This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

See REAGENTS section for a full explanation of symbols used in reagent component naming.
The lung is the last vital organ of the fetus to mature to the extent necessary to support extrauterine life. Studies have shown that, in the process of maturation, the lungs begin to secrete a surfactant. This surfactant prevents the collapse of the alveoli at end-expiration by decreasing surface tension. In cases of premature birth, in which insufficient amounts of pulmonary surfactant are present, the newborn infant develops respiratory distress syndrome (RDS), a serious and life-threatening condition requiring aggressive and long-term respiratory support. RDS is a complication of prematurity birth and is a cause of morbidity and mortality in the premature infant. The major surface-active constituent of pulmonary surfactant is phosphatidylcholine (lecithin). As the fetus matures, the synthesis of lecithin increases, and by 34-36 weeks the amount of lecithin present is sufficient to prevent alveolar collapse. The surfactant is transferred to the amniotic fluid for assessment of lung maturity of the fetus. Amniotic fluid also contains a number of other phospholipids (e.g., sphingomyelin), and proteins (e.g., albumin). The level of protein is independent of lung function and remains relatively constant throughout the later months of pregnancy. The onset of lung maturity results in an increase in the amount of surfactant relative to other components of amniotic fluid. The Fetal Lung Maturity II assay measures lung surfactants relative to albumin and the results are expressed as the surfactant/albumin ratio.

**PRINCIPLES OF THE PROCEDURE**

In the Fetal Lung Maturity II procedure, a fluorescent dye is added to the amniotic fluid sample. The dye partitions between albumin and the aggregates formed by the surfactant. Dye molecules associated with albumin are restricted in movement and are exposed to a polar environment. Consequently, the fluorescence lifetime is increased, and a high level of polarization is obtained. When associated with the surfactant, the dye is in a much lower polarity environment, has a longer fluorescence lifetime, and therefore, a lower polarization. The overall fluorescence polarization measured for a sample reflects the distribution of the dye between the protein and surfactant components of the amniotic fluid, and serves as a way of determining the ratio of surfactant to albumin present in the sample. The precise relationship between the surfactant/albumin ratio and the results are expressed as the surfactant/albumin ratio.

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**SUMMARY AND EXPLANATION OF TEST**

The Fetal Lung Maturity II assay utilizes Fluorescence Polarization Assay (FPA) technology. The TDx/TDxFLx Fetal Lung Maturity II (FLM II) assay is a reagent system for the quantitative measurement of the ratio of surfactant to albumin in amniotic fluid for assessment of lung maturity of the fetus. The TDx/TDxFLx Fetal Lung Maturity II (FLM II) assay is a reagent system designed for the quantitative measurement of the ratio of surfactant to albumin in amniotic fluid for assessment of lung maturity of the fetus. The major surface-active constituent of pulmonary surfactant is phosphatidylcholine (lecithin). As the fetus matures, the synthesis of lecithin increases, and by 34-36 weeks the amount of lecithin present is sufficient to prevent alveolar collapse. The surfactant is transferred to the amniotic fluid for assessment of lung maturity of the fetus. Amniotic fluid also contains a number of other phospholipids (e.g., sphingomyelin), and proteins (e.g., albumin). The level of protein is independent of lung function and remains relatively constant throughout the later months of pregnancy. The onset of lung maturity results in an increase in the amount of surfactant relative to other components of amniotic fluid. The Fetal Lung Maturity II assay measures lung surfactants relative to albumin and the results are expressed as the surfactant/albumin ratio.

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Fetal Lung Maturity

Before beginning the initial calibration and subsequent analysis with the Fetal Lung Maturity assay, press ASSAY, 88 and PRINT on the control panel. Verify the parameters with the following assay illustration:

88. FLM
88.1 SPL VOL 150.0
88.2 SPL REP 1
88.3 LOQUM 0.00
88.4 HELM 160.00
88.5 CAL VOL 150.0
88.6 CAL REP 2
88.7 CONC A 0.00
88.8 CONC B 10.00
88.9 CONC C 20.00
88.10 CONC D 40.00
88.11 CONC E 80.00
88.12 CONC F 160.00
88.13 UNITS 19
88.14 CRV FIT 2
88.15 MX DEV 5.0
88.16 MN POLA *
88.17 MN SPAN #
88.18 MODE 23
88.19 GAIN *
88.20 MX BKG 8000.00
88.21 MN TR *

To edit a parameter, follow these steps:
1. Press ASSAY, 88... plus the parameter number you wish to change, and EDIT.
2. Enter the new parameter value. Press STORE after each parameter is edited.
3. After editing has been completed, press STOP.

The instrument will return to operational status and the display panel will read: READY

NOTES:
• Parameters cannot be edited.
• Parameter can be edited and may vary with reagent pool changes.

Refer to your operation manual for a discussion of:
• Quality Control
• Installation Procedures
• Methods of Operation
• Performance Characteristics
• Assay Procedures
• Barcode Override
• Dilution Protocol
• Calibration Procedures
• Operational Precautions and Limitations
• Maintenance and Component Replacement

SAMPLE COLLECTION AND PREPARATION FOR TESTING

Analyzing

A single Fetal Lung Maturity determination requires at least 1.0 mL of amniotic fluid. Amniotic fluid for testing should be collected via trans-abdominal amniocentesis or from a free flowing or carefully tapped vaginal sample. Do not centrifuge samples; centrifugation falsely decreases results. The samples should be filtered before testing using the filters provided. Samples can be stored at 2-8°C up to 72 hours prior to testing, or can be kept frozen at -12°C or colder and tested within 72 hours. Frozen samples should be thoroughly mixed, preferably by vortexing, after thawing. Repeated freeze-thawing is not recommended.

NOTE: Samples suspected of being contaminated with maternal urine should not be used with the FLM assay.

Before testing, each Fetal Lung Maturity patient sample must be filtered. Calibrators and controls should not be filtered. The filters provided with FLM Reagents consist of a glass fiber matrix which holds back solid materials that could interfere with pipetting.

NOTE: The package of three reusable filter holders must be ordered separately (9453-02). See the Materials Not Provided section of this assay insert for more information.

Place a filter in the reusable filter holder. Ensure the filter gasket is properly seated into the Luer lock side of the filter assembly before inserting the 13 mm filter. The filter holder is then fitted to a syringe. A minimum volume of 1 mL of amniotic fluid is placed in the syringe and passed through the filter into a clean glass container, from which 250 μL can be pipetted into the sample well. The filter should be discarded, and the filter holder and gasket must be cleaned and decontaminated before reusing. Refer to the Interferences section for decontamination procedures.

For additional information, refer to your operation manual in the System Description section under Operational Precautions and Limitations.

Interferences

Interference in the Fetal Lung Maturity assay was tested with substances which may affect test results. Interference was determined by adding the test material to pooled amniotic fluid of several maturity levels, then assaying with the Fetal Lung Maturity assay.

Bilirubin may be elevated in samples from pregnancies in which Rh isoimmunization is a problem. Visibly icteric samples should not be used. Meconium in amniotic fluid is usually a result of fetal distress. Samples with visible meconium contamination should not be used.

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CAUTION: Extreme caution should be used in the evaluation of fetal lung maturity in cases of multiple births (twins, triplets, etc.). Manual dilution of samples for the Fetal Lung Maturity II assay is not recommended. The Dilution Protocol outlined in the operation manual cannot be used with the Fetal Lung Maturity II assay. Samples from a fetus with kidney or urinary anomalies visible by ultrasound may give erroneous results. Results from these samples should be confirmed by other test methods.

For the infrequent sample with a background intensity greater than the MX BLK value, the BLK I reading will be printed followed by "HI". The patient sample must not be diluted and rerun. An alternate test method for measuring fetal lung maturity is recommended.

EXPECTED VALUES

Although each laboratory should establish their own reference ranges for the interpretation of immature and mature results, the following ranges may be used as guidelines.

Immature results with the FLM II assay are less than or equal to 39 mg/g and mature results range from 55 mg/g and above. A result of "HI" indicates a surfactant/albumin ratio greater than 160 mg/g; therefore, a high level of maturity. Results between 40 mg/g and 54 mg/g cannot be declared "mature" or "immature" and should be evaluated with caution.

SPECIFIC PERFORMANCE CHARACTERISTICS

Specificity

The Fetal Lung Maturity II assay responds to surfactants in amniotic fluid including phospholipids, free fatty acids and cholesterols. Phospholipids and free fatty acids form aggregates with which the fluorescent dye in the FLM II assay interacts. In amniotic fluid, phospholipids are the major components of the aggregate. Non-phospholipid surfactants at the levels found in amniotic fluid have not been found to adversely affect the assay.

Sensitivity

Based on a linear dilution of the FLM II Calibrator B, thelowest reportable result was determined to be 4 mg/g.

Precision

Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) Protocol EP1-T2 using the FLM II Low, Medium and High Controls (surfactant and albumin in buffer) at 25.0, 50.0 and 100.0 mg/g. Results from these studies typically yielded CV's of less than or equal to 3.0%. Representative data follow.

Accuracy by Correlation

The Fetal Lung Maturity II assay was evaluated with respect to clinical outcome. Samples were tested within 24 hours of collection. For purposes of comparing a FLM II result with the outcome of the pregnancy, only those cases in which delivery occurred within 72 hours of sample collection were considered valid. The results of this evaluation follow.

Materials Provided

7A76-59 TDX/TDX-Fx FLII Assay System
(50 Tests) Includes:
• REAGENT KIT
• REAGENT REFERENCE
• SYSTEMS
• SAMPLE CONTAINERS

5518 Pipelines

Calibration

An acceptable Fetal Lung Maturity II assay calibration curve should meet the following criteria:

a) Polarization Error (PERR) -2.00 to +2.00 for all calibrators.

b) Root Mean Squared Error (RMSE) less than or equal to 1.00.

c) All controls are within the acceptable ranges.

When to Recalibrate

Recalibration is required when:

• The memory circuit board (Board 4D) is replaced.

• An assay activation (new reagent pool) is issued.

Recalibration may be necessary when:

• Assay control values fall outside of the acceptable range specified in the REAGENTS section of this insert.

• PERR or RMSE values are not acceptable as specified above.

• A new lot number of reagent is used.

• A new lot number of buffer is used.

• Any dispense component is replaced.

• Any instrument calibration procedure is performed.

To determine whether recalibration is required, this reagent system should be checked by assaying all the controls. If the control results are within range, patient samples may be run without need for recalibration. If the control results are not within range, refer to your operation manual in the Troubleshooting section.

Quality Control

The recommended control requirement is one Fetal Lung Maturity II Control level tested once every 8 hour shift, no less than two different controls per 24 hours. Controls should be run as patient samples and may be placed in any position on the Sample Carousel. If the quality control procedures in your laboratory require more control testing to verify test results, follow those procedures.

Ensure that assay control values are within the concentration ranges specified in the REAGENTS section of this insert. At the discretion of the laboratory, selected quality control rules may be applied to the quality control data. As an example, a Levey-Jennings plot may be used to track the assay's performance. Refer to your laboratory Standard Operating Procedure and/or Quality Assurance Plan for additional details.

When an assay control does not meet the established criteria for acceptability, associated test results should be considered suspect. Your laboratory should evaluate results and take remedial corrective action prior to reporting test results. In conjunction with your laboratory's Quality Assurance Plan, refer to your operation manual in the Troubleshooting section.

RESULTS

The TDX and TDX-Fx software calculates a best-fit curve equation that is used to generate a calibration curve. This curve is stored in memory and concentrations of analyte in unknown samples are calculated from this curve using polarization values generated for each sample in the assay. For additional details in reporting results refer to your operation manual in the Operation section for printouts.

LIMITATIONS OF THE PROCEDURE

As with all analyte determinations, the Fetal Lung Maturity II value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
Results in the intermediate category cannot be declared “mature” or “immature.” Therefore, these results must be reviewed with caution. A mature result has good correlation with a normal clinical outcome. An immature result should be regarded with caution, since both normal and RDS outcomes were associated with immature FLM II values.

REFERENCES


Related Readings

TDx, TDxFLx and XSYSTEMS are trademarks of Abbott Laboratories, Abbott Park, IL 60064 USA.