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PP-289 [Beekeeping Technology and Quality]

Innovative 4-in-1 Rapid Test for Screening Residues of Nitrofurans (AOZ, AMOZ, AHD, SEM) in Honey

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Overview

Nitrofurans are banned antibiotics that leave toxic metabolites in the form of SEM, AOZ, AHD, and AMOZ that are linked to cancer and genetic damage. Though prohibited in many regions, residues can persist through illegal use, cross-contamination, or environmental exposure. Therefore Governing bodies have established regulatory Reference Points for Action (RPA) of 0.5ppb.

Detecting Nitrofurans in honey has always been challenging, traditional methods like LC-MS/MS and ELISA while accurate, are costly, slow and require specialist expertise. Existing screening options are impractical for beekeepers and small producers with multiplex systems demanding expensive analysers and ELISA needing 1 kit for each individual antibiotic (4 in total). As a result, many producers and suppliers are unable to carry out testing at all or cannot test at the frequency and scale that is truly needed.

Cost-Effective Nitrofuran Monitoring:

To address this issue Biorex Food Diagnostics have developed an innovative multiplex lateral flow device, capable of detecting four key Nitrofuran metabolites in one simple rapid test. Cost-effective, highly sensitive and simple to use, the test enables producers to uphold quality standards, comply with regulations and prevent contaminated honey from reaching the market.

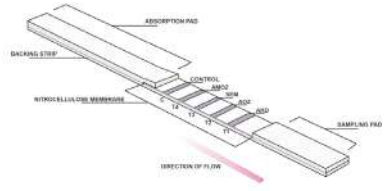

Figure 1: Design of Nitrofuran 4-in-1 lateral flow device

Honey exporters face strict regulations, with many markets requiring each batch sold to be tested for banned nitrofurans. LC-MS/MS testing can cost up to €150 per sample and take days to deliver results, meaning annual testing can reach tens of thousands of euros for high-volume producers.

With results available in just 10 minutes, this lateral flow device enables on-site screening of all batches, sending only positives for lab confirmation, cutting costs by up to 90% while ensuring compliance.

Honey Sample Preparation

Weigh 2g honey

↓

Add 4ml distilled water, 0.5ml 1M HCL and 200µl derivatisation reagent

↓

Vortex 3 minutes

↓

Incubate 15 minutes at 60-C

↓

Cool samples, add 5ml 0.1M K2HPO4, 400µl 1M NaOH and 6ml ethyl acetate

↓

Vortex 2 minutes

↓

Centrifuge 10 minutes at 3,000RCF

↓

Transfer 3ml from top layer into glass vial

↓

Evaporate to dryness at 60-C under stream of Nitrogen

↓

Dissolve with 0.5ml n-Hexane and vortex 1 minute

↓

Add 0.5ml 1X Sample Diluent and vortex 1 minute

↓

Centrifuge 2 minutes at 4,000RCF

Innovative Derivatisation reagent

In order to detect nitrofuran metabolites, a derivatisation step is required due to its chemical composition. Traditionally, this is time consuming, with the reaction taking 1 -2 hours. As part of the Flowsense test a lyophilised derivatisation agent has been developed that enables full detection from 15 minute incubation saving significant labour time and reducing time to result.

FlowSense Test Method

Test protocol

Apply 200µl to test microwell and mix 5-8 times

↓

Incubate well for 5 minutes at 25-C

↓

Place Test strip directly into microwell

↓

Incubate well for 5 minutes at 25-C

↓

Read test strip visually or Flowsense reader

Results

Detection limit/CCβ testing

Target Antibiotic	FlowSense Reader LOD (ppb)	Visual Detection LOD (ppb)	CCB achieved (ppb)
Nitrofurantoin (AHD)	0.25	0.5	0.25
Furazolidone (AOZ)	0.25	0.5	0.25
Nitrofurazone (SEM)	0.25	0.5	0.25
Furaltadone (AMOZ)	0.5	0.5	0.5

Table 1: CCβ performance summary of Nitrofuran 4-in-1 test

Figure 2: Flowsense test result screen for AHD

CCβ is a validation process used to confirm a method's ability to reliably detect the target analytes. It involves testing 20 different honey samples, both blank and spiked.

A level is considered acceptable if there is no overlap between the blank and spiked results. This test kit complies with EU regulatory limits for nitrofurans.

Precision

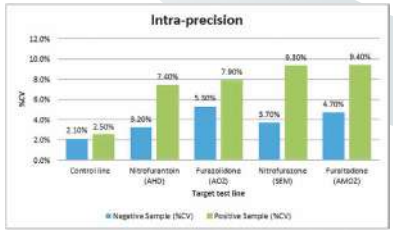

Figure 3: Precision data (%CV) of Nitrofuran 4-in-1 test kit.

Intra-assay precision was evaluated by analysing a single honey sample (blank and spiked) across 20 test strips. The coefficient of variation (CV) for all test lines was below 10%.

FAPAS (Certified Reference Honey)

	Fapas sample 02496	
Target	FAPAS Assigned Concentration/ expected diluted (ppb)	Flowsense Result
AHD	2.1	Positive
	1.05	Positive
	0.53	Positive
	0.26	Positive
	0.13	Negative
	0.07	Negative
AMOZ	4.2	Positive
	2.1	Positive
	1.05	Positive
	0.52	Positive
	0.26	Positive
	0.13	Negative

Table 2: FAPAS test results obtained from Nitrofuran 4-in-1 test

A reference honey sample from FAPAS containing quantified levels of AMOZ and AHD was examined as part of our test validation. This material was serially diluted with a confirmed negative honey sample to evaluate assay performance at very low concentrations.

As expected, the AMOZ and AHD test lines produced positive and negative results at the corresponding dilution points, while SEM and AOZ remained negative since they were absent from the reference material. These results demonstrate excellent correlation of the test with the FAPAS reference sample.

Chloramphenicol FlowSense

Chloramphenicol is a banned antibiotic with zero tolerance limits worldwide, yet it continues to be detected in honey making routine monitoring also essential. Globally, chloramphenicol is often tested alongside nitrofurans due to their shared regulatory importance in export markets. With a limit of detection of 0.1 ppb using the Flowsense reader (0.5 ppb visual), excellent precision (<5% CV) and a rapid result time of 8 minutes, the Chloramphenicol Flowsense provides a complementary solution to the Nitrofuran Flowsense for comprehensive honey safety screening.

Conclusion

The FlowSense 4-in-1 multiplex test provides a rapid, sensitive and dependable method for detecting all four banned nitrofuran metabolites in honey. With a streamlined 15-minute sample Derivatisation and 10-minute assay, it delivers visual results at the EU MRPL of 0.5 ppb and enhanced sensitivity down to 0.25 ppb with the FlowSense reader. High precision and semi-quantitative analysis make it an effective tool for beekeepers, exporters, and regulators to ensure compliance.

Utilising both Chloramphenicol and Nitrofuran Flowsense kits enables rapid on detection of two key banned antibiotic groups in honey, through just 2 tests compared to 5 separate ELISA's.

5 ELISA kits can be replaced by only 2 FlowSense kits

Figure 4: Chloramphenicol Test Strip (BXEFB59A) *Figure 5: Nitrofuran 4-in 1 Test Strip (BXEFB60A)*

Contact Us

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Honey is a widely consumed natural product subject to strict food safety regulations. However, contamination with veterinary drug residues, particularly Nitrofurans, remains a concern. These synthetic antibiotics formerly used in beekeeping, leave metabolites such as Semicarbazide (SEM), 3-amino-2-oxazolidinone (AOZ), 1-aminohydantoin (AHD), and 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), which are genotoxic and carcinogenic. Their use in food production is banned in many regions, but illegal use, cross-contamination, or environmental exposure can still lead to residues in honey. Conventional detection methods, such as LC-MS/MS and ELISA, are accurate but costly, time-consuming and require specialised training. A rapid, cost-effective on-site test is needed to support compliance with food safety standards.

Existing Nitrofuran screening tests are impractical for routine use by beekeepers and small-scale producers. Multiplex screening systems often require expensive analysers, while ELISA methods demand four separate test kits for comprehensive results. A simple, rapid, sensitive, and cost-effective method would improve monitoring and prevent contaminated honey from reaching the market.

Here, we present a multiplex lateral flow device (LFD) developed for detecting four key Nitrofuran metabolites in honey. The test uses specific antibodies with optimised detection chemistry and rapid sample preparation. A 2g honey sample undergoes acid hydrolysis and derivatisation at 60°C for only 15 minutes followed by solvent extraction and evaporation. The reconstituted extract is applied to a supplied microwell, incubated for 5 minutes followed by another 5-minute incubation with the LFD in place. The single test provides a visual result for all 4 antibiotics, with an optional semi-quantitative FlowSense reader measurement.

The test kit exhibits high sensitivity, detecting residues below regulatory limits. The visual detection limit (LOD) was 0.5 ppb, while the FlowSense reader improved detection down to 0.25ppb. Intra-assay precision ranged from 2.1% to 5.3%, confirming reliability. The FlowSense reader also shows potential for future quantitative testing.

This rapid, user-friendly test enables multiplex screening with an assay time of only 10 minutes, reducing reliance on costly lab tests. Beekeepers, exporters, and regulators can use it to ensure compliance, improve food safety, and enhance market confidence in honey products.