GOLD STANDARD DIAGNOSTICS

Patulin Lateral Flow Test Kit Validation

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Patulin Lateral Flow Test Kit Validation

Patulin, a commonly occurring mycotoxin, is a metabolite produced by the fungus *Penicillium expansum* and can occur in infected fruits, grains, and other foods. Its stability in acidic environments (such as in apple juice or cider) is one reason it persists even after processing, making it more difficult to eliminate completely.

As various international studies have proven, this toxin has been linked to a variety of health issues, including gastrointestinal disturbances and potential carcinogenic effects, especially with long-term exposure, and can lead to large economic losses to the food/agriculture industry.

Test kit validation provides assurance of reliability during normal use and is the process of providing documented evidence that the method performs as intended. Validations have been completed to ensure that Gold Standard Diagnostics' ABRAXIS® Patulin Test Strip Kits are accurate, precise, specific, reproducible, and robust.

The performance results below demonstrate

- Sensitivity
- Limit of Quantitation
- Specificity
- Performance near regulatory level
- Lot-to-lot variability
- Correlation to traditional analytical (LC-MS/MS and ELISA) methods

Patulin Test Method

Lateral Flow Test Strip

The test is based on the recognition of Patulin by specific antibodies. The sample to be tested is derivatized and then added to the conical test vial containing specific antibodies for Patulin labeled with colloidal gold. The Patulin conjugate on the strip membrane competes with the Patulin that may be present in the sample for antibody binding sites.

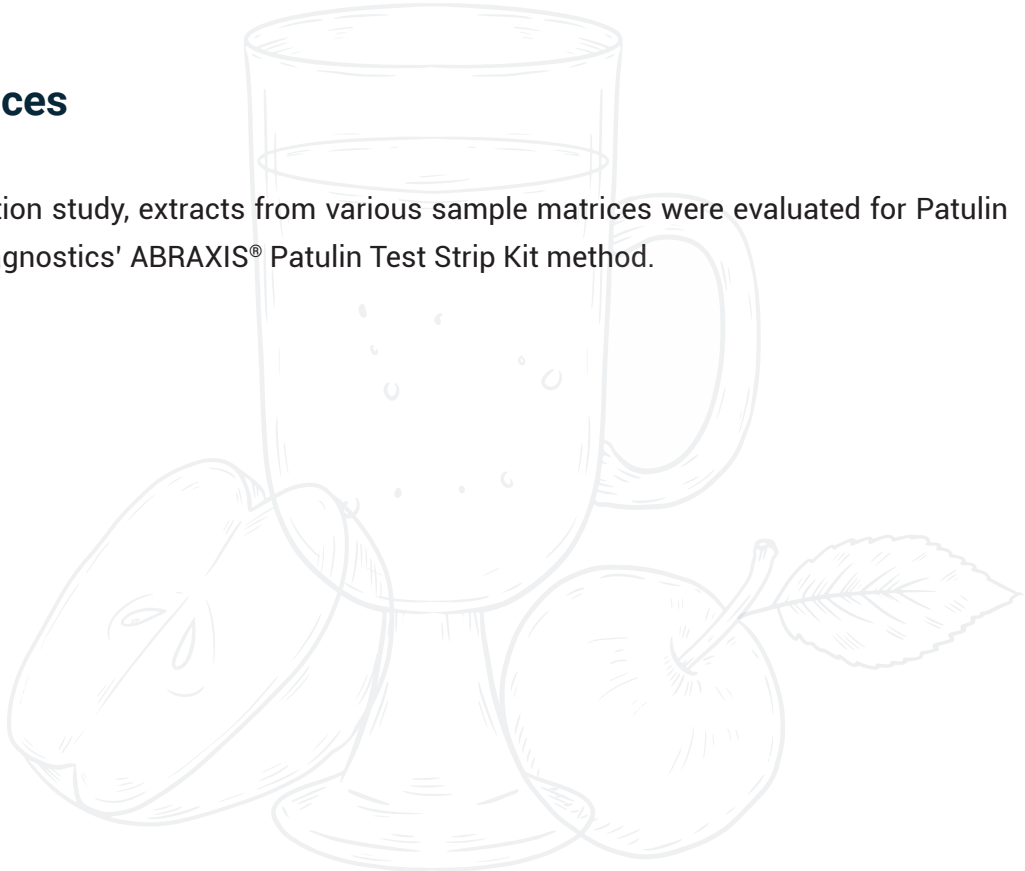
A control line, produced by a different antibody/antigen reaction, is also present on the strip membrane. The control line is not influenced by the presence or absence of Patulin in the sample and, therefore, should be present in all reactions. In the absence of Patulin in the sample, the colloidal gold-labeled antibody complex moves with the sample by capillary action to react with the immobilized Patulin conjugate.
An antibody-antigen reaction forms a visible line in the 'test' area. The formation of two visible lines of similar intensity indicates a negative test result, meaning the test did not detect Patulin or that the Patulin present is below the limit of detection.

If Patulin is present in the sample, it competes with the immobilized Patulin conjugate in the test area for the antibody binding sites on the colloidal gold labeled complex. If a colored line is not visible in the test line region, or if the test line is lighter than the control line, Patulin is present at a detectable level.

Sample Matrices

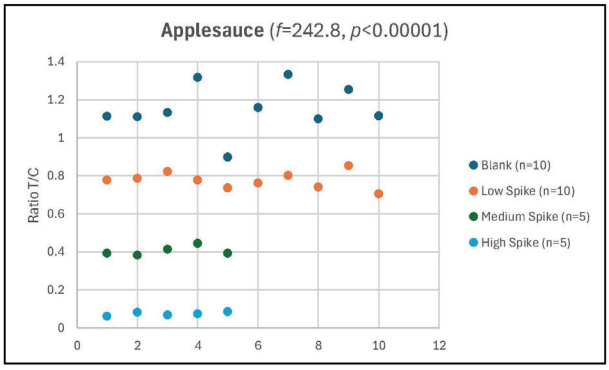
For the performance validation study, extracts from various sample matrices were evaluated for Patulin using the Gold Standard Diagnostics' ABRAXIS® Patulin Test Strip Kit method.

- Apple Juice
- Apple Cider
- Applesauce
- Pear Juice
- Pear Puree

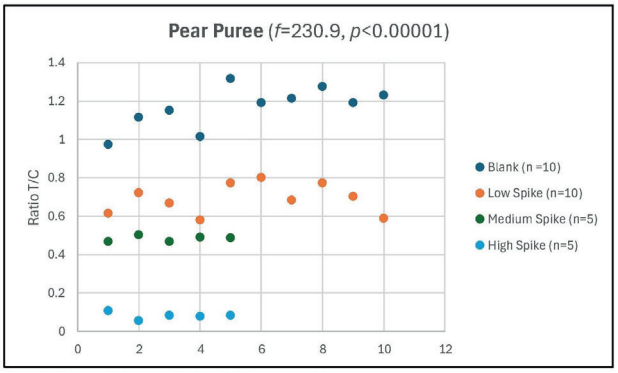


Sensitivity

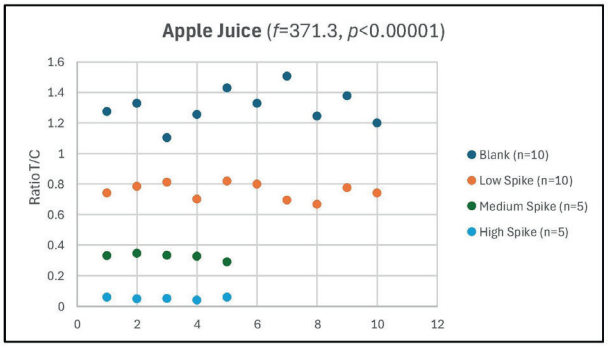
Validated sensitivity values were determined by spiking gravimetric Patulin into residue matrices at the levels indicated below each graph. Ten replicate test portions (n=10) were prepared for the Blank and Low levels, and five replicates (n=5) for the Medium and High levels. All samples were derivatized and analysed using the Gold Standard Diagnostics' ABRAXIS Patulin Test Strip Kit method.



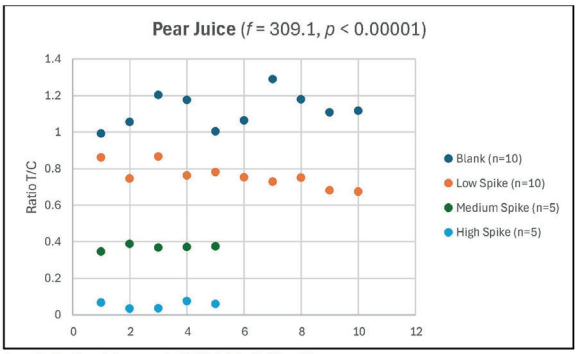
Low Spike 4 ppb in sample (50-fold = 0.08 ppb)
Medium Spike 10 ppb in sample (50 -fold =0.20 ppb)
High Spike 45 ppb in sample (50 -fold = 0.90 ppb)



Low Spike 5 ppb in sample (50-fold = 0.10 ppb)
Medium Spike 10 ppb in sample (50 -fold =0.20 ppb)
High Spike 45 ppb in sample (50 -fold = 0.90 ppb)



Low Spike 8 ppb in sample (100-fold = 0.08 ppb)
Medium Spike 20 ppb in sample (100 -fold =0.20 ppb)
High Spike 90 ppb in sample (100 -fold = 0.90 ppb)



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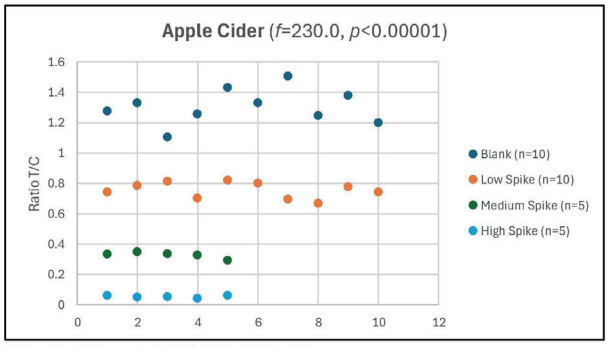

Spike 15 ppb in sample (100-fold = 0.15 ppb)

Results: The ABRAXIS® Patulin Test Strips have a demonstrated sensitivities of 4 ppb in applesauce, 5 ppb in pear puree, 8 ppb in apple or pear juice, 15 ppb in apple cider based on the matrix dilution factor as shown across all tested matrices.

The test line/control line (T/C) values at different concentrations were statistically distinct by Analysis of Variance (ANOVA), confirming the test's robust sensitivity using the F-statistic *(f)* and the probability *(p)* (f = 230.0–371.3, $p < 0.00001$) which indicates that the four groups (blank, low positive, mid positive and high positive) are significantly different.

Specificity

The cross reactivity, to other common mycotoxins/compounds, was tested at the concentrations shown below and presented no false positives.

Interferant	ppb
5-(Hydroxymethyl) Furfural	10000
Aflatoxin B1	10000
Deoxynivalenol	1000
FumonisinB1	500
Ochratoxin A	100

Correlation

The aim of a correlation study is to assess the agreement between the results from the ABRAXIS® Patulin Test Strip Kit method and the results from either ABRAXIS® Patulin ELISA Test Kit or LC-MS/MS methods for the determination of Patulin in apple juice and applesauce.

Apple Juice Samples	T/C ratio (n=5)	% CV (n=5)	Dilution/sensitivity	Strip Interpretation	ELISA result, ppb (n=5)	ELISA % CV
Sample 1	1.63	9.3	100-fold/8 ppb	<8 ppb (negative)	6.3 (negative)	4.6%
Sample 2	1.69	17.4	100-fold/8 ppb	<8 ppb (negative)	7.0 (negative)	11.8%
Sample 3	0.15	13.3	100-fold/8 ppb	20 ppb <C< 90 ppb	43.9 (positive)	5.8%

Applesauce Samples	T/C ratio (n=3)	% CV (n=3)	Dilution/sensitivity	Strip Interpretation	LC-MS/MS, ppb
Sample 4	0.48	11.2	50-fold/4 ppb	< 15 ppb	22
Sample 5	0.11	3.6	50-fold/4 ppb	> 25 ppb	42

Results: A strong correlation was demonstrated between the test strips and ELISA for apple juice and between the test strips and LC-MS/MS for applesauce. All the apple juice samples below the test strip Limit of Detection, LOD (4.0 ppb), which would be reported as non-detect, were confirmed as negative by ELISA analysis. Applesauce and apple juice samples with detectable Patulin tested positive on the test strips and were confirmed by both ELISA and LC-MS/MS, supporting the reliability of the test strip method.

Lot-To-Lot Variation

Lot-to-lot variation is a frequent challenge that limits a user or laboratory's ability to produce consistent results over time. Assuring lot-to-lot consistency is important to a successful testing program. The ABRAXIS® Patulin Test Strips were tested across 3 lots for lot-to-lot variability by testing each of the matrices using blank, low, mid, and high spike samples.

Applesauce 3 lot-averages

Sample	Ratio	T/C, %CV	Dil Fact	LOQ ppb	Control	Control, %CV	Test	Test, %CV
Blank (n=30)	**1.04**	4.9	**50**	**4.0**	311453	8.6	323630	8.9
Low (n=30)	**0.84**	6.1	**50**	**4.0**	341225	11.6	285876	12.3
Med (n=15)	**0.62**	11.9	**50**	**4.0**	354398	5.9	218951	11.4
High (n=15)	**0.19**	34.7	**50**	**4.0**	452399	7.1	85019	30.6

Apple Juice 3 lot-averages

Sample	Ratio	T/C, %CV	Dil Fact	LOQ ppb	Control	Control, %CV	Test	Test, %CV
Blank (n=30)	**0.92**	3.9	**50**	**4.0**	339641	9.5	312135	7.5
Low (n=30)	**0.66**	5.0	**50**	**4.0**	397452	8.2	263581	10.4
Med (n=15)	**0.32**	16.0	**50**	**4.0**	416749	9.4	132162	16.5
High (n=15)	**0.06**	24.2	**50**	**4.0**	484690	8.7	31182	29.1

Apple Cider 2 lot-averages

Sample	Ratio	T/C, %CV	Dil Fact	LOQ ppb	Control	Control, %CV	Test	Test, %CV
Blank (n=20)	**1.16**	4.8	**50**	**4.0**	321070	11.7	373014	15.1
Low (n=20)	**0.73**	9.8	**50**	**4.0**	403084	14.4	290128	6.9
Med (n=10)	**0.32**	18.7	**50**	**4.0**	454585	10.3	142537	11.0
High (n=10)	**0.06**	16.3	**50**	**4.0**	532997	11.4	30182	14.1

Pear Juice 3 lot-averages

Sample	Ratio	T/C, %CV	Dil Fact	LOQ ppb	Control	Control, %CV	Test	Test, %CV
Blank (n=30)	**0.98**	3.9	**50**	**4.0**	303054	8.5	298341	9.7
Low (n=30)	**0.61**	9.5	**50**	**4.0**	385591	6.1	236548	13.7
Med (n=15)	**0.31**	16.5	**50**	**4.0**	396858	9.3	122136	19.3
High (n=15)	**0.06**	21.8	**50**	**4.0**	472385	5.1	26625	23.6

Pear Puree 2 lot-averages

Sample	Ratio	T/C, %CV	Dil Fact	LOQ ppb	Control	Control, %CV	Test	Test, %CV
Blank (n=20)	**0.93**	4.2	**50**	**4.0**	342099	7.0	318456	8.8
Low (n=20)	**0.81**	4.4	**50**	**4.0**	398022	7.0	323204	10.5
Med (n=10)	**0.61**	4.4	**50**	**4.0**	372324	4.1	227858	6.0
High (n=10)	**0.18**	3.8	**50**	**4.0**	474097	6.9	87536	6.6

Results: Lot-to-lot variability across the five tested matrices demonstrated good reproducibility at blank and low spike levels, with a percent coefficient of variation (%CV) values consistently below ~12%. Moderate variability was observed at medium spikes (%CV up to ~20%), which remains acceptable for semi-quantitative testing. At high spike levels, %CV increased in some matrices (up to ~30%), primarily due to very low T/C ratios at signal saturation rather than assay inconsistency. Overall, the assay shows robust lot-to-lot performance across diverse food matrices, supporting its reliability for routine testing.

Performance Near Regulatory Level

For customers wishing to evaluate samples around the regulatory limit, a 0.5 ppb positive control is available (equivalent to 50 ppb in the sample prior to 100-fold dilution). This control enables users to compare their test results directly with a known reference, facilitating an assessment of how their samples align with the regulatory threshold. The performance of the 0.5 ppb control is shown below.

Sample	Control	Test	T/C Ratio	Average T/C	St Dev	% CV
Apple Juice (non-spiked)	241578	376123	1.56			
Apple Juice (non-spiked)	275791	403219	1.46			
n=2				1.51	0.067	4.4
Apple Juice (spike with 50	330639	37836	0.11			
ppb)	393059	46726	0.12			
	384700	46094	0.12			
	384169	41383	0.11			
	380929	38032	0.10			
	456658	74897	0.16			
n=6				0.12	0.022	18.6

Result	T/C Ratio
Blank/ND	0.88 +
Low Positive	0.55 - 0.87
Mid Positive	0.20 - 0.54
High Positive	0.00 - 0.19

ABRAXIS® Patulin Dipstick Test Kits

The ABRAXIS® Patulin Dipstick Test Kits include materials and reagents for sample derivatizing and analysis. Other materials needed are listed below. Contact us to discuss your specific needs and to learn more about available applications.

Product Description	Part Nr.
ABRAXIS® Patulin Dipstick, 5-Test	500130
ABRAXIS® Patulin Dipstick, 5-Test, Starter Bundle For Juice	500132
ABRAXIS® Patulin Dipstick, 5-Test, Starter Bundle For Sauce	500133
ABRAXIS® Patulin Dipstick, 20-Test	500135
ABRAXIS® Patulin Dipstick, 20-Test, Starter Bundle For Juice	500137
ABRAXIS® Patulin Dipstick, 20-Test, Starter Bundle For Sauce	500138

Additional materials not provided with test kits	Part Nr.
ABRAXIS® Patulin Dipstick Control Solution, 1 mL	500140
Heating Block, Digital 115V	709003
Heating Block Accessory, Modular Vial Block	709004
Pocket Scale	709049
Timer	709055
Vortex (for Apple Cider/Apple Juice/Pear Juice)	709045

Starter Bundles include the Heater Block, Modular Vial Block, Timer, Scale and either the Vortex or the Mini centrifuge.

Test Strip Readers

Gold Standard Diagnostics offers the RapidScan ST5-W and AbraScan test strip readers to provide more objective interpretation of test strip results than visually comparing test and control line intensities as an integrated solution to customers.

Part Number	Product Description
475025B	Abrascan® Dipstick Reader III
475035	RapidScan ST5-W Test Strip Reader
475032	RapidScan ST5 ABRAXIS® Strip Cassette

GOLD STANDARD DIAGNOSTICS

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