2024/10/31
A02A00018DEN

Yumizen G AT

REF 1300036390
THROMBIN 4 x 3 mL
SUBSTRATE 4 x 3mL
DILUENT 4 x 7mL

IVD CE

HORIBA ABX SAS
Parc Euromédecine
Rue du Caducée
BP 7290
34184 Montpellier Cedex 4
FRANCE

- **Yumizen G200**
- **Yumizen G400 DDi/G405**
- **Yumizen G800/G800h/G850h**
- **Yumizen G1500/G1550/G1500h/G1550h**

In vitro diagnostic reagent for determination of antithrombin activity by chromogenic assay.

Application Release [a] [b]

	Test name
Yumizen G1500/G1550	AT
Yumizen G1500h/G1550h	AT
Yumizen G800	AT
Yumizen G800h/G850h	AT
Yumizen G405	AT
Yumizen G400 DDi	AT
Yumizen G200	AT

Intended Use (1, 2) [c]

For *in vitro* diagnostic use only.
Yumizen G AT is a chromogenic assay used for the quantitative determination of Antithrombin (AT) activity in human citrated plasma.

Clinical Interest (3) [d]

Antithrombin functions as a key regulator of blood coagulation and is essential for effective heparin therapy.
Antithrombin and thrombin form a stoichiometric complex.
The velocity of the complex formation is highly increased by the presence of heparin, thus the antithrombotic effect appears immediately when heparin is present.

Method (1) [e]

Yumizen G AT assay for determination of the AT level consists of two steps:

1. The plasma sample is incubated with a known, excess amount of thrombin in the presence of heparin.
2. After the complex formation, the activity of the residual thrombin is determined by a chromogenic thrombin substrate (absorbance measurement at 405 nm).
   The amount of the inhibited thrombin is proportional to AT level of the sample.

The result of the absorbance measurement is obtained in optical density per unit time (OD/min). It is converted to a percentage (%) by a calibration equation.

Reagents [b]

Reagent 1

Yumizen G AT Thrombin is freeze-dried.
This reagent is bovine thrombin in buffered medium which contains heparin and preservative.

Bovine thrombin	< 1%
Heparin	< 0.1 g/L
Bovine albumin	< 10 g/L
Sodium azide	< 1 g/L

Reagent 2

Yumizen G AT Substr. is freeze-dried.

[a]Modification: new instrument added.
[b]Modification: chapter added.
[c]Modification: new leaflet form.
[d]Modification: § "Clinical Interest" changed.
[e]Modification: § "Method" changed.

Yumizen G AT

This reagent is chromogenic thrombin substrate which contains preservative.

Thrombin substrate	< 2 g/L

Reagent 3

Yumizen G AT Dil. is ready-to-use.
This reagent is a buffer which contains preservative.

Sodium azide	< 1 g/L

Yumizen G AT should be used according to this notice.
The manufacturer cannot guarantee its performance if used otherwise.

Handling [f]

1. Allow the vials to stand for 5 min (20 - 25°C) before reconstitution.
2. Reconstitute the content of the vials with deionized or purified water as follows:
   - **Yumizen G AT Thrombin**: 3 mL
   - **Yumizen G AT Substr.**: 3 mL
   - **Yumizen G AT Dil.**: Ready to use

   Be careful when opening the rubber cap as some lyophilized material may be lost.
3. Replace the caps and gently invert the bottles (8 - 10 times) to disperse the contents (avoid foaming).
4. Allow the vials to stand for at least 30 min (20 - 25°C).
5. Mix thoroughly the vials once more before use.
6. **For automated analyzers only:** place the vials in the instrument without cap as follows:
   - **Yumizen G AT Thrombin**: Reagent holder
   - **Yumizen G AT Substr.**: Reagent holder
   - **Yumizen G AT Dil.**: Auxiliary holder

For optimal performance remove the reagent from the instrument after use, close the vial and store at 2 - 8°C.
An analysis of the control must be carried out on a daily basis at the same time as the patient samples, including each time a calibration is carried out.
The frequency of the controls depends on the laboratory requirements.
Each laboratory must establish the quality assurance procedures to be followed. These must conform to the current accreditation requirements and pertinent regulations.

Care should be taken not to interchange the caps with others products.

Calibrator [b]

Calibrator Information
Yumizen G CAL (1300036146) (not included)
This assay reported dimension is calculated from a lin-lin calibration curve.
Every AT determination requires local calibration with the given lot of reagent on the given instrument.

Calibration Procedure for Semi-Automated Analyzers
The calibration is a calibrator dilution based process that can be used with HORIBA (Yumizen G Line).

1. Prepare a serial dilution of the calibrator **Yumizen G CAL** as follows:

	Point 1	Point 2	Point 3	Point 4
Dilution rate	1/20	1/40	1/60	0
Yumizen G CAL	20 µL	100 µL Point 1 (1/20)	100 µL Point 1 (1/20)	0 µL
Yumizen G AT Dil.	380 µL	100 µL	200 µL	100 µL
Total volume	400 µL	200 µL	300 µL	100 µL

2. Run each diluted calibrator according to the *Semi-Automated Analyzers Procedure* chapter.
   Duplicated measurement is recommended.
3. Prepare the calibration curve as follows:

Dilution rate	%	OD/min
1/20	X[(a)]	Result 1
1/40	X/2	Result 2
1/60	X/3	Result 3
0	0	Result 4

[a]: **Yumizen G CAL** value from the enclosed annex

4. Report the % and OD/min results in the calibration menu of you instrument.
5. Enter the data by using the "points" icon.
   Refer to the user manual of your instrument.

In case of determination by any other hemostasis analyzers, please follow the instructions of the manual.

[f]Modification: § "Handling" changed.
[b]Modification: chapter added.

Yumizen G AT

Control [b]

For internal quality control, use:

- **Yumizen G CTRL I & II** (1300036412) (not included)
  5 x 1 mL + 5 x 1 mL

The frequency of controls and the confidence intervals should correspond to laboratory guidelines and country-specific directives. You should follow federal, state and local guidelines for testing quality control materials. The results must be within the range of the defined confidence limits. Each laboratory should establish a procedure to follow if the results exceed these confidence limits.
Each control should be assayed daily and/or after a calibration.

Semi-Automated Analyzers Procedure [g]

Yumizen G AT can be used on semi-automated analyzers (Yumizen G Line), according to the following procedure.
Duplicated measurement is recommended.

1	Dilute the sample with **Yumizen G AT Dil.**.	1:20
2	Add the diluted sample into the cuvette.	50 µL
3	Incubate at 37°C.	30 s
4	Add the **Yumizen G AT Thrombin**.	50 µL
5	Incubate at 37°C.	2 min
6	Add the **Yumizen G AT Substr.**.	50 µL
7	Start immediately the measurement at 405 nm.	10 - 40 s

In case of determination by any other hemostasis analyzers, please follow the instructions of the manual.

Materials Required but not Provided [h]

- HORIBA analyzers (Yumizen G Line) are recommended.
- Third-party analyzers can also be used where chromogenic based method at 405 nm and free test setup access are allowed.
- Calibrator: **Yumizen G CAL** (1300036146)
- Control: **Yumizen G CTRL I & II** (1300036412)
- Deionized or purified water
- Standard laboratory equipment

Specimen [i]

Plasma

- 3.2% (109 mmol/L) sodium-citrate anticoagulated plasma in primary tube.
- 3.2% (109 mmol/L) sodium-citrate, theophylline, adenosine and dipyramidole (CTAD) anticoagulated plasma in primary tube.

Mix the blood carefully.

Specimen centrifugation

Speed	Time	Temperature
1500 g	15 min (not less)	room temperature

Specimen Stability (4)

- At 20 - 25°C: 4 hours
- At 2 - 8°C: 4 hours
- At -24°C: 24 months (only the plasma)

To thaw plasma:

1. Thaw rapidly the frozen plasma specimen at 37°C.
2. Gently mix the sample.
   Resuspend any precipitation by thorough mixing immediately after thawing and before testing.
3. Test immediately the sample.

Mixing is critical before testing, as precipitation of certain proteins may occur with freezing.
For additional information, please refer to CLSI document H21-A5.

Reference Range [b]

Each laboratory should establish its own reference ranges.
The values given here are used as guidelines only.

	Mean (%)	From (%)	To (%)
Normal adult range	100	80	120

[b]Modification: chapter added.
[g]Modification: § "Semi-Automated Analyzers Procedure" changed.
[h]Modification: § "Material Required but not Provided" changed.
[i]Modification: § "Specimen" changed.

Yumizen G AT

Storage and Stability [j]

Stability before opening

Stable up to the expiry date on the label if stored at 2 - 8°C.

Stability after reconstitution

	2 - 8°C
Yumizen G AT Thrombin	7 days
Yumizen G AT Substr.	7 days
Yumizen G AT Dil.	7 days

Stability on board

Different on-board containers are required for reagents and diluent. Please follow the instructions of the manual of automated analyzers.

Automated Analyzers

	20 - 25°C	15 - 19°C
Yumizen G AT Thrombin	-	3 days
Yumizen G AT Substr.	-	3 days
Yumizen G AT Dil.	3 days	-

Do not freeze.

Yumizen G AT Substr.

Store protected from light.

Waste Management [b]

- Please refer to local legal requirements.
- This product contains less than 0.01% of sodium azide as a preservative. Sodium azide may react with lead and copper to form explosive metal azides.

General Precautions [k]

- This product is for professional *in vitro* diagnostic use only.
  For laboratory use.
- For prescription use only.
- This reagent is classified as non-hazardous in compliance with regulation (EC) N°.1272/2008.
- **Reagent 1 (Yumizen G AT Thrombin)**
  Warning: This product is obtained from substances of animal origin. Consequently, it should be treated as potentially infectious and handled with the appropriate cautions in accordance with good laboratory practices (5).
- Do not pipette by mouth.
- Do not replenish the products.
- Do not swallow. Avoid contact with skin and mucous membranes.
- Observe the standard laboratory precautions for use.
- The product vials should be discarded after use. Disposal of all waste material should be in accordance with local guidelines.
- Please refer to the SDS associated with the product.
- Do not use the product if there is visible evidence of biological, chemical or physical deterioration.
- Do not use the product if the recommended storage conditions, including temperature, are not followed.
- User must be trained by a HORIBA representative before attempting to operate the device.
- It is the user's responsibility to verify that this document is applicable to the product used.
- For technical assistance, you can call +33 (0)4 67 14 15 16.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.
- Using of third-party hemostasis analyzers may cause a risk of system un-harmonization.
- It is the user's responsibility to evaluate the risk of using a third-party hemostasis analyzers.

Performance (2, 6)

The performance data listed below are representative of performance on HORIBA systems.

Lot to Lot Variability [b]

The lot to lot variability was tested with sample group analysis between consecutive lots. The unit difference of different sample groups on Yumizen G Line analyzers give the following result.

Sample group	Normal	Pathological
Difference	1.5%	6.4%

[j]Modification: § "Storage and Stability" changed.
[b]Modification: chapter added.
[k]Modification: § "General Precautions" changed.

Yumizen G AT

Sample Volume [b]

Instrument	Volume
Yumizen G1500/G1550	50 µL (of diluted sample)
Yumizen G1500h/G1550h	50 µL (of diluted sample)
Yumizen G800	50 µL (of diluted sample)
Yumizen G800h/G850h	50 µL (of diluted sample)
Yumizen G405	50 µL (of diluted sample)
Yumizen G400 DDi	50 µL (of diluted sample)
Yumizen G200	50 µL (of diluted sample)

Limit of Detection [b]

The limit of detection according to the recommendations found in the CLSI (NCCLS) EP17-A2 (7) is 10%.

Limit of Quantitation [b]

The limit of quantitation according to the recommendations found in the CLSI (NCCLS) EP17-A2 (7) is 10%.

Precision

Repeatability (on automated analyzers) [l]

Repeatability according to the recommendations found in the CLSI (NCCLS), H57-A (8), EP05-A3 (9) (data obtained on internal study).

- 2 controls (10 runs)
- 2 specimens (20 runs)

	Mean value %	CV %
Control specimen 1	108.8	1.730
Control specimen 2	50.5	4.621
Specimen 1	108.4	2.224
Specimen 2	68.8	2.997

Maximum acceptance criteria (CV %): < 8%.

Reproducibility (on automated analyzers) [m]

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP05-A3 (9) (data obtained on internal study).

- 2 controls (10 runs)

	Mean value %	CV %
Control specimen 1	108.4	2.154
Control specimen 2	47.2	6.326

Maximum acceptance criteria (CV %): < 15%.

Measuring Range [b]

The measuring range and linearity are determined up to 150% on the Yumizen G Line instruments.

Correlation [b]

Specimens are correlated with a commercial reagent taken as reference on HORIBA analyzers (Yumizen G Line).

- Passing-Bablok regression: 1.061 (slope)
- Bland et Altman plot procedure: 0.63 (% difference)

Interferences (10) [b]

Haemoglobin: No significant influence is observed up to 16.7 g/L.
Triglycerides: No significant influence is observed up to an Intralipid® concentration (representative of lipemia) of 40 mmol/L.
Bilirubin: No significant influence is observed up to 750 µmol/L.

Yumizen G AT assay cannot be applied in case of the presence of thrombin inhibitors (e.g. hirudin and New Oral Anticoagulants (NOAC), like direct thrombin inhibitors) in the patient sample.

Clinical Performance [b]

Clinical sensitivity and specificity, positive predictive value and negative predictive value are not commonly reported for this test.
This is largely attributed to the fact that this antithrombin assay is a functional test.
To arrive at a diagnosis and a course of treatment, results from others routine coagulation tests should be used in conjunction with other diagnostic information and the attending health-care professional's evaluation of the patient's condition.

Precautions of Characteristics

The measurement data was generated during a performance evaluation and is not recommended as an acceptance criterion.

[b]Modification: chapter added.
[l]Modification: modification of repeatability.
[m]Modification: modification of reproducibility.

Yumizen G AT

Reference

1. Blombäck M, Blombäck B, Olsson P and Svendsen L. The Assays of Antithrombin Using a Synthetic Chromogenic Substrate for Thrombin. Thromb Res (1974) **5** (5): 621-632.
2. Khor B, Van Cott EM. Laboratory tests for antithrombin deficiency. Am J Hematol (2010) **85**: 947-950.
3. One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test. Approved Guideline, 2nd ed., CLSI (NCCLS) document H47-A2 (2008) 28:20.
4. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. Approved Guideline, 5th ed., CLSI (NCCLS) document H21-A5 (2008).
5. Council Directive (2000/54/EC). Official Journal of the European Communities. No. L262 from October 17, 2000: 21-45.
6. Meijer P, Haverkate F, Kluft C. Performance goals for the laboratory testing of antithrombin, protein C and protein S. Thromb Haemost (2006) **96**: 584-589.
7. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012).
8. Protocol for the Evaluation, Validation, and Implementation of Coagulometers. Approved Guideline, 1th ed., CLSI (NCCLS) document H57-A (2008).
9. Evaluation of Precision of Quantitative Measurement Procedures. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP05-A3 (2014).
10. Interference Testing in Clinical Chemistry. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP07-A2 (2005).