Dade® Hepzyme®

Revision bar indicates update to previous version.

Intended Use

Dade® Hepzyme® is a supplementary reagent used for heparin degradation in human citrated plasma from patients under heparin therapy or heparin contamination during blood withdrawal to be assayed in coagulations assay, such as activated partial thromboplastin time (APTT), prothrombin time (PT) or thrombin time (TT), by means of automated, semi-automated and/or manual methods to rule out heparin influence in these coagulation tests.

Summary and Explanation

Heparin is a potent and widely used anticoagulant that catalyzes the inactivation of thrombin and other serine proteases by antithrombin (AT)[1,2]. It is used therapeutically to reduce thrombotic disease and prophylactically for patients undergoing major surgical procedures or during dialysis to prevent clotting in indwelling catheters and extracorporeal devices. The presence of heparin in a specimen may interfere with the interpretation of various coagulation tests. Heparinase I, the active component of Dade® Hepzyme®, is specific for heparin. Heparinase cleaves heparin at multiple sites per molecule, including the ATIII binding site, producing oligosaccharides with average molecular weights of 1 000 Da that have lost their antithrombotic activity[3]. Heparin contained in plasma samples can be digested by the addition of heparinase prior to prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time assays[4,5]. Cartridges coated with heparinase have been used in conjunction with ACT assays to monitor by-pass surgery and obtain accurate baseline clotting information on heparinized whole blood[6].

Dade® Hepzyme® is a stable, lyophilized preparation of purified bacterial heparinase I (E.C. 4.2.2.7) produced in Flavobacterium heparinum. The availability of this enzyme in large quantities was made possible by purification and fermentation methods. Dade® Hepzyme® can neutralize up to 2 USP units of unfractionated heparin in 1 mL of citrated plasma. Dade® Hepzyme® is easy to use, requiring minimal plasma handling. Other available methods for removing heparin contamination have limitations. The use of protamine to neutralize heparin requires careful titration to prevent the effects of incomplete neutralization or of protamine's own anticoagulant properties[7]. Heparin adsorbents and filters that remove heparin from plasma have been shown to bind FIX and FX and frequently prolong coagulation tests[7,8,9].

Dade® Hepzyme® may be used to:

a. Rule out heparin contamination as responsible for abnormal coagulation results of activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and Thrombin Clotting Time (TCT) assays.
b. Compare the APTT results for heparinized samples when monitoring heparin therapy.
c. Evaluate patients on combined heparin and coumadin therapy.

Reagents

Reagent	Description	Storage	Stability
Dade® Hepzyme®	Lyophilized reagent containing: • purified bacterial heparinase I (reconstituted: >125 IU/mL) • Stabilizer	2–8 °C May be used up to the expiry date indicated on the label if stored unopened[a].	15–25 °C[b]: reconstituted

[a] Neutralized plasma samples should be stored at room temperature (15 to 25 °C) and tested as soon as possible without exceeding the permissible sample storage time for individual tests.

[b] closed original vial

Signs of Spoilage: No vacuum when opening the vial; insufficient neutralization of the control (see section "Internal Quality Control", page 3).

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Preparing Reagents

Reconstitute each vial with 1 mL patient citrated plasma. Allow to stand at room temperature (15 to 25 °C) for 15 minutes before testing.

Mix carefully once more before using.

Stability after reconstitution

Neutralized plasma samples should be stored at room temperature (15 to 25 °C) and tested as soon as possible without exceeding the permissible sample storage time for individual tests.

Sample Collection and Preparation

Use citrated plasma only. To prepare, mix nine parts of freshly collected patient blood with one part 0.11 or 0.13 mol/L (3.2 % or 3.8 %) sodium citrate. Evacuated tubes containing the desired anticoagulant are commercially available and may be used with caution in blood coagulation assays.

Centrifuge the blood specimen for a minimum of 10 minutes at 1 500 × g as soon as possible after collection and within 60 minutes. Transfer plasma using a plastic pipette to a plastic centrifuge tube and centrifuge again for 10 minutes at 1 500 × g to insure platelet-poor plasma. Transfer supernatant plasma into another tube using a plastic transfer pipette. Close the tube with a rubber stopper until ready for test.

Please refer to CLSI-document H21-A5[10] for further details.

Neutralization with Dade® Hepzyme® should be done at room temperature (15 to 25 °C) as soon as possible without exceeding the permissible sample storage time for the coagulation test.

Procedure

Materials Provided

REF	Contents	
B4240-10	Dade® Hepzyme®	10 × → 1 mL

Materials Required but not Provided

Item	Description
REF 291070 REF B4244-10	Dade® Ci-Trol® 1, or Ci-Trol CONTROL 1, Dade® Ci-Trol® Coagulation Control Level 1, as control for the normal range
REF 291071 REF B4244-20	Dade® Ci-Trol® 2, or Ci-Trol CONTROL 2, Dade® Ci-Trol® Coagulation Control Level 2, as control for the pathological/therapeutical range
REF 291072 REF B4244-30	Dade® Ci-Trol® 3, or Ci-Trol CONTROL 3, Dade® Ci-Trol® Coagulation Control Level 3, as control for the pathological/therapeutical range
REF B4224-50	Ci-Trol HEPARIN CONTROL 1, Dade® Ci-Trol® Heparin Control, Low
REF B4224-60	Ci-Trol HEPARIN CONTROL 2, Dade® Ci-Trol® Heparin Control, High
–	For blood collection, use sodium citrate (0.11 mol/L or 0.13 mol/L/3.2 % or 3.8 %), or Standard commercial blood collection systems
Coagulation analyzers[c], such as:	• Automated Blood Coagulation Analyzer CA-600 series (CA-600 series) • AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) • AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System) • Automated Blood Coagulation Analyzer CN-3000/CN-6000 (CN-3000/CN-6000 System)

[c] Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

Test Procedure

1. Add 1 mL platelet-poor citrated plasma to each Dade® Hepzyme® vial, re-close with a rubber stopper, and invert gently 5 to 10 times.

   Note: If plasma volume is limited, add no less than 0.5 mL plasma. All the lyophilized material must be dissolved. This applies to APTT and PT testing only.
2. Allow vials to stand at room temperature (15 to 25 °C) for 15 minutes.

   Note: If heparin level in plasma is greater than 2 USP units but less than 4 USP units, sequential neutralization may be performed. This applies to APTT and PT testing only. Neutralization is carried out by subjecting the plasma to a second heparinase treatment after the first treatment with Dade® Hepzyme®. This is done by transferring at least 0.5 mL of the fluid from the first vial treated with Dade® Hepzyme® after 15 minutes to a second vial and allowing it to stand for another 15 minutes at room temperature (15 to 25 °C).
3. Perform the desired coagulation test(s).

Internal Quality Control

	for APTT	PT	TCT
Dade® Ci-Trol® 1, or Ci-Trol CONTROL 1 (≙ Dade® Ci-Trol®, Level 1)	yes	yes	yes
Dade® Ci-Trol® 2, or Ci-Trol CONTROL 2 (≙ Dade® Ci-Trol®, Level 2)	yes	yes	–
Dade® Ci-Trol® 3, or Ci-Trol CONTROL 3 (≙ Dade® Ci-Trol®, Level 3)	yes	yes	–

	for APTT	PT	TCT
Ci-Trol HEPARIN CONTROL 1	yes	–	–
Ci-Trol HEPARIN CONTROL 2	yes	–	–

See the respective Instructions for Use. The controls should be processed just like the patient samples.
Each laboratory should determine its own quality control range by means of its own confidence level established in the laboratory.
This normally amounts to ±2.0 to ±2.5 standard deviations (SD) from the mean control value. If a value outside of the permissible confidence range is encountered, the coagulation analyzer and reagents should be checked.

Neutralization

1. Use the Ci-Trol HEPARIN CONTROL 1: Reconstitute one vial as specified in the Instructions for Use.
   a. Perform APTT determination. The result must be within the confidence range established in each laboratory.
   b. Neutralize remaining Ci-Trol HEPARIN CONTROL 1, with Dade® Hepzyme®, handling it in the same manner as a patient sample.
   c. Perform APTT with neutralized Ci-Trol HEPARIN CONTROL 1. The results should lie within an allowable confidence range established in each individual laboratory for this control when treated with Dade® Hepzyme®. Because of stabilizers in the Ci-Trol HEPARIN CONTROL 1, the neutralized control may not be within the normal range for APTT.
2. As an alternative, Dade® Ci-Trol®, Level 1, may be used: Reconstitute two vials of the same lot as specified in the package insert and combine.
   a. Perform APTT determination. The result must fall within the determined confidence range.
   b. Prepare a heparin stock solution with approximately 19.6 U/mL heparin (use the same heparin as administered for heparin therapy) in physiological saline as follows: For heparin with 1 000 USP units/mL, add 0.1 mL heparin to 5.0 mL saline and mix well.
   c. To 1.5 mL Dade® Ci-Trol®, Level 1, add 0.02 mL (19.6 U/mL) heparin stock solution. The control will contain approximately 0.26 U/mL heparin. Gently invert to mix.
   d. Perform APTT determinations of heparin-spiked Dade® Ci-Trol®, Level 1. The results must lie within the allowed confidence range established within each laboratory for this control when spiked with heparin from a specific source/manufacturer.
   e. Neutralize 1 mL of the heparin-spiked Dade® Ci-Trol®, Level 1, with Dade® Hepzyme®, handling it in the same manner as a patient plasma.
   f. Perform APTT determination of the neutralized Dade® Ci-Trol®, Level 1. The results should lie within the confidence range established in the laboratory for an APTT or within an allowable confidence range for this control (also calculated in each individual laboratory) when neutralized with Dade® Hepzyme®.

Results

The results of APTT and TCT should be reported in seconds (see the Instructions for Use for the respective coagulation tests). The PT may be reported in seconds, INR or in percent (see Instructions for Use of the PT reagent used).

If unfractionated heparin is present in the specimen at a level of ≤2 USP units/mL, Dade® Hepzyme® will eliminate the heparin effect. Differences in test values before and after plasma treatment with Dade® Hepzyme® indicate the presence of heparin in the sample. The results should lie within the laboratory's normal range or within 15 % of the patient's baseline for APTT testing, within 10 % of the patient's baseline for PT, and be at the baseline for TCT testing. For patients on oral anticoagulant therapy, Dade® Hepzyme® will neutralize heparin for PT testing.

Limitations

1. Dade® Hepzyme® will neutralize up to 2 USP units of unfractionated heparin when reconstituted with 1.0 mL citrated plasma. The addition of plasma volumes between 0.5 and 1.0 mL will not alter Dade® Hepzyme® neutralization.
2. In evaluating Dade® Hepzyme® results of patients during heparin therapy, the presence of TFPI (Tissue Factor Pathway Inhibitor) should be considered. The levels of TFPI increase several-fold following heparin injection[11]. An increase in TFPI activity has been associated with

an anticoagulant effect that is not removed by heparin neutralization and may result in prolonged clotting times[12,13].

3. For patients receiving oral anticoagulants, thrombolytic therapy, or patients with circulating inhibitors or anti-phospholipid antibodies, the medication and clinical histories should be considered when interpreting results. These patients may have underlying conditions that result in a prolongation of the APTT and/or PT in the absence of heparin. Abnormal APTTs and/or PTs should be followed by additional coagulation studies to determine the source of the abnormal results.
4. If Dade® Hepzyme® neutralization does not return the APTT to within the normal range and high levels of heparin are suspected, sequential neutralization may be tried. A plasma specimen should not be subjected to more than two sequential neutralizations, and heparin levels should not exceed 4 USP units.
5. Thrombin Clotting Times are subject to variation in sensitivity, depending on the thrombin concentration in the reagent and the instrument used. Stabilizers in Dade® Hepzyme® can interfere with TCT results. A shift in the normal range may be expected. TCT testing should not be performed on samples that have been neutralized by sequential vial passages.
6. If a heparinized sample is run after a Dade® Hepzyme® treated sample the result may be shorter due to some Dade® Hepzyme® remaining in the sample probe of an automated analyzer. In order to prevent this, Dade® Hepzyme® treated samples should be tested separately from other samples on the analyzer. After the run of Dade® Hepzyme® treated samples is completed, a system specific rinse program must be manually requested using CA CLEAN I (REF 964-0631-3 or B4265-1 in the US) for the Sysmex CA Systems and CS Systems, or CN-COAGWASHER (REF AZ700649) for the Sysmex CN Systems.

   Please refer to the system specific operators manuals for additional information regarding the operation of the rinse programs.
7. Refer to the Instructions for Use of the respective coagulation test to be performed for additional limitations.

Expected Values

The reference values may vary from laboratory to laboratory depending on the technique and the instrument used. Therefore, each laboratory should determine its own reference range for APTT, PT and TCT.

Acceptable limits for correction should be established by each laboratory for the testing systems used.

Performance Characteristics

Studies have shown that Dade® Hepzyme® can neutralize up to 2 USP units/mL of unfractionated heparin in normal samples spiked *in-vitro*. In 34 samples of unheparinized plasma samples, the APTT was 25.7 ±1.7 seconds before and 25.2 ±1.7 seconds after Dade® Hepzyme® treatment.

Tests were performed on untreated and on Dade® Hepzyme® treated plasma samples from a) patients treated with heparin (frozen samples), and from b) normal blood donors (heparin added *in-vitro*). The results showed no significant change in levels of factors FII, FV, FVII, FVIII, FIX, FX, FXI, FXII or fibrinogen.

Plasma samples from 157 patients were examined in clinical studies. Of these, 47 specimens were from patients who had been treated with 0.1 to 1.1 U/mL of unfractionated heparin (cardiac, orthopedic surgery and DVT patients). Treatment with Dade® Hepzyme® showed a reversal of the heparin effect with APTT results returning to within the APTT reagent's normal range or within 15 % of the patient's baseline before treatment with heparin. The APTT values of 60 apparently healthy donors also lay within this range. PT testing was also done on 32 of the patients treated with heparin.

Results after Dade® Hepzyme® treatment were within 10 % of the patient's baseline PT before treatment with heparin. Samples from 11 patients on low molecular weight heparin therapy were also neutralized by Dade® Hepzyme®.

Thirty specimens from patients receiving coumadin had an APTT mean value of 53.5 ±7.2 seconds. Addition of heparin (0.3 to 1.2 U/mL) increased the value to >200 seconds. After Dade® Hepzyme® treatment, the APTT mean value was 54.6 ±8.7 seconds. Also, 9 plasma samples taken during the induction phase of oral anticoagulation (coumadin) while patients were still on heparin showed an APTT mean value of 75.7 ±28.6 seconds. Dade® Hepzyme® treatment reduced this value to

31.8 ±4.2 seconds. PT values before and after Dade® Hepzyme® treatment were prolonged, as expected. Factor assays and/or chromogenic substrate tests for residual heparin proved that the prolongation of these values even after Dade® Hepzyme® treatment was not due to heparin.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

Current Version of Application Sheets

Dade® Hepzyme® can be used in combination with various automated coagulation analyzers. Sysmex provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 3 under the dedicated link below:

sysmex-ifu.com/ag

As the manufacturer continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.

References

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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community	CH REP	Authorized representative in Switzerland
	Contains sufficient for <n> tests		Biological Risks
IVD	*In Vitro* Diagnostic Medical Device		Temperature Limitation
	Consult instruction for Use		Non-sterile
CE	CE marking of conformity	CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.
CONTENTS	Contents	→	Reconstitution volume
LEVEL	Level		Keep away from sunlight and heat
WARNING	Warning	DANGER	Danger
RxOnly	Prescription device (US only)	UDI	Device Identification (UDI) barcode
REACH xx/xx/xx	REACH Authorization Number		

Legal Information

Siemens Healthcare Diagnostics Products GmbH
Emil-von-Behring-Str. 76
35041 Marburg
Germany
siemens-healthineers.com