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Comparing the sterility and visibility of surgical marking pens available in Australia

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Abstract

Background: Surgical site marking is an important safety procedure prior to surgery. Visibility of pen marks is affected by surgical wash which increases the risk of wrong-site surgery. Additionally, multiple patient contact with a single pen is a potential source of bacterial transmission. In this study we compare pens commonly used for surgical marking in Australia.

Methods: We conducted an unblinded, prospective cohort study comparing 12 marking pens. Six volunteers' thighs were marked with each pen. Standardized photographs were taken before and after wash with four prep solutions. Ink visibility was analysed using gray-scale images, comparing the pen mark tone before and after wash. The pen tips were swabbed for culture.

Results: Red tinted 2% chlorhexidine gluconate (w/v) with 70% isopropyl alcohol (v/v) was shown to reduce pen mark visibility significantly more than the other solutions used. The Pentel N50 permanent marker and Aspen WriteSite Plus were least affected by wash. No pen tip cultured any bacteria.

Conclusions: When marking the correct site for surgery, we recommend the use of either the Pentel N50 permanent marker or Aspen Writesite Plus pen. A 2-min interval between patient contact limits bacterial transmission.

Introduction

Marking the correct site prior to surgery is recommended practice in Australia.[1] The mark should be visible after surgical wash and draping.[2] Past studies have demonstrated that ink visibility is influenced by the alcohol-based liquid used and the pen ink.[3–6] Reduction of visibility predisposes to surgical error, as ink may be completely washed from the skin prior to incision.[7] Pen tips have also been implicated as a vector for bacterial transmission, which poses a risk when marking occurs over the surgical incision site.[8,9] This study aims to objectively quantify these risks for pens commonly used in Australia.

Methods

We conducted an unblinded, prospective cohort study using 12 surgical pens available in Australian hospitals. All gentian violet pens that were available for purchase and three permanent markers known to be used by surgical personnel for site marking were included. Twenty-three pens were sourced. One fine-tip pen was excluded, and one pen was excluded in favour of the 'plus' version of the same product. We noted some brands sold identical pens, and these were regarded as the same pen for study purposes. One pen was randomly selected to represent duplicate groups. After excluding nine duplicates, 12 pens were therefore selected for the trial; nine gentian violet marking pens and three black permanent marking pens (Table 1). The surgical solutions used were brown tinted 1% iodine (w/v) with 70% isopropyl alcohol (v/v), pink tinted 0.5% chlorhexidine gluconate (w/v) with 70% isopropyl alcohol (v/v), red tinted 2% chlorhexidine gluconate (w/v) with 70% isopropyl alcohol (v/v) and untinted 70% isopropyl alcohol (v/v). For simplicity, these will be abbreviated to 1% IA, 0.5% CGA, 2% CGA and 70% alcohol, respectively.

Six voluntary participants of Caucasian descent with fair skin, aged between 21 and 24 were recruited. A private surgical theatre (Linacre Private Hospital, Melbourne, Victoria, Australia) was

Table 1 List of pens that were sourced and included.

Pen brand (manufacturer)	Ink	Included in study
Artline	Black Permanent	**Yes**
Sharpie	Black Permanent	**Yes**
Pentel	Black Permanent	**Yes**
Covidien	Gentian Violet	**Yes, as Covidien (Duplicate #1)**
Richard Allan	Gentian Violet	As Covidien (Duplicate #1)
Nuvasive	Gentian Violet	As Covidien (Duplicate #1)
DeRoyal	Gentian Violet	**Yes, as DeRoyal (Duplicate #2)**
Symmetry	Gentian Violet	As DeRoyal (Duplicate #2)
Medline	Gentian Violet	As DeRoyal (Duplicate #2)
Secureline	Gentian Violet	As DeRoyal (Duplicate #2)
Codman	Gentian Violet	As DeRoyal (Duplicate #2)
ArcRoyal	Gentian Violet	As DeRoyal (Duplicate #2)
Viscot	Gentian Violet	**Yes**
Linear (Concord Medical)	Gentian Violet	**Yes, as Concord (Duplicate #3)**
Multigate (Concord Medical)	Gentian Violet	As Concord (Duplicate #3)
Liberty (Concord Medical)	Gentian Violet	As Concord (Duplicate #3)
Sandel	Gentian Violet	**Yes**
Matrix (Advanced Medical)	Gentian Violet	**Yes, As Matrix**
Aspen WriteSite Plus	Gentian Violet	**Yes**
Aspen WriteSite	Gentian Violet	Excluded (WriteSite Plus used)
Medline	Gentian Violet	**Yes**
SMI	Gentian Violet	**Yes**
Precise Medical (Viomedex)	Gentian Violet	Excluded (Fine Tip only accessible)

Bold represents pens actually tested. Exclusions are listed with reason. The manufacturer of some duplicate pens remains unclear.

used. A Nikon D5500 camera and lens (Nikon AF-S DX 18-140 mm) without flash was mounted above the operating table in the same position in every instance. A line was drawn with each of the 12 pens on all 12 thighs. Lines were drawn in parallel, separated by 2 cm and measuring 7 cm in length. A standardized photo (1/160, f/4.0, ISO 100) of each thigh was taken prior to surgical prep. All thighs were washed with surgical prep; three thighs with each solution. A standardized technique simulating clinical practice was performed, with forward and backward strokes, using sterile prep sponges soaked in solution. A single coat was applied and allowed to dry followed by a second coat. Consistent, light pressure was applied to the skin and horizontal strokes were used to wash each ink mark once per coat. Another standardized photo of each thigh was taken after prep.

Every pen tip was swabbed and sent for microscopy and culture (Alfred Health Pathology, Melbourne, Victoria, Australia). Each swab was taken 2 min after the pen made final contact with skin.

All photos were converted to grayscale in Adobe Photoshop (Version 19.1.5.722). The average (mean) tone was calculated for each ink mark using the histogram function (Fig. 1). The RGB (red, green, blue) tone ranged between 255 (pure white) and 0 (pure black). The average tone of the skin beside each pen mark was calculated, of a similar surface area to the pen mark. The skin tone was subtracted from the ink tone to account for lighting and angle (which affected the tone of the marks in different parts of the photo). This calculated the corrected ink tone prior to and following surgical wash. The values were averaged for each prep solution used (Fig. 3).

Results

Two-tailed Student's *t*-tests were performed for statistical analysis. A *P*-value of less than 0.05 was considered statistically significant. The sample size was based on similar cohort studies.[3–5]

All prep solutions reduced ink visibility significantly (Fig. 2). Ink was most affected by 2% CGA which decreased the tone by 56% on average ($P < 0.05$). The other solutions reduced tone by 43% ($P < 0.05$).

Fig. 1. The histogram function was used to calculate the mean tone value. This ranged between 255 (pure white) and 0 (pure black). A value was obtained for every pen mark and skin adjacent to the marks, before and after wash.

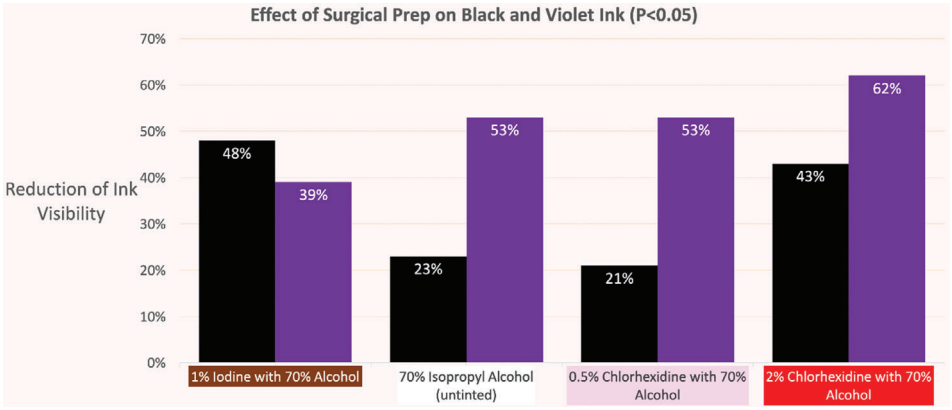

Fig. 2. The effect of each surgical prep solution was different for black and gentian violet inks. Brown tinted 1% IA had the least effect on gentian violet, but the most significant effect on black ink. Red tinted 2% CGA had a significant effect on both ink types.

Gentian violet

There was a clear difference between the effect of 1% IA compared with other solutions on gentian violet ink. Subjectively, gentian violet ink appeared darker and did not smear after 1% IA application. The average reduction in tone was 39% ($P < 0.05$). Significant visibility loss and smearing occurred when the other solutions were applied to gentian violet ink. The most significant effect was 2% CGA, causing a 62% reduction in tone ($P < 0.05$).

Black permanent

Black ink was most affected by 1% IA and 2% CGA, reducing their tone by 48% and 43%, respectively ($P < 0.05$). Subjectively, black ink did not significantly smear. Unlike gentian violet, black ink appeared to become lighter when washed with 1% IA.

Each pen was analysed individually with each prep solution to assess its performance (Table S1).

Three pens performed well with all solutions. Pentel N50 performed the best in all instances. It had no statistically significant

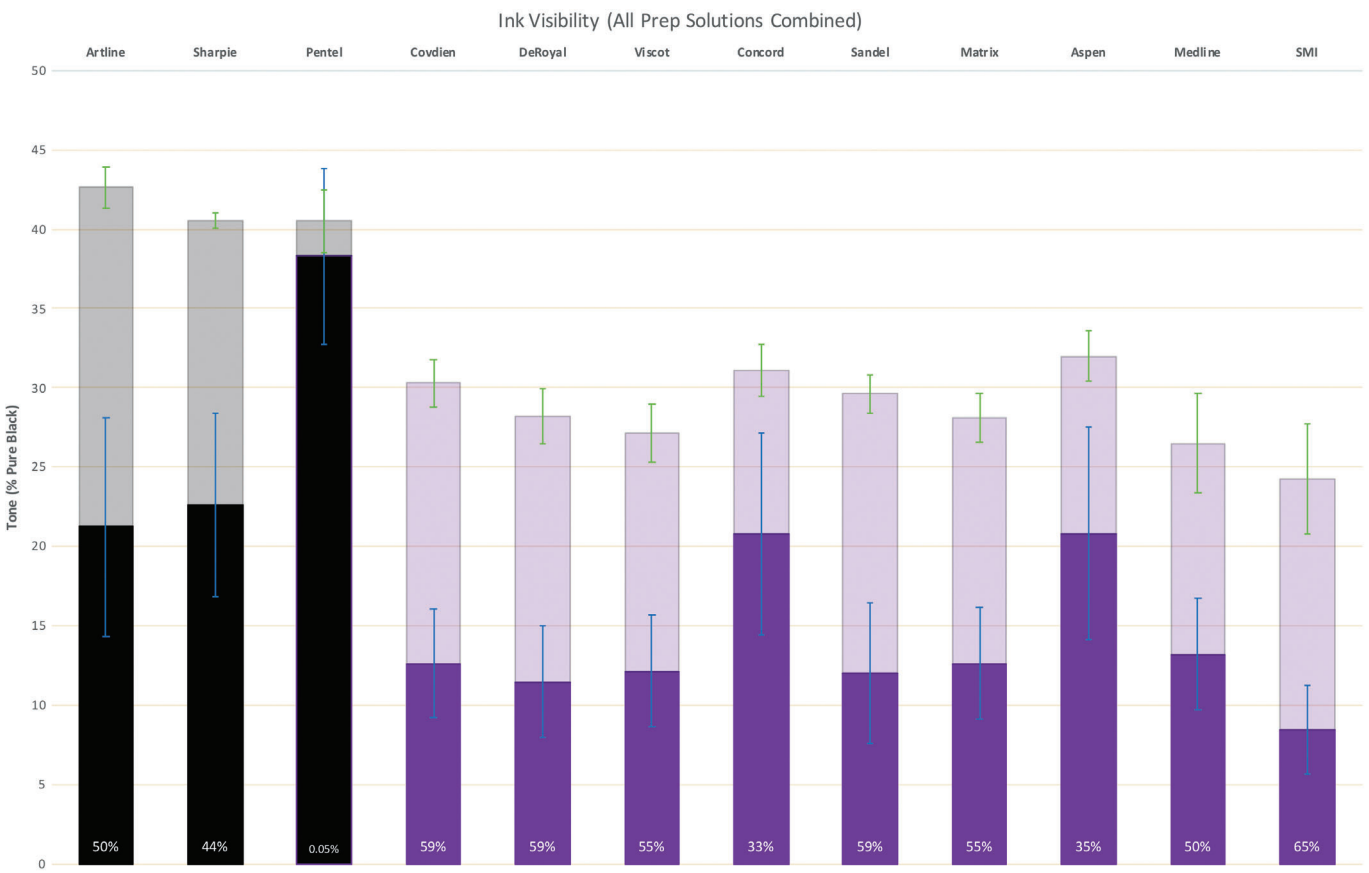

Fig. 3. All prep solutions were pooled to estimate the average reduction in visibility for each pen. All values were statistically significant ($P < 0.05$) except the Pentel N50. This graph demonstrates the tone value for ink before and after wash with standard deviations, as well as a percentage reduction in tone at the bottom of each graph. (■) After prep solution, (■) before prep solution, (□) reduction of ink visibility.

reduction in visibility for all solutions combined and subjectively did not smear. It had the darkest tone of any pen, both before and after wash. It was most affected by 1% IA, showing a reduction of 22% in tone ($P < 0.05$), followed by a 13% reduction with 2% CGA ($P < 0.05$). The reduction was not subjectively significant. Aspen WriteSite Plus was the best performing gentian violet ink pen and second-best performing pen overall. Subjectively it did not smear. It had the equal darkest tone after application of 1% IA with a 17% reduction in visibility which was not subjectively significant ($P < 0.05$). When red tinted 2% CGA was applied, it had a 42% reduction in tone which was second only to Pentel N50 ($P < 0.05$). This reduction was not subjectively significant. Concord was the second-best gentian violet and third best performing pen overall. The ink appeared to smear even prior to wash. Despite smearing, it had very little reduction in visibility when washed with 70% alcohol (29%) or 0.5% CGA (31%). It had the third least reduction in visibility after prep with 2% CGA or 1% IA.

Some gentian violet ink marks were completely removed by prep. Pens by SMI, DeRoyal, Sandel and Covidien were barely visible after wash with chlorhexidine-based solutions.

All pen tips were swabbed with Copan E-Swabs 2 min after their final use. The swabs were sent to a pathology laboratory for culture (Alfred Hospital, Melbourne, Victoria, Australia). No swabs grew any bacteria after a standard incubation period of 7 days.

Discussion

Surgical marking pens serve a range of purposes. Pens are used to mark the correct limb for surgery, delineate an intended incision, assist tissue approximation and identify anatomical landmarks. Ink should be resistant to erasure by alcohol-based surgical prep. When ink comes in contact with deep tissue, the ink should be 'bio-compatible' to avoid tattooing and tissue necrosis.[10] Pen tips should possess anti-microbial properties to prevent bacterial transmission between patients. A sterile packaged pen should be available to allow intra-operative marking.

Visibility

The World Health Organization states that the surgical mark should be unambiguous, clearly visible and made with permanent ink.[11] However, many pens are highly susceptible to erasure by alcohol-based solutions. This can contribute to an event such as wrong-site surgery.[12] Visibility is dependent on patient skin colour, the pen ink and surgical prep solution used.

Skin colour is known to affect the visibility of pen marks. Bathla *et al*.[3] compared the visibility of different pens on patients with different Fitzpatrick skin types and demonstrated that ink was more difficult to see on darker skin tones. The study recommended using ink resistant to erasure for patients with Fitzpatrick grade 4 and 5 skin tones.

Mears *et al*.[4] demonstrated in a cadaveric study that chlorhexidine solutions were far more likely to decrease ink visibility than iodine solutions and this was reproduced by Thakkar *et al*.[5] in a clinical setting.

Mears noted that manufacturer guidelines for chlorhexidine solutions recommend application using forward and backward strokes, whilst iodine solutions recommend applying a single layer without scrubbing. This was proposed as a possible explanation for the difference in visibility. We applied all preparations in the same manner to prevent this confounding factor.

Our results were consistent with their findings. The 1% IA solution affected ink visibility the least and appeared to darken gentian violet ink. The reduction in visibility when using a brown tint may be partly due to a 'dye effect'; black ink was not darkened by brown tint, but the skin was. It is difficult to interpret the extent that the tint, alcohol content and antimicrobial agent affected ink visibility in isolation. It was evident that alcohol alone had a significant effect on ink, and all solutions had the same concentration of alcohol. Despite the alcohol content of 1% IA, it affected gentian violet ink less than untinted alcohol, which may be due to the brown tint rather than a property of iodine. Chlorhexidine solutions had the most effect on visibility. The 2% CGA had more effect than 0.5% CGA, which may be due to the darker tint or chlorhexidine concentration.

As skin tone, prep solution and prep method were standardized, we were able to assess each pen. While black ink pens were most visible on unwashed skin, the Artline and Sharpie marks were greatly affected by prep and were therefore no better than the best performing gentian violet pens.

Bio-compatibility

A risk inherent to marking incisions is 'tattooing'; where ink is permanently incorporated into the scar. This complication is higher when using black permanent markers.[10] Gentian violet ink is regarded as 'bio-compatible' for use on human tissues, while the use of black permanent markers is not recommended due to an inflammatory response and tissue necrosis.[10,13] However, the effect of gentian violet ink on living tissues is not inert, as inflammation and necrosis still occurs.[10,14]

We reviewed the effect of surgical wash on pen marks used to identify the correct limb. If the mark is not over an incision site, there should be no risk of tattooing. Nonetheless, a versatile marking pen would also be bio-compatible for use at the incision site or on subdermal tissue.

Bacterial transmission

Marking pens have been identified as a potential vector for bacterial transmission between patients.[7,8] Tadiparthi *et al*. assessed the bacterial growth from marking pen tips on agar plates at 1-min interval to 5 min. They assessed 'fresh' permanent markers with ethanol in the tip and 'dry' permanent markers that may have been over-used. The 'fresh' pens demonstrated bactericidal action within 2 min, while the 'dry' pen tips had bacterial colonization for at least 30 min after use. The study recommended a 2-min interval between patient marking and old or 'dry' pens be discarded to prevent bacterial transmission. We followed this recommendation and ensured at least a 2-min interval between each study participant and swabbed the pen tips 2 min after final use. There is no evidence to suggest a

difference in infection potential between gentian violet or black permanent marking pens.

Limitations

There are several limitations to this study that should be acknowledged.

The study was conducted in a simulated setting, meaning usual pre-operative preparation was not followed. There may be other factors that affect pen mark visibility during this period. While theatre lights were considered, the lighting negatively affected photography and objective measurement of visibility. It is possible that ink visibility is improved under surgical lights. The measurement of visibility was an objective measurement using software rather than subjective measurement by surgical staff. The clinical significance of our results is therefore difficult to assert.

We used participants with light skin tones to control confounding factors. We assumed that pen visibility would be more important on darker skin tones but did not specifically examine this.

The pens selected were either available for purchase during the study period, or black permanent markers known to be used in this setting by our colleagues. There may be other pens not tested that perform well.

The choice to use one swab per pen was dictated by cost. There is a significant chance of false-negative results. Multiple swabs or direct culture of the pen tip may have improved diagnostic utility, but the efficacy of each method remains unclear.[15]

Conclusion

Surgical marking pens should be resistant to erasure from surgical prep solutions, particularly when used to mark the correct site. Pens may be used on multiple patients for this purpose provided that 2 min elapse between patient contact and the pen tip has not dried out. A permanent marking pen is appropriate for this purpose. Pens with a biocompatible ink should be used for incision site marking or use on human tissue other than skin, thereby decreasing the risk for tattooing, tissue necrosis and transmission of pathogens. Pen selection based on ink visibility is most important when used on dark skin tones and when chlorhexidine-based solutions are used.

For surgical site marking where the pen mark will not contact the incision site or exposed wounds, we recommend the Pentel N50.

We recommend the Aspen WriteSite Plus or Concord pens when a bio-compatible ink is required.

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Conflicts of interest

None declared.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. A table outlining the absolute tone value and percent reduction after wash for every pen with every prep solution and *P*-values. This table shows the average tone (% pure black) after surgical wash and the total reduction in visibility for each pen and each prep solution.