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Technique for Processing and Routing Staining of Hair Samples for Histological Study

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ABSTRACT

The staining of free hair samples is considered as one of the biggest problem by the Histologists. This technique has been developed for processing and routine staining of free hair samples without the skin. It is based on paraffin embedded blocks of hair samples. The blocks were cut in the rotary microtome at 6 µ thickness. After obtaining the sections of 6 µ thickness in rotary microtome, the sections were not required to be put in water bath as the hair samples were hard and did not get folded. Further, if water bath was used, this could result in the loss of the hair part from the section. The cuticle did not take any color in the routine staining as it was composed of enucleated or keratinized cells. The cortex had taken the pink colour of eosin which might be due to the composition of cortex with flattened cornified cells and numerous air spaces. The medulla showed a mixture of pink and blue color on staining with Hematoxylin and Eosin solutions.

Key words: Cortex, Cuticle, Free Hair, Staining.

There is a myth amongst the researchers that the hair samples cannot be stained when received without skin. Through this work, we developed the technique to stain the free hair samples without skin and would also contribute to erase the myth from the scientific world that the hair samples without skin cannot be stained. The histology of hair was usually reported along with the hair follicle of skin. However, processing, cutting and staining of free hair samples were difficult when compared to other tissue samples. Considering the uniqueness of the technique, the following technique was developed which facilitates the scientists to go for study of free hair samples without the need of attached skin. However, this technique could be of great value for sectioning animal as well as human hairs received without skin for histological, histopathological and histochemical study in both the human and animal health care.

The experiement was carried out in Department of Anatomy, College of Veterinary Science, Guwahati, Assam. In the development of the technique, the hair samples were plucked to keep the root of the hair intact. After plucking the following steps to be followed:

1. 10-60 numbers of hair were cut in short considering the part of hair desirable for study.
2. Hairs were arranged in parallel and wrapped in paper and put it in the tissue capsule. The size of the wrapped paper was made same to the size of tissue capsule so that it does not get misplaced during the entire process. The tissue capsule was placed for washing under running tapewater (dropbydrop) for 2hrs to clean the dirt adhered to it.
3. The tissue capsule was then put in Diethyl ether for 1hr.
4. Then it was putinthe70% for overnight.
5. Next morning, it was processed in 80% and 90% alcohol respectively1 hr each.
6. Then kept in Cedarwood oil and xylene combination (1:1ratio) for 1 day.
7. The cedar wood oil was then removed with xylene of two changes.
8. Hair samples were embedded in the melted paraffin at 60°C. Orientation of the hair samples was horizontal. Depending on the section of the hair desired for study, cut the block longitudinally or transversely.
9. Blocks were cut into sections of 6 µ thickness. Do not put the section in water bath. Directly take the sections in the egg albumen painted microslides.
10. Allow the slides to dry for 1 day at least. Heat fix the slides.
11. Deparaffinise the section with two changes of xylene.
12. It was hydrated with distilled water for 10 mins.
13. Dipped in Hematoxylin for 10-15 mins.
14. Then keep in distilled water for 5 mins.

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15. Treated with eosin for 2 minutes.
16. Treated with xylene directly.
17. Slides mounted in DPX medium.

The area that proved to be difficult in developing the technique was the cutting. The placement of the samples in diethyl ether for 1 hr showed us good results. Diethyl ether was used for lipid removal (Shelley, 1969). Cedar wood oil in combination with xylene cleared the hair to have a clear view under microscope. Cedar wood oil in combination with xylene was used so that the hair does not get hardened resulting in difficulty to cut the tissue. Trial was also conducted without using the cedar wood oil, but the result was not appreciable. The embedding of the hair samples was somewhat done in a different manner. First a layer of melted paraffin was poured with the help of a small spoon and then the hairs were placed which was again followed by a layer of melted paraffin with spoon and then hair samples again. This was done to accommodate as much of hair samples in a block and use of spoon could control the pouring of the melted paraffin. After obtaining the sections of 6 µ thickness in rotary microtome, the sections were not required to be put in water bath as the hair samples were hard and did not get folded. Further, if water bath was used, this could result in the loss of the hair part from the section. The cut sections were directly put in the egg albumen painted slides. However, the slides with egg albumen were dipped in water before placing the sections to it for ensuring proper attachment of the section to the slides. In staining process, slow dipping of slides was followed throughout the procedure so that the hairs do not get washed away. The use of alcohol in various percentages was not appreciable in the staining process. The use of various percentile alcohols made the slides hazy due the remaining fat or lipid in the hairs which could not be removed by diethyl ether. The routine staining of the free hair samples showed that the hair consisted of three regions; outer cuticle, inner cortex and a central medulla (Fig 1). The root of the hair showed the hair bulb and dermal papilla (Fig 2). The cuticle did not take any color in the routine staining as it was composed of enucleated or keratinized cells [Banks, 1993] (Fig 1).The cortex had taken the pink colour of eosin which might be due to the composition of cortex with flattened cornified cells and numerous air spaces (Banks, 1993) (Fig 1). The medulla showed a mixture of pink and blue color on staining with Hematoxylin and Eosin solutions which might due to the presence of cuboidal cells and air spaces [Banks, 1993] in the medulla (Fig 3).

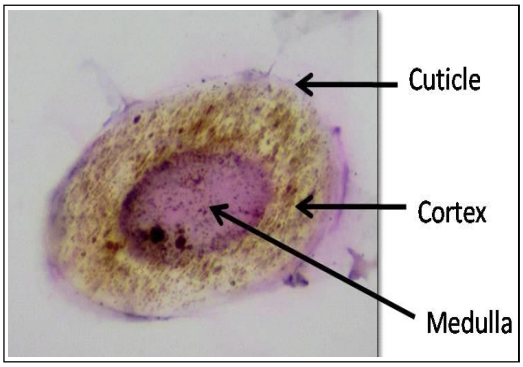

Fig 1: Cross section showing the cuticle,cortex and medulla part of the hair, H&E,40X.

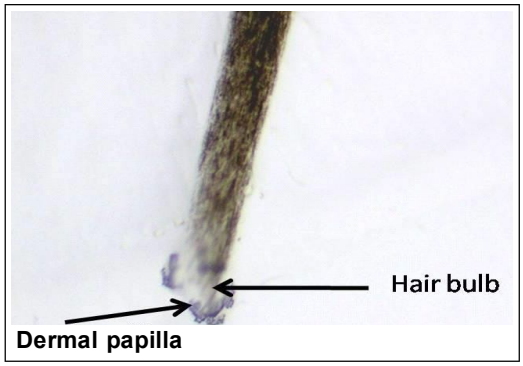

Fig 2: Longitudinal section of root part showing the hair bulb and dermal papilla, H&E,10X.

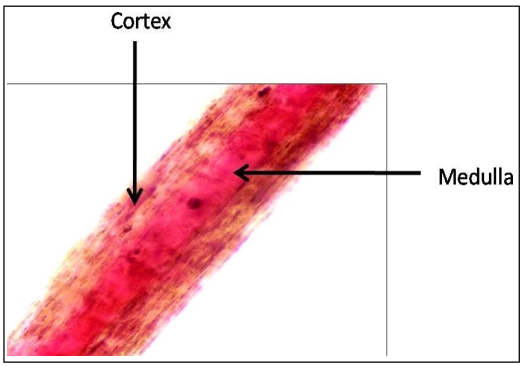

Fig 3: Longitudinal section showing cortex and medulla, H&E, 40X.

CONCLUSION

This technique could be of great value for sectioning animal as well as human hairs received without skin for histological, histopathological and histochemical study in both the animal and human health care

Conflict of interest: None.

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