Patulin ELISA 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. As various international studies have proven, the main route of exposure to Patulin is through the ingestion. Patulin, which poses major health risks to humans and animals, can lead to large economic losses to the food/agriculture industry.

Test Kit Validation

Test kit validation provides an assurance of reliability during normal use and is the process of providing documented evidence that the method does what it is intended to do.

Validations have been completed to ensure that Gold Standard Diagnostics' Patulin ELISA 96 well test kits are accurate, precise, specific, reproducible and robust. The performance results below demonstrate:

- Sensitivity
- Specificity
- Lot-to-lot reproducibility
- Limit of Quantitation
- Correlation to traditional analytical (LC-MS/MS and HPLC) methods

Patulin Test Method

Enzyme-Linked Immunosorbent Assay (ELISA) 96 well plate kit

Patulin ELISA Test Validation

Patulin Test Method

This method is a direct competitive ELISA based on the recognition of Patulin by specific monoclonal antibodies. The standards and samples are derivatized and then analyzed in the ELISA microtiter plate. This method allows for the detection of Patulin between of 7.0 to 300 ppb and including sample preparation, can be performed and results obtained in only 3 hours.

Sample Matrices

For the performance validation study, extracts from various sample matrices were evaluated for Patulin using the Gold Standard Diagnostics Patulin ELISA test kit method.

- Apple Juice
- Apple Cider
- Applesauce
- Orange Juice
- Various fruit purée

Sensitivity

The Patulin ELISA has an estimated limit of detection (LOD) (90% B/B0) of 0.028 ppb (µg/L) or 7.0 ppb after sample dilution. The middle of the test (50% B/B0) is approximately 45 ppb compensating for matrix dilution. Determinations closer to the middle of the calibration curve give the most accurate results.
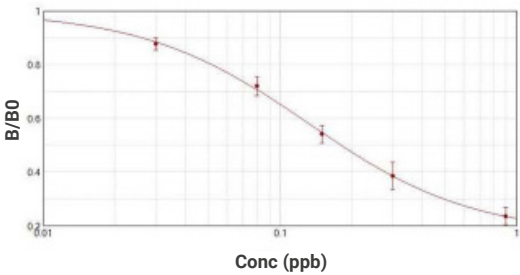
Test reproducibility: Coefficients of variation (CVs) for standards: <10%; for control and samples: <15%

Specificity

The cross reactivity to other common mycotoxins/compounds tested at the concentrations below presented no false positives as shown in the data.

Interferant	ppb	detection
5-(Hydroxymethyl) Furfural	10000	<0.0376
Aflatoxin B1	10000	<0.0375
Deoxynivalenol	1000	0.04
Fumonisin B1	500	0.04
Ochratoxin A	100	<0.0375

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.

A commercially available, apple cider sample was tested alongside Patulin standards and controls to evaluate product consistency through quantitation in two different ELISA test kit lots. All samples, standards and controls were analyzed in duplicate following derivatization per kit instructions.

Results: The % CV for each kit run of standards and controls are statistically consistent between the two kit lots showing excellent reproducibility.

Lot 1

HRP # Groups	600	912	600	912	Mean	%CV
Std #	312	312	320	320		
Control	0.102	0.102	0.088	0.09	0.0955	8%
Apple Cider P9	58.3	62.07	56.18	65.12	65.4175	7%

Lot 2

HRP # Groups	600	912	600	912	Mean	%CV
Std #	312	312	320	320		
Control	0.082	0.09	0.099	0.105	0.094	11%
Apple Cider P9	52.79	58.32	56.88	59.89	56.97	5%

Limit of Quantification (LOQ)

Validated LOQ values were determined by spiking gravimetric Patulin into residue matrix (Apple Juice, Orange Juice and Apple Sauce) to approximate these concentrations. At least ten replicate test portions were derivatized then analyzed by the ELISA method.

Results: All the 10 ppb samples were detected with a % CV less than 20%.

Sample P5

Sample	Blank	Spiked 10 ppb	% recover from Control
	6.8	14.20	135.4
	4.3	14.80	141.1
	6.1	11.10	106.0
	6.0	12.70	121.4
Apple Juice	5.6	14.20	135.1
	6.1	14.50	138.7
	7.0	12.40	118.6
	7.7	12.10	115.0
	6.7	13.70	130.2
	4.8	13.50	128.8
Avg ppb	6.1	13.3	
Stdev	1.020	1.197	
%CV	16.7	9.0	

LOD=7.0 ppb (Based on 90% B/Bo)
LOQ=10.0 ppb

Control spike 10 ppb: 10.48

Sample P11

Sample	Blank	Spiked 10 ppb	% recover from Control
	2.8	12.4	118.8
	2.5	11.8	113.0
	3.7	11.0	105.5
	2.9	11.4	108.9
Orange Juice	2.5	13.1	125.1
	2.7	13.2	126.0
	4.8	11.6	110.6
	3.6	11.5	110.0
	3.4	13.5	128.8
	2.7	12.4	119.0
Avg ppb	3.1	12.2	
Stdev	0.722	0.849	
%CV	23.1	7.0	

LOD=7.0 ppb (Based on 90% B/Bo)
LOQ=10.0 ppb

Control spike 10 ppb: 10.44

Sample P1

Sample	Blank	Spiked 10 ppb	% recover from Control
	3.7	17.3	110.6
	4.0	17.8	113.7
	2.0	14.1	90.2
	2.0	13.9	89.1
Apple Sauce	4.7	18.0	115.0
	4.3	18.0	115.3
	3.1	16.5	105.5
	2.9	13.7	87.6
	4.4	18.2	116.5
	4.0	18.5	118.4
Avg ppb	3.5	16.6	
Stdev	0.969	1.943	
%CV	27.6	11.7	

LOD=7.0 ppb (Based on 90% B/Bo)
LOQ=10.0 ppb

Control spike 10 ppb: 15.64

Patulin ELISA Test Validation

LC-MS/MS and HPLC Correlation

The aim of the correlation study is to assess the closeness of agreement between results from the ELISA Test Kit, LC/MS and HPLC methods for the determination of Patulin in specific matrices. The study was to evaluate the amount of Patulin in different matrix samples by running each sample using the Patulin ELISA Test Kit and LCMS or HPLC.

Results: The data shows good correlation with both LCMS and HPLC performance. All the samples below the ELISA LOD (7.0 ppb) that would be reported as Non-Detect were confirmed with either LC/MS or HPLC. The samples with Patulin presence were confirmed by either LC/MS or HPLC with a Spearman's correlation coefficient of rs = -0.7, p (2-tailed) = 0.19 attesting that instrumental analysis and ELISA are not statistically significantly different.

Type	ELISA ppb	LC/MS ppb
Apple Sauce	<7 ND	<5 ND
Apple Cider P6	<7 ND	<5 ND
Apple Juice	<7 ND	<5 ND
Apple Puree	21.2	19.8

Type	ELISA ppb	LC/MS ppb
Apple Sauce Sample 2	39.5	42
Apple Sauce Sample 3	18.75	22

Matrix	HPLC ppb	ELISA ppb
Apple - Peach Purée (Sample 8)	D <5 ppb	<7 ND
Apple - Pear Purée (Sample 2)	6.41	7.47
Apple - Rapsberry Purée (Sample 1)	<2.5 ND	<7 ND
Apple - Rapsberry Purée (Sample 6)	<2.5 ND	<7 ND
Apple - Rapsberry Purée (Sample 7)	D <5 ppb	8.09
Apple - Strawberry Purée (Sample 5)	<2.5 ND	<7 ND
Apple - Strawberry Purée (Sample 9)	<2.5 ND	<7 ND
Apple Mango Passion Fruit Purée (Sample 3)	5.62	<7 ND
Apple Mango Passion Fruit Purée (Sample 20)	7.31	7.41

Matrix	HPLC ppb	ELISA ppb
Apple Purée (Sample 13)	5	<7 ND
Apple Purée (Sample 16)	D <5 ppb	<7 ND
Apple Purée (Sample 18)	7.6	7.34
Apple Purée (Sample 19)	5.92	7.68
Apple Purée (Sample 21)	6.71	9.18
Apple Purée (Sample 24)	D <5 ppb	8.50
Apple Purée (Sample 26)	<2.5 ND	<7 ND
Apple Purée with Red Fruits (Sample 14)	<2.5 ND	<7 ND
Apricot Apple Purée (Sample 17)	<2.5 ND	<7 ND
Apricot Apple Purée (Sample 22)	<2.5 ND	<7 ND
Apricot Apple Purée (Sample 27)	<2.5 ND	<7 ND

Matrix	HPLC ppb	ELISA ppb
Apricot Apple Purée (Sample 29)	<2.5 ND	<7 ND
Cherry Apple Purée (Sample 25)	<2.5 ND	<7 ND
Chestnut Apple Purée (Sample 15)	D <5 ppb	<7 ND
Chestnut Apple Purée (Sample 23)	D <5 ppb	<7 ND
Crushed Apple (Sample 10)	<2.5 ND	<7 ND
Crushed Apple (Sample 11)	<2.5 ND	<7 ND
Crushed Apple (Sample 12)	<2.5 ND	<7 ND
Letchi Apple Purée (Sample 4)	<2.5 ND	<7 ND
Peach and Apple Purée (Sample 28)	<2.5 ND	<7 ND

Some data was provided in collaboration with NOVAKITS

Manual Test Procedure

Patulin ELISA tests can be run manually with laboratory equipment that includes pipettors and a microplate reader among other.

Part Number	Product Description
500106	Patulin ELISA 96-test kit
500110	Patulin 1X6 ELISA test kit