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The Effect of Skin Cleansing Agents on Ethanol Results Measured with the Du Pont Automatic Clinical Analyzer

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ABSTRACT: The effect of various prepackaged skin cleansers on the results of serum ethanol analyses performed with the Du Pont automatic clinical analyzer has not been previously reported. When added directly to serum in concentrations of either 0.625 or 1.56% (v/v), neither polyvinylpyrrolidone iodine nor benzalkonium chloride affected the ethanol results. The cross-reactivity of isopropanol with the automatic clinical analyzer ethanol procedure was 3.9%. The greatest interference in the measured ethanol concentration was from the addition of green soap tincture, which contained 30% ethanol. The effect of improper phlebotomy technique on ethanol measurements was also investigated by performing venipunctures through a pool of 100% ethanol on the skin. No ethanol was detected in these samples unless an ethanol-soaked sponge was pressed over the venipuncture site while the needle was withdrawn from the skin. When correct phlebotomy technique is used, skin cleansing agents should not affect the results of ethanol measurements determined with the Du Pont automatic clinical analyzer.

KEYWORDS: criminalistics, skin cleansers, alcohol, ethanol, phlebotomy, automatic clinical analyzer (ACA)

Forensic scientists are frequently called to give expert testimony regarding the effects of ethanol on the human body. A question frequently asked is whether the skin cleansing agent used (particularly isopropanol) may produce a positive interference with a measured ethanol result. When a chromatographic method, which separates alcohols,[2] is used for ethanol analysis, the answer is self-evident. However, if the serum ethanol concentration is measured as part of the medical evaluation of a patient by a hospital laboratory, a less specific method may be used. An instrument widely used for this purpose is the Du Pont automatic clinical analyzer (ACA). This instrument measures ethanol using a spectrophotometric technique, and little information on the effect of skin cleansing agents on this instrument is available. Goldfinger [*1*] found no significant differences between the "blood alcohol concentration" measured with an ACA after the donor's skin had been cleansed with either 70% isopropanol or a povidone-iodine (Betadine®) impregnated swab. The manufacturer of the ACA states that their ethyl alcohol method reacts with less than 6% of the isopropyl alcohol present in the specimen [*2*], but this claim has not been independently verified. We are aware of no reports that quantitate the

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[2]In this article the term "alcohol" is used in the generic sense. Specific alcohols are differentiated by name.

interference of skin cleansing agents on serum ethanol concentrations measured with the Du Pont ACA.

This lack of information may affect legal proceedings. When blood is to be collected for ACA ethanol measurements, Du Pont specifies the use of a "nonalcohol germicidal solution to cleanse the skin" [2]. The basis for this precaution is the theoretical possibility that the alcohol used to cleanse the skin may falsely increase the measured serum ethanol concentration. Failure to follow the manufacturer's directions in this regard may adversely affect the admissibility of ethanol results as evidence in court. In a recent Indiana criminal case [3], the Court of Appeals sustained a motion to suppress the evidence, based on the possible contribution of an isopropanol skin cleanser to the serum ethanol concentration indicated by the test results. During legal proceedings in which the test results have been admitted into evidence, cleansing agents may have an impact on the weight a judge or jury gives to that evidence.

For this study we examined the potential of various skin cleansing agents, including "alcohol-containing skin cleansing agents" [2], to influence the serum ethanol concentration as measured by the Du Pont ACA. Where possible, we present this information in quantitative form.

Materials and Methods

Reagents

Isopropanol was Baker Analyzed®, reagent grade, from J. T. Baker Chemical Co. Absolute (200 proof, USP) ethanol, reagent quality, was obtained from AAPER Alcohol and Chemical Co.

Skin Cleansing Agents

The following prepackaged skin cleansing solutions were used in this study: (1) A 10% polyvinylpyrrolidone iodine solution (PVP iodine solution, 10% U.S.P., Marion Scientific); (2) polyvinylpyrrolidone iodine scrub solution (povidone-iodine Redi-Prep® scrub pad, Professional Disposables, Inc.); (3) benzalkonium chloride 1:250 (Tomac® antiseptic skin towelette, distributed by American Hospital Supply); (4) 70% isopropanol plus 10% acetone (antiseptic applicator, Marion Scientific); (5) 70% isopropanol (PDI alcohol prep, medium pad, Professional Disposables, Inc.); (6) 70% isopropanol (antiseptic applicator, Marion Scientific); and (7) green soap tincture, (30% alcohol, Marion Scientific). Cleansing Agents 1, 4, 6, and 7 were supplied as solutions. Samples of Agents 2, 3, and 5 were obtained by squeezing the pad and collecting the expressed liquid in a clean tube.

Quantitative Ethanol Determinations

Ethanol concentrations in serum were measured using a Du Pont ACA-III, which enzymatically oxidizes ethanol to acetaldehyde, with the simultaneous conversion of nicotinamide adenine dinucleotide (NAD) to the reduced cofactor, NADH. The appearance of NADH is measured by monitoring the rate of change in absorbance at 340 nm. The ACA was calibrated according to the manufacturer's instructions using ethanol standards purchased from MCB Reagents (MCB Manufacturing Chemists, Inc.). Our mean and standard deviation between days for a serum control was 100 ± 1.3 mg/dL.[3]

Qualitative Alcohol Determinations

Identification and confirmation of the ethanol and isopropanol concentrations in serum and other solutions was performed on a Varian Aerograph model 550 gas chromatograph equipped

[3]An ethanol concentration of 100 mg/dL is equivalent to an ethanol concentration of 0.10% (w/v).

with a flame ionization detector (Varian Associates, Inc.). The 2-m by 1-mm (internal diameter) stainless steel column was packed with 5% Hallcomid M-18 and 0.5% Carbowax-600 on Chromosorb T, 40 to 60 mesh (Applied Science Laboratories, Inc.).

Effect of Improper Phlebotomy Technique

We examined the potential for ethanol contamination of the blood specimen if a less-than-optimal phlebotomy technique was used when obtaining blood specimens for ethanol analysis. Our protocol was to swab and saturate liberally the skin of the antecubital fossa of the nine volunteers with 100% ethanol. A venipuncture was immediately performed through a standing pool of ethanol, with the beveled portion of the multisample needle oriented upward. Four blood samples were obtained, with the first three 10-mL evacuated tubes filled completely. The needle was not withdrawn between samples. The fourth tube was less than half filled when the needle was withdrawn slowly from the skin; a cotton swab saturated with 5 mL of ethanol was held against the skin directly over the venipuncture site. During withdrawal, an audible suction sound confirmed that ethanol and air were being drawn into the partially filled blood sampling tube. This is similar to the protocol used by Muller and Hundt [*4*].

Results

Interference by extraneous substances in the ACA ethyl alcohol procedure is proportional to both the amount of the substance present in the serum sample and to any nonspecificity of the alcohol dehydrogenase used in the reagent packs. We examined each of these possibilities individually.

Specificity of the ACA Ethanol Procedure

We examined the potential for skin cleansers to interfere with the ACA ethanol procedure by adding relatively large amounts of these agents directly to serum that contained either no ethanol, or to serum that had been tainted with ethanol to a concentration of 224 mg/dL. Adding benzalkonium chloride or polyvinylpyrrolidone iodine solution to serum had no effect on the serum ethanol concentration as measured by the ACA (Fig. 1).

Green soap tincture with "30% alcohol" caused the greatest interference of any skin cleansing preparation added directly to serum. Although the package label did not specify which alcohol was incorporated with the soap in this preparation, gas chromatographic analysis showed that the alcohol used in the green soap tincture preparation was ethanol. The interference obtained by adding green soap tincture to sera could be reproduced by substituting a 30% ethanol solution and adding a similar volume.

When added directly to the serum, each of the 70% isopropanol preparations, whether originally packaged as solutions, swabs, or combined with 10% acetone, increased the measured ethanol results only slightly. The magnitude of this increase was the same for all three of these isopropanol preparations, depending entirely on the isopropanol content of the serum samples. The effect of isopropanol in the ACA ethanol determination was reproducible from lot to lot of ACA ethanol packs. We examined the results obtained after adding isopropanol in concentrations up to 1260 mg/dL to pooled serum supplemented with ethanol concentrations of either 100, 200, or 300 mg/dL. At each of the serum ethanol concentrations used, we found an identical percentage of isopropanol interference with the test (Fig. 2). The magnitude of this interference was 3.9% of the isopropanol concentration added.

Will Skin Cleansers Contaminate Blood?

Phlebotomy was performed as described. The concentration of ethanol in Tube 4 is shown in Table 1. Ethanol was not detected in specimen Tubes 1, 2, or 3 from any volunteer, but signifi-

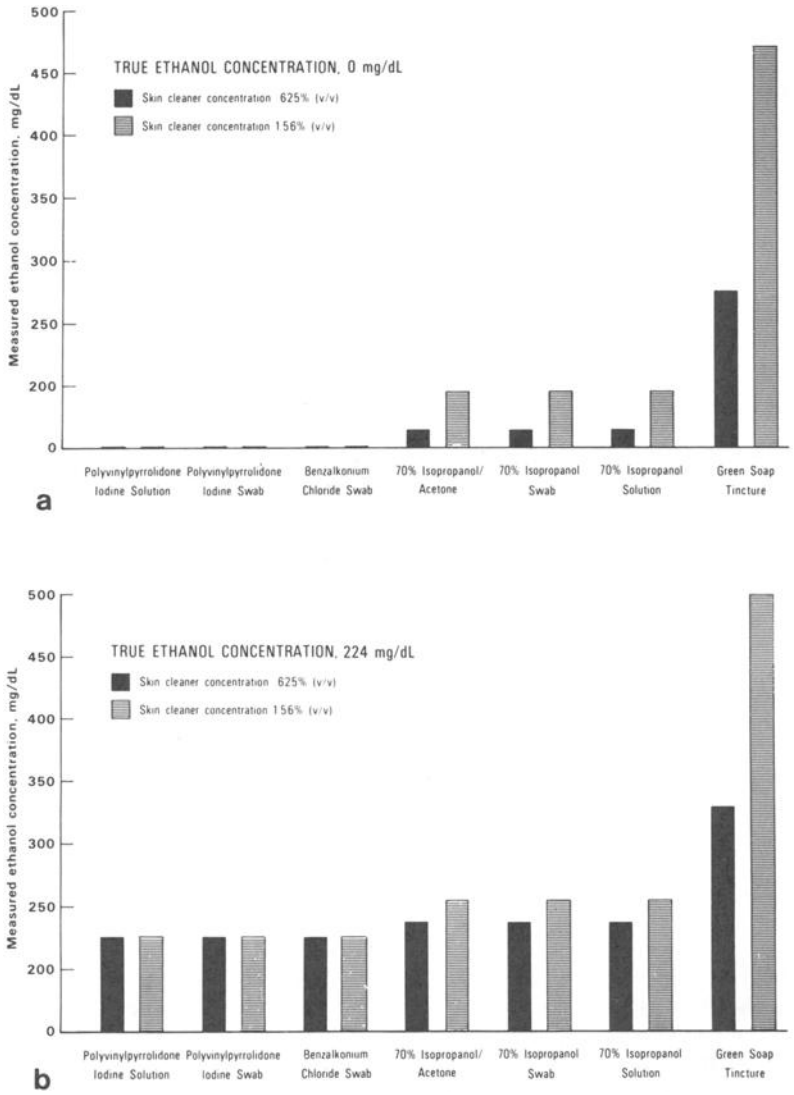

FIG. 1—*The effect of adding two different concentrations of skin cleansing agents (0.625%, 1.56%) to serum containing no ethanol* (a), *and to serum with an ethanol concentration of 224 mg/dL* (b), *on the ethanol result measured with the Du Pont ACA.*

cant amounts of ethanol were incorporated into the blood specimens during withdrawal of the needle from the skin under the conditions used to obtain Tube 4. The results in Table 1 are similar to those in earlier reports [*4,5*].

Discussion

When the effect of the skin cleansing agent on the observed ethanol concentration is questioned in court, the answer depends on the skin cleanser used. Neither benzalkonium chloride nor polyvinylpyrrolidone iodine solutions affect the ethanol result measured with the ACA. Similarly, skin cleansers containing either isopropanol or ethanol will not affect the accuracy

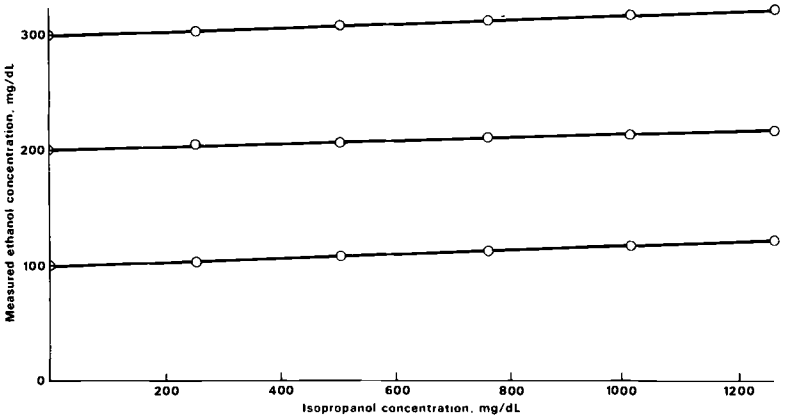

FIG. 2—*The effect of adding 100% isopropanol to sera with ethanol concentrations of 100, 200, and 300 mg/dL on the ethanol result measured with the Du Pont ACA.*

TABLE 1—*Serum ethanol concentration in Tube 4.*

Subject	Ethanol Concentration, mg/dL
1	102
2	272
3	310
4	10
5	21
6	71
7	638
8	88
9	526

of the ACA ethanol result if proper phlebotomy technique (letting the skin dry before venipuncture) has been used [*6*]. We found no detectable ethanol in serum even when the phlebotomy had been performed through a standing pool of 100% ethanol. If poor phlebotomy technique was used, however, and either the isopropanol or ethanol skin cleanser was aspirated into the sample, then the possibility of contamination exists. We found (see Table 1) that the amount of contamination varied between subjects. The magnitude of expected changes using skin cleansers other than absolute ethanol would be less because a dilute solution is used in the skin cleanser. Using green soap tincture (30% ethanol), the expected contamination would be 30% of the amount observed for the specimens drawn into Tube 4 (Table 1). If 70% isopropanol were the skin cleansing agent, the apparent serum ethanol increase would be only 2.7% (0.70 × 3.9%) of that shown in Table 1 because of the 3.9% reactivity of isopropanol in the ACA ethanol procedure.

A strict prohibition against the use of alcohol-containing skin cleansing agents when obtaining blood for ethanol measurements—as recommended by the manufacturer of the ACA [*2*], as well as Dubowski [*5*] and Kaye [*7*]—will avoid even the theoretical possibility of contamination of the specimen. We have shown, however, that inadvertantly cleansing the skin with an ethanol- or isopropanol-containing agent (not in accord with manufacturer's recommendations) does not necessarily invalidate the ethanol result measured with the ACA. Such results must be interpreted both in light of the skin cleansing agent and the phlebotomy technique used.

References

[1] Goldfinger, T. M. and Schaber, D., "A Comparison of Blood Alcohol Concentration Using Nonalcohol- and Alcohol-Containing Skin Antiseptics," *Annals of Emergency Medicine*, Vol. 11, No. 12, Dec. 1982, pp. 665-667.

[2] *ACA Ethyl Alcohol Test Methodology*, Du Pont Co., Clinical Systems Division, Wilmington, DE, 1981.

[3] *State v. Alderson*, 435 N.E.2d 614 (Ind. App. 1982).

[4] Muller, F. O. and Hundt, H. K. L., "Ethyl Alcohol: Contamination of Blood Specimens," *South African Medical Journal*, Vol. 50, 24 Jan. 1976, p. 91.

[5] Dubowski, K. M. and Essary, N. A., "Contamination of Blood Specimens for Alcohol Analysis During Collection," *Abstracts & Reviews in Alcohol & Driving*, Vol. 4, No. 2, April-June 1983, pp. 3-8.

[6] *Standard Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (ASH-3)*, National Committee for Clinical Laboratory Standards, Villanova, PA, 1977.

[7] Kaye, S., "The Collection and Handling of the Blood Alcohol Specimen," *American Journal of Clinical Pathology*, Vol. 74, No. 5, Nov. 1980, pp. 743-746.

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