Determination of Antiviral Activity of Royale Health Shield against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

Rajiv Gandhi Centre for Biotechnology (RGCB)
Bio-Innovation Centre, KIFRA Film & Video Park, Sainik School Post, Trivandrum – 695585, Kerala, India

Study Director
Dr. Radhakrishnan R. Nair, Scientist EII

Study Coordinator
Dr. S. Dayakar, Program Scientist

Customer Name: Asian Paints Limited
Contact Name: Sunil Jambhale – Sr Manager-Technology
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Address: Research and Technology Centre,
Plot No C -3, B1, TTC MIDC Pawane
Thane Belapur Road – Navi Mumbai-400703
Maharashtra -INDIA

Sample's Information: Royale Health shield (RHS1)
Sample Submission Date: 26/08/2020
Study date: 27/08/2020
Report date: 14/09/2020
1. Brief Note on Test Virus SARS-CoV-2

SARS-CoV-2 is a positive-sense, single-stranded RNA (ssRNA), group IV virus. It comprises of four structural proteins, namely, spike (S), nucleocapsid (N) envelope (E), and membrane (M). The S protein is responsible for virus attachment to the receptor and fusion with cell membrane. The N protein interacts with the viral RNA to form the ribonucleoprotein. The E protein helps in virions assembly and comprises ion channel actions; the M protein shares in the assembly of new virus particles. The structural genes of SARS-CoV-2 comprises the S, E, M, and N genes, while the nonstructural genes include the RNA-dependent RNA polymerase (RdRP) and main protease (Mpro) genes.

2. Study protocol

- Test panel (20mmX 20mm square) was obtained by punching a hole in the sheet.
- 50 µl of virus (SARS-CoV-2- RGCB Isolate) was spotted on the sheet (Δ Ct 22).
- The samples were incubated for 120 minutes.
- 150 µl of neutralization buffer (1X) was added to retrieve the virus.
- RNAase treatment performed as per manufacturers instruction (Genelink,40-5101-01)
- RNA was isolated as per manufacturers instruction (ADT Biotech-Malaysia,811801/811803)
- qRT-PCR was performed to quantify the RNA content using Kit (Real Star SARS-CoV-2 RT-PCR kit 1.0, altona Diagnostics GmbH-Germany, 023005) as per manufacturers instruction.

3. Sample and experimental details

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Sample Number</th>
<th>Product Code</th>
<th>Batch Number</th>
<th>Identification marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>--</td>
<td>MSB-162319-49</td>
<td>![Blank Image]</td>
</tr>
<tr>
<td>2</td>
<td>RHS 1</td>
<td>5664</td>
<td>AG01-CC 726</td>
<td>![RHS 1 Image]</td>
</tr>
</tbody>
</table>

Note: Generally, in antiviral efficacy determination protocols, the virus post exposure to the test samples are allowed to grow on mammalian cells and the plaques are counted. This step is modified in the above protocol as growing COVID-19 wouldn't be safe.
4. Study result
In the present study SARS-CoV-2 specific RNA (E&S target gene) was not detected after 30 minutes (Royale Luxury Emulsion- Health Shield 5664) of treatment indicating rupturing of viral envelope whereas Blank panel shows non-significant reduction. It has significantly enhanced the antiviral log reduction and reduces viral infectivity by 99% reduction of virus in 30 minutes. The study was performed as per modified ISO18184 protocol.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Time (min)</th>
<th>Royale Luxury Emulsion- Health Shield – 5664</th>
<th>SARS-CoV-2</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E Gene</td>
<td>S Gene</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ct Value* Delta Ct** %Reduction in Viral load</td>
<td>Ct Value Delta Ct %Reduction in Viral load</td>
<td>Ct Value Delta Ct %Reduction in Viral load</td>
</tr>
<tr>
<td>1</td>
<td>BE</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>21 -</td>
<td>20 -</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>23 2</td>
<td>23 3</td>
<td>25 1</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>26 2 &gt;95</td>
<td>26 3 &gt;95</td>
<td>25 0</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>28 2 99</td>
<td>28 2 99</td>
<td>26 1</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>ND End point</td>
<td>End point</td>
<td>26 0</td>
</tr>
<tr>
<td>7</td>
<td>120</td>
<td>ND End point</td>
<td>End point</td>
<td>26 1</td>
</tr>
<tr>
<td>8</td>
<td>PC</td>
<td>21 -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>NC</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

mean of triplicates. ** Delta Ct of 3-4 corresponds to 1 log difference. ND-Not detected NS- non-significant PC-Positive control NC-Negative control

BE-Before exposure

5. Quality Control

In accordance with the ISO15189:2012-certified Quality Management System, each lot of SARS-CoV-2 RT-PCR assay is tested against predetermined specifications to ensure consistent product quality

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Study Director

Dr. S. Dayakar  
Study Co-ordinator

Ms Heera R. Pillai  
Quality Manager
LAB NO.: 13278/ 1 - 2

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ADDRESS : R & T Center - Turbhe, Plot No C - 3B/1,
TTC Industrial Area, MIDC Pawane,
Thane Belapur Road, Navi Mumbai 400703

REFERENCE : Letter Ref. No. Nil dated September 27, 2017
K. Attention: Mahesh Chavan

DATE OF RECEIPT : 27/09/2017
DATE OF INITIATION : 27/09/2017
DATE OF COMPLETION : 02/12/2017

SAMPLE DESCRIPTION : SAMPLE LABELLED AS –
1. AP Royale Health Shield AG 2 – Blank
2. AP Royale Health Shield AG 2 - Hygiene

Name of Test:
Evaluation of Antimicrobial Activity of Paint sample

Name of Test Protocol:
JIS Z 2801: 2010
Test Organisms used for evaluating Antimicrobial activity:
1. Acinetobacter baumanniiATCC 19606
2. Bacillus cereus ATCC 6633
3. Burkholderia cepacia ATCC 25416
4. Corynebacterium minutissimum ATCC 23348
5. Corynebacterium xerosis ATCC 7711
6. Enterobacter aerogenes ATCC 13048
7. Enterobacter aerogenes ATCC 13048
8. Enterococcus faecalis ATCC 10541
9. Escherichia coli ATCC 25922
10. Klebsiella pneumoniae ATCC 1705 – KPC
11. Klebsiella pneumoniae ATCC 2146 - MBL strain
12. Klebsiella pneumoniae ATCC 4352
13. Klebsiella pneumoniae ATCC 700603 ESBL strain
14. Listeria monocytogenes ATCC 23074
15. Meticillin Resistant Staphylococcus aureus ATCC 43300
16. Micrococcus luteus ATCC 10240
17. Proteus mirabilis ATCC 14153
18. Proteus vulgaris ATCC 13315
19. Pseudomonas aeruginosa ATCC 9027
20. Pseudomonas Putida ATCC 12633
21. Salmonella typhi ATCC 10749
22. Serratia marcescens ATCC 14756
23. Shigella flexneri ATCC 9199
24. Staphylococcus aureus ATCC 6538
25. Streptococcus faecalis ATCC 9790
26. Streptococcus mutans ATCC 25175
27. Vancomycin Resistant Enterococcus AATCC 51299
28. Vancomycin Resistant Enterococcus ATCC 2006
29. Vancomycin Resistant Enterococcus ATCC 2006

Test Conditions:
Neutraliser used : Buffered Saline with Tween 80 - 0.01 %
Contact Time : 24 hours at 37°C
Incubation Temperature : 37°C
Media and Reagent : Soyabean-casein digest agar
### RESULTS:

#### ANTIBACTERIAL ACTIVITY

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Test Organism</th>
<th>Exposure Time</th>
<th>Count of Test Organism</th>
<th>Antimicrobial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial Count (CFU/ml)</td>
<td>After Exposure CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>Log</td>
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<td></td>
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<td></td>
<td>Percentage Reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Log Reduction</td>
</tr>
<tr>
<td>1</td>
<td><em>Acinetobacter baumanii</em> ATCC 19606</td>
<td>2 hours</td>
<td>Log = 4.48</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus cereus</em> ATCC 8633</td>
<td>2 hours</td>
<td>Log = 4.20</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td><em>Burkholderia cepacia</em> ATCC 25416</td>
<td>2 hours</td>
<td>Log = 4.29</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4</td>
<td><em>Corynebacterium minutissimum</em> ATCC 23348</td>
<td>2 hours</td>
<td>Log = 5.00</td>
<td>8.70 x 10^2</td>
</tr>
<tr>
<td>5</td>
<td><em>Corynebacterium xerosis</em> ATCC 7711</td>
<td>2 hours</td>
<td>Log = 5.19</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6</td>
<td><em>Enterobacter aerogenes</em> ATCC13048</td>
<td>2 hours</td>
<td>Log = 4.52</td>
<td>&lt;10</td>
</tr>
<tr>
<td>7</td>
<td><em>Enterobacter aerogenes</em> ATCC 13048</td>
<td>2 hours</td>
<td>Log = 5.50</td>
<td>1.40 x 10^3</td>
</tr>
<tr>
<td>8</td>
<td><em>Enterococcus hirae</em> ATCC 10541</td>
<td>2 hours</td>
<td>Log = 4.43</td>
<td>&lt;10</td>
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<tr>
<td>9</td>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>2 hours</td>
<td>Log = 5.69</td>
<td>1.00 x 10^3</td>
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<tr>
<td>10</td>
<td><em>Klebsiella pneumoniae</em> ATCC 1705 - KPC</td>
<td>2 hours</td>
<td>Log = 5.19</td>
<td>&lt;10</td>
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<tr>
<td>11</td>
<td><em>Klebsiella pneumoniae</em> ATCC 2146 - MBL strain</td>
<td>2 hours</td>
<td>Log = 4.79</td>
<td>&lt;10</td>
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<tr>
<td>12</td>
<td><em>Klebsiella pneumoniae</em> ATCC 4352</td>
<td>2 hours</td>
<td>Log = 5.89</td>
<td>&lt;10</td>
</tr>
<tr>
<td>13</td>
<td><em>Klebsiella pneumoniae</em> ATCC 700603 ESBL strain</td>
<td>2 hours</td>
<td>Log = 4.09</td>
<td>&lt;10</td>
</tr>
<tr>
<td>14</td>
<td><em>Listeria monocytogenes</em> ATCC 23704</td>
<td>2 hours</td>
<td>Log = 5.00</td>
<td>2.80 x 10^2</td>
</tr>
<tr>
<td>15</td>
<td><em>Methicillin Resistant Staphylococcus aureus</em> ATCC 43300</td>
<td>2 hours</td>
<td>Log = 5.77</td>
<td>4.00 x 10^3</td>
</tr>
<tr>
<td>Sr No</td>
<td>Test Organism</td>
<td>Exposure Time</td>
<td>Count of Test Organism</td>
<td>Antimicrobial activity</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Initial Count (CFU/ml)</td>
<td>After Exposure</td>
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<tr>
<td>16</td>
<td>Micrococcus luteus ATCC 10240</td>
<td>2 hours</td>
<td>Log = 5.38</td>
<td>5.20 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>17</td>
<td>Proteus mirabilis ATCC 14153</td>
<td>2 hours</td>
<td>Log = 4.26</td>
<td>&lt;10</td>
</tr>
<tr>
<td>18</td>
<td>Proteus vulgaris ATCC 13315</td>
<td>2 hours</td>
<td>Log = 4.44</td>
<td>20</td>
</tr>
<tr>
<td>19</td>
<td>Pseudomonas aeruginosa ATCC 9027</td>
<td>2 hours</td>
<td>Log = 6.38</td>
<td>1.36 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>Pseudomonas Putida ATCC 12633</td>
<td>2 hours</td>
<td>Log = 4.71</td>
<td>&lt;10</td>
</tr>
<tr>
<td>21</td>
<td>Salmonella typhi ATCC 10749</td>
<td>2 hours</td>
<td>Log = 5.85</td>
<td>&lt;10</td>
</tr>
<tr>
<td>22</td>
<td>Serratia marcescens ATCC 14758</td>
<td>2 hours</td>
<td>Log = 5.85</td>
<td>1.20 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>23</td>
<td>Shigella flexneri ATCC 8199</td>
<td>2 hours</td>
<td>Log = 5.25</td>
<td>2.00 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>24</td>
<td>Staphylococcus aureus ATCC 6538</td>
<td>2 hours</td>
<td>Log = 5.85</td>
<td>&lt;10</td>
</tr>
<tr>
<td>25</td>
<td>Streptococcus faecalis ATCC 9790</td>
<td>2 hours</td>
<td>Log = 5.62</td>
<td>&lt;10</td>
</tr>
<tr>
<td>26</td>
<td>Streptococcus mutansATCC 25175</td>
<td>2 hours</td>
<td>Log = 5.36</td>
<td>1.62 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>27</td>
<td>Vancomycin Resistant Enterococcus ATCC 51299</td>
<td>2 hours</td>
<td>Log = 4.59</td>
<td>&lt;10</td>
</tr>
<tr>
<td>28</td>
<td>Vancomycin Resistant Enterococcus ATCC 2006</td>
<td>2 hours</td>
<td>Log = 4.47</td>
<td>&lt;10</td>
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<tr>
<td>29</td>
<td>Vancomycin Resistant Enterococcus ATCC 2006</td>
<td>2 hours</td>
<td>Log = 4.39</td>
<td>2.40 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

For BIOTECH TESTING SERVICES

Dr Shilpa U. Nair  
Quality Manager  
(Authorized Signatory)

13278/1 - 2  
Page 4 of 4

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An ISO / IEC 17025:2005 Accredited Testing Services  
www.biotechts.in
This is in reference to the association of Asian Paints Ltd and their product Royale Health Shield with the Indian Medical Association.

Indian Medical Association constantly works towards the welfare of community at large and association with Asian Paints Royale Health Shield – an interior paint is one such step towards it. Royale Health Shield which contains Silver Ion Technology, has shown anti-bacterial activity through the tests recommended by the IMA.

The Indian Medical Association hereby grants the usage of Indian Medical Association logo to Asian Paints Ltd for their product Royale Health Shield with a mandatory disclaimer as below.

Fomites (bacteria, viruses) infections can spread through infected walls among other indoor surfaces in homes and offices. Silver Ion Technology based paints kills 99% bacteria when they come in contact with the walls.