

**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: 04	Replaces: Rev 03 dated 09/01/05	Effective: 03/01/08

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

**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. Reagent controls are added when required.

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 of Procedures**

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



**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: 04	Replaces: Rev 03 dated 09/01/05	Effective: 03/01/08

Attachment 1, Current and Historical QA Control Strain Information

Attachment 2, QC Control Failure Reporting Form

**5. References**

5.1. ISO/IEC 17025 Guidelines

5.2. A2LA Food Microbiology Program Requirements, subsection 5.10, Reporting the Results, June 2001

5.3. Memorandum, Requirement to Notify MPO of QA Control Failure, February 3, 2004

5.4. Memorandum, Quality Assurance Control Requirements, January 21, 2004

**6. Specific Procedures**

6.1. Controls Required per Set

6.1.1. Each analytical batch/set of samples shall include uninoculated media controls, a negative cultural control, a positive cultural control, and a positive produce control for each method used to test that batch/set of samples.

6.1.1.1. The uninoculated media controls are intended to demonstrate the sterility of the medium and the results also may be used as a baseline within the analytical system.

6.1.1.2. 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.

6.1.1.3. 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 that no inhibitory effects occur from the produce.

6.1.2. Characteristics of control strains are detailed in Attachment 1, Current QA Control Strain Information.

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

6.2.1. A separate area away from the general microbiological work is required for processing samples for polymerase chain reaction (PCR). If space is limited a hood or a chamber or a Biosafety cabinet can be used. To avoid



**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: 04	Replaces: Rev 03 dated 09/01/05	Effective: 03/01/08

cross-contamination, transfer of cultures and DNA samples should be performed with extreme care.

### 6.3. Acceptability Criteria for QA Controls

6.3.1. 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 shall be corrected. If the problem is not readily identified or able to be corrected, that batch of samples must be re-analyzed. Complete Attachment 2, QC Control Failure Reporting Form and fax to the MPO laboratory liaison at (703) 369-0678, with a copy to the Deputy Director. Fax notification shall be followed by a telephone call to the MPO laboratory liaison at (703) 330-2300. Alternatively, Portable Document Format (PDF) copies of Attachment 02 with the signature may be e-mailed to the both the liaison and the Deputy Director.

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).



**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: 04	Replaces: Rev 03 dated 09/01/05	Effective: 03/01/08

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.

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 or 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, corrective action on file” 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 corrective action on file” and provide any additional details.

6.5.5. Data for samples associated with unacceptable controls will be excluded from the MDP central database.





**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: 04	Replaces: Rev 03 dated 09/01/05	Effective: 03/01/08

Revision 04                      March 03, 2008                      Monitoring Programs Office

- Updated MDP-QA-03 Attachment 1 Current QA Control Strain Information to include positive and negative controls for SOPs MDP MTH-01A, MTH-01B, MTH-01C and MTH-08

Revision 03                      September 2005                      Monitoring Programs Office

- Added control strains for MDP-MTH-07, revised attachments

Revision 02                      January 2005                      Monitoring Programs Office

- Replaced control strains, revised attachments

Revision 01                      September 2004                      Monitoring Programs Office

- 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 response to failures to meet acceptability criteria
  - Added reporting data associated with Failed Controls
  - 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-01A</b>	<b>Positive</b>	<b>MDP-017</b>	<b>E. coli</b>  ATCC 25922 $\Delta ybiK$ -KanR, Kanamycin resistant	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey EMB agar	dark pink/red green sheen, dark center	Lac(+) Lac(+)	positive positive
				Durham tube Indole test	gas production red, hot pink	Gas[+] Trptophanase	positive positive
				NA+kanamycin Media + Novobiocin	growth growth	kan resistance Novobio.resistant	positive positive
<b>E. coli MPN MTH-01A</b>	<b>Negative</b>	<b>MDP-005</b>	<b>E. coli</b>  CGSC#7923. $\Delta(\arg F$ - lac)169, $\Delta uidA3::pir$ , rpoS396 (Am)	Chromagar	colorless	MUG(-)	negative
				LST MUG	No fluorescence	MUG(-)	negative
				MacConkey EMB agar	colorless colorless	Lac negative Lac negative	negative negative
<b>TCO MPN MTH-01B</b>	<b>Positive</b>	<b>MDP-017</b>	<b>E. coli</b>  ATCC 25922 $\Delta ybiK$ -KanR, Kanamycin resistant	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey EMB agar	dark pink/red green sheen, dark center	Lac(+) Lac(+)	positive positive
				Durham tube Indole test	gas production red, hot pink	Gas[+] Trptophanase	positive positive
				NA+kanamycin Media + Novobiocin	growth growth	kan resistance Novobio.resistant	positive positive
<b>TCO MPN MTH-01B</b>	<b>Negative</b>	<b>MDP-005</b>	<b>E. coli</b>	Chromagar	colorless	MUG(-)	negative

**Current QA Control Strain Information**

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
			CGSC#7923. Δ(argF-lac)169, ΔuidA3::pir, rpoS396 (Am)	LST MUG	No fluorescence	MUG(-)	negative
				MacConkey EMB agar	colorless colorless	Lac negative Lac negative	negative negative
			serotype	EMB agar	green sheen, dark center	Lac(+)	positive
				Durham tube Indole test NA+kanamycin Media + Novobiocin ELFA (VIDAS)	gas production red, hot pink growth growth negative	Gas[+] Trptophanase kan resistance Novobio.resistant	positive positive positive positive negative
TVC MPN MTH-01C	Positive	MDP-017	<i>E. coli</i>  ATCC 25922 ΔybiK-KanR, Kanamycin resistant	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey EMB agar	dark pink/red green sheen, dark center	Lac(+) Lac(+)	positive positive
				Durham tube Indole test NA+kanamycin Media + Novobiocin	gas production red, hot pink growth growth	Gas[+] Trptophanase kan resistance Novobio.resistant	positive positive positive positive
				<b>For negative control</b>	Use sterile growth medium		
Salmonella Cultural MTH-03	Negative	MDP-017	<i>E. coli</i>  ATCC 25922 ΔybiK-Kan, Kanamycin resistant	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey EMB agar	dark pink/red green sheen, dark center	Lac(+) Lac(+)	positive positive
				Durham tube Indole test NA+kanamycin Media + Novobiocin	gas production red, hot pink growth growth	Gas[+] Trptophanase kan resistance Novobio.resistant	positive positive positive positive

**Current QA Control Strain Information**

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
<b>Salmonella</b> <b>BAX PCR MTH-04</b>	Positive	MDP-014	<b>Salmonella typhimurium</b>  <i>Δasd/pYA3553</i> ; the plasmid carries the <i>asd</i> gene along with GFP	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(-)	
				Chromagar O157 without antibiotics	mauve		
				BA, use of UV light BAX (Salmonella)	fluorescence positive amplification	GFP expression characteristic melting curves	positive positive
PCR							
<b>Salmonella</b> <b>BAX PCR MTH-04</b>	Negative	MDP-017	<b>E. coli</b>  <b>ATCC 25922 ΔybiK-Kan, Kanamycin resistant</b>	Chromagar E. coli	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey	dark pink/red	Lac(+)	positive
				EMB agar	green sheen, dark center	Lac(+)	positive
				Durham tube	gas production	Gas+	positive
				Indole test	red, hot pink	Trptophanase	positive
				NA+kanamycin	growth	kan resistance	positive
Media + Novobiocin	growth	Novobio.resistant	positive				
BAX (Salmonella)	no amplification	No characteristic melting curves	negative				
PCR							

**Current QA Control Strain Information**

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
<b><i>E. coli</i> O157:H7 BAX PCR MTH-05</b>	Positive	MDP-004	<i>E. coli</i> O157:H7 ATCC 43890-GFP  <i>Note: Adding 1 mM IPTG to the medium will enhance GFP expression</i>	Chromagar <i>E. coli</i>	white	MUG(-)	negative
				MacConkey EMB agar NA+kan NA, use of UV light	dark pink/red green sheen growth fluorescence	Lac(+) Lac (+) kan resistance GFP expression	positive positive positive positive
			PCR	BAX (EC O157:H7)	positive amplification	characteristic melting curves	positive
<b><i>E. coli</i> O157:H7 BAX PCR MTH-05</b>	Negative	MDP-017	<i>E. coli</i>  <b>ATCC 25922 <math>\Delta</math>ybiK-Kan, Kanamycin resistant</b>	Chromagar <i>E. coli</i>	blue	MUG (+)	positive
				LST MUG	blue fluorescence	MUG (+)	positive
			MacConkey EMB agar	dark pink/red green sheen, dark center	Lac(+) Lac(+)	positive positive	
			Durham tube Indole test NA+kanamycin Media + Novobiocin	gas production red, hot pink growth growth	Gas+ Trptophanase kan resistance Novobio.resistant	positive positive positive positive	
			PCR	BAX ( <i>E. coli</i> O157:H7)	no amplification	No characteristic melting curves	negative
<b><i>E. coli</i> O157 IMS MTH-06</b>	Positive	MDP-004	<i>E. coli</i> O157:H7 ATCC 43890-GFP  IMS	Chromagar <i>E. coli</i>	white	MUG(-)	negative
				MacConkey EMB agar NA+kan NA or BA, use of UV light	dark pink green sheen growth fluorescence	Lac(+) Lac (+) kan resistance GFP expression <sup>1</sup>	positive positive positive positive
			IMS	CHROMagar O157 with and without antibiotics	mauve	O157(+)	positive



**Current QA Control Strain Information**

Method SOP	Control Type	MDP Strain No.	Strain Description	Test Results			Expected result
				Media	Result	Characteristic	
				Hektoen (HE) agar	light green to greenish blue w/o dark centers red clonies red slant, yellow butt, w/o gas or hydrogen sulfide production yellow	GFP expression	positive
			XLD agar	positive			
			TSI slant	positive			
			Lysine decarboxylase	negative			
			GFP expression - no need to add IPTG	Exposure to UV light	colonies fluoresce	GFP expression	positive
<b>Shigella rtPCR MTH-08</b>	<b>Negative</b>	<b>MDP-017</b>	<b><i>E. coli</i></b>	Chromagar <i>E. coli</i>	blue	MUG (+)	positive
			<b>ATCC 25922 <math>\Delta</math>ybiK-Kan, Kanamycin resistant</b>	LST MUG	blue fluorescence	MUG (+)	positive
				MacConkey EMB agar	dark pink green sheen, dark center	Lac(+) Lac(+)	positive positive

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

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

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MDP Group Identification Number	
Internal Lab number	
Commodity Identification	
Test Method Used (SOP etc.)	
Control Strain (s) Used	
Type of Control Failure	
Reported Result	

**Corrective Action Planned / Completed:** (Include identifying tracking information.)

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Signature

Date