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Review Article

Contemporary art of cell-block preparation: Overview

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ABSTRACT

Cell blocks (CBs) are paraffin-embedded versions of cytology specimens. These versions are contrasted with tissues made from surgical pathology specimens of formalin-fixed paraffin-embedded (FFPE) tissue. CBs enable various elective ancillary studies of a range of specimens. These studies include the potential to perform molecular tests with the enhanced cytopathological interpretation. CBs are increasingly reported in cytology specimens. The enhanced role of CBs incorporates additives with new markers for immunohistochemistry (IHC), including the multicolored approach to IHC, and the subtractive coordinate immunoreactivity pattern. Even when archived material is retrospectively retrieved, CBs are a major tissue source for many supplementary studies. The CBs have been qualitatively and quantitatively improved. CBs are significant since they have increased molecular markers standardized on FFPE tissue. High-quality CBs can serve as useful additions to cytological smear preparations and touch imprint cytology. Most cytological specimens, such as fine-needle aspirations, cavitary effusion, washings, brushings, and gynecological and non-gynecological liquid specimens, may be used to produce CBs. This review deals with the CB-making process and discusses various historical limitations with an emphasis on recent advances.

Keywords: Cell-block, Cell blocking, Fine Needle Aspiration, FFPE

INTRODUCTION

Cell block (CB) is a processing method used to create a "button" in cells, which is then processed as histopathology. The CB processes routine cytology samples, such as fine-needle aspiration (FNA) and fluid samples. The CB provides additional techniques for cytological slides prepared from cavitary effusions, needle aspirates, and liquid-based cytology (LBC). Instead of placing a sample on a slide, the LBC takes a sample, transfers it to a transport medium, and processes it for inclusion in a slide. In 1896, traditional CB techniques were first described with collodion (nitrocellulose) bag techniques. However, it was only in 1947 that these techniques became widely used.[1,2]

Specimens of cytology can be used for preparing CB-embedded paraffin material. The process is called CB preparation. This terminology can be further simplified as the CB-making process. The tissue blocks of surgical pathology specimens that are formalin-fixed and paraffin-embedded (FFPE) are similar to the CBs. They enable performing a variety of tests, including molecular tests, immunohistochemistry (IHC), and special stains for the detection and confirmation of various microbial and deposit types. They facilitate performance. However, in the research literature, the term "cell block" usually refers to prison cells. Any attempt to find information on the internet, as a result, is typically focused on "prison cells," with only a few cytopathology-related searches.[3]

CBs have already been established in cytopathology. However, as new IHC markers and technical advancements, such as those in multicolor IHC and the subtractive coordinate immunoreactivity pattern approach, are made the role of CBs continues to grow.[4] Many new molecular markers in the tissue of FFPE are also standardized. All these tests could be performed on CBs that are properly prepared. Since the CBs can be filed, the retrieved material is available if new testing is implemented when the tumor diagnostic material cannot be obtained.[5-8]

The significant role of CBs in tissue diagnosis protocols should be highlighted. These protocols are continually improved for excellence in patient care. With scientific efforts, the complexity of this science, at the qualitative and quantitative levels, could be examined for potential innovation. Cell-blocking science is evolving for the study of chemical science and quantitative and qualitative CB development. The evolution of this science allows for enhanced patient care.[3] A further benefit associated with improved sampling by the CB includes the assessment of diagnostic architecture patterns, such as gland configurations, psammoma bodies, and stromal invasion.[9-11]

CELL-BLOCKING METHODS

CBs are important when treating cytology specimens. The main goal is to use the best methods for cytology preparation so that diagnostic components in cytological specimens are evaluated by cytomorphological evaluation by the laboratory policies. It is advisable to process the remaining specimen – including the coagulated component – by cell blocking. Many approaches for CB preparation have been previously used.[12]

The residual material for the cytological specimen is sedimented before being added to the medium and processed and embedded as FFPE. Numerous challenges may arise, depending on the methods, including questions about the procedure itself. Doubts can arise from an indiscriminate approach without proper control of cell diagnostic cell spreading, where qualitative interference due to exposure to several fixatives or reactive substances cannot be reproduced. Because of these problems, the common random and indiscriminate CB-processing methods often compromise the quantitative and qualitative completeness of various CB components.

For more than a century, various CB techniques have been used. In the diagnostic work of patients with FNA-aspirated masses, CBs have been widely used as diagnostic information, complementing FNA smears.[13] The CB technique was more suitable for immunocytochemical analysis and the pap-stained FNA smear was the best method for routine diagnosis due to the superior conservation of the nuclear and cytoplasmic properties.[14] The samples from CBs work best when added to immunocytochemistry (ICC) rather than as the sole source of cytological diagnosis.

Delay in immersion in fixative CBs immediately following collection and variation in FNA techniques between personnel can be the cause of cell degeneration in the CB samples. This technical variability can mean that sufficient or insufficient CB samples are achieved, depending on the aspirant's ability and the high cellularity of the aspirator.[15,16]

Hanley *et al.* (2009)[17] have described preserving the antigenicity of tumor cells for accurate ICC analyses. It is crucial to use optimum fixative parameters. The fixative and tissue processing schedules used are considered appropriate when optimum preservation is noted in the remaining samples of the CB.

A well-prepared CB could be obtained from hypocellular specimens using Shandon's cytoblock cell preparation system. Varsegi and Shidham (2009)[18] have designed an enhanced technology for CB preparation and cell capture that could be useful for the existing method. The Varsegi and Shidham technique increases the possibility of capturing cells dispersed by *HistoGel*™ (HG; Thermo Shandon) individually. This capture prevents the histotechnologist from cutting into the block too deeply, risking the key area, and missing the cells of particular interest. Despite statistical insignificance, collected samples for the technique of cell blocking could be disadvantaged by not receiving an original, specific suction, which could have affected cellularity. To improve output, it is important to explore material from several dedicated CB needle aspirations.

The Shidham's method

Traditional CB-making techniques lack reproducibility. This lack of reproducibility is problematic because diagnostic cells are indiscriminately distributed across CBs without any control of the histotechnologist's paraffin-block-cutting depth. Recent developments have included efforts to improve the quantitative and qualitative measures for CBs. Some of these enhancements can achieve quantitative improvement but the qualitative integrity of formalin-fixed surgical pathology specimens may not be comparable to FFPE.[19,20]

Shidham is a standardized method used for these enhanced features, which provides histotechnologists with an AV marker to spot the final paraffin-embedded block's cutting depth as a dark-colored guide beacon.[18] Substantial skill, however, is needed for this practice, which may be difficult to adapt to the cytology laboratory's routine workflow. It is desirable to develop commercially available kits that are ready-to-use, cheap, and convenient. These kits should include a precisely set, built-in AV marker, along with all the benefits of the originally published Shidham method.

Cell tube blocks

The cell tube block technique involves inserting the material into a simple microhematocrit tube with a clay-covered hole. Giotto clay (Giotto Pongo, Fila Hispania, Barcelona,

Spain) tends to dissolve when fixed with formalin. The authors, therefore, recommend the use of Jovi clay (Jovi Plastilina, Rubi, Spain), which is a modeling clay that is resistant to xylene. Before adding the microhematocrit tube, centrifugation (2,000 g for 5 min) may be used to concentrate the sediment if the sample is of a low volume. The microhematocrit tube is filled about three-quarters full to boost the recovery of the sample.

By rocking the tubes in a parallel position, a small volume of air is added, and about 10 μL is then added to Percoll or Ficoll. The sample creates a bubble between the Percoll and Ficoll. Clay is stitched around the tube before it is centrifuged at 14,500 g for 5 min. The tube is broken after centrifugation and marked close to the liquid-air interface. The surrounding capillary tube and cellular component of the sample are preserved in 10% buffered formalin for 24 h. The sample is then extruded into the paraffin block using a clip of paper from a capillary tube.

All cells, from red blood cells to nuclear cells, are horizontally embedded in each section. A single block provides about 100 sections that are 5 μm thick and suitable for special and IHC stains. The ability to separate nucleated cells from red blood cells and the ease with which blood-contaminated samples can access sample treatment materials are two benefits of this technique.[21]

Needle rinse method

In the needle rinse method, 20–30 mL of normal saline, formalin, paraformaldehyde ethanol, or a special medium such as Roswell Parks Memorial Institute medium, are sequentially rinsed with the needle for sampling and centrifugation.[22,23] Pre-fixing rinses with balanced saline may provide ancillary testing flexibility.[24] Alternatively, it is possible to rinse the needle contents with 4% buffered paraformaldehyde, which provides better ICC results than the 7.5% buffered formalin initially proposed.[25,26]

After needle rinsing, cells are pelleted for 10 min at 1,000 revolutions per minute (RPM) in a soft plastic and tapered centrifuge tube. The supernatant is decanted, and the sediment is filled with 70% ethyl alcohol. At 1,000 RPM, the sediment is restarted and centrifuged again for 10 min. This step is repeated before the sediment is removed with a spatula. After brief xylene processing, the sediment is wrapped in lens paper and placed in a tissue cassette. The main drawbacks of this approach are the potential loss of specimens and the potential harm of ethyl alcohol fixation for IHC.

Tissue clot method

Two well-defined approaches are available for the tissue clot. The material is permitted to coagulate in the needle lumen. It is then pressed with a syringe or a wire from the needle and collected on filtered paper with a tight circular movement to build a cone. This cone can then be slightly dried to guarantee the congealment of the coagulum. The coagulum is then carefully wrapped in paper tissue and transmitted as a histological sample to the formalin.[23]

The second method for collecting effusion samples is more documented. This method can also be used to collect FNA samples in ordinary saline, following needle rinsing. In both cases, the cell suspension is centrifuged and the supernatant is discarded. The sample is replenished in 0.1 mL of pooled plasma, followed by 0.2 mL of reconstituted thrombin at room temperature, and is mixed well quickly.[27-29]

When no clot forms after 5 min, an extra 0.2 mL of thrombin may be added.[29] The coat is then slid over and wrapped in a piece of filter paper. The pellet is finally embedded in paraffin after being fixed in 10% neutral buffered formalin (NBF). Some reagents (such as Simplastin Excel S, BioMerieux, Durham, NC, USA) prepared in rabbit lungs or brains may contain epithelial cells that lead to incorrect interpretations. It is advisable to use cell-free thrombin.[30] Some authors recommend that the pellet is stabilized using other media because plasma or thromboplastin may cause cross-reactions when stained with human proteins.[31,32]

CB embedding material

There are a variety of materials for embedding concentrated cell matrixes, such as agar, HG, gelatin albumin, collodion sacks, pregelatinized starch, sodium alginate, gelatin foam, and acetone-melted paraffin. There are many types of materials available.[18,33-44] The concentrated sample (pellet or sediment) is physically supported by embedded material before paraffin embedding. The collodion bag or Shidham method is recommended for low-cellularity samples. Materials commonly used are discussed in the following sections.

Agarose gel method

In the agarose gel method, the sample is discharged into a 70% ethanol tube from Eppendorf with centrifugation following conventional FNA (2,817 g for 10 min). The material is centrifuged once more (2,817 g for 10 min) after decanting and adding 2% liquid agarose gel. After being embedded in paraffin, the pellet is processed as a histological standard. This technique is used in veterinary medicine to preserve architectural designs, which provides an example of how to use CBs for additional testing (cytochemical, ICC, molecular, and proteomic analysis).[33]

Histogel™ Method

HistoGel (Thermo Fisher Scientific, Inc., Waltham, MA, USA) is an aqueous processing gel that is non-fixed, inert, and can be used on both unfixed and formalin-fixed tissues. Paraffin is incorporated into the sample and HG mix, which allows for a double-embedding process. *HistoGel* has been used

to produce biopsies of friable tissue in veterinary medicine; biopsies for specially oriented, highly mobile fluids; urine sediments;[2,45] bone marrow; and peripheral blood samples. The reports by Chapman and Whalen (1947)[2] and Craig *et al.* (2008)[45] show that the samples have excellent cytokeratin, vimentin, glial fibrillary protein, and muscle-specific actin immunosuppression. The benefit of HG is that it uses an easily accessible medium and does not require any special tools or treatment once the tissue has been integrated. However, it is not certain that it is useful in low-cellular fluids. An alternative approach is recommended for low-cellular samples.

Histogel™ with Shidham's method

The HG with Shidham's technique modifies the standard approach to HG. The purpose of this technique is to process samples with low cellularity and particularly samples with individually dispersed cells or small groups of cells. This method is based on liquid, low-cellularity human cervicovaginal specimens and is optimized for them. It can also be applied to non-gynecological samples, such as effusions, FNAs, brushed matter, and cyst contents. By producing a pellet that the histotechnician can section more effectively, this technique compensates for samples with low cellularity.[18] Concentration cells are parallel to the section plane and level markings of the paraffin. This concentrates cells so that the histotechnician's cut is not too slender or too deep in comparison to the cells. The specifications for this modified version of Shidham's are flat-bottom glass tubes and a swiveling centrifuge, as opposed to traditional HG techniques.[18]

Gelatin foam method

For the gelatin foam technique, the sample is centrifuged in a conical 1.5 mL tube, and the cell sediment is saved. The top of the sediment is then covered with a piece of gelatin foam that is approximately 4 mm by 4 mm and is prodded to encourage absorption. The gel foam is then covered with methanol for 30 s, which "seals" the cells into the foam and causes the foam to dislodge from the tube. With successful results, CBs without methanol have been created. The foam is fixed in 10% tamponized formalin for at least 6 h after the methanol has been removed. The gelatin foam then becomes a normal specimen of histology.[46]

Collodion cell bag method

The nitrocellulose-based film is used to create collodion cell bags inside a 10–15 mL test tube. The sample is sealed with either 90% alcohol or 10% formalin once the material used to make the bag inside the tube has solidified. The sample is centrifuged for 10 min at 3,000 RPM, and the supernatant is discarded. The excess bag material is carefully cut off after the sediment bag is twisted over the sediment and removed from the tube. To make it simpler to identify the cell pellet, the bag is whirled into a 1% alcohol-eosin solution before being processed as a histologic example.[47]

Several other techniques

The alginate (sodium alginate/calcium chloride) CB-making technique is gaining popularity in several centers, especially in Asian countries. It involves the use of sodium alginate and calcium chloride to create a gel matrix that can encapsulate cells for further analysis. To begin, the cells of interest are harvested and washed to remove any residual media or enzymes. Sodium alginate powder is then dissolved in sterile distilled water or buffer to create an alginate solution. The cell suspension is mixed with the alginate solution to ensure uniform distribution of cells. The resulting mixture is drawn into a syringe and extruded dropwise into a calcium chloride solution. The calcium ions in the solution react with the alginate, causing it to crosslink and form gel beads that encapsulate the cells. After allowing the beads to harden, they are washed to remove any residual calcium chloride or alginate solution. The washed beads are then transferred to a mold or embedding cassette for further processing, such as dehydration, paraffin embedding, sectioning, and staining. This technique provides a platform for downstream histopathological and molecular studies [Table 1].[48]

FIXATIVE MATERIALS AND METHODS

The preparation of CBs in cytopathology involves the use of various fixative materials and methods to ensure proper preservation of cellular morphology and antigenicity. NBF is widely considered to be the universal fixative for CB preparation. NBF fixation involves immersing the cytology specimen in a solution of formaldehyde buffered with a neutral pH buffer. This fixative provides excellent preservation of cellular architecture and facilitates subsequent histological and IHC analysis.

In addition to NBF, other fixatives are employed in specific situations. For example, alcohol-based fixatives, such as methanol or ethanol, are commonly used in LBC preparations, such as pap smears and urine specimens. These fixatives aid in cellular preservation and can be compatible with certain molecular techniques. Nathan alcohol formalin substitute consists of a mixture of 100% ethanol and 40% formaldehyde and is a less toxic fixative alternative, which offers comparable fixation capabilities. The choice of fixative depends on the specific requirements of the downstream analyses. The preferred format for many ancillary testing platforms is FFPE tissue. These testing platforms include IHC, fluorescence *in situ* hybridization, and molecular genetic testing. Formalin fixation provides optimal antigen preservation and allows for long-term storage of specimens. However, non-formalin fixation methods, such as alcohol-based fixatives, may affect antigenicity and require validation for immunocytochemical and molecular techniques.[49]

Vohra *et al*. (2016)[50] have demonstrated a high concordance between FNA CBs and paired histologic specimens in the expression of the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2).The authors reported a concordance rate of 95.5%, 95.2%, and 97.0% for ER, PR, and HER2, respectively. These results indicate that immunostaining performed on CBs can provide reliable and accurate information on the molecular characteristics of breast cancer, comparable to that obtained from histologic specimens. The high concordance observed in this study supports the utility of FNA CBs as a valuable tool in the diagnostic and prognostic evaluation of breast cancer.

SUITABILITY FOR MOLECULAR TECHNIQUES

Several studies have highlighted the advantages of using CBs for molecular analysis. For instance, CBs offer a higher cellular yield compared to LBC preparations, enabling the retrieval of adequate material for DNA or RNA extraction. The preserved cellular architecture in CBs facilitates the interpretation of molecular results in conjunction with histomorphological features. The paraffin embedding process enhances the stability of nucleic acids, allowing for prolonged storage and enabling retrospective analysis. It can be emphasized that, since the CBs can be filed, diagnostic material is readily available. Additional cytopathology material offers many advantages over a relatively low-cost, minimally invasive procurement. The CBs are mainly concentrated, with no significant proportion of stroma in diagnostic tumor cells. In comparison, tissue biopsies frequently contain stroma, a non-tumor tissue component, which could complicate molecular testing. Therefore, rather than using core biopsies, properly prepared CBs should be used for molecular pathology testing.[51]

In a study conducted by Lin *et al*. (2019),[52] the authors assessed the utility of CBs for epidermal growth factor receptor (EGFR) mutation testing in lung cancer.They reported a concordance rate of 96% between the CB samples and corresponding surgical specimens, demonstrating the reliability of CBs for molecular analysis. Another study by Roy-Chowdhuri *et al*. (2017)[53] evaluated the use of CBs for next-generation sequencing (NGS) in solid tumors.The authors found that CBs provided sufficient DNA for NGS analysis, yielding accurate and clinically relevant molecular profiles.

Table 1: Highlights the differences between the various cell block-making techniques.

Technique	Description	Advantages	Disadvantages
Shidham	This technique involves mixing cytological material with plasma-thrombin mixture, allowing it to clot and form a solid cell block. The block is then processed, embedded, and sectioned for analysis.	- Retains architectural features of cells and tissues. - Allows immunohistochemical staining.	- Requires expertise in preparing plasma-thrombin mixture. - Time-consuming process.
Agarose Gel	Cells are mixed with a warm agarose gel solution, which solidifies to form a gel matrix. The gel containing the cells is then processed, embedded, and sectioned for examination.	- Provides good structural preservation of cells and tissues. - Facilitates immunohistochemical staining.	- Gel formation may cause distortion of cellular morphology. - Requires special equipment for gel preparation.
HistoGel™	HistoGel™ is a water-soluble gelatinous substance. Cells are mixed with HistoGel™, which solidifies on cooling. The resulting gel block is processed, embedded, and sectioned for analysis.	- Easy to use and handle. - Preserves cellular and tissue architecture. - Compatible with immunohistochemical staining.	- May cause distortion of cellular morphology. - Limited availability and higher cost compared to other techniques.
Gelatin Foam	This technique involves mixing cytological material with a gelatin solution, which is then whipped to create a foam. The foam is allowed to solidify, and the resulting foam block is processed, embedded, and sectioned for examination.	- Provides good structural preservation of cells and tissues. - Allows immunohistochemical staining.	- Foam formation may cause distortion of cellular morphology. - Requires special equipment and expertise for foam preparation.
Collodion Cell Bag	Cells are mixed with a collodion solution, which forms a thin film when spread on a glass slide or surface. The film is then peeled off, resulting in a cell sheet. The sheet is processed, embedded, and sectioned for analysis.	- Retains cellular and tissue architecture. - Easy to handle and process.	- May cause distortion of cellular morphology during the peeling process. - Requires expertise in preparing and handling the collodion solution.
Alginate	Cells are mixed with an alginate solution, which forms a gel when exposed to calcium ions. The gel containing the cells is then processed, embedded, and sectioned for examination.	- Provides good structural preservation of cells and tissues. - Compatible with immunohistochemical staining.	- Gel formation may cause distortion of cellular morphology. - Alginate may interfere with some histological stains.

However, the use of CBs in various molecular techniques may exhibit challenges. One limitation is the heterogeneity within the tissue sample. Different areas of the CB may contain varying proportions of different cell types, which could affect the accuracy of molecular analysis. Tissue-processing artifacts, such as folding, fragmentation, or loss of cellular morphology, can occur during the embedding and sectioning processes, potentially affecting the interpretation of molecular findings. Extracting DNA or RNA from CBs can be more challenging than extraction from fresh or frozen tissue. The limited sample volume in CBs may restrict the number and types of molecular tests that can be performed.[53] A lack of standardization in protocols and the associated costs and time required for CB preparation and molecular analysis are further considerations. However, in some countries, such as India, recommendations for CB preparation, such as the *Effusion Guidelines Committee of IAC Guidelines drafting and finalization committee*, have been incorporated into daily practice.[54] Despite these limitations, CBs remain a valuable tool for molecular analysis in cytopathology. Ongoing research and advancements in techniques for DNA/RNA extraction, standardization of protocols, and quality control measures aim to address these challenges and further enhance the utility of CBs for molecular diagnostics.

COST AND TIME CONSIDERATIONS

When comparing various methods for preparing cytopathology CBs in terms of cost and time considerations, several factors come into play. The plasma thrombin method is relatively cost-effective, requiring minimal additional equipment and reagents. However, the process of clot formation can be time-consuming, taking several hours. Formalin fixation using NBF is widely used and cost-effective. While the cost of NBF is relatively low, the standard processing schedule typically takes 6–24 h. The Shidham method overcomes the majority of issues concerning the qualitative and quantitative integrity of the final CBs. This method is, however, intensive in terms of work and skill and can be difficult to practice in the routine laboratory of clinical cytology since the home protocol has been developed. Celloidin embedding, which uses a gelatinous matrix, may have higher initial reagent costs but provides good preservation of cellular morphology. The processing time for celloidin embedding is shorter than for formalin fixation. The Cellient system, an automated method, offers increased efficiency, but it incurs higher initial equipment costs. However, the overall time required for CB preparation using Cellient is significantly reduced. *HistoGel*, a commercially available matrix, is cost-effective and has a relatively short processing time. Specific cost and time considerations can vary depending on laboratory workflows, sample volume, and downstream processing requirements.[55,56]

SUMMARY

Cell blocking can be carried out on almost any specimen containing loosely dispersed cell and tissue fragments. For various elective auxiliary studies, such as IHC and molecular tests, including markers for targeted therapy and predictive biomarkers, CB is an excellent FFPE tissue resource. Any method may be used to treat the sediment-rich specimens. Any CB should, however, be prepared with precaution to prevent fastening and processing interference. For the results to be compared to those obtained with FFPE surgical tissue, the procedure should mimic a FFPE protocol to ensure the highest possible quality and integrity of diagnostic cells.

CBs require a longer turnaround time than FNA, and not all of them have the cellularity or volume necessary for this technique. The regular use of CBs may be constrained by the expense and excess material required to produce a good-quality cell pellet. However, CBs offer distinct advantages for supplying histological specimens and ancillary test material. These advantages allow for the introduction of more laboratories as part of routine practice.

In diagnostic cytopathology, the role of CB preparation is extremely important because it enables numerous ancillary studies and renders a more accurate cytological interpretation. The methodology can be further improved by shortening the time between the collection and fixation of the samples and standardizing the FNA technique used by staff. Direct FNA and CBs are mutually complementary and both are required to assess morphology and for the best immunocytochemical outcomes in patients' diagnostic work.

ABBREVIATIONS

CB – Cell block
ER – Estrogen receptor
FFPE – Formalin-fixed and paraffin-embedded
FNA – Fine-needle aspiration
HER2 – Human epidermal growth factor receptor 2
HG – HistoGelTM
IHC – Immunohistochemistry
LBC – Liquid-based cytology
NGS – Next-generation sequencing
PR – Progesterone receptor
RPM – Revolutions per minute
SCIP – Subtractive coordinate immunoreactivity pattern.

AUTHOR CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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CONFLICT OF INTEREST

The author declare no conflict of interest and amongst authors, the author affiliated in King Abdulaziz University and King Abdulaziz University Hospital claims no conflict of interest.

EDITORIAL/PEER REVIEW

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