

Q is for Quik Diff

A-Z of Staining - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.



Welcome to our A-Z of Staining where we feature the letter Q for the Quik-Diff (QD) stain, a rapid dip and dunk method that uses the dyes eosin and methylene blue (Figure 1). Although this stain is more commonly known as the Diff-Quik, my first memory of it as the QD was in the identification of *Campylobacter pylori* (now reclassified as the genus *Helicobacter*) which often colonize the mucosal surfaces of the stomach. Infection by *H pylori* is not only associated with gastritis and peptic ulcers but continues to be a significant risk factor for the development of gastric cancer. As a result, precise identification of the curved, S-shaped bacteria in these biopsies is imperative. The QD prevails as a rapid and reproducible method that is superior to the often complex and time-consuming alternatives employed in histology for identifying *H*

pylori in paraffin sections. With the QD method, *H pylori* stain blue and appear as helical structures on gastric mucosal surfaces (Figure 2).



Figure 1. QD stain reagents (left) displaying the dip and dunk technique (right)

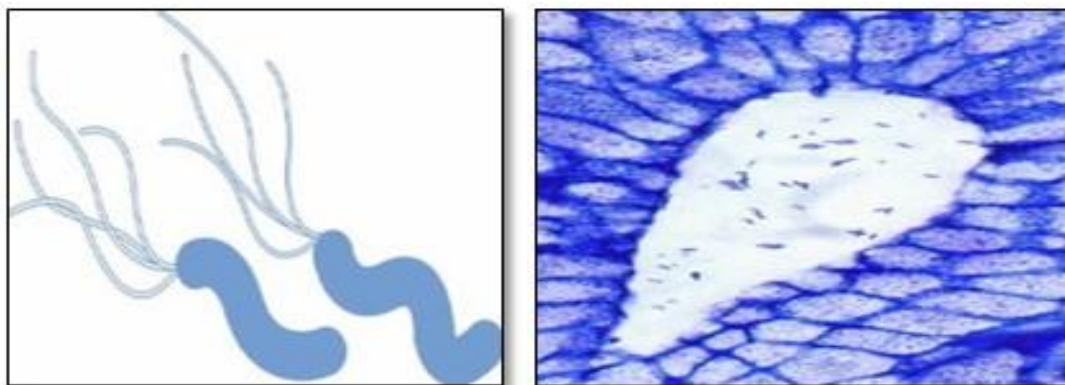


Figure 2. Structure of *H pylori* and identification in gastric mucosa using the QD stain

In addition to its use in histology, the QD stain is frequently used to rapidly stain and differentiate a variety of samples which include cytology smears, aspirates and blood films. The method is based on a modification of the Romanowsky stain which is essentially a three-step system employing a fixative solution for air-dried smears (methanol), an eosinophilic solution (eosin) and a basophilic solution (methylene blue). The stains are buffered at neutral pH and the method is carried out rapidly by dipping the slides into each of the solutions 5 or 6 times to obtain even staining. With this method, the nuclei of cells stain red to purple and the cytoplasm pink to yellowish red. By manipulating protocols, preferential intensities of staining can be personalized. Staining using the QD method can also be enhanced by using

polychrome methylene blue which is obtained by degradation of the methylene blue into threonine and azure dyes by a demethylation reaction.

While there are countless modifications of the Romanowsky stain created by the likes of Giemsa, Wright, Leishman, May and Grunwald, it is the QD that remains the ideal, cost-effective alternative for urgent laboratory diagnosis of preparations from cytological fluids, fine needle aspirates, semen analysis, blood films, bone marrows and tissue sections (Figure 3). In addition to staining *H pylori* bacteria, the QD method has proved useful in identifying the fungus *Pneumocystis* (a causative agent of pneumonia) and protozoa such as *Plasmodium* (malaria), *Trichomonas* and *Chlamydia* (causes of genital infections). Extracellular material such as ground substance and mucin also stain metachromatically and the method has been used as a simple alternative to Papanicolaou for staining endocervical specimens. Rapid on-site evaluations (ROSE) can also be performed at locations where assessment of sample adequacy during procedures such as endoscopy and bronchoscopy is vital. Consequently, optimum cellular yields are ensured and these help to determine a preliminary diagnosis.

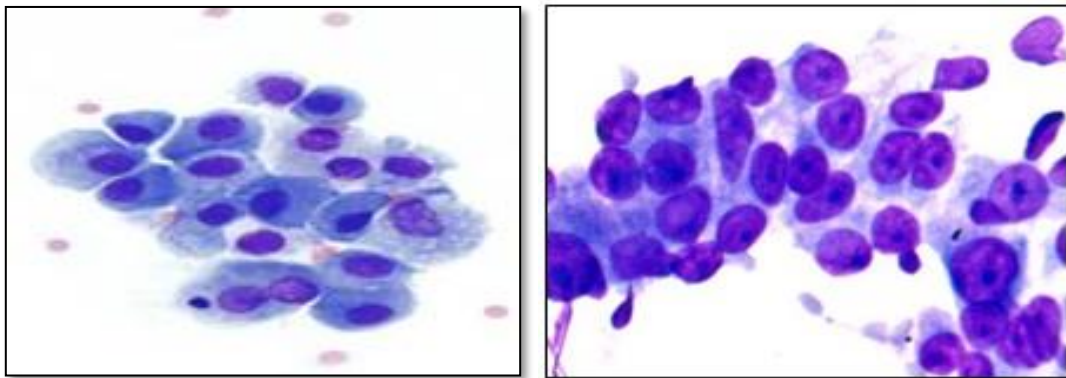


Figure 3. QD stain showing epithelial cells and macrophages in a bronchoalveolar lavage (left) and adenocarcinoma cells in a washing from lung (right)

Commercially, there are many proprietary QD staining kits available for differentiating a variety of pathological samples. Some are offered in containers designed specifically for direct staining without the need for transfer to staining jars. The convenience of these kits provides both ROSE and laboratory procedures with a rapid turnaround that is especially significant for samples requiring immediate diagnostic interpretation.

Further reading

1. How Romanowsky stains work and why they remain valuable (Horobin). *Biotechnic and Histochemistry* 2011;86(1):36-51. doi: [10.3109/10520295.2010.515491](https://doi.org/10.3109/10520295.2010.515491)
2. Attempt to fulfil Koch's postulates for pyloric *Campylobacter* (Marshall et al). *Medical Journal of Australia* 1985;142(8):436-9. doi: [10.5694/j.1326-5377.1985.tb113443.x](https://doi.org/10.5694/j.1326-5377.1985.tb113443.x)
3. Evaluating Diff-Quik cytology smears for large-panel mutation testing in lung cancer - Predicting DNA content and success with low-malignant cellularity samples (Fielding et al). *Cancer Cytopathology* 2023;1-10. doi: [10.1002/cncy.22690](https://doi.org/10.1002/cncy.22690)

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