Diazyme Procalcitonin (PCT) Customer Installation Guidelines

AU480, AU680, AU5800 and DxC 700 AU Clinical Systems

Purpose:

The purpose of this document is to provide a standard set of instruction guidelines to ensure consistency with the installation of Diazyme Procalcitonin (PCT) on the AU480, AU680, AU5800 and DxC 700 AU Clinical Systems.

This guideline covers three (3) specific studies: Within run precision, linearity and methods comparison.

Requirements:

- Data analysis software
- PCT Instructions for Use, http://www.diazyme.com/BCI-applications (registration required for first time access).
- Volumetric or Calibrated pipettes (for reconstituting Cals/QC)
- Study material required (minimum):

REF	Description	Number of kits required	Used in
DZ558A-K	PCT Reagent	1	All studies
DZ558A-CAL	PCT Calibrator	3	Calibration, Linearity
DZ558A-CON	PCT Control	2	QC, Precision

PCT Customer Installation Guidelines

WARNINGS AND PRECAUTIONS

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate the instrument.

Beckman Coulter, Inc. urges its customers and employees to comply with all national health and safety standards such as the use of barrier protection. This may include, but is not limited to, protective eyewear, gloves and suitable laboratory attire when operating or maintaining this or any other automated laboratory equipment.

The procedures described are basic guidelines for Diazyme PCT performance verification. Laboratories must follow current policies and/or their local regulatory agency for acceptable performance limits and specifications.

INTENTION FOR USE

The instructions are to provide a standard set of guidelines to ensure consistency with the installation of Diazyme Procalcitonin (PCT) on the AU480, AU680, AU5800 and DxC 700 AU Clinical Systems.

Always follow product labeling and manufacturer’s recommendations for intended use.

TRADEMARKS

Diazyme is a trademark of Diazyme Laboratories, Inc.

Within Run Precision

Introduction:

Precision studies should be completed prior to linearity and methods comparison studies, to verify optimal performance of the instrument. A minimum of 20 replicates is required for both the low and high level controls.

Pre-requisite:

- ✓ Analyzer maintenance is up to date
- ✓ PCT reagent calibration will not expire during the test procedure*
- ✓ PCT QC control recovery are within acceptable limits

Note: The OD values for calibrator levels 1 to 5 should not be negative

Acceptance Criteria:

Compare results to the manufacturer's acceptance criteria based on the mean concentration of the controls:

- **For mean ≤ 1.0 ng/mL, use SD ≤ 0.1 ng/mL**
- **For mean > 1.0 ng/mL, use CV% ≤ 10%**

Procedure:

1. Calibrator and Control preparation precautions:
    - Ensure pipette(s) used can accurately deliver 1 mL deionized water for calibrator and 3 mL for control
    - Carefully remove the cap to minimize loss of lyophilized materials
    - Gently invert bottle after reconstitution to ensure lyophilized materials from rubber stopper are mixed into solution
    - Do not vortex. Let stand for a minimum of 5 minutes and ensure contents are fully dissolved prior to use
2. Run a minimum of twenty (20) replicates in the same run for both low and high level controls
3. Determine mean, SD and %CV
4. Compare results to the manufacturer's acceptance criteria based on the mean concentration of the controls
5. If precision run fails acceptance criteria, troubleshoot as follows:

    Parameter Settings/Calibration on analyzer

    A. Verify parameter settings are entered correctly
    B. Verify calibrator set points (note: the zero level calibrator is not zero. Refer to Instrument specific assay parameter sheets for value)
    C. Verify the OD values for calibrator levels 1 to 5 are not negative

Investigate/troubleshoot hardware:

A. Sample dispense - Inspect probe and syringe for leaks; prime/clean probe if necessary
B. Reagent dispense - Inspect probes and syringes for leaks; prime/clean probes if necessary.
C. Mixing - Inspect mixers; clean if necessary
D. Cuvettes - Perform W2 1N HCl wash and Photocal
E. Evaluate QC and precision performance (N=5 to 10) for other assays that are similar in principle or pipetting volume to PCT (creatinine and iron)

6. Contact Beckman Coulter Technical Support or local Field Service for advanced troubleshooting of persistent precision run failures

Linearity

Introduction:

Linearity studies are performed to determine the linear analytical measuring range of 0.20 to 52 ng/mL for PCT. It is performed with five or more interrelated samples, with three (3) replicates for each level. Currently, no commercially available PCT linearity set is available for the Diazyme PCT assay on AU platforms.

Pre-requisite:

- ✓ Analyzer maintenance is up to date
- ✓ PCT reagent calibration will not expire during the test procedure*
- ✓ PCT QC control recovery are within acceptable limits
- ✓ PCT passed within-run precision

Note: The OD values for calibrator levels 1 to 5 should not be negative

Acceptance Criteria:

The manufacturer's acceptance criteria for linearity is **Slope = 0.95 – 1.05 and R^2 > 0.95**. For EP users, manufacturer's acceptance criteria can be recorded under "comments"

Procedure:

Verify analytical measuring range: 0.20 to 52 ng/mL

1. Ensure the pipettes used can accurately deliver the volumes required in sample preparation
2. Reconstitute two bottles each of PCT Cal levels "0" (zero) and 5 *(refer to Calibrator and Control preparation precautions under Within Run Precision)*
3. For each calibrator levels, pool the two reconstituted bottles prior to linearity sample preparation to minimize bottle-to-bottle variability
4. Program samples in triplicate
5. Prepare linearity samples using PCT Cal levels 0 and 5 as follows:

Linearity level	% of Level 5	PCT CAL Level 5 (µL)	PCT CAL Level 0 (µL)
1	0	0	500
2	20	100	400
3	40	200	300
4	60	300	200
5	80	400	100
6	100	500	0

6. Plot the mean of the triplicates against the % pool for all 6 levels. Determine linear regression. Compare results to the manufacturer's acceptance criteria

Verify low end measuring range: 0.20 to 5 ng/mL

If your laboratory requires verifying linearity at the low end of the analytical measuring range, 0.2 to 5 ng/mL

Due to matrix effect, do not use saline or deionized water to dilute

1. Prepare a low PCT human serum pool (~0.2 ng/mL)
   A. Pool human serum containing PCT < 0.2 ng/mL.
   B. Determine PCT concentration using Diazyme's PCT Assay REF DZ558A-K in triplicates.
   C. Verify the low PCT Pool is < 0.2 ng/mL.
2. Prepare a high PCT human serum pool (~5 ng/mL)
   A. Spike the low PCT human serum pool with PCT calibrator level 5 (For example: add 200 µL of PCT calibrator level 5 to 1800 µL of low PCT pool and mix thoroughly).
   B. Determine PCT concentration using Diazyme's PCT Assay REF DZ558A-K in triplicates.
   C. Determine the mean of the triplicates. This value is used as the PCT concentration for the high sample pool.
3. Prepare linearity samples using a low PCT Pool (<0.2 ng/mL) and high PCT Pool (~5 ng/mL) as follows:

Linearity level	% of High PCT Pool	High PCT Pool (µL)	Low PCT Pool (µL)
1	0	0	500
2	20	100	400
3	40	200	300
4	60	300	200
5	80	400	100
6	100	500	0

4. Plot the mean of the triplicates against the % pool for all 6 levels. Determine linear regression. Compare results to the manufacturer's acceptance criteria

Methods Comparison

Introduction:

Methods comparison studies are performed to evaluate the bias between two (2) methods that measure the same analyte. The test method is the Diazyme PCT assay and the reference method is the current PCT method used by the laboratory. Patient samples are analyzed with both reference and test method. Sample population should cover the entire analytical measuring range which encompasses normal low and abnormal high range for patients. A concordance study to clinical outcome is recommended if the laboratory is currently not running PCT or to resolve discrepancies between methods.

Pre-requisite:

✓ Analyzer maintenance is up to date
✓ PCT reagent calibration will not expire during the test procedure
✓ PCT QC control recovery are within acceptable limits
✓ PCT passed within-run precision
✓ PCT has passed linearity performance evaluation
Note: The OD values for calibrator levels 1 to 5 should not be negative

Acceptance Criteria:

Concordance: Defined by the laboratory based on regulatory and clinical requirements
Methods Comparison: Manufacturer's acceptance criteria is **Slope = 0.90-1.10 and R^2 > 0.90**

Testing Tips:

General

1. Do not use lipemic or hemolyzed samples
2. Mix samples prior to testing (especially if sample is previously frozen, vortexing optional)
3. Analyze a minimum of 40 samples across the analytical measuring range
4. Perform testing on the two methods within two (2) hours of each other to preserve sample stability and integrity
5. Perform testing in multiple runs to reduce the systematic error that can occur in a single run
6. Particular attention should be made to keep spiked or diluted sample matrix as close to the original sample matrix as possible
7. Do not dilute more than the recommended ratio specified in the IFU of 1:4 at the high end. Dilutions above 1:4 have not been validated
8. All test samples should be the same matrix (serum or plasma) and stored under the same condition (room temperature, refrigerated or frozen) to minimize sample variability

Sample Stability

9. Sample degradation affects both Latex enhanced Immunoturbidimetric assay (Diazyme PCT) and Immunoluminometric (IA assay) methods. Maximal sample degradation occurs during first hours after sample collection*
10. Fresh samples must be used for optimal results
    - Add AU Diazyme PCT testing to current routine PCT testing as the samples arrive in the laboratory
    - If immediate testing is not possible, samples may be stored up to 3 days at 2-8° or freeze the samples within 3 days of blood collection at -20°C to avoid sample degradation
11. Limit the freeze-thaw cycles to less than four (4). Test samples at the same freeze thaw cycle on both methods. Centrifuge samples after thawing if the sample is visibly turbid

Procedure:

Concordance

✓ Preferred for Labs running PCT on an Immunoassay (IA) analyzer

1. Compare Diazyme's PCT results to alternate IA PCT results for samples in the diagnostic range of **≤ 0.5 ng/mL, between 0.5 to 2.0 ng/mL and > 2.0 ng/mL**
    a. If the results are discrepant, check sample integrity and review patient's clinical history/outcome
2. Labs currently not running PCT test
    a. Follow current laboratory policy and regulatory requirements for methods comparison performance verification

Methods Comparison

✓ Labs comparing Diazyme's PCT between AU models

1. Compare Diazyme's PCT results between AU models for samples covering the assay's analytical measuring range. Run a minimum of 40 samples with replicate of one for each sample.
2. Plot reference method (X) against test method (Y). Determine Deming regression.
3. If results do not agree between methods:
    - Repeat discrepant sample(s) on both methods
    - Investigate patient history (disease/diagnosis, drug usage) and sample integrity (turbidity/hemolysis/matrix, "spiking" with outside material)
    - Monitor direction of subsequent PCT measurements (ascending or descending) and their clinical outcomes for diagnostic accuracy
    - Consider concordance comparison testing between Diazyme AU PCT against positive/negative clinical outcome
    - Refer to the "Limitations" section of the PCT assay instructions for use

*Meisner M. Procalcitonin – Influence of Temperature, Storage, Anticoagulation and Arterial or Venous Asservation of Blood Sample on Procalcitonin Concentrations. *Eur J Clin Chem Clin Biochem 1997; 35(8):597 – 601*