Measuring Histamine Levels in Food Samples with the Azure Ao Absorbance Microplate Reader

Introduction

Histamine is a vasoactive amine derived from the decarboxylation of the amino acid histidine. It is a mediator of inflammation closely associated with the initial phase of anaphylaxis and is involved in the regulation of gastric acid, the permeability of blood vessels, muscle contraction and brain function. Histamine appears naturally in humans, with the highest concentrations found in the lungs, stomach and skin, and also occurs to various degrees in food. In normal individuals, the enzyme diamine oxidase works to metabolize and balance produced and ingested histamine in the body. However, individuals with reduced diamine oxidase activity may suffer from histamine toxicity resulting from excess ingested histamine.[1] Symptoms of histamine excess may present similarly to allergic reactions – headache, asthma, hypotension, flushing, diarrhea, itching, swelling, and arrhythmia.[2] Histamine-free diets are currently the remedy of choice for those suffering from histamine toxicity[3], and thus, the accurate measurement of histamine levels in various foods is vital to furthering research on histamine toxicity.

This application note describes how the level of histamine can be measured in food samples using an enzyme immunoassay. A microplate coated with a monoclonal antibody to histamine is incubated with several food samples containing unknown concentrations of histamine. Samples are mixed with an enzyme conjugate of horseradish peroxidase-labeled histamine creating a competitive environment. During incubation, the HRP-labeled histamine competes with the histamine in the food samples for binding sites on the anti-histamine coated plate. The bound conjugate is then detected using a TMB substrate that reacts with the horseradish peroxidase causing color development that is inversely proportional to the amount of histamine present in the sample. The color development reaction was stopped using 1N hydrochloric acid resulting in a color change from blue to yellow, and the assay was read at 450nm.

Materials and Methods

The Azure Ao Absorbance Microplate Reader was used for all measurement and analysis.

The Histamine EIA Kit (Catalog #FS35) from Oxford Biomedical Research was used to measure histamine levels in biological samples. The kit provided PBS, wash buffer, substrate, histamine conjugate, histamine standards, and a 96-well plate pre-coated with monoclonal anti-histamine antibody. Unknown samples were prepared by diluting food samples in the kit provided PBS.

The food samples tested were dilution series of red wine, apple juice, blueberries, spinach, coffee and walnuts.

The experiment was a competitive ELISA. The plates were pre-coated with anti-histamine antibody and the food samples were incubated in the wells to allow binding of the histamine to the antibody. Horseradish peroxidase (HRP) labeled histamine was added with the samples, creating competition between binding of the labeled histamine conjugate and any unlabeled histamine occurring in the samples. After incubation and washing, the substrate was added to the wells to allow the reaction of the HRP enzyme with the substrate to produce a colored substance. The reaction was stopped using 1N Hydrochloric Acid and the change in color produced a change in absorbance that was read at 450nm with a substrate reference read at 630nm using the Azure Ao Microplate Reader.

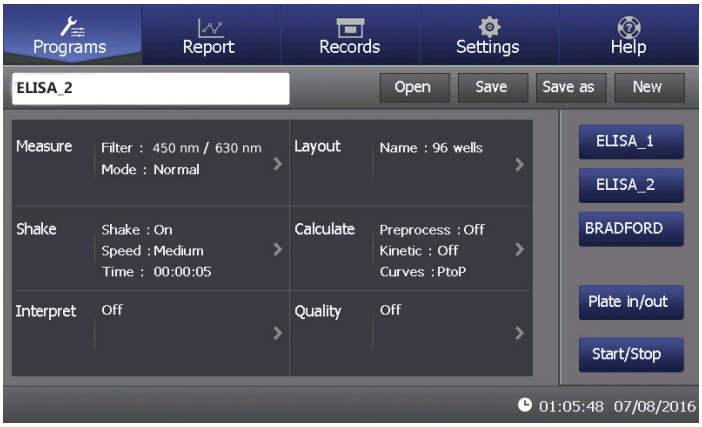

Figure 1. The ELISA_2 pre-programmed protocol was used to read the assay on the Azure Ao Absorbance Microplate Reader.

The histamine concentration of each samples was then measured and analyzed using the calibration curve of histamine standards.

Results and Conclusion

All results of this Histamine detection assay were calculated using the software onboard the Azure Ao Absorbance Microplate Reader. A standard curve was generated by plotting the absorbance values against the assigned concentration values of the kit provided standards. The histamine concentration of the food samples was then automatically calculated by the Azure Ao software.

On the Azure Ao Absorbance Microplate Reader results can be viewed, exported and printed by selecting **ANALYSIS** or **RAW DATA** in the Report tab. Figure 3 shows both raw data and analyzed results interpolated from the standard curve.

	1	2	3	4	5	6	7	8
A	Kit Standard 1		Red Wine 1:100		Blueberries 1:100		Coffee 1:10	
B	Kit Standard 2		Red Wine 1:200		Blueberries 1:200		Coffee 1:20	
C	Kit Standard 3		Red Wine 1:400		Blueberries 1:400		Coffee 1:40	
D	Kit Standard 4		Red Wine 1:800		Blueberries 1:800		Coffee 1:80	
E	Kit Standard 5		Apple Juice 1:100		Spinach 1:100		Walnuts 1:2	
F	Kit Standard 0		Apple Juice 1:200		Spinach 1:200		Walnuts 1:4	
G	Water		Apple Juice 1:400		Spinach 1:400		Walnuts 1:8	
H	Blank		Apple Juice 1:800		Spinach 1:800		Walnuts 1:16	

Table 1. Plate layout.

Figure 2. Setting up the plate and assigning standard values using the Ao software. A) The plate layout interface set to designate where the standards, blank wells, and unknowns are located. Each sample was loaded in duplicate. B) Standard concentration values and units (as provided by manufacturer) were entered into the system in order to generate a standard curve and interpolate sample values.

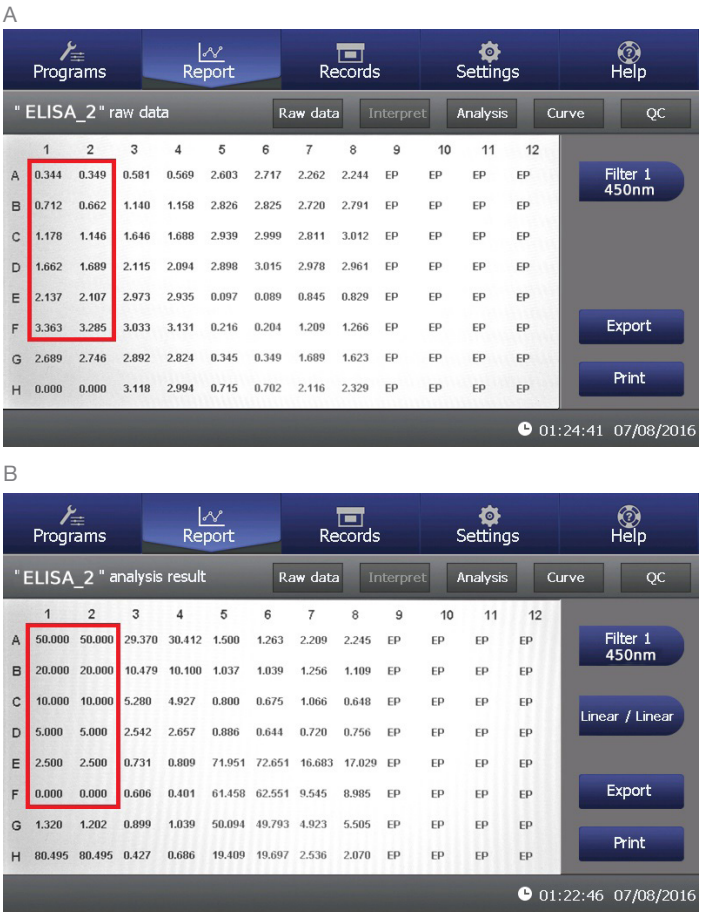

Figure 3. View raw absorbance values, analyzed results and analysis curve easily in the **REPORT** tab. A) Raw absorbance values of the standards and samples. The absorbance values of kit provided standards are shown inside the red box. B) Interpolated concentration values of samples. The standard concentrations are shown inside the red box.

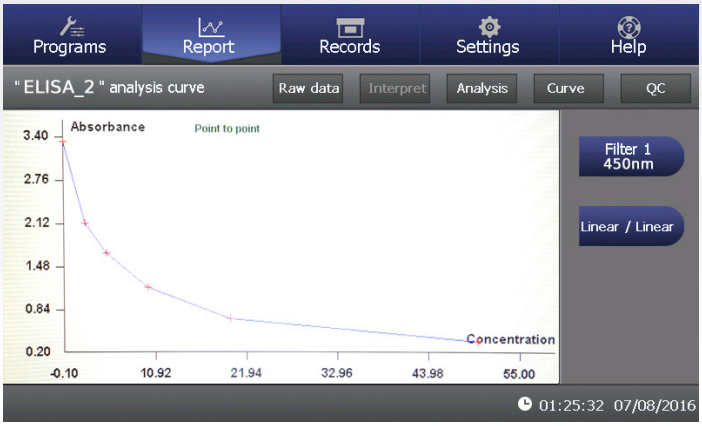

Figure 4. The absorbance values of the standards plotted against the kit designated concentration values.

Samples with calculated concentrations well below the lowest kit standard value of 2.5ng/mL tended to have variable resulting interpolated histamine concentrations, showing that the effective limit of quantification of the Histamine EIA Kit is about 2.5ng/mL. Unsurprisingly, spinach and red wine showed the highest levels of histamine while the lowest levels were present in coffee and walnuts.

Measuring the levels of histamine present in food is made quick and easy using an EIA kit such as the Histamine EIA Kit from Oxford Biomedical Research and the Azure Ao Absorbance Microplate Reader. The 96-well format allows for the testing of multiple samples simultaneously while still allowing for a broad detection range. The Azure Ao Absorbance Microplate Reader makes measurement and analysis easy by creating a standard curve and performing sample calculations automatically.

References

1. Maintz L, Novak N. Histamine and histamine intolerance. *The American Journal of Clinical Nutrition.* 2007. 85:1185-1196
2. Skypala I, Williams M, Reeves L, Meyer R, Venter C. Sensitivity to Food additives, vaso-active amines and salicylates: a review of the evidence. *Clinical and Translational Allergy.* 2015. 5:34
3. Wantke F, Gotz M, Jarisch R. Histamine-free diet: treatment of choice for histamine-induced food intolerance and supporting treatment for chronical heachaches. *Clinical and Experimental Allergy.* 1993. 23:982-985

Sample	Measured Abs.	Calculated Conc.	Dilution Factor	Histamine Conc.
Red Wine 1:100	0.575	29.89	100	2989
Red Wine 1:200	1.149	10.29	200	2058
Red Wine 1:400	1.667	5.104	400	2041
Red Wine 1:800	2.105	2.600	800	2080
Apple Juice 1:100	2.954	0.770	100	77.00
Apple Juice 1:200	3.082	0.504	200	100.7
Apple Juice 1:400	2.858	0.969	400	387.6
Apple Juice 1:800	3.056	0.557	800	445.2
Blueberries 1:100	2.660	1.382	100	138.2
Blueberries 1:200	2.826	1.038	200	207.6
Blueberries 1:400	2.969	0.738	400	295.0
Blueberries 1:800	2.957	0.765	800	612.0
Spinach 1:100	0.093	72.23	100	7223
Spinach 1:200	0.210	62.00	200	12401
Spinach 1:400	0.347	49.94	400	19977
Spinach 1:800	0.709	19.55	800	15642
Coffee 1:10	2.435	2.227	10	22.27
Coffee 1:20	2.756	1.183	20	23.65
Coffee 1:40	2.912	0.857	40	34.28
Coffee 1:80	2.970	0.738	80	59.04
Walnuts 1:2	0.837	16.86	2	33.71
Walnuts 1:4	1.238	9.220	4	36.88
Walnuts 1:8	1.656	5.214	8	41.71
Walnuts 1:16	2.223	2.303	16	36.85

Table 2. Histamine concentration of analyzed food samples in ng/mL.

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