Original Article

Laboratory Errors In Pathology And Troubleshooting Methods

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Abstract

Pathologists play an important role in the final diagnosis for the patient and help the clinician in appropriate treatment planning. Both clinicians and pathologists have a crucial role in the laboratory process and it should be monitored properly for accurate and timely reporting.

There are many steps in the laboratory process, where errors can occur which can affect the final diagnosis and prognosis for the patient. Errors can be major or minor but it eventually affects the patient's outcome.

The laboratory process is broadly classified into three categories- pre-analytic, analytic and post-analytic phases. The errors that could frequently occur in each phase and the troubleshooting methods are broadly discussed in this article.

Errors that have occurred should be investigated properly by identifying the main source of error and preventive action should be taken to avoid it in the future.

Keywords: laboratory errors, pre-analytic, analytic, post-analytic

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INTRODUCTION

Pathology is the discipline that aims at the most appropriate and timely diagnosis for the patient and to assist the clinician in further treatment planning. However, there are possibilities of several errors that could occur in the laboratory on a daily basis.[1]

This can have a direct effect on histopathological diagnosis which can in turn affect the patient's treatment and prognosis. Therefore it is very essential for the pathologists to be aware of all the possible errors that can occur in the laboratory and the ways to minimize them.

The goal of surgical pathology is to provide complete and correct diagnosis to the right patient. Accurate and Timely Reporting is the key element. This article gives an insight to the possible laboratory errors and the troubleshooting steps that can be done in such instances.

Errors

Errors in the laboratory can be broadly classified into Major errors and Minor errors.

A major error in pathology is an error that can have a major effect on the treatment of the patient that can alter the prognosis of a disease.

A minor error is an error that does not have a major effect on the treatment of the patient or that cannot alter the prognosis of a disease.

Laboratory Errors

Surgical pathology laboratory process is a complex process and it can be broadly divided into three phases - based on the stage of process/test cycle.[2]

Pre-analytic phase

Analytic phase

Post-analytic phase

Errors Related To Pre-Analytic Phase

Clinician error is the major source of error in the pre-analytic phase, and this ultimately impacts as a major laboratory error. Unfortunately, the laboratory team is often held accountable for these errors.[3] The following table describes the various errors that could occur and the ways to minimize them.(Table.1)

Table 1: Table showing the various errors in pre-analytic phase, its causes and troubleshooting procedures

STEP IN LABORATORY PROCESS	ERROR	CAUSE OF ERROR	TROUBLESHOOTING
Specimen selection and labelling	Clinician error	Obtaining specimen from wrong patient Performing wrong surgical procedure Providing inadequate tissue for diagnosis Placing specimen in wrong fixative or without proper fixation Ordering wrong tests Mislabelling samples Inadequate clinical information	Check if the specimen name is identical to that written on the request form Check if the specimen is received in formalin Check for the patient details in biopsy request form
Transport	Clinician error	Specimen loss Environmental factors that cause specimen destruction Untimely or delay in delivery of a specimen Delivery of a specimen to wrong site	Ensure proper transportation in appropriate transport medium
Specimen accession number	Clinician labeling error Incorrect order entry	Assigning a specimen accession number to the wrong patient Misidentifying the site of origin	Proper identification and checking of details Initial rejection of the specimen, if proper details are not provided at the time of receipt .

Analytic Phase

Analytical phase in laboratory process involves all the steps starting from the fixation of specimen to staining. The following table shows the various errors in the analytical phase and the troubleshooting methods.(Table.2)

Table 2: Table showing the various errors in the analytic phase, its causes and troubleshooting procedures

STEP IN LABORATORY PROCESS	ERROR	CAUSE OF ERROR	TROUBLESHOOTING
Grossing	Errors related to grossing	Incomplete or incorrect gross examination Poor description of the sample Poor or incomplete sampling	Note the following gross findings Check if specimen is fixed in formalin Number of fragments of received tissue Colour Consistency Dimensions Cut surface Orientation if required Macroscopic description
	Improper fixation	Specimen not completely covered with fixative	Change with 10% neutral buffered formalin and ensure complete fixation of the specimen
Fixation	Autolysis In H&E section, tissue shows loss or total disappearance of nuclear chromatin	Delayed fixation	The specimen should be placed into fixative, as soon as possible
	Cells characterised by smudged nuclei, indistinct nuclear pattern, nuclear bubbling	Incomplete fixation	Prolong fixation time Thin gross section Fresh formalin solution Cassettes should not be tightly packed Agitation of cassettes in fixation should be prevented
Dehydration	Micro-chatter around the tissue in H&E stain	Over dehydration	Small biopsies should be processed separately Shorten the dehydration time
	Weak or absent staining of cells	Incomplete dehydration - Repeated use of alcohols for longer periods without replacement	No cassette condensation Dehydration reagents should be changed according to the schedule
Clearing	Variation in staining procedure	Contaminants in Xylene	Clearing agents should be changed according to the schedule
Infiltration	Artefacts in sections	Variation in temperature of paraffin wax	Maintain the temperature of the paraffin wax right around 60 degrees Celsius
Embedding and specimen orientation	Soft mushy tissue	Thin sections at gross examination or tissue compressed between top and bottom of the cassette	Take appropriate section thickness Adequate fixation time Wax replacement according to schedule
	Incorrect orientation	Incorrectly oriented at the embedding station	Marking the side of tissue to be embedded facing up with ink

	Tissue carryover	Small pieces or fragments of tissue may be carried from one sample to next sample at the embedding table resulting in cross-contamination	Carefully clean forceps used at the embedding table between specimens The tissue cassettes of single case should be opened, embedded and discarded before moving onto the next sample.
	Tissue not embedded at same level	Tissue is not properly flattened by pressing it down uniformly when placed in the mould	Press tissue uniformly Keep paraffin molten enough to get all pieces embedded at the same level Work very fast while embedding multiple tissue bits
Decalcification	Bone dust	When obtaining section of bone using bone saw, bone dust is pressed into bone surface	Use saw with diamond blade Trim the bone surface after decalcification
	Under - decalcification	Tissue still hard and sectioning is difficult	Ensure adequate decalcification endpoint tests before tissue processing Surface decalcification could be attempted before sectioning
	Over decalcification (Poorly stained section)	Occurs when the endpoint of decalcification is not checked carefully	Choose correct decalcification agent Develop good method to detect the endpoint of decalcification
Frozen section	Freezing artefact	Ice crystals formation due to improper freezing of tissue	Snap freezing Make sure that tissue was not immersed in saline before freezing
	Tissue not embedded flat	If tissue is not embedded flat on the cryostat chuck, the section trimming may lead to wastage of important tissue components.	Place tissue in the slide, surround it with medium, when the the medium turns white(begin), Coat the Cryostat chuck with an embedding medium and invert over the tissue and remove the slide.
Frozen section	Block loosens from the cryostat Chuck while sectioning	Occurs if the chuck was too cold, when the embedding medium was applied	Reattach the tissue block to a clean chuck with additional embedding medium
Microtomy	Crooked ribbons	Results when horizontal edges of the block are not parallel Lower block edge not parallel to cutting edge of blade	Upper and lower edge of the tissue block should be parallel to the cutting edge No discrepancies in blade edge
	Block face unevenly sectioned	Occurs when block holder is not parallel to the blade	Ensure block face and blade are perfectly parallel

	Thick section	Discrepancies in setup	Adjust the thickness while sectioning
	Vertical scratches	Caused by defects in the blade edge, Presence of calcification, bone or hard material within the specimen	Ensure block holder is adjusted that the block face and blade are perfectly parallel
	Holes in the section	Occurs when block is faced too aggressively Specimen is excessively dehydrated Improperly processed specimens	Ensure to chill the block with ice before cutting and discard ribbon until the holes disappear Facing the block less aggressively - small micrometer advances for each section removed
	Failure of ribbon to form	Caused by dull blade Hard paraffin Excessive blade tilt	Paraffin with lower melting point Decreased blade tilt Changes in room temperature
	Wash boarding or indulation in section	Occurs in over fixed tissue Macroscopic type of chatter commonly caused by loose clamping of blade or block	Proper clamping of blade and block Block holder shaft is not over extended Good working of microtome
	Chatter or microscopic vibration	Caused by over dehydration Dull blade Excessive blade tilt	Proper processing Restore moisture Decrease cutting speed
Deparaffinization	Incomplete deparaffinization-white spots seen	Water left in the tissue Incomplete drying Not leaving in xylene long enough	Dry the section properly before deparaffinization Allow sufficient time in xylene Avoid contaminated xylene
Staining	Pale nuclear staining	Slide not exposed to hematoxylin long enough Exhausted(over oxidised or depleted) hematoxylin	Leave the slide longer Use fresh hematoxylin
	Dark nuclear staining	Slide exposed too long Sections too thick	Decrease hematoxylin exposure time Use thinner sections
	Red or brown nuclei	Hematoxylin is breaking down Blueing step not properly done	Use freshly prepared hematoxylin Proper blueing
	Blue black precipitate	Hematoxylin precipitate	Filter the hematoxylin

	Pale cytoplasmic staining	Eosin pH over 5 Section too thin Left long in dehydration	Check eosin pH Completely remove blueing agent before transferring to eosin
	Dark cytoplasmic staining	Overstaining	Avoid over concentration of the stain, dilute eosin
	Dark basophilic staining of nuclei and cytoplasm, especially arcing the tissue edges	Laser and electrocautery techniques denature macromolecules and produce heat artefact	It is an integral part of surgical procedures and could not be avoided.

Post-Analytic Phase Errors

In the post-analytic phase of testing, the results of the analytic phase of testing are delivered to the clinician and the patient for further treatment planning.

The errors in this phase can result in malpractice litigation. Billing issues, patient safety issues and total turnaround time are all monitored during this phase. Careful monitoring should be done to avoid errors.(Table.3)

Table 3: Table showing the various errors in post-analytic phase, its causes and troubleshooting procedures

STEP IN LABORATORY PROCESS	ERROR	CAUSE OF ERROR	TROUBLESHOOTING
Reporting	Typographic errors	Errors during interpretation Errors in patient details	Proof reading the report before
Delivery of report	Delivery of report	Delivery to wrong clinician Untimely delivery of report	TurnAround Time to be strictly monitored.

Approach To Error Investigation

All the errors that occur in laboratory should be investigated and documented.[4]

Type of error

Timing of discovery

Discoverer

Report revision

Mechanism of discovery

Outcome of error: Initial vs Late

Audits To Minimise Errors

Regular audits can be conducted in the laboratory to minimise errors.[5]

Intradepartmental consultation - Review of selected cases by colleagues

Intraoperative consultation - Review of frozen section diagnosis in the light of final paraffin section diagnosis.

Random case review - Re-reporting of random samples from all the cases submitted

Clinical indicator audit - Cases selected on a clinical basis are checked over a given period to ensure consistency in diagnosis and reporting

Intra and Interdepartmental conferences - Review of cases presented and compared with provisional diagnosis against reported

diagnosis.

Inter-institutional review - Comparison of local diagnosis with outside review diagnosis

Surgical Pathology turnaround time - Audit of time taken to produce a report.

Specimen adequacy - Monitor specimen identification before tissue processing.

Lost specimen - Monitor number of lost tissue specimens before receipt and during processing.

Histology quality control - Assess time of delivery of slides, adequacy and quality of staining.

Conclusion

Major or minor errors could adversely affect the patient's life. To err might be human but these laboratory and clinical human errors will definitely impact patient prognosis. Hence, sincere efforts must be taken to minimize these errors as much as possible.

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Conflict of Interest

All the authors declare no conflict of interest.

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