

Test report R-LV001

Determination of virucidal activity of DUTRION TABLET _ Quantitative suspension test according to NF EN 14476 (medical area, phase 2 step 1_2015)

| | |
|-------------------------------|--|
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This test report includes 22 pages

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I. CONCLUSION

The product shall be deemed to have passed the NF EN 14476 standard if it demonstrates in a valid test at least a 4 log reduction within 5 minutes or less at 20°C, with the chosen interfering substances under the conditions defined by this standard when the test microorganisms are murine norovirus and type-5 adenovirus.

For the product DUTRION TABLET, the minimal virucidal concentration determined according to NF EN 14476 under clean and dirty conditions is 1000 ppm.

The 3000 ppm concentration is also virucidal under the conditions defined by this standard.

| Product | Concentration | Interfering substance | Compliance to NF EN 14476 for virucidal activity | |
|----------------|---------------|-----------------------|--|------------------|
| | | | Norovirus | Adenovirus |
| DUTRION TABLET | 1000 ppm | Clean | Compliant | Compliant |
| | | Dirty | Compliant | Compliant |
| | 3000 ppm | Clean | Compliant | Compliant |
| | | Dirty | Compliant | Compliant |

II. TEST SUMMARY

The aim of this test was to determine the virucidal activity of DUTRION TABLET according to NF EN 14476 medical area experimental conditions. The virucidal activity of the product DUTRION TABLET has been tested against viruses: murine norovirus (MNV-1) and type-5 adenovirus (Ad5).

Results have shown that the minimal virucidal concentration of DUTRION TABLET was 1000 ppm under the conditions defined by this standard (5min, 20°C, clean and dirty conditions).

The 1000 ppm and 3000 ppm concentrations induce a logarithmic reduction greater than 4 against the viruses: murine norovirus and type-5 adenovirus.

III. CONTRACTUAL DOCUMENTS

VIRHEALTH was hired to perform tests according to the French standard:

- NF EN 14476

On behalf of: **NOXIMA**

The present service is defined by the following contractual documents:

- Quotation: 1612TPLB001
- Order : good for agreement dated 13/12/2016

IV. TEST CONDITIONS AND SAMPLES DATA

IV.I Samples identification

| Disinfectant product | |
|-----------------------|---|
| Name | DUTRION TABLET |
| Batch number | Unknown |
| Manufacturer | Duka Production Ltd |
| Manufacture date | Unknown |
| Expiry date | Unknown |
| Concentrations tested | 100, 1000 and 3000 ppm |
| Diluent | Hard water |
| Product appearance | White solid effervescent tablet |
| Storage conditions | RT, dark, dry, cool and well ventilated place |
| Active substances | 1g at 12% of sodium chlorite |

Table 1: disinfectant product characteristics

IV.II Experimental conditions

| Test conditions | |
|-------------------------------|---|
| Viral strains | - Murine norovirus - Type-5 adenovirus |
| Product diluent | Hard water |
| Product aspect after dilution | Liquid, yellow, chlorine smell |
| Test concentrations | C1 = 3000 ppm C2 = 1000 ppm C3 = 100 ppm |
| Temperature | 20 ± 1°C |
| Contact time | 5 min |
| Interfering substances | Clean conditions: 0,3g/l BSA Dirty conditions: 3g/l BSA + 3ml/l erythrocytes |
| Neutralization technique | Ultrafiltration with MicroSpin S-400HR columns |
| Quantification technique | Endpoint titration TCID50 on permissives cells |
| Number of well/dilution | 8 |
| Incubation temperature | 37 °C |
| Tests dates | 11/01/2017 |

Table 2: Experimental conditions to assess virucidal activity

Special remarks regarding the results:

- All controls and validation were within the basic limits.
- The formation of a precipitate is observed when the dirty condition is mixed with the two highest concentration of the product (C1 and C2). This observation did not seem to impact the results obtained.

V. RESULTS

V.I Virucidal activity of DUTRION TABLET

A. Type 5 adenovirus

a. Determination of cytotoxicity

The disinfectant effect of the product is determined by reading of cytopathic effect (CPE) on permissive cells A549 and quantified by TCID50 technique.

| Product | Concentration | Interfering substances | Dilution factor | | | | | |
|----------------|---------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ |
| DUTRION TABLET | 3000 ppm | clean | 0 | 0 | 0 | 0 | 0 | 0 |
| | | dirty | 0 | 0 | 0 | 0 | 0 | 0 |
| DUTRION TABLET | 1000 ppm | clean | 0 | 0 | 0 | 0 | 0 | 0 |
| | | dirty | 0 | 0 | 0 | 0 | 0 | 0 |
| DUTRION TABLET | 100 ppm | clean | 0 | 0 | 0 | 0 | 0 | 0 |
| | | dirty | 0 | 0 | 0 | 0 | 0 | 0 |

Explanations :

- 1-4 : degrees of CPE in 8 cell culture unit (microtiter plate)
- 0 : no virus present
- C: cytotoxicity observed on cells
- n.a : not applicable

The cytotoxicity of the tests solutions which are indispensable to show 4 log reduction did not affect the morphology and the growth of cells.

Under test conditions with interfering substances, the three tested concentrations of DUTRION TABLET have no cytopathic effects on A549 cells.

The test results are dependent and take into account the cytotoxicity results.

b. Method validation

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID50 technique on A549 cells.

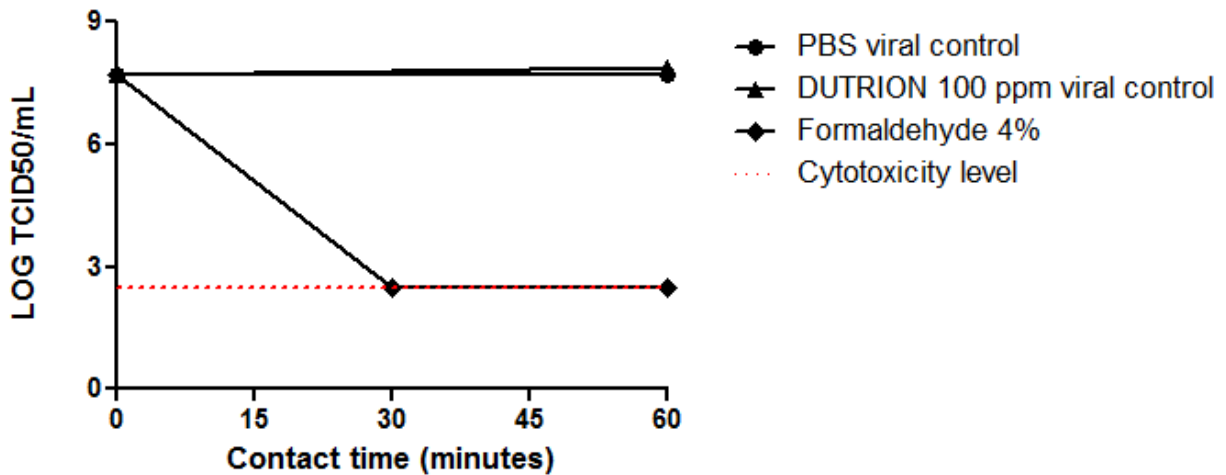


Figure 1: graphic representation of controls for virucidal activity of DUTRION TABLET on type 5 adenovirus

Raw data of controls results for virucidal activity are presented on appendices.

- Control of cell susceptibility

| Product | Log TCID50/ml |
|---|---------------|
| DUTRION TABLET 100 ppm | 7,9 |
| PBS solution | 7,7 |
| Difference <1 log <input checked="" type="checkbox"/> yes <input type="checkbox"/> no | |

Comparative titer of cells treated with dilutions of 100 ppm concentration and with PBS solution shows a difference of less than 1 log.

Results shown that cytotoxicity of test solutions did not affect cell susceptibility to type-5 adenovirus.

- Formaldehyde inactivation of Ad5

| Product | Cytotoxicity (log TCID50/ml) | Results (log TCID50/ml) | Reduction (log) |
|--|---------------------------------|---|-----------------------------|
| Formaldehyde 4% 30minutes | 2,7 | 2,5 | 5,0 |
| Formaldehyde 4 % 60 minutes | 2,5 | 2,5 | 5,2 |
| Reduction between 3 and 5 log after 30 min | | <input checked="" type="checkbox"/> yes | <input type="checkbox"/> no |
| Reduction between 3,5 and 5,5 log after 60 min | | <input checked="" type="checkbox"/> yes | <input type="checkbox"/> no |

Under test conditions when PBS solution is used as an interfering substance, formaldehyde 4 % shows a slight cytotoxicity on A549 cells.

Results shown that the titer difference between viral control and virus used for the inactivation test was 5 log after 30min and 5,2 log after 60min.

c. Test

Raw data of tests results for virucidal activity of DUTRION TABLET are presented on appendices.

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID50 technique on A549 cells.

- Test under clean conditions (0,3g/l BSA)

| Product | Concentrations | Interfering substance | Cytotoxicity level | Log TCID50 after 5 min | | Log R | > 4 log reduction after ... min |
|------------------|----------------|-----------------------|--------------------|------------------------|-----|-------|---------------------------------|
| | | | | 0 | 5 | | |
| Dutrition tablet | 3000 ppm | 0,3 g/L BSA | 0,5 | 0,5 ⁺ | 0,5 | 6 | <1 |
| | 1000 ppm | | 0,5 | 0,5 ⁺ | 0,5 | 6 | <1 |
| | 100 ppm | | 0,5 | 4,2 | 4,2 | 2,3 | >5 |
| Viral control | n.a | 0,3 g/L BSA | n.a | 6,7 | 7 | n.a | n.a |

Explanations:

n.a : not applicable

Cytotoxicity level is expressed in log TCID50/ml and determined by Spearman and Karber method.

Test suspension is expressed in log TCID50/ml and determined by Spearman and Karber method.

Viral control is expressed in log TCID50/ml and determined by Spearman and Karber method.

R = reduction expressed in logarithm (logR = log viral control – log test suspension)

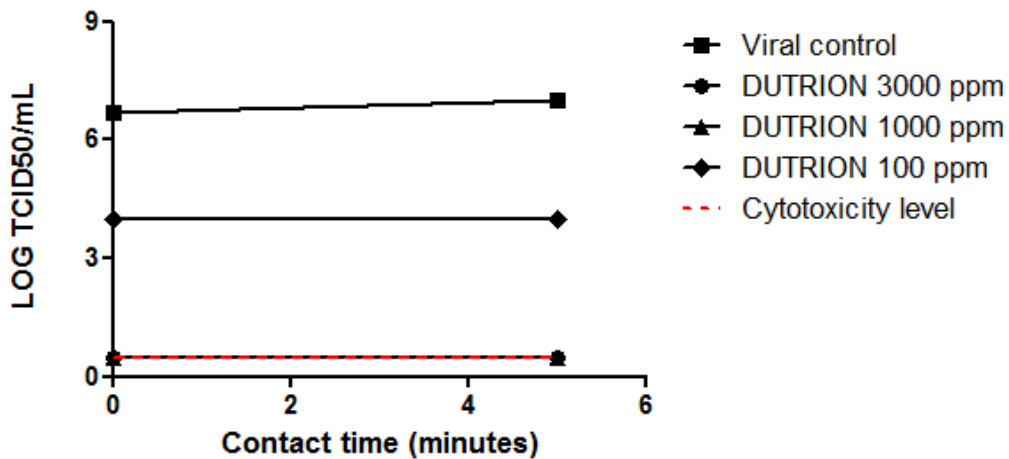


Figure 2: graphic representation of virucidal activity of DUTRION TABLET on type 5 adenovirus under clean conditions

* DUTRION TABLET action is less than 1 minute for 3000 ppm and 1000 ppm concentrations. Use of MicroSpin S-400 HR columns did not stopped product activity.

The product DUTRION TABLET at 3000 ppm and 1000 ppm under NF EN 14476 conditions shows a virucidal activity against type 5 adenovirus greater than 4 LOG (6LOG TCID50/ml) under clean conditions for a 5 minutes contact time.

The concentrations 3000 and 1000 ppm induce a logarithmic reduction greater than 6 under clean conditions against type 5 adenovirus.

The concentration 100 ppm induces a logarithmic reduction of 2,3 under clean conditions against type 5 adenovirus.

- Test under dirty conditions (3g/l BSA + 3ml/l erythrocytes)

| Product | Concentrations | Interfering substance | Cytotoxicity level | Log TCID50 after 5 min | | Log R | > 4 log reduction after ... min |
|------------------|----------------|-------------------------------|--------------------|------------------------|-----|-------|---------------------------------|
| | | | | 0 | 5 | | |
| Dutrition tablet | 3000 ppm | 3g/L BSA + 3mL/L erythrocytes | 0,5 | 0,5 | 0,5 | 5,7 | < 1 |
| | 1000 ppm | | 0,5 | 0,5 | 0,5 | 5,7 | < 1 |
| | 100 ppm | | 0,5 | 4,4 | 4,4 | 1,8 | > 5 |
| Viral control | n.a | 3g/L BSA + 3mL/L erythrocytes | n.a | 7 | 6,7 | n.a | n.a |

Explanations:

n.a : not applicable

Cytotoxicity level is expressed in log TCID50/ml and determined by Spearman and Karber method.

Test suspension is expressed in log TCID50/ml and determined by Spearman and Karber method.

Viral control is expressed in log TCID50/ml and determined by Spearman and Karber method.

R = reduction expressed in logarithm (logR = log viral control – log test suspension)

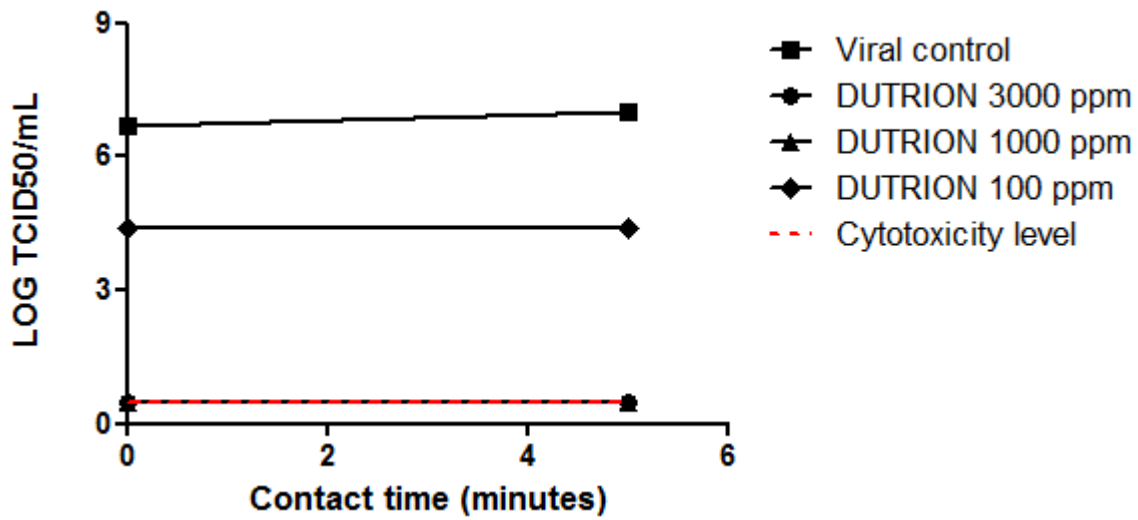


Figure 3: graphic representation of virucidal activity of DUTRION TABLET on type 5 adenovirus under dirty conditions

* DUTRION TABLET action is less than 1 minute for 3000 ppm and 1000 ppm concentrations. Use of MicroSpin S-400 HR columns did not stopped product activity.

The product DUTRION TABLET at 3000 ppm and 1000 ppm under NF EN 14476 conditions shows a virucidal activity against type 5 adenovirus greater than 4 LOG (5,7 LOG TCID50/ml) under dirty conditions for a 5 minutes contact time.

The concentrations 3000 and 1000 ppm induce a logarithmic reduction greater than 5,7 under dirty conditions against type-5 adenovirus.

The concentration 100 ppm induces a logarithmic reduction of 1,8 under dirty conditions against type-5 adenovirus.

For the virucidal activity, at least one concentration of DUTRION TABLET shows a logarithmic reduction of 4 or more and at least one concentration shows a logarithmic reduction of less than 4. The test is compliant under the NF EN 14476 requirements.

B. Murine type 1 norovirus

a. Determination of cytotoxicity

The disinfectant effect of the product is determined by reading of cytopathic effect (CPE) on permissive cells RAW and quantified by TCID50 technique.

| Product | Concentration | Interfering substances | Dilution factor | | | | | |
|----------------|---------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ |
| DUTRION TABLET | 3000 ppm | clean | 0 | 0 | 0 | 0 | 0 | 0 |
| | | dirty | 0 | 0 | 0 | 0 | 0 | 0 |
| DUTRION TABLET | 1000 ppm | clean | 0 | 0 | 0 | 0 | 0 | 0 |
| | | dirty | 0 | 0 | 0 | 0 | 0 | 0 |
| DUTRION TABLET | 100 ppm | clean | 0 | 0 | 0 | 0 | 0 | 0 |
| | | dirty | 0 | 0 | 0 | 0 | 0 | 0 |

Explanations :

- 1-4 : degrees of CPE in 8 cell culture unit (microtiter plate)
- 0 : no virus present
- C: cytotoxicity observed on cells
- n.a : not applicable

The cytotoxicity of the tests solutions which are indispensable to show 4 log reduction did not affect the morphology and the growth of cells.

Under test conditions with interfering substances, the three tested concentrations of DUTRION TABLET have no cytopathic effects on RAW cells.

The test results are dependent and take into account the cytotoxicity results.

b. Method validation

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID50 technique on RAW cells.

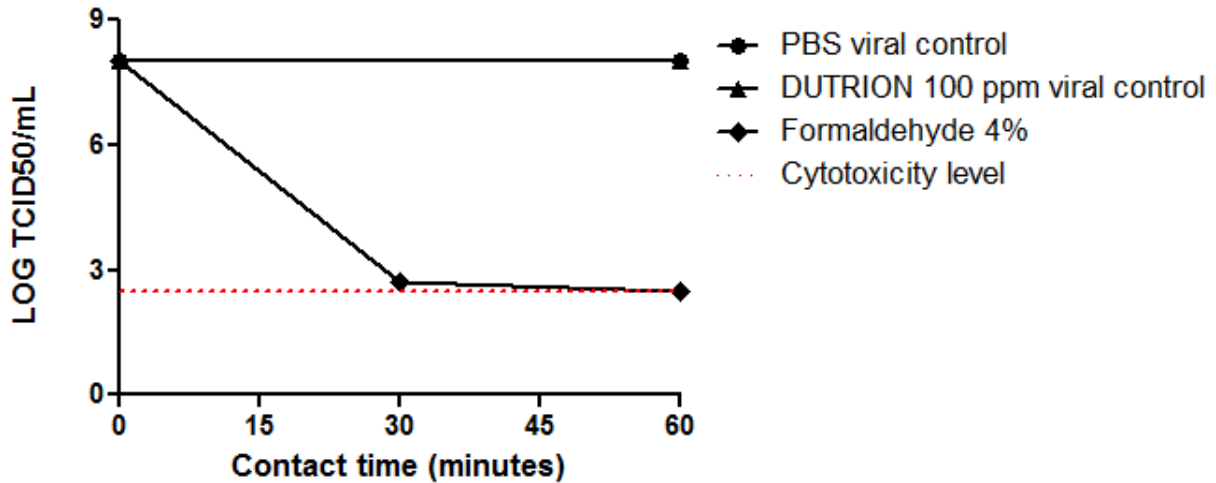


Figure 4: graphic representation of controls for virucidal activity of DUTRION TABLET on murine norovirus

Raw data of controls results for virucidal activity are presented on appendices.

- Control of cell susceptibility

| Product | Log TCID50/ml |
|---|---------------|
| DUTRION TABLET 100 ppm | 8 |
| PBS solution | 8 |
| Difference <1 log <input checked="" type="checkbox"/> yes <input type="checkbox"/> no | |

Comparative titer of cells treated with dilutions of 100 ppm concentration and with PBS solution shows a difference of less than 1 log.

Results shown that cytotoxicity of test solutions did not affect cell susceptibility to murine norovirus.

- Formaldehyde inactivation of MNV-1

| Product | Cytotoxicity (log TCID50/ml) | Results (log TCID50/ml) | Reduction (log) |
|--|---------------------------------|---|-----------------------------|
| Formaldehyde 4% 30minutes | 2,7 | 2,5 | 5,0 |
| Formaldehyde 4 % 60 minutes | 2,5 | 2,5 | 5,2 |
| Reduction between 3 and 5 log after 30 min | | <input checked="" type="checkbox"/> yes | <input type="checkbox"/> no |
| Reduction between 3,5 and 5,5 log after 60 min | | <input checked="" type="checkbox"/> yes | <input type="checkbox"/> no |

Under test condition when PBS solution is used as an interfering substance, formaldehyde 4 % shows a slight cytotoxicity on RAW cells.

Results shown that the titer difference between viral control and virus used for the inactivation test was 5 log after 30min and 5,2 log after 60min.

c. Test

Raw data of tests results for virucidal activity of DUTRION TABLET are presented on appendices.

Results have been determined by visual reading of cytopathic effects (CPE) and quantified by TCID50 technique on RAW cells.

- Test under clean conditions (0,3g/l BSA)

| Product | Concentrations | Interfering substance | Cytotoxicity level | Log TCID50 after 5 min | | Log R | > 4 log reduction after ... min |
|------------------|----------------|-----------------------|--------------------|------------------------|-----|-------|---------------------------------|
| | | | | 0 | 5 | | |
| Dutrition tablet | 3000 ppm | 0,3 g/L BSA | 0,5 | 0,5 | 0,5 | 6 | < 1 |
| | 1000 ppm | | 0,5 | 0,5 | 0,5 | 6 | < 1 |
| | 100 ppm | | 0,5 | 5,2 | 5,2 | 1,3 | > 5 |
| Viral control | n.a | 0,3 g/L BSA | n.a | 7,4 | 7 | n.a | n.a |

Explanations:

n.a : not applicable

Cytotoxicity level is expressed in log TCID50/ml and determined by Spearman and Karber method.

Test suspension is expressed in log TCID50/ml and determined by Spearman and Karber method.

Viral control is expressed in log TCID50/ml and determined by Spearman and Karber method.

R = reduction expressed in logarithm (logR = log viral control – log test suspension)

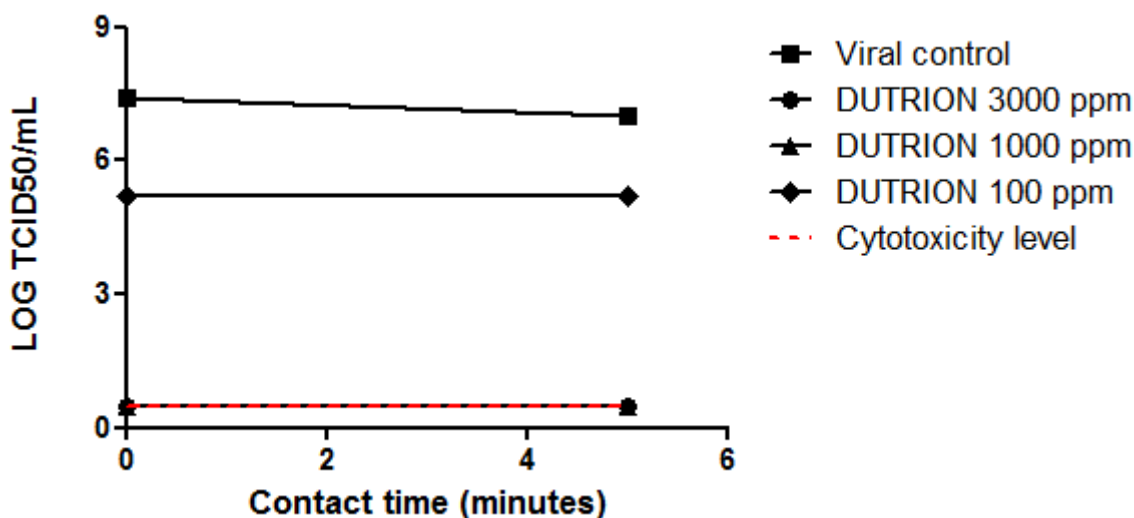


Figure 5: graphic representation of virucidal activity of DUTRION TABLET on murine norovirus under clean conditions

* DUTRION TABLET action is less than 1 minute for 3000 ppm and 1000 ppm concentrations. Use of MicroSpin S-400 HR columns did not stopped product activity.

The product DUTRION TABLET at 3000 ppm and 1000 ppm under NF EN 14476 conditions shows a virucidal activity against murine norovirus greater than 4 LOG (6LOG TCID50/ml) under clean conditions for a 5 minutes contact time.

The concentrations 3000 and 1000 ppm induce a logarithmic reduction greater than 6 under clean conditions against murine norovirus.

The concentration 100 ppm induces a logarithmic reduction of 1,3 under clean conditions against murine norovirus.

- Test under dirty conditions (3g/l BSA + 3ml/l erythrocytes)

| Product | Concentrations | Interfering substance | Cytotoxicity level | Log TCID50 after 5 min | | Log R | > 4 log reduction after ... min |
|------------------|----------------|-------------------------------|--------------------|------------------------|-----|-------|---------------------------------|
| | | | | 0 | 5 | | |
| Dutrition tablet | 3000 ppm | 3g/L BSA + 3ml/L erythrocytes | 0,5 | 0,5 | 0,5 | 6 | <1 |
| | 1000 ppm | | 0,5 | 0,5 | 0,5 | 6 | <1 |
| | 100 ppm | | 0,5 | 5,4 | 5,5 | 1 | >5 |
| Viral control | n.a | 3g/L BSA + 3ml/L erythrocytes | n.a | 7,1 | 7 | n.a | n.a |

Explanations:

n.a : not applicable

Cytotoxicity level is expressed in log TCID50/ml and determined by Spearman and Karber method.

Test suspension is expressed in log TCID50/ml and determined by Spearman and Karber method.

Viral control is expressed in log TCID50/ml and determined by Spearman and Karber method.

$R = \text{reduction expressed in logarithm (logR = log viral control} - \text{log test suspension)}$

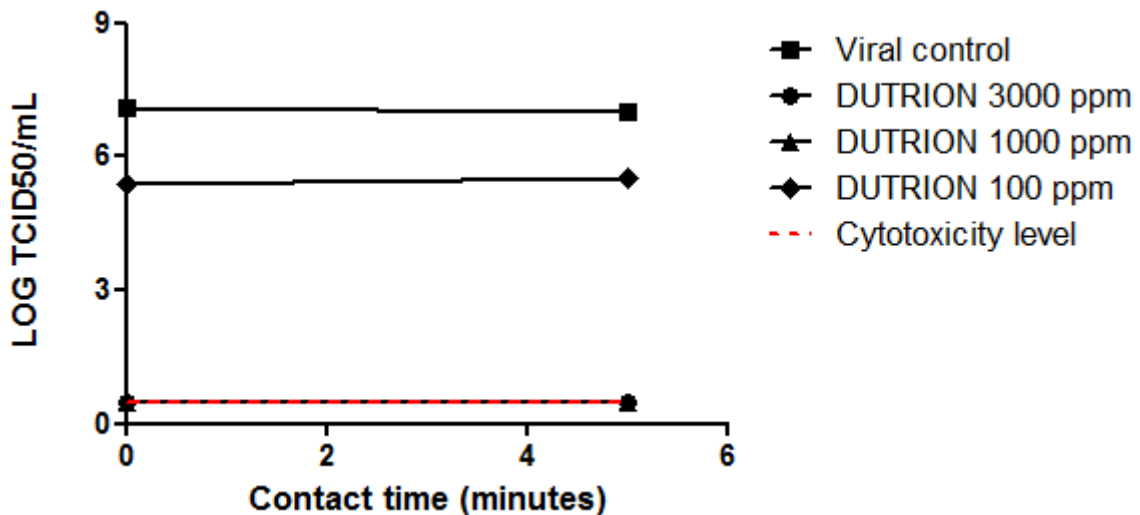


Figure 6: graphic representation of virucidal activity of DUTRION TABLET on murine norovirus under dirty conditions

* DUTRION TABLET action is less than 1 minute for 3000 ppm and 1000 ppm concentrations. Use of MicroSpin S-400 HR columns did not stopped product activity.

The product DUTRION TABLET at 3000 ppm and 1000 ppm under NF EN 14476 conditions shows a virucidal activity against murine norovirus greater than 4 LOG (5,7 LOG TCID50/ml) under dirty conditions for a 5 minutes contact time.

The concentrations 3000 and 1000 ppm induce a logarithmic reduction greater than 5,7 under dirty conditions against murine norovirus.

The concentration 100 ppm induces a logarithmic reduction of 1 under dirty conditions against murine norovirus.

For the virucidal activity, at least one concentration of DUTRION TABLET shows a logarithmic reduction greater than 4 or more and at least one concentration shows a logarithmic reduction of less than 4. The test is compliant under the NF EN 14476 requirements.

VI. APPENDICES

VI.I Materials and reagents

A. Virucidal activity

a. Test suspension

Cell line

Name: RAW 264.7 (macrophage) ATCC®-TIB-71™ (batch n°323142)

Number of passages: 8

Culture medium: DMEM 1,0 g/L (Lonza, batch n°000515663, 11/2017) complemented with 10% of FCS (Dutscher, batch n° S11971S1810), 1% of antibiotics (Lonza, batch n° 6MB152, 09/2018) and 1% of L-glutamine (Lonza, batch n° 6MB149, 08/2018)

Name: A549

Number of passages: 10

Culture medium: DMEM 1,0 g/L (Lonza, batch n°000515663, 11/2017) complemented with 10% of FCS (Dutscher, batch n° S11971S1810), 1% of antibiotics (Lonza, batch n° 6MB152, 09/2018) and 1% of L-glutamine (Lonza, batch n° 6MB149, 08/2018)

Viral strains

Name: murine type 1 norovirus (MNV-1)

Batch number: 1611N_004

Quantification technique:

- Successive tenfold dilutions in infection medium DMEM 1,0 g/L (Lonza, batch n°000515663, 11/2017) complemented with 10% of FCS (Dutscher, batch n° S11971S1810), 1% of antibiotics (Lonza, batch n° 6MB152, 09/2018) and 1% of L-glutamine (Lonza, batch n° 6MB149, 08/2018)
- Add 100µL of each dilution into 8 wells of 96 microtitration plate (RAW 264.7)
- Incubate 3-5 days at 37°C, 5% CO₂

Name: type 5 adenovirus (Ad5)

Batch number: 1610Ad_002

Quantification technique:

- Successive tenfold dilutions in infection medium DMEM 1,0 g/L (Lonza, batch n°000515663, 11/2017) complemented with 2% of FCS (Dutscher, batch n° S11971S1810), 1% of antibiotics (Lonza, batch n° 6MB152, 09/2018) and 1% of L-glutamine (Lonza, batch n° 6MB149, 08/2018)
- Add 100µL of each dilution into 8 wells of 96 microtitration plate (A549)
- Incubate 7 days at 37°C, 5% CO₂

b. Reagents preparation

Hard water

- prepare solution A: dissolve 1,984 g magnesium chloride (MgCl₂) and 4,624 g calcium chloride (CaCl₂) in 100 ml of water. Sterilize by membrane filtration or in the autoclave
- prepare solution B: dissolve 3,502 g sodium bicarbonate (NaHCO₃) in 100 ml of water. Sterilize by membrane filtration.
- add 6,0 ml of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with water. The pH of the hard water shall be $7,0 \pm 0,2$.

Interfering substance

- Clean conditions

Dissolve 0,30 g of bovine albumin fraction V (Sigma Aldrich, batch n°SLMB162) in 100 ml of sterile water (Aguettant, batch n°3011178). Sterilize by membrane filtration.

- Dirty conditions

Dissolve 3,00 g of bovine albumin fraction V (Sigma Aldrich, batch n° SLMB162) in 97 ml of sterile water (Aguettant, batch n° 3011178). Sterilize by membrane filtration.

Prepare at least 8,0 ml fresh defibrinated sheep blood (Oxoid, batch n°32301300, 17/01/2017). Centrifuge the erythrocytes at 800 gN for 10 min. After discarding the supernatant, resuspend erythrocytes in PBS. Repeat this procedure at least 3 times, until the supernatant is colorless.

Resuspend 3 ml of the packed sheep erythrocytes in the 97 ml of sterilized bovine albumin solution.

VI.II Type 5 adenovirus : TCID50 technique (8wells/dilution)

a. Controls

| Controls | Product | Concentration | Interfering substance | Contact time (minutes) | Dilution factor | | | | | | | | |
|--------------------------|----------------|---------------|-----------------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ | 10 ⁻⁷ | 10 ⁻⁸ | 10 ⁻⁹ |
| Inactivation du virus | Formaldehyde | 4 % (v/v) | PBS | 30 | CCCCCCCC | 11000000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 60 | CCCCCCCC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Cytotoxicité | Formaldehyde | 4 % (v/v) | PBS | 60 | CCCCCCCC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sensibilité des cellules | PBS | n.a | n.a | 60 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 10000001 | 0 | 0 |
| | DUTRION TABLET | 100 ppm | n.a | 60 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 10100001 | 0 | 0 |

Explanations:

- 1-4 : degrees of CPE in 8 cell culture unit (microtiter plate)
- 0 : no virus present
- C: cytotoxicity observed on cells
- N.a : not applicable

b. Test

Clean conditions

| TEST | Product | Concentration | Interfering substance | Contact time (minutes) | Dilution factor | | | | | | | | |
|---------------|----------------|---------------|-----------------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ | 10 ⁻⁷ | 10 ⁻⁸ | 10 ⁻⁹ |
| TEST | DUTRION TABLET | 3000 ppm | 0,3 g/L BSA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | DUTRION TABLET | 1000 ppm | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | DUTRION TABLET | 100 ppm | | 0 | 44444444 | 33333333 | 22022022 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 5 | 44444444 | 33333333 | 22222200 | 0 | 0 | 0 | 0 | 0 | 0 |
| Viral control | HARD WATER | n.a | 0,3 g/L BSA | 0 | 44444444 | 44444444 | 44444333 | 33333333 | 3330332 | 20000010 | 0 | 0 | 0 |
| | HARD WATER | | | 5 | 44444444 | 44444444 | 43333434 | 34433333 | 33334333 | 32000022 | 0 | 0 | 0 |

Dirty conditions

| TEST | Product | Concentration | Interfering substance | Contact time (minutes) | Dilution factor | | | | | | | | |
|---------------|----------------|---------------|--------------------------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | | | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ | 10 ⁻⁷ | 10 ⁻⁸ | 10 ⁻⁹ |
| TEST | DUTRION TABLET | 3000 ppm | 3 g/L BSA + 3mL/L erythrocytes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | DUTRION TABLET | 1000 ppm | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | DUTRION TABLET | 100 ppm | | 0 | 44444444 | 33333333 | 22022222 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 5 | 44444444 | 33333333 | 22222220 | 0 | 0 | 0 | 0 | 0 | 0 |
| Viral control | HARD WATER | n.a | 3 g/L BSA + 3mL/L erythrocytes | 0 | 44444444 | 44444444 | 43334443 | 33333344 | 33333333 | 33000330 | 0 | 0 | 0 |
| | HARD WATER | | | 5 | 44444444 | 44444444 | 44334343 | 33334434 | 33330333 | 20010000 | 0 | 0 | 0 |

Explanations:

- 1-4 : degrees of CPE in 8 cell culture unit (microtiter plate)
- 0 : no virus present
- C: cytotoxicity observed on cells
- N.a : not applicable

VI.III Murine norovirus: TCID50 technique (8wells/dilution)

a. Controls

| Controls | Product | Concentration | Interfering substance | Contact time (minutes) | Dilution factor | | | | | | | | | |
|---------------------|----------------|---------------|-----------------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|---|
| | | | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ | 10 ⁻⁷ | 10 ⁻⁸ | 10 ⁻⁹ | |
| Virus inactivation | Formaldehyde | 4 % (v/v) | PBS | 30 | CCCCCCCC | 20000001 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | 60 | CCCCCCCC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cytotoxicity | Formaldehyde | 4 % (v/v) | PBS | 60 | CCCCCCCC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cells suceptibility | PBS | n.a | n.a | 60 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 30030303 | 0 | 0 | |
| | DUTRION TABLET | 100 ppm | n.a | 60 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 44444444 | 33000333 | 0 | 0 | |

Explanations:

- 1-4 : degrees of CPE in 8 cell culture unit (microtiter plate)
- 0 : no virus present
- C: cytotoxicity observed on cells
- N.a : not applicable

b. Test

Clean conditions

| TEST | Product | Concentration | Interfering substance | Contact time (minutes) | Dilution factor | | | | | | | | | | |
|------|----------------|---------------|-----------------------|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|---|---|
| | | | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ | 10 ⁻⁷ | 10 ⁻⁸ | 10 ⁻⁹ | | |
| TEST | DUTRION TABLET | 3000 ppm | 0,3 g/L BSA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | DUTRION TABLET | 1000 ppm | | 0 | 44444444 | 33333333 | 33332223 | 22022022 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | | | 5 | 44444444 | 33333333 | 33332223 | 22222200 | 0 | 0 | 0 | 0 | 0 | | |
| | | | | DUTRION TABLET | 100 ppm | 0 | 44444444 | 44444444 | 44444333 | 33333333 | 3333332 | 20220110 | 10110000 | 0 | 0 |
| | | | | | | 5 | 44444444 | 44444444 | 4333434 | 34433333 | 33334333 | 32000002 | 10000000 | 0 | 0 |

Dirty conditions

| TEST | Product | Concentration | Interfering substance | Contact time (minutes) | Dilution factor | | | | | | | | | | | |
|------|----------------|---------------|-----------------------------------|------------------------|------------------|------------------|-----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|----------|----------|---|
| | | | | | 10 ⁻¹ | 10 ⁻² | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻⁶ | 10 ⁻⁷ | 10 ⁻⁸ | 10 ⁻⁹ | | | |
| TEST | DUTRION TABLET | 3000 ppm | 3 g/L de BSA + 3mL/L erythrocytes | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | | | | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| | DUTRION TABLET | 100 ppm | | 0 | 44444444 | 33333333 | 22212222 | 22111111 | 0 | 0 | 0 | 0 | 0 | | | |
| | | | | 5 | 44444444 | 20000002 | 22112222 | 10111111 | 0 | 0 | 0 | 0 | | | | |
| | | | | Viral control | HARD WATER | n.a | 3 g/L de BSA + 3mL/L erythrocytes | 0 | 44444444 | 44444444 | 43334443 | 33333344 | 33333333 | 33220030 | 0 | 0 |
| | | | | | | | | 5 | 44444444 | 44444444 | 44334343 | 33334343 | 33333333 | 20010200 | 10000000 | 0 |

Explanations:

- 1-4 : degrees of CPE in 8 cell culture unit (microtiter plate)
- 0 : no virus present
- C: cytotoxicity observed on cells
- n.a : not applicable