# Preparing Cdiff Positive and Negative Controls

**PURPOSE**

* This procedure provides instructions for preparing the Cdiff positive and negative matrix controls

## SAFETY CONSIDERATIONS

* Standard precautions. Refer to MB 2.02 Biohazard Containment
* Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination

## ABBREVIATIONS

Area/Room 1: Clean room

Area/Room 2: Processing room

Area/Room 3: Amplification room

NEGC: negative control

POSC: positive control

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| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 2* Refrigerator 2 – 8° C
* -70⁰ C freezer
* BSC BSL-2
* VWR Vortex Mixer
* Vortex mixer
* 10 µl pipette

Room 3* + BD MAX instrument
 | BD MAX Cdiff Assay kit: Product No. 443418 | Orange barrier wipes |
| Previous positive stool sample (Ct value approx. 26 – 28) | Nitrile gloves (powder-free) |
| Previous negative stool sample | BD MAX PCR cartridges |
| Bleach Sani-Cloth | Test tube rack |
| 70% alcohol | 50 ml sterile conical tube |
| Tap water | Nalgene cryogenic vial holder |
| Nuclease Free Water (NFW) | 30 µl pipette tips |
|  |  |

####  MATERIALS REQUIRED

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | **Temperature** | **F/T cycles** | **Stability** |
| Cdiff matrix control (POSC & NEGC) | 2 – 8° C | NA | 7 days |
| Cdiff matrix control (POSC & NEGC) | – 70° C | 1 | 1 year |

**Storage and Stability of Prepared Controls**

**PROCEDURE A:** Follow the activities for preparing the positive Cdiff control in the table below

**Positive Matrix Control Preparation**

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Prepare in** | 1 | Prepare stock solution in Microbiology BSC (BioSafety Cabinet)  | MB 7.08 Equipment and Room Decontamination |
| **Microbiology** | 2 | Wipe down hood before preparation with Bleach Sani-Wipe followed by water and then 70% alcohol |
|  | 3 | Prepare a 1:10 suspension of a known positive sample (approx. Ct 26 – 28) in NFW to achieve a Ct value of 30 – 31* ***Note:*** Each 10 fold dilution will increase the Ct value by approx 3 Ct.
 |  |
|  | 4 | Vortex well |  |
|  | 5 | Change gloves; move to room 2 |  |
| **Determine Ct value** | 6 | Test suspension according to Cdiff Assay procedure to determine the approximate Ct value after dilution* If in the desired range, aliquot suspension and freeze
* If not in the desired range, adjust and retest
 | MB 7.05Cdiff Assay |
| Room 2, 3 | 8 | Print PCR analysis curve for QC records |  |
| **Freeze Aliquots** | 9 | Aliquot POSC:* Label and date cryo-tubes
* Pipette 400 µl of final suspension to each tube
 |  |
| Room 2 | 10 | Freeze aliquots at –70°C |  |
| **Clean** **Hood** | 11 | Decontaminate BSC * Wipe with bleach Sani-Cloth; allow to sit 4 – 5 min
* Wipe with water followed by 70% alcohol
 | MB 7.08 Equipment and Room Decontamination |
|  | 12 | UV hood for 15 min  |  |
| **Reactivity and Stability** | 13  | Before use, thaw one new POSC aliquot; test in parallel 7 days with old POSC for appropriate reactivity and stability | MB 7.09.F4 Cdiff POSC Reagent Verification |
| 14 | Print PCR analysis curves after F/T for QC records |  |
|  | 15 | Record results in *New Lot Inventory and QC* manual |  |
|  | 16 | Once thawed, POSC control is stable for 7 days at refrigerated temperature |  |
|  | 17 | Do not refreeze (only 1 F/T cycle) |  |

**PROCEDURE B:** Follow the activities for preparing the negative Cdiff control in the table below

**Negative Matrix Control Preparation**

| **Activity** | **Step** | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Prepare in** | 1 | Prepare stock solution in Microbiology BSC (BioSafety Cabinet) | MB 7.08 Equipment and Room Decontamination |
| **Microbiology** | 2 | Wipe down hood before preparation with bleach Sani-Cloth followed by water and 70% alcohol |
|  | 3 | Prepare a 1:10 suspension of a known negative sample in NFW |  |
|  | 4 | Vortex well |  |
|  | 5 | Change gloves; move to room 2 |  |
| **Confirm reactivity** | 6 | Test suspension according to Cdiff Assay procedure 5 times in parallel to old NEGC for appropriate reactivity | MB 7.05Cdiff AssayMB 7.09.F6Cdiff NEGC Reagent Verification |
| **Freeze Aliquots** | 8 | Aliquot NEGC:* Label and date cryo-tubes
* Pipette 400 µl of final suspension to each tube
 |  |
| Room 2 | 9 | Freeze aliquots at –70°C |  |

**REFERENCES**

1. BD MAX™ Cdiff Assay, REF: 443418 P0137(03), 2015-05, GeneOhm Sciences, 2555 boul. Parc-Technologique, Quebec, Qc, Canada, G1P 4S5
2. CLSI *Molecular Diagnostic Methods for Infectious Diseases;* Approved Guideline – Second Edition, CLSI document MM3-A2, Wayne, PA, Clinical and Laboratory Standards Institute; 2006

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| Historical Record |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Pat Ackerman | 6/1/2011 | Initial Version |
| 2 | Pat Ackerman | 6/3/2013 | Revised: 1:10 extraction suspension in NFW |
| 3 | Pat Ackerman | 04.18.14 | Omitted Extraction control, added Proc. B |
|  | 4 | P. Ackerman | 08.31.2016 | Reformatted for CMS upload; changed logo; prev number CDT 004 |
|  | 5 | J. Laramie | 08.31.2016 | -Added testing of negative control 5 times-Biennial review: 02.09.2018 |