J7 Microscopic Examination of Blue Gel Inks After Cellulase Digestion of Paper

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The goal of this presentation is to teach an assay which allows for the separation of gel inks from paper substrates so they can be studied using microscopic methods.

This presentation will impact the forensic community by providing a procedure which will allow the separation of non-soluble pigmented inks from paper so the further instrumental methods can be applied to their identification.

Gel pens or pigmented inks have been difficult to investigate due to their insoluble nature. Microscopic techniques[1] have shown promise in the pursuit of differentiation, but may only be applicable if the inks can be removed from a paper substrate.

This study focuses on the use of an enzymatic assay to dissolve paper offering a potential method for removing ink from paper and thus allowing microscopic examination of the ink without interference from the paper substrate. Two types of cellulase from the species *Trichoderma viride* and *reesei,* respectively, were used to digest one millimeter hole punches of paper samples containing gel inks from 36 different blue gel pens. The enzyme was used in a concentration of 2.5 mg/ml and diluted to 1:2 with citrate buffer at a pH of 4.8. The units of activity were 9.285 unit/ml for *Trichoderma viride* and 8.125 unit/ml for *Trichoderma reesei*. The digestion of the paper was performed at 50 °C directly on depression slide enabling samples to be observed microscopically without having to transfer sample. Comparisons were made with standards of gel inks placed directly on glass depression slides and put through the same digestion process. Examination of digested material was performed with bright field, dark field and phase contrast microscopy.

Ten soluble inks were tested but in all cases ink standards were either washed away or significantly diluted to where they could not be examined. For test samples, six of the soluble inks turned paper fibers blue under all three applied methods. Using phase contrast, some of these fibers appeared lime green and purple and under dark field the fibers appeared bright blue. Black particles were observed in two of the soluble gel inks with no other indication of ink. Two soluble inks were completely washed away during the digestion process.

Twenty-six non-soluble blue gel inks were similar digested and examined microscopically. After digestion, these inks appeared as scattered particles some of which were free floating in the digestion solution and some were still attached to fibers. There was a relationship between the amount of standard ink left on the depression slide after digestion and the size of the ink particles on the digested paper samples. In particular, gel inks from Zebra tended to yield smaller ink particles. Non-soluble gel inks that were not affected by the digestion process as observed on standard samples were more likely to remain attached to paper fibers. The colors of the non-soluble gel inks varied from blue, blue-green and purple when visualized under bright field. Phase contrast either increased the amount of colors visible (as in the case of some blue-green inks appearing purple and green under phase contrast) or made the ink appear a darker shade. Dark field microscopy enabled the visualization of colors along the edge of gel inks and increased the variation in color allowing for better discrimination of samples. Several of the gel inks contained black clumps or angular particles, which were visibly present in the sample and not washed away by digestion.

Digestion time of twenty-four to forty-eight hours was used with no indications that longer incubation times increases discrimination. Problems with the formation of crystals due to precipitation of citrate buffer sometimes occurred, but diluting the sample with deionized water and reducing drying time diminished the amount and size of these crystals.

Gel inks, Cellulase, Microscopic

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