mild smokiness. The dehydration results in loss of transparency. In dehydration, the gel becomes more viscous, rubbery, gradually hardens, and loses its elasticity. In severe dehydration, the gel becomes plasticized, rigid, distorted and may compress and deform the preserved tissue.

Table-1 (Spleen Infarct)										
Sr no	Study type	Medium	Color	Turbid	pH	Specific gravity	Organism growth	Specimen color	Disintegration	Histopathology
1	Pre study	Kaiser-Ling III	Color less	nil	8.0	1.060 1:3Diln.	Nil	Dark Brown	Nil	Well stained
2	Post study	Kaiser-Ling III	Color less	nil	7.5	1.055 1:3 Diln	Nil	Dark Brown	Nil	Well stained
3	Pre study	Polyvinyl alcohol gel	Color less	nil	8.5	1.055	Nil	Dark Brown	Nil	Well stained
4	Post study	Polyvinyl alcohol gel	Focal dark brown	Mild turbid	8.5	1.056	Nil	Dark Brown	Nil	Well stained

Figure- 1.0 (Appearance of spleen specimen and mounting media before study and after study)



Figure 1.1 Gross appearance of spleen infarct 7.5x8.0x1.5 cms. pre study in Kaiserling -III. The organ is dark brown. Medium is colorless. Paper print easily read in the background⁽²⁾



Figure 1.2 Gross appearance of spleen infarct 7.5x8.0x1.5 cms. post study in Kaiserling - III. The organ is dark brown. Medium is colorless. Paper print easily read in the background⁽²⁾



Figure 1.3 Gross appearance of spleen infarct 7.5x8.0x1.5 cms. pre study in PVA gel. The organ is dark brown. Medium is colorless. Paper print easily read in the background



Figure 1.4 Gross appearance of spleen infarct 7.5x8.0x1.5 cms. post study in PVA gel. The organ is dark brown. Medium is dark brown. Paper print visible.

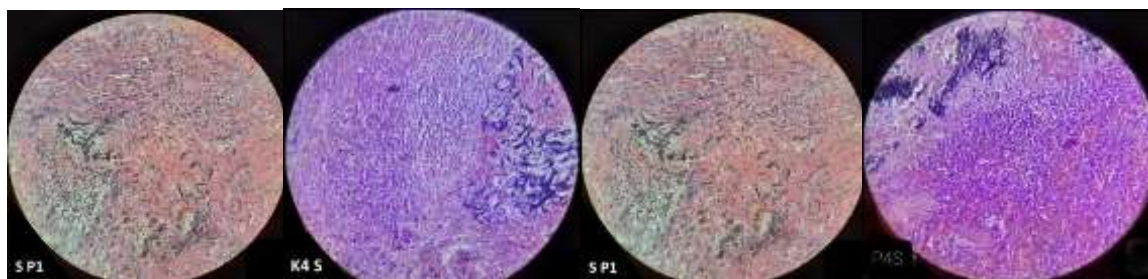


Figure 1.5 Spleen Histology H/E stain 400X magnification. Lymphoid tissue with hemorrhage and Gamna gandy bodies. Prestudy. Kaiserling III solution⁽²⁾.

Figure 1.6 Spleen Histology H/E stain 400X magnification. Lymphoid tissue with hemorrhage and Gamna gandy bodies. Post study. Kaiserling III solution.⁽²⁾

Figure 1.7 Spleen Histology H/E stain 400X magnification. Lymphoid tissue with hemorrhage and Gamna gandy bodies. Pre study.⁽²⁾

Figure 1.8 Spleen Histology H/E stain 400X magnification. Lymphoid tissue with hemorrhage and Gamna gandy bodies. Post study. Polyvinyl alcohol gel.

Table-2 .0 (Lipoma)

Sr no	Study type	Medium	Color	Turbid	pH	Specific gravity	Organism growth	Specimen color	Disintegration	Histopathology
1	Pre study	Kaiser-Ling III	Color less	nil	8.0	1.060 1:3Diln	Nil	Pale yellow	Nil	Well stained
2	Post Study	Kaiser-Ling III	Color less	nil	7.5	1.055 1:3Diln	Nil	Pale yellow	Nil	Well stained
3	Pre study	Polyvinyl alcohol gel	Color less	Nil	8.5	1.055	Nil	Pale yellow	Nil	Well stained
4	Post Study	Polyvinyl alcohol gel	Pale white	Focal turbid	8.5	1.056	Nil	Pale yellow	Mild focal	Well stained

Figure-2.0(Appearance of Lipoma specimen and mounting media before study and after study)

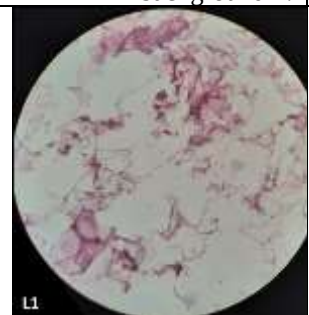
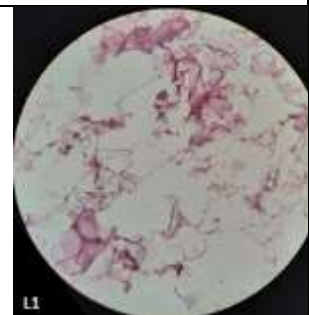
			
Figure 2.1 Gross appearance of Lipoma 6.5x5.0x3.0 cms. pre study in Kaiserling III. The tissue is yellow Medium is clear, colorless. Paper print easily read in the background⁽²⁾.	Figure 2.2 Gross appearance of Lipoma 6.5x5.0x3.0 cms. post study in Kaiserling III. The tissue is yellow Medium is clear, colorless. Paper print easily read in the background⁽²⁾.	Figure 2.3 Gross appearance of Lipoma 7.0x5.0x3.0 cms. pre study in PVA gel. The tissue is yellow Medium is clear, colorless. Paper print easily read in the background.	Figure 2.4 Gross appearance of Lipoma 7.0x5.0x3.0 cms. post study in PVA gel. The tissue is yellow Medium is hazy, colorless. Print not read in the background.
			
Figure-2.5 Lipoma histology, H/E stain 400X magnification. It shows fat cells with clear cytoplasm and surrounding fibro-collagenous strands. Prestudy. Kaiserling III soln.⁽²⁾.	Figure-2.6 Lipoma histology, H/E stain 400X magnification. It shows fat cells with clear cytoplasm and surrounding fibro-collagenous strands. Post study. Kaiserling III soln.⁽²⁾.	Figure-2.7 Lipoma histology, H/E stain 400X magnification. It shows fat cells with clear cytoplasm and surrounding fibro-collagenous strands. Pre study⁽²⁾	Figure-2.8 Lipoma histology, H/E stain 400X magnification. It shows fat cells with clear cytoplasm and surrounding fibro-collagenous strands. Post study. PVA gel.

Table-3.0 (Gallbladder)

Sr no	Study type	Medium	Color	Turbid	pH	Specific gravity	Organism growth	Specimen color	Disintegration	Histopathology
1	Pre study	Kaiser-Ling III	Color less	nil	8.0	1.060 1:3Diln	Nil	Brown green	Nil	Well stained
2	Post study	Kaiser-Ling III	Color less	nil	7.5	1.055 1:3Diln	Nil	Brown green	Nil	Well stained
3	Pre study	Polyvinyl alcohol	Color less	nil	8.5	1.055	Nil	Brown green	Nil	Well stained
4	Post study	Polyvinyl alcohol	Color less	nil	8.5	1.056	Nil	Brown green	Nil	Well stained

Figure-3.0 (Appearance of gallbladder specimen and mounting media before study and after study)

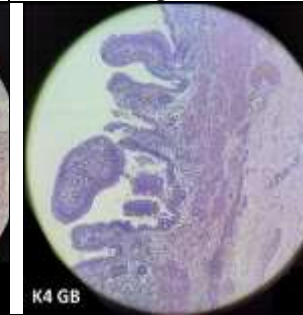
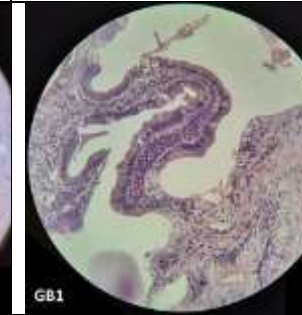
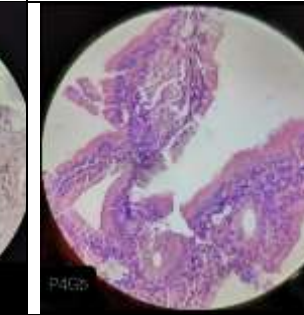
			
Figure 3.1 Gross Appearance Gallbladder 5.0x2.0x2.0 cms. in Kaiserling III solution, Pre study. Focal congestion seen grossly. Medium clear colorless. Paper print easily read in the background⁽²⁾.	Figure 3.2 Gross Appearance Gallbladder 5.0x2.0x2.0 cms. in Kaiserling III solution, Post study. Focal congestion seen grossly. Medium clear colorless. Paper print easily read in the background⁽²⁾.	Figure 3.3 Gross Appearance Gallbladder 7.5x2.0x2.0 cms in PVA gel, Pre study. Focal congestion seen grossly. Medium clear colorless. Paper print easily read in the background.	Figure 3.4 Gross Appearance Gallbladder 7.5x2.0x2.0 cms. in PVA gel, Post study. Focal congestion seen grossly. Medium clear colorless. Paper print easily read in the background.
			
Figure 3.5 Gallbladder histology H/E stain 400X magnification. Papilla lined by columnar cells with visible blue nucleus. Pre study Kaiserling solution⁽²⁾.	Figure 3.6 Gallbladder histology H/E stain 400X magnification. Papilla lined by columnar cells with visible blue nucleus. Post study Kaiserling solution⁽²⁾	Figure 3.7 Gallbladder histology H/E stain 400X magnification. Papilla lined by columnar cells with visible blue nucleus. Pre study⁽²⁾	Figure 3.8 Gallbladder histology H/E stain 400X magnification. Papilla lined by columnar cells with visible blue nucleus. Post study Polyvinyl alcohol gel

Table-4.0 (Kidney)

Sr no	Study type	Medium	Color	Turbid	pH	Specific gravity	Organism growth	Specimen color	Disintegration	Histopathology
1	Pre study	Kaiser-Ling III	Color less	nil	8.0	1.060 1:3Diln	Nil	Pale Brown	Nil	Well stained
2	Post study	Kaiser-Ling III	Color less	nil	7.5	1.055 1:3Diln	Nil	Pale Brown	Nil	Well stained
3	Pre study	Polyvinyl alcohol gel	Color less	nil	8.5	1.055	Nil	Pale Brown	Nil	Well stained
4	Post study	Polyvinyl alcohol gel	Color less	Minimal smoky	8.5	1.056	Nil	Pale Brown	Nil	Well stained

Figure-4 (Appearance of kidney specimen and mounting media before study and after study)

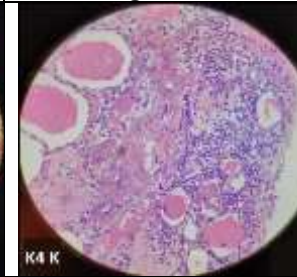
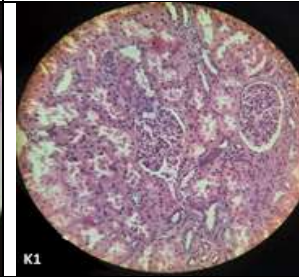
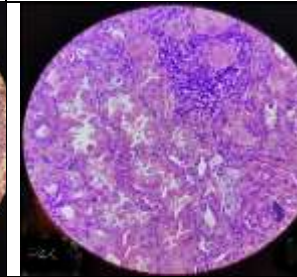
			
Figure 4.1 Gross appearance of kidney 6.5x5.0x2.5 cms.in Kaiserling III. Pre study. Dilated calyces present. Medium is clear, colorless. Paper print easily read in the background⁽²⁾.	Figure 4.2 Gross appearance of kidney 6.5x5.0x2.5 cms. in Kaiserling III. Post study. Dilated calyces present. Medium is clear, colorless. Paper print easily read in the background⁽²⁾	Figure 4.3 Gross appearance of kidney 8.5x4.0x2.5 cms in PVA gel. Pre study. Dilated calyces present. Medium is clear, colorless. Paper print easily read in the background.	Figure 4.4 Gross appearance of kidney 8.5x4.0x2.5 cms. in PVA gel. Post study. Dilated calyces present. Medium shows minimal smokiness of media and minimal hazy paper print
			
Figure 4.5 Kidney histology H/E stain, 400X magnification. Glomeruli and tubules with mild interstitial infiltrate. Pre study, Kaiserling III solution⁽²⁾.	Figure 4.6 Kidney histology H/E stain, 400X magnification. Interstitial lymphocytes infiltrate. Post study, Kaiserling III soln.⁽²⁾.	Figure 4.7 Kidney histology H/E stain, 400X magnification. Glomeruli and tubules with mild interstitial infiltrate. Pre study⁽²⁾,	Figure 4.8 Kidney histology H/E stain, 400X magnification. Interstitial lymphocytes infiltrate. Post study, Polyvinyl alcohol gel.

Table-5.0 (Ovarian cyst)

Sr no	Study type	Medium	Color	Turbid	pH	Specific gravity	Organism growth	Specimen color	Disintegration	Histopathology
1	Pre study	Kaiser-Ling III	Color less	nil	8.0	1.060 1:3Diln	Nil	Brown white	Nil	Well stained
2	Post study	Kaiser-Ling III	Color less	nil	7.5	1.055 1:3Diln	Nil	Brown white	Nil	Well stained
3	Pre study	Polyvinyl alcohol gel	Smoky	nil	8.5	1.055	Nil	Pale white	Nil	Well stained
4	Post study	Polyvinyl alcohol gel	Smoky	Turbid	8.5	1.056	Present gram (+) bacilli	Pale white	Nil	Well stained

Figure-5.0 (Appearance of ovary specimen and mounting media before study and after study)

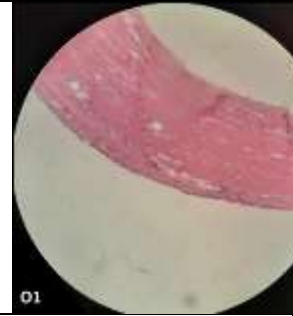
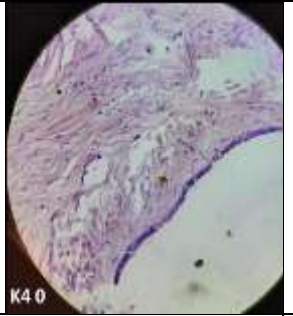
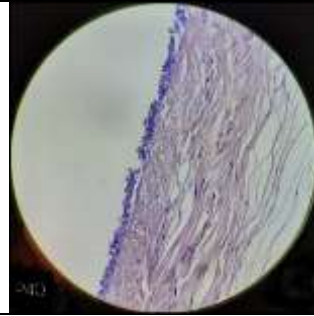
			
Figure 5.1. Gross appearance of ovary serous cyst. 10.0x8.5x3.5 cms. in Kaiserling III solution. Pre study. Brown white color cyst. Medium clear, colorless. Paper print easily read in the background ⁽²⁾.	Figure 5.2. Gross appearance of ovary serous cyst. 10.0x8.5x3.5 cms.in Kaiserling III solution. Post study. Brown white color cyst. Medium clear, colorless. Paper print easily read in the background ⁽²⁾.	Figure 5.3. Gross appearance of ovary serous cyst 7.5x5.0x2.5 cms in PVA gel. Pre study. Pale white color cyst. Medium smoky, colorless. Paper print slightly blurred.	Figure 5.4. Gross appearance of ovary serous cyst 7.5x5.0x2.5 cms in PVA gel. Post study. Pale white color cyst. Medium opaque in upper part. Paper print not easily read.
			
Figure-5.5. Ovary serous cyst histology. H/E stain. 400X magnification. Cyst wall lined by columnar cells with blue stained nuclei. Wall is pink fibro-collagenous. Pre study. Kaiserling III solution.⁽²⁾	Figure-5.6. Ovary serous cyst histology. H/E stain. 400X magnification. Cyst wall lined by columnar cells with blue stained nuclei. Wall is pink fibro-collagenous. Post study. Kaiserling III solution⁽²⁾.	Figure-5.7. Ovary serous cyst histology. H/E stain. 400X magnification. Cyst wall lined by columnar cells with blue stained nuclei. Wall is pink, fibro-collagenous. Pre study⁽²⁾.	Figure-5.8. Ovary serous cyst histology. H/E stain. 400X magnification. Cyst wall lined by columnar cells with blue stained nuclei. Wall is pink fibro-collagenous. Post study Polyvinyl alcohol gel.

Table-6.0 (Uterine Fibroid)

Sr no	Study type	Medium	Color	Turbid	pH	Specific gravity	Organism growth	Specimen color	Disintegration	Histopathology
1	Pre study	Kaiser-Ling III	Color less	nil	8.0	1.060 1:3Diln	Nil	Pale Brown	Nil	Well stained
2	Post study	Kaiser-Ling III	Color less	nil	7.5	1.055 1:3Diln	Nil	Pale Brown	Nil	Well stained
3	Pre study	Polyvinyl alcohol gel	Color less	nil	8.5	1.055	Nil	Pale Brown	Nil	Well stained
4	Post study	Polyvinyl alcohol gel	Color less	Slight turbid	8.5	1.056	Nil	Pale Brown	Nil	Well stained

Figure-6.0 (Appearance of uterus specimen and mounting media before study and after study)

			
Figure 6.1 Uterine fibroid 7.5x3.0x2.0 cms. in Kaiserling III solution. Prestudy. Submucosal fibroid seen with whorled appearance cut surface. Medium is clear, colorless. Paper print easily read in the background⁽²⁾.	Figure 6.2 Uterine fibroid 7.5x3.0x2.0 cms in Kaiserling III solution, Post study. Submucosal fibroid seen with whorled appearance cut surface. Medium is clear, colorless. Paper print easily read in the background⁽²⁾	Figure 6.3 Uterine fibroid 8.0x3.5x2.5 cms. in PVA gel. Prestudy. Submucosal fibroid seen with whorled appearance cut surface. Medium is slight smoky, colorless. Paper print easily read in background.	Figure 6.4 Uterine fibroid 8.0x3.5x2.5 cms in PVA gel. Post study. Submucosal fibroid seen with whorled appearance cut surface. Medium is slight smoky, colorless. Paper print easily read in the background.
			
Figure 6.5 Fibroid histology, H/E stain 400X magnification. Spindle smooth muscle cells . Prestudy. Kaiserling III soln.⁽²⁾.	Figure 6.6 Fibroid histology, H/E stain 400X magnification. Spindle smooth muscle cells Post study. Kaiserling III soln.⁽²⁾.	Figure 6.7 Fibroid histology, 400X magnification. H/E stain Spindle smooth muscle cells Pre study⁽²⁾.	Figure 6.8 Fibroid histology, H/E stain 400X magnification. Spindle smooth muscle cells Post study. PVA gel.

Figure 7.0 (Culture and Gram Stain of smear)

	
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Figure-7.1 Nutrient agar culture plate inoculation. Growth seen in O-ovarian cyst medium. No growth in media in specimens of L-lipoma, F-fibroid uterus, Gb-gallbladder, S-spleen, K-Kidney.	Figure-7.2 Gram positive bacilli seen singly and in clusters.
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The pH of medium: The pH of the Kaiserling solution was high. This was because the sodium acetate imparted an alkaline pH to the medium. The pH was carried out in the undiluted Kaiserling III solution. The Polyvinyl alcohol gel itself was formed at a high pH value of 8.5

Specific gravity of medium: The Kaiserling III solution had to be diluted to get necessary reading on the scale because of high salt concentration. The specific gravity was beyond 1.070, hence not readable on the visual scale. Dilution 1:3 with distilled water showed a specific gravity 1.060, which could be read on the scale (Table 1 to 6). The specific gravity of the polyvinyl alcohol gel was 1.055-1.056. Since the gel was of high consistency, it was aspirated by a syringe with a cut nozzle, and minimal 0.3 ml quantity gel was ejected on the surface of the objective plate of refractometer. The overlying transparent plate was pressed to flatten the surface without forming bubbles, and the reading was taken.

Growth of organisms: Contaminants introduced within the media may be bacterial or fungal organisms. They may also be introduced by the specimens harboring them, in case the specimens are not properly fixed by 10 % formalin. Alternatively, addition of phenoxylethanol in concentration less than 1% in the gel may prevent growth of bacteria or fungal organisms. *Bacillus cereus* is a common contaminant in the atmosphere, soil and object surfaces. The polyvinyl alcohol during preparation may pick up the contaminant within its porous structure, if proper sterility is not maintained. In our case, in the medium of the ovarian cyst (Table 5.0, Figure 5.4) the contaminant bacteria were identified as gram-positive rod-shaped bacillus (Figure 7.1, 7.2). Further identification was not done. The specimen jars were kept at room temperature, which locally varied from 24 degrees centigrade to 40 degrees centigrade according to the seasons, and this may have led to the growth of the contaminating organism.

Specimen color: Freshly preserved tissue may show varied color due to the native tissue which may be brownish white to whitish in color. Gall bladder may be greenish brown due to bile pigment. Presence of purulent exudate in inflammation may show greenish white to dark color. Autolysis due to improper fixation by 10% formalin, or presence of contamination by bacteria or fungus may result in disintegration of the tissue. The tissue may become purple to brown to black in color due to putrefaction. Few pigmented bacteria or fungal organisms may produce a dark, blackish pigment. Congestion of the organ or presence of hemorrhage may impart a red to brownish black color. In our case, the slice of the spleen organ showed large dilated vascular spaces on the cut surface engorged with dark colored blood (Table 1.0, Figure 1.8).

Specimen histopathology: Disintegrated tissue shows loss of architecture, absence of nuclear staining, and fading of the stains with absence of blue and pink differentiation. Well preserved tissue will show sharply stained blue nucleus, and pink cytoplasm and matrix, with regular tissue architecture. Hence histological examination of the tissues help in determining whether the tissues are well preserved or not⁽²⁾. The histological appearances remained consistently well preserved for one year study period of time. Kaiserling III mounted specimens have lasted for many years. Stability of the medium polyvinyl alcohol gel as well as the tissue mounted in it, have not been confirmed beyond one year.

4. Conclusion

In conclusion, polyvinyl alcohol gel may be used as a preservative medium for biological specimens in museums, with certain precautions which include better sealing of the jar to prevent dehydration of the medium, maintaining proper sterility of the sample and medium, and trimming loose fatty tissue and necrotic tissue which could lead to opacity of the medium. Lastly, an air-conditioned museum with room temperature of 25 degrees centigrade will preserve the specimens better and longer. Trials with other hydrogels as preservative media are envisaged in the future.

Conflict Of Interest: Nil

Source of support: Indian Council of Medical Research (I.C.M.R.)

Acknowledgement: I express my gratitude to the Indian Council of Medical Research for sponsorship of this study. I am grateful to Dr. R.K. Tandon, Head, Department of Pathology, and Dr. Bakul

Javdekar, The Dean, SBKSMIRC, Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia Road, Vadodara, for providing me necessary facility and help to complete this study.

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