RESEARCH ARTICLE

Sudanese traditional stains for staining some biological samples

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Ibrahim HS and Kafi SK. Sudanese traditional stains for staining some biological samples. Curr Res Integr Med 2017;2(1):54-56.

Taifta, cloth dye and paints were considered as one of the Sudanese colours that was extracted from Sudanese rocks; it used to colour traditional handworks such as Palm Sunday (local name; saaf), prayers carpets while the second dye used to stain clothes in addition to the third one ElMohandis paints (water base painting) that used for synthetic enamel and emulsion paints. All those dyes were used in this study to stain some bacteriological smears in addition to blood films to study the possibility of using these stains in the future to stain some medical samples in case of safe stain, ruler areas to avoid travels cost, good staining, availability of stain, and low cost too.

In this study, we have used different staining colours with the different procedures to study the possibility of staining of some biological samples with those traditional Sudanese dyes; beside ability to uses those stains in the future to stain other kind of biological samples such as histopathological samples, in cosmetics, medical tabs and also to colour food.

Key Words: *Taifta, S. pyogens, K. pneumoniae, Coomassie blue.*

Staining is an old procedure that humans used from the beginning of life until now by different techniques for different uses, but in this study is concerned about medical stains especially local traditional stains such as taifta, paints and fabric paints to stain some biological samples such as bacterial smears and human blood films as a way to use these traditional stains in the faraway laboratories in rural areas.

The importance of staining technique in the medical fields

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image, stains they were frequently used in biology and medicine to highlight structures in biological tissues for viewing muscle fibers or connective tissue, cell populations such as different blood cells, organelles within individual cells, DNA, proteins, lipids, carbohydrates compound, to mark cells in flow cytometry, and to flag proteins or nucleic acids in gel electrophoresis, lamellar structures of semi-crystalline polymers or the domain structures of block copolymers (1,2).

Staining can be simple that's contains only one stain/dye, or complicated that contains more the one dye or stains, such as; counterstaining or negative staining, differential staining, or both. Also, staining can be used *in vivo;* which is the process of dyeing living tissues (vital stain) or *in vitro* staining that used to colour cells or structures that have been removed from their biological context (1,2).

Most common used laboratory stains

1) Romanowsky stain that's named after the Russian physician Dmitri Leonidovich Romanowsky (1861–1921), who invented it, in 1891. It based on a combination of eosinate (chemically reduced eosin) and methylene blue (sometimes with its oxidation products azure A and azure B for staining and examination of blood or bone marrow films (1,3,4).
2) Gram stains named after the Danish bacteriologist who originally devised it in 1882 (published in 1884), Hans Christian Gram, it is one of the most important staining techniques in microbiology. It is almost; always the first test that performed for the identification of bacteria because it separates almost all bacteria into two large groups: Gram-positive bacteria that stain blue and the Gram-negative bacteria that stain pink (2,5-9).
3) Others laboratory staining are: Endospore staining, Ziehl-Neelsen stain, Haematoxylin and eosin (H&E) staining, Papanicolaou staining or pap stain, PAS (Periodic acid-Schiff) staining, Masson's trichrome, Silver staining, Sudan staining, Conklin's staining, Acridine orange (AO), Bismarck brown or Manchester brown, Carmine, Coomassie blue etc. (1).

METHODS

Preparation of bacteriological smear

Two chocolate agars plates that contained either *S. pyogens* or *K. pneumoniae* were brought out of the incubator for madding two different kinds of smears from both organisms. Dust free slide were brought and passed three times under the benzene burner for sterilization, waiting to cool, a colony from zig zag area was taken by loop then added to slides that contains a drop of normal saline, mix, waiting to dry and fixed by passing through the flame three times for fixations.

Preparation of blood film

Venous blood from cubical vein was collected in EDTA tube after sterilizing the collection area with 70% alcohol; a blood drop was added in dust clean free slide, spreading by spreader at 45 angels, drying and fixation with absolute alcohol for few seconds or until alcohol evaporation.

Preparation of taifta

Two gram from taifta were measured by sensitive balance in clean sterilized containers followed by adding 100 ml of tape water, mix until all stains powered dissolved, now the stain was ready to use.

Preparation of paints

A little amount from paints paste (2 g) were added to clean sterile containers followed by adding 50 ml of tape water, mix then the stain was ready to be used.

Preparation of fabrics colour

A little amount from fabrics colour paste (2 g) were added to clean sterile containers followed by addition of water to complete the volume to 40 ml and mix, the stain was ready to use.

Tafita staining procedure

Eight slides were brought into the staining rack, 2ml from each Tafita were added to each slides, waiting 3 minutes, after that washed the slides with tape water and leave them to dried by air; the same procedure was applied with tafita mix after mixing equal volume from tafita staining solutions or tafita red was added first to slides, waiting 3 minutes for staining, after that washed the slide with tape water, then tafita blue was added for 2 minutes, washed by tape water and drying by air; while for Tafita-zn, tafita red was added to the slides first followed by heating until presence of steam, waiting for 3 minutes, washed with tape water, tafita blue were added for 2 minutes, washed by tape water and drying by air.

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Paints staining procedure

Three Slides were brought to the staining racks, 3 ml from Paints colour were added to each slides, waiting for 10 minutes, washed the film with tape water and drying by air.

For mixed paints coloured, the same procedure as above were applied after mixing equal amount from both red and green paints solutions.

Fabric colour staining procedure

Two slides were brought to the staining racks, 3 ml from Fabric colour were added to each slide and waiting for 10 minutes then washed the films with tape water, drying by air.

RESULTS

Traditional local Sudanese stains showed a good staining results within a different kind of biological samples and organisms, please review the Tables 1 and 2 for more details.

TABLE 1
Illustrate smears and blood films results with traditional stains

Types of stain	Total number of cocci smear	Total Number of bacilli smear	Total number of blood smear	Stain results for cocci	Stain results for bacilli	Stain results for blood film	Total number of slides
Taifta red	5	5	5	Positive red colour cocci	Positive red colour bacilli	Positive with different degree red colour for both RBCs and WBCs	15
Taifta blue	5	5	5	Positive blue colour cocci with little deposit	+ve faint blue colour bacilli	Positive with different degree blue/ green RBCs and WBCs colour	15
Taifta brown	5	5	5	Positive brown colour cocci	Positive brown colour bacilli (less in number and mix with cocci	Positive brown or yellow RBCs colour + red WBCs colour	15
Taifta mix 1	5	5	5	Positive red colour cocci	Unclear/ faint film	Positive red/ violet WBCs colour with blue back	15
Taifta-zn_2	5	5	-	Positive red colour cocci with blue back ground	Unclear film/ faint blue bacilli	-	10
Black dye	5	5	-	Positive black colour cocci	Positive black colour bacilli	-	10
Red paints	-	5	5	-	Positive red colour	Positive pale red or yellowish RBCS Positive to violet WBCS colour	10
Green paints	-	5	5	-	Positive green colour bacilli	Positive pale red or yellowish RBCs + green WBCs colour	10
Mix paints-3	-	-	5	-	-	Positive yellowish or pale red RBCS + red to violet WBCS colour	5
Red fabric colour	-	-	5	-	--	Positive red RBCs	5
Blue fabric colour	-	-	5	-	-	Positive Blue RBCs Positive violet WBCs	5
Total	30	40	45				115

1: Taifta mix (red+ blue),

2: Taifta - zn (staining procedure similar to Zn stain), 3: Mixed paints (red + green).

TABLE 2
Illustrate traditional stains quality

Types of stain	Quality of stain for cocci	Quality of stain for bacilli	Quality of stain blood film
Taifta red	Good	Good	Good
Taifta blue	Good + little amount of deposit	Faint	Neither good nor bad + blue or green background make it the film difficult to interrupt
Taifta brown	Good	Good	Good
Taifta mix	Good	Faint	Good + blue background
Taifta-zn	Good	Faint	-
Black dye	Good + moderate amount of deposit	Good	-
Red paints			Good
Green paints		-	Good
Mix paints	-	-	Good
Red fabrics colour	-	-	Neither good nor bad
Blue fabrics colour	-	-	Neither good nor bad

DISCUSSION AND CONCLUSION

Dying and staining procedure were considered the most important techniques in medical filed, industries, foods, decoration, cosmetics and artist, here I was concerned for the first one; the medical filed specially laboratories due to ability of dye for staining different kinds of cells, tissues and organisms; as I mentioned before the main aim of this study is to study the possibility of staining some biological samples by using traditional Sudanese dye/stains; according to these, I found that, traditional Sudanese dye can be used to stain some biological samples with a good results except in some cases with gram negative bacteria it can gives a good stain or faint coloured sometimes; according culture old, ability of bacteria to change the PH in the medium, or may be due to absence of buffer that can fix the PH of staining solution nor the staining times was too short and needs to be prolonged with gram-negative bacilli another things are that; absence of substances that can enhance taken up of stain by organism or may be due to bacteria itself, but those things nearly were not happens with a blood films instead of presence of different kinds of blood cells with a different charges inside the cells; further more blue tafita was made a very strong background colour that makes differential blood films very hard to distinguish between different kinds of blood cells, fabrics coloured when compare with tafita and paints showed neither good nor bad blood film staining technique that is

may be due to thickness of blood films or its unsuitable to staining biological samples, now I tried to use tafita with malaria parasite but it needs more times to cover all malaria parasite stages.

ACKNOWLEDGEMENT

The author would like to thank Allah, her family for always supporting her, especially her father and also The National Ribat University; microbiology department members.

STATEMENT OF COMPETING INTERESTS

The authors declare that they have no competing interests.

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