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

SOP No.: MDP-QA-03		Page 1 of 8
Title: Quality Assurance (QA) Controls		
Revision: 07	Replaces: 09/25/09	Effective: 10/01/10

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

3.1 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.

3.2 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.

3.3 If any control yields an unacceptable result, appropriate investigative/re-testing measures, as outlined in subsection 5.3 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 5.4 of this SOP.

4. References

4.1 AOAC International Guidelines for Laboratories performing Microbiological and Chemical Analyses of Food and Pharmaceuticals, An Aid to Interpretation of ISO/IEC 17025:2005 (Rev 09/2006), Section: General requirements for the competence of testing and calibration laboratories

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

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

4.4 Memorandum, Quality Assurance Control Requirements, January 21, 2004

5. Procedures

5.1 Controls Required per Set

5.1.1 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.

5.1.2 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.

5.1.3 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.

5.1.4 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 that no inhibitory effects occur from the produce.

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

5.2 Procedures for Handling QA Controls for All PCR-Based Methods

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

5.2.2 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 and/or does not fall within the categories as described in Sections 5.2.3 through 5.2.5, that control does not meet MDP acceptability criteria and is considered unacceptable.

5.2.3 For Internal Control (IC) failures associated with SOP MDP MTH-11, the laboratories should check the PCR amplification results for target genes (Stx-1 and/or Stx-2). If the target gene(s) are amplified and the non-template control has worked as expected, the laboratories may treat the IC control failure as acceptable. Samples and controls need not be restested/repeated; however, laboratories are encouraged to determine appropriate concentrations of genomic DNA preparations from the control strains for PCR assays and to document those procedures in internal laboratory SOPs.

5.2.4 For *uidA* gene amplification failure in the positive produce culture control associated with SOP MDP MTH-11, the laboratories shall check PCR amplification results from the positive culture control and positive DNA control. If these results are positive for *uidA* amplification and the non-template control has worked as expected the laboratories may treat this failure as acceptable. Samples and controls need not be retested/repeated; however, laboratories are encouraged to determine appropriate spike levels for setting up overnight positive produce control cultures for use in PCR assays (refer to section 6.4 Controls in SOP MDP-LABOP-02).

5.2.5 For PCR results yielding non-specific amplification(s) in the non-template control or suspicious amplifications (specific or non-specific) in several test samples, all samples along with appropriate controls must be repeated. In addition, the laboratory must institute a plan for cleanup of the pipettes used in the PCR assay, the PCR workstation, and other surfaces that may come in contact with aerosols and amplicons. Other steps must include discarding suspect buffers and reagents used in the PCR assay and initiation of retesting with new or freshly reconstituted reagents and buffers. All investigative steps and results must be reported to MPO.

5.3 **Response to Failure to Meet Acceptability Criteria** (Comments, observations, corrective actions, etc., should be recorded as part of sample data transmitted to MPO)

5.3.1 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.

5.3.2 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, and does not fall under situations as described in Section 5.2, 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(s) at (703) 330-2300. Alternatively, Portable Document Format (PDF) copies of Attachment 02 with the signature may be e-mailed to both the liaison(s) and the Deputy Director.

5.3.3 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.

5.3.4 The recommended timeframe for initiating investigation/re-testing is within two business days of control failure. 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.

5.3.5 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, etc.).

5.3.6 All corrective actions taken as described in the preceding subsections (5.3.1 to 5.3.5) must be properly documented in internal records and the MDP RDE system.

5.4 **Reporting Data Associated with Failed Controls** (Comments, observations, corrective actions, etc., should be recorded as part of sample data transmitted to MPO)

5.4.1 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.

5.4.2 For controls yielding acceptable results for the initial testing, the results shall be reported as “acceptable.”

5.4.3 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, etc.” and any additional details.

5.4.4 For controls yielding unacceptable results for a rerun test, the result shall be reported as "unacceptable." In the comments field enter "re-tested, corrective action on file, etc." and provide any additional details.

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

Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.

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Revision 07 August 2010 Monitoring Programs Office

- Merged Sections 5.2.2 and 5.2.3 and added **bold** portion, "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 **and/or does not fall within the categories as described in Sections 5.2.3 through 5.2.5**, that control does not meet MDP acceptability criteria and is considered unacceptable."
- Added new Section 5.2.3, "For Internal Control (IC) failures associated with SOP MDP MTH-11, the laboratories should check the PCR amplification results for target genes (Stx-1 and/or Stx-2). If the target gene(s) are amplified and the non-template control has worked as expected, the laboratories may treat the IC control failure as acceptable. Samples and controls need not be restested/repeated; however, laboratories are encouraged to determine appropriate concentrations of genomic DNA preparations from the control strains for PCR assays and to document those procedures in internal laboratory SOPs."
- Added new Section 5.2.4, "For *uidA* gene amplification failure in the positive produce culture control associated with SOP MDP MTH-11, the laboratories shall check PCR amplification results from the positive culture control and positive DNA control. If these results are positive for *uidA* amplification and the non-template control has worked as expected the laboratories may treat this failure as acceptable. Samples and controls need not be retested/repeated; however, laboratories are encouraged to determine appropriate spike levels for setting up overnight positive produce control cultures for use in PCR assays (refer to section 6.4 Controls in SOP MDP-LABOP-02)."
- Added new Section 5.2.5, "For PCR results yielding non-specific amplification(s) in the non-template control or suspicious amplifications (specific or non-specific) in several test samples, all samples along with appropriate controls must be repeated. In addition, the laboratory must institute a plan for cleanup of the pipettes used in the PCR assay, the PCR workstation, and other surfaces that may come in contact with aerosols and amplicons. Other steps must include discarding suspect buffers and reagents used in the PCR assay and initiation of retesting with new or freshly reconstituted reagents and buffers. All investigative steps and results must be reported to MPO."
- Revised Section 5.3.2 as shown in **bold**, "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, **and does not fall under situations as described in Section 5.2,** 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(s) at (703) 330-2300. Alternatively, Portable Document Format (PDF) copies of Attachment 02 with the signature may be e-mailed to both the liaison(s) and the Deputy Director."

- Revised Attachment 1, Current QA Control Strain Information
- Revised Attachment 2, QC Control Failure Reporting Form

Revision 06 September 2009 Monitoring Programs Office

- Revised Section 3, Principle
- Revised Sections 5.1 through 5.4
- Updated Attachment 02

Revision 05 March 30, 2009 Monitoring Programs Office

- Updated MDP-QA-03 Attachment 1 Current QA Control Strain Information by deleting the negative control strain used for SOPs MDP MTH-01A, MTH-01B. Deleted procedures and strains related to SOPs MDP MTH-01A, MTH-01B, MTH-01C and MTH-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