Novel 'half-and-half' design of staining jars for comparative assessment of stains

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Early-stage innovation report

Novel 'half-and-half' design of staining jars for comparative assessment of stains

Sanpreet Singh Sachdev , Tabita Joy Chettiankandy

Oral Pathology and Microbiology, Government Dental College and Hospital, Mumbai, Maharashtra, India

Correspondence to
Dr Sanpreet Singh Sachdev, Oral Pathology and Microbiology, Government Dental College and Hospital, Mumbai, Maharashtra, India; sunpreetss@yahoo.in

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CONTEXT

At present, Coplin jars are used universally for histological staining. They are grooved glass jars which allow microscopic slides to stand separated while staining procedure. They were invented by William Coplin in 1897 and remain in use to date.[1] Except for a few modifications such as use of plastic materials, or screw-capping to avoid evaporation or contamination, the design of Coplin jars has remained mostly unchanged.[2]

Many times, situations arise in histopathological laboratories wherein multiple special stains are to be applied on the tissue sections for histochemical confirmation of the nature of cells or material.[3] The scenario is fairly common in researches that compare two or more histological stains.[4] Certain study designs also revolve around comparing the effect of fixation or embedding on staining characteristics.[5] Demonstrating suitability of histological stains in detecting microbes, pigments, or tissue elements such as muscle, cartilage, etc aids in making the diagnostic pathological procedures more accurate and efficient.

In such scenarios, the extent of materials such as microscopic slides, cover slips, staining dyes and other chemicals required for the procedures correspond to the number of samples to be stained. The need may not seem significant in routine cases, but the financial toll is amplified exponentially when a large number of samples are involved in studies. Many laboratories, particularly in low-income and middle-income countries, are not able to afford such exuberant expenditure and are ultimately unable to conduct such large-scale comparative studies due to non-feasibility.[6] They either refrain from conducting the study or select a smaller sample size. A smaller sample size ultimately makes the finals results of their study less valid.

The chemicals and materials required for research may also be difficult to procure at times. The problem is fairly common in rural areas of low-income and middle-income countries, such as India. The problem was even further amplified in COVID-19 pandemic, wherein much of the funds were focused on countering the pandemic. There was a decrease in manufacturing of laboratory materials, and their supply to institutions was cut-off temporarily.[7] The lockdown measures

Summary box

⇒ The novel design of half-and-half' staining jars allows simultaneous staining of two halves of a slide with different stains. It would, therefore, minimise the time-dependent variation in the staining procedures by enabling both to be conducted synchronously.
⇒ Similarly, they allow a more easy comparison between two stains or staining procedures since the sections are on the same slide.
⇒ They also halve the number of slides needed in a comparative study. To some extent, they also aid in preserving the chemicals. These qualities are extremely beneficial in developing countries in which researchers are unable to pursue research projects with large sample size.
⇒ The jars could possibly be set as a standard in future, particularly when comparative studies in histopathology are to be conducted.

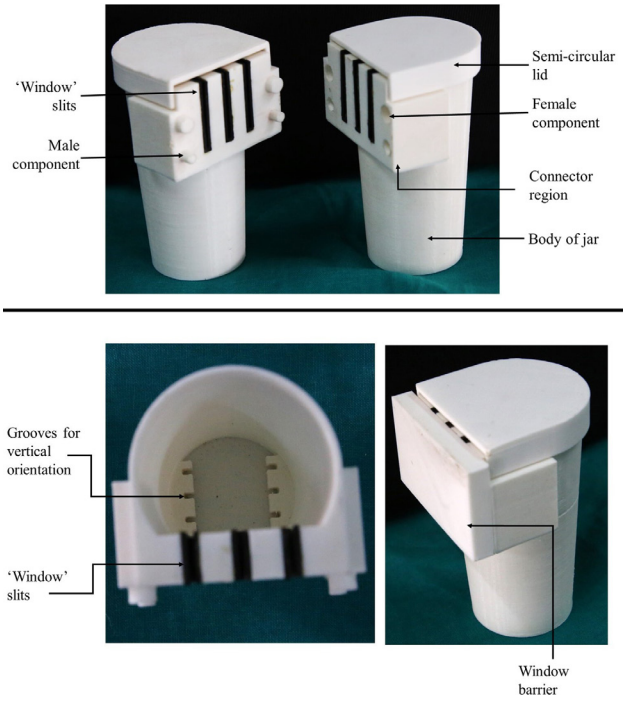

Figure 1 Components of the novel half-and-half staining jars.

made it more difficult to procure the materials required for research projects.

An additional problem that arises in such study designs, is the variability of staining characteristics for different batches depending on the environmental factors. Climatic factors such as temperature and humidity may influence histopathological procedures such as embedding, sectioning, deparaffinisation and ultimately alter the staining, directly or indirectly.[8] These factors may also vary depending on the time of the day during which the procedure is conducted. This would result in variation in the results produced among different batches of staining, even though the same procedure is followed. Therefore, the results produced by comparative studies in which the procedures are performed at different times may be unreliable to some extent.

To overcome these drawbacks, we developed a new 'half-and-half' design of staining jars by incorporating certain modifications in the standard Coplin jars.

THE DESIGN OF 'HALF-AND-HALF' STAINING JARS

The design is registered in Indian Patent Office under the number: 344808-001. Principally, the assembly comprises a jars that have three window slots on one side (figure 1). The jars either have a projection 'male' component or a space 'female' component of corresponding shape and size. The jars can be joined tightly by fitting together 'male' and 'female' components. Once joined, the jars form a connection in between, wherein the windows of one jar align with those of the other (figure 2). Microscopic slides can be inserted in the slots present in the connecting region such that

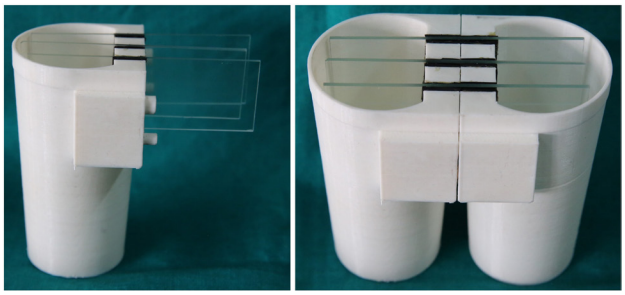

Figure 2 Assembly of jars with glass slides placed in the 'window' slits.

each half of the slide lies in either of the respective jars. The slots are equipped with rubber gaskets of customisable diameter to ensure snug fit of the slides, completely restricting any leakage of the stains from one jar to another.

A maximum of three slides can be stained at a time. However, when less than three slides are to be stained, a blank slide must be inserted in the empty slots, to completely avoid leakage from the connection between the two jars. The lids of the jars are semicircular and are inserted by sliding motion. The diameter of jars is slightly larger towards the top along one perimeter, allowing a tight friction grip for the lids.

The jars are filled with staining solutions only up to the level below the windows. The slides with sections are inserted in the slots in such a way that one section is present in either of the jars. The lids are then closed, and the jars are inverted. The staining solution is now present in the 'window' half of the jars. However, the slides present in the connected slots of the jars do not allow any leakage of the staining solutions from one jar to another. Furthermore, the lids do not allow leakage of the staining solutions from over the connection. The entire working process of jars with end result produced are depicted in figure 3.

Overall, a closed compartment is produced in both the jars, in which the sections take up the respective stains. Once the desired time is elapsed, the jars can be reverted back, which allows the fluid to return to the base of the jars. The lids can now be removed, followed by the slides. In case, if the staining procedure is to be continued using different chemicals, the jars can be separated and the corresponding jars (male or female) filled with the desired chemicals can be put in place. The staining procedure may now be continued by again inverting the jars with new chemicals. Once the staining procedure is completed, the jars are reverted, followed by removal of lids and the slides.

Conventionally, simultaneous staining of two halves of a slide has been performed by dispensing stains by means of by droppers. However, the solutions dispensed by droppers may not always have uniform concentration of staining dyes. The staining dye tends to be darker towards the periphery of the dispensed solution, making the staining uneven. Furthermore,

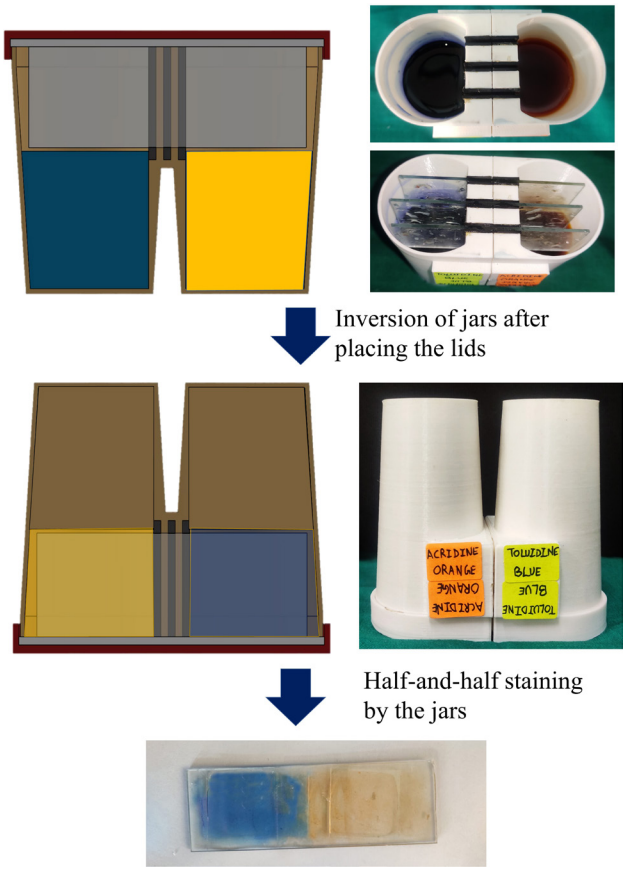

Figure 3 Working Principle of the jars with the produced result.

the staining rack needs to be absolutely plane. Any inclination would change the concentration of dyes by movement of fluids, changing the intensity of staining across the slide. The chemicals also evaporate over time. The dispensed chemicals may get contaminated in the open and are generally washed off once the prescribed time for staining is completed. This further adds to the wastage of chemicals.

A scenario may occur where one of the staining procedures is completed while the other is yet remaining. Should such a need arise, the jars may be separated, and the slides are now inserted vertically in the respective staining jar. The jars contain three grooved slots at their base for holding the slides vertically, similar to a conventional Coplin jar. The section to be stained must be towards the base of the jar. Since the staining solution is present only in the lower half of the jar (below the window level), the other section will be unaffected. Care must be taken, however, that the tissue section not in the fluid does not dry out due to a prolonged procedure.

In addition, the jars are provided with a barrier that allows closing of the windows, allowing a jar to be used singly; similar to a conventional Coplin jar. Thus, when both the sections are to be immersed in the same chemical, or conventional staining is to be carried out, the slides can be inserted vertically and stained with the barrier in place.

ADVANTAGES OFFERED BY THE NOVEL DESIGN

1. The environmental influences on the staining characteristics are minimised by carrying out both the methods simultaneously. Therefore, a more valid comparison can be made between two stains, staining procedures, fixatives or embedding techniques.
2. Allows simultaneous assessment of two different stains on a single slide.
3. The number of glass slides required is halved, which is particularly beneficial in studies involving a large number of samples to be stained.
4. Much less chemicals are used as compared with conventional Coplin jars or dropper methods, reducing their contamination and wastage.
5. The jars can function both, as conventional Coplin jars for routine/conventional staining, as well as half-and-half jars for comparative/simultaneous staining.

CONCLUSION

The 'half-and-half' staining jars allow a more valid comparison of staining characteristics by minimising the time-based environmental influences on the histopathological procedures. They also make the study designs more feasible by reducing the materials and chemicals required. The novel design could be considered as a standard part of protocol for comparative histopathological studies instead of conventional Coplin jars.

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ORCID iD
Sanpreet Singh Sachdev http://orcid.org/0000-0001-7655-8180

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