

A simple differential stain of blood smears using black Quink®

Chris Thomas, 3 Hall End, Milton, Cambridge CB24 6AQ (Email: chris AT miltoncontact DOT com)

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Summary

Dried and fixed peripheral blood smears can easily be differentially stained with Parker's black Quink® fountain pen ink. It successfully increases the contrast of erythrocytes, stained orange/yellow. It differentially stains white blood cells and platelets blue. It is possible to distinguish between neutrophils, small and large lymphocytes, monocytes and eosinophils.

A simple method using common and economical ingredients is given.

The method can be used in schools, by amateur microscopists, and by professionals in situations where conventional stains are either temporarily unavailable or unobtainable.

Contents

Summary.....	1
Introduction.....	1
Results.....	2
Discussion.....	5
Method.....	7
Bibliography.....	8

Introduction

“What does blood look like under the microscope?” My 82 year old mother asked during a visit, as I was looking at some slides. With a promise to show her, I prepared some smears, yet they were very faint when viewed under transmitted light. Fountain pen ink is a known stain, both of writer's fingers and samples for the microscope. I therefore tried a quick stain with Parker's Quink® black ink and was surprised by the increased contrast obtained, for erythrocytes (red blood cells), platelets and the differential staining of five different types of white blood cells that I could find. Staining was initially variable, so I continued to try a variety of different conditions, based on other researcher's experience and the nature of Quink® ink. The results and discussion of these experiments are given below, followed by the final method. My mother enjoyed her look at the stained blood cells.

Results

An unstained blood smear is clearly seen by eye on the microscope slide, however, the blood cells are barely visible under the microscope (figure 1).

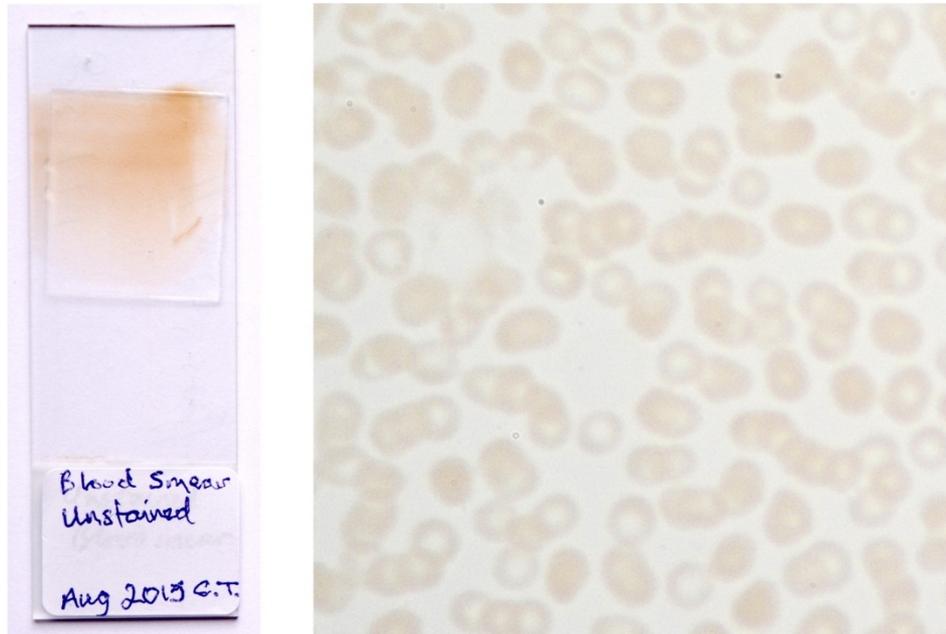


Figure 1. Left - unstained blood smear on slide. Right – blood as seen at 400x under light microscope.

After one minute staining with black Quink®, the walls of the red blood cells (erythrocytes) are sufficiently stained grey or black and the contents appear yellow or orange (figure 2). White blood cells and platelets appear in dramatic blue.

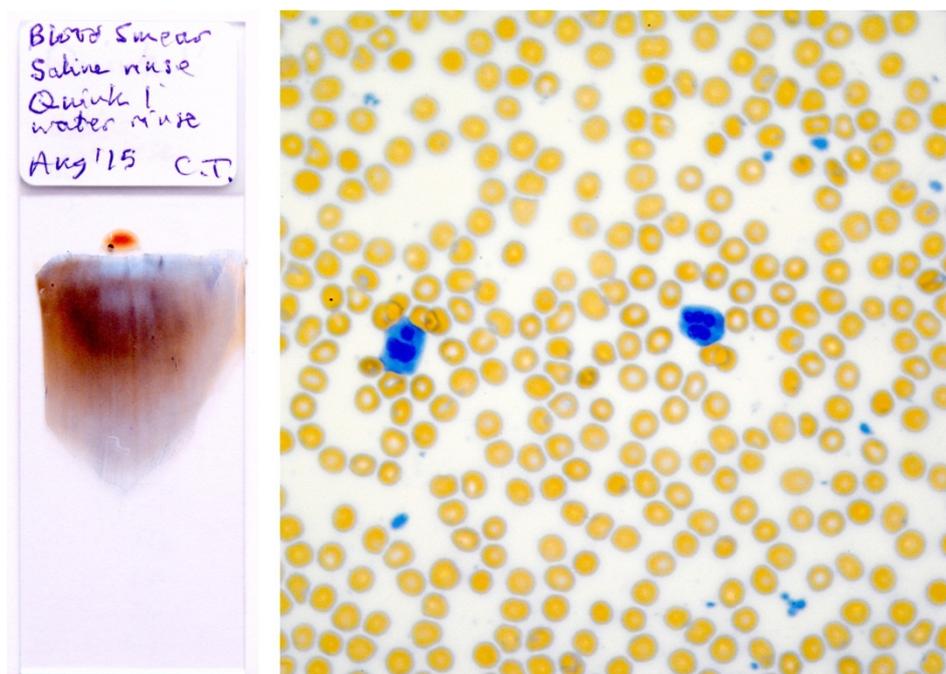


Figure 2. Left - blood smear stained with Quink®. Right - stained blood as seen at 400x under light microscope.

Figure 3 shows examples of five different white blood cell types and platelets viewed with a 100x oil immersion lens.

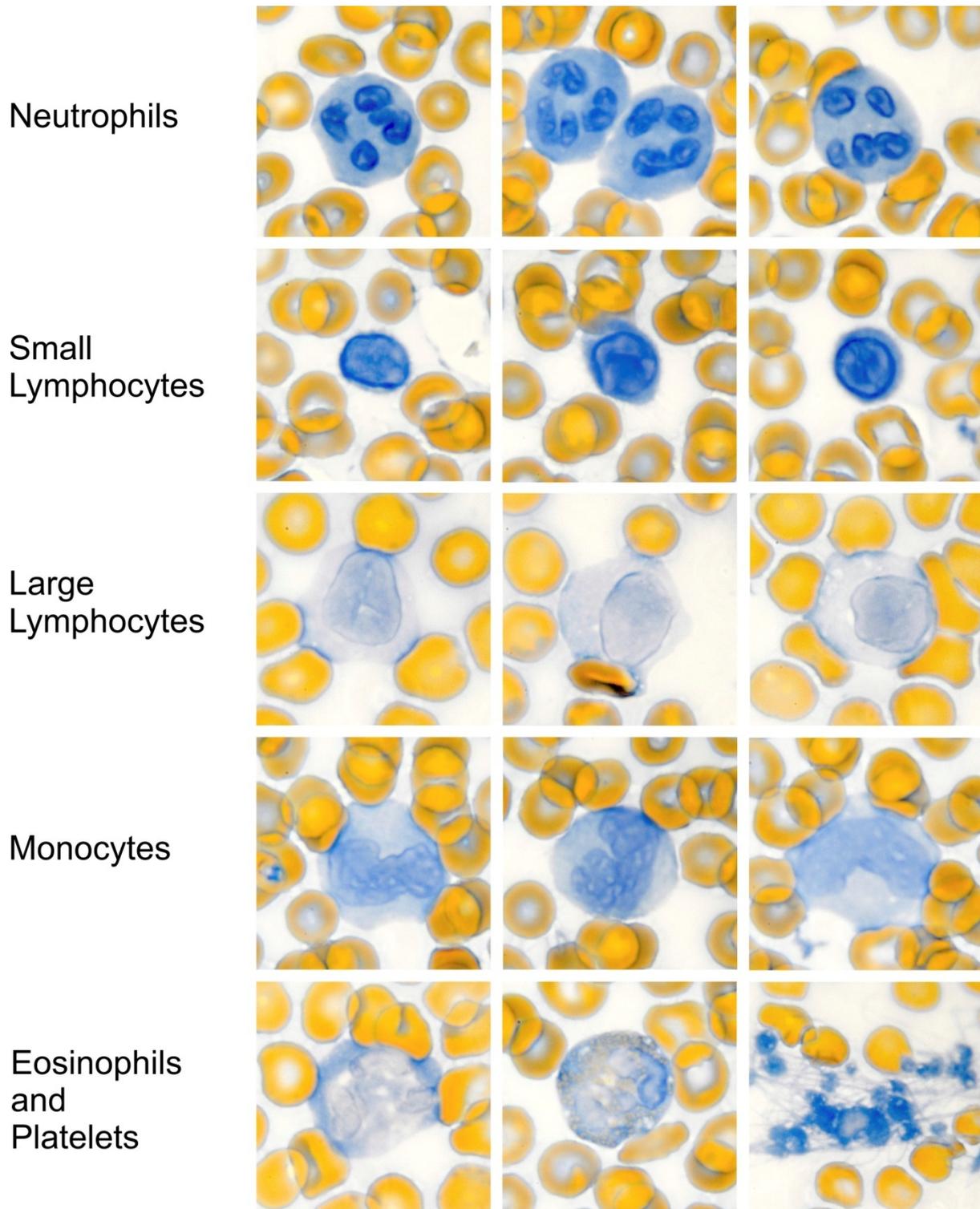


Figure 3. Examples of white blood cell types seen after Quink® staining. Viewed at 1000x using oil immersion light microscopy

The cell types are in approximate order of abundance: Erythrocytes (red blood cells – appear orange); platelets (strongly stained blue); neutrophils with multiple lobed nuclei (strong blue staining); small lymphocytes (strong blue staining. Ovoid nucleus, little cytoplasm); large lymphocytes (weaker

staining. Larger ovoid nucleus with visible cytoplasm); monocytes with sausage shaped or bilobed nuclei (weaker blue staining); and eosinophils (irregular blue staining and granulation obscuring the cell contents). A rarer type of white blood cell, the basophil, was not seen or identified.

The key element in staining was pre-wetting the fixed blood smear with the 0.8% salt solution. Simply wetting with water also worked, but resulted in uneven staining. Using 0.1% sodium bicarbonate (baking soda, alkali) or ascorbic acid (Vitamin C, acidic) to wet the slides also worked better than water.

Reasonable staining could still be achieved with a twofold dilution of black Quink®.

Staining for 3 minutes gave equally intense staining of the white blood cells. However, the red blood cells took on more stain too, appearing darker.

Staining blood smears with dilutions of 10x or 20x in either water, saline, dilute sodium bicarbonate or vitamin C only gave faint blue staining of white blood cells after 3'. This was the case even after 30 minutes staining (figure 4). The differentiation between red blood cells and white was also lost as the red blood cells became grey blue. If the slide was then restained with undiluted black Quink®, the white blood cells were stained a darker blue (figure 4), as seen with slides originally stained with undiluted Quink®. However, the yellow-orange of the red blood cells was not regained.

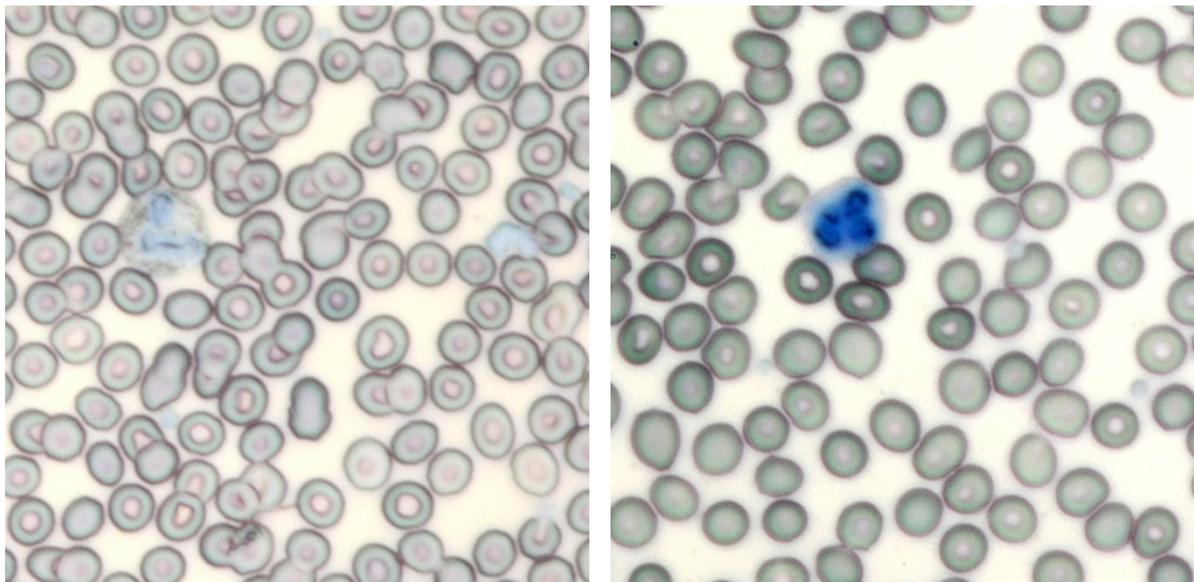


Figure 4. Left blood smear after staining for 30 minutes with 1:20 dilution of Quink® in saline. Right - Same slide restained using undiluted Quink® for 1 minute.

Staining worked with a 20+ year old bottle of Parker black Quink® and one purchased in August 2015, the ink seems remarkably stable and consistent (figure 5).

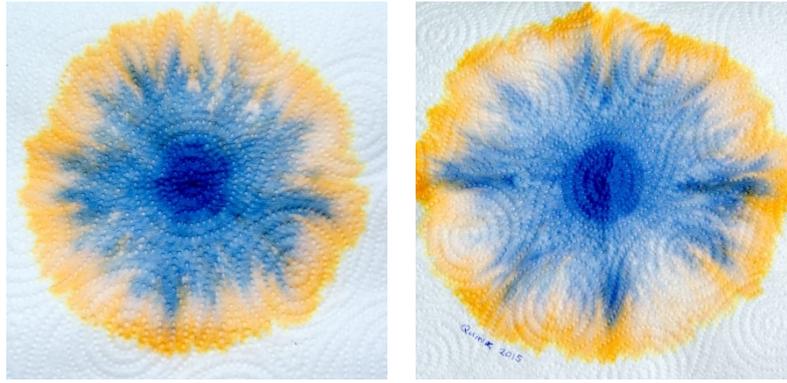


Figure 5. Paper towel chromatography of 20+year old Quink® (left) and 2015 Quink® (right). About 0.3ml ink applied to centre of single layer of paper towel, followed by several millilitres of water till orange dye visible.

Discussion

This report appears to be the first on the successful use of Quink® as a stain for peripheral blood smears. The stain is simple to use and consistent.

Quink® appears to make red blood cells (erythrocytes) more visible by weakly binding to the outer surface. In white blood cells, the stain penetrates into the cells and preferentially stains nuclear material, with weaker staining of the cytoplasm. Neutrophils, small lymphocytes and platelets stain most intensely. Large lymphocytes, monocytes and eosinophils stain a paler blue.

The main benefit of the Quink® stain for peripheral blood smears is for use either:

- In schools or teaching locations where fountain pen ink is seen as a psychologically safer material to other chemical stains
- In places or under conditions where it is difficult to obtain commercial stains
- Where you are found short of your existing stain but do find a bottle of Quink®!

Quink® staining is not seen as a replacement for conventional differential blood stains. It is simply another useful tool in the microscopist's toolbox.

The current preferred differential stains are Wright's stain, Wright-Giemsa stain and the May-Grünwald stain (1). A faster (15 second) stain, Diff-Quik, is also available. It is based on a modified Romanowsky stain (2). They all stain red blood cells a pale red or similar colour and the white blood cells and platelets in shades of blue or violet. The nuclei are more intensely stained. The Diff-Quik also stains the granules in some white blood cell types red or violet. Platelets are stained blue, violet or purple according to commercial stain used.

Parker's Quink® ink has been used for a number of different staining techniques for more than 50 years. Examples include:

- 1971, Buckley – Fungi pathogenic to man and animals (3)
- 2005, Walker - Fungal mycorrhiza in roots (4)
- 2009, Wikibooks – Staining onion cells (5)
- 2010, Schmitz – Cartilage lesions (6)
- 2012, Mizutani et al – Herpes virus diagnosis (7)

Quink® is a fountain pen ink available in a range of colours. It was developed by Parker as a quick drying ink not requiring the use of blotting paper, using an investment of \$68,000 over the three year period 1928-31 (8). The original ink was strongly alkaline and contained isopropanol. A modern Material Safety Data Sheet indicates that it now contains diethylene glycol instead of isopropanol, as well as dyes and preservative (9).

As can be seen from the chromatograms in figure 5, the black ink is created by combining two dyes of unknown composition, one blue and one a complimentary orange. The dyes are most likely to be derived from aniline salts (10). Other black fountain pen inks that give a similar chromatogram might also work in this procedure.

The Quink® differential staining of blood smears appears to work best using undiluted or twofold diluted Quink®. Mizutani (7) was able to stain the nuclei of herpes giant cells using a 5% solution (twenty fold dilution) of Quink® in phosphate buffered saline. Walker stained fungal mychorriza in an acidified 2% (50 fold dilution) of Quink® in dilute acetic acid or HCL (4).

Undiluted Quink® as used in my trials is said to be alkaline. However, staining with a 1:20 dilution of Quink® in either acidic solution, weak alkali or simply 0.8% saline did not give the same strong differential staining as undiluted Quink®. The ability to restain with undiluted Quink® and obtain intensely stained neutrophils suggests that the stainable material within the cells still remains.

The most important other factor for even staining with Quink® was pre-wetting the dried blood smear. Acidic, alkali or saline washes before staining all seemed to give more consistent staining than wetting with tap water. It was for this reason that I decided that 0.8% salt solution was the simplest pre-wetting liquid.

Post-staining rinsing also appeared to be uninfluenced by either weak acid, alkali or saline solution. Hence the decision to use a water rinse.

Parker's black Quink® ink can be used as a reproducible and reliable stain for peripheral blood smears. It colour differentiates between red and white blood cells. Erythrocytes, neutrophils, large and small lymphocytes, monocytes, eosinophils and platelets can be identified. The stain can be used with generally available ingredients, making it accessible to schools, amateur microscopists and professionals who are either out of stock or unable to obtain conventional laboratory stains.

The method should be transferable for use with blood from other animals. I look forward to your news and results.

Method

Using my own blood samples, this was the final procedure adopted after several trials

- Wash hand to be sampled with warm soap and water (11)
- Prick the side of a finger near the nail with a sterile needle or blood lance (1)
- Make an air dried blood smear as described by Schall (12)
- Fix by flooding slide with either alcohol or methylated spirits for 1 minute
- Discard alcohol into waste container
- Allow slide to air dry
- Wet the slide with a few millilitres 0.8% NaCl (salt) dissolved in tap or bottled water. Collect runoff in waste container
- Place slide on flat surface or hold horizontally
- Add 0.5ml to 1.0ml of Parker Black Quink® fountain pen ink to cover wetted blood smear. Use undiluted or diluted 1:1 with 0.8% salt solution
- Stain for 1 minute
- Rinse ink off slide with water to into waste container
- Stand slide vertically on tissue paper and allow to air dry
- The stained smear is coloured blue-black to blue grey
- Once the slide is dry, it can be viewed under the microscope at 100x and 400x magnification in air or at 100x using oil immersion
- For correct use of a microscope see *Understanding and using the light microscope (3)*
- Red blood cells appear yellow to dirty orange, in high contrast. White blood cells and platelets are stained blue
- Permanent slides can be made by adding a drop of Canada balsam on the slide and adding a cover slip. Warm the slide to help the Canada balsam spread. Store flat and allow to harden over several days.

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