

Zadar, 14.7.2021.

## ANALYTICAL REPORT br. 1491/21

**Robosol Anolitni dezificijens serija: 006-05-/21 (Robosol Anolyte disinfectant)**

### GENERAL INFORMATION

|                                  |   |
|----------------------------------|---|
| Client                           | <b>Robotic Solution d.o.o., A.G. Matoša bb, 21000 Split</b> |
| Section                          | /   |
| Sampled and submitted by         | Delivered by customer                                       |
| Type of request                  | Order   |
| Delivery date                    | 27.05.2021.   |
| Analysis started                 | 27.05.2021.   |
| Temperature at sampling/delivery | Not specified °C / Not specified °C                         |
| Analysis ended                   | 14.07.2021.   |

### SAMPLE DESCRIPTION

Batch: 006-05/21  
Appearance: Colourless transparent liquid in commercial container  
Packaging: Original polymer packaging  
Storing conditions: 22°C  
Active substances: Hypochlorous Acid  
Usage: Surface  
Objections: No objections  
Concentration: Ready-to-use at 500ppm

### CONFORMITY STATEMENT

Not requested/applicable.

  **HAMILTON  
CROATIA**

Luka Beretin, mag.chem  
Quality manager

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## ANALYTICAL RESULTS

### 1. Testing method

Procedure DESIN-1078 (NF EN 14476:2013+A2:2019 Standard)

### 2. Experimental conditions

|  |   |
|--|---|
| Assay period:  | 21.06.2021. - 30.06.2021.   |
| Assay temperature:   | 37°C ± 1°C  |
| Titration method:  | TCID <sub>50</sub> (Tissue Culture Infective Dose 50%)  |
| Product concentrations for the assay:  | 80%, 50% and 0.1%   |
| Contact time:  | 3 minutes   |
| Contact temperature:   | 22°C ± 1 °C   |
| Procedure to stop product cytotoxicity:  | Molecular sieving (< 4 columns)   |
| Procedure to stop product activity:  | Cooling with ice  |
| Solvent of the product used in the assay:  | Sterile distilled water   |
| Aspect of the dilutions of the product:  | Transparent dilutions   |
| Stability of the mixture (interfering substance and product diluted in sterile distilled water): | Stabile   |
| Interfering substance:   | - Clean conditions in the presence of bovine serum albumin 0.3 g/L.   |
| Identification of the origin of viral strains and number of passes:                              | Poliovirus aliquot: 2020/01/07 passage 2<br>Adenovirus aliquot: 2020/01/14 passage 2<br>Norovirus aliquot: 2020/02/11 passage 2       |
| Cell lines (name, origin, number of passes):   | Vero, ref: FTVE, working aliquot 2, passages 19 and 21<br><br>Raw 264.7, Public health England, working aliquot 2, passages 18 and 19 |

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### 3. Validation of assay results

#### Poliovirus type 1 (ATCC VR-192)

|  |                         |
|--|-------------------------|
| Titre of the viral suspension for the virus control (at the requested time):   |                         |
| Clean conditions:  | log 10 <sup>-6.91</sup> |
| Cytotoxicity level (80%)   | log 10 <sup>-0.50</sup> |
| Maximum level of virus inactivation detectable<br>(difference between the titre of the viral suspension and the cytotoxicity level): |                         |
| Clean conditions:  | log 10 <sup>-6.41</sup> |

#### Adenovirus type 5 (ATCC VR-5)

|  |                         |
|--|-------------------------|
| Titre of the viral suspension for the virus control (at the requested time):   |                         |
| Clean conditions:  | log 10 <sup>-6.08</sup> |
| Cytotoxicity level (80%)   | log 10 <sup>-0.50</sup> |
| Maximum level of virus inactivation detectable<br>(difference between the titre of the viral suspension and the cytotoxicity level): |                         |
| Clean conditions:  | log 10 <sup>-5.58</sup> |

#### Murine norovirus (Strain S99 Berlin)

|  |                         |
|--|-------------------------|
| Titre of the viral suspension for the virus control (at the requested time):   |                         |
| Clean conditions:  | log 10 <sup>-6.41</sup> |
| Cytotoxicity level (80%)   | log 10 <sup>-0.50</sup> |
| Maximum level of virus inactivation detectable<br>(difference between the titer of the viral suspension and the cytotoxicity level): |                         |
| Clean conditions:  | log 10 <sup>-5.91</sup> |

#### Reference test (Formaldehyde 1.4%)

|   |                         |
|---|-------------------------|
| Cytotoxicity level of formaldehyde 0.7%   | log 10 <sup>-0.50</sup> |
| Viral quantification in the reference test (formaldehyde) after 60 minutes and with Poliovirus type 1 | log 10 <sup>-2.91</sup> |
| Viral quantification in the reference test (formaldehyde) after 60 minutes and with Adenovirus type 5 | log 10 <sup>-1.82</sup> |
| Viral quantification in the reference test (formaldehyde) after 60 minutes and with Murine norovirus  | log 10 <sup>-2.90</sup> |

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## Confidence interval

|  |                            |
|--|----------------------------|
| Titre of virus with 95% confidence interval with Poliovirus type 1 (at the requested time) |                            |
| Clean conditions:  | $\log 10^{-6.91 \pm 0.33}$ |
| Titre of virus with 95% confidence interval with Adenovirus type 5 (at the requested time) |                            |
| Clean conditions:  | $\log 10^{-6.08 \pm 0.34}$ |
| Titre of virus with 95% confidence interval with Murine norovirus (at the requested time)  |                            |
| Clean conditions:  | $\log 10^{-6.41 \pm 0.39}$ |
| Reduction with the confidence interval of 95%  | See annex                  |

## Sensitivity of cells to virus

|   |                   |
|---|-------------------|
| Viral quantification of Poliovirus type 1 with cells not treated by the test solution with the test product | $\log 10^{-7.33}$ |
| Viral quantification of Poliovirus type 1 with cells treated by the test solution with the test product     | $\log 10^{-6.83}$ |
| Viral quantification of Adenovirus type 5 with cells not treated by the test solution with the test product | $\log 10^{-6.41}$ |
| Viral quantification of Adenovirus type 5 with cells treated by the test solution with the test product     | $\log 10^{-5.91}$ |
| Viral quantification of Murine norovirus with cells not treated by the test solution with the test product  | $\log 10^{-6.41}$ |
| Viral quantification of Murine norovirus with cells treated by the test solution with the test product      | $\log 10^{-5.83}$ |

Note: only can be used to determine the infectivity of cells, those dilutions which:

- a) show a low degree of cellular destruction (< 25% of cell monolayer)
- b) produce a reduction of the titre of the virus < 1 log<sub>10</sub>

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## Control of the effectivity of the disinfectant detection activity

|   |                   |
|---|-------------------|
| Viral quantification of Poliovirus type 1 after 30 minutes on bath ice without exposing the virus to the test product | $\log 10^{-7.41}$ |
| Viral quantification of Poliovirus type 1 exposing the virus to the test product and incubated 30 minutes on ice bath | $\log 10^{-6.91}$ |

|   |                   |
|---|-------------------|
| Viral quantification of Adenovirus type 5 after 30 minutes on bath ice without exposing the virus to the test product | $\log 10^{-6.41}$ |
| Viral quantification of Adenovirus type 5 exposing the virus to test product and incubated 30 minutes on ice bath     | $\log 10^{-6.08}$ |

|  |                   |
|--|-------------------|
| Viral quantification of Murine norovirus after 30 minutes on bath ice without exposing the virus to the test product | $\log 10^{-6.41}$ |
| Viral quantification of Murine norovirus exposing the virus to the test product and incubated 30 minutes on ice bath | $\log 10^{-5.91}$ |

Note: The difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension should be  $\leq 0.5$

### 4. Special remarks

- The product is tested at 80%, 50% and 0.1%. The highest concentration that can be tested in the test is 80%, because of the mixtures made during the test.
- All controls and validation were between the basic limits.
- One concentration at least showed a log reduction less than 4 log.
- One concentration at least showed a log reduction equal or higher than 4 log

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## 5. Assay results

### 5.1. Description of the results under the requested test conditions

| Virus of assay    | Test concentrations, reduction obtained with the confidence interval of 95% and virucidal activity |  |   |
|-------------------|--|--|---|
|                   | 80%  | 50%  | 0.1%  |
| Poliovirus type 1 | $\geq 6.41 \pm 0.33$ TCID <sub>50</sub><br>Shows   | $\geq 6.41 \pm 0.33$ TCID <sub>50</sub><br>Shows | $0.09 \pm 0.49$ TCID <sub>50</sub><br>Does not show |
| Adenovirus type 5 | $\geq 5.58 \pm 0.34$ TCID <sub>50</sub><br>Shows   | $\geq 5.58 \pm 0.34$ TCID <sub>50</sub><br>Shows | $0.09 \pm 0.48$ TCID <sub>50</sub><br>Does not show |
| Murine Norovirus  | $\geq 5.91 \pm 0.39$ TCID <sub>50</sub><br>Shows   | $\geq 5.91 \pm 0.39$ TCID <sub>50</sub><br>Shows | $0.25 \pm 0.51$ TCID <sub>50</sub><br>Does not show |

Note: Virucidal activity exists when the titre of virus shows a reduction  $\geq 4$  log  
TCID<sub>50</sub>: Tissue Culture Infectious Dose 50%

### 5.2. Tables of results and graphics

- See Annex 1 below

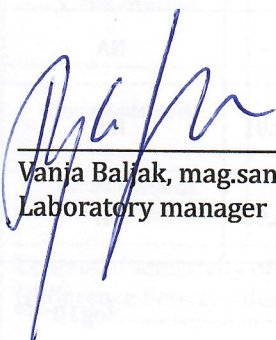
## 6. Conclusion

The disinfectant product „Robosol Anolitni Dezinficijens / Robosol Anolyte Disinfectant“, batch: 006-05/21, at 80% concentration, requested by the client, under clean conditions (bovine serum albumin 0.3 g/L) and during 3 minutes of contact time and 22°C of temperature, **shows virucidal activity** against the three mandatory viruses (Poliovirus type 1 , Adenovirus type 5 and Murine norovirus) when the activity is assayed according with the **NF EN 14476:2013+A2:2019 Standard**.

Therefore, the disinfectant tested **shows general virucidal activity**, diluted at **80%**, when the activity is evaluated according with the **NF EN 14476:2013+A2:2019 standard**.

### Reference:

NF EN 14476:2013+A2:2019 Guideline. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine. Test method and requirements (Phase 2/Step 1). AFNOR.



Vanja Baljak, mag.sanit.ing.  
Laboratory manager

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**Table 1.** Results of activity of the test sample, with Poliovirus type 1 (ATCC VR-192) under clean conditions:

| Assay   | Concentration | Interfering substance | Cytotoxicity level | log <sub>10</sub> TCID <sub>50</sub> after: |       |        |        | Reduction with the confidence interval of 95 % |
|---|---------------|-----------------------|--------------------|---|-------|--------|--------|--|
|   |               |                       |                    | 0 min                                       | 3 min | 30 min | 60 min |  |
| Test sample   | 80%           | 0.3 g/L BSA           | 0.50               | -   | 0.50  | -      | -      | $\geq 6.41 \pm 0.33$                           |
|   | 50%           |                       | 0.50               | -   | 0.50  | -      | -      | $\geq 6.41 \pm 0.33$                           |
|   | 0,1%          |                       | 0.50               | -   | 6.82  | -      | -      | $0.09 \pm 0.49$                                |
| Virus control   | NA            | 0.3 g/L BSA           | NA                 | 6.99  | 6.91  | -      | -      | NA   |
| Formaldehyde  | 0.7% (w:v)    | NA                    | 0,5                | NR  | NR    | 5.66   | 2.91   | NA   |
| Virus control Formaldehyde  | 0.7% (w:v)    | NA                    | 0,5                | 7.07  | NR    | NR     | 6.90   | NA   |
| Control of sensitivity of cells to virus<br>(difference between decimal logarithm of titre using treated and untreated cells).....log <sub>10</sub> <sup>-0.50</sup>  |               |                       |                    |   |       |        |        |  |
| Control of the effectiveness of the disinfectant detection activity<br>(difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension).....log <sub>10</sub> <sup>-0.50</sup>  |               |                       |                    |   |       |        |        |  |
| NA: not applicable; NR: not realized<br>Times recommended by Guideline for surfaces: maximum 5 or 60 minutes<br>Times recommended by Guideline for instruments: maximum 60 minutes<br>Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds<br>PBS: phosphate buffered saline; BSA: bovine serum albumin.<br>Viricidal activity exists when the titre of virus shows a reduction $\geq 4$ log. |               |                       |                    |   |       |        |        |  |

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**Table 2.** Results of activity of the test product, with Adenovirus type 5 (ATCC VR-5) under clean conditions:

| Assay   | Concentration | Interfering substance | Cytotoxicity level | log <sub>10</sub> TCID <sub>50</sub> after: |       |        |        | Reduction with the confidence interval of 95 % |
|---|---------------|-----------------------|--------------------|---|-------|--------|--------|--|
|   |               |                       |                    | 0 min                                       | 3 min | 30 min | 60 min |  |
| Test sample   | 80%           | 0.3 g/L BSA           | 0.50               | -   | 0.50  | -      | -      | $\geq 5.58 \pm 0.34$                           |
|   | 50%           |                       | 0.50               | -   | 0.50  | -      | -      | $\geq 5.58 \pm 0.34$                           |
|   | 0,1%          |                       | 0.50               | -   | 5.99  | -      | -      | $0.09 \pm 0.48$                                |
| Virus control   | NA            | 0.3 g/L BSA           | NA                 | 6.24  | 6.08  | -      | -      | NA   |
| Formaldehyde  | 0.7% (w:v)    | NA                    | 0,5                | NR  | NR    | 2.07   | 1.82   | NA   |
| Virus control Formaldehyde  | 0.7% (w:v)    | NA                    | 0,5                | 5.99  | NR    | NR     | 5.82   | NA   |
| Control of sensitivity of cells to virus<br>(difference between decimal logarithm of titre using treated and untreated cells).....log <sub>10</sub> <sup>-0.50</sup><br><br>Control of the effectiveness of the disinfectant detection activity<br>(difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension).....log <sub>10</sub> <sup>-0.33</sup>  |               |                       |                    |   |       |        |        |  |
| NA: not applicable; NR: not realized<br>Times recommended by Guideline for surfaces: maximum 5 or 60 minutes<br>Times recommended by Guideline for instruments: maximum 60 minutes<br>Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds<br>PBS: phosphate buffered saline; BSA: bovine serum albumin.<br>Viricidal activity exists when the titre of virus shows a reduction $\geq 4$ log. |               |                       |                    |   |       |        |        |  |

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**Table 3.** Results of activity of the test product, with Murine norovirus (Strain S99 Berlin) under clean conditions:

| Assay   | Concentration | Interfering substance | Cytotoxicity level | log <sub>10</sub> TCID <sub>50</sub> after: |       |        |        | Reduction with the confidence interval of 95 % |
|---|---------------|-----------------------|--------------------|---|-------|--------|--------|--|
|   |               |                       |                    | 0 min                                       | 3 min | 30 min | 60 min |  |
| Test sample   | 80%           | 0.3 g/L BSA           | 0.50               | -   | 0.50  | -      | -      | $\geq 5.58 \pm 0.34$                           |
|   | 50%           |                       | 0.50               | -   | 0.50  | -      | -      | $\geq 5.58 \pm 0.34$                           |
|   | 0,1%          |                       | 0.50               | -   | 6.16  | -      | -      | $0.25 \pm 0.51$                                |
| Virus control   | NA            | 0.3 g/L BSA           | NA                 | 6.24  | 6.41  | -      | -      | NA   |
| Formaldehyde  | 0.7% (w:v)    | NA                    | 0,5                | NR  | NR    | 3.82   | 2.90   | NA   |
| Virus control Formaldehyde  | 0.7% (w:v)    | NA                    | 0,5                | 6.07  | NR    | NR     | 5.91   | NA   |
| Control of sensitivity of cells to virus<br>(difference between decimal logarithm of titre using treated and untreated cells).....log <sub>10</sub> <sup>-0.58</sup><br><br>Control of the effectiveness of the disinfectant detection activity<br>(difference between decimal logarithm of titre without exposing the virus to the product and of the test suspension).....log <sub>10</sub> <sup>-0.50</sup>  |               |                       |                    |   |       |        |        |  |
| NA: not applicable; NR: not realized<br>Times recommended by Guideline for surfaces: maximum 5 or 60 minutes<br>Times recommended by Guideline for instruments: maximum 60 minutes<br>Times recommended by Guideline for Hygienic treatment of hands by friction and hygienic handwashing: between 30 or 120 seconds<br>PBS: phosphate buffered saline; BSA: bovine serum albumin.<br>Viricidal activity exists when the titre of virus shows a reduction $\geq 4$ log. |               |                       |                    |   |       |        |        |  |

End of annex

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