REVIEW ARTICLE

Arvand Journal of Health & Medical Sciences

Laboratory and pathological diagnostic assessment of each Gastroenterology Research Center

Farnoush Barzegar, Fariba Aliverdi, Maryam Pirayesh, Danial Amiri

Research Institute for Gastroenterology and Liver Disease, Gastroenterology and Liver Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

✉ **Reprint or Correspondence**: Farnoush Barzegar MSc; Research Institute for Gastroenterology and Liver Disease, Gastroenterology and Liver Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
@✉ farnoosh_b21@yahoo.com.

ABSTRACT

New techniques of gastric, small bowel and colonoscopic biopsy followed and added to the abundance of tissue available to pathologists for diagnosis and study of the pathogenesis of GI disease. Today gastrointestinal pathology is accepted as one of the largest sub-specialties within general histopathology and there has been a steady movement away from old style morbid anatomy and histology during the past 50 years to a greater appreciation of cellular pathology. Although new techniques, in particular immunocytochemistry and molecular analysis, have become popular, it is remarkable how the old technique of H&E staining remains the standard for diagnostic purposes, especially in research projects. In this short review we will introduce the laboratory and pathological assessments available at each Gastroenterology and Liver Diseases Research Center.

Keywords: Gastroenterology, Molecular typing, Pathology

***Received*: 1 November 2015 *Accepted*: 1 January 2016**

Introduction

The primary aim of examining pathologic specimens is to provide physicians and surgeons with essential diagnostic and prognostic information, however, this is not the only purpose of pathological examination, and these specimens serve as important sources of materials for investigating disease processes and also for teaching students (1). In each gastroenterology research center the biopsies and other bodies' fluids samples from every parts of the digestive system, from mouth to colon, in order to diagnose and score the grade and stage of various gastrointestinal (GI) diseases will be collected (Figure 1). In this short review we will discussed the laboratory and pathological assessments for each Gastroenterology and Liver Diseases Research Center.

I. Histochemical sectioning and staining

Most GI mucosal biopsies are ting samples of a large surface area of suspected disease (2). Section paraffin blocks of a small mucosal biopsies (Figure 2), are stained routinely with hematoxylin and eosin (H&E). Additionally, for gastric biopsies, stain for helicobacter-like organisms with Gimsa are also applied.

Please cite as: **Barzegar F, Aliverdi F, Pirayesh M, Amiri D. Laboratory and pathological diagnostic assessment for each Gastroenterology Research Center. Arvand J Health Med Sci 2016;1(1):2-8.**

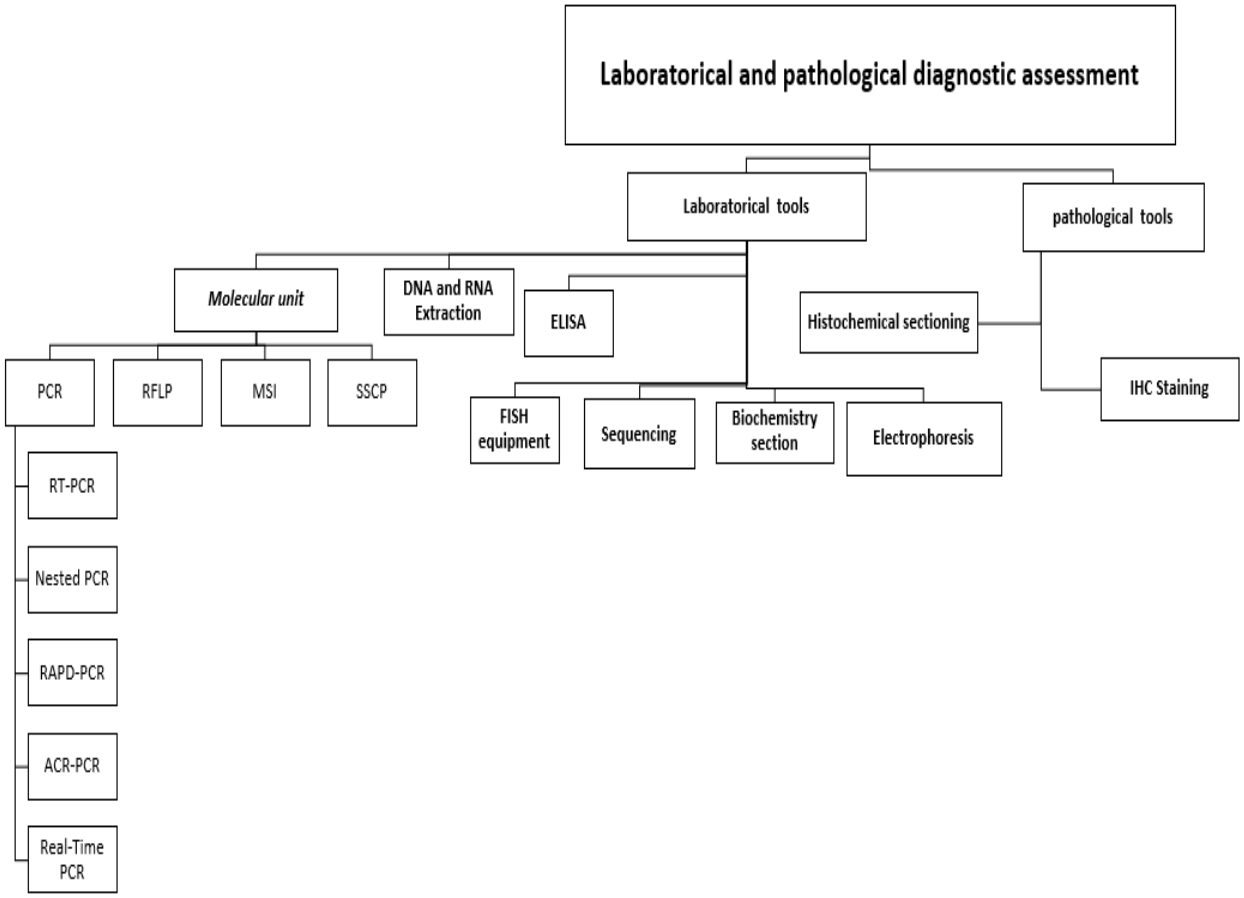

Figure 1. Laboratory and pathological diagnostic assessments in each GI research center

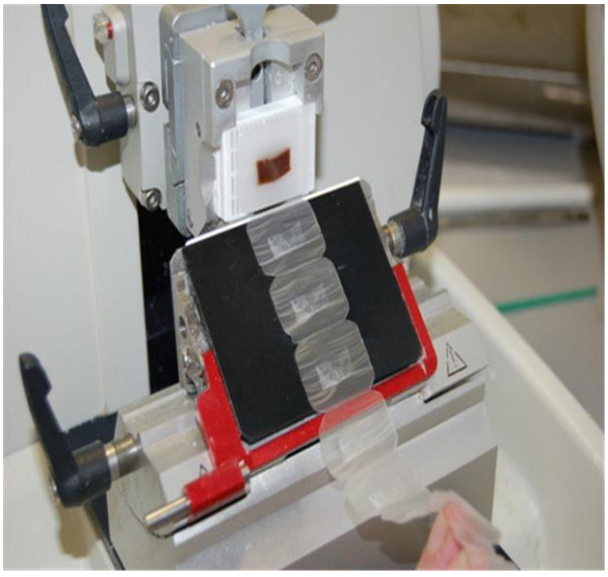

Figure 2. Histochemical sectioning

Meanwhile, trichrome and reticulin staining will use routinely for liver biopsies.

Other techniques revealed to be helpful for diagnostic approaches are include: PAS (periodic acid-Schiff), Amyloid staining (Congo-Red), Argentaffin and argyrophilic stains, PTAH, stains for hemosiderin (Pearls), melanin (Fontanamasson) and calcium (Von Kossa), mucin stain, Verhoeff-Van Gieson and etc.

II. Immunohistochemical (IHC) Staining

IHC is the application of immunologic principles and techniques for studying antigenic components of cells and tissues (3). Examples of diagnostically antigens that can be recognized include: endocrine cells and neuronal granules,

vesicles and peptides, cytokeratine and intermediate filaments, leukocyte markers, immunoglobulines, oncofetal antigens, proliferation markers, mucin core proteins and numerous enzymes (Figure 3) (4-11).

Several procedures are available for IHC, however, currently the two most commonly used are proxidase-antiproxidase immune complex method and the biotin-auidin immunoenzymatic technique (12-16).

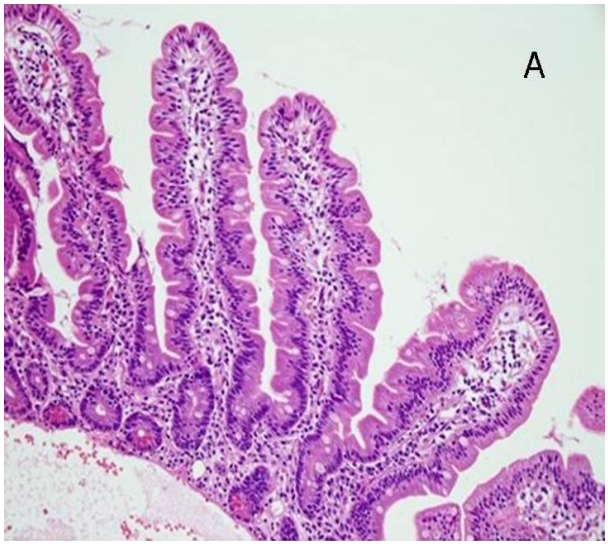

Figure 3. Immunohistochemical (IHC) Staining

III. Molecular unit

Amplification DNA technology has provided an important investigative tool in the field of GI pathology (17-19). Its value has been most obvious in explaining the pathogenesis of neoplastic disorders and uncovering the genetic basis of neoplasia, for which GI cancer served as an important model for solid tumors (20).

1. Polymerase chain reaction (PCR)

PCR is a simple, in vitro, chemical reaction that permits millions of copies of any specific DNA sequence to be generated within a few hours (21). This Technique is capable of amplifying a sequence 105-to 106 folds from nanogram amounts of template DNA using a heat-stable DNA polymerase and using an automated thermocycler (Figure 4).

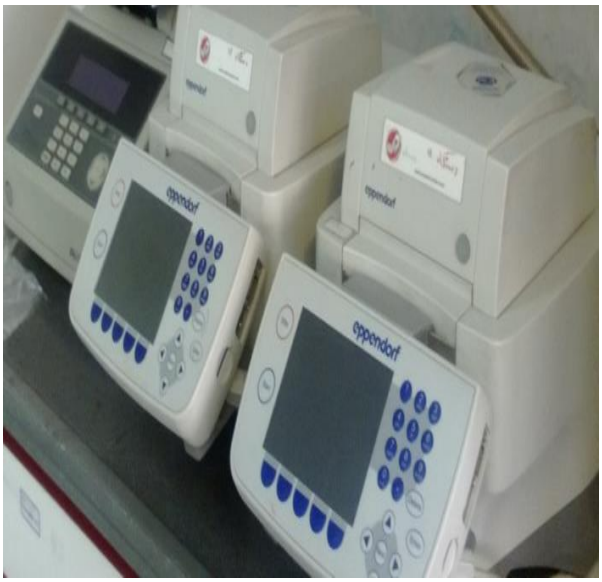

Figure 4. Thermocycler machines.

In our laboratory, there are several researchers and gradient thermocyclers (Eppendorf Company, Germany) which enable us to easily detect the target DNA. PCR itself is consisted of different types, all experienced in each research center is including:

A. RT-PCR: Reverse Transcriptase PCR was developed to amplify RNA targets. In this process, complementary DNA (cDNA) is first produced from RNA targets by reverse transcription, and then the cDNA is amplified by PCR (22).

B. Nested PCR: Nested PCR was developed to increase both the sensitivity and specificity of PCR (23). It employs two pairs of amplification primers and two rounds of PCR for a single locus.

C. RAPD-PCR: Random Amplified Polymorphic DNA is a method of producing a biochemical fingerprinting of a particular species (24). Relationships between species may be

determined by comparing their unique fingerprint information.

D. ACR-PCR: In Amplification Created Restriction PCR a restriction site is created (25). In near future another PCR method will be utilized in this laboratory called "Real-Time PCR" that is a new method of PCR quantification which allows actually viewing the increment in the amount of DNA as it is amplified.

E. Real-Time PCR: describes methods by which the target amplification and detection steps occur simultaneously in the same tube (25). These methods require special thermal circlers with precision optics that can monitor the florescence emission from the sample wells (Figure 5).

Figure 5. Real-Time PCR machine

2. RFLP (Restriction Fragment Length polymorphism)

Assays that utilize the sequence recognition property of restriction enzymes to demonstrate variations or polymorphisms in the DNA sequence of two samples are known as RFLP (26). Genomic DNA or RNA produced from a PCR reaction is digested with a restriction enzyme and the fragments analyzed by gel electrophoresis followed by band visualization or southern blot analysis with a site specific probe.

3. MSI (Micro Satellites Instability) study in HNPCC

Mutation in genes that repair damaged DNA cause regions called micro satellites to get longer or shorter, a phenomenon the scientists call micro satellite instability (MSI) (27-30). Testing for MSI helps us determining whether a person is likely to have a gene mutation, as described in hereditary non-polyposis colorectal cancer (31).

4. SSCP: Single Stranded Conformational Polymorphism

To scan a disease gene for any number of unknown mutations several screening techniques are available. SSCP is a process utilizing gel-electrophoresis to identify a segment of DNA containing a mutation. It can be used as the first step in mutation scanning (32).

5. DNA and RNA Extraction

In DNA extraction unit, serum, plasma and buffy coat will be isolated from the whole blood samples, then, the optimum density (DD) of the samples will be checked by spectrophotometer. One of the innovations of each GI center providing a DNA bank of patients with GI-related diseases, GI cancers, liver diseases, Celiac disease, IBD and etc. is require.

6. ELISA

Enzyme – linked immunosorbent assay is a useful and powerful method in estimating ng/ml to pg/ml ordered materials in the solution, such as serum, urine and culture super nations (33). ELISAS combine the specificity of antibodies with or antigens coupled to an easily- assayed enzyme (Figure 6).

7. Sequencing

In the sequencing unit, genotyping, determining mutations and drug resistance for viral hepatitis, small intestine and large intestine abnormalities

and other GI-related disorders and liver disorders will be evaluated by DNA sequencing in those research center that have accessibility to this machine (Figure 7).

Figure 6. ELISA machine

Figure 7. Sequencing machine

8. Biochemistry section

Different biochemical tests such as liver function tests, CBC diff, Ferritin, Fe, Transferrin, etc. are performed in this section as.

9. Electrophoresis

In this part, agarose gel, polyacrylamide gel (PAGE) and SDS-PAGE (for protein detection) should be prepared and instruments such as horizontal and vertical electrophoresis tanks and the Gel-Documentation system are used for viewing and detection of PCR bands (figure 8).

Figure 8. Electrophoresis and gel running

10. FISH equipment

The absence of an elastin gene from a chromosome is detected by the FISH technique (34). FISH is an acronym for the technical expression "fluorescence in situ hybridization" (34). For example, a blood sample is taken from a patients and then treated with two specific colored markers that give off a "fluorescent" light when exposed to ultra-violet light.

One of the markers attaches to each of the two copies of chromosome number seven in a cell (35). When both copies of chromosome possess the elastin gene, an additional fluorescence of another color is seen attached at another location to each of the two chromosome 7s.

But, as is the case in over 95% of the Williams syndrome individuals tested, only one copy of chromosome seven, not two, will show the fluorescent spot for the elastin gene (36). For pictures illustrating chromosome seven and this test, check the article in the December, 1997 Scientific American magazine (http://www.ucd.ie/artspgs/langimp/williams.pdf). If the FISH test shows the elastin gene to be missing from one copy of chromosome seven, then the individual tested can be said to have Williams's syndrome.

Conclusion

Blood factors play a precarious role in diagnosis, assessing development, and in the classification of disease and phenotypes in clinical and research circumstances. The accuracy and reliability of the whole blood parameter analysis depends on identification and control or eradication of variables that may disturb these results. Clinical pathology deals with the use of laboratory methods (clinical chemistry, microbiology, hematology and molecular diagnostics) for the diagnosis and treatment of different human diseases are areas of active research and development. Pathology as a medical subject and an investigative scientific discipline, is concerned with understanding the essential nature of human GI disorders. We suggest to use a wide range of routine diagnostic services such as haematology, biochemistry, microbiology, cytology and pathology, as well as more specialised services like molecular methods in specific areas to investigate the prognosis, diagnosis, prevention and treatment of different disorders in the field of gastroenterology and liver diseases.

References

1. Bernardini N, Ippolito C, Segnani C, Mattii L, Bassotti G, Villanacci V, et al. Histopathology in gastrointestinal neuromuscular diseases: methodological and ontological issues. Adv Anat Pathol. 2013;20:17-31.

2. Odze RD. Pathology of eosinophilic esophagitis: what the clinician needs to know. Am J Gastroenterol. 2009;104:485-90.

3. Ajura AJ, Sumairi I, Lau SH. The use of immunohistochemistry in an oralpathology laboratory, Malays. J Pathol. 2007;29:101–105.

4. Edgar MA, Rosenblum MK. The differential diagnosis of central nervous system tumors: A critical examination of some recent immunohistochemical applications. Arch Pathol Lab Med. 2008;132:500–509.

5. Garcia CF, Swerdlow SH. Best practices in contemporary diagnostic immunohistochemistry: Panel approach to hematolymphoid proliferations. Arch Pathol Lab Med. 2009;133:756–65.

6. Leong AS, Wright J. The contribution of immunohistochemical staining in tumor diagnosis. Histopathology. 1987;11:1295–305.

7. Bernardi FD, Saldiva PH, Mauad T. Histological examination has a majorimpact on macroscopic necropsy diagnoses. J Clin Pathol. 2005;58:1261–64.

8. Roulson J, Benbow EW, Hasleton PS. Discrepancies between clinical andautopsy diagnosis and the value of post mortem histology; A meta-analysisand review. Histopathology. 2005;47:551–59.

9. Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. Proc Soc Exp Biol. 1941;47:200–202.

10. Duraiyan J, Govindarajan R, Kaliyappan K, Palanisamy M. Applications of immunohistochemistry. J Pharm Bioallied Sci. 2012;4:S307-S309.

11. Nakane PK, Pierce GB. Enzyme labeled anti-bodies: Preparation and application for the localization of antigens. J Histochem Cytochem. 1966;14:929–31.

12. Mason DY, Sammons R. alkaline phosphatase and peroxidase for double immunoenzymatic labeling of cellular constituents. J Clin Pathol. 1978;31:454–60.

13. Faulk WP, Taylor GM. An immunocolloid method for the electron microscope. Immunochemistry. 1971;8:1081–83.

14. Rajendran. Shafer's textbook of oral pathology. 6th edition. India: Elsevier; 2009. p. 932.

15. Harsh Mohan. Essential pathology for dental students. 3rd edition. New Delhi: Jaypee brother's medical publishers; 2005. p. 14.

16. Vainzof M, Zata M. Protein defects in neuromuscular diseases. Braz J Med Biol Res. 2003;36:543-55.

17. Lima G, Panduro A. [The PCR in gastroenterology]. Rev Gastroenterol Mex. 1993;58:108-18. [Article in Spanish]

18. Mima K, Nishihara R, Qian ZR, Cao Y, Sukawa Y, Nowak JA, et al. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. Gut. 2015 [In Press].

19. Halstead DC, Abid J, Sloan L, Meza D, Ramsey-Walker D, Hata DJ. A multi-laboratory comparison of two molecular methods for the detection of toxigenic Clostridium difficile. J Infect Dev Ctries. 2016;10:62-67.

20. Graf EH, Simmon KE, Tardif KD, Hymas W, Flygare S, Eilbeck K, et al. Unbiased Detection of Respiratory Viruses Using RNA-seq-Based Metagenomics: A Systematic Comparison to A Commercial PCR Panel. J Clin Microbiol. 2016 [In Press].

21. Polymerase chain reaction / PCR. Available at: http://www.nature.com/scitable/definition/polymerase-chain-reaction-pcr-110

22. Overbergh L, Giulietti A, Valckx D, Decallonne R, Bouillon R, Mathieu C. The use of real-time reverse transcriptase PCR for the quantification of cytokine gene expression. J Biomol Tech. 2003;14:33-43.

23. Kim EJ, Bauer C, Grevelding CG, Quack T. Improved PCR/nested PCR approaches with increased sensitivity and specificity for the detection of pathogens in hard ticks. Ticks Tick Borne Dis. 2013;4:409-16.

24. Balajee SA, Sigler L, Brandt ME. DNA and the classical way: identification of medically important molds in the 21st century. Med Mycol. 2007;45:475-90.

25. González-Ballester D, de Montaigu A, Galván A, Fernández E. Restriction enzyme site-directed amplification PCR: a tool to identify regions flanking a marker DNA. Anal Biochem. 2005;340:330-35. Erratum in: Anal Biochem. 2006;353:302.

26. Hu Z, Zhang SH, Wang Z, Bian YN, Li CT. Progress of DNA-based Methods for Species Identification. Fa Yi Xue Za Zhi. 2015;31:129-31.

27. Prakash O, Pandey PK, Kulkarni GJ, Mahale KN, Shouche YS. Technicalities and Glitches of Terminal Restriction Fragment Length Polymorphism (T-RFLP). Indian J Microbiol. 2014;54:255-61.

28. Schlotterer C. Evolutionary dynamics of microsatellite DNA. Chromosoma. 2000;109:365-71.

29. Kim KM, Kwon MS, Hong SJ, Min KO, Seo EJ, Lee KY, et al. Genetic classification of intestinal-type and diffuse-type gastric cancers based on chromosomal loss and microsatellite instability. Virchows Arch. 2003;443:491-500.

30. Bachtrog D, Agis M, Imhof M, Schlotterer C. Microsatellite variability differs between dinucleotide repeat motifs- evidence from Drosophila melanogaster. Mol Biol Evol. 2000;17:1277-85.

31. Bhargava A, Feuntes FF. Mutational dynamics of microsatellites. Mol Biotechnol. 2010;44:250-66.

32. Mahdieh N, Rabbani B. An overview of mutation detection methods in genetic disorders. Iran J Pediatr. 2013;23:375-88.

33. Meagher RJ, Hatch AV, Renzi RF, Singh AK. An integrated microfluidic platform for sensitive and rapid detection of biological toxins. Lab on a Chip. 2008;8:2046-53.

34. Lowery MC, Morris CA, Ewart A, Brothman LJ, Zhu XL, Leonard CO, et al. Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: evaluation of 235 patients. Am J Hum Genet. 1995;57:49-53.

35. Brøndum-Nielsen K, Beck B, Gyftodimou J, Hørlyk H, Liljenberg U, Petersen MB, et al. Investigation of deletions at 7q11.23 in 44 patients referred for Williams-Beuren syndrome, using FISH and four DNA polymorphisms. Hum Genet. 1997;99:56-61.

36. Elçioglu N, Mackie-Ogilvie C, Daker M, Berry AC. FISH analysis in patients with clinical diagnosis of Williams syndrome. Acta Paediatr. 1998;87:48-53.