



GUIDELINES:

The science behind **FUELSTAT^{Plus}**

FUELSTAT® *Plus* is an immunoassay antibody test similar to the pregnancy tests that we are all familiar with. But instead of searching for the markers of pregnancy hormones (HCG), FUELSTAT® *Plus* searches for the markers of the microorganisms that thrive and do damage to fuel systems.

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What is Immunoassay antibody testing?

An immunoassay antibody test is a biochemical technique that uses the same technology as your immune system to identify the presence of a specific microorganism.

A marker, known as the antigen, is selected to target the microorganism in question. It is usually a part of the microorganism or a chemical they produce during their growth.

An antibody is developed to bind to a specific antigen. The antibody-antigen combination is unique as the two fit together like jigsaw pieces.

Antibodies or antigens can be attached to surfaces and used to collect and measure the amount of organism present in a sample. The collected antigen is used to confirm whether the organism is present and determine the approximate quantity of it. This is a common tool for detecting levels of specific chemicals in clinical settings for medical diagnosis. For example, it is used to precisely measure blood insulin levels to check for hypoglycaemia, hormones for prostate cancer or drugs used in sports doping.

Immunoassay antibody testing is used all over the world and is being especially highlighted now as we use it for covid-19 testing. This important technology has a huge market, with millions of tests being done daily for coronavirus worldwide.

Immunoassay – a biochemical test that measures the amount of a specific substance with antibodies and antigens.

Antigen – a specific marker for the organism.

Antibody – a binding protein that attaches to its specific antigen.

Competition assay – the *antigen* in the sample competes with the *antigen* on the test line for binding.



How is immunoassay antibody testing used in FUELSTAT® Plus?

Immunoassay antibody test technology was discovered in the 1950s and has evolved into being a key method in cutting-edge scientific research. FUELSTAT® Plus has adapted this technology into a semi-quantitative test to identify specific microorganisms that contaminate and actively break down fuel as well as the biodegraded products they produce.

We have developed antigens that are used as markers for key microorganisms known to contaminate fuel. These are grouped into three categories for detection by the test kit:

- **Bacteria**
- **Fungi** – yeasts and moulds
- **H.res** (*Hormoconis resinae*) – a filamentous fungi particularly prevalent and problematic in fuel

FUELSTAT® *Plus* test plates contain 6 tests, testing for each separate group at high (heavy contamination) and low (moderate contamination) levels as per the IATA guidance material¹. Testing each separate group at two levels allows us to determine the type and severity of contamination present in the fuel, when both tests are negative a negligible level of contamination is recorded.

The principles of antibody testing in fuels using FUELSTAT® *Plus*: A, B, C

For simplicity let us take a very simple view of microbiological activity in fuels. There are 3 categories of microorganisms that may occur in fuel:

A: This consists of what is classed as primary fuel degraders (**A**), microorganisms that are known to thrive in jet fuel and feed on fuel components, i.e. degrade fuel.

B: Microorganisms that are classed as secondary degraders (**B**) and cannot directly feed on fuel. The principle here is that secondary degraders do NOT exist unless (**A**) group microbes are present to break down the fuel in the first place. They will not multiply, unless significant amounts of **A** group microbes are present to create food; these organisms are not targeted by FUELSTAT® *Plus*.

C: Human, Plant-based airborne and other “stray” microorganisms (**C**), that are all around us and could enter the sample during sampling, handling and transport stages. Such organisms are not targeted by FUELSTAT® *Plus*.

FUELSTAT® *Plus*, put simply, searches for **A** group of microorganisms only. Alternative tests based on culture methods or Adenosine Triphosphate (ATP) search for potentially **A**, **B** and/or **C**.

This means that Alternative Tests could have higher counts depending on the total microbial presence of **A+B+C** in variable quantities. Unless the sample is then further analysed by, for example, molecular technology, there is no way of understanding what the result seen by culture or ATP tests may represent.

The FUELSTAT® *Plus* test may give a result that is different from alternative tests, but this result would be indicative of the presence of microorganisms in group **A**, that are the primarily responsible for operational and engineering issues.

A **Primary fuel degraders**
feed and thrive in jet fuel

B **Secondary degraders**
rely on presence of primary fuel degraders

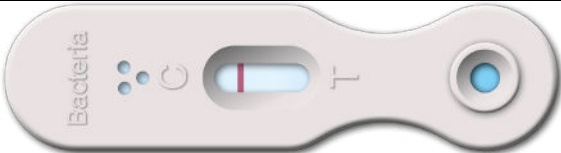
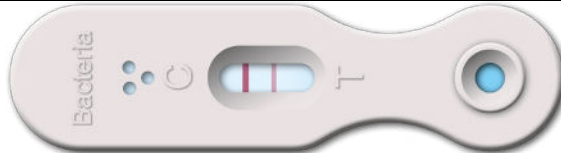
C **“Stray” microorganisms**
other organisms that can contaminate a sample but do not feed on fuel

FUELSTAT® *Plus* uses a “competition assay” type test:

1. The blue reagent is used to extract the antigens in the sample.
2. This is then applied onto the lateral flow device (LFD) and mixes with our antibodies that are specially designed to bind the antigens of fuel degrading microorganisms. These antibodies have been labelled with a gold dye that has a red colour.

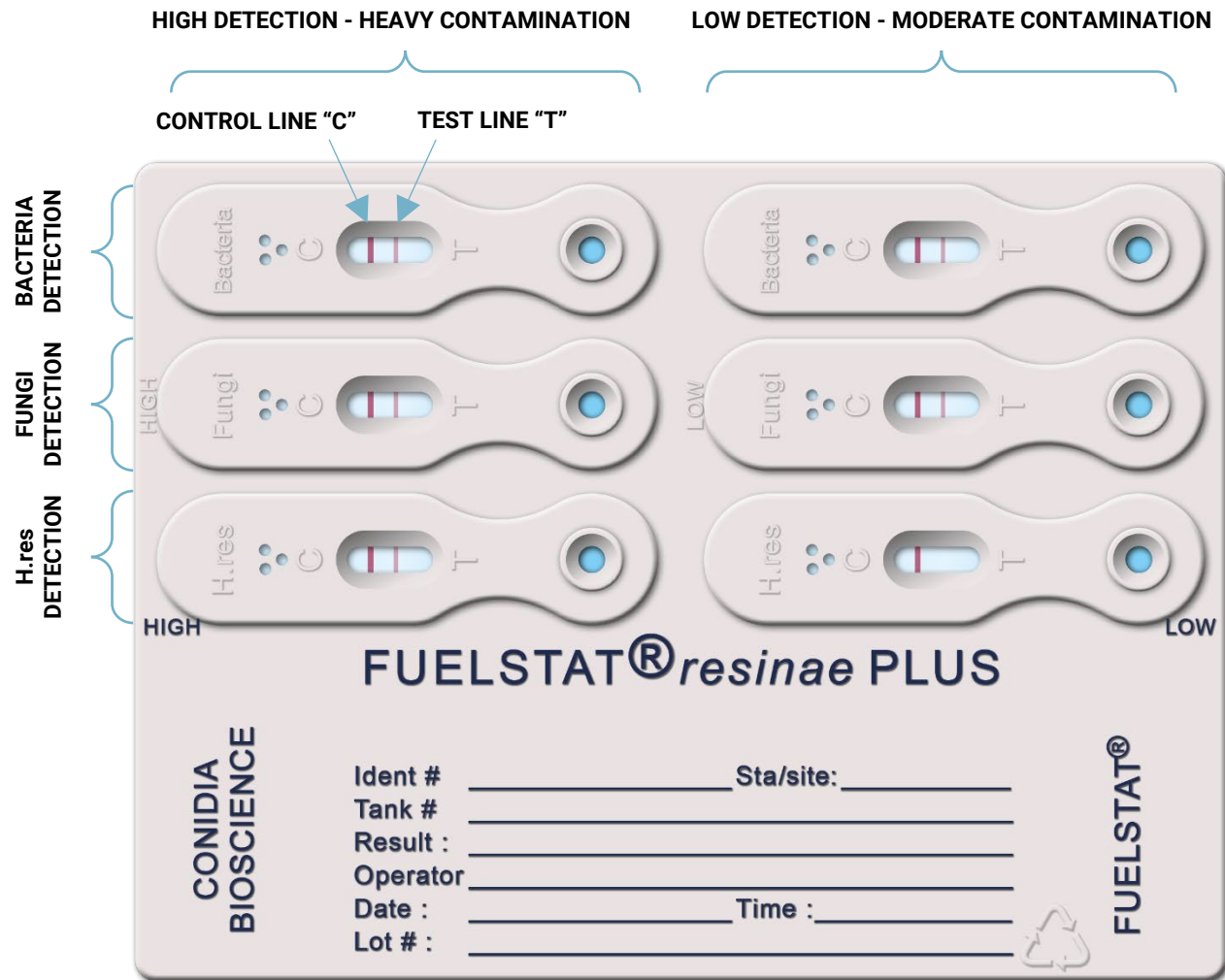


3. The liquid will soak into the enclosed pad and the mixture of dyed antibodies and antigens will flow along the device.
4. On the device there is a test line “T”, this has a known quantity of antigen embedded in it. The dyed antibodies bind to the antigen in the sample first, then any leftover unbound dyed antibodies will bind to the test line.

Positive	Negative
There is more antigen in the sample than on the test line. The sample antigen binds all the dyed antibodies and none is left to bind to the test line.	There is less antigen in the sample than on the test line. There are plenty of dyed antibodies left to bind to the test line.
A red test “T” line is <u>NOT</u> formed	A red test “T” line is formed
	

5. There are two thresholds of test on each kit for each of the 3 detected contamination groups. The “**low**” side of the test will show a positive (no line) when moderate levels of contamination are present, the “**high**” side will show a positive (no line) with a heavy contamination. When all six of the tests on the plate give negative results (all “T” lines visible) it shows negligible levels of contamination.
6. To verify validity of the test and ensure that there is sufficient reagent flow along the test strip there is a built-in control line “**C**”. When the reagent flow reaches that point a strong line should appear on all tests to indicate proper flow.

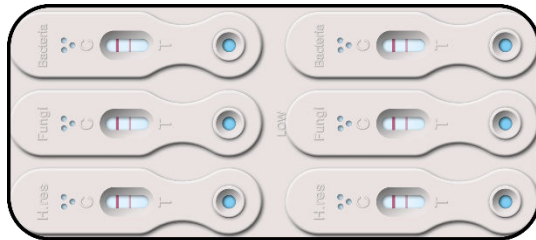
The FUELSTAT® Plus test kit plate



*Note: the above example has just one positive result on the low side for H.res
 = moderate contamination of Hormoconis resinae*

Reading results of FUELSTAT® Plus test

If all 6 'Control lines' are visible the test result is now ready to be interpreted. **Read results within 15-30 minutes** after placing sample fluid into sample wells. How to manually interpret the test is shown below:

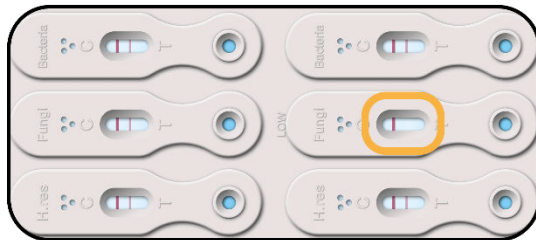


Negligible Result

NEGLECTIBLE CONTAMINATION

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.

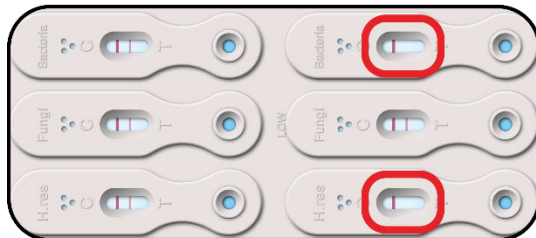


Low Positive Result

MODERATE CONTAMINATION

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

This means that there is contamination present and action should be taken - refer to OEM manuals and industry guidance



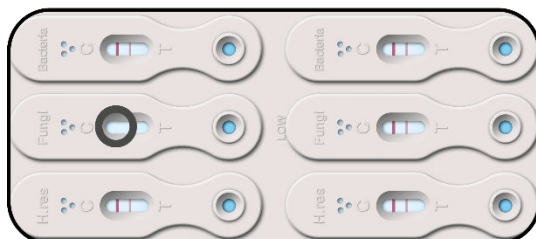
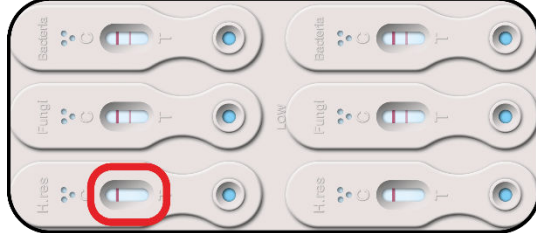
High Positive Result

HEAVY CONTAMINATION

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

*note: the combined amount of contamination present when 2 or more Low Positive results are obtained equates to a heavy total level of contamination.

This means that there is higher levels of contamination present and urgent action should be taken - refer to OEM manuals and industry guidance



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).

Interpretation of test results

Phase	Target antigen limits	Alert level
Fuel	Up to 150 µg/L	Negligible
Water	Up to 33 µg/ml	
Fuel	Between 150-750 µg/L	Moderate
Water	Between 33-166 µg/ml	
Fuel	Greater than 750 µg/L	Heavy
Water	Greater than 166 µg/ml	

What advantages does FUELSTAT® technology give me?

FUELSTAT® *Plus* identifies the presence of microorganisms known to contaminate and degrade fuel by detecting their antigens. Antigens act as markers that can be assessed using immunoassay antibody tests. Immunoassay antibody technology is specific which allows FUELSTAT® *Plus* to only identify the presence of fuel degrading microorganisms; other types, such as those that may be found on humans and the surrounding environment and could easily contaminate a test, will not be detected. **Sterility and cross-contamination of tests is therefore less of an issue than other techniques.**

FUELSTAT® *Plus* will give results as negligible, moderate, or heavy level of contamination, reflecting IATA guidelines¹. Each group of organisms; bacteria, fungi (moulds and yeasts) and H.res are tested on high and low LFD assays, giving a clear picture as to which group of microorganisms are present in the fuel and the level of contamination. Detailed test procedure can be found at reference².

Immunoassay antibody tests are inherently **quick, simple and reliable**. This technology is prepared and enclosed in the plate allowing the test to be **done in 15 minutes, on site and with minimal training**. By measuring the presence and intensity of test lines, the FUELSTAT® *Result* app is available to help read the tests, record and communicate data, so that work can be done in solo conditions and multiple locations.



FUELSTAT® PROTECTS

tests sold
120,000
per annum

over
130
countries
globally

more than
400
airlines

more than
10
major oil
companies

FUELSTAT® SAVES

cost reduction, less
effort, less time
50%
savings

labour hours saved
+ 1,000,000

TRIED. TESTED. TRUSTED.



Who we are:

FUELSTAT® fuel tests are developed, manufactured and marketed by Conidia Bioscience Limited. Based in UK, Conidia Bioscience Limited was founded in early 2000's by experts in immunoassay techniques and holds the internationally patented intellectual property for FUELSTAT®.

FUELSTAT® Result is hosted by Conidia Bioscience Limited and its service partners on secure servers and does not share any data with third parties.

Where to find us:

FUELSTAT® is distributed globally by a network of specialist distributors covering the major sectors. Contact info@conidia.com who will arrange for a distributor to support you.

References:

¹ The International Air Transport Association (IATA): [Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks](#), 5th Edition

² [International Standard ASTM D8070](#) details the standard test method for screening of fuels and fuel associated aqueous specimens for microbial contamination by lateral flow immunoassay.

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