

# **Supplementary material**

# Are mild cleansers appropriate for hand hygiene in the COVID era? An investigation of the anti-viral efficacy of different hand hygiene products

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## 2 Non-enveloped viruses remain resilient after longer 'hand washing' period.

Additional time points were also investigated to determine if longer hand washing regimes would improve the anti-viral properties of the test products. All enveloped viruses similar to results to Fig 1, whereby all products minus the SFC 2 demonstrate virucidal activity (S1Fig). Again, similar to Fig 1 non-enveloped viruses demonstrated greater resistance across all products, with only Ad exhibiting susceptibility to the natural soap at 97% (S1.G Fig )



S1 Fig: Only non-enveloped viruses displayed resistance against soaps and skin friendly cleansers compared to non-enveloped viruses at a later time point. The anti-viral efficacy of a range of wash products (at 20 and 97% concentrations) was determined under simulated clean and dirty hand washing conditions (0.3% BSA -'clean' and 3% BSA with 20% Zerobase)-'dirty'), with soft water, for 40 seconds. The wash products were incubated with HSV (A-B), HCoV (C-D), IVA (E-F), Ad (G-H) and MNV (I-J). Viral counts were obtained by plaque assay or TCID50 and expressed as pfu/ml (mean  $\pm$  SD for n=3). The washing conditions were considered anti-viral if a log4 or greater reduction in viral count was observed (indicated by X). Positive control consists of 1% bleach instead of a wash product and the negative control consists of virus and respective interference substance (no wash product), and otherwise performed under the same conditions.



#### 3 Time dependent hand hygiene's make no difference to viral titer in soft water

Results at 60 seconds mimic the data seen at both 20 and 40 seconds, with enveloped viruses more susceptible to tests products, when compared with non-enveloped viruses. This showed similar results, to that seen, in S1 Fig



## S2 Fig: The efficiency of the anti-viral properties of test products doesn't not improve with

time in soft water. The anti-viral efficacy of a range of wash products (at 20 and 97% concentrations) was determined under simulated clean and dirty hand washing conditions (0.3% BSA -'clean' and 3% BSA with 20% Zerobase)-'dirty'), with soft water, for 60 seconds. The wash products were incubated with HSV (A-B), HCoV (C-D), IVA (E-F), Ad (G-H) and MNV (I-J). Viral counts were obtained by plaque assay or TCID50 and expressed as pfu/ml (mean  $\pm$  SD for n=3). The washing conditions were considered anti-viral if a log<sub>4</sub> or greater reduction in viral count was observed (indicated by X). Positive control consists of 1% bleach instead of a wash product and the negative control consists of virus and respective interference substance (no wash product), and otherwise performed under the same conditions.

#### 4 Water hardness improved the anti-viral properties of natural soap against adenovirus

Similar to soft water at additional time points, the data generated in hard water showed the same trend at 40 seconds as they do at 20 seconds. The remaining products, minus SFC 2, showed viricidal activity against non-enveloped viruses (Fig S3 C-F). Ad displayed susceptibility towards the natural soap at high concentrations (97%) (Fig S3G), whereas MNV remained resistant against all products.





S3 Fig: Hard water reduced the antiviral properties to natural soap for Herpes simplex virus, whilst improving its virucidal activity in Adenovirus. The anti-viral efficacy of a range of wash products (at 20 and 97% concentrations) was determined under simulated clean and dirty hand washing conditions (0.3% BSA - 'clean' and 3% BSA with 20% Zerobase)- 'dirty'), with hard water, for 40 seconds. The wash products were incubated with HSV (A-B), HCoV (C-D), IVA (E-F), Ad (G-H) and MNV (I-J). Viral counts were obtained by plaque assay or TCID50 and expressed as pfu/ml (mean  $\pm$  SD for n=3). The washing conditions were considered anti-viral if a log<sub>4</sub> or greater reduction in viral count was observed (indicated by X). Positive control consists of 1% bleach instead of a wash product and the negative control consists of virus and respective interference substance (no wash product), and otherwise performed under the same conditions.

# 5 Longer hand washing regimes make little difference to viral titre compared with other time points

Results at 60 seconds mimic the data seen at 40 seconds, with enveloped viruses more susceptible to tests products, when compared with non-enveloped viruses. This showed similar results to that seen S3 Fig





S4 Fig: Longer hand washing time points, do not improve the test products activity in soft. The anti-viral efficacy of a range of wash products (at 20 and 97% concentrations) was determined under simulated clean and dirty hand washing conditions (0.3% BSA -'clean' and 3% BSA with 20% Zerobase)-'dirty'), with hard water, for 60 seconds. The wash products were incubated with HSV (A-B), HCoV (C-D), IVA (E-F), Ad (G-H) and MNV (I-J). Viral counts were obtained by plaque assay or TCID50 and expressed as pfu/ml (mean  $\pm$  SD for n=3). The washing conditions were considered anti-viral if a log<sub>4</sub> or greater reduction in viral count was observed (indicated by X). Positive control consists of 1% bleach instead of a wash product and the negative control consists of virus and respective interference substance (no wash product), and otherwise performed under the same conditions.

### 6 Surfactant content in Skin friendly cleanser can generate a greater antiviral response

Additional products including an additional synthetic soap (commercially bought) and two more SFC were tested on one enveloped (HSV) and non-enveloped (Ad) virus. Results showed that HSV in soft or hard water either in both 'clean' and 'dirty' still demonstrated susceptibility against all additional test products (S5.A-D Fig). Moreover, Ad still showed the general trend that non-enveloped viruses are more resilient across all products, except for SFC4. The data suggested that both diluted and concentrated test products of SFC 4 were effective as long as the virus was in 'dirty' conditions and hard water (S5.H Fig).



S5 Fig: Herpes simplex virus remained susceptible against all additional products, whilst Adenovirus showed greater susceptibility. The anti-viral efficacy of a range of additional wash products (at 20 and 97% concentrations) was determined under simulated clean and dirty hand washing conditions (0.3% BSA -'clean' and 3% BSA with 20% Zerobase)-'dirty'), with soft and hard water, for 20 seconds. The wash products were incubated with HSV (A-D) and Ad (E-H). Viral counts were obtained by plaque assay or TCID50 and expressed as pfu/ml (mean  $\pm$  SD for n=3). The washing conditions were considered anti-viral if a log<sub>4</sub> or greater reduction in viral count was observed (indicated by X). Positive control consists of 1% bleach instead of a wash product and the negative control consists of virus and respective interference substance (no wash product), and otherwise performed under the same conditions