

## ABSTRACT

Previously published research contends whether ethanol production can occur in blood tubes. This project investigates whether unusual conditions can result in ethanol production within blood tubes. This project investigated three aspects: 1) storage time, 2) adverse storage conditions, and 3) exposure of blood samples to microorganisms that may exist in the laboratory.

## INTRODUCTION

Blume and Lakatua published a study in 1973 demonstrating ethanol production within a blood tube [1]. Chang and Kollman also demonstrated the issue in 1989 [2]. These publications are often cited by defense lawyers during cross-examination of blood alcohol analysts, contending that the reported blood alcohol concentration (BAC) is elevated. Multiple studies have been published regarding this issue. Many of these studies were conducted under acceptable laboratory conditions. This project investigated the possibility of ethanol production in blood tubes when conditions are not ideal.

The first part of the study investigated storage time. Blood tubes from 2014 to 2021 were analyzed in July 2021 to determine their current BACs. This experiment investigated whether ethanol production was possible over multiple years.

The second part of the study investigated the effect of temperature and was conducted while the laboratory was undergoing air conditioning repair, during which no air conditioning was operable in the area the blood tubes for this study were stored. Houston, Texas is an area prone to hurricanes, which can cause loss of electricity. This part of the study investigated the scenario under which the Houston lab had no air conditioning during the summer heat. Blood tubes were divided into two groups to study what would occur if there was a total loss of air conditioning. In one group, tubes were left on the benchtop without air conditioning, while in the second group tubes were stored in a refrigerator in a room without air conditioning.

The third part of the study investigated whether exposure to the laboratory would result in ethanol production. Laboratory surfaces may contain microorganisms. These microorganisms may or may not be capable of producing ethanol. This part of the study investigated whether, if any ethanol-producing microorganisms existed on those surfaces, any increase in BAC would result. Areas of the laboratory that blood tubes interact with most were swabbed. Blood samples used in this portion of the study were stored either in a refrigerator or at normal room temperature with air conditioning working.

## RESULTS AND DISCUSSION

**Table 1.** Summary table for storage time.

Original year	Within uncertainty limits	Outside uncertainty limits
2014	2	1
2015	2	2
2016	3	2
2017	2	6
2018	6	3
2019	6	11
2020	9	4
2021	8	4

The BACs from the storage time measurement were compared to the original BACs and classified as within or outside uncertainty limits. Blood tubes are organized by year the blood tube was received by the lab.

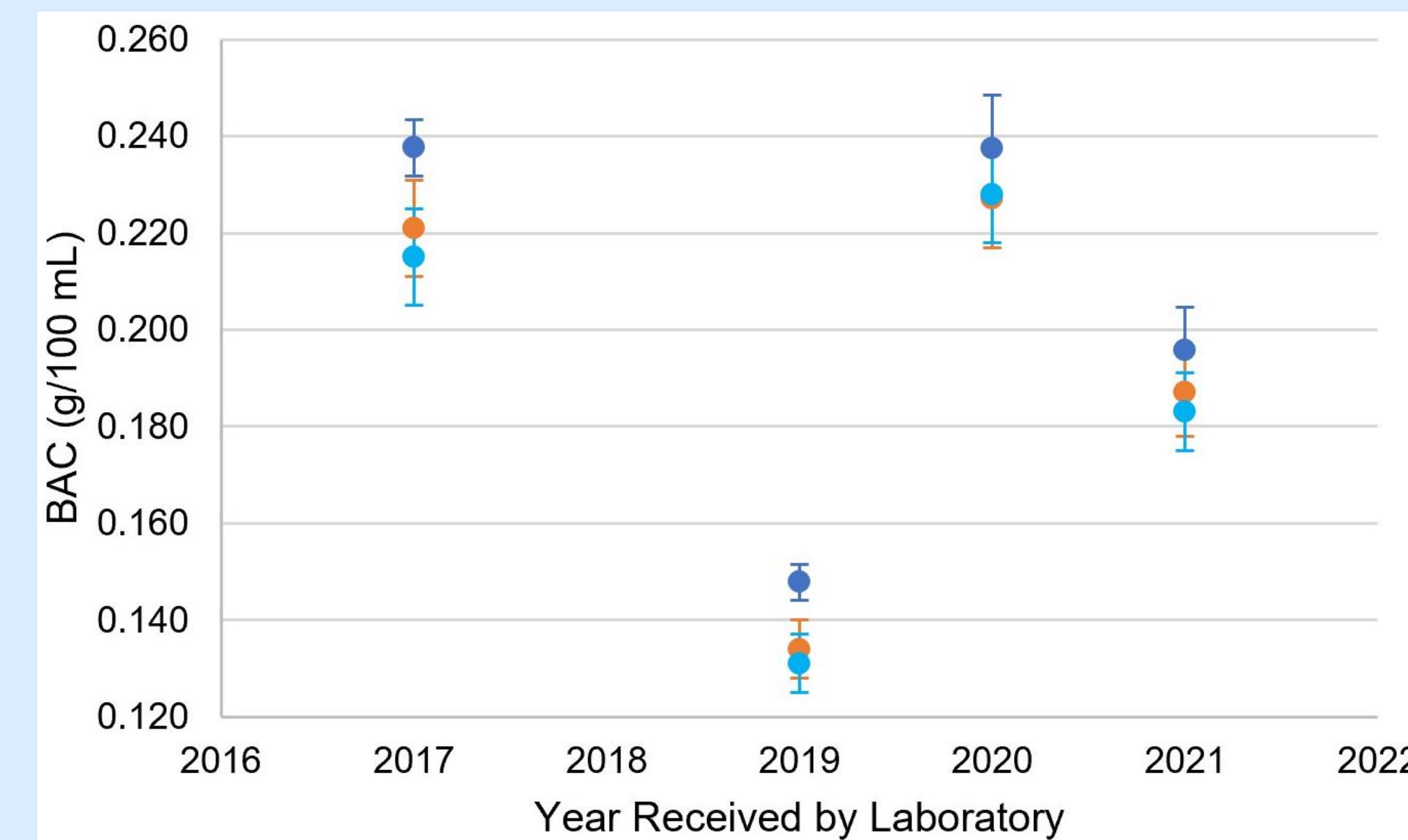
- ❖ Blood tubes with BACs from different time points with overlapping uncertainties are classified as within uncertainty limits, as shown by comparing dark blue and orange error bars for the tubes from 2020 and 2021 in Figure 1.
- ❖ Blood tubes with BACs from different time points with uncertainties that do not overlap are classified as outside uncertainty limits, as shown by comparing dark blue and orange error bars for the tubes from 2017 and 2019 in Figure 1.
- ❖ Blood tubes outside uncertainty limits from Table 1 experienced ethanol loss ranging between 0.004 and 0.021 g/100 mL.
- ❖ For the adverse storage conditions, both the benchtop and refrigerated blood tubes were within uncertainty limits after more than 10 days, as shown by comparing the orange and light blue error bars in Figure 1.

**Table 2.** Laboratory exposure results after 3 days.

Sample	Lab exposure type	Condition	BAC (g/100 mL)	
			Initial	3 days
1	Left open in biosafety cabinet next to Sample 2 for 10 min	Refrigerated	0.228	0.226
		Room temp.	0.228	0.225
2	Left open in biosafety cabinet next to Sample 1 for 10 min	Refrigerated	0.008	0
		Room temp.	0.008	0
3	Exposed to swab from biosafety cabinet surface	Refrigerated	0.065	0.056
		Room temp.	0.065	0.043
4	Exposed to swab from inside of disposable tube stored in biosafety cabinet	Refrigerated	0.183	0.172
		Room temp.	0.183	0.159
5	Exposed to swab from Hamilton diluter pipette	Refrigerated	0.266	0.258
		Room temp.	0.266	0.252
6	Exposed to swab from rocker	Refrigerated	0.149	0.143
		Room temp.	0.149	0.132

The benchtop condition for this experiment was normal room temperature with air conditioning.

- ❖ No increase in BAC was observed for any blood sample under refrigeration or benchtop conditions.
- ❖ Generally, the blood samples under benchtop conditions experienced a greater decrease in BAC than the corresponding refrigerated blood sample.



**Figure 1.** Comparison of BACs of exemplar blood tubes across years. Dark blue dots indicate the BAC measured at the time the blood tube was received by the laboratory. Orange dots indicate the BAC measured for the storage time experiment. Light blue dots indicate the BAC measured after the adverse storage conditions experiment. Blood tubes with BACs from different time points with overlapping uncertainties are classified as within uncertainty limits, as shown by the tubes from 2020 and 2021. Blood tubes with BACs from different time points with uncertainties that do not overlap are classified as outside uncertainty limits, as shown by tubes from 2017 and 2019.

## MATERIALS AND METHODS

### Storage Time

Refrigerated blood tubes from 2014 to 2021 were analyzed in July 2021. The BACs measured in July 2021 were compared to the BACs measured at the time the blood tubes were originally received by the laboratory.

### Adverse Storage Conditions

Half of the blood tubes were stored on the benchtop at elevated temperatures and the other half were refrigerated for more than 10 days before analysis. The BACs measured from this part of the study were compared to the BACs measured from the first part of the study.

### Laboratory Exposure

Four areas of the laboratory were swabbed, each with a separate cotton-tipped applicator. Each sample was swirled into a corresponding blood tube. Two blood tubes were left uncapped in the biosafety cabinet for 10 minutes to test whether ethanol could transfer from one tube to another.

### Instrumentation and Data Analysis

A Shimadzu GC-FID was used to analyze the headspace of the blood samples. Each sample was run in duplicate. Microsoft Excel was used to calculate average BACs and uncertainty estimates with a 99.7% confidence interval.

## CONCLUSIONS

- ❖ No ethanol production was observed at any point of the study, only ethanol loss.
- ❖ During storage time, ethanol losses of 0.004-0.021 g/100 mL were observed.
- ❖ No significant change in BAC occurred when blood tubes were stored at elevated temperatures for more than 10 days.
- ❖ No increase in BAC occurred in blood tubes exposed to certain areas of the lab or another blood tube.

## REFERENCES

- [1] P. Blume, D.J. Lakatua, The Effect of Microbial Contamination of the Blood Sample on the Determination of Ethanol Levels in Serum, *Am. J. Clin. Pathol.* 60(5) (1973) 700-702.
- [2] J. Chang, S.E. Kollman, The Effect of Temperature on the Formation of Ethanol by *Candida Albicans* in Blood, *J. Forensic Sci.* 34(1) (1989) 105-109.

## ACKNOWLEDGEMENTS

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