

# Technical Data

## INTRODUCTION

There are a number of different types of microorganisms that can grow in certain types of fuel. The biggest problem is presented by a filamentous fungus called *Hormoconis resiniae*, (H Res). Previously called *Cladosporium resiniae*, and more commonly referred to as "the diesel bug".

H Res is a fungus that thrives in diesel fuel. It requires only a minimal amount of water content in the fuel to grow and will cause filter blockages, component failure and tank corrosion if left unchecked. Bacteria and other types of fungi, particularly some yeasts can also cause problems in fuel tanks, usually acting as a consortium.

The objective of this test is to provide rapid screening of fuel samples (water in fuel or fuel), giving a quick and accurate assessment of H Res, bacteria & other fungi including yeasts in the fuel tank. 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 actions and alert levels.

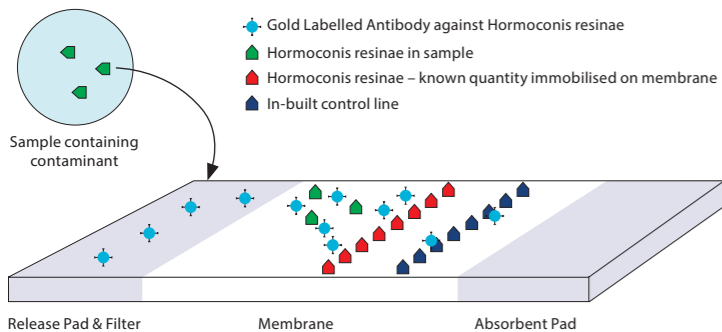
The FUELSTAT® *resinae* PLUS DIESEL test measures the amount of different types of contamination: H Res, bacteria and fungi actively growing in the sample and reports that as the weight of material in the sample. This is a newer, more accurate measurement system than the old Colony Forming Unit (CFU) count.

The test provides results based on a traffic light scenario:

- Negligible (green) – negligible contamination
- Low Positive (amber) – moderate contamination
- High Positive (red) – heavy contamination

## ASSAY DESCRIPTION

The FUELSTAT® *resinae* PLUS DIESEL test utilises lateral flow technology and antibodies to H Res, bacteria and fungi which grow in diesel fuel.

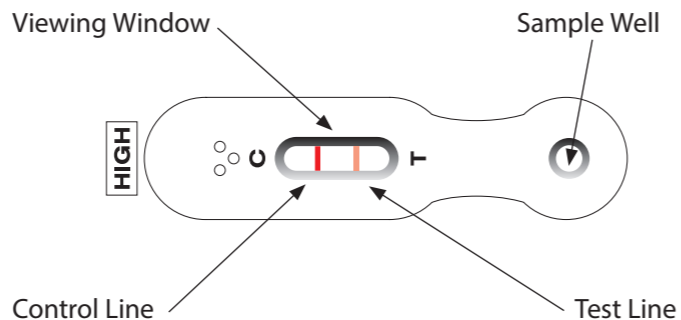


The gold-labelled antibody is immobilised in the Release Pad under the sample well. The sample containing an unknown amount of contamination is added to the sample well and this re-hydrates, allowing the reagents to flow up (wicking) the membrane towards the absorbent pad. Any large particles in the sample, which may block the reaction, are blocked by the filtering action of the pad. During the wicking, the contamination in the sample will bind to the specific antibodies.

As the liquid reaches the Test Line (T) any free gold-labelled antibodies will bind to the test line. This means if more contamination is in the sample than the threshold engineered, there will be no antibodies to bind to the Test Line, no red line will appear, and this is a POSITIVE result.

If the amount of contamination in the sample is lower than the threshold, there will be free antibodies to bind to the Test Line, a red line will appear, and this is a NEGLIGIBLE result. The quantities of materials immobilised in the device are engineered to provide results at the different thresholds of H Res, bacteria and fungi in each of the test devices on the Test Paddle.

## DIAGRAM ILLUSTRATING A HIGHDEVICE

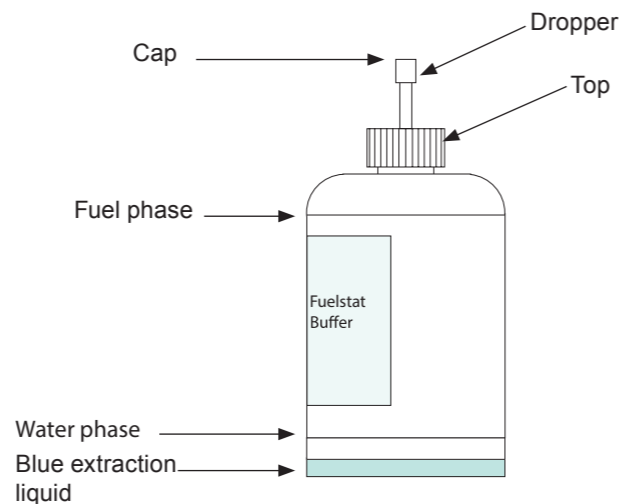


There is an in built Control in the test and for all samples run; a line should appear against the C (Control) point in the viewing window. If no line appears the test has failed and should be repeated.

## SIX DEVICES ARE INCLUDED IN EACH TEST:

**Right side of paddle (LOW)** 3 devices with cut off levels for H Res, bacteria and fungi at a level derived from IATA Guidance Material where the microbes have established themselves as a consortium and a fuel treatment is required.

**Left side of paddle (HIGH)** 3 devices with cut off levels for H Res, bacteria and fungi at a level derived from IATA Guidance Material where the microbes are growing so rapidly, or have been growing for a long time that they may be causing damage to the structure of the tank or are likely to cause filter blockage and component failure and a tank clean and fuel treatment are required.



# Diesel Test Instructions

**Open foil pack contents**

**Take a sample from the lowest point in the tank**

**Once settled, is there any free water?**

**Water Phase Test**

**Mixed Water and Fuel Phase Test**

**Fuel Phase Test**

**If enough water is free in the sample use the pipette to fill the bottle to lower line**

**Use pipette to collect free water and put into bottle. If water does not reach lower line, fill to top line with fuel from the same sample**

**If no water is visible in the sample, fill the bottle to the top line with fuel**

**Shake for 5 seconds**

**For all types of test, secure dropper cap and shake sample vigorously for 5 seconds**

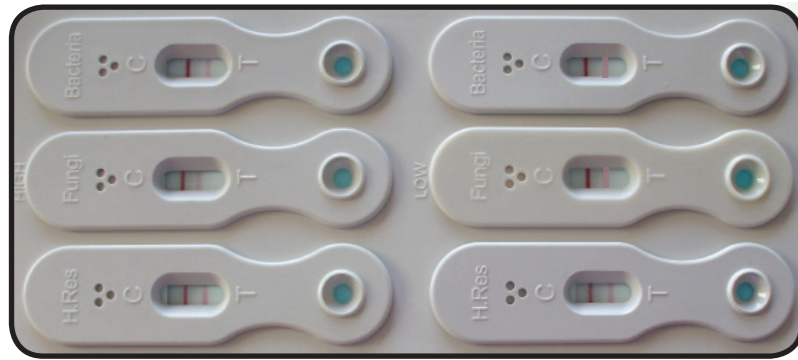
**Invert bottle and allow blue fluid to settle out of the fuel sample. NOTE: For a water phase test the blue fluid will not separate from the sample**

**Allow 3 drops to spill onto a tissue to clear the dropper then carefully allow 4 drops of blue fluid into each sample well ensuring no spillage into the viewing window**

**Keep the paddle flat: the blue fluid will be seen to flow along the viewing window and, after a few minutes, a dark red Control line to the left of each viewing window will appear**



# The Results



## Negligible Result

If all 6 Control lines and all 6 Test lines are visible, this is a Negligible result, no action required.

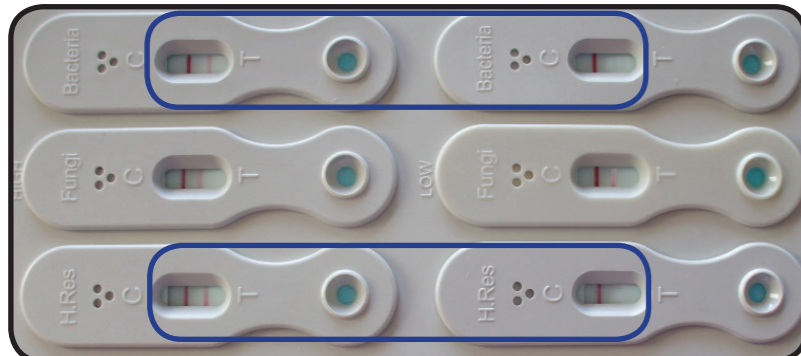
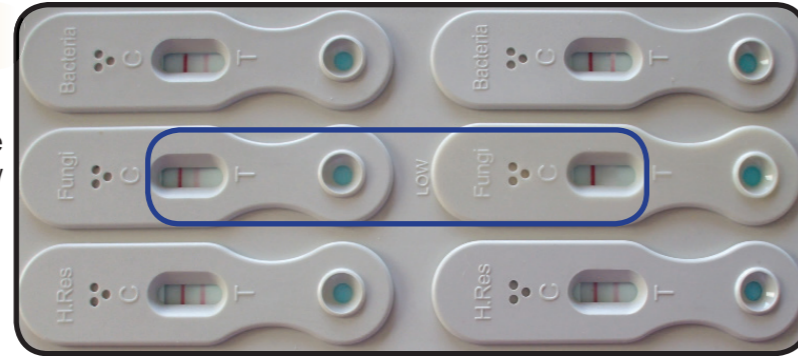
This means that there is either no contamination or, if there is contamination, it is at such a low level that it requires no action.

## Low Positive Result

### MODERATE CONTAMINATION

If 1 Test Line is missing, here the Test Line in the Fungi field is not visible, this is a Low Positive result.

This means that there is contamination present and it is at a level that requires fuel treatment.

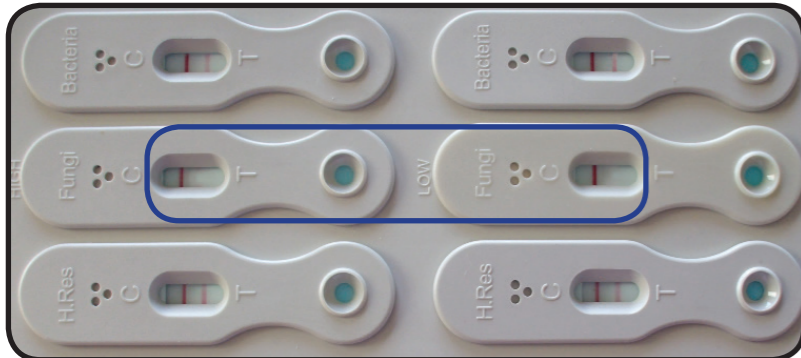


## High Positive Result

### HEAVY CONTAMINATION

If 2 or more Low Test Lines (on the right side of the paddle) or any High Test Lines (on the left side of the paddle) are not visible, this is a High Positive result.

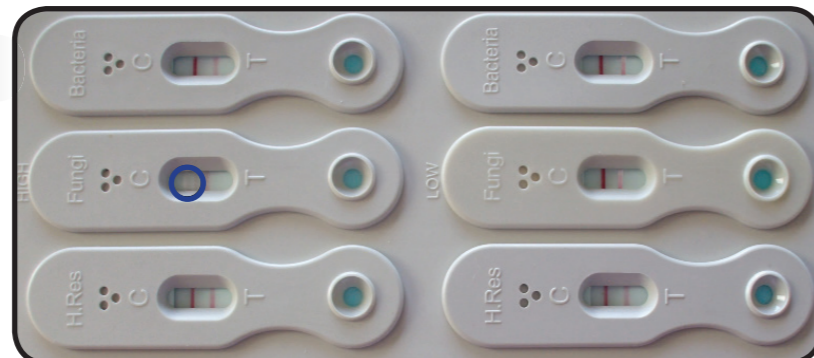
This means that there is contamination present and it is at a level that requires tank cleaning and fuel treatment



## Test Not Valid

If there is no Control Line visible on any of the 6 devices, then the test is invalid and must be run again using a new test kit.

Retest even if there are lines opposite the 'T' (Test Line)



## TEST CONTENTS

Each heat sealed foil pouch contains a Paddle with desiccant sachet and pipette in one section and Sample Extraction Bottle with flat cap, dropper cap and instructions for use in the other section.

- Paddle: Plastic base with 6 lateral flow devices affixed
- Preparation Bottles: 175ml plastic bottle with flat cap and "dropper" cap containing 3.0ml of Sample Extraction Liquid
- Disposable, single use, plastic pipette
- Instruction leaflet

## STORAGE AND STABILITY

- No special transport precautions
- Store below 30°C
- Use between 10° and 30°C
- Do not use after the stated expiry date
- Long term freezing is not recommended

## WARNINGS AND PRECAUTIONS

- Caution should be exercised in the handling of fuel or other hazardous materials in accordance with Health and Safety procedures.
- Optimum results will be obtained by strict adherence to this protocol.
- Each paddle is disposable. Use only once.
- The paddle in the foil pack should be kept sealed until ready for use. Once the foil pack is opened the shelf life of the device is not guaranteed. It should be used as soon as possible.
- The viewing window of the test device should not be touched.
- The paddle should be kept dry at all times. DO NOT USE if any of the devices become wet.
- If the paddle appears damaged, scratched or marked in any way please contact Conidia Bioscience.

## SAMPLE PREPARATION

Take a sample from the fuel tank into a clean container. Allow the sample to "settle" and any water to accumulate at the bottom of the sampling container. Ideally, the sample should be drawn from the tank sump or lowest point from a water drain point. Alternatively, draw a tank bottom sample using a dipped sample tube from the tank fill aperture. For vehicle tank testing, if it is impossible to harvest a tank bottom sample then the contents of the primary fuel filter is the next best place. This sample however may not represent the tank bottom. Any sample taken should be tested as soon as possible after collecting and, in any event within 6 hours to maintain the integrity of the sample.

**NOTE : When possible, test the water phase of the sample taken from the fuel tank. Testing the water phase will provide more accurate results than testing the fuel phase.**

## ACTIONS FOLLOWING TESTING

Based on extensive research, Conidia Bioscience Recommends:

**NOTE: Each user should define their own policy on test frequency and actions following a positive test result.**

1. **Low Positive Result.** The fuel should be treated as soon as possible. Treatments include the application of a biocide, passing the fuel through a UV light device, the use of a centrifuge, filters or fuel conditioning additives (i.e. water removal products).
2. **High Positive Result.** The fuel tank should be drained and cleaned as soon as is practicable. The replacement fuel in the tank should then be treated.
3. **After Treatment.** The fuel tank should be retested 7 to 10 days after treatment to ensure that the action taken was totally effective. If the retest returns a Low or a High Positive result then the tank should be drained and cleaned as for a High Positive Result. The contamination may have stuck to the tank surfaces, most probably above the current fuel level.
4. **General.** If the detection test shows contamination from H Res, bacteria or fungi then carry out the scheduled inspection test more often.

## FUEL HUSBANDRY

Rather than treat contamination when significant operational or fuel quality problems arise it is better to prevent microbial growth occurring, much can be done to ameliorate problems associated with microbial contamination in fuels, these include:

- Allowing fuel deliveries to settle in the tank for 24 hours before putting the tank back into service.
- Drain any accumulated water from the bottom of the tank weekly.
- Routinely test bottom samples for microbial contamination.
- Treat fuel if required, according to test results.

## TROUBLESHOOTING

Problem	Cause/Remedy
No drops from bottle	Particulate material in sample may be blocking the dropper nozzle. Shake bottle again, allow to settle and then gently squeeze the bottle until drops appear.
No blue dye flow	Add an additional drop, one at a time, until flow is achieved.
No control line visible	Too much sample added or fuel flooded device and test flooded. Repeat with new Paddle. Flow components exposed to wet or damp. Repeat test using new Paddle.
Faint red test lines	Low level of contaminant present or uneven flow of sample. This may be due to insufficient sample added or sample not mixed vigorously enough. If Test Line is very faint, appears to be a shadow and is only visible at close range, then it should be considered to be a Positive result.
Control and test lines are blue in colour	Extraction liquid not mixed with Fuel/Water sample properly or Fuel/Water added to sample well. Repeat test using a new Paddle.
Damaged devices or bottles	Contact Conidia Bioscience. Please quote batch number for reference.
Lines appear before sample added	Test device made wet. Repeat test with new Paddle.