

**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

SOP No.: MDP-QA-03		Page 1 of 6
Title: Quality Assurance (QA) Controls		
Revision: 01	Replaces: 05/15/04	Effective: 10/01/04

**1. Purpose:**

To provide minimum requirements and standard procedures for quality assurance (QA) controls used in the USDA/AMS Microbiological Data Program (MDP).

**2. Scope:**

This standard operating procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program.

**3. Principle:**

QA controls have known values that ensure the accuracy and reliability of a test system. MDP QA controls consist of an uninoculated media control, a negative cultural control, a positive cultural control, and a positive produce control for each batch/set of samples analyzed by each test method.

QA controls are expected to exhibit known well-characterized results. If a QA control does not exhibit the expected result, that control does not meet the MDP acceptability criteria and is considered unacceptable.

If any control yields an unacceptable result, appropriate investigative/re-testing measures, as outlined in subsection 6.4 of this SOP, must be taken. If a control result is unacceptable for either the original or rerun analysis, the Monitoring Programs Office (MPO) shall be notified. Results associated with unacceptable controls shall be appropriately coded in Remote Data Entry (RDE) as described in subsection 6.5 of this SOP.

**4. Outline:**

Controls Required per Set	Section 6.1
Procedures for Handling QA Controls for All PCR-Based Methods	Section 6.2
Acceptability Criteria for QA Controls	Section 6.3
Response to Failure to Meet Acceptability Criteria	Section 6.4
Reporting Data Associated with Failed Controls	Section 6.5

**5. References:**

- 5.1** Attachment 1, Current and Historical QA Control Strain Information
- 5.2** Attachment 2, QC Control Failure Reporting Form



**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

SOP No.: MDP-QA-03		Page 2 of 6
Title: Quality Assurance (QA) Controls		
Revision: 01	Replaces: 05/15/04	Effective: 10/01/04

- 5.3 Memorandum, Requirement to Notify MPO of QA Control Failure, February 3, 2004
- 5.4 Memorandum, Quality Assurance Control Requirements, January 21, 2004
- 5.5 ISO/IEC 17025 Guidelines
- 5.6 A2LA Food Microbiology Program Requirements, subsection 5.10, Reporting the Results, June 2001

**6. Specific Procedures:**

This SOP represents minimum MDP requirements and is presented as a general procedure. Each laboratory shall have written operating procedures that provide specific details concerning the manner in which the procedures have been implemented in that laboratory.

**6.1 Controls Required per Set**

- 6.1.1 Each analytical batch/set of samples shall include an uninoculated media control, a negative cultural control, a positive cultural control, and a positive produce control for each method used to test that batch/set of samples. Refer to Attachment 1 for control strain characteristics.
- 6.1.2 The uninoculated media control is intended to demonstrate the sterility of the medium and the results also may be used as a baseline within the analytical system.
- 6.1.3 The negative cultural control is intended to demonstrate suitable microbial conditions for growth, but differing biochemical reactions than the target organism in a given environment.
- 6.1.4 The positive cultural and positive produce controls are intended to reflect the expected behavior of a target organism in a given environment (e.g., substrate, temperature, pH) within the analytical system. An additional intent of the positive produce control is to demonstrate no inhibitory effects from the produce.
- 6.1.5 Characteristics of control strains are detailed in Attachment 1, Current and Historical QA Control Strain Information.

**6.2 Procedures for Handling QA Controls for All PCR-Based Methods**

- 6.2.1 Separate areas for pre- and post-polymerase chain reaction (PCR) work are essential. These areas must be separated from general microbiological work.
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**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

SOP No.: MDP-QA-03		Page 3 of 6
Title: Quality Assurance (QA) Controls		
Revision: 01	Replaces: 05/15/04	Effective: 10/01/04

DNA extraction procedures should be performed in a PCR chamber or in a Biosafety cabinet if possible.

- 6.2.2** To avoid cross-contamination, transfer of cultures and DNA should be performed with extreme care.

**6.3 Acceptability Criteria for QA Controls**

QA controls are expected to exhibit known values as specified in Attachment 1. If a QA control does not exhibit the expected result, that control does not meet MDP acceptability criteria and is considered unacceptable.

**6.4 Response to Failure to Meet Acceptability Criteria**

- 6.4.1** If any of the controls (media, negative cultural, positive cultural, or positive produce control) fail to yield the expected results, the situation must be investigated.
- 6.4.2** If the problem is easily identified (e.g., typographical error), it should be corrected. If the problem is not readily identified or able to be corrected, that batch of samples must be re-analyzed. At this point, MPO must be notified (refer to Attachment 2, QC Control Failure Reporting Form).
- 6.4.3** For all MDP procedures, the appropriate sample wash/broth or aliquot of the wash/broth for each test must be saved under refrigeration; each laboratory should determine the best method for accomplishing this requirement (e.g., transfer to sterile centrifuge tube). Discard samples only after controls have yielded satisfactory results.
- 6.4.4** The recommended timeframe for investigation/re-testing is 24-48 hours. It is recognized that there could be a change (e.g., growth of bacteria) in the sample wash/broth; however, re-testing is required and re-sampling is not acceptable.
- 6.4.5** Contact MPO for further guidance immediately if control results for the re-analysis are again unacceptable. For methods using automated PCR instrumentation, MPO may require further investigation (e.g., instrument parameters, melting curves, gel electrophoresis data).
- 6.4.6** All corrective actions taken as described in the preceding subsections (6.4.1 through 6.4.5) must be properly documented in internal records and the MDP RDE system.
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**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

SOP No.: MDP-QA-03		Page 4 of 6
Title: Quality Assurance (QA) Controls		
Revision: 01	Replaces: 05/15/04	Effective: 10/01/04

**6.5 Reporting Data Associated with Failed Controls**

- 6.5.1** Results for each of the media, negative, positive, and positive produce controls shall be reported as acceptable or unacceptable on the QA Results screen in RDE. **Note:** the current RDE system does not contain a separate field for the positive produce control. Results and any comments for the positive produce control should be entered into the comments field.
  - 6.5.2** For controls yielding acceptable results for the initial testing, the results shall be reported as “acceptable.”
  - 6.5.3** For controls yielding acceptable results for a rerun test triggered by initially unacceptable results, the result shall be reported as “acceptable.” In the comments field enter “re-tested” and any additional details.
  - 6.5.4** For controls yielding unacceptable results for a rerun test, the result shall be reported as “unacceptable.” In the comments field enter “re-tested” and any additional details.
  - 6.5.5** Data for samples associated with unacceptable controls will be excluded from the MDP central database.
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**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

SOP No.: MDP-QA-03		Page 5 of 6
Title: Quality Assurance (QA) Controls		
Revision: 01	Replaces: 05/15/04	Effective: 10/01/04

*Cindy Koschmann*

*09/24/04*

Approved by: Cindy Koschmann

Date

MDP Technical Advisory Committee

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**United States Department of Agriculture  
Agricultural Marketing Service, Science & Technology  
Microbiological Data Program**

SOP No.: MDP-QA-03		Page 6 of 6
Title: Quality Assurance (QA) Controls		
Revision: 01	Replaces: 05/15/04	Effective: 10/01/04

Revision 01

September 2004

MPO

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- Revised/condensed Excel attachments into one document
  - Removed NSL as only reference laboratory
  - Replaced specific references to BAX with generic terminology to apply to other DNA-based techniques
  - Added option to save broth instead of wash
  - Added media and positive produce controls to response and RDE reporting requirements subsections 6.4 and 6.5
  - Revised RDE comments required to reflect current RDE reporting system
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### Current QA Control Strain Information

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
<b>E. coli MPN MTH-01</b>	<b>Positive</b>	<b>MDP-001</b>	E. coli 0137:H41/pKt-Kan GFP  Strain MW421 USDA/ARS/PW #RM2375	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey	dark pink	Lac(+)	positive
				EMB agar	green sheen, dark center	Lac(+)	positive
				NA+kan	growth	pres of plasmid kan resistance	positive
NA, use of UV light	fluorescence	GFP expression	positive				
<b>E. coli MPN MTH-01</b>	<b>Negative</b>	<b>MDP-003</b>	Enterobacter aerogenes ATCC 13048 AND 35029	Chromagar	colorless	MUG(-)	negative
				LST MUG	No blue fluorescence	MUG(-)	negative
				MacConkey EMB agar	light pink light green	Lac variable Lac variable	
<b>Salmonella VIDAS MTH-02</b>	<b>Positive</b>	<b>MDP-002</b>	Salmonella enterica serovar poona/pKT-kan-GFP CDHS/MDL #00A 3563  serotype	BS agar	black, gray with metallic sheen	typical	positive
				HE agar	blue, blue green w, /o black center	typical	positive
				XLD agar	red, pink w/ black center	typical	positive
				MacConkey	colorless	Lac(-)	
				EMB agar	colorless	Lac(-)	
				NA+kan	growth	kan resistance	positive
				NA, use of UV light	fluorescence	GFP expression	positive
ELFA (VIDAS)	Group G		positive				

**Current QA Control Strain Information**

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
Salmonella VIDAS MTH-02	Negative	MDP-003	Enterobacter aerogenes ATCC 13048 AND 35029  serotype	Chromagar	colorless	MUG(-)	negative
				MacConkey EMB agar ELFA (VIDAS)	light pink light green none	Lac variable Lac variable	negative
Salmonella Cultural MTH-03	Positive	MDP-002	Salmonella enterica serovar poona/pKT-kan-GFP CDHS/MDL #00A 3563	BS agar	black, gray with metallic sheen	typical	positive
				HE agar	blue, blue green w, /o black center	typical	positive
				XLD agar	red, pink w/ black center	typical	positive
				MacConkey EMB agar NA+kan	colorless colorless growth	Lac(-) Lac(-) kan resistance	positive positive
				NA, use of UV light	fluorescence	GFP expression	positive
Salmonella Cultural MTH-03	Negative	MDP-003	Enterobacter aerogenes ATCC 13048 AND 35029	Chromagar	colorless	MUG(-)	negative
				MacConkey EMB agar	light pink light green	Lac variable Lac variable	

### Current QA Control Strain Information

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
Salmonella BAX PCR MTH-04	Positive	MDP-002	Salmonella enterica serovar poona/pKT-kan-GFP CDHS/MDL #00A 3563  PCR	BS agar	black, gray with metallic sheen	typical	positive
				HE agar	blue, blue green w, /o black center	typical	positive
				XLD agar	red, pink w/ black center	typical	positive
				MacConkey	colorless	Lac(-)	positive
				EMB agar	colorless	Lac(-)	
				NA+kan	growth	kan resistance	
			NA, use of UV light	fluorescence	GFP expression	positive	
			BAX (Salmonella)	positive amplification		positive	
Salmonella BAX PCR MTH-04	Negative	MDP-003	Enterobacter aerogenes ATCC 13048 AND 35029  PCR	Chromagar	colorless	MUG(-)	negative
				MacConkey	light pink	Lac variable	negative
				EMB agar	light green	Lac variable	
			BAX (Salmonella)	no amplification		negative	

**Current QA Control Strain Information**

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
<b>E. coli O157:H7 BAX PCR MTH-05</b>	<b>Positive</b>	<b>MDP-004</b>	E. coli O157:H7 ATCC 43890-GFP	Chromagar E. coli	white	MUG(-)	negative
				MacConkey	dark pink	Lac(+)	positive
				EMB agar	green sheen	Lac (+)	positive
				NA+kan	growth	kan resistance	positive
NA, use of UV light	fluorescence	GFP expression <sup>1</sup>	positive				
PCR	BAX (EC O157:H7)	positive amplification	positive				
<b>E. coli O157:H7 BAX PCR MTH-05</b>	<b>Negative</b>	<b>MDP-008</b>	E. coli (any except O157): ATCC strains 25922 AND 35218	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				EMB	green sheen	Lac (+)	positive
				MacConkey	dark pink	Lac (+)	positive
PCR	BAX (EC O157:H7)	no amplification	negative				
<b>E. coli O157 IMS MTH-06</b>	<b>Positive</b>	<b>MDP-004</b>	E. coli O157:H7 ATCC 43890-GFP	Chromagar E. coli	white	MUG(-)	negative
				MacConkey	dark pink	Lac(+)	positive
				EMB agar	green sheen	Lac (+)	positive
				NA+kan	growth	kan resistance	positive
NA, use of UV light	fluorescence	GFP expression <sup>1</sup>	positive				
IMS	CHROMagar O157	mauve	O157(+)	positive			

### Current QA Control Strain Information

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
E. coli O157 IMS MTH-06	Negative	MDP-008	E. coli (any except O157): ATCC strains 25922 AND 35218  IMS	Chromagar E. coli	Blue	pres of plasmid	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				EMB	green sheen	Lac (+)	positive
				MacConkey CHROMagar O157	dark pink colorless or blue	Lac (+) O157(-)	positive negative

1. Addition of 1.0 mM IPTG to the medium is needed for maximal expression of GFP

**USDA/AMS Microbiological Data Program (MDP)  
QC Control Failure Reporting Form**

**TO:** Monitoring Programs Office      **ATTENTION:**  
**FAX:** 703-369-0678                      **DATE:**  
**FROM:**

MDP Group Identification Number	
Commodity Identification	<input type="checkbox"/> CL <input type="checkbox"/> LT <input type="checkbox"/> CN <input type="checkbox"/> PY <input type="checkbox"/> GO <input type="checkbox"/> TO
Test Method	
Type of Control	<input type="checkbox"/> Uninoculated media control <input type="checkbox"/> Negative cultural control <input type="checkbox"/> Positive cultural control <input type="checkbox"/> Positive produce control
Control Strain	<input type="checkbox"/> MDP-001 <input type="checkbox"/> MDP-004 <input type="checkbox"/> MDP-002 <input type="checkbox"/> MDP-008 <input type="checkbox"/> MDP-003
Expected Result	
Reported Result	

**Corrective Action Planned / Initiated:**

Signature

Date