



## Test report

### Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C According to ASTM E 1259-10

**Customer:** LIQUI MOLY GmbH  
 Jerg-Wieland-Straße 4  
 89081 Ulm  
 Germany

**Sample:** Marine Diesel Protect (Batch no.: LA 298-18-7)

<b>Project No. (cust.):</b>		<b>Method:</b>	ASTM E 1259-10
<b>No. of order (TM):</b>	A248/19 (A764/18)	<b>Start of the test:</b>	2018-11-09
<b>Date of order:</b>	2019-04-04 (2018-10-29)	<b>End of the test:</b>	2018-12-12
<b>Lab No.:</b>	11.10.18-4378	<b>Respons. person:</b>	S. Horn
<b>Date of delivery:</b>	2018-10-11	<b>Evaluation:</b>	S. Horn

### Results and conclusion

This report represents a transcription of the original results and conclusion of report A764/18 (Technische Mikrobiologie Dr. Jutta Höffler GmbH, Ahrensburger Straße 162, D-22045 Hamburg, dated 2018-12-12). All tests mentioned below were conducted using a product sample with the batch number "LA 298-18-7" (laboratory number: 11.10.18-4378). Due to the confirmation of the manufacturer, that "LA 298-18-7" is identical to "Marine Diesel Protect" all results and conclusions of the original report A764/18 can be transcribed to "Marine Diesel Protect". Consequently, this product name is now used.

The test sample "Marine Diesel Protect" (Batch no.: LA 298-18-7; Lab. No.: 11.10.18-4378) was provided by the customer and tested 1:200 in Diesel fuel (Lab.No.: 17.09.18-4025) according to ASTM 1259-10. Germs were determined in the water and the fuel phases.

After a contact time of 4 weeks the water phases of the samples showed the following biocidal activities:

Against *Pseudomonas aeruginosa* "Marine Diesel Protect" (Batch no.: LA 298-18-7) was effective up to the detection limit, showing the required log reduction according to ASTM E 1259 (log reduction >50% of maximum difference compared with the control).

Against *Hormoconis resinae* "Marine Diesel Protect" (Batch no.: LA 298-18-7) was effective up to the detection limit, showing the required log reduction according to ASTM E 1259 (log reduction >50% of maximum difference compared with the control).

Against *Yarrowia tropicalis* "Marine Diesel Protect" (Batch no.: LA 298-18-7) was effective up to the detection limit, showing the required log reduction according to ASTM E 1259 (log reduction >50% of maximum difference compared with the control).

After a contact time of 4 weeks none of the samples showed growth in their fuel phases.

**Technische Mikrobiologie  
 Dr. Jutta Höffler GmbH**

**Hamburg, 2019-04-08**

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 S. Horn  
 Certified Biologist

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 C. Ludwig  
 Quality management

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 IBAN DA 12 2005 0550 1208 1153 · BIC: HASPDEHH XXX · Geschäftsführerin: Dr. rer. nat. Jutta Höffler  
 Amtsgericht Hamburg Handelsregister B Nr. 58 682 · St.Nr.: 51/762/00075 · UST.IdNr. DE 171622643

## 1.1 Growth of *Pseudomonas aeruginosa* in DWEF (Diesel Water Emulsion Fuel) samples

### Study of *Pseudomonas aeruginosa* in the water phase

Drawn from:  
ASTM E 1259

Name of the products: LA 298-18-7

Laboratory numbers: 11.10.18-4378

Diesel Fuel: Sulfur-free, DIN EN 590, up to 7% Bio-Diesel  
Lab.No.: 17.09.18-4025

Remarks: none

Diluent for product test solutions: Diesel fuel, water phase 0.5 % hard water (300 ppm CaCO<sub>3</sub>)

Appearance of the test setups: Yellow, clear (fuel phase)  
Colourless, clear solution (aqueous phase)

Test temperature: 18-25°C Incubation temperature: 18-25°C Incubation period: 4 weeks

Test organism: *Pseudomonas aeruginosa*, DSM 15980 cfu /ml of initial bacterial inoculum: 3.1 x10<sup>5</sup>

Start of test:	2018-11-09
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Responsible person: S. Horn sign:

**Results are shown in the following tables: 1.1a and 1.1b**

**Table 1.1a: Efficacy of preserved Diesel Fuels against *Pseudomonas aeruginosa* in a repetitive challenge test (challenges at the beginning and every 7 days after start of the test). Total viable count (TVC) of *Pseudomonas aeruginosa* in water phase after different incubation times. Numeric evaluation**

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
30 min. (TVC)	$3.1 \times 10^5$	$2.4 \times 10^4$
1 week (TVC)	$2.2 \times 10^7$	<10
2 <sup>nd</sup> Challenge after 7 days		
2 weeks (TVC)	$1.1 \times 10^8$	<10
3 <sup>rd</sup> Challenge after 14 days		
3 weeks (TVC)	$2.0 \times 10^7$	<10
4 <sup>th</sup> Challenge after 21 days		
4 weeks (TVC)	$1.2 \times 10^7$	<10

n.d. = none detected (<10 colony forming units /ml)

**Table 1.1b: Efficacy of preserved Diesel Fuels against *Pseudomonas aeruginosa***

Logarithmic evaluation / lg- Reduction

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 30 min.	no reduction	1.11
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 1 week	no reduction	>6.34
2 <sup>nd</sup> Challenge after 7 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 2 weeks	no reduction	>7.04
3 <sup>rd</sup> Challenge after 14 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 3 weeks	no reduction	>6.30
4 <sup>th</sup> Challenge after 21 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 4 weeks	no reduction	>6.08

TVC<sub>c</sub> = TVC Diesel fuel control

TVC<sub>TS</sub> = TVC Test Sample

## 1.2 Growth of *Pseudomonas aeruginosa* in DWEF (Diesel Water Emulsion Fuel) samples

### Study of *Pseudomonas aeruginosa* in the fuel phase

Drawn from: Name of the products: LA 298-18-7  
 ASTM E 1259  
 Laboratory numbers: 11.10.18-4378  
 Diesel Fuel: Sulfur-free, DIN EN 590, up to 7% Bio-Diesel  
 Lab.No.: 17.09.18-4025

Remarks: none

Diluent for product test solutions: Diesel fuel, water phase 0.5 % hard water (300 ppm CaCO<sub>3</sub>)

Appearance of the test setups: Yellow, clear (fuel phase)  
 Colourless, clear solution (aqueous phase)

Test temperature: 18-25°C Incubation temperature: 18-25°C Test temperature: 18-25°C

Test organism: *Pseudomonas aeruginosa*, DSM 15980 cfu /ml of initial bacterial inoculum: 3.1 x10<sup>5</sup>

Start of test:	2018-11-09	Responsible person:	S. Horn	sign:
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Results are shown in the following table 1.2

**Table 1.2: Efficacy of preserved Diesel Fuels against *Pseudomonas aeruginosa***

Total viable count (TVC) of *Pseudomonas aeruginosa* in fuel phase after different incubation times.

TVC was determined with membrane filtration according to IP 385/99

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
30 min. (TVC)	n.d.	n.d.
1 week (TVC)	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days		
2 weeks (TVC)	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days		
3 weeks (TVC)	n.d.	n.d.
4 <sup>th</sup> Challenge after 21 days		
4 weeks (TVC)	n.d.	n.d.

n.d. = none detected (total viable count is 0)

## 2.1 Growth of *Hormoconis resiniae* in DWEF (Diesel Water Emulsion Fuel) samples

### Study of *Hormoconis resiniae* in the water phase

Drawn from: Name of the products: LA 298-18-7  
 ASTM E 1259  
 Laboratory numbers: 11.10.18-4378  
 Diesel Fuel: Sulfur-free, DIN EN 590, up to 7% Bio-Diesel  
 Lab.No.: 17.09.18-4025

Remarks: none

Diluent for product test solutions: Diesel fuel, water phase 0.5 % hard water (300 ppm CaCO<sub>3</sub>)  
 Appearance of the test setups: Yellow, clear (fuel phase)  
 Colourless, clear solution (aqueous phase)

Test temperature: 18-25°C Incubation temperature: 18-25°C Test temperature: 18-25°C  
 Test organism: *Hormoconis resiniae*, DSM 1203 cfu /ml of initial bacterial inoculum: 1.0 x10<sup>5</sup>

Start of test:	2018-11-09	Responsible person:	S. Horn	sign:
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**Results are shown in the following tables: 2.1a and 2.1b**

**Table 2.1a: Efficacy of preserved Diesel Fuels against *Hormoconis resiniae*** in a repetitive challenge test (challenges at the beginning and every 7 days after start of the test).

Total viable count (TVC) of *Hormoconis resiniae* in water phase after different incubation times.

Numeric evaluation

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
30 min. (TVC)	$1.0 \times 10^5$	$1.0 \times 10^5$
1 week (TVC)	$2.4 \times 10^3$	<10
2 <sup>nd</sup> Challenge after 7 days		
2 weeks (TVC)	$1.0 \times 10^3$	<10
3 <sup>rd</sup> Challenge after 14 days		
3 weeks (TVC)	$2.1 \times 10^3$	<10
4 <sup>th</sup> Challenge after 21 days		
4 weeks (TVC)	$1.9 \times 10^4$	<10

n.d. = none detected (<10 colony forming units /ml)

**Table 2.1b: Efficacy of preserved Diesel Fuels against *Hormoconis resiniae***

Logarithmic evaluation / lg- Reduction

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 30 min.	no reduction	no reduction
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 1 week	no reduction	>2.36
2 <sup>nd</sup> Challenge after 7 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 2 weeks	no reduction	>2.00
3 <sup>rd</sup> Challenge after 14 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 3 weeks	no reduction	>2.32
4 <sup>th</sup> Challenge after 21 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 4 weeks	no reduction	>3.28

TVC<sub>c</sub> = TVC Diesel fuel control

TVC<sub>TS</sub> = TVC Test Sample

## 2.2 Growth of *Hormoconis resiniae* in DWEF (Diesel Water Emulsion Fuel) samples

### Study of *Hormoconis resiniae* in the fuel phase

Drawn from: Name of the products: LA 298-18-7  
 ASTM E 1259

Laboratory numbers: 11.10.18-4378

Diesel Fuel: Sulfur-free, DIN EN 590, up to 7% Bio-Diesel  
 Lab.No.: 17.09.18-4025

Remarks: none

Diluent for product test solutions: Diesel fuel, water phase 0.5 % hard water (300 ppm CaCO<sub>3</sub>)

Appearance of the test setups: Yellow, clear (fuel phase)  
 Colourless, clear solution (aqueous phase)

Test temperature: 18-25°C Incubation temperature: 18-25°C Test temperature: 18-25°C

Test organism: *Hormoconis resiniae*, DSM 1203 cfu /ml of initial bacterial inoculum: 1.0 x10<sup>5</sup>

Start of test:	2018-11-09	Responsible person:	S. Horn	Start of test:
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**Results are shown in the following table: 2.2**



**Table 2.2: Efficacy of preserved Diesel Fuels against *Hormoconis resiniae***

Total viable count (TVC) of *Hormoconis resiniae* in fuel phase after different incubation times.

TVC was determined with membrane filtration according to IP 385/99

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
<b>30 min.</b> (TVC)	n.d.	n.d.
<b>1 week</b> (TVC)	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days		
<b>2 weeks</b> (TVC)	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days		
<b>3 weeks</b> (TVC)	n.d.	n.d.
4 <sup>th</sup> Challenge after 21 days		
<b>4 weeks</b> (TVC)	n.d.	n.d.

n.d. = none detected (total viable count is 0)

### 3.1 Growth of *Yarrowia tropicalis* in DWEF (Diesel Water Emulsion Fuel) samples

#### Study of *Yarrowia tropicalis* in the water phase

Drawn from: Name of the products: LA 298-18-7  
 ASTM E 1259

Laboratory numbers: 11.10.18-4378

Diesel Fuel: Sulfur-free, DIN EN 590, up to 7% Bio-Diesel  
 Lab.No.: 17.09.18-4025

Remarks: none

Diluent for product test solutions: Diesel fuel, water phase 0.5 % hard water (300 ppm CaCO<sub>3</sub>)

Appearance of the test setups: Yellow, clear (fuel phase)  
 Colourless, clear solution (aqueous phase)

Test temperature: 18-25°C Incubation temperature: 18-25°C Test temperature: 18-25°C

Test organism: *Yarrowia tropicalis*, DSM 11953 cfu /ml of initial bacterial inoculum: 9.0 x10<sup>5</sup>

Start of test:	2018-11-09	Responsible person:	S. Horn	sign:
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**Results are shown in the following tables: 3.1a and 3.1b**

**Table 3.1a: Efficacy of preserved Diesel Fuels against *Yarrowia tropicalis*** in a repetitive challenge test (challenges at the beginning and every 7 days after start of the test).

Total viable count (TVC) of *Yarrowia tropicalis* in water phase after different incubation times.

Numeric evaluation

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
30 min. (TVC)	9.0 x 10 <sup>5</sup>	7.0 x 10 <sup>5</sup>
1 week (TVC)	5.6 x 10 <sup>4</sup>	<10
2 <sup>nd</sup> Challenge after 7 days		
2 weeks (TVC)	4.3 x 10 <sup>4</sup>	<10
3 <sup>rd</sup> Challenge after 14 days		
3 weeks (TVC)	3.2 x 10 <sup>4</sup>	<10
4 <sup>th</sup> Challenge after 21 days		
4 weeks (TVC)	8.0 x 10 <sup>5</sup>	<10

n.d. = none detected (<10 colony forming units /ml)

**Table 3.1b: Efficacy of preserved Diesel Fuels against *Yarrowia tropicalis***

Logarithmic evaluation / lg- Reduction

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 30 min.	no reduction	0.11
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 1 week	no reduction	>3.75
2 <sup>nd</sup> Challenge after 7 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 2 weeks	no reduction	>3.63
3 <sup>rd</sup> Challenge after 14 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 3 weeks	no reduction	>3.51
4 <sup>th</sup> Challenge after 21 days		
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 4 weeks	no reduction	>4.90

TVC<sub>c</sub> = TVC Diesel fuel control

TVC<sub>TS</sub> = TVC Test Sample

### 3.2 Growth of *Yarrowia tropicalis* in DWEF (Diesel Water Emulsion Fuel) samples

#### Study of *Yarrowia tropicalis* in the fuel phase

Drawn from: ASTM E 1259      Name of the products: LA 298-18-7

Laboratory numbers: 11.10.18-4378

Diesel Fuel: Sulfur-free, DIN EN 590, up to 7% Bio-Diesel  
Lab.No.: 17.09.18-4025

Remarks: none

Diluent for product test solutions: Diesel fuel, water phase 0.5 % hard water (300 ppm CaCO<sub>3</sub>)

Appearance of the test setups: Yellow, clear (fuel phase)  
Colourless, clear solution (aqueous phase)

Test temperature: 18-25°C      Incubation temperature: 18-25°C      Test temperature: 18-25°C

Test organism: *Yarrowia tropicalis*, DSM 11953      cfu /ml of initial bacterial inoculum: 9.0 x10<sup>5</sup>

Start of test:	2018-11-09	Responsible person:	S. Horn	Start of test:
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**Results are shown in the following table: 3.2**

**Table 3.2: Efficacy of preserved Diesel Fuels against *Yarrowia tropicalis***

Total viable count (TVC) of *Fusarium solani* in fuel phase  
after different incubation times.

TVC was determined with membrane filtration according to IP 385/99

Sample	Diesel fuel Control	LA 298-18-7 1:200
1 <sup>st</sup> Challenge at test start		
<b>30 min.</b> (TVC)	n.d.	n.d.
<b>1 week</b> (TVC)	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days		
<b>2 weeks</b> (TVC)	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days		
<b>3 weeks</b> (TVC)	n.d.	n.d.
4 <sup>th</sup> Challenge after 21 days		
<b>4 weeks</b> (TVC)	n.d.	n.d.

n.d. = none detected (total viable count is 0)