

Comparative Benefits of Tissue Marking by Poster Ink in Histopathology

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ABSTRACT

Background: Tissue margin marking with India ink is important in decision making for surgeons. The present study was conducted to examine the reliability of different shades of locally available poster colours in tissue marking and to evaluate the colour perceptibility microscopically in comparison with similar tissues marked by India ink.

Methodology: This experimental study was conducted at Department of Pathology, HBS Medical & Dental College & Hospital, Islamabad from 27th February 2021 to 29th April 2021. Sample size was fifty, collected through convenient sampling technique. Five types of formalin fixed tissue specimens were selected for the study so as to evaluate the effectiveness of poster ink marking on different tissue surfaces. From each specimen, four sections were taken from the margins. Three shades of poster colour (black, blue and green) were used to ink three sections while one section from each tissue type was marked with India ink. After complete tissue processing and routine Haematoxylin and Eosin (H&E) staining, slides were examined microscopically. Scoring was done on a scale 0 to 3 on the basis of visibility.

Results: The present study showed that poster colours inking of the tissues was quite reliable as compared to India ink. Most consistent results were achieved with black and blue colours as compared to green colour.

Conclusion: Poster colours are reliable tool for tissue marking when India ink is not available. Their availability in a variety of colours provides them an edge over India ink.

Key words: Eosin, India ink, Resection Margins

Authors' Contribution:

¹Conception; Literature research; manuscript design; ²Critical analysis and manuscript review; ³Data collection; ⁴Manuscript Editing; ⁴Data analysis.

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Introduction

The first step in the evaluation of a surgical specimen is its gross or macroscopic examination. It is not infrequent during this process that there is a need to identify the resection margins or to identify small tissue pieces at embedding station and also to accurately orient a specimen. Use of tissue marking ink greatly facilitates all these processes. Accurate evaluation of stained tissue is essential for many

clinical decisions and also improves intra observer and inter observer reliability.^{1,2}

India ink is routinely used for this purpose. But India ink is available only in black colour. During routine histopathology, frequently there is a need for different colour ink when different surfaces/margins are to be studied or laterality of specimens needs to be identified. Differently coloured inks also help in reducing identification error that may happen as in

prostate needle biopsy when multiple samples are taken from different sites in an enlarged prostate gland or during research similar specimens of different patients can be coloured differently and put in one cassette.³

Tissue marking dyes (TMD) have been used as an alternate to India ink in tissue marking. They are available in many colours. But they are expensive and not always readily available especially in small laboratories in developing countries.⁴ Other alternate marking techniques are gelatin, oil paints, acrylic paints, and also eosin and alcian blue which are commonly used dyes in histopathology lab.⁵

The accurate histopathological evaluation of surgical margins is very important especially when there is a need to decide the type of surgery to be performed like whether an organ sparing or complete resection is required based on the fact that the tumour borders are infiltrative, irregular, or excision margins are close to the line of resection.⁴

The present study aims to examine the reliability of locally available poster colours in different shades for tissue inking and to evaluate the colour perceptibility microscopically in comparison with similar tissues marked using India ink. This will lead to generation of more reliable histopathology reports which is beneficial for the patients.

Methodology

The study was conducted in the department of Pathology, HBS Medical & Dental College & Hospital, Islamabad from 27 February 2021 to 29 April 2021 after approval of the ethical review board of HBS Medical and Dental College, Islamabad. Five different tissue specimens fixed in formalin were included in the study (breast, colon, skin, thyroid and testis) to represent different consistency and

surfaces for inking while improperly processed or oriented specimens or improperly stained slides were excluded from the study. For the study, sections were taken from the selected specimen (breast, colon, skin, thyroid and testis) after routine grossing for histopathology was done. From each of the selected specimen, four sections were taken from the margins. Three shades of poster colour (black, blue and green) were used to ink the three sections while one section from each of the tissue type was marked with India ink. Fifty samples were collected by convenient sampling technique. The tissues were wiped dry using a piece of gauze before and after applying the ink. No other technique or chemical was used for inking. Colours were directly applied with a brush. Cassettes were processed in automated tissue processor and blocks were prepared after standard paraffin embedding. Sections were cut at 3mm thickness and routine haematoxylin and eosin (H&E) was used for staining purpose. To evaluate the colour perceptibility and sharpness, slides were examined microscopically. Scoring was done on a scale 0 to 3 as (0-No colour visible; 1- colour is perceptible only faintly; 2- colour is perceptible more clearly than 1 but less than 3, 3- colour perceptibility is clear and sharp)⁶.

Results

Four sections were taken, one each from colon, breast, skin, thyroid and testis. There were 9 specimen of breast, 5 of colon, 14 of skin, 4 of testis and 18 of thyroid. Sections from each of the organ was coloured with black, blue, green and India ink. The colour perceptibility as observed under microscope is shown in the Table 1

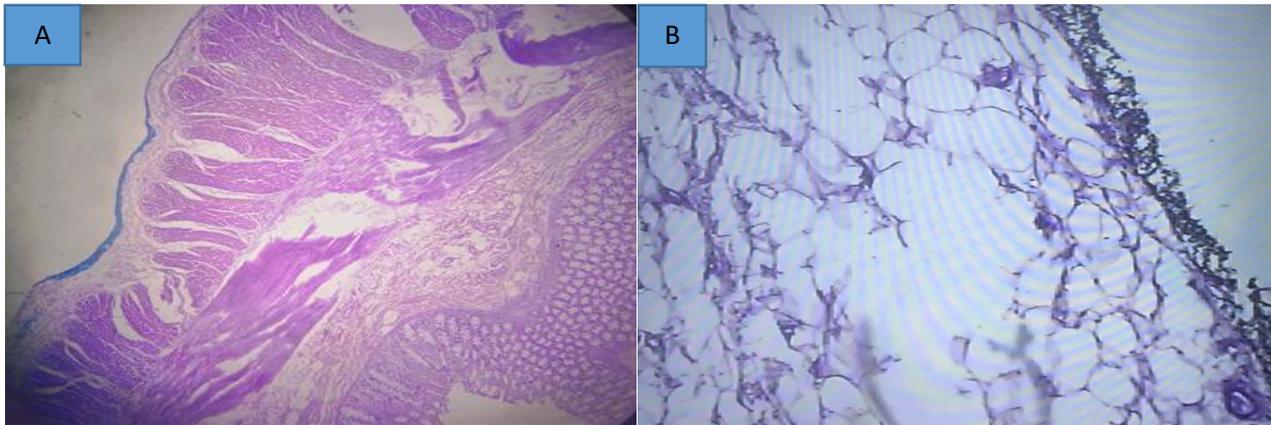
Tissue Type	Colours	Poster Colour visibility score	India ink visibility score
Breast	Black	3 (100% cases)	2 (55% cases)
	Blue	3 (100% cases)	

	Green	1 (77% cases)	
Colon	Black	3 (100% cases)	2 (60% cases)
	Blue	3 (100% cases)	
	Green	0	
Skin	Black	3 (100% cases)	3 (100% cases)
	Blue	3 (100% cases)	
	Green	1	
Testis	Black	2 (50% cases)	1 (75% cases)
	Blue	1 (75% cases)	
	Green	0	
Thyroid	Black	3 (100% cases)	3 (100% cases)
	Blue	3 (100% cases)	
	Green	0	

Score: 0-No colour visible; 1- colour is perceptible only faintly; 2- colour is perceptible more clearly than 1 but less than 3, 3-colour perceptibility is clear and sharp

The results of black and blue colour were comparable to that of India ink in all types of tissue; in fact poster colours were sharper and easily visible in breast and colon specimen than India ink (all

sections of blue and black poster colour gave score 3 visibility as compared to India ink in which only 4/9 (45%) cases of breast and 2/5 (40%) cases of colon gave score 3 visibility) Blue colour yielded the most consistent results in all types of tissues and the results of green colour were poor. Figure 1 shows the visibility of different poster colours in different tissues.



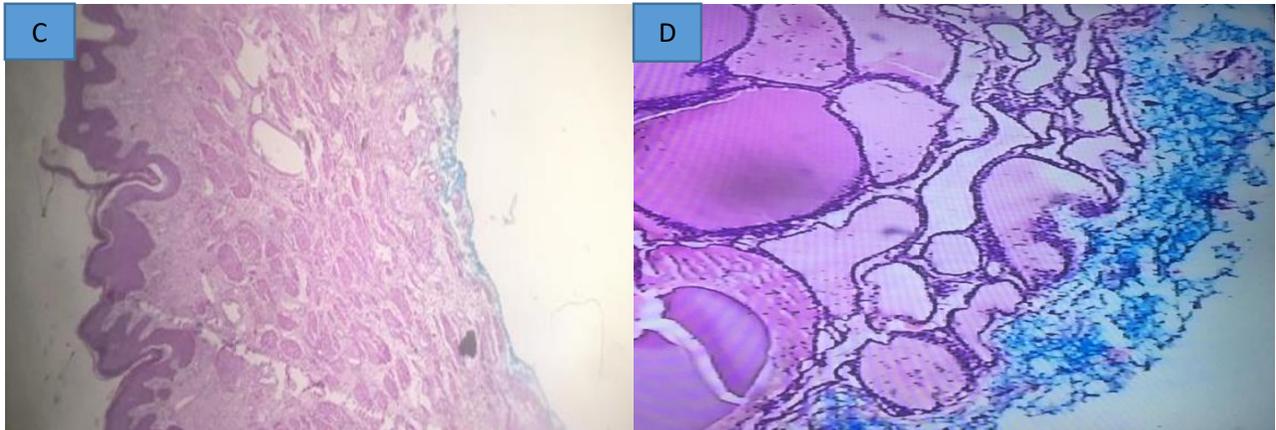


Fig1: (H&E 100x) shows the surgical specimen margins marked with poster colours as seen under the microscope. A: colon (blue), B: fat (black), C: skin (green), D: thyroid (blue)



Fig2: The poster paint of blue, black and green colours is clearly visible by the naked eye on the section margins

Discussion

In the present study, the poster colours successfully marked the tissue margins of routine histopathology specimens and yielded results comparable to that of similar tissues marked with India ink. India ink is the most well known tissue marking dye. Kizhakkootu S (2021)⁷ conducted a large scale study on 1325 specimens to compare the properties of India ink,

Acrylic colours and Tissue Marking Dyes in inking of tumour resection margins. They concluded that India ink is the best tissue marking dye but the advantages of acrylic paints compete with that of India ink and require much larger studies to prove their reproducibility. Our study also yielded similar results with additional benefit of less cost and multiple colours.

Poster colour is a type of acrylic paint and it is commonly used in artwork and painting. It is a distemper paint which has gum water as its binder and has water soluble characteristics; while acrylic paints are particulate pigments suspended in acrylic

polymer emulsion which along with a mixture of multiple chemicals forms a thick emulsion. Poster paints can be used in the same way as acrylic paints; except poster paints are more economical and dries more quickly than regular acrylic paints. Acrylic paints were first introduced around 1950, and have become increasingly popular with multiple uses, as these paints are easy to apply, dry and are long lasting.⁸

Aziz A (2019) & Pursnani D (2015) used acrylic colours on breast and colon specimen and concluded that use of acrylic colours has many benefits over India ink. They also studied three different methods of colour application and found that best results were achieved when the sections were taken after inking and overnight fixation.^{6,9} Tampi C. (2012) concluded that the easy availability and application of acrylic colours mark them as a good alternate inking technique in India that gives reliable results on microscopic examination and use of coloured inks provides precision to the examination of the tissue margins.¹⁰ Sarode SC et al (2015) used acrylic paint for margin inking in oral squamous cell carcinoma and concluded that acrylic colours are better than India ink as surgical ink because they are available in a variety of colours, are easy to apply, quickly dry, and withstand the processing procedures. They also have an advantage of being easily available and of being clearly visible on paraffin blocks and under microscope. They further suggested use of multiple colours will aid in reassessment of resection margins of the gross specimens in the future.¹¹ We also experienced similar advantages as the studies mentioned though our sample size was much less.

Vatsyayan A (2019)¹², Criswell (2021)¹³, Mardiana AA (2019)¹⁴ are of the same view regarding the usefulness of poster colours in marking of surgical specimen. Mardiana AA (2019) conducted his study in Malaysia and used poster colours which is a cheaper version of acrylic paints and found it to be a reliable alternate to India ink and strongly recommended its use in routine histopathology.

Many studies are available that showed the benefits of acrylic paints for margin inking but only a few; as the present study, are about the application of poster paints for this purpose. We favour poster paints as their less cost and a good comparable yield makes them more suitable for long term use. However, Tampi C. (2012) showed that not all shades of acrylic colours suffice as grossing ink because of their poor visibility in slides as happened in the present study, the green colour was not visible on colonic serosa and testicular biopsy.¹⁰

Much larger studies are needed, especially in tissues requiring decalcification to assess the reliability of poster paints as tissue markers in routine histopathology, as is pointed out by Williams AS et al (2014)¹⁵ and Keifer S (2021)¹⁶ in their research.

Jennifer S (2019)¹⁷ and Kamat M (2019)¹⁸ studied the importance of resection margin in oral squamous cell cancer. They recommended the use of acrylic paints as substitute for india ink and tissue marking dyes. According to them, coloured ink is more useful for better orientation, post grossing reconstruction of the specimen and reducing the identification error. The ease of use, quick drying and easy availability of acrylic paints favours their use. Fei B (2017)¹⁹ and Hon JD (2019)²⁰ emphasized the need of accurate resection margins in oral cancers. Molecular assessment of resection margins is a novelty. Among the available tools they preferred the use of coloured inks for surgical resection margin assessment.

Local data mentions the use of India ink for evaluation of surgical resection margins but not the use of any other multicolour inks like acrylic paints or poster paints.²¹ But there are studies from India, Singapore and Malaysia which highlight the utility of coloured inks in surgical specimens.^{6,9,10}

We used poster paints for inking of surgical resection margins while mostly available data is of the use of acrylic paints. The advantages of using poster colours in this study were that black poster colour was more reliable than India ink in case of colonic mucosa, there was minimal seepage of colour into

the underlying tissue and margin staining with poster colours did not affect the quality of tissue sections or staining when compared with other non-inked tissues processed on the same day. The limitations of the study are it is a single centred study with a small sample size. The use of poster colours as a routine technique requires a much larger study with a greater sample size.

Conclusion

Poster colours are available in most stationery shops. They seem to be a reliable tool for tissue marking when India ink is not available. Their availability in a variety of colours provides them an edge over India ink. To ensure reproducibility of the results these alternative paints should be tested in individual laboratories before applying in routine use.

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