

Proposal Subject:	Thermazyme™ ACP Test
Specific NSSP Guide Reference:	NSSP Section IV Guidance Documents Chapter II. Growing Areas .10 Approved Laboratory Tests
Text of Proposal/ Requested Action	Advanced Instruments, Inc. request ISSC adoption of this method for use in the National Shellfish Sanitation Program
Public Health Significance:	Thermazyme™ ACP Test will provide the basis for determining if shellfish have been thermally processed. This test will allow decisions to be based on a rapid, quantitative method rather than sensory related methods.
Cost Information (if available):	Not available
Action by 2005 Laboratory Methods Review Committee	Recommended the Conference direct the ISSC Executive Office to continue to investigate the issue of standards and pursue the development of standards and report back to the Laboratory Methods Committee with progress on the issue in six (6) months.
Action by 2005 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation for Proposal 05-115.
Action by 2005 General Assembly	Adopted recommendation of 2005 Task Force I.
Action by USFDA	Concurred with Conference action.
Action by 2007 Laboratory Methods Review Committee	Recommended referral of Proposal 05-115 to the Executive Board for consideration for interim approval. Insufficient data at this time to approve this method under Procedure XVI. Need AP curves at 145 for 15 seconds for each type of shellfish.
Action by 2007 Task Force I	Recommended adoption of the Laboratory Methods Review Committee recommendation on Proposal 05-115.
Action by 2007 General Assembly	Adopted recommendation of 2007 Task Force I.
Action by USFDA	December 20, 2007 Concurred with Conference action.
Action by 2009 Laboratory Methods Review Committee	Recommended referral of Proposal 05-115 to the appropriate Committee as determined by the Conference Chairman to review new data as it becomes available.
Action by 2009 Task Force I	Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-115.

- Action by 2009
General Assembly** Adopted recommendation of 2009 Task Force I on Proposal 05-115.
- Action by USFDA
02/16/2010** Concurred with Conference action on Proposal 05-115.
- Action by 2011
Laboratory
Methods Review
Committee** Recommended referral of Proposal 05-115 to the appropriate committee as determined by the Conference Chairman to continue the validation of the Thermazyme ACP Test for possible use in the NSSP. LMRC further Recommended the information requested by the testing lab and Advanced Instruments for validation be submitted within 6 months to be considered as an emerging method.
- Action by 2011
Task Force I** Recommended adoption of Laboratory Methods Review Committee recommendation on Proposal 05-115.
- Action by 2011
General Assembly** Adopted recommendation of 2011 Task Force I on Proposal 05-115.
- Action by FDA
February 26, 2012** Concurred with Conference action on Proposal 05-115.



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June 25, 2004

Ken Moore
Executive Director
Interstate Shellfish Sanitation Conference
209-2 Dawson Drive
Columbia, SC 29223

Dear Mr. Moore:

Due to the advice of the USFDA Office of Seafood, I am writing this letter to request a review and approval of the Thermazyme™ ACP Test for use on thermally processed (cooked versus raw) shellfish products in order to make decisions based on a rapid, quantitative method rather than sensory related methods.

I have enclosed some literature and materials to demonstrate how the Fluorophos® ALP Test and Thermazyme ACP Test have brought value to the dairy and meat processing industries as an assessment tool for determining lethality of the kill step and finished product analysis.

Catherine Cutter, Ph.D documents the current situation in the seafood industry and has scientifically demonstrated that the Thermazyme ACP Test could also be utilized to advance the cause of food safety, thereby protecting consumers by minimizing the potential of under processed products making it into distribution channels.

Please have this method reviewed and approved for its use by seafood processors and agencies interested in maintaining the highest level of public safety.

I will be out of the office from June 28-July 6th. For assistance you may contact Eileen Garry, R&D Lab Manager, Advanced /Instrument, Inc. at 781-320-9000 X2118 or email eileeng@aicompanies.com or Gary Wolf, Regional Shellfish Specialist, FDA Office of Seafood, Vorhees, NJ, at 856-783-1420 X13 or Email - gwolf@ora.fda.gov.

I look forward to speaking with you about this exciting opportunity for the industry and thank you for your attention to this important development.

Sincerely,


Kenneth F. Micciche
Director of Marketing
Advanced Instruments, Inc.
Office – 781-471-2145
Facsimile 781-320-8181
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TECHNICAL REPORT

NUMBER: TR203

DATE: 04 May 24

TITLE: Performance characteristics of the ThermaZyme® acid phosphatase ("ACP") measurement system on seafood.

AUTHOR: R. A. LaBudde

ABSTRACT: Data from a recent study of the use of the ThermaZyme® acid phosphatase measurement system on seafood was analyzed to assess relevant performance characteristics such as accuracy and precision, false positive and false negative error rates and other parameters. Although the data in the study were limited, some quantitative assessment of these parameters was possible.

KEYWORDS: 1) THERMAZYME 2) ACP 3) EPT

REL.DOC.:

REVISED: 04 May 28

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INTRODUCTION

The use of heat-labile enzymes in the determination of cook endpoint temperatures has a long history in the food industry [1-13]. Heat lethality in bacteria is believed to be due primarily to denaturation of cellular enzymes, so verification of destruction of such enzymes is highly correlated to bacterial destruction.

Catalase (meat), alkaline phosphatase (milk) and acid phosphatase (various foods) have been used as surrogates to verify post-process that adequate pasteurization has taken place [2-12].

The ThermaZyme® system, distributed by Advanced Instruments, is based on the fluorometric measurement of acid phosphatase (“ACP”) enzyme. Several validation studies have been published for the system involving a variety of food products.

Recently, a study by Cutter and Miller [1] has investigated ACP for endpoint temperature verification in seafood.

BACKGROUND ASSUMPTIONS RELATED TO ACP IN SEAFOOD

The performance characteristics and inferences with respect to ACP in seafood are based entirely on the work done by Cutter and Miller [1]. In particular, the population of inference is limited to those sources of supply locally available to these authors. However, in recommended use, the method may be calibrated using samples of control raw material and cook EPT determination to establish a standard curve for the relevant population of inference.

PERFORMANCE CHARACTERISTICS IN SEAFOOD

The ThermaZyme system may be used to verify endpoint temperatures in two different ways:

1. **INFERENCE:** Was the food processed to a specific minimum endpoint temperature (“EPT”) or higher?
2. **ESTIMATION:** What was the highest equivalent endpoint temperature to which the food was exposed?

A. ACCURACY:

The ThermaZyme Test System can detect as low as 0.1 U/kg of sample, based on a 1:3 initial dilution.

Based on the Cutter and Miller data [1], estimation of endpoint temperature is subject to the following precisions, based on each test comprising the average of 5 replications:

PRECISION OF ENDPOINT TEMPERATURE			
<i>Seafood</i>	<i>Range</i>	<i>Standard error of fit</i>	<i>95% Confidence Interval of EPT</i>
Clams	130-165 F	9.1 F	+/- 8.6 – 14.9 F
Lobster	140-165 F	5.4 F	+/- 6.1 – 13.9 F
Oysters	140-175 F	3.8 F	+/- 3.4 – 5.9 F
Shrimp	140-165 F	5.4 F	+/- 6.1 – 13.9 F

B. SPECIFICITY:

For the inference that raw seafood has been cooked to a specified minimum EPT:

RAW SEAFOOD COMPARED TO MINIMUM ENDPOINT TEMPERATURE		
<i>Seafood</i>	<i>Minimum EPT</i>	<i>False Positive Rate</i>
Clams	130 F	0.0064%
Lobster	140 F	0.0987%
Oysters	150 F	1.7385%
Shrimp	140 F	0.3711%

C. PRECISION:

Based on the Cutter and Miller data [1], estimation of endpoint temperature is subject to the following precisions, based on each test comprising the average of 5 replications:

PRECISION OF ENDPOINT TEMPERATURE			
<i>Seafood</i>	<i>Range</i>	<i>Standard error of fit</i>	<i>95% Confidence Interval of EPT</i>
Clams	130-165 F	9.1 F	+/- 8.6 – 14.9 F
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Shrimp	140-165 F	5.4 F	+/- 6.1 – 13.9 F

D. SENSITIVITY:

The ThermoZyme Test System can detect as low as 0.1 U/kg of sample, based on a 1:3 initial dilution.

E. SELECTIVITY:

The test is specific for the ACP enzyme involved and has no interferences from other compounds.

F. ASSAY INTERVAL:

Each test involves comminution of the bulk sample, possible draining, weighing of a 0.8 g specimen, dilution with standard reagents, homogenization and measurement in the fluorometer. Total time expended per sample is less than 10 minutes for one replicate and an additional 5 minutes for each further replicate.

G. ASSAY COST:

Reagent costs per replicate are approximately \$3.00 with approximately 1/6 hr of analyst time.

H. COMPARABILITY:

Alternative methods of verifying EPT are limited. The most obvious being Aerobic Plate Count ("APC") microbial determination. In this case, the analysis cost is approximately \$1.00-\$2.00 in supplies and 1/6 hr of analyst time per replicate. For viral determinations, the cost would be significantly higher (\$30-\$100).

I. OTHER STUDIES:

See references [2-13] for studies based on acid or alkaline phosphatase as a means of cook endpoint temperature determination in various meat and dairy products.

J. REGULATORY APPROVALS:

1. AOAC First Action, 1991.
2. AOAC Final Action, 1995. Method 979.13.
3. International Dairy Federation, 1992.
4. Interstate Milk Shippers, 1993.
5. ISO/DIS 11816-2, 2001.
6. FDA, 1995. (Cheese)
7. NCIMS, 2001. (Cream)

REFERENCES

1. Cutter CN and Miller BJ. 2003. Use of an acid phosphatase assay to detect deviations in thermal processing of seafood. *J Assoc Food and Drug Officials* 67(4):1-14.
2. Davis CE. 1998. Fluorometric determination of acid phosphatase in cooked, boneless, nonbreaded broiler breast and thigh meat. *J AOAC Inter* 81(4):887-906.
3. Davis CE and Townsend WE. 1994. Rapid fluorometric analysis of acid phosphatase activity in cooked poultry meat. *J Food Prot* 57:1094-1097.
4. Lyon BG et al. 2001. Acid phosphatase activity and color changes in consumer-style griddle-cooked ground beef patties. *J Food Prot* 64:1199-1205.
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6. Wilson CE et al. 2002. Influence of *Listeria* spp. contamination on end-point temperature determination in broiler breast patties by a fluorometric acid phosphatase assay.
7. Cohen EH. 1969. Determination of acid phosphatase activity in cooked hams as an indicator of temperature attained during cooking. *Food Tech* 23:101-104.
8. Kormendy L et al. 1987. Determination of the extent of heat treatment in canned hams by use of the phosphatase test. *Meat Sci* 19:77-?.
9. Lint J. 1965. The determination of the acid phosphatase activity in canned hams. Report 25/65A, Danish Meat Products Laboratory, Royal Veterinary and Agricultural College, Denmark.
10. Rocco RM. 1990. Fluorometric analysis of alkaline phosphatase in fluid dairy products. *J Food Prot* 53:588-591.
11. Rocco RM. 1990. Fluorometric determination of alkaline phosphatase in fluid dairy products: collaborative study. *J AOAC* 73:842-849.
12. Anonymous. 1986. Determination of internal cooking temperature (acid phosphatase activity). Revised Basic Chemistry Laboratory Guidebook (rev. March 1986), USDA-FSIS No. 3.018:3-49.
13. Jones DR et al. 2002. Variations in levels of acid phosphatase present in chicken whole leg meat. *Poultry Sci* 81(10):1567-1570.