



**Proudly Presents**

**ConidiaBioscience**

**FUELSTAT™ Marine test kit**

**For the Detection of  
Microbial Contamination in Diesel fuel**



# CONTENTS:

|  |          |
|--|----------|
| <b>Executive Summary - Testing of Marine and Land Based Diesel Fuel Systems.....</b> | <b>3</b> |
| <i>Hormoconis resinae</i> , Bacteria and Other Fungi in Diesel Fuel.....             | 4        |
| Clarification – List of Microbes Growing in Fuels.....                               | 5        |
| What’s So Special About the FUELSTAT™ Test Kits.....                                 | 6        |
| A Quick, Easy and Accurate Test for Microbial Contamination.....                     | 7        |
| Basic Design information, Layout and Packaging.....                                  | 8        |
| <b>Monitoring and Treatment Regimes for Storage Tanks Containing Diesel.....</b>     | <b>9</b> |
| Contact Information.....   | 10       |



## Conidia Bioscience

*Based in the UK at Egham in Surrey, Conidia Bioscience has been trading since 2003 and is fully established in the aviation industry providing a “rapid test kit” to the world’s leading and regional airlines. Currently serving over 290 airlines, Conidia Bioscience is an International Air Transport Association (IATA) Strategic Partner (member of microbial working group) and an IMarEST Partner. The current Aviation Test Kit (as seen on the left) is recommended by Boeing, Airbus, Embraer, BAE Systems, Raytheon, Fokker and other OEMs and Maintenance, Repair &*

*Overhaul organisations (MRO’s). Conidia Bioscience’s lab resources at CAB International are fully accredited and have great expertise in microbiology across temperate and tropical areas. They currently hold one of the world’s largest culture collections and have particular expertise in environmental biology, bio deterioration, and bio remediation.*



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## Executive Summary - Testing of Marine and Land Based Diesel Fuel Systems:

There are a number of microbes that can degrade fuels. These microbes tend to work together as a consortium, which often leads to compounded contamination throughout the tank and entire fuel delivery system.

The most dominant of these microbes is *Hormoconis resiniae* (*H.res*). Previously known as *Cladosporium resiniae*, *H.res* is more commonly referred to these days as “**The Diesel Bug**”. Circumstances for *H.res* to be present in diesel vary, but include temperature, humidity, quality of fuel and water content (water can migrate into the fuel from a variety of sources including condensation etc).

Monitoring fuel systems for contamination on a regular basis is extremely important; particularly for *H.res* as it is the one organism that will stick to the surface of a tank and is highly corrosive, causing more physical damage to the tank and hull structures than any of the other microbes. Other problems resulting from *H.res* include filter blockage, component failure and gauging errors.

Over the years **Conidia Bioscience** (and other scientific organisations) has noticed that *H.res* is present in approximately 70% of all cases of contamination in diesel fuel. The remaining 30% is made up of bacteria and/or fungi (including some yeasts). This consortium works together in the vast majority of cases of diesel fuel contamination. However recent introduction of biodiesels appears to be shifting the balance in such consortia towards yeasts and other filamentous fungi, so it is important to detect all organisms present.

In response to requests from civil and naval marine operators for a **real-time test** for microbial contamination in diesel fuel delivery systems and tanks, **Conidia Bioscience Ltd** has developed and manufactured the **FUELSTAT™ Marine test kit**.

The **FUELSTAT™ Marine test kit** measures the amount of different types of contamination **actively growing** in the sample and reports this as the weight of material in the sample. This is a newer, **more accurate measurement system** than the old Colony Forming Unit (CFU) count used by most testing laboratories and other fuel testing systems on the market.

The objective of the test is to **provide rapid screening of fuel samples** (free water in fuel or just fuel), giving a quick and accurate assessment of *H.res*, bacteria and other fungi in the fuel tank and associated fuel system. This test is unlike current growth-based tests, which require a minimum of 72 hours to provide any results. The test measures the amount of active growth in the sample and provides action and alert levels.

The **FUELSTAT™ Marine test kit** is quick and simple to use and requires very little training to operate and interpret the results. It comes complete with instructions and does not require sterile/lab conditions. It can be used “on the shop floor” and **results are obtained in 10 minutes**.

**Conidia Bioscience Ltd** recommends testing fuel tanks using the **FUELSTAT™ Marine test kit** at least once a year, but because conditions vary nationally, testing will have to be carried out more regularly in some parts of the country. Engineers should do a risk assessment to identify if their tank is at a higher than normal risk of contamination and therefore requires more regular monitoring.

The new **FUELSTAT™ Marine test kit** carries the **NATO Stock No.:** 4940-99-907-9225  
The *Fuel Test* part number for a **Kit** will be **FMD8**. A **Kit** is a **box of 8 Individual Tests**.

### Orders can be placed in one of four ways:

- 88 Kits = 704 individual tests (Pallet Lot)
- 44 Kits = 352 individual tests (Transit Box)
- 10 Kits = 80 individual tests (Box of 10)
- 1 Kit = 8 individual tests

**Individual tests will not be sold separately.**



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## ***Hormoconis resiniae*, Bacteria and Other Fungi in Diesel Fuel:**

Microorganisms can grow in fuels by using the Alkanes present as a foodstuff and, in some cases, may also utilise some of the additives in the fuel. Mid-to-light distillate fuels (diesels, kerosene etc) are particularly susceptible to contamination by microorganisms.

The type of organisms and the damage inflicted depend on the fuel and the additives. All contamination is important when considering the quality of a fuel, particularly when monitoring stored products and reserves. However, whilst a wide range of microorganisms can be found in diesel fuels, the most serious organism is the filamentous fungus, ***Hormoconis resiniae* (H.res)**. It is the most damaging of all contaminants for a number of reasons.

Firstly, its size and bulk. When compared to single cell yeasts and bacteria *H.res* produces far more biomass (slime, sludge etc) and is thus more likely to cause blockage problems in filters and fuel lines.

Secondly, it is by far the most common cause of microbial corrosion in fuel tanks. Other organisms are more important in certain applications i.e. in long term storage – these other corrosive organisms include the anaerobic bacteria, collectively known as *Sulphate Reducing Bacteria* (SRB) or, more accurately, *Sulphide Generating Bacteria* (SGB). These can also be found in marine fuel tanks, especially if “sludge” is present at the bottom of the tank. Other filamentous fungi may be emerging as important, but these tend not to occur without *H.res* being present and, in any case, are not currently very common.

Thirdly, because of the way *H.res* grows between the fuel and water, it usually starts on small water droplets. It then covers the droplet, holding it in place, and continues its growth, actually generating more water under the mat due to its metabolism. In the process, it firmly attaches itself to the tank surface.

***Bacteria and yeasts*** tend to require free water and are found mainly floating in the water phase. This means they are less likely to adhere to tank surfaces and will therefore be significantly reduced each time water is drained. However, once established *H.res* continues to **multiply in situ**.

High levels of bacteria and yeasts often indicate that you have picked up poor quality fuel and, as such, are useful indicators. However, this does not necessarily mean that they are causing any problem in the tank and they will probably be significantly reduced at the next water drain. However, in the marine environment drainage is often not possible and yeasts and bacteria become established; the **FUELSTAT™ Marine test kit** tells the whole story by detecting *H.res*, bacteria and fungi (including yeasts) in the fuel.

Currently available fuel tests involving total microbial counts simply tell us that there are microorganisms present in the fuel and that they are alive. Conidia Bioscience's new **FUELSTAT™ Marine test kit** goes beyond this. It detects **active *H.res***, bacteria and other fungi including yeasts and tells us not only that contamination is present and alive, but also that it is growing and therefore the potential for damage is real. **The FUELSTAT™ Marine test kit** measures the amount of active growth in the sample and provides action and alert levels.

A point worth noting for any organisation that undertakes sampling and microbial testing of fuel is that changes can and do occur in the sample as soon as the fuel has been removed from the tank. For this reason **samples should be put on test within 12 hours** to accurately report the level of contamination in the tank. Hard to do if a sample has to be transferred off site, packaged, possibly stored and then transported to the lab. The **FUELSTAT™ Marine test kit** avoids this whole issue as samples can be tested on-site as soon as the fuel sample has been drawn from the tank.



## List of Microbes Growing in Fuel

– Clarification of potentially misleading information currently in circulation:

**Conidia Bioscience** is aware that there is some confusion over a list of organisms found in hydrocarbon fuels published in a presentation on the “types of contamination in aircraft fuel tanks”. We would like to take the opportunity to clarify the situation.

The list includes 125 microbes (30 bacteria, 12 yeasts and 83 filamentous fungi). The list is credited to a peer-reviewed scientific paper authored by “CC Gaylarde et al” and Conidia Bioscience’s Technical Director, Dr J Kelley. The paper quoted is called “Microbial Contamination of Stored Hydrocarbon Fuels and Its Control”. Please note the following:

- The paper lists organisms reported at that time as having been isolated from **ALL** fuel types. This does not mean that the organism was growing in the fuel or was able to degrade it. In fact, the paper reduces the list of organisms that **CAN** grow in the various types of fuels to 8 bacteria (from the original 30), 5 yeasts (from 12) and 16 filamentous fungi, including *Hormoconis resiniae* (*H.res*), (from 83). I.E. a total of 29 out of the original list of 125 are potentially problem organisms for hydrocarbon fuels in general.
- The paper discusses fuel storage systems **NOT** on-board fuel tanks.
- The paper discusses **ALL** fuels from gasoline to heavy diesel. There is no suggestion in the paper that the 29 listed will grow in aviation kerosene, but the paper makes the point that “*diesel is the fuel which suffers from the most varied microbial contamination problems*”.



Microbial contamination in underground tanks

(Note the scrape marks where the maintenance crew have started cleaning the tank)



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## What's So Special About the FUELSTAT™ Test Kit?

The **FUELSTAT™ test kit** does not utilise the traditional growth methods employed by all other “rapid” tests currently on the market. **FUELSTAT™** is an **immunoassay test** (rather like a pregnancy test). This means that it detects contamination by "**finding**" **material that is produced** by the 3 different types of contaminants that grow in fuel. Therefore, there is no need to capture a part of the living organism and grow it up.

This is important as the fungus the test kit detects, *Hormoconis resiniae* (*H.res*) or “The Diesel Bug” does not just float around at the water/fuel interface like the other microbes; it actually sticks to the bottom or sides of the tank. This style of testing is also important as the ‘produced material’ is spread throughout the liquid of the tank, not just at the interface. Therefore, there is a better chance of finding that ‘produced material’ in a small sample. The result is a more accurate and consistent answer.

### **The objective of the test kit:**

The objective of the test is to provide rapid screening of fuel samples (free water in fuel or just fuel), giving a quick and accurate assessment of *H.res*, bacteria, and other fungi including yeasts in the fuel tank. The **FUELSTAT™ Marine** test is unlike current growth-based tests, which require a minimum of 72 hours to provide any results. The test measures the amount of active growth in the sample and **provides actions and alert levels**.

The kit will ignore any microorganisms that have been blown in from outside or have been growing on trees or other food sources. Other tests on the market will grow whatever they find in the sample - whether it came from the fuel or not. Where they require sterile sampling conditions, we just require that the sample equipment is clean (i.e. has no residue from the last test sample).

The **FUELSTAT™ Marine test takes 10 minutes to operate**. Most other tests take a minimum of 2 to 3 days to give a full picture of bacterial and fungal contamination. As fungal spores will not show any significant growth before 4 days, a complete answer using traditional growth techniques takes five to seven days. During this time the competitor's products must be incubated, and many need to be monitored daily. ATP based tests while rapid, show total life, and do not relate to actively growing organisms.

The results from most of the Fuelstat's competitor's tests are deduced either by comparing colours or spot numbers with a chart or, if you need to be very accurate, counting under a microscope. With Fuelstat™ you look at the six lateral flow devices on the test kit paddle and read off whether result is negligible (we never say nil), moderate or heavy contamination. **The FUELSTAT™ Marine test kit** results correspond to the recommended limits set out in the International Air Transport Association (IATA) guidelines for negligible, moderate and heavy contamination.

### **Negligible - Negative**

Fuel - Below 4,000 cfu/l or **150 µg/l**

Water - Below 1,000 cfu/ml or **33 µg/ml**

### **Moderate - Low Positive**

Fuel – 4,000 – 20,000 cfu/l or **150 - 750 µg/l**

Water – 1,000 – 10,000 cfu/ml or **33 – 166 µg/ml**

### **Heavy - High Positive**

Fuel – Above 20,000 cfu/l or **750 µg/l**

Water - Above 10,000 cfu/ml or **166 µg/ml**

The **FUELSTAT™ test kits** measure in the more modern and accurate measurement of **micro-grams per litre of fuel** (µg/l) or **micro-grams per milli-litre of water** (µg/ml).



A point worth noting is that while **cfu** (Colony Forming Unit) is a very common measurement in microbiology, particularly in growth type testing methods, it becomes highly inaccurate when measuring or counting filamentous fungi. This is because unlike bacteria, where a single cell normally gives rise to a single cfu filamentous fungi consist of a mass of threadlike structures and masses of conidia (or 'seeds'). These structures fragment leading to cfu numbers which are difficult to relate to the original contamination.

Most of Fuelstat's™ competitor test kits require special handling, certainly for safe disposal. The Fuelstat™ test kit (apart from the fuel itself of course) can be disposed of in the nearest bin when the test is finished or recycled if your company has a plastics recycling policy.

### A Quick, Easy and Accurate Test for Microbial Contamination



Open foil pack and remove test kit



Read instructions and record sample data



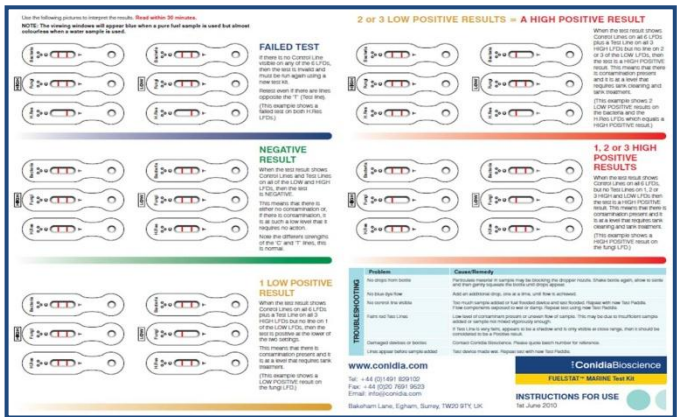
If any Free Water use it instead of fuel\*



Shake & put four drops into each test well\*\*



Control & Test lines appear (or not!)



Wait 10 minutes then interpret the results

\*If **Free Water IS present** drawn it out from the fuel sample with the pipette and fill up the kit bottle to the bottom line. If **NO Free Water is present** then fill the kit bottle up to the top line with fuel.

\*\* Once shaken **only the blue fluid** should be put into the test wells. With fuel this will take time to drop into the cap.



## Basic Design Information, Layout and Packaging:

### Packaging

The **FUELSTAT™ Marine test kit** will be delivered in a **box of 8 tests** and will use the part number FMD8. Individual tests come in a single sealed foil pouch containing a test paddle, pipette, a 160 ml sample extraction bottle, instructions for use and a desiccant sachet. This will allow customers to issue the test to maintenance engineers as a single test unit.

### The 160ml Buffer Extraction Fluid Bottle

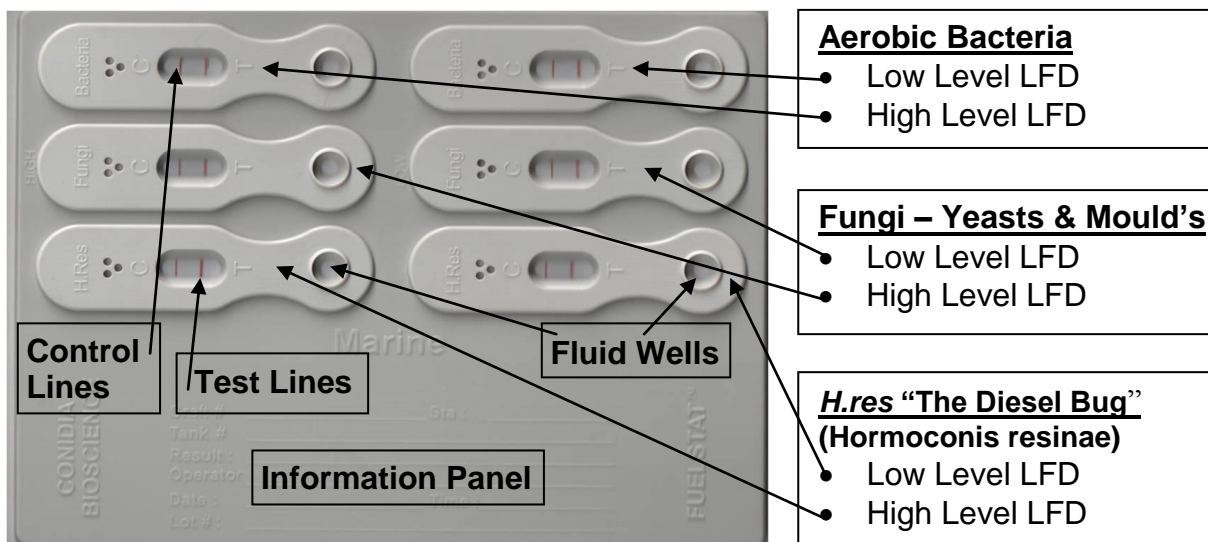
The Buffer Extraction bottle contains 3ml of buffer extraction fluid. (Material Safety Data Sheets can be supplied on request).

### Instructions for Use, Information & Material Data Safety Sheets (MSDS)

Full instructions are included, but can also be viewed online along with the MSDS and **FUELSTAT™ Marine** information sheets. All can be downloaded from the websites: [www.fueltest.com.au](http://www.fueltest.com.au) or [www.fueltest.co.nz](http://www.fueltest.co.nz) or [www.conidia.com](http://www.conidia.com)

### The Test Paddle

The test paddle incorporates 6 Lateral Flow Devices (LFDs). 2 LFDs are for *H.res*, 2 for bacteria, and 2 for other fungi. Each pair of LFDs will detect for HIGH and LOW levels of contamination. The **FUELSTAT™ Marine test kit** contamination levels and subsequent advice is based on the IATA guidance material.



Individual **FUELSTAT™ Marine test kit** for diesel

### Orders can be placed in one of four ways:

- 88 Kits = 704 individual tests (Pallet Lot)
- 44 Kits = 352 individual tests (Transit Box)
- 10 Kits = 80 individual tests (Box of 10)
- 1 Kit = 8 individual tests

### Minimum orders may apply

*Individual tests will not be sold separately*

**Kit containing 8 individual **FUELSTAT™** test kits:**

Part No. FMD8

NATO Stock # 4940-99-907-9225



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## Monitoring and Treatment Regimes for Storage Tanks Containing Diesel:

**Q: We routinely biocide our storage tanks, what added value would the FUELSTAT™ test offer us?**

*This question supposes that the system you currently operate includes draining water and then biociding the fuel in the storage tank. Draining the water from the tanks is the key basis for any control system. However, there are problems associated with maintenance dosing (routine biociding). Firstly, it involves costs in terms of manpower and the biocide itself. Secondly, there can be problems with warranties from engine manufacturers if there is any doubt about the ppm levels of biocide in the fuel. Thirdly, there are health, safety and environmental considerations when using biocides and finally, unless the correct ppm level is maintained continuously, there is a danger that a resistant strain or strains of microbes will be produced. Therefore we suggest that the use of biocides should be restricted to the minimum required to maintain clean fuel i.e. used only when test results indicate live microbial growth is occurring.*

The introduction of a monitoring regime using the **FUELSTAT™ Marine test kit** allows the inspection or maintenance engineers to have real-time information on the state of the tank on which to base decisions for biociding. No resource time or expense is wasted on unnecessary treatment. As the **FUELSTAT™ Marine test kit** requires only a sample from the tank and takes just 10 minutes to operate, it is a quick, easy and an accurate way of determining when and what treatment is required.

**Q: How many kits do we need to test our fuel tanks?**

A minimum of one test per tank is required in any monitoring regime. However, more will be required depending on the size and application of a tank. Multiple sample points i.e. top, middle and bottom along with fuel lines and/or dispensing nozzles should be included in the monitoring regime.

The issue for the maintenance and inspection engineers is the frequency of testing. The **FUELSTAT™ Marine test kit** is based on contamination levels set out by IATA in their microbial contamination guidelines. IATA recommends a minimum frequency of once a year, but that frequency should be increased in relation to the risk. If in a high risk area or a tank has had contamination in the past then the frequency should be increased to between one to three months. Conidia Bioscience advises that testing more frequently than once a month is unnecessary.

*Fuel Test* offers a consultancy service to carry out these risk assessments.

**Q: Any other 'specialist' equipment required?**

The only equipment needed is the normal safety equipment (gloves etc) required for handling diesel and of course clean sample bottles necessary for gathering and holding the sample of fuel for testing. Other than that, the test is "stand-alone".

**Q: Does it have any reaction with the biocides?**

The basic answer to this is "no it does not". There should be a delay between biociding and retesting to see whether the biocide has been totally effective. We would suggest retesting a week after the biocide has been introduced into a contaminated tank. That would mean looking for traces of any surviving microbes, not the residue from that which has already been killed.

Further Q & A's on the **FUELSTAT Marine test kit** can be found on the FAQ sheet or our website.



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## Contact Details:



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## Proudly Supported By

**ConidiaBioscience**

**Conidia Bioscience Ltd** offers a full backup service and can carry out a comprehensive lab test in extreme cases at their base in Egham, Surrey UK. Contact can be made by visiting their website at [www.conidia.com](http://www.conidia.com)



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