FUELSTAT® 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® searches for the markers of the microorganisms that thrive and do damage to fuel systems.
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.

How is immunoassay antibody testing used in FUELSTAT®?

Immunoassay antibody test technology was discovered in the 1950s and has evolved into being a key method in cutting-edge scientific research. FUELSTAT® 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® test paddles contain 6 tests, testing for each separate group at high (heavy contamination) and low (moderate contamination) levels as per the IATA guidance material\(^1\). 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.

**FUELSTAT® 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.

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
<td>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.</td>
</tr>
<tr>
<td>A red test “T” line is NOT formed</td>
<td>A red test “T” line is formed</td>
</tr>
</tbody>
</table>

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 paddle 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® test kit paddle

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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® test

Negligible Result

**NEGLIGIBLE 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 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).

**Interpretation of test results**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Target antigen limits</th>
<th>Alert level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuel</td>
<td>Up to 150 µg/L</td>
<td>Negligible</td>
</tr>
<tr>
<td>Water</td>
<td>Up to 33 µg/ml</td>
<td></td>
</tr>
<tr>
<td>Fuel</td>
<td>Between 150-750 µg/L</td>
<td>Moderate</td>
</tr>
<tr>
<td>Water</td>
<td>Between 33-166 µg/ml</td>
<td></td>
</tr>
<tr>
<td>Fuel</td>
<td>Greater than 750 µg/L</td>
<td>Heavy</td>
</tr>
<tr>
<td>Water</td>
<td>Greater than 166 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>

The science behind FUELSTAT®
What advantages does FUELSTAT® technology give me?

FUELSTAT® 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® 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® will give results as negligible, moderate, or heavy level of contamination, reflecting IATA guidelines. 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 reference.

Immunoassay antibody tests are inherently quick, simple and reliable. This technology is prepared and enclosed in the paddle 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® 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.