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██████████████████ ████████	Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe
Report Version: 2.0	Report Date: 3rd June 2020

To:

███████████████

From:
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Study Title: *In vitro* test to determine the impact of four whitening treatments versus deionised water on enamel erosion and surface microhardness

1. Objectives

- To compare the impact of four whitening treatments versus deionised water on enamel erosion and surface microhardness.

2. Overview

Samples of human enamel were sectioned, set into resin moulds and polished to 2400 grit. A ProFilm 3D surface profilometer was used to measure the baseline surface profiles of the enamel samples in order to ensure the samples were sufficiently flat. A Tukon 1202 surface microhardness machine was used to measure the baseline Vickers microhardness of the enamel samples.

Tape was used to cover part of each enamel sample in order to provide a baseline reference area for post treatment surface profilometry assessments.

Each enamel sample was subjected to 6 consecutive applications of the assigned treatment. The treatments comprised;

- 6 x 10-minute applications of a HISMILE Teeth Whitening Gel PAP Formula A
- 6 x 10-minute applications of a whitening gel containing 35% Carbamide Peroxide
- 6 x 10-minute applications of a whitening gel containing 6% Hydrogen Peroxide
- 6 x 10-minute applications of a whitening gel containing 35% Hydrogen Peroxide
- 6 x 10-minute applications of deionised water (negative control)

A ProFilm 3D surface profilometer was used to measure the erosion of the enamel samples by comparing the treated areas with the reference areas that were protected from the treatment. A Tukon 1202 surface microhardness machine was used to measure the post treatment Vickers microhardness of the enamel samples.

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███████████████████ ████████████	Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe
Report Version: 2.0	Report Date: 3rd June 2020

3. Treatments

Table 1: Products Used in Study

Treatment	Duration of Treatments
Negative Control Fisher Analytical Reagent Grade Water Code: W/0100/25 Lot: 1919526	6 x 10-minute applications
HISMILE Teeth Whitening Gel PAP Formula A Lot: 333112019	6 x 10-minute applications
Whitening Gel containing 35% Carbamide Peroxide Lot: X000UX3HGR	6 x 10-minute applications
Whitening Gel containing 6% Hydrogen Peroxide	6 x 10-minute applications
Whitening Gel containing 35% Hydrogen Peroxide	6 x 10-minute applications

4. Study Preparation

Sample Preparation

Six samples of enamel were prepared for each treatment group, shaped from extracted human teeth. Enamel samples (4x4mm) were prepared with a dental abrader from the coronal portion of the tooth.

Samples of enamel were set into resin discs using EpoxiCure2 resin. Enamel surfaces were machine polished using a Saphir 550 polishing machine to a final grade of P2400. Reference areas were formed in the enamel samples by covering part of the samples with tape.

Baseline Assessments

A calibrated ProFilm 3D surface profilometer was used to measure the surface of the enamel samples (Figure 1).

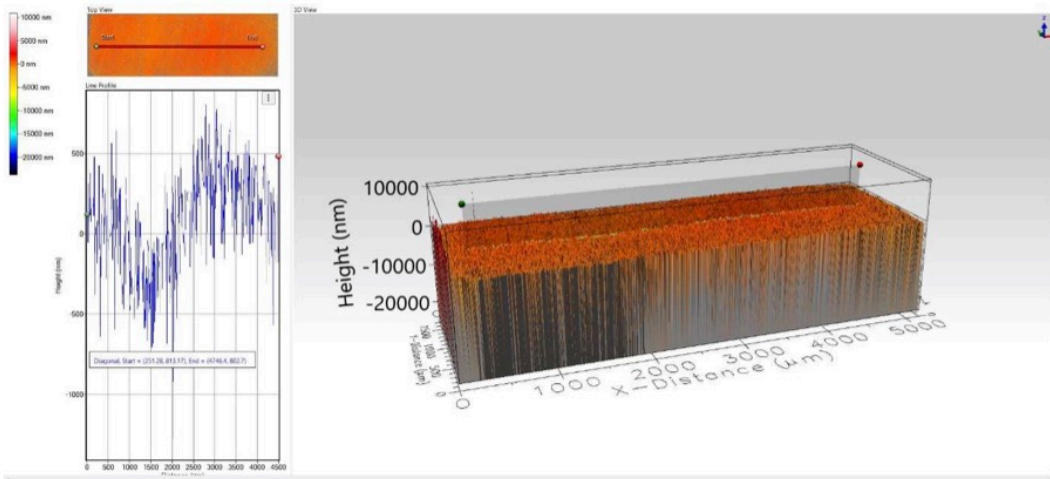| ![intertek — Total Quality. Assured.]	**Laboratory Report**
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Report Version: 2.0	Report Date: 3rd June 2020

Figure 1: Example of a Baseline 3D Surface Measured During the Study.

A calibrated Tukon 1202 surface microhardness machine was used to measure the Vickers surface microhardness of the enamel samples. Three surface microhardness measurements were measured for each sample, under a 50-gram load.

5. Treatment

Six consecutive applications of each treatment were applied. No saliva immersions were performed between treatments in order to create a worst-case scenario and to better understand the propensity of the treatments to damage enamel.

The following treatment procedure was followed for the HISMILE Whitening Gel PAP Formula A group:

- Approximately 0.5g of whitening gel PAP Formula A was applied to the end of a cotton bud.
- The gel was then applied to the moist enamel surface of the block using the cotton bud in a gentle, swiping motion, ensuring the entire surface was evenly covered.
- The gel was left on the blocks for 10 minutes, before being rinsed off with deionised water.
- The blocks were dabbed with tissue to remove excess moisture from the surface.
- The process was repeated until 6 consecutive treatment applications were performed.

The following treatment procedure was followed for the whitening gel containing 35% Carbamide Peroxide group:

- Approximately 0.5g of whitening gel containing 35% carbamide peroxide was applied to the end of a cotton bud.
- The gel was then applied to the moist enamel surface of the block using the cotton bud in a gentle, swiping motion, ensuring the entire surface was evenly covered.
- The gel was left on the blocks for 10 minutes depending on the group, before being rinsed off with deionised water.
- The blocks were dabbed with tissue to remove excess moisture from the surface.

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████████████████████ ███████████	Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe
Report Version: 2.0	Report Date: 3rd June 2020

- The process was repeated until 6 consecutive treatment applications were performed.

The following treatment procedure was followed for the whitening gel6% Hydrogen Peroxide group:

- Approximately 0.5g of whitening gel containing 6% Hydrogen Peroxide was applied to the end of a cotton bud.
- The gel was then applied to the moist enamel surface of the block using the cotton bud in a gentle, swiping motion, ensuring the entire surface was evenly covered.
- The gel was left on the blocks for 10 minutes, before being rinsed off with deionised water.
- The blocks were dabbed with tissue to remove excess moisture from the surface.
- The process was repeated until 6 consecutive treatment applications were performed.

The following treatment procedure was followed for the whitening gel containing 35% Hydrogen Peroxide group:

- Approximately 0.5g of whitening gel containing 35% Hydrogen Peroxide was applied to the end of a cotton bud.
- The gel was then applied to the moist enamel surface of the block using the cotton bud in a gentle, swiping motion, ensuring the entire surface was evenly covered.
- The gel was left on the blocks for 10 minutes, before being rinsed off with deionised water.
- The blocks were dabbed with tissue to remove excess moisture from the surface.
- The process was repeated until 6 consecutive treatment applications were performed.

The following treatment procedure was followed for the deionised water group:

- Approximately 0.5g (one drop) of deionised water was applied to the surface of the block, ensuring the entire enamel surface was covered.
- The water was left on the blocks for 10 minutes, before being dabbed dry with tissue.
- The process was repeated until 6 consecutive treatment applications were performed.

6. Efficacy Assessments

A calibrated ProFilm 3D surface profilometer measured the erosion of the enamel samples by comparing treated areas against the protected reference areas (Figure 2). Enamel erosion was measured after the final treatment.
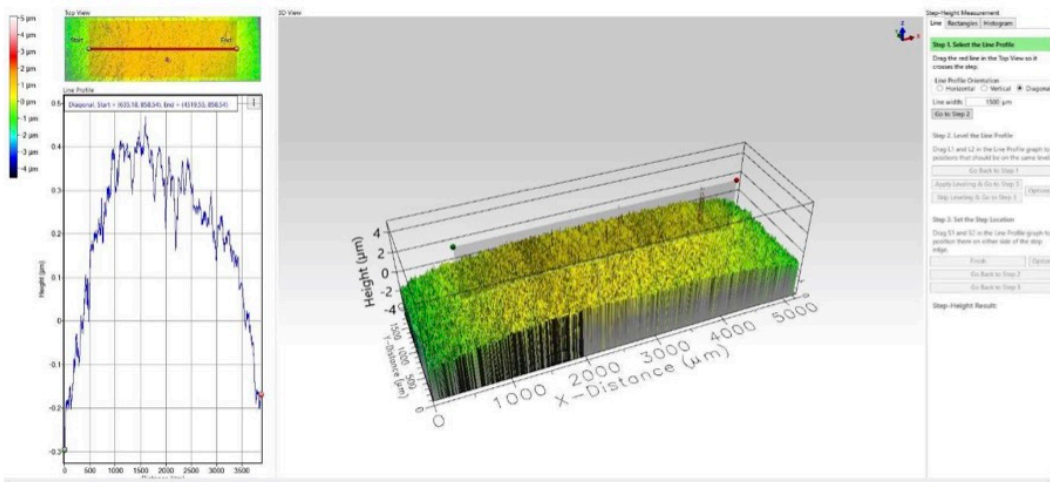
Figure 2: Example of a Post Treatment 3D Surface Measured During the Study.

A calibrated Tukon 1202 surface microhardness machine was used to measure the post treatment Vickers surface microhardness of the enamel samples. Three surface microhardness measurements were measured for each sample, under a 50-gram load. Post treatment surface microhardness was measured after the final treatment.
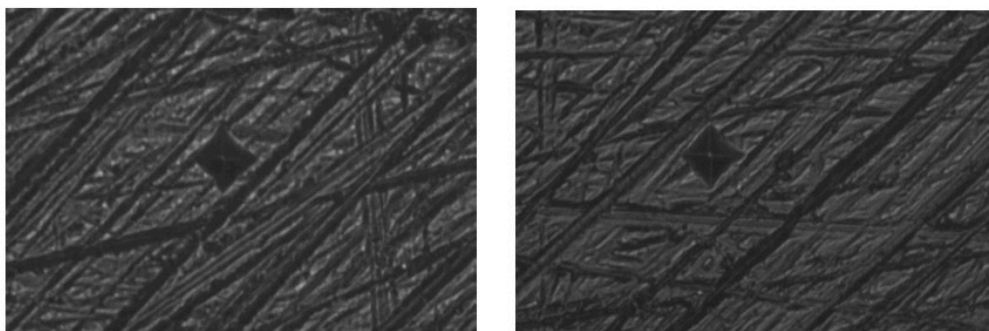

Figure 3: Example baseline and post-treatment SMH indents for deionised water.

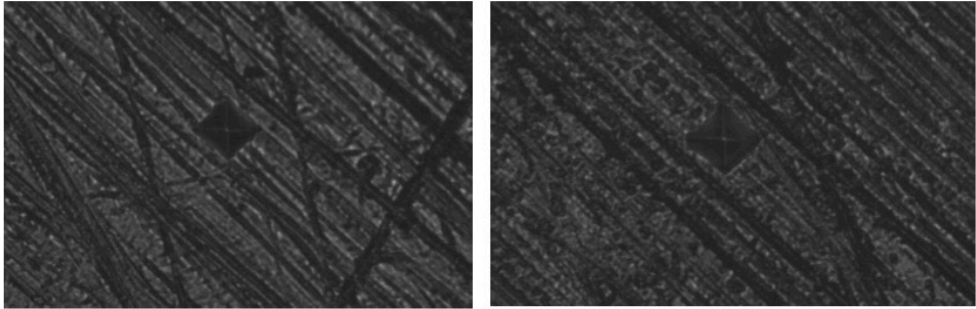| ![intertek logo] Total Quality. Assured.	**Laboratory Report**
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Report Version: 2.0	Report Date: 3rd June 2020

Figure 4: Example baseline and post-treatment SMH indents for whitening gel containing 35% Carbamide Peroxide (6 x 10 mins).
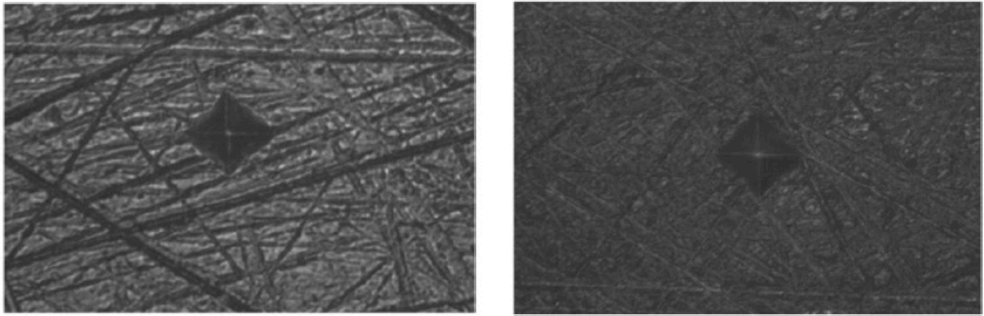

Figure 5: Example baseline and post-treatment SMH indents for HISMILE Teeth Whitening Gel PAP Formula A.
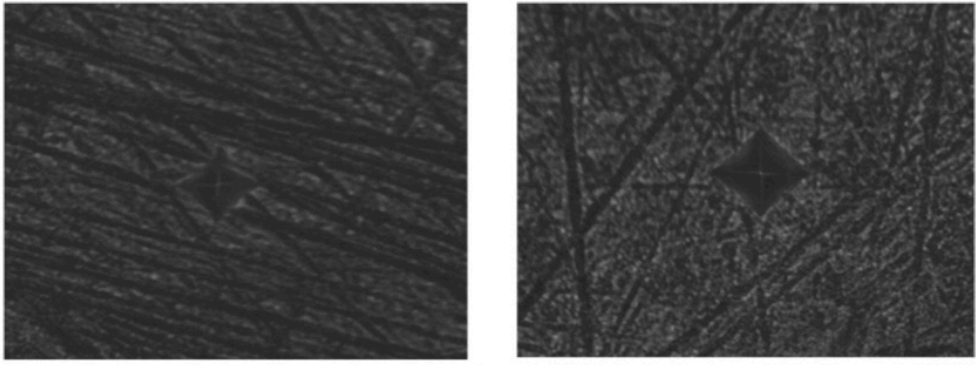

Figure 6: Example baseline and post-treatment SMH indents for whitening gel 6% Hydrogen Peroxide (6 x 10 mins).

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Report Version: 2.0	Report Date: 3rd June 2020

Figure 7: Example baseline and post-treatment SMH indents for whitening gel 35% Hydrogen Peroxide (6 x 10 mins).

7. Data Management

The surface microhardness data and formulae were entered into Excel and subjected to a randomised 10% data check, which was signed by the data checkers.

Minitab18 was used to generate descriptive statistics for the post treatment changes in surface microhardness. A 2-sample t-test or a Mann-Whitney test was used to statistically compare the whitening achieved by each treatment.

8. Results and Discussion

The statistical analysis outputs for the enamel surface microhardness data can be found in Appendix 1. The post treatment erosion data can be seen in Table 2, and the post treatment changes in enamel surface microhardness can be found in Table 3.

Table 2: Post Treatment Enamel Erosion

Treatment	Sample Number	Enamel Erosion (um)	Mean Enamel Erosion (um)
Deionised Water	5	0.00	0.00
	7	0.00	
	8	0.00	
	9	0.00	
	14	0.00	
	28	0.00	

Treatment Group	Sample	Value	Mean
35% Carbamide Peroxide	3	0.00	0.00
	6	0.00	
	12	0.00	
	13	0.00	
	16	0.00	
	20	0.00	
HISMILE Teeth Whitening Gel PAP Formula A	2	0.00	0.00
	5	0.00	
	11	0.00	
	21	0.00	
	28	0.00	
	20	0.00	
6% Hydrogen Peroxide	1	0.1832	0.1140
	12	0	
	10	0.1152	
	11	0.1401	
	2	0	
	3	0.2455	
35% Hydrogen Peroxide	6	0.149	0.0969
	7	0.1211	
	13	0	
	5	0.1246	
	8	0.1864	
	14	0	

Six applications of the negative control (deionised water), the HISMILE Teeth Whitening Gel PAP Formula A and the whitening gel containing 35% carbamide peroxide caused no measurable amounts of enamel erosion. This contrasted with the whitening gels containing 35% and 6% hydrogen peroxide, which caused measurable amounts of enamel erosion after six applications.

No statistical analysis was performed on the erosion data because only two treatments caused measurable enamel erosion.

Table 3: Post Treatment Change in Surface Microhardness

Treatment Group	Sample Number	Change in SMH	Mean Change in SMH	StDev
Deionised Water	5	6.3	4.4	7.6
	7	-9.7		
	8	4.3		
	9	6.7		

	14	5.7		
	28	13.3		
35% Carbamide Peroxide	3	-65.7	-55.3	24.6
	6	-100.7		
	12	-47.3		
	13	-35.0		
	16	-39.3		
	20	-43.7		
HISMILE Teeth Whitening Gel PAP Formula A	2	-7.0	12.9	11.7
	5	14.0		
	11	7.3		
	21	23.0		
	28	25.0		
	20	15.3		
6% Hydrogen Peroxide	1	-42.67	-62.22	19.52
	12	-54.33		
	10	-45.00		
	11	-68.67		
	2	-67.33		
	3	-95.33		
35% Hydrogen Peroxide	6	-97.00	-94.28	27.09
	7	-75.00		
	13	-80.67		
	5	-147.33		
	8	-79.00		
	14	-86.67		

Six applications of the negative control (deionised water) and the HISMILE Teeth Whitening Gel PAP Formula A did not cause a reduction in the surface microhardness of the enamel samples. Statistical analysis of the microhardness data showed the negative control and the HISMILE Teeth Whitening Gel PAP Formula A were statistically significantly less damaging to enamel than the whitening gels containing carbamide peroxide and hydrogen peroxide.

Six applications of the whitening gels containing hydrogen peroxide and carbamide peroxide reduced the surface microhardness of the enamel samples. Statistical analysis of the data showed six applications of the whitening gel containing 35% hydrogen peroxide caused statistically significantly larger reductions in enamel surface microhardness when compared to all other whitening gels.

9. Conclusions

The HISMILE Teeth Whitening Gel PAP Formula A was not damaging to the enamel samples and had an impact comparable to deionised water. The whitening gels containing hydrogen peroxide were the most damaging, causing a reduction in enamel microhardness and measurable amounts of enamel erosion. The whitening gels containing carbamide peroxide caused reductions in enamel microhardness but no erosion of the enamel surfaces.

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Report Version: 2.0	**Report Date:** 3rd June 2020

Appendix 1: Statistical Analysis Outputs for Enamel Surface Microhardness Data

Descriptive Statistics: Mean Change in SMH
Statistics

Variable	Treatment	N	Mean	StDev	Minimum	Median	Maximum
Mean Change in SMH	35% Carbamide Peroxide	6	-55.3	24.6	-100.7	-45.5	-35.0
	35% Hydrogen Peroxide	6	-94.3	27.1	-147.3	-83.7	-75.0
	6% Hydrogen Peroxide	6	-62.2	19.5	-95.3	-60.8	-42.7
	Deionised water	6	4.4	7.6	-9.7	6.0	13.3
	PAP	6	12.9	11.7	-7.0	14.7	25.0

General Linear Model: Mean Change in SMH versus Treatment
Method

Factor coding   (-1, 0, +1)
Factor Information

Factor	Type	Levels	Values
Treatment	Fixed	5	35% Carbamide Peroxide, 35% Hydrogen Peroxide, 6% Hydrogen Peroxide, Deionised water, PAP

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment	4	50673	12668.2	33.07	0.000
Error	25	9578	383.1		
Total	29	60251			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
19.5734	84.10%	81.56%	77.11%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-38.88	3.57	-10.88	0.000	
Treatment					
35% Carbamide Peroxide	-16.40	7.15	-2.29	0.030	1.60
35% Hydrogen Peroxide	-55.40	7.15	-7.75	0.000	1.60

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	Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe
Report Version: 2.0	Report Date: 3rd June 2020

6% Hydrogen Peroxide	-23.34	7.15	-3.27	0.003	1.60
Deionised water	43.32	7.15	6.06	0.000	1.60

Regression Equation

$$\text{Mean Change in SMH} = -38.88 - 16.40 \text{ Treatment\_35\% Carbamide Peroxide} - 55.40 \text{ Treatment\_35\% Hydrogen Peroxide} - 23.34 \text{ Treatment\_6\% Hydrogen Peroxide} + 43.32 \text{ Treatment\_Deionised water} + 51.82 \text{ Treatment\_PAP}$$

Fits and Diagnostics for Unusual Observations

Obs	Mean Change in SMH	Fit	Resid	Std Resid	
12	-100.67	-55.28	-45.39	-2.54	R
28	-147.33	-94.28	-53.06	-2.97	R

R Large residual

Residual Plots for Mean Change in SMH

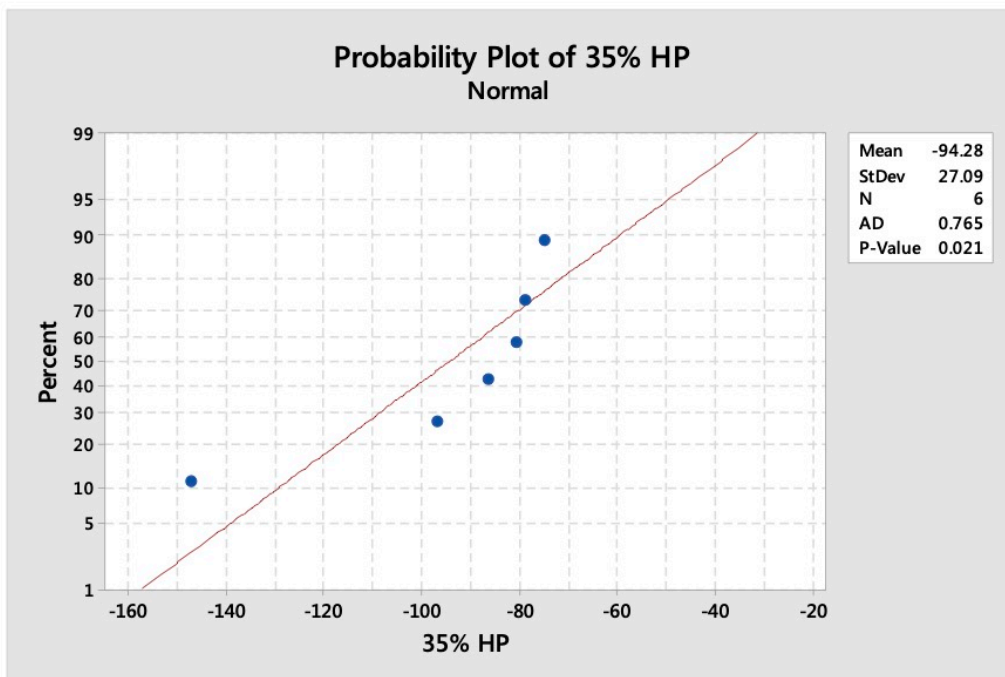| ![intertek logo] **intertek** Total Quality. Assured.	**Laboratory Report**
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Report Version: 2.0	Report Date: 3rd June 2020

Probability Plot of 35% CP — Normal

Mean −55.28
StDev 24.62
N 6
AD 0.540
P-Value 0.094

Probability Plot of 35% HP — Normal

Mean −94.28
StDev 27.09
N 6
AD 0.765
P-Value 0.021

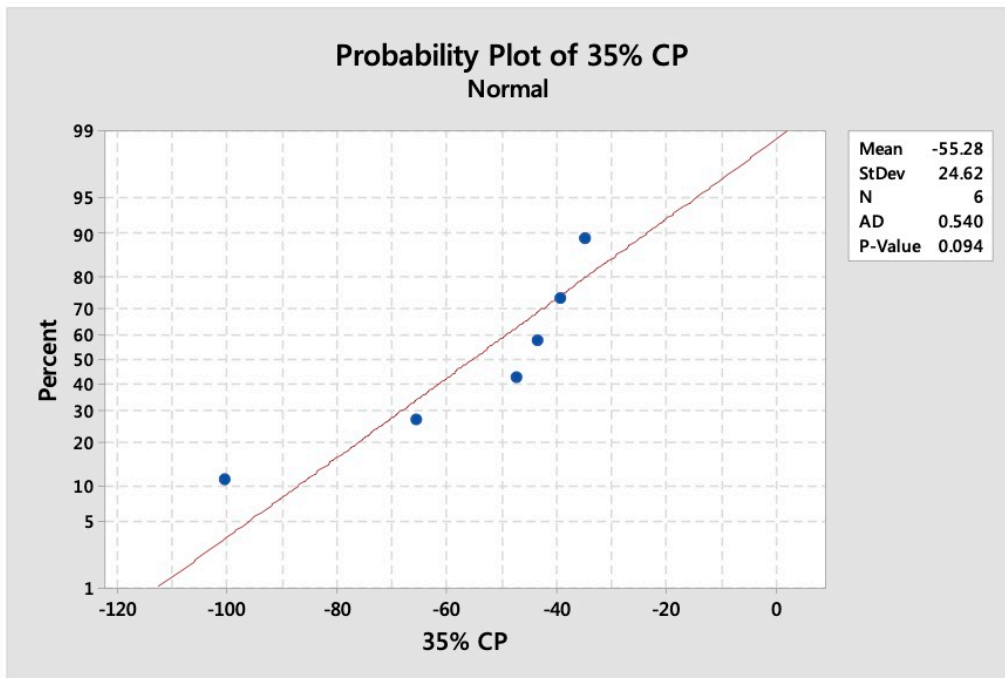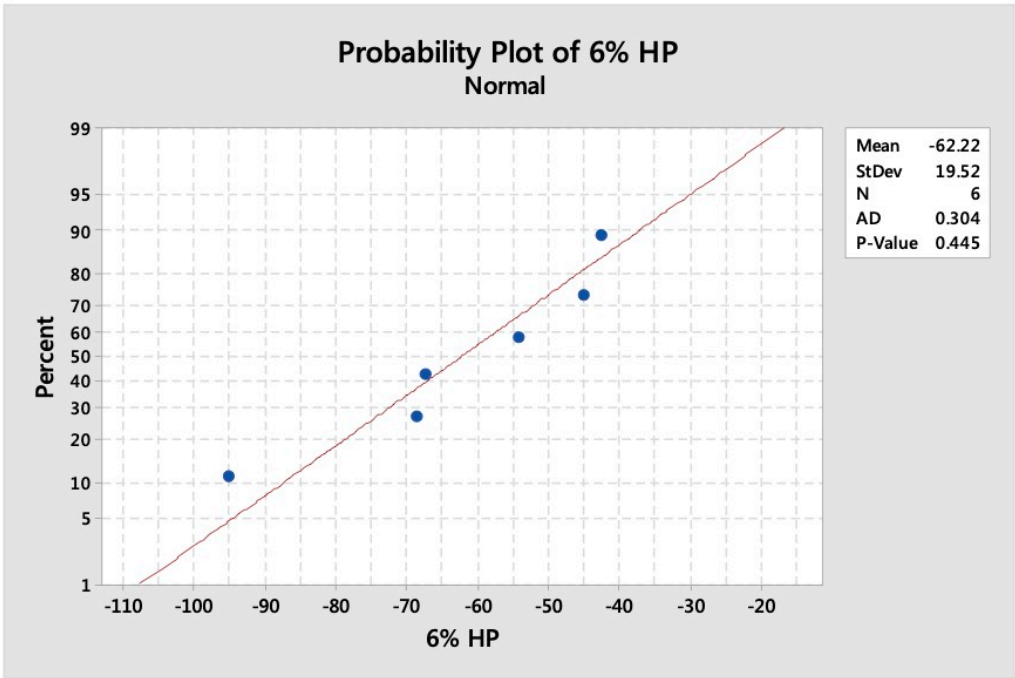| intertek Total Quality. Assured.	**Laboratory Report**
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Report Version: 2.0	Report Date: 3rd June 2020

Probability Plot of 6% HP — Normal

Mean	-62.22
StDev	19.52
N	6
AD	0.304
P-Value	0.445

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Report Version: 2.0	Report Date: 3rd June 2020

Probability Plot of Water — Normal

Mean 4.444
StDev 7.591
N 6
AD 0.609
P-Value 0.059

Probability Plot of PAP — Normal

Mean 12.94
StDev 11.68
N 6
AD 0.281
P-Value 0.509

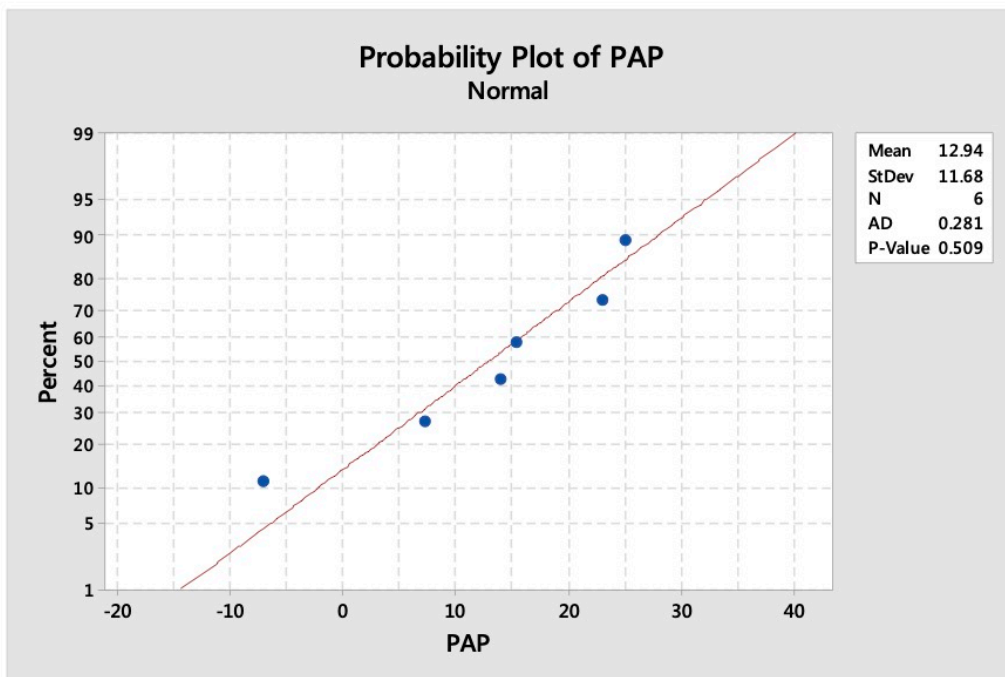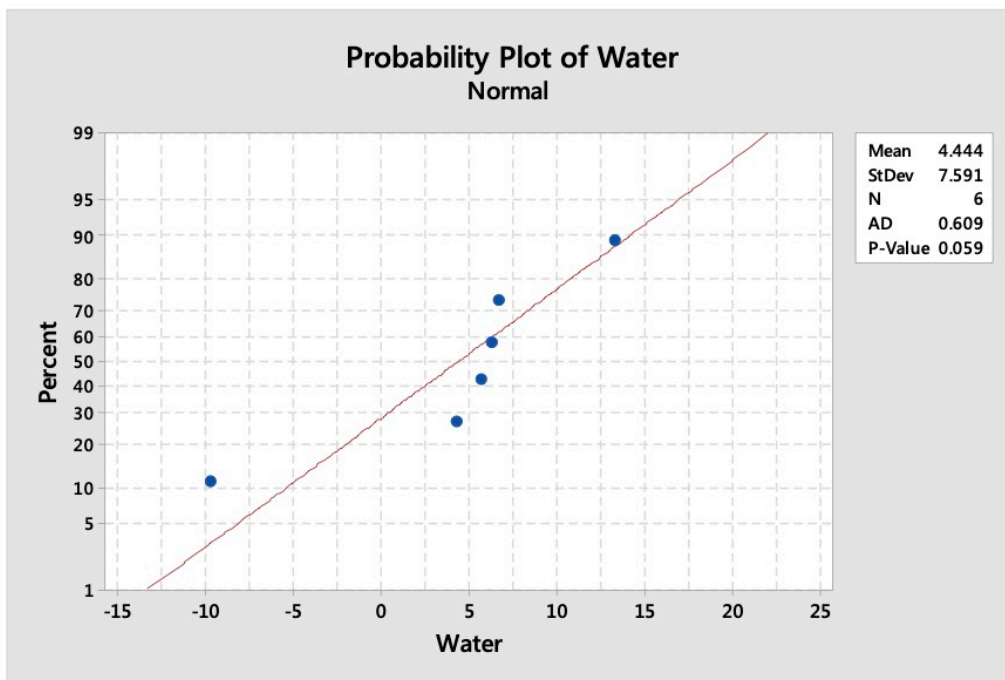| ![intertek logo] **intertek** Total Quality. Assured.	**Laboratory Report**
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Report Version: 2.0	Report Date: 3rd June 2020

Mann-Whitney: 35% CP, 35% HP
Method

η_1: median of 35% CP

η_2: median of 35% HP

Difference: $\eta_1 - \eta_2$
Descriptive Statistics

Sample	N	Median
35% CP	6	-45.5000
35% HP	6	-83.6667

Estimation for Difference

Difference	CI for Difference	Achieved Confidence
39.5	(9.33333, 62)	95.47%

Test

Null hypothesis	$H_0: \eta_1 - \eta_2 = 0$
Alternative hypothesis	$H_1: \eta_1 - \eta_2 \neq 0$

W-Value	P-Value
52.00	0.045

Two-Sample T-Test and CI: 35% CP, 6% HP
Method

μ_1: mean of 35% CP

μ_2: mean of 6% HP

Difference: $\mu_1 - \mu_2$
Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
35% CP	6	-55.3	24.6	10
6% HP	6	-62.2	19.5	8.0

Estimation for Difference

Difference	95% CI for Difference

     6.9   (-22.1, 36.0)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$

Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.54	9	0.601

Two-Sample T-Test and CI: 35% CP, Water

Method

μ_1: mean of 35% CP

μ_2: mean of Water

Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
35% CP	6	-55.3	24.6	10
Water	6	4.44	7.59	3.1

Estimation for Difference

Difference	95% CI for Difference
-59.7	(-86.8, -32.7)

Test

Null hypothesis H_0: $\mu_1 - \mu_2 = 0$

Alternative hypothesis H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-5.68	5	0.002

Two-Sample T-Test and CI: 35% CP, PAP

Method

μ_1: mean of 35% CP

μ_2: mean of PAP

Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

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████████████████████ ████████████	Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe	
Report Version: 2.0	Report Date: 3rd June 2020	

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
35% CP	6	-55.3	24.6	10
PAP	6	12.9	11.7	4.8

Estimation for Difference

Difference	95% CI for Difference
-68.2	(-94.5, -41.9)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$

Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-6.13	7	0.000

Mann-Whitney: 35% HP, 6% HP

Method

η_1: median of 35% HP

η_2: median of 6% HP

Difference: $\eta_1 - \eta_2$

Descriptive Statistics

Sample	N	Median
35% HP	6	-83.6667
6% HP	6	-60.8333

Estimation for Difference

Difference	CI for Difference	Achieved Confidence
-29.8333	(-54.3333, -6.33333)	95.47%

Test

Null hypothesis $H_0: \eta_1 - \eta_2 = 0$

Alternative hypothesis $H_1: \eta_1 - \eta_2 \neq 0$

W-Value	P-Value
25.00	0.031

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Report Version: 2.0	Report Date: 3rd June 2020

Mann-Whitney: 35% HP, Water

Method

η_1: median of 35% HP

η_2: median of Water

Difference: $\eta_1 - \eta_2$

Descriptive Statistics

Sample	N	Median
35% HP	6	-83.6667
Water	6	6.0000

Estimation for Difference

Difference	CI for Difference	Achieved Confidence
-89.6667	(-137.667, -80.6667)	95.47%

Test

Null hypothesis	$H_0: \eta_1 - \eta_2 = 0$
Alternative hypothesis	$H_1: \eta_1 - \eta_2 \neq 0$

W-Value	P-Value
21.00	0.005

Mann-Whitney: 35% HP, PAP

Method

η_1: median of 35% HP

η_2: median of PAP

Difference: $\eta_1 - \eta_2$

Descriptive Statistics

Sample	N	Median
35% HP	6	-83.6667
PAP	6	14.6667

Estimation for Difference

Difference	CI for Difference	Achieved Confidence
-101.333	(-140.333, -86.3333)	95.47%

Test

Null hypothesis $H_0: \eta_1 - \eta_2 = 0$

Alternative hypothesis $H_1: \eta_1 - \eta_2 \neq 0$

W-Value	P-Value
21.00	0.005

Two-Sample T-Test and CI: 6% HP, Water

Method

μ_1: mean of 6% HP

μ_2: mean of Water

Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
6% HP	6	-62.2	19.5	8.0
Water	6	4.44	7.59	3.1

Estimation for Difference

Difference	95% CI for Difference
-66.67	(-87.59, -45.74)

Test

Null hypothesis $H_0: \mu_1 - \mu_2 = 0$

Alternative hypothesis $H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-7.80	6	0.000

Two-Sample T-Test and CI: 6% HP, PAP

Method

μ_1: mean of 6% HP

μ_2: mean of PAP

Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean

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Laboratory Report

Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe

Report Version: 2.0

Report Date: 3rd June 2020

6% HP	6	-62.2	19.5	8.0
PAP	6	12.9	11.7	4.8

Estimation for Difference

Difference	95% CI for Difference
-75.17	(-96.58, -53.75)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-8.09	8	0.000

Two-Sample T-Test and CI: Water, PAP

Method

μ_1: mean of Water

μ_2: mean of PAP

Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Water	6	4.44	7.59	3.1
PAP	6	12.9	11.7	4.8

Estimation for Difference

Difference	95% CI for Difference
-8.50	(-21.62, 4.62)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-1.49	8	0.173

intertek Total Quality. Assured.	**Laboratory Report**
██████████████████ ███████████	Report Authors: Gavin Thomas, Thomas Badrock, Michael Lloyd & Rebecca Metcalfe
Report Version: 2.0	Report Date: 3rd June 2020

Report signature:

I declare that this report constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study.

Gavin Thomas

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(Laboratory Project Manager, Intertek CRS)   Date....3rd June 2020......

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Last page of document