

SJMLS-3(1)-001

**The Staining Variations on The Kidney and Liver Tissue Sections with Periodic Acid Schiff and Lemon Juice Solution**

Okorie Nnaemeka\* <sup>1</sup>, Fasogbon Samuel Ayobami <sup>2</sup>, Ajileye Ayodeji Blessing<sup>3</sup>, Aba Terseer David <sup>4</sup>  
NKST Len Gabrielse School of Medical Laboratory Science, Mkar Benue State <sup>1</sup>, Public Health In-vitro Diagnostic Control Laboratory, Medical Laboratory Science Council of Nigeria <sup>2</sup>, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti <sup>3</sup>, NKST Len Gabrielse School of Medical Laboratory Science, Mkar Benue State <sup>4</sup>  
Author for Correspondence: ayobless05@gmail.com/+234-8030445624

**Abstract**

**Periodic acid–Schiff (PAS)** is a staining method used to detect polysaccharides such as glycogen, and mucosubstances such as glycoproteins, glycolipids and mucins in tissues. To establish the staining variations between tissue sections stained with Periodic Acid Schiff's method and that of tissue section stained with Lemon Schiff's staining method. Tissue samples were gotten from the liver and kidney of adult Wistar rats. The tissues obtained were histological processed and sectioned with a rotary microtome. Lemon orange was obtained from a local market in Anyigba, Kogi State. The juice was extracted locally and used to oxidize tissue section obtained from the liver and kidney from adult Wistar rats before staining the tissue section with Schiff reagent and then counterstaining with Cole's haematoxylin. The control tissue sections were correspondingly stained with Periodic Acid Schiff's stain for the demonstration of kidney and liver tissues. Liver and kidney tissue sections oxidized with lemon juice extracts stained more intensely with Schiff's reagents when compared with the other liver and kidney tissue sections oxidized with Periodic Acid and then stained with Schiff's reagent. Considering the result generally, lemon juice Schiff reagent is a good substitute for Periodic Acid Schiff reagent.

**Keywords:** Kidney, Liver, Lemon juice extract, Periodic, Schiff's, Wistar Rats.

**Introduction**

Periodic Acid Schiff (PAS) is a special stain that present details of tissue and cell structures as well as differentiation of cellular constituents,

especially the cytoplasm and nucleus (Jackson and Blythe, 2013). The stain is a technique for the demonstration of carbohydrates in tissue sections (Avwioro, 2002). The purpose of the periodic acid is to oxidize some of the tissue carbohydrates (Avwioro, 2002). These produce aldehyde groups, which can then react strongly with Schiff's reagent forming a bright red colouration and demonstrating the tissue component, to which the carbohydrate is attached (Culling, 1974). PAS is mainly used for staining structures containing a high proportion of carbohydrate macromolecules (glycogen, glycoprotein, proteoglycans), typically found in connective tissues (Ochei, 2005), mucus, the glycocalyx, basement membrane, basal laminae and Kidney tubules (Barbara et al., 2006). The use Haematoxylin and Eosin is historically and nearly all tissue specimens are treated with haematoxylin and Eosin today. There have been great laboratory changes in haematoxylin stains (Bancroft and Lauston, 2013). However, the Haematoxylin and Eosin are inefficient in that not all features of a substance can be received and special stain must be used (Carlton, 1967). The liver and kidney are extremely active organs, the former plays important roles in metabolism of carbohydrates, fat and protein while the kidney regulate and excrete metabolic waste of the body (Barbara et al., 2006). The liver has four lobes, the two most obvious are the large right lobe and the smaller, wedge-shaped, left lobe. The other two, the caudate and quadrate lobes, are areas on the posterior surface. It is made up of portal tissues, a region on the posterior surface (Carson and Hladik, 2009). It is made up of portal fissure, a region on the posterior surface of

the liver where various structures such as portal vein, inferior vena cava, Hepatic artery enters and leaves the gland (Barbara *et al.*, 2006). The kidney is made up of an outer fibrous capsule, the cortex, a reddish –brown layer of tissue immediately below the capsule and outside the renal pyramids, the medulla, the innermost layer, consisting of pair conical-shaped striation, the renal pyramids. The hilum is a concave medial border of the kidney where the renal blood lymph vessels, the ureter and nerves enter. The kidney is also known to have the calyce, pelvis and the nephron which is the functional unit of the kidney (Barbara *et al.*, 2006). The aforementioned stains will help to highlight the important features of the selected tissue sections of a Wistar rat.

## Materials and Methods

### Study Area

This research study was carried out at the Histopathology Laboratory of N.K.S.T. Len Gabrielse School of Medical Laboratory Science Mkar, Gboko Benue State, Nigeria.

### Experimental Animals

Fifteen (15) adult Wistar rats of both gender were obtained from the animal house of the Department of Animal Production; University of Agriculture Makurdi, Benue State. The animals were in healthy conditions and weighed between 160g-250g. On transfer to the experimental location, the rats were allowed to acclimatize for 2 weeks under a good climatic condition and were properly fed.

### Ethical Clearance

Ethical approval for this research study was obtained from the Research Ethics Committee of the N.K.S.T. Len Gabrielse School of Medical Laboratory Science, the experiment was carried out in strict accordance with the guidelines for the care and use of animals for research.

### Sample Collection and Preparation for Histopathology

At the end of the acclimatization period, the rats were allowed to fast overnight, and then sacrificed by spinal dislocation. The liver and the kidney were harvested. The tissues were immediately fixed in 10% formol saline for 24

hours and standard manual tissue processing techniques were used. Sections of about 3-5 $\mu$ m were cut by a Leica medical rotary microtome. The tissue sections gotten from the liver and the kidney were divided into two (2) groups. The first group of the tissue sections (control) from the liver and kidney were first oxidized with Periodic Acid, then with Schiff's reagent and then counter stained with Cole's Haematoxylin Stain, while the other part of the tissue section (test) from the liver and kidney were first oxidized with extract of Lemon Juice and then continued with Schiff's reagent and then counter stained with Cole's Haematoxylin. The stained slides of both groups were viewed and analysed with the aid of a light microscope for histological (Carlton, 1967) similarities and differences, thereafter captured with a Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular).

### Extraction of Lemon Juice.

Unripe lemon fruits were plucked from the lemon tree, the rind was peeled off. The juice was extracted using an electric juice extractor, which was further filtered through four-layer folds of sterile gauze and the juice pH was measured using a pH meter immediately after extraction, then measured again after 24 and 28 hours post extraction.

### Periodic Acid Schiff Staining Method

#### Procedure

1. Sections were deparaffinised using xylene for 5 minutes each x3 changes
2. Sections were taken to alcohol for 5 minutes each x2 changes.
3. Sections were brought to water.
4. Sections were oxidized with periodic acid for 10 minutes.
5. They were thoroughly washed in running tap water then, rinsed in distilled water.
6. Sections were stained with Schiff's solution for 20 minutes.
7. They were thoroughly washed in tap water.
8. They were counter stained progressively in Cole's haematoxylin for 10 minutes.
9. Sections were thorough washed in running tap water.
10. They were blued in tap water.

11. They were dehydrated, cleared, and mounted with DPX.
12. Sections were examined under the microscope using x100 objective (Baker *et al.*, 2001).

**Staining procedure for test (lemon juice)**

1. Sections were deparaffinised in three changes of xylene for 5 minutes each.
2. Sections were hydrated in descending grades of alcohol (100%, 90%, 70%) for 5 minutes each.
3. Sections were taken to water for 5 minutes.
4. Sections were oxidized with Lemon juice for 10 minutes immediately after extraction at pH 3.2 (Test 1), then another after 24 and 48 hours at pH 3.1 and 2.9 (Test 2 and 3) respectively.
5. They were thoroughly washed in running tap water.
6. Sections were stained with Schiff's solution for 20 minutes.
7. They were thoroughly washed in running tap water.

8. Sections were counterstained progressively in Cole's haematoxylin for 10 minutes.
9. Sections were thoroughly washed in running tap water.
10. Sections were blued in tap water for 5 minutes.
11. They were dehydrated, cleared and mounted with DPX.
12. Sections were examined systematically under the microscope using x100 objective (Method adopted from Culling, 1963).

**Slide Examination**

Mounted slides were examined and photographed under a light microscope coupled with an image analyser. Figure 1-4 shows the Kidney sections periodic acid oxidation at pH 3.9 (control) compared with Tests 1, 2, 3 Lemon juice oxidation at pH: 3. 2, 3. 1 and 2.9 respectively. Figure 5-8: Liver sections periodic acid oxidation at pH 3.9 (control) compared with Tests 1, 2, 3 Lemon juice oxidation at pH: 3. 2, 3. 1 and 2.9 respectively.

Figure 1-4: Kidney sections periodic acid oxidation at pH 3.9 (control) compared with Tests 1, 2, 3 Lemon juice oxidation at pH: 3. 2, 3. 1 and 2.9 respectively

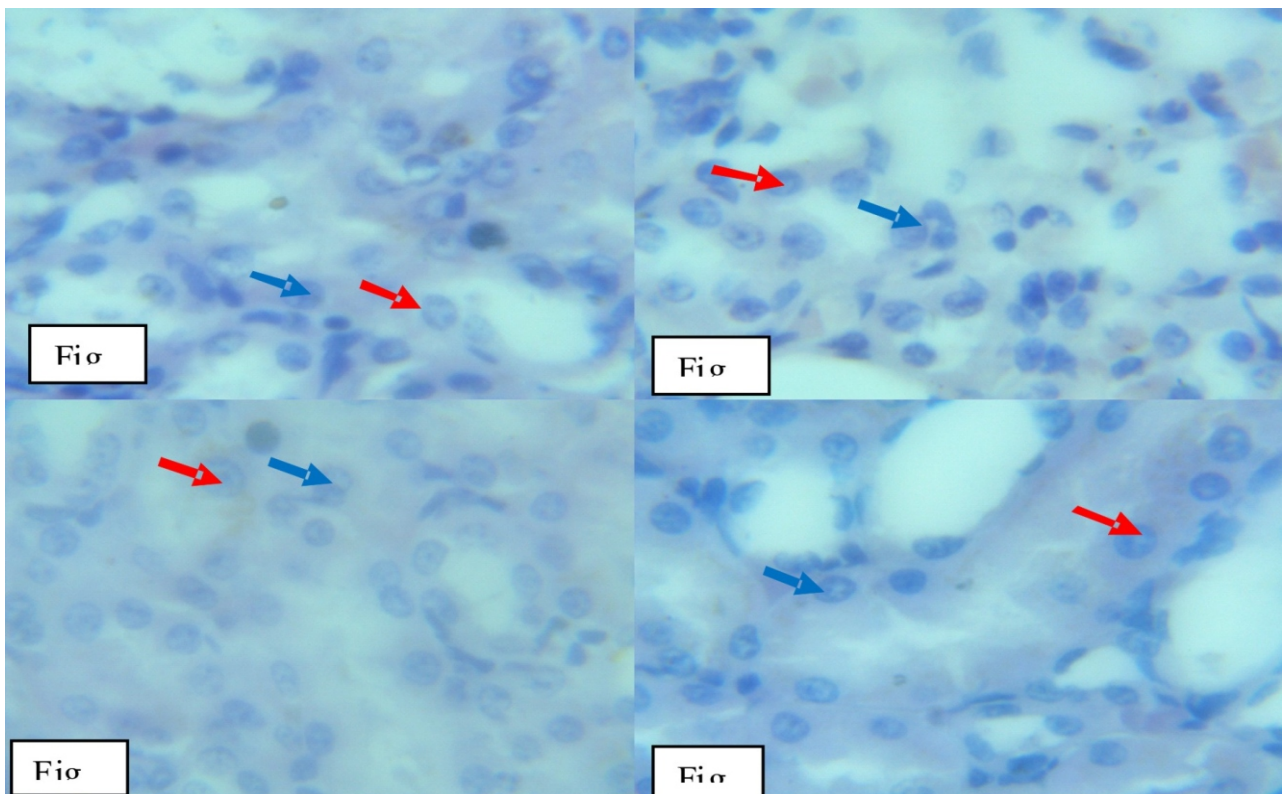
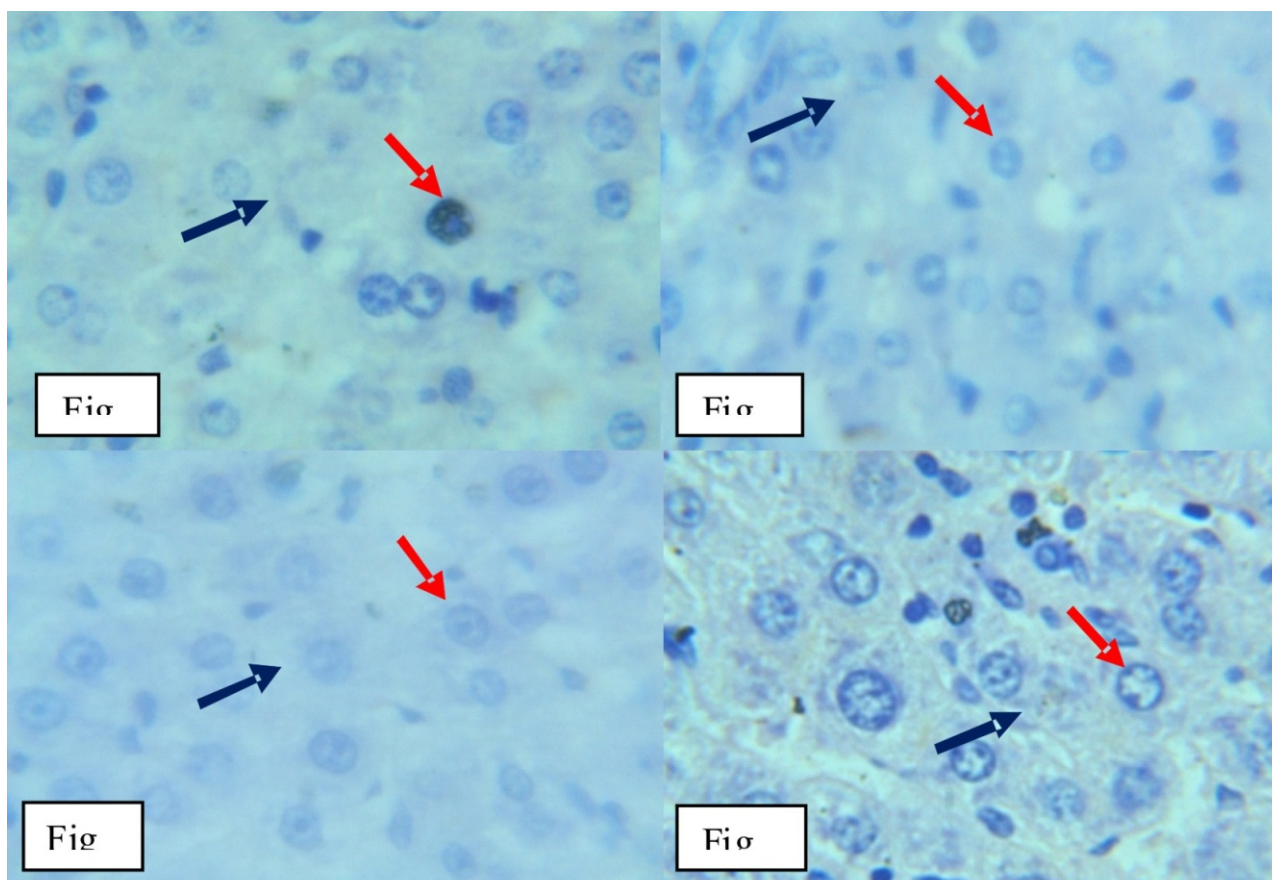




Figure 5-8: Liver sections periodic acid oxidation at pH 3.9 (control) compared with Tests 1, 2, 3 Lemon juice oxidation at pH: 3. 2, 3. 1 and 2.9 respectively



**Fig 1:** Kidney section oxidized with Periodic Acid (control) at pH 3.90 and stained with Schiff's reagents, slides showing Distal convoluted tubule (Red arrow) and podocytes within the Glomerulus (Blue Arrow) at high magnification  $\times 400$ .

**Fig 2:** Kidney section (Test 1) oxidized with Lemon juice extract (Test 1) at pH 3.20 and stained with Schiff's reagents, slides showing Distal convoluted tubule (Red arrow) and podocytes within the Glomerulus (Blue Arrow) at high magnification  $\times 400$ .

**Fig 3:** Kidney section (Test 2) oxidized with Lemon juice extract at pH 3.3 then stained with Schiff's reagent, slide showing Distal convoluted tubule (Red arrow) and podocytes within the Glomerulus (Blue arrow) at high magnification  $\times 400$ .

**Fig 4:** Kidney section (Test 3) oxidized with lemon juice extract at pH 2.90 then stained with Schiff's reagent, slide showing Distal convoluted tubule (Red arrow) and podocytes within the

Glomerulus (Blue arrow) at high magnification  $\times 400$ .

**Fig 5:** Liver section oxidized with Periodic Acid (control) at pH 3.90 and stained with Schiff's reagents, slides showing hepatocytes (blue arrow) and sinusoids (red Arrow) at high magnification  $\times 400$ .

**Fig 6:** Liver section oxidized with Lemon Juice extract (Test 1) at pH 3.20 and stained with Schiff's reagents, slides showing hepatocytes (blue arrow) and sinusoids (red Arrow) at high magnification  $\times 400$ .

**Fig 7:** Liver section oxidized with Lemon juice extracts (Test 2) at pH 3.10 and stained with Schiff's reagents, slides showing hepatocytes (blue arrow) and sinusoids (red Arrow) at high magnification  $\times 400$ .

**Fig 8:** Liver section oxidized with Lemon juice extracts (Test 3) at pH 2.90 and stained with Schiff's reagents, slides showing hepatocytes (blue arrow) and sinusoids (red Arrow) at high magnification  $\times 400$ .

## Discussion

According to Culling (1974), Periodic acid is not the only oxidizing agent that has been recommended for the oxidation of carbohydrates. Although it is certainly the most used and arguably the most effective (Culling, 1974). With quality staining reaction exhibited by the use of lemon juice as an oxidizing agent, it is more favourable and reasonable that lemon juice should be adopted as a renowned histochemical approach. Indubitably, slides or sections oxidized with lemon juice stained more intensely with Schiff solution indicating the presence of carbohydrate and other structures which were initially oxidized by the lemon juice as shown by the magenta colouration and as well as blue colouration of the nuclei in both liver and kidney tissue sections. Lemon juice Schiff staining technique has been carried out in conformity with the laid down procedure and criteria for demonstration of periodic acid positive materials as stated by Culling (1963) and Pearse (1972). The lemon juice Schiff's approach has demonstrated the list of periodic acid positive materials such as Basement membrane, Bowman's capsule, and Capillaries of the glomeruli as reviewed by Culling (1963). Lemon Juice being more acidic than periodic acid has yielded a better result as compared to Periodic Acid. It can be deduced that Lemon Juice has the properties to oxidize Periodic Acid Schiff positive materials even more effectively especially when allowed to ripe after extraction for a period of 48 hours as observed in this study. The pH of lemon juice decreases from 3.2 to 2.9 after 48 hours hence, this could be said to account for better oxidation property than Periodic acid. Considering the generated result, Lemon Juice Schiff reagent is a good substitute for Periodic Acid Schiff reagent.

## References

- Avwioro, O. G. (2002). Histochemistry and Tissue Pathology. Ibadan: Claverianum Centre: 31-140.
- Baker, F.J., Silverton, R.E. and Pallister, C.J. (2001). Introduction to Medical Laboratory Technology. London: Butterworths: 191 – 218.
- Bancroft, J.D. and Lauston, C. (2013): The Haematoxylin and Eosin. In theory and Practice of Histological Technique: 7th ed. Ch. 10 and 11. Philadelphia: Churchill Livingstone of El Sevier; 179–220.
- Barbara, Y., Lowe, J.S., Stevens, A. and John, W. H. (2006): Wheater's Functional Histology, a text and colour atlas. Elsevier, China: 288-298.
- Carlton, S.M. (1967). Text Book of Histochemical Techniques 4th Edition, Oxford University. Press: 48-137.
- Carson, F.L. and Hladik, C. (2009). Histotechnology: A Self-Instructional Text (3 ed.). Hong Kong: American Society for Clinical Pathology Press: 137–139.
- Culling, C.F.A. (1963). Hand book of histopathological technique 2<sup>nd</sup> Ed. 3 Butterworth, London UK: 154–167.
- Culling, C.F.A. (1974). Handbook of histopathological technique. Ed. 2 Butterworth, London United Kingdom: 107-135.
- Jackson, P., Blythe, D. (2013). Immunochemical technique Theory and Practice of histochemical techniques: 54-99.
- Ochei, J. K. (2005). Medical Laboratory Science Theory and Practical. London: 220–400.
- Pearse, A.G.E. (1973). Histochemistry; theoretical and applied, Ed. 3 Churchill Livingstone, Edinburgh, London UK: 34(3):191–203.

**Citation:** Okorie Nnaemeka, Fasogbon Samuel Ayobami, Ajileye Ayodeji Blessing, Aba Terseer David. The Staining Variations on The Kidney and Liver Tissue Sections with Periodic Acid Schiff and Lemon Juice Solution. *Sokoto Journal of Medical Laboratory Science*; 3(1): 5 - 9

**Copyright.** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.