Comparison of Various Repurposing Methods for Laboratory Grade Pipette Tips

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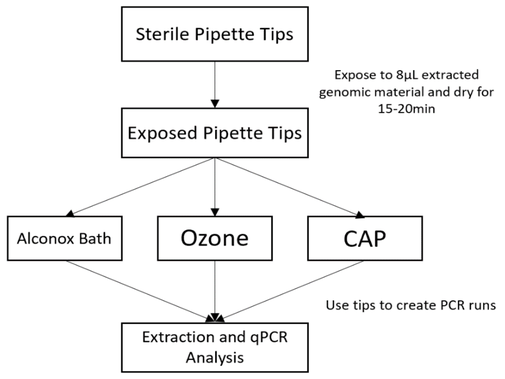
Abstract

The goal of this investigation was to assess reusability of decontaminated pipette tips by analyzing traces of replicable DNA with real-time quantitative PCR (qRT - PCR). Decontamination methods consisted of the following: (1) washing tips with a 2.5% detergent solution (Alconox) along with steam sterilization, (2) exposure of ozone vapor at 250 and 14400 PPM * minute, and (3) upright and inverted tip exposure of cold atmospheric plasma (CAP). Negative controls consisted of unused tips as a reference to compare DNA extracts of *Aeromonas hydrophila* (ATCC-23211) from experimental groups. Efficacy was determined by turnover ratio (ratio between decontaminated tips and total treated tips) and log reduction in detectable genomic material of the contaminated products via real-time quantitative PCR (qRT - PCR). Although the detergent solution and steam sterilization method had the highest log reduction (5.943) the residue in these pipette tips, following cleaning (including, inner filters and tip box), suggested that washing with a detergent was an unfavorable method. Ozone vapor at 14400 PPM * minute showed the second highest turnover ratio (98.4 %) and log reduction (4.511). CAP exposure in inverted tip orientation (tip end) 1 minute showed turnover ratio (68.3 %) and log reduction (4.002). Therefore, CAP exposure could be further optimized to mitigate human error and to develop a time - efficient method, (1-5 minutes for CAP versus overnight exposures for ozone). The conclusion resulting from this study included the following: from these modalities, the least change to pipette tip performance, based on subjective analysis, was CAP, while ozone vapor decontamination demonstrated the greatest clearance of nucleic acids and tip performance without destroying the tips.

Materials and Methods

Schematic Layout and Sample Size / Selection

Protocol Outline **Figure 1.**



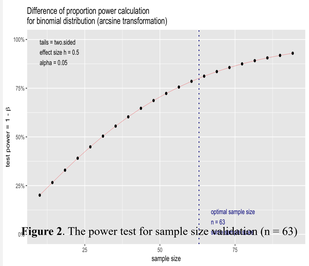


Figure 2. The power test for sample size validation (n = 63)

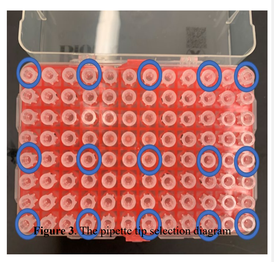
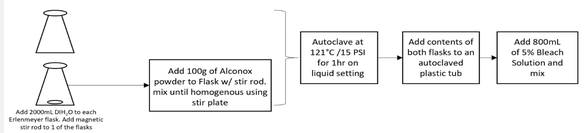


Figure 3. The pipette tip selection diagram

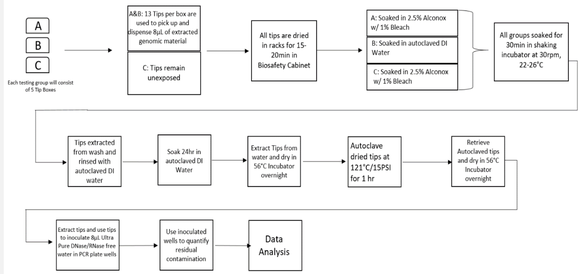
Materials and Methods

Layouts of Repurposing Methods

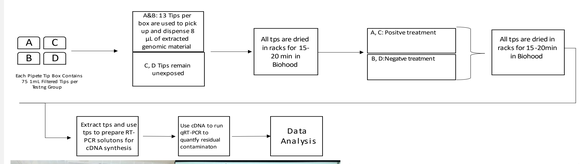
Alconox/Bleach Wash Solution **Figure 4.**



Alconox/Bleach Wash Protocol **Figure 5.**



Ozone / CAP Protocol **Figure 6.**



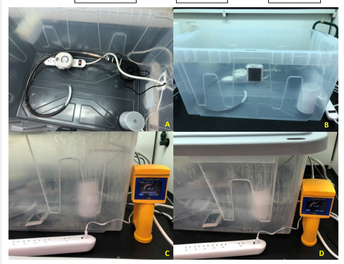


Figure 7.
A) Top view of the OSC from left are Carulite 200 packed tubing using glass wool, ozone generator, pump with tubing, and humidifier.
B) Side view of the OSC,
C) Side view of the OSC during exposure, Ozone sensor: 14.2 PPM (Parts per million),
D) Side view of the OSC post neutralization, Ozone sensor: 0.0 PPM.

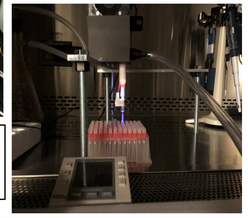


Figure 8. Layout of pipette tips during the CAP exposure
Input voltage: 25 V Regulated voltage: 12 V
Discharge voltage: 6.5 kV Frequency: 12.5 kHz
Helium gas flowrate range of 5 LPM (liters per minute)
Distance from exit to exposure site: 2~5 cm

Primer Validation for A. hydrophila							
			Primer BLAST		IDT Oligoanalyzer		
Target Gene	FP/RP	Primer Sequence (5'-3')	Tm (°C)	GC%	Hairpin ΔG (kCal/mol)	Self-Dimer ΔG (kCal/mol)	Hetero-Dimer ΔG (kCal/mol)
16s rRNA	Forward Primer	GCGGCGGACGGGTGAGTA	64.44	72.22	-1.58	-3.61~-0.96	-11.09~-1.6
	Reverse Primer	CCCACTGCTGCCTCCCGT	64.41	72.22			

Validation and Results

qRT – PCR Data Processing and Log Reduction Validation

"CDC 2019 Novel Coronavirus (nCoV) Real-Time RT-PCR Diagnostic Panel - Instructions for Use" reported: the limit of detection for the samples was $10^{0.5}$ RNA copies / µL (~ 3162 copies / mL) and the mean threshold cycle value was 32 with 100% positive detection test results (20 / 20), (Positive / Total).

The threshold number of DNA copies / mL present in a detectable sample =

Initial amount of DNA copies / mL X ($2^{\text{Mean Threshold Cycle}}$)

$$\text{Expected mean threshold cycle} = \log_2 \frac{\text{The threshold number of DNA copies / mL present in a detectable sample}}{\text{Initial amount of DNA copies / mL}}$$

Example Calculation:

Initial number of copies : 3.544×10^5 DNA copies / mL

Threshold number of DNA copies / mL present in a detectable sample : 1.358×10^{13} RNA copies / mL

Expected mean threshold cycle of a detectable sample : 25.19 cycles

Log reduction = Log_{10} (concentration of inoculated) – Log_{10} (concentration of harvested)

DNA concentration = Threshold number of DNA copies / mL in a detectable sample / ($2^{\text{mean threshold cycle}}$)

Turnover ratio = Number of samples considered as repurposed / total sample number

Log Reduction Data and Graph

	Conditions									
	Positive Control	Negative Control	Alconox Control	Alconox 2.5 %	Ozone Control	Ozone 250 PPM*min	Ozone 14400 PPM*min	CAP Control	CAP Upright	CAP Inverted
Average Threshold Cycle	16.316	30.213	37.130	36.058	31.039	27.440	31.301	31.439	25.047	29.611
Cleaned Sample	0	36	71	70	58	13	62	59	9	43
Total Sample Number	63	63	73	73	63	63	63	63	63	63
Turnover Ratio	0.000	0.571	0.973	0.959	0.921	0.206	0.984	0.937	0.143	0.683
Log Reduction	-4.183	0.000	2.082	5.943	0.248	3.349	4.511	0.369	2.628	4.002
Threshold Cycle Standard Deviation	2.093	1.080	5.659	4.679	1.198	4.145	0.660	1.302	4.236	2.467
Percent Error	0.128	0.036	0.152	0.130	0.039	0.151	0.021	0.041	0.169	0.083
Error Bar (Log)	0.537	0.000	0.317	0.771	0.010	0.506	0.095	0.015	0.445	0.333
Processed Error Bar (Log)	0.537	0.009	0.317	1.308	0.010	1.042	0.632	0.015	0.981	0.870

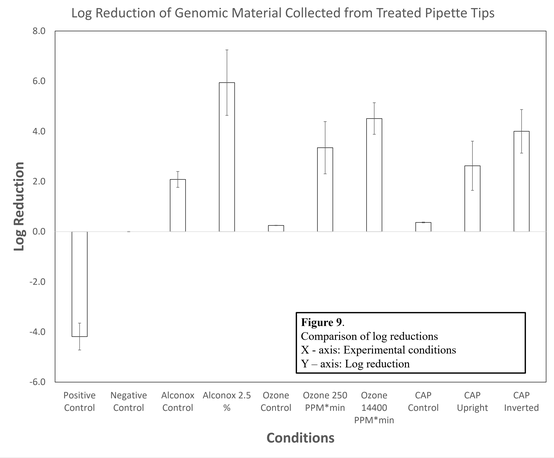


Figure 9.
Comparison of log reductions
X - axis: Experimental conditions
Y – axis: Log reduction

Results and Discussion

Statistical Analysis of Data to Determine Decontamination Efficacy

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Negative Control vs. Alconox Control	-2.082	-2.484 to -1.680	****	<0.0001
Negative Control vs. Ozone Control	-0.248	-0.6645 to 0.1685	ns	0.6757
Negative Control vs. CAP Control	-0.369	-0.7855 to 0.04754	ns	0.1343
Alconox 2.5 % vs. Ozone 250 PPM*min	2.594	2.192 to 2.996	****	<0.0001
Alconox 2.5 % vs. Ozone 14400 PPM*min	1.432	1.030 to 1.834	****	<0.0001
Alconox 2.5 % vs. CAP Upright	3.315	2.913 to 3.717	****	<0.0001
Alconox 2.5 % vs. CAP Inverted	1.941	1.539 to 2.343	****	<0.0001
Alconox Control vs. Ozone Control	1.834	1.432 to 2.236	****	<0.0001
Alconox Control vs. CAP Control	1.713	1.311 to 2.115	****	<0.0001
Ozone 250 PPM*min vs. Ozone 14400 PPM*min	-1.162	-1.579 to -0.7455	****	<0.0001
Ozone 250 PPM*min vs. CAP Upright	0.721	0.3045 to 1.138	****	<0.0001
Ozone 250 PPM*min vs. CAP Inverted	-0.653	-1.070 to -0.2365	****	<0.0001
Ozone 14400 PPM*min vs. CAP Upright	1.883	1.466 to 2.300	****	<0.0001
Ozone 14400 PPM*min vs. CAP Inverted	0.509	0.09246 to 0.9255	**	0.0045
Ozone Control vs. CAP Control	-0.121	-0.5375 to 0.2955	ns	0.9957
CAP Upright vs. CAP Inverted	-1.374	-1.791 to -0.9575	****	<0.0001

The positive and negative controls demonstrated that active DNA residue can interfere with qRT - PCR results. The calibration curve of *A. hydrophila* followed a growth pattern previously observed and the concentration of extracted DNA were appropriately normalized into a range of 110 to 150 ng/µL with acceptable absorbance ratio.

Alconox (Least favorable)
- Highest log reduction (5.943)
- Excessive residue, notable degradation
- Statistically significant difference in Positive Control versus Negative Control

Ozone 14400 PPM * minute (Most optimized)
- The second highest turnover ratio (98.4 %)
- Log reduction (4.511)
- Minimal degradation

CAP inverted 1 minute (Greatest potential)
- The turnover ratio of 68.3 %
- Log reduction (4.002)
- Efficient and minimal degradation, further optimization needed.

Conclusions

These results demonstrate detergent wash, ozone and CAP treatments resulted in different outcomes with regards to genomic material removal, product integrity, and therefore reuse. Overall, ozone exposure showed the most inactivation of genetic material while retaining product efficacy. CAP optimization, relative to the product could result into an efficient decontamination treatment, based on these preliminary findings.

Disclaimer

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Acknowledgement

This research was supported by OCS/MCMi, CDRH/OCET, and DBCMS/OSEL