

# pDST Calculator - Final Results (Step 4)

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## **Final Instructions:**

1. Review all calculated values below and distinguish between different drugs' workflows
2. Follow your laboratory's standard operating procedures for phenotypic drug susceptibility testing
3. Ensure proper sterile technique throughout the preparation process
4. Label all solutions clearly
5. Store solutions according to manufacturer recommendations and laboratory protocols
6. Dispose of any unused drug solutions following your institution's hazardous waste disposal guidelines

## **Special Notes for Bedaquiline (BDQ):**

Use polystyrene tubes (1.5ml or 5ml) as bedaquiline binds strongly to glass surfaces, which can cause loss of drug and inaccurate (lower) effective concentrations in solution.  
Do not invert tubes as BDQ will attach to sides, if crystal doesn't dissolve after 1 hour, use sonicator for ~3 minutes

## **Special Notes for DMSO:**

Wrap the tube in foil or use a light-resistant container to protect DMSO from degradation caused by light exposure.

## **Stock Solution Preparation**

Drug	Diluent	Drug Weight (mg)	Total Stock Volume (ml)	Stock Concentration ( $\mu\text{g}/\text{ml}$ )	Dilution Factor
Amikacin (AMK)	WATER	10.00	13.20	739.39	8.8
Bedaquiline (BDQ)	DMSO	0.80	5.50	145.45	1.7

## **Preparation Steps:**

1. Label a clean container
2. Record the drug details:
  - Date: 2025-11-04
  - Drug
  - Diluent
  - Concentration
  - Initials
3. Wrap the tube in foil or use a light-resistant container to protect DMSO from degradation caused by light exposure.
4. Add the weighed drug powder to a clean container
5. Add Diluent to the same container
6. Mix thoroughly

For bedaquiline: Cap the tube securely. Do not invert tubes as drug will attach to sides. If crystal doesn't dissolve after 1 hour, use sonicator for ~3 minutes

For other diluents: Cap the tube securely. Invert tube gently 2-4 times or vortex briefly. Do not shake vigorously to avoid foam formation. Check that drug powder is completely dissolved. Ensure no visible particles remain.

## **Working Solution Preparation**

Drug	Diluent	Stock to Add (ml)	Diluent to Add (ml)	Volume of WS (ml)	Conc. WS. ( $\mu\text{g}/\text{ml}$ )

Amikacin (AMK)	WATER	0.5000	3.8800	3.88	84.00
Bedaquiline (BDQ)	DMSO	0.1600	0.1200	0.12	84.00

### **Preparation Steps:**

1. Label a clean container
2. Record the drug details:
  - Date: 2025-11-04
  - Drug
  - Diluent
  - Concentration
  - Initials
3. Wrap the tube in foil or use a light-resistant container to protect DMSO from degradation caused by light exposure.
4. Add the weighed drug powder to a clean container
5. Add Diluent to the same container
6. Mix thoroughly

For bedaquiline: Cap the tube securely. Do not invert tubes as drug will attach to sides. If crystal doesn't dissolve after 1 hour, use sonicator for ~3 minutes

For other diluents: Cap the tube securely. Invert tube gently 2-4 times or vortex briefly. Do not shake vigorously to avoid foam formation. Check that drug powder is completely dissolved. Ensure no visible particles remain.

### **Aliquoting Remaining Stock**

Drug	Number of Aliquots	Volume Stock per Aliquot (ml)
Amikacin (AMK)	4	3.0
Bedaquiline (BDQ)	5	1.0

### **Preparation Steps:**

1. Label a clean container
2. Record the drug details:
  - Date: 2025-11-04
  - Drug
  - Diluent
  - Concentration
  - Initials
3. Add the stock solution to aliquot

### **Special Notes for Aliquots:**

- Cap tubes tightly and check for proper sealing

#### Storage instructions:

- Store aliquots at -20°C or -80°C
- Valid for up to 6 months from preparation date
- Avoid repeated freeze-thaw cycles

### **MGIT Tubes Preparation**

Drug	Number of MGITs	Volume WS per MGIT (ml)	Volume OADC (growth suppl) per MGIT (ml)	Volume Culture per MGIT (ml)
Amikacin (AMK)	5	0.1	0.8	0.5
Bedaquiline (BDQ)	4	0.1	0.8	0.5

### **Preparation Steps:**

1. Label a clean container
2. Record the drug details:
  - Date: 2025-11-04
  - Drug
  - Diluent
  - Concentration
  - Initials

For each MGIT tube:

3. Pipette 0.1 ml (= 100 µl) of working solution
4. Add 0.8 ml (= 800 µl) of OADC (growth supplement)
5. Add 0.5 ml (= 500 µl) of culture

After adding all components to each tube:

6. Mix thoroughly

For bedaquiline: Cap the tube securely. Do not invert tubes as drug will attach to sides. If crystal doesn't dissolve after 1 hour, use sonicator for ~3 minutes

For other diluents: Cap the tube securely. Invert tube gently 2-4 times or vortex briefly. Do not shake vigorously to avoid foam formation. Check that drug powder is completely dissolved. Ensure no visible particles remain.

7. Place in MGIT machine as soon as possible after adding culture