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## Test report

Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C  
 According to ASTM E 1259-05

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

**Sample:** **Sample 1: Marine Diesel Schutz/Marine Diesel Protect (1:1000 and 1:200),  
 Lab.No.: 29.03.16-1419;**  
**Sample 2: Marine Diesel Bakterien Stop (1.1000 and 1:4000),  
 Lab.No.: 29.03.16-1420;**  
**Diesel Fuel: Lab.No.: 29.03.16-1418**

<b>Project No. (cust.):</b>		<b>Method:</b>	ASTM E 1259-05
<b>No. of order (TM):</b>	A218/16	<b>Start of the test:</b>	2016-04-15
<b>Date of order:</b>	2016-03-24	<b>End of the test:</b>	2016-06-09
<b>Lab No.:</b>	29.03-16-1419 29.03.16-1420 29.03.16-1418	<b>Respons. person:</b>	S. Horn
<b>Date of delivery:</b>	2016-03-29 / 2016-04.04	<b>Evaluation:</b>	S. Horn

### Results and conclusion

The Diesel fuel samples (Lab.No.: 29.03.16-1418) with the above mentioned different biocide concentrations were tested according to ASTM 1259-05. Germs were determined in the water and the fuel phases.

After a contact time of 21 days the water phases of the samples showed the following biocidal activities:  
 Against *Pseudomonas aeruginosa* all the tested samples were effective up to the detection limit.  
 Against *Hormoconis resiniae* all the tested samples were effective up to the detection limit.  
 Against *Yarrowia tropicalis* all the tested samples were effective up to the detection limit.

After a contact time of 21 days, none of the samples showed growth in their fuel phases.

In summary all tested samples showed a complete bactericidal and fungicidal efficacy according to ASTM 1259-05 during the test period.

Technische Mikrobiologie  
 Dr. Jutta Höffler GmbH

Hamburg, 2016-06-09

S. Horn  
 Certified Biologist

C. Ludwig  
 Quality management

## 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 product: **Marine Diesel  
Schutz/Marine Diesel  
Protect**

Laboratory number: 29.03.16-1419

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Pseudomonas aeruginosa*, DSM 15980 cfu /ml of initial bacterial inoculum:  $7.2 \times 10^7$

Start of test: 2016-04-15

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 7 and 14 days after start of the test). Total viable count (TVC) of *Pseudomonas aeruginosa* in water phase after different incubation times. Numeric evaluation**

Sample	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	$6.0 \times 10^7$	$7.1 \times 10^7$	$8.8 \times 10^7$
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	$5.3 \times 10^8$
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	$7.8 \times 10^8$

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	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
$TVC_c/TVC_{TS}$ (lg) 30 min.	0.166	0.093	--
2 <sup>nd</sup> Challenge after 7 days			
$TVC_c/TVC_{TS}$ (lg) 7 days	>7.724	>7.724	--
3 <sup>rd</sup> Challenge after 14 days			
$TVC_c/TVC_{TS}$ (lg) 21 days	>7.892	>7.892	--

$TVC_c$  = TVC Control

$TVC_{TS}$  = 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 product: **Marine Diesel  
ASTM E 1259 Schutz/Marine Diesel  
Protect**

Laboratory number: 29.03.16-1419

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Pseudomonas aeruginosa*, DSM 15980 cfu /ml of initial bacterial inoculum: 7.2 x10<sup>7</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign: 

#### Results:

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

Total viable count (TVC) of *Pseudomonas aeruginosa* in fuel phase after incubation time of 30 minutes, 7 days and 21 days.

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

Sample	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	n.d.	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	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: ASTM E 1259  
Name of the product: **Marine Diesel  
Schutz/Marine Diesel  
Protect**

Laboratory number: 29.03.16-1419

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Hormoconis resiniae*, DSM 1203 cfu /ml of initial bacterial inoculum: 1.4 x 10<sup>6</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign:



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 7 and 14 days after start of the test). Total viable count (TVC) of *Hormoconis resiniae* in water phase after different incubation times. Numeric evaluation**

Sample	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	1.6 x 10 <sup>6</sup>	1.2 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	4.2 x 10 <sup>7</sup>
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	5.0 x 10 <sup>7</sup>

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	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 30 min.	no reduction	no reduction	--
2 <sup>nd</sup> Challenge after 7 days			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 7 days	>6.623	>6.623	--
3 <sup>rd</sup> Challenge after 14 days			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 21 days	>6.699	>6.699	--

TVC<sub>c</sub> = TVC 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 product: **Marine Diesel**  
 ASTM E 1259 **Schutz/Marine Diesel**  
**Protect**

Laboratory number: 29.03.16-1419

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Hormoconis resiniae*, DSM 1203 cfu /ml of initial bacterial inoculum: 1.4 x 10<sup>6</sup>

Start of test:	2016-04-15
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Responsible person: S. Horn

sign:



**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 incubation time of 30 minutes, 7 days and 21 days.

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

Sample	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	n.d.	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	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:  
ASTM E 1259

Name of the product: **Marine Diesel  
Schutz/Marine Diesel  
Protect**

Laboratory number: 29.03.16-1419

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Yarrowia tropicalis*, DSM 11953 cfu /ml of initial bacterial inoculum: 1.3 x 10<sup>6</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign:



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 7 and 14 days after start of the test). Total viable count (TVC) of *Yarrowia tropicalis* in water phase after different incubation times. Numeric evaluation**

Sample	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	1.0 x 10 <sup>6</sup>	1.0 x 10 <sup>6</sup>	1.6 x 10 <sup>6</sup>
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	6.0 x 10 <sup>7</sup>
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	2.4 x 10 <sup>8</sup>

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	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
TVC <sub>c</sub> /TVC <sub>ts</sub> (lg) 30 min.	0.204	0.204	--
2 <sup>nd</sup> Challenge after 7 days			
TVC <sub>c</sub> /TVC <sub>ts</sub> (lg) 7 days	>6.778	>6.778	--
3 <sup>rd</sup> Challenge after 14 days			
TVC <sub>c</sub> /TVC <sub>ts</sub> (lg) 21 days	>7.380	>7.380	--

TVC<sub>c</sub> = TVC 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 product: **Marine Diesel  
Schutz/Marine Diesel  
Protect**

Laboratory number: 29.03.16-1419

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Yarrowia tropicalis*, DSM 11953 cfu /ml of initial bacterial inoculum: 1.3 x 10<sup>6</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign: 

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 *Yarrowia tropicalis* in fuel phase  
after incubation time of 30 minutes, 7 days and 21 days.

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

Sample	Marine Diesel Schutz/Marine Diesel Protect 1:200	Marine Diesel Schutz/Marine Diesel Protect 1:1000	Control Diesel fuel
Laboratory number (29.03.16)	1419	1419	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	n.d.	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	n.d.

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

#### 4.1 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 product: **Marine Diesel  
Bakterien Stop**

Laboratory number: 29.03.16-1420

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT      Incubation temperature: RT      Incubation period: 21 days

Test organism: *Pseudomonas aeruginosa*, DSM 15980      cfu /ml of initial bacterial inoculum: 7.2 x10<sup>7</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign:



Results are shown in the following tables: 4.1a and 4.1b

**Table 4.1a: Efficacy of preserved Diesel Fuels against *Pseudomonas aeruginosa* in a repetitive challenge test (challenges at the beginning and 7 and 14 days after start of the test). Total viable count (TVC) of *Pseudomonas aeruginosa* in water phase after different incubation times. Numeric evaluation**

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	$6.8 \times 10^7$	$7.2 \times 10^7$	$6.5 \times 10^7$
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	$4.2 \times 10^8$
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	$6.9 \times 10^8$

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

**Table 4.1b: Efficacy of preserved Diesel Fuels against *Pseudomonas aeruginosa* Logarithmic evaluation / lg- Reduction**

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
$TVC_c/TVC_{TS}$ (lg) 30 min.	no reduction	no reduction	--
2 <sup>nd</sup> Challenge after 7 days			
$TVC_c/TVC_{TS}$ (lg) 7 days	>7.623	>7.623	--
3 <sup>rd</sup> Challenge after 14 days			
$TVC_c/TVC_{TS}$ (lg) 21 days	>7.839	>7.839	--

$TVC_c$  = TVC Control  
 $TVC_{TS}$  = TVC Test Sample

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

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

Drawn from: Name of the product: **Marine Diesel  
ASTM E 1259** **Bakterien Stop**

Laboratory number: 29.03.16-1420

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Pseudomonas aeruginosa*, DSM 15980 cfu /ml of initial bacterial inoculum:  $7.2 \times 10^7$

Start of test: 2016-04-15

Responsible person: S. Horn

sign: 

#### Results:

#### Table 4.2: Efficacy of preserved Diesel Fuels against *Pseudomonas aeruginosa*

Total viable count (TVC) of *Pseudomonas aeruginosa* in fuel phase after incubation time of 30 minutes, 7 days and 21 days.

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

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	n.d.	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	n.d.

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

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

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

Drawn from: Name of the product: **Marine Diesel  
ASTM E 1259 Bakterien Stop**

Laboratory number: 29.03.16-1420

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Hormoconis resiniae*, DSM 1203 cfu /ml of initial bacterial inoculum:  $1.4 \times 10^6$

Start of test: 2016-04-15

Responsible person: S. Horn

sign:



Results are shown in the following tables: 5.1a and 5.1b



**Table 5.1a: Efficacy of preserved Diesel Fuels against *Hormoconis resiniae* in a repetitive challenge test (challenges at the beginning and 7 and 14 days after start of the test). Total viable count (TVC) of *Hormoconis resiniae* in water phase after different incubation times. Numeric evaluation**

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	$1.1 \times 10^6$	$1.3 \times 10^6$	$9.3 \times 10^5$
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	$4.2 \times 10^7$
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	$5.4 \times 10^7$

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

**Table 5.1b: Efficacy of preserved Diesel Fuels against *Hormoconis resiniae* Logarithmic evaluation / lg- Reduction**

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 30 min.	no reduction	no reduction	--
2 <sup>nd</sup> Challenge after 7 days			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 7 days	>6.623	>6.623	--
3 <sup>rd</sup> Challenge after 14 days			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 21 days	>6.732	>6.732	--

TVC<sub>c</sub> = TVC Control

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

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

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

Drawn from: Name of the product: **Marine Diesel  
ASTM E 1259** **Bakterien Stop**

Laboratory number: 29.03.16-1420

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Hormoconis resiniae*, DSM 1203 cfu /ml of initial bacterial inoculum:  $1.4 \times 10^6$

Start of test: 2016-04-15

Responsible person: S. Horn

sign: 

Results are shown in the following table: 5.2

**Table 5.2: Efficacy of preserved Diesel Fuels against *Hormoconis resiniae***Total viable count (TVC) of *Hormoconis resiniae* in fuel phase

after incubation time of 30 minutes, 7 days and 21 days.

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

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	n.d.	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	n.d.

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

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

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

Drawn from:  
ASTM E 1259

Name of the product: **Marine Diesel  
Bakterien Stop**

Laboratory number: 29.03.16-1420

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Yarrowia tropicalis*, DSM 11953 cfu /ml of initial bacterial inoculum: 1.3 x 10<sup>6</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign: 

Results are shown in the following tables: 6.1a and 6.1b

**Table 6.1a: Efficacy of preserved Diesel Fuels against *Yarrowia tropicalis* in a repetitive challenge test (challenges at the beginning and 7 and 14 days after start of the test). Total viable count (TVC) of *Yarrowia tropicalis* in water phase after different incubation times. Numeric evaluation**

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	1.2 x 10 <sup>6</sup>	9.8 x 10 <sup>5</sup>	1.1 x 10 <sup>6</sup>
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	4.2 x 10 <sup>7</sup>
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	3.1 x 10 <sup>8</sup>

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

**Table 6.1b: Efficacy of preserved Diesel Fuels against *Yarrowia tropicalis* Logarithmic evaluation / lg- Reduction**

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 30 min.	no reduction	0.050	--
2 <sup>nd</sup> Challenge after 7 days			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 7 days	>6.623	>6.623	--
3 <sup>rd</sup> Challenge after 14 days			
TVC <sub>c</sub> /TVC <sub>TS</sub> (lg) 21 days	>7.491	>7.491	--

TVC<sub>c</sub> = TVC Control

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

## 6.2 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 product: **Marine Diesel  
Bakterien Stop**

Laboratory number: 29.03.16-1420

Diesel Fuel: 29.03.16-1418

Remarks: Diesel fuels were provided by customer.

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

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

Test temperature: RT Incubation temperature: RT Incubation period: 21 days

Test organism: *Yarrowia tropicalis*, DSM 11953 cfu /ml of initial bacterial inoculum: 1.3 x 10<sup>6</sup>

Start of test: 2016-04-15

Responsible person: S. Horn

sign: 

Results are shown in the following table: 6.2

**Table 6.2: Efficacy of preserved Diesel Fuels against *Yarrowia tropicalis***

Total viable count (TVC) of *Yarrowia tropicalis* in fuel phase after incubation time of 30 minutes, 7 days and 21 days.

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

Sample	Marine Diesel Bakterien Stop 1:1000	Marine Diesel Bakterien Stop 1:4000	Control Diesel fuel
Laboratory number (29.03.16)	1420	1420	1418
1 <sup>st</sup> Challenge at test start			
30 min. (TVC)	n.d.	n.d.	n.d.
2 <sup>nd</sup> Challenge after 7 days			
7 days (TVC)	n.d.	n.d.	n.d.
3 <sup>rd</sup> Challenge after 14 days			
21 days (TVC)	n.d.	n.d.	n.d.

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