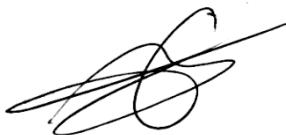
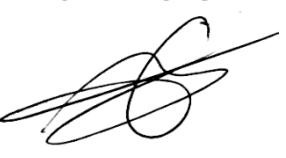


**MCBRIDE SAS**  
**Mme Pauline MIOSSEC**  
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29140 ROSPORDEN

**DETERMINATION OF THE VIRUCIDAL  
ACTIVITY OF THE  
Désinfectant 16052024  
PRODUCT ACCORDING TO THE  
EN 14476+A2:07-2019 STANDARD**

**ANALYSIS REPORT  
R/20/198601 GB**

Version #	Validation - Scientific & Technical Direction / Quality Direction	Verification – Assistant of the Scientific & Technical Direction	Edition date of the version	Date of amendment
1	J.-F. LACROIX 	J.-F. LACROIX 	27/06/2020	/

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F-63 – V2 – 22/04/2009

27/06/2020  
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## APPENDIX

**Appendix : pages 9 to 16**

## SAMPLES IDENTIFICATION

Our references	Information provided by the client		Date of sampling	Date of analysis
	Your references	Description		
E/20/80120	<b>Désinfectant 16052024</b>	Disinfectant	/	From 15/05 to 11/06/2020

**Your order : 2201827728**

## 1. Mission

The firm MCBRIDE SAS asks the firm Analytice to undertake a study to test the virucidal activity of the product **Désinfectant 16052024** according to the NF EN 14476+A2 (July 2019) standard: chemical antiseptics and disinfectants – virucidal quantitative suspension tests for chemical disinfectants and antiseptics used in human medicine.

The tests and validations were carried out on the **Désinfectant 17076801** formula (Report R/20/19860G GB), which is an identical formula to the **Désinfectant 16052024** product.

In order to validate the equivalence between the two products, only the tests at 80% and 97% and the cytotoxicity of the product **Désinfectant 16052024** on the cells were carried out.

## 2. Sample identification

- Manufacturer:	MCBRIDE SAS.
- Manufacturing date:	Non communicated.
- Expiration date:	Non communicated.
- Storage condition:	room temperature.
- Active substances:	ethanol 70% + 1% quaternary ammoniums.
- Appearance of the product:	clear, colorless.
- Product diluent recommended by the manufacturer for use:	none, ready-to-use product.
- Date of delivery of the product:	15/05/2020.
- Date of test:	from 15/05/2020 to 11/06/2020.

## 3. Experimental conditions

- Temperature used during the assays:	20°C ± 1°C.
- Titration units:	log TCID <sub>50</sub> and log UFP/mL for Poliovirus.
- Exposure time:	15 min.
- Final concentrations tested:	97% (modified method) and 80%.
- Diluent used for the product:	distilled water.
- Viral strains:	<b>adenovirus</b> type 5, adenoid strain 75, grown on HEp-2 cells, under 5% CO <sub>2</sub> atmosphere, <b>murine norovirus</b> , strain S99, grown on RAW 264.7 cells, under 5% CO <sub>2</sub> atmosphere and <b>poliovirus</b> type 1, strain LSc-2ab, grown on VERO cells, under 5% CO <sub>2</sub> atmosphere.
- Organic soil load:	0,3 g/L BSA and 3 g/L BSA + 3 mL/L sheep erythrocytes.
- Product stability:	good.
- Stop solution:	cold shock.

### Viral titre:

**Viral titers are expressed in log TCID<sub>50</sub> for adenovirus and norovirus, log UFP/mL for poliovirus:**

- For adenovirus, titer = 6,875 log TCID<sub>50</sub>
- For norovirus, titer = 6,750 log TCID<sub>50</sub>
- For poliovirus, titer = 6,667 log UFP/mL

## **4. Method validation**

### **4.1. Cytotoxicity**

- For adenovirus, the cell toxicity was observed until to the dilution 10<sup>-1</sup>.
- For poliovirus, the cell toxicity was observed until to the dilution 10<sup>-1</sup>.
- For norovirus, the cell toxicity was observed until to the dilution 10<sup>-1</sup>.

### **4.2. Susceptibility to viruses**

See Report R/20/19860G GB concerning Désinfectant 17076801 product.

### **4.3. Validations of the cold shock method**

See Report R/20/19860G GB concerning Désinfectant 17076801 product.

### **4.4. Inactivation assays of the virus with a control solution**

See Report R/20/19860G GB concerning Désinfectant 17076801 product.

## **5. Testing - Calculation of the virucidal activity**

### **5.1. FOR ADENOVIRUS**

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is ≥ 4,0 log.

#### **5.1.1. In clean conditions**

##### **Trial 1**

The viral suspension was titrated at **6, 875 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	1, 875	<b>5,000</b>

*The tested concentrations of product have a virucidal effect if the reduction in the viral titer is greater than or equal to 4.0 log.*

##### **Trial 2**

The viral suspension was titrated at **6, 875 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	1, 875	<b>5,000</b>

*The tested concentrations of product have a virucidal effect if the reduction in the viral titer is greater than or equal to 4.0 log.*

### 5.1.2. In dirty conditions

#### Trial 1

The viral suspension was titrated at **6, 875 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	2,500	4,375

*The tested concentrations of product have a virucidal effect if the reduction in the viral titer is greater than or equal to 4.0 log.*

#### Trial 2

The viral suspension was titrated at **6, 875 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	2,625	4,250

*The tested concentrations of product have a virucidal effect if the reduction in the viral titer is greater than or equal to 4.0 log.*

## 5.2. FOR POLIOVIRUS

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is  $\geq 4,0$  log.

### 5.2.1. In clean conditions

#### Trial 1

The viral suspension was titrated at **6,667 log UFP/mL**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log log UFP/mL)	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	2,395	4,272

#### Trial 2

The viral suspension was titrated at **6,667 log UFP/mL**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log log UFP/mL)	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	2,206	4,461

### 5.2.2. In dirty conditions

#### Trial 1

The viral suspension was titrated at **6,667 log UFP/mL**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log log UFP/mL)	Viral titer reduction
DÉSINFECTANT 16052024	97%	15 min	20°C	2,949	<b>4,173</b>

#### Trial 2

The viral suspension was titrated at **6,667 log UFP/mL**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log log UFP/mL)	Viral titer reduction
DÉSINFECTANT 16052024	97%	15 min	20°C	2,515	<b>4,152</b>

### 5.3. FOR NOROVIRUS

The concentrations of the product demonstrated a virucidal activity on the virus tested if the viral titer reduction is  $\geq 4,0$  log.

#### 5.3.1. In clean conditions

#### Trial 1

The viral suspension was titrated at **6,750 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	1,875	<b>4,875</b>

#### Trial 2

The viral suspension was titrated at **6,750 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	1,875	<b>4,875</b>

### 5.3.2. In dirty conditions

#### Trial 1

The viral suspension was titrated at **6,750 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	2,375	<b>4,375</b>

#### Trial 2

The viral suspension was titrated at **6,750 log TCID<sub>50</sub>**.

PRODUCT	Concentration	Time of exposure	Temperature	Viral titer (log TCID <sub>50</sub> )	Viral titer reduction
DÉSINFECTANT 16052024	80%	15 min	20°C	2,500	<b>4,250</b>

## 6. Methodology validation

The assays were validated as required by the European standard EN 14476+A2:07-2019:

- The viral titers of the suspension tests were sufficient in order to observe a reduction of 4 log after time exposure with the product:
  - 6,875 log TCID<sub>50</sub> for adenovirus.
  - 6,667 log UFP/mL for poliovirus
  - 6,750 log TCID<sub>50</sub> for norovirus.
- The DÉSINFECTANT 16052024 product does not have a cytotoxic effect on the HEp-2 cells, the VERO cells and the RAW cells.

## **7. Conclusion**

The assays performed with the **DÉSINFECTANT 16052024** product:

### **In clean conditions:**

- **Demonstrated a virucidal activity on the adenovirus, poliovirus and norovirus from the concentration 80%,** as required by the European standard EN 14476+A2:07/2019, following a **15 min** exposure period, at **20°C, in clean conditions.**
- That in accordance with the standard EN 14476+A2:07/2019, the product DÉSINFECTANT 16052024, is therefore **effective on all viruses (including H1N1 and coronavirus)**, from **80%** following a **15 min** exposure period, at **20°C, in clean conditions** (*point 4, table 1 page 9*)

### **In dirty conditions:**

- **Demonstrated a virucidal activity on the adenovirus, poliovirus and norovirus from the concentration 97%,** as required by the European standard EN 14476+A2:07/2019, following a **15 min** exposure period, at **20°C, in dirty conditions.**
- That in accordance with the standard EN 14476+A2:07/2019, the product DÉSINFECTANT 16052024, is therefore **effective on all viruses (including H1N1 and coronavirus)**, from **97%** following a **15 min** exposure period, at **20°C, in dirty conditions** (*point 4, table 1 page 9*)

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## **APPENDIX**

## APPENDIX 1

### **Adenovirus:**

- Cell line: HEp- 2 cells (RD-Biotech ref. 84011, batch n°110315-118)
- Viral strain: adenovirus type 5, adenoïd strain 75 (ATCC ref. VR-5, batch n°3679877

### **Norovirus:**

- Cell line: RAW 264.7 cells (ATCC TIB-71)
- Viral strain: Murine norovirus, STRAIN S99 (batch n° 4/200409/220409- Friedrich Loeffler Institut)

### **Poliovirus:**

- Cell line: VERO cells (RD-Biotech ref. 84009, batch n°110118-110V)
- Viral strain: poliovirus type 1, strain LSc-2ab (ref RVB-1260 - batch n° 2/10121998- Friedrich Loeffler Institut)

### **Buffers and media:**

- PBS buffer: sodium chloride, Panreac, ref. 141659.1211, batch n° 0000204679; sodium phosphate dibasic, Sigma Aldrich, ref. S5136, batch n° BCBC7067V; sodium phosphate monobasic, Sigma Aldrich, ref. S5011, batch n° 1019K01021V
- MEM media, Sigma Aldrich, ref. 0268, batch n° 040M8301
- DMEM media, Sigma Aldrich, ref. D5796, batch n° RNBB9336
- Fetal calf sera, Sigma Aldrich, F7524, batch n° 098K3397

### **Reagents:**

- Albumine bovine sera, Sigma Aldrich, ref. 05479, batch n° STBB7838V
- Sheep erythrocytes, Analytic Lab, ref. 08449, batch n°bcbj3984V

### **Inactivation solution:**

- Formaldehyde, Sigma Aldrich, ref. F-1635, batch n° BCBB3510

## APPENDIX 2

**Table A1 - Adenovirus titer, by Spaerman-Kärber method:**

Log DICT<sub>50</sub> = 6,875

Dilution (- log)	Result	% positive results
-3	44444444	100
-4	44444444	100
-5	44444444	100
-6	44444444	100
-7	44400000	37,5
-8	00000000	0
-9	00000000	0
-10	00000000	0
Sum of the % of positives cultures		437,5

**Table A2 - Results on Adenovirus in clean and dirty conditions**

Product	Concentration	Organic soil load	Cytotoxicity level	Lg TCID <sub>50</sub>					Reduction
				0	5 min	15 min	30 min	60 min	
DÉSINFECTANT 16052024 TRIAL 1 CLEAN	80,00%	0,3 g/l BSA	1,500	6,875	N.T.	1,875	N.T.	N.T.	15 min R = 5,000
DÉSINFECTANT 16052024 TRIAL 2 CLEAN	80,00%	0,3 g/l BSA	1,250	6,875	N.T.	1,875	N.T.	N.T.	15 min R = 5,000
DÉSINFECTANT 16052024 TRIAL 1 DIRTY	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	1,250	6,875	N.T.	2,500	N.T.	N.T.	15 min R = 4,375
DÉSINFECTANT 16052024 TRIAL 2 DIRTY	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	1,125	6,875	N.T.	2,625	N.T.	N.T.	15 min R = 4,250

**Table A3 - Raw results for trials 1 and 2****In clean conditions****TRIAL 1**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 1	80,00%	0,3 g/l BSA	15 min	4444	4440	0000	4000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	0,3 g/l BSA	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000

**TRIAL 2**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 2	80,00%	0,3 g/l BSA	15 min	4444	4440	0000	0000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	0,3 g/l BSA	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4400	0000	0000	0000	0000	0000	0000	0000	0000

**In dirty conditions****TRIAL 1**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 1	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	15 min	4444	4440	0000	4000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4400	0000	0000	0000	0000	0000	0000	0000	0000

**TRIAL 2**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 2	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	15 min	4444	4444	4000	0000	0000	0000	0000	0000	0000
				4444	4444	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4000	0000	0000	0000	0000	0000	0000	0000	0000

**Table A4 - Norovirus titer, by Spaerman-Kärber method:**

Log DICT50 = 6,750

Dilution (- log)	Result	% positive results
-3	44444444	100,0
-4	44444444	100,0
-5	44444444	100,0
-6	44444444	100,0
-7	44000000	25,0
-8	00000000	0,0
-9	00000000	0,0
-10	00000000	0,0
Sum of the % of positives cultures		425,0

**Table A5 - Results on *Norovirus* in clean and dirty conditions**

Product	Concentration	Organic soil load	Cytotoxicity level	Lg TCID <sub>50</sub>					Reduction
				0	5 min	15 min	30 min	60 min	
DÉSINFECTANT 16052024 TRIAL 1 CLEAN	80,00%	0,3 g/l BSA	1,375	6,750	N.T.	1,875	N.T.	N.T.	15 min R = 4,875
DÉSINFECTANT 16052024 TRIAL 2 CLEAN	80,00%	0,3 g/l BSA	1,375	6,750	N.T.	1,875	N.T.	N.T.	15 min R = 4,875
DÉSINFECTANT 16052024 TRIAL 1 DIRTY	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	1,000	6,750	N.T.	2,375	N.T.	N.T.	15 min R = 4,375
DÉSINFECTANT 16052024 TRIAL 2 DIRTY	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	1,000	6,750	N.T.	2,500	N.T.	N.T.	15 min R = 4,250

**Table A6** - Raw results for trials 1 and 2**In clean conditions****TRIAL 1**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 1	80,00%	0,3 g/l BSA	15 min	4444	4440	0000	0000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	0,3 g/l BSA	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4440	0000	0000	0000	0000	0000	0000	0000	0000

**TRIAL 2**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 2	80,00%	0,3 g/l BSA	15 min	4444	4440	0000	0000	0000	0000	0000	0000	0000
				4444	0000	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	0,3 g/l BSA	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				4440	0000	0000	0000	0000	0000	0000	0000	0000

**In dirty conditions****TRIAL 1**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 1	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	15 min	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4440	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				0000	0000	0000	0000	0000	0000	0000	0000	0000

**TRIAL 2**

	Concentration	Organic soil load	Exposure time	Dilutions								
				-1	-2	-3	-4	-5	-6	-7	-8	-9
DÉSINFECTANT 16052024 TRIAL 2	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	15 min	4444	4444	0000	0000	0000	0000	0000	0000	0000
				4444	4440	0000	0000	0000	0000	0000	0000	0000
DÉSINFECTANT 16052024 cytotoxicity	80,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	N.A.	4444	0000	0000	0000	0000	0000	0000	0000	0000
				0000	0000	0000	0000	0000	0000	0000	0000	0000

**Table A7 - Poliovirus titer**

Dilution (- log)	PUITS 1	PUITS 2	PUITS 3	TOTAL PAR DILUTION
-4	448,00	437,00	489,00	1374,00
-5	52,00	49,00	57,00	158,00
-6	5,00	5,00	7,00	17,00
TOTAL				1549,00

**Table A10 - Results on *Poliovirus* in clean and dirty conditions**

Product	Concentration	Organic soil load	Cytotoxicity level	Lg UFP/mL				Reduction
				0	5 min	15 min	60 min	
DÉSINFECTANT 16052024 TRIAL 1 CLEAN	80,00%	0,3 g/l BSA	1,457	6,667	N.T.	2,395	N.T.	15 min R = 4,272
DÉSINFECTANT 16052024 TRIAL 2 CLEAN	80,00%	0,3 g/l BSA	1,230	6,667	N.T.	2,206	N.T.	15 min R = 4,461
DÉSINFECTANT 16052024 TRIAL 1 DIRTY	97,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	1,342	6,667	N.T.	2,494	N.T.	15 min R = 4,173
DÉSINFECTANT 16052024 TRIAL 2 DIRTY	97,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	1,114	6,667	N.T.	2,515	N.T.	15 min R = 4,152

**Table A11 - Raw results for trials 1 and 2****In clean conditions****TRIAL 1**

	Concentration	Organic soil load	Exposure time	Dilutions					
				-1	-2	-3	-4	-5	-6
DÉSINFECTANT 16052024 TRIAL 1	80,00%	0,3 g/l BSA	15 min	21	3	0			
				24	3	0			
		VIRAL CONTROL	6,667	28	3				
DÉSINFECTANT 16052024 TRIAL 1 Cytotoxicity	80,00%	0,3 g/l BSA	N.A.	28	3				
				24	4				
				24	3				

**TRIAL 2**

	Concentration	Organic soil load	Exposure time	Dilutions					
				-1	-2	-3	-4	-5	-6
DÉSINFECTANT 16052024 TRIAL 2	80,00%	0,3 g/l BSA	15 min	15	2	0			
				14	2	0			
		VIRAL CONTROL	6,667	18	2	0			
DÉSINFECTANT 16052024 TRIAL 2 Cytotoxicity	80,00%	0,3 g/l BSA	N.A.	14	2				
				12	2				
				19	2				

**In dirty conditions****TRIAL 1**

	Concentration	Organic soil load	Exposure time	Dilutions					
				-1	-2	-3	-4	-5	-6
DÉSINFECTANT 16052024 TRIAL 1	97,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	15 min	28	3	0			
				32	4	0			
		VIRAL CONTROL	6,667	33	3	0			
DÉSINFECTANT 16052024 TRIAL 1 Cytotoxicity	97,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	N.A.	21	3				
				19	2				
				19	2				

**TRIAL 2**

	Concentration	Organic soil load	Exposure time	Dilutions					
				-1	-2	-3	-4	-5	-6
DÉSINFECTANT 16052024 TRIAL 2	97,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	15 min	30	3	0			
				34	4	0			
			VIRAL CONTROL	33	4	0			
DÉSINFECTANT 16052024 TRIAL 2 Cytotoxicity	97,00%	3 g/l BSA + 3 mL/L sheep erythrocytes	N.A.	6,667					
				11	2				
				12	1				
				12	1				