Customer Name	Gabriel Scientific Ltd.,
Customer Address	Paramount Court, Corrig Road, Sandyford, Dublin 18
Contact	David Woolfson
Test Requested	Barrier Test
Sample Description	SleepAngel™ pillow
Number of Samples	3
Date of Receipt	24/09/2015
ASC Code	ASC003246
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Contents

1. Purpose

To assess the ability of the SleepAngel™ pillow to act as a barrier against Human Coronavirus 229E.

2. Background

Coronaviruses are enveloped RNA viruses belonging to the *Coronaviridae* family. They are responsible for mild and acute respiratory illness in humans.

Human Coronavirus 229E is responsible for up to one-third of common colds and may also be involved in more serious diseases (Myint 1995; Falsey et al. 1997). Interest in Coronaviruses was boosted by the outbreaks of severe acute respiratory syndrome (SARS) that started in China in 2003 and then spread to other parts of the world (Poutanen et al. 2005) because the aetiological agent was proven to be a novel coronavirus named SARS-CoV. About 8,000 cases of infection were registered in almost 30 countries, with a case fatality rate of about 10% (Peiris et al. 2003). In 2012 another novel coronavirus that emerged in the Middle East was also associated with acute respiratory syndrome (MERS coronavirus).

SARS and MERS coronaviruses are highly pathogenic. Therefore for this study Human Coronavirus 229E (HCoV 229E) was used as a representative of the Coronavirus family.

The *Coronaviridae* family viron structure is depicted in Figure 2.1.

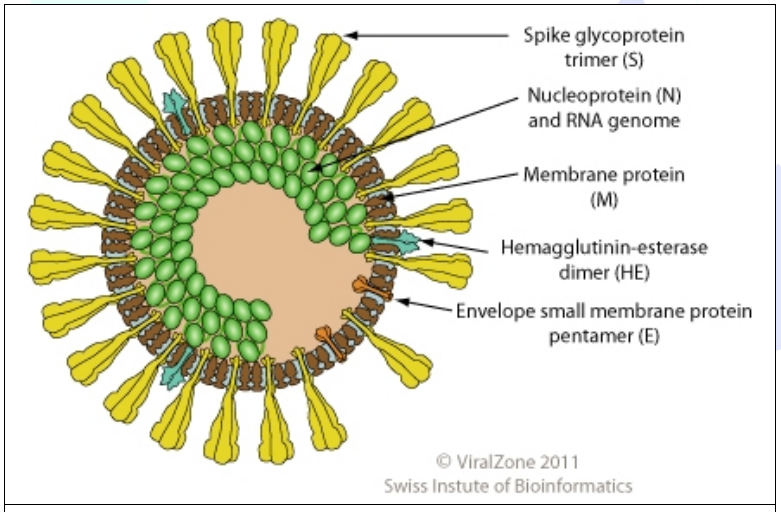

Figure 2.1. Coronavirus virion

3. Test Item Description

Three SleepAngel™ pillows sent by Gabriel Scientific to airmid healthgroup were received on 24/09/2015 (Figure 3.1).

Figure 3.1. SleepAngel™ Pillow.

4. Materials and Methods

4.1. Materials

4.1.1. Human Coronavirus 229E (HCoV 229E)

4.1.2. Real time PCR kit for Coronaviruses detection

4.1.3. Vacuum pump and BioStage impaction apparatus

4.1.4. Laminar flow cabinet

4.2. Methods

4.2.1. Barrier test

- The seams, filter and bulk fabric of each of the three pillows were tested for their ability to act as a barrier to HCoV 229E using a BioStage impactor. 15 x 15 cm pillow portions were cut as shown in Figure 4.1 and positioned on the BioStage impactor as required.
- The positive control consisted of cotton fabric. Impermeable latex glows were used as the negative control. Duplicate positive and negative controls were tested.

- The virus sample was placed in the upper chamber of the apparatus, on the test material or control, and then the vacuum was applied for 30 seconds. Any virus that passed through the test material or control was recovered in a collection buffer placed in the lower part of the impactor. The collected samples were then analysed for the presence of the virus by quantitative PCR.

Figure 4.1. SleepAngel™ Pillow investigated portions:
a – Long seam (top and bottom)
b – Filter and filter seam
c – Bulk fabric
d – Short seam (left hand side)

4.2.1. Quantitative PCR

Quantitative PCR (qPCR) is a form of polymerase chain reaction (PCR) where data are collected and monitored in real time, hence it is also known as real time PCR. Thanks to fluorescent dyes or probes and the use of standards, a targeted product of unknown concentration can be precisely quantified. The technique is very sensitive: the limit of detection is 2 nucleic acid copies per microlitre of sample. In this case a reverse transcriptase qPCR (RT-qPCR) was performed as HCoV229E is an RNA virus so an initial step in which the RNA is converted into DNA was introduced.

5. Results

5.1. Sleep Angel pillow barrier test for Human Coronavirus 229E

Different pillow areas were tested as outlined in Section 4 for their permeability to Human Coronavirus 229E (HCoV229E). Table 5.1 shows the results of the qPCR analysis. Sample values represent the average of three replicates, control values represent the average of two replicates.

Table 5.1 Total number of viral RNA copies recovered from the barrier test.

Pillow Portion	Total number of RNA copies	Standard Deviation
Long Seam	<LOD[a] [b]	/
Short Seam	<LOD [b]	/
Filter & Filter seam	<LOD [b]	/
Bulk Fabric	<LOD [b]	/
Positive Control (Cotton)	$8.6x10^8$ [c]	$1.3 x10^8$
Negative Control (Latex)	<LOD [c]	/

[a] <LOD = below the limit of detection (<10^4 **total** RNA copies)
[b] Values represent the average of three replicates
[c] Values represent the average of two replicates

6. Discussion

Samples of the SleepAngel™ pillow were tested for their ability to act as a barrier to Human Coronavirus 229E (HCoV 229E). HCoV 229E was chosen as a representative for the Coronavirus family due to the relevant role Coronaviruses play in mild and severe respiratory syndrome.

Pillow seams, filters and bulk fabric (Figure 4.1) were tested for their permeability to the virus using a BioStage impactor. The effectiveness of the test was proved by using cotton fibre as the positive control. The positive control material allowed the transfer of the virus through to the collection buffer where an average of 8.6 x 10^8 total RNA viral copies were detected. Analysis of the collection buffer for the negative controls (latex fibre) by qPCR gave values below the limit of detection.

The results of the analysis of the test samples show that the pillow does not allow the transfer of viral particles through the seams, the filter and the fabric itself. Analysis of the collection buffer by qPCR gave values below the limit of detection for every replicate examined.

7. Conclusions

The SleepAngel™ pillow was proven to be effective in acting as barrier for Human Coronavirus 229E, used as a representative for the Coronavirus family. The test carried out using a BioStage impactor demonstrated that the pillow was able to block 99.99% of virus.

8. References

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End of Report