

Microbiological Method Validation Guidelines and Claim Verification in Selected Foods – Pet Foods

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Introduction



- Microbiological methods for detection of pathogens can be validated by a variety of approaches
 - In the US, AOAC's Official Methods of Analysis (OMA) and Performance Tested Methods (PTM) are generally recognized by food industry

Introduction



- Guidelines define requirements for study parameters allowing for validation of new microbiological detection methods
 - However there are many different guidelines; e.g.; AOAC, ISO 16140, FDA, NMKL, USDA, Health Canada and other governmental bodies

Introduction



- Regardless of the validation, each approach evaluates selected matrices and the claim for validation only covers these selected matrices
 - A variety of matrices within a food category may be evaluated
 - Although, it is impossible to validate all types of products within a food category
 - A verification study is required for detection of *Salmonella* when a method has thoroughly studied a food category and testing as an extension of this category is required

Background



- There has been recent agreement in stakeholders to replace “All Foods” with the claim “Broad Range of Foods”
 - This agreement along with verification of methods by the user confirm that the method will be applicable to the matrix

What is the Study Design to Use?



	Validation	Verification
Number of Replicates	20	7
Inoculation Level	Low (1-5 cells/sample) Target “fractional positive”	Medium (<30 cells/sample) Expected positive
Interpretation	Probability of Detection (POD) Statistics	Pass = all 7 positive Fail = <7 positive
Objective	Method does not have validation for product category Example: Pet Food Category is not in the claim.	Method is validated for product category of interest Need for extension evaluation (i.e., pet food is validated at 375 g, verification necessary for different formulation)

Four Examples of Detection of *Salmonella* in Pet Foods



- bioMérieux VIDAS UP *Salmonella* (SPT)
- Dupont Real Time BAX System Assay
- Roka Bioscience Atlas SEN Detection Assay
- bioMérieux GENE-UP *Salmonella*

VIDAS UP *Salmonella* (SPT)



- bioMérieux validated detection of *Salmonella* by VIDAS UP (OMA 2013.01) in dry pet food using their VIDAS SPT method at 1:4 dilution in 375 g test portions
 - Reported examples of product absorbing a great amount of media liquid during enrichment so further studies were conducted at 1:4.6 ratio
- Results from their internal study (dated April 1, 2014) showed detection of *Salmonella* with 1:4.6 media ratio
 - Additional media provided a great amount of available enrichment broth with 1:4.6 ratio to perform the test

Verification Study – VIDAS UP



- Study design

- 375 g test portion
- 7 replicate samples
 - Biscuit
- Inoculated at target level of <math><10</math> cells / test portion (375 g)
- Enriched in 1:4.6 media dilution ratio
 - 1350 mL of BPW with supplement
- Enriched in 1:5 media dilution ratio
 - 1500 mL of BPW with supplement
- Incubated at 41-43°C for 22 - 26 h



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	<u>1:4.6 Dilution</u>		<u>1:5 Dilution</u>	
	375 g enriched in pre-warmed 1350 mL BPW + 5 mL of supplement incubated at 42°C for 22-26 h		375 g enriched in pre-warmed 1500 mL BPW + 6.67 mL of supplement incubated at 42°C for 22-26 h	
Replicate	Assay	Culture Confirmation	Assay	Culture Confirmation
1	<i>Not Tested*</i>	<i>Not Tested</i>	+	+
2	<i>Not Tested</i>	<i>Not Tested</i>	+	+
3	<i>Not Tested</i>	<i>Not Tested</i>	+	+
4	<i>Not Tested</i>	<i>Not Tested</i>	+	+
5	<i>Not Tested</i>	<i>Not Tested</i>	+	+
6	<i>Not Tested</i>	<i>Not Tested</i>	+	+
7	<i>Not Tested</i>	<i>Not Tested</i>	+	+
# of Positives	NA	NA	7	7

Verification Study – VIDAS UP



- Study design

- 3 different pet food products at 375 g test portion
- 7 replicate samples
- Inoculated at target level of approx. 10 cells / test portion (375 g)
- Enriched in 1:4.6 media dilution ratio
 - 1350 mL of BPW with supplement
- Incubated at 41-43°C for 22 - 26 h



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VIDAS UP *Salmonella* (SPT)

Detection of *Salmonella* in Three Other Pet Food Products



1:4.6 dilution

375 g
enriched in
pre-warmed
1350 mL
BPW
+ 5 mL of
supplement
incubated
at 42°C for
22-26 h

	Product #1		Product #2		Product #3	
Replicate	Assay	Culture Confirmation	Assay	Culture Confirmation	Assay	Culture Confirmation
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
# of Pos.	7	7	7	7	7	7

VIDAS UP *Salmonella* (SPT)



- It is recommended to use 1:5 media ratio for pet foods analyzed by bioMérieux's VIDAS UP SPT
 - Use up to 1:8 when analyzing 375 g test portions if the laboratory is observing issues with consistency

Dupont RT BAX *Salmonella*



- Dupont validated detection of *Salmonella* by Real Time PCR BAX System (OMA 2013.12) in dry pet food (375 g) at 1:10 media dilution
- Dupont also showed with internal studies using their previous method (*Salmonella* BAX) that detection of *Salmonella* in dry pet food, pig ears and rawhide was effective with 1:10 dilution media enrichment
 - Dupont internal technical papers dated 11/29/10, and papers numbered 2-1096-1011, 2-1088-0810, 2-1094, 0811
 - With this validation and subsequent internal work, further study within this category at 1:10 dilution was recommend by verification studies

Verification Study – Dupont BAX



- Study design

- 7 replicate samples
 - Same as VIDAS UP study (Biscuit plus 3 other pet food products)
- Inoculated at target level of approx. 10 cells / test portion (375 g)
- Enriched in 1:10 media dilution ratio
 - 3375 mL of prewarmed BPW
- Incubated at 34-36°C for 22 - 26 h

Dupont BAX *Salmonella*

Detection of *Salmonella* in a Pet Food Biscuit



	<u>Biscuit</u> <u>1:10 Dilution</u> 375 g enriched in pre-warmed 3375 mL BPW incubated at 35°C for 22-26 h	
Replicate	Assay	Culture Confirmation
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	+	+
# of Positives	7	7



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Dupont BAX *Salmonella*

Detection of *Salmonella* in a Three Pet Food Products



1:10 dilution

375 g
enriched in
pre-warmed
3375 mL
BPW
incubated at
35°C for
22-26 h

	Product #1		Product #2		Product #3	
Replicate	Assay	Culture Confirmation	Assay	Culture Confirmation	Assay	Culture Confirmation
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+
5	+	+	+	+	+	+
6	+	+	+	+	+	+
7	+	+	+	+	+	+
# of Pos.	7	7	7	7	7	7



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Dupont RT BAX *Salmonella*



- It is recommended to use 1:10 media ratio for pet foods analyzed by Dupont RT BAX
 - Reduced media enrichment ratios will require further study for 375 g test portions



- Roka validated detection of *Salmonella* by Atlas SEN Salmonella Assay (PTM 031201)
 - Dry pet food (25 g) at 1:10 dilution
- Demonstrated the detection of *Salmonella* in 375 g test portions
 - Contracted study



- Study design

- 375 g test portion
- 20 replicate low level inoculated samples
 - Target level 1-2 cells per test portion
- 5 replicate high level inoculated samples
- 5 replicate uninoculated (control) samples
- Enriched in 1:6 media dilution ratio (1875 mL BPW)
- Enriched in 1:10 media dilution ration (3375 mL BPW)
- Incubated at 42°C for 18, 20 and 24 h

Roka Bioscience Atlas *Salmonella*

Detection of *Salmonella* in a Pet Food Kibble



Pet Food Kibble Media Dilution Ratio	Incubation Time at 42C	ATLAS	Reference	POD*
1:6 dilution	18 h	13	13	Pass
	20 h	13	13	Pass
	24 h	13	13	Pass
1:10 dilution	18 h	12	12	Pass
	20 h	12	12	Pass
	24 h	12	12	Pass

* No significant difference for detection of target organism using Atlas as compared to reference method (POD = Probability of Detection)



- It is recommended to use as low as 1:6 media ratio for pet foods analyzed by Roka ATLAS
 - Reduced media enrichments were studied for 375 g test portions and showed no significant differences in detection of *Salmonella* as early as 18 h.



- bioMérieux validated detection of *Salmonella* GENE-UP *Salmonella* Assay (PTM 061504)
 - Dry pet food (25 g) at 1:10 dilution
- Demonstrated the detection of *Salmonella* in 375 g test portions
 - Contracted study



- Study design
 - 375 g test portion
 - 20 replicate low level inoculated samples
 - Target level 1-2 cells per test portion
 - Two media dilution ratios
 - 1:4 media dilution ratio (1125 mL BPW)
 - Incubated 42C for 18 h
 - 1:6 media dilution ratio (1875 mL BPW)
 - Incubated 42C for 18, 20 and 22 h
 - Two comparison methods
 - Enriched in 1:10 media dilution ration (3375 mL BPW)
 - Incubated at 35°C for 18 h (method 1) and 22 h (method 2)

bioMérieux GENE-UP *Salmonella*

Detection of *Salmonella* in a Pet Food Product



Pet Food Matrix Media Dilution Ratio	Incubation Time	GENE-UP (42°C)		Method 1 (1:10 ratio; 35°C)	Method 2 (1:10 ratio; 35°C)
1:4 dilution	18 h	13		14	19 (22h)
1:6 dilution	18 h	15		14	<i>Not applicable</i>
	20 h	15		<i>Not applicable</i>	<i>Not applicable</i>
	22 h	15		<i>Not applicable</i>	14

* Number of positive assays out of 20 replicate samples inoculated at a low level to achieve partial positive results.



- It is recommended to use as low as 1:6 media ratio for pet foods analyzed by GENE-UP
 - Reduced media enrichments were studied for 375 g test portions and showed detection similar to 2 other methods on the market as early as 18 h.

Conclusions



- Pet foods can vary in complexity
 - Fat
 - Inhibitory substances
- Increased test portion size and reduced media enrichment ratios add to this complexity
- Complexity can affect ability of a validated method to detect target organisms
 - A method may have enhanced performance for a particular sample and may be incompatible with a particular matrix or set of parameters
- Verification studies allow for laboratories to understand method performance in specific food types
 - Small changes to a method allow for detection
 - Changes require validation

Conclusions



- “All Foods” claims can be misleading
 - Recommend the use of “Broad Range of Foods” to represent food categories
 - Validated method claims need to specify the foods claimed
- Harmonization of food category tables among organizations will help minimize confusion
- Laboratories need to validate methods for new matrices (extension of foods originally studied)
 - Begin with small studies to understand
 - Validation studies to be completed

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