FREND TSH Neo

Quantitative assay for Neonatal Thyroid Stimulating Hormone

REF FRTHN 020

IVD For *in vitro* diagnostic use only

Intended use

The FREND TSH Neo is intended for the quantitative determination of thyroid-stimulating hormone(TSH) in neonatal blood specimens collected onto blood collection filter paper based on Fluorescence immunoassay (FIA). It is designed to aid in screening newborns for congenital hypothyroidism.

Summary and explanation of test

Measurement of thyrotropin (TSH), a glycoprotein that has a molecular weight of 28,000 daltons and is secreted from the anterior pituitary gland, is generally considered to be the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism[1,2].
Thyroid hormone produced under TSH's direction bring into play negative feedback on the pituitary gland, which regulates the secretion of TSH[3]. This negative feedback relationship between the thyroid gland and the pituitary gland means that TSH is always elevated, frequently at very high levels, in primary hypothyroidism. Although the typical concentration of TSH in the blood is especially low, it is essential for maintaining ordinary thyroid function[4].

The earliest signs of primary hypothyroidism are elevated TSH concentrations in infant blood. TSH testing is a screening method for the detection of neonatal hypothyroidism due to its high specificity and sensitivity.

The American Thyroid Association has declared that the most important diagnostic test for neonates is the identification of hypothyroidism during the first few days of life. The federal and state health departments have established screening centers in response to the need for early detection and treatment.

Temporary and mild hypothyroidism can also occur, especially in very premature or very underweight infants[5,6]. Studies have shown that early clinical diagnosis of this disorder, mostly within the first two weeks of birth, and subsequent treatment tends to prevent irreversible mental retardation, neurological dysfunction, or impairment[7, 8, 9,10].

The most effective techniques for determining if thyroid illness occurred in babies have been suggested. Serum T3, T4, and TSH should be used to inform these choices. It has been demonstrated that there are minor diversifications in the concentrations of demographic diversity in body weight, preterm newborns, TSH, and T4. As a result, each laboratory needs to establish its own cutoff and normal values.

FREND TSH Neo has created a kit utilizing immunoassay methods and a way for gathering dried blood spot samples. TSH levels in newborns can be quantitatively measured with this kit in a sensitive, accurate, safe, and reliable manner. Determining the thyroid status of newborns is a crucial and useful tool that helps prevent infant mental impairment.

Principle of the assay

The FREND TSH Neo assay is a sandwich type of fluorescence immunoassay using fluorescent nanoparticles in microfluidic flow and is based on two monoclonal antibodies with different epitope specificities for the β-subunit of the TSH molecule.

A 35 µL drop of specimen solution (DBS eluted dilution buffer) is placed in the FREND TSH Neo cartridge inlet port, where the sample interacts with a proprietary mix of dry-loaded reagent. One of these reagents includes antibody-conjugated fluorescent nanoparticles, which form immune complexes with TSH in the infant sample. Capillary action moves the sample to the detection region, where capture antibodies grab the TSH-nanoparticle.

The concentration of TSH is calculated by the FREND™ System when the ration of Test/Reference fluorescence in an unknown is compared to that same ratio for standards of known concentration. The result is calculated using information stored on the lot specific FREND TSH Neo Code chip and then is displayed on the FREND System screen. A hard copy printout can be obtained if desired. A ratio calculated between the Reference zone and the Test zone corrects for test-to-test variations.

TSH concentration in a sample analyzed with the FREND TSH Neo on the FREND™ System correlates directly with the fluorescence intensity the higher the TSH concentration, the greater the fluorescence ratio. The FREND TSH Neo has a measuring range determined as 1.35 µIU/mL Blood to 60.0 µIU/mL Blood (3.0 ~ 130.0 µIU/mL serum, Hematocrit approximately 50-55%)

The FREND TSH Neo uses single-use transparent plastic cartridges in which all required reagents are stored within the cartridge itself. All that is added by the user is a 35 µL test sample. The cartridge is inserted into the FREND™ System in a prescribed fashion indicated with a black arrow on the cartridge. The reaction is read multiple times as the sample moves via capillary action through the cartridge. This type of assay system is sometimes referred to as one which incorporates laminar flow.

Material provided

Q'ty	Contents	Catalogue number
20	FREND TSH Neo cartridge(s)	FRTHN 020
20	FREND TSH Neo Dilution tube(s)	
20	FREND TSH Neo DBS filter paper(s)	
30	Disposable pipette tips	
01	FREND TSH Neo Code chip	
01	FREND TSH Neo Package Insert	

Materials required but not provided

- The FREND™ System
- Micro-pipette capable of delivering 35 µL

Warning and Precautions

- The FREND TSH Neo cartridges are intended for in vitro diagnostic use only.
- The FREND TSH Neo cartridges are only to be used on the FREND™ System.
- Allow sealed cartridges and dilution tubes come to room temperature for 15-30 minutes prior to use.
- Assure the humidity in the laboratory is in the 10-80% range when tests are run.
- Assure the room temperature remains in the range of 22~30°C when tests are run.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
- Inaccurate results are possible if the sample used is contaminated in any way.

- Using specimens containing clotted fibrin could result in erroneous results.
- Over or under loading the cartridge with sample may result in inaccurate results.
- Cartridges should not be frozen.
- Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the laboratory may be derived from human materials, Use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples.
- Do not use the cartridges beyond the expiration date on the pouch.
- Do not use the cartridge if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package Insert and User manual.
- The FREND TSH Neo cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Keep the cartridge sealed in the pouch until ready for use.
- Use the cartridge immediately after opening the pouch.
- Wear disposable gloves when handling the cartridges and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- Do not ingest the silica gel packet found in the cartridge pouch.
- To avoid cross contamination, wash and clean hole punch before punching a new test sample.
- All samples must be collected on sample collection card.
- Do not punch blood dots from areas that are printed or that are near the edge of the blood spot.
- During the incubation period make sure all blood spots are within the conjugate to ensure accurate and reliable results.

Storage and Stability

Store at 2-8 °C. All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for twelve months from the date of manufacture. The expiration date is clearly indicated on the product box and the cartridge pouches.

Materials	Catalogue number
FREND TSH Neo cartridges	FRTHN 020
FREND TSH Neo Dilution tubes	None

* Store at 2-8 °C (35.6-46.4 °F)

Specimen collection and handling

DBS (Dried blood spot) is required for the assay. Neonatal screening programs differ from one another in the type of specimen required, the recommendation is a blood spot.
Allow a sufficient quantity of blood to seep through to completely fill a pre-printed circle on the filter paper. Examine both sides of the filter paper to make sure that the blood has penetrated and saturated the paper. Allow the filter paper to dry at room temperature overnight away from heat and moisture. The sample paper should be punched from similar areas to 6 mm in diameter. Put the one punched sample in the provided dilution buffer and placed on the table 3 hours to allow the sample to permeate sufficiently into the buffer.
The sample required to test the FREND TSH Neo is a DBS with a diameter of 6 mm. When pipetting 35 µL of DBS eluted sample into the FREND TSH Neo cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete.

Procedure

1) Reagent preparation

There is no reagent preparation required to measure TSH using the FREND TSH Neo cartridge on the FREND™ System. However, the cartridges and Dilution buffer tubes needed for a particular run should be removed from the refrigerator and allowed to reach room temperature for 15~30 minutes before they are used.

Procedure

2) Calibration

The calibrators used during the reagent manufacture process to create the information placed electronically on the FREND TSH Neo Code chip are prepared gravimetrically and are compared to international reference standards (WHO International Thyroid Stimulating Hormone, Human, Pituitary, NIBSC code: 81/615). However, for the end user, there is no need for calibration as is generally performed on other automated laboratory equipment. All calibration statistics and information have been electronically stored on the FREND TSH Neo Code chip included in each box of FREND TSH Neo. The FREND TSH Neo Code chip is specific for that manufactured lot of FREND TSH Neo cartridges.
The appropriateness of the calibration information should always be checked by running sufficient external quality control materials as samples to verify that the results obtained for TSH Neo on the FREND™ System using the FREND TSH Neo cartridges of a particular lot meet the laboratory criterion for acceptability.

3) Code chip installation

There are two models available for the FREND™ System: F10 and FREND 2.0. Please refer to the specific model's user manual for more detailed instructions on Code chip installation. Abbreviated instructions are as follows:

(1) FREND™ System (Model: F10)

1. Insert the FREND™ System (F10) electrical cord into an appropriate outlet.
2. Press the 'Setup' button on the 'Main' screen.
3. Insert the Code chip into the Code chip slot at the rear of the system, following the arrow.
4. Press the 'Code chip' button on the 'Setup' screen.
5. The information embedded in the FREND TSH Neo Code chip is automatically saved in the FREND™ System.

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6. When the Code chip installation is complete, press the ‘OK’ button to return to the ‘Setup’ screen.
7. Press the ‘Item’ button on the ‘Setup’ screen.
8. Click the FREND TSH Neo cartridge and check the installed lot number and the installation date of the Code chip.
9. Press the ’Home’ button to go to the ‘Main’ screen to begin running the external quality control and the specimens.

(2) FREND™ System (Model: FREND 2.0)

1. Insert the FREND™ System (FREND 2.0) electrical cord into an appropriate outlet.
2. Press the ‘Setting’ button on the ‘Main’ screen.
3. Press the ‘Code’ button on the ‘Setting’ screen.
4. When installing with the Code chip, insert the Code chip into the Code chip slot on the right side of the system following the arrow. Then, press the ‘Code chip’ button.
5. When installing with the QR code, press the ‘QR Code’ button, then scan the QR code with the barcode scanner on the front of the system.
6. The information embedded in the FREND TSH Neo Code chip is automatically saved in the FREND™ System.
7. When the Code chip installation is complete, press the ‘X’ button to close the window.
8. Press the ‘Item’ button on the ‘Setting’ screen.
9. Click the FREND TSH Neo cartridge and check the installed lot number and the installation date of the Code chip.
10. Press the ‘←Back’ button to exit ‘Setting’ menu and begin running the external quality control and the specimens.

4) Quality control

- **FREND™ System QC cartridges**

  FREND QC Cartridge contains multiple controls to check optic part of the system. By testing QC Cartridge, part of analytical components of the system of (1) laser power, (2) alignment, and (3) mechanical integrity are confirmed.

For each day of patient testing perform QC Cartridge testing. Refer to the quality control procedures section in the User manual of FREND™ System. In brief, perform QC Cartridge testing for the following conditions:

1) Upon initial setup of the system
2) Each day of patient testing,
3) When the system has been transported or moved,
4) Whenever there is uncertainty about the performance of the system,
5) Whenever required by your laboratory's quality control requirements.

- **Specimen measurement processing**

1) Preparation

Remove from the refrigerator sufficient cartridges of FREND TSH Neo to test the number of patient samples. Allow the tubes and the sealed pouches containing the cartridges to come to room temperature for 15-30 minutes prior to the start of the testing sequence. For consistent results, all testing should be done when room temperature is 22~30°C.

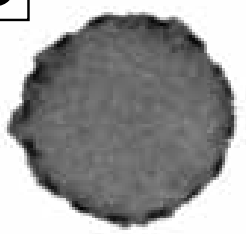
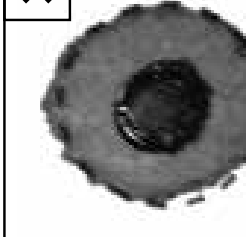
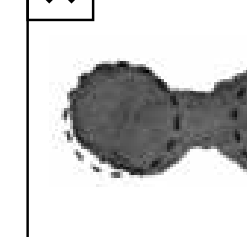
2) Assay procedure (For more detailed instructions, please refer to the FREND™ System User Manual)

- **Preparation of draw blood**

1. Wipe the heel prick to be collected with alcohol swap, dry it, and bleed.
2. The first blood is wiped and then used.
3. Fill a pre-printed circle on the DBS filter paper with the specimen completely to soak.

⚠ ***Caution:*** *Examine both side of the filter paper to make sure that the blood has penetrated and saturated the paper.*

Sample collection examples

Valid	Insufficient	Layering	Super saturated
O 	× 	× 	× 

- **Preparation of DBS samples**

1. Dry the DBS filter paper at room temperature for overnight to avoid heat and moisture.
2. The dried DBS filter paper should be punched from to 6 mm in diameter.

⚠ ***Caution:*** *Do not punch blood dots from areas that are printed or near the edge of the circle. Please make sure entire punched sample is soaked with blood.*
It is recommended to punch the center of the printed circle.

3. Put the one punched DBS sample in the provided dilution buffer.
4. Make sure the paper is completely submerged in the buffer.
5. Placed on the table for 3 hours at room temperature.

- **Sample loading**

1. After 3 hours DBS incubation, prepare the cartridge.
2. Tapping the tube twice for sample mixing.
3. After pipetting, drop the mixed sample (35 μL) into the sample inlet on the cartridge using a micro-pipette equipped with a fresh pipette tip.

- **Sample reading**

1. Prepare the FREND TSH Neo cartridge and sample.
2. Record the Sample ID on the cartridge in the designated area.
3. Press the ‘Test’ button on the ‘Main’ screen of the FREND™ System.
4. And then press the ‘Patient’ button, the system moves to the Patient ID screen automatically.
5. Type the Patient ID and press the ‘Enter’ button to begin the test.
6. Insert the loaded cartridge into the cartridge slot using the cartridge arrows as a guide.

⚠ ***Caution:*** *Please check the direction of the cartridge before insertion and assure that insertion is complete.*

7. When the reaction in the cartridge is completed, the FREND™ System will automatically begin the reading process.
8. When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

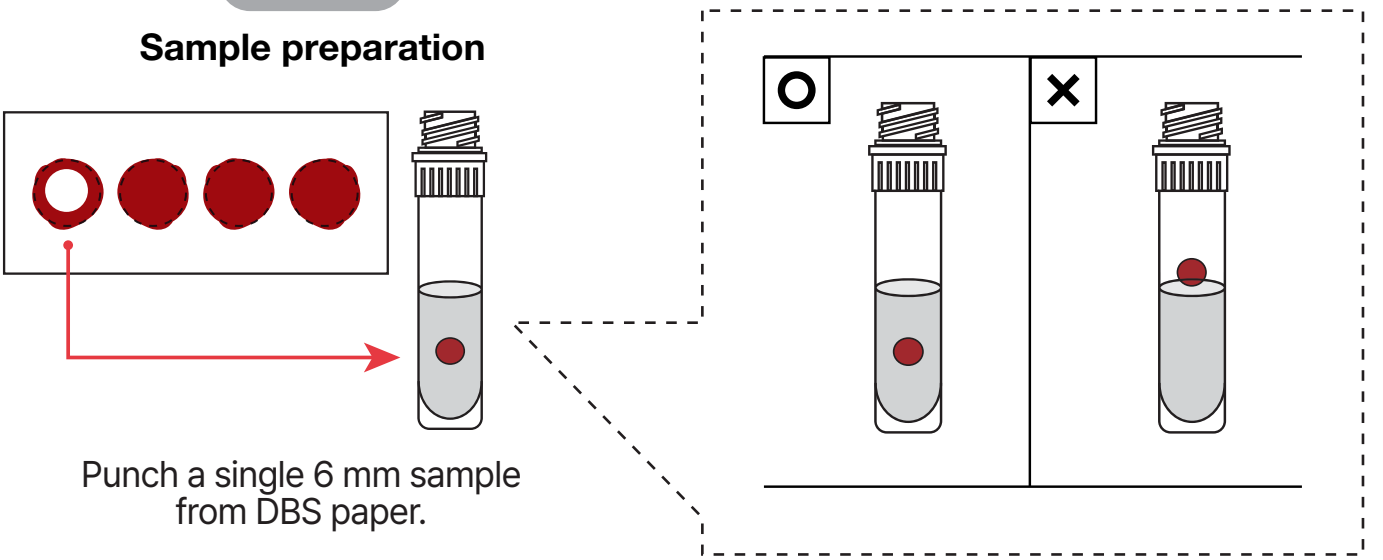
⚠ ***Caution:*** *Do not remove power from the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.*

9. If the FREND™ System is connected to the optional printer, press the ‘Print’ button and the results will be printed on the printer paper.
10. For more detailed instructions, please refer to the FREND™ System User Manual.

Summary of FREND TSH Neo test procedure

STEP 1.

Sample preparation



Punch a single 6 mm sample from DBS paper.

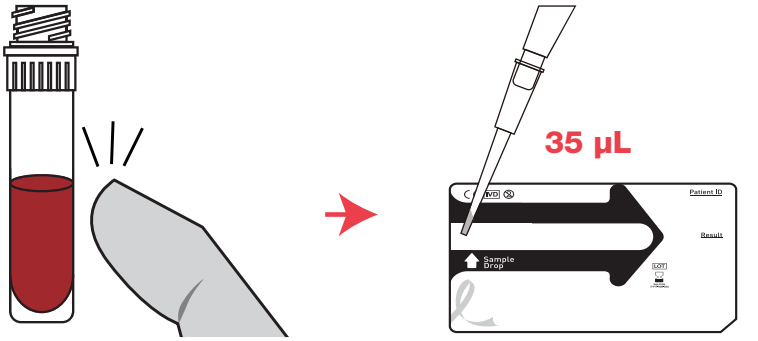
STEP 2.

Incubation

Place it in a dilution tube for 3 hours at RT.

STEP 3.

Sample loading

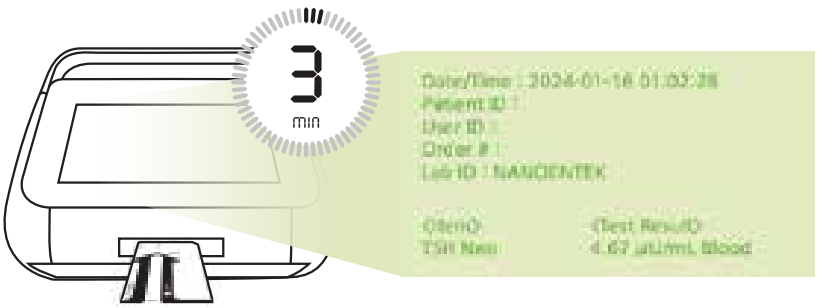


After 3 hours incubation, tapping the tube for mixing well. Load 35 µL of the on the cartridge.

STEP 4.

Sample reading

Insert the cartridge and get the results.



Calculation of results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been manually loaded to the sample inlet in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process, blank readings are subtracted after which the net rate is automatically converted to Thyroid Stimulating Hormone (TSH) concentration in µIU/mL Blood based upon information stored on the FREND TSH Neo Code chip. This result is then displayed on the screen and can be sent to the optional printer. It is also stored in memory on the FREND™ System.

Screen displays for various concentration scenarios

Displayed result	Description
	TSH Neo concentration Less than 1.35 µIU/mL Blood
	TSH Neo concentration Not less than 1.35 µIU/mL Blood and not higher than 60.00 µIU/mL Blood

	TSH Neo concentration Higher than 60.00 µIU/mL Blood

Limitations of the procedure

1. When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of other tests, clinical impressions, medical history, therapy, etc).
2. The FREND™ system paired with a FREND TSH Neo cartridge, is programmed to report 60.0 µIU/mL Blood as the highest concentration of TSH measurable without dilution. The lowest measurable concentration is TSH 1.35 µIU/mL Blood– the assay sensitivity limit.
3. The concentration of TSH in a given sample determined using assays from different manufacturers can vary due to differences in assay methods, calibration, and antibody specificity.
4. Please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.
5. FREND TSH Neo has not been validated in point-of-care settings.
6. Performance of this assay has not been established with neonatal specimens or specimens from pregnant patients.
7. FREND TSH Neo is to be used in licensed clinical laboratories with trained technologists.

Performance characteristics

Precision

Precision of the FREND TSH Neo was evaluated using dried blood spot specimens according to the CLSI guideline EP05-A3, following a protocol: 2 runs per day, in duplicate, over 20 days (n=80).

	TSH (μIU/mL Blood)	Repeatability		With-in laboratory	
		SD	%CV	SD	%CV
Low	5.00	0.21	4.2%	0.28	5.5%
Med	20.00	1.11	5.6%	1.23	6.2%
High1	40.00	2.33	5.8%	2.36	5.9%
High2	60.00	3.57	6.0%	3.65	6.2%

Analytical sensitivity

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) were determined in accordance with the CLSI guideline EP17-A2.

LoB (μIU/mL Blood)	**LoD** (μIU/mL Blood)	**LoQ** (μIU/mL Blood)
0.09	0.16	0.55

Linearity

The range of linearity in DBS specimen was established using CLSI guideline EP06-A2. FREND TSH Neo was found to have linearity within the analytical measuring range of 1.35 – 60.00 μIU/mL Blood within the deviation of ±10%.

Comparative analysis

FREND TSH Neo was compared to the predicate device A using guidelines outlined in CLSI document EP09-A3. According to the result of the study, FREND TSH Neo has a high correlation with the predicate devices. The result of the study are as follows.

1) FREND TSH Neo vs Predicate device A

Samples(n)	Slope	Intercept	Correlation coefficient (R)
111	1.0099	- 0.149	0.9925

Comparative analysis

FREND TSH Neo was evaluated with 'FREND 2.0' and predicate device 'F10' using CLSI document EP09-A2. According to the result of the study, FREND 2.0 has a high correlation with the predicate devices in FREND TSH Neo. The result of the study are as follows.

1) F10 vs FREND 2.0

Samples(n)	Slope	Intercept	Correlation coefficient (R)
111	0.982	0.2144	0.9989

Interference

The interference of the endogenous and pharmaceutical substances listed below was evaluated on the FREND TSH Neo assay following the CLSI guideline EP07-A3. No interference was observed up to the specified concentrations.

No.	Substance	Concentration
1	D-Glucose	120 mg/dL
2	Bilirubin	20 mg/dL
3	Bilirubin (unconjugated)	39 mg/dL
4	Hemoglobin	500 mg/dL
5	Cholesterol	700 mg/dL
6	L-Ascorbic acid	5.25 mg/dL
7	Biotin	3500 ng/mL
8	triglycerides	500 mg/dL

Cross reactivity

The potential cross-reactants, selected based on structural similarity or known for cross-reaction, were tested following the CLSI guideline EP07-A3. The effect of the cross-reactants listed below were evaluated for potential cross-reactivity with the FREND TSH Neo assay. No cross-reactivity was observed at the specified concentrations.

No.	Substance	Concentration
1	Follicle-stimulating hormone, FSH	500 mIU/mL
2	Luteinizing hormone, LH	500 mIU/mL
3	Human chorionic gonadotropin, hCG	200,000 mIU/mL

Accuracy

The accuracy studies was evaluated on the FREND TSH Neo assay following the CLSI guideline EP15-A2. As a result of the accuracy test for FREND TSH Neo, all samples met the criteria.

Hook effect

No hook effect was observed with TSH concentration up to 2000 μIU/mL Blood.

References

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Glossary of symbols

	Caution, warning, Consult accompanying documents
	Catalogue number/Reference number
 www.nanoentek.com/eifu.php	Consult Instructions for Use An electronic instructions for use (eIFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.
	Lot number/Batch number
	Use by YYYY-MM-DD or YYYY-MM
	Manufacturer
	CE marking
	*In vitro* diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests
	Do not reuse
	Do not use if package is damaged

Rx Only	For prescription use only CAUTION: Federal (U.S.) law restricts this device to sale by or on order of a physician.
US Corporation	US Corporation
Patient ID	Patient ID
Result	Result
Sample Drop	Sample Drop
EC REP	Authorized representative in the European Community
UK Representative	Authorized representative in United Kingdom
CH REP	Authorized representative in Switzerland
BRH	Authorized representative in Brazil

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