DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

P92048

Memorandum

Date SEP 21 1995

From Director, Office of Device Evaluation (HFZ-400)
Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Adeza Biomedical Corporation's Fetal Fibronectin Enzyme Immunoassay Kit - ACTION

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Susan Alpert, Ph.D., M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved _____ Disapproved _____ Date _____

Prepared by CDRH, HFZ-440. V. Calvin, 9-19-95, 594-1243.

DRAFT

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

[DOCKET NO.]

Adeza Biomedical Corporation; PREMARKET APPROVAL OF Fetal Fibronectin Enzyme Immunoassay Kit

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Adeza Biomedical Corporation, Sunnyvale, CA, for premarket approval, under section 515 of the Federal Food, Drug, and Cosmetic Act (the act), of Fetal Fibronectin Enzyme Immunoassay Kit. After reviewing the recommendation of the Clinical Chemistry and Clinical Toxicology Devices Advisory Panel, FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter on September 29, 1995, of the approval of the application.

DATE: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESS: Written requests for copies of the summary of safety

and effectiveness data and petitions for administrative review to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 1-23, 12420 Parklawn Drive, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Cornelia B. Rooks
Center for Devices and Radiological Health (HFZ-440)
Food and Drug Administration
2098 Gaither Road
Rockville, MD 20850
301-594-1243.

SUPPLEMENTARY INFORMATION: On October 31, 1994, Adeza Biomedical corporation, Sunnyvale, CA 94089, submitted to CDRH an application for premarket approval of Fetal Fibronectin Enzyme Immunoassay Kit.

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is a device to be used as an aid in assessing the risk of preterm delivery in $\leq$ 7 days or $\leq$ 14 days from the time of sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (< 3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5% and 99.2%, for delivery in $\leq$ 7 and $\leq$ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7% and 16.7% for delivery in $\leq$ 7 and $\leq$ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

B

On April 6, 1995, the Clinical Chemistry and Clinical Toxicology Devices Advisory Panel, an FDA advisory panel, reviewed and recommended approval of the application.

On September 21, 1995, CDRH approved the application by a letter to the applicant from the Director of the office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

OPPORTUNITY FOR ADMINISTRATIVE REVIEW

Section 515(d)(3) of the act (21 U.S.C. 360e(d)(3)) authorizes any interested person to petition, under section 515(g) of the act (21 U.S.C. 360e(g)), for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under part 12 (21 CFR part 12) of FDA's administrative practices and regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 10.33(b) (21 CFR 10.33(b)). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition

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and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h), (21 U.S.C. 360e(d), 360j(h)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated:_____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

SEP 21 1995

David Casal, Ph.D.
Vice-President, Clinical and Regulatory Affairs
Adeza Biomedical Corporation
1240 Elko Drive
Sunnyvale, California 94089

Re: P920048
Fetal Fibronectin Enzyme Immunoassay Kit
Filed: October 31, 1994
Amended: January 31, March 13, April 6, April 25, May 19, May 31, June 19, August 2, August 16, September 11, September 13, and September 20, 1995

Dear Dr. Casal:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Fetal Fibronectin Enzyme Immunoassay Kit.

This device is to be used as an aid in assessing the risk of preterm delivery in ≤ 7 days or ≤ 14 days from the time of sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (< 3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5% and 99.2%, for delivery in ≤ 7 and ≤ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7% and 16.7% for delivery in ≤ 7 and ≤ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

Expiration dating for this device has been established and approved at 1 year from date of manufacture for unopened reagents refrigerated at 2° to 8°C. This is to advise you

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that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Cornelia Rooks at (301) 594-1243.

Sincerely yours,

Susan Alpert, Ph.D., M.D.
Director
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Device Generic Name: Fetal Fibronectin Enzyme Linked Immunosorbent Assay (ELISA) for the Qualitative Detection of Fetal Fibronectin in Cervicovaginal Secretions

Device Trade Name: Fetal Fibronectin Enzyme Immunoassay Kit

Applicant's Name and Address: Adeza Biomedical Corporation
1240 Elko Drive
Sunnyvale, CA 94089

Premarket Approval Application (PMA) Number: P920048

Date of Panel Recommendation: April 6, 1995

Date of Notice of Approval to Applicant: September 21, 1995

II. Indications for Use

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay, hereinafter referred to as the fFN Immunoassay, is a device to be used as an aid in assessing the risk of preterm delivery in ≤ 7 days or ≤ 14 days from the time of sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes, and minimal cervical dilatation (< 3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5 percent and 99.2 percent, for delivery in ≤ 7 and ≤ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7 percent and 16.7 percent for delivery in ≤ 7 and ≤ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

Background:

Fetal fibronectin (fFN), the protein detected by the fFN Immunoassay, is an isoform of fibronectin, a complex,

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multifunctional heterodimer with a molecular weight between 450-500 kD (Ruoslahti, 1988). It was first described in 1985 as an extracellular matrix protein of rapidly proliferating cell populations, e.g., tumor and placental cells (Matsuura, 1985). Fetal fibronectin is largely insoluble and functions primarily as an extracellular matrix protein. Fetal fibronectin differs from other isoforms of fibronectin in that it has the insertion of a unique epitope within the domain known as III-CS. III-CS is a glycosylated 120 amino acid peptide positioned between the heparin and fibrin binding domains of the carboxyl-terminus of the molecule. A murine monoclonal antibody, known as FDC-6, which is specific for a unique epitope within the III-CS region found only in fFN has been characterized (Matsuura, 1985, 1988).

In pregnancy, fFN is found in very high concentrations in the amniotic fluid and the extracellular matrix (ECM) of the placenta and fetal membranes (Lockwood, 1991). Immunohistological studies of placental tissues have shown that fFN is preferentially expressed in the ECM of extravillous cytotrophoblasts of both the placenta and chorion (Lockwood, 1991). As fetal cells, extravillous trophoblasts have contact with the maternal cells of pregnancy, the decidua. Because of the selective immunolocalization of fFN along the maternal-fetal

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interface, it is thought that fFN plays a unique role in maintaining intercellular contact between the extravillous trophoblast and decidual cell populations (Lockwood, 1991).

The fFN produced by the extravillous trophoblasts of the chorion is apparently shed into the amniotic fluid in the first 16 to 20 weeks of pregnancy. The concentration of fFN in amniotic fluid peaks during the second trimester, reaching levels as high as 100 μg/mL. At 20 weeks, the amniotic fluid concentration of fFN begins to decrease and continues to decrease gradually throughout the remainder of pregnancy. The concentration of fFN in amniotic fluid at term is approximately 20-30 μg/mL (Lockwood, 1991).

Clinical studies have demonstrated that fFN can be detected in cervicovaginal secretions of a large proportion of pregnant women prior to 20 weeks gestation (Lockwood, 1991). It has been suggested that fFN detected in cervicovaginal secretions after 24 weeks gestation indicates that the amniotic membranes have either ruptured (Eriksen, 1992) or are significantly weakened, resulting in transcervical extravasation of chorionic fFN into the vagina and elevated risk for preterm delivery (Lockwood, 1991). The presence of fFN

in cervicovaginal secretions has been demonstrated as a risk factor for preterm delivery in patients with intact amniotic membranes and symptoms associated with risk for preterm delivery (e.g., uterine activity or abdominal discomfort), even when the cervix is minimally dilated (Lockwood, 1991). Previous studies (Lockwood, 1991) have shown that women self-selecting for obstetrical care related to idiopathic preterm pregnancy complications associated with prematurity had a high probability of preterm delivery when fFN was detected in cervicovaginal secretions. Conversely, when fFN was not detected in cervicovaginal secretions, women with similar clinical symptoms had a significantly lower probability of preterm delivery (Lockwood, 1991).

III. Device Description

A. fFN Immunoassay

The fFN Immunoassay is a qualitative test designed for determining the presence of fFN in cervicovaginal secretions. The format is a solid-phase immunosorbent device utilizing a murine monoclonal antibody as the solid-phase "capture" antibody and a goat polyclonal antibody conjugated

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to alkaline phosphatase as the "label" antibody in a sequential "sandwich" assay configuration.

Replicate patient samples and reference samples containing fFN are incubated in adjacent microtiter plate wells coated with a murine monoclonal antibody directed against a unique antigenic determinant on the fFN molecule. Following the initial sample incubation, "captured" fFN is detected by a second incubation with affinity purified goat anti-human fibronectin polyclonal antibody conjugated to alkaline phosphatase. The monoclonal antibody-fFN-polyclonal antibody conjugate sandwich is then spectrophotometrically measured following a third incubation with phenolphthalein monophosphate substrate. The mean absorbance of the duplicate samples is approximately proportional to the amount of fFN in the sample. Samples having an average absorbance greater than or equal to the average absorbance of the Positive Reference (contained in the fFN Immunoassay) are defined as positive for the presence of fFN.

B. Fetal Fibronectin Enzyme Immunoassay Control Kit

The fFN Immunoassay Control Kit ("Control Kit") is an accessory to the fFN Immunoassay and is available separately. The Control Kit consists of a set of two Control Samples, one positive and one negative, for monitoring the performance of the fFN Immunoassay. The recommended frequency of use of the Control Kit is once per plate per assay.

C. Specimen Collection Kit

The Adeza Biomedical Specimen Collection Kit, also a separate product, was used for collecting cervicovaginal specimens.

IV. Alternative Practices and Procedures

Alternative practices and procedures associated with the identification of symptomatic women at elevated risk for preterm delivery include:

- Establishing if the amniotic membranes are ruptured using:
  - pooling (visual examination of the vagina for presence of amniotic fluid),

- ferning (microscopic assessment of a characteristic crystallization pattern produced by amniotic fluid),

- qualitative or quantitative determination of vaginal pH (normal vaginal secretions are acidic, while amniotic fluid has a neutral pH),

- ultrasonography to determine if amniotic fluid volume is diminished (oligohydramnios) and,

- transabdominal, intrauterine instillation of small molecular weight dyes (e.g., indigo carmine or methylene blue).

- Assessment of cervical dilatation and effacement by digital examination or ultrasonography.

- Measurement of uterine activity using external tocodynamometry or abdominal palpation.

- Assessment of preterm delivery risk using population derived algorithms.

These practices and procedures, along with the clinical evaluation of the patient, are currently used by clinicians to assess the risk for preterm delivery.

V. Marketing History

The fFN Immunoassay has been marketed in Australia, Austria, Belgium, Brazil, Canada, Denmark, France, Hong Kong, Germany, Italy, Japan, Mexico, the Netherlands, Spain, Sweden, Switzerland, and the United Kingdom. The fFN Immunoassay has never been withdrawn from the market in any country for any reason related to the safety and effectiveness of the device.

VI. Adverse Effects of the Device on Health

Any adverse effects of the device on health would be indirect, since the device does not come in contact with the patient and is used as an aid in assessing the risk of preterm delivery. A false-negative result could delay intervention in a woman with signs of preterm labor. In a case such as this, the pregnancy could be adversely affected by a delay in early beneficial treatment or resumption of treatment. A false positive result could contribute to a medical decision which might cause a patient to undergo needless treatment or an unnecessary

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change in treatment. Risk of these events is low if the conventional "standard of care" procedures are also performed for assessing the risk of preterm delivery. The reliability of this test in assessing the risk of preterm delivery in pregnant women who are not symptomatic has not been evaluated. This test has not been assessed as a screening tool for the general population of pregnant women.

Contraindications, Warnings, and Precautions:

Contraindications

The fFN Immunoassay is not intended for use in the management of patients with medium or gross vaginal bleeding. The presence of vaginal bleeding judged by the caregiver to be medium or gross in amount may produce false positive results. Additionally, results from specimens with trace amounts of blood must be interpreted with caution.

Warnings

The safety and effectiveness of the fFN Immunoassay has not been evaluated as a screening procedure for the general population of pregnant women.

The fFN Immunoassay should not be used if a patient has bacterial vaginosis because a higher false positive rate occurs in this patient population.

Precautions

Manipulations of the cervix may lead to a false-positive fFN Immunoassay result. Therefore, the fFN Immunoassay specimen should be obtained prior to any digital examination or manipulation of the cervix.

Sexual intercourse may also disturb the cervix and produce false positive results; therefore the fFN Immunoassay should not be used for patients reporting sexual intercourse in the previous 24 hours.

Collection of vaginal specimens for microbiologic culture frequently requires aggressive collection techniques which abrade the cervical or vaginal mucosa. Since cellular debris may potentially interfere with sample preparation, specimens for the fFN Immunoassay should be collected prior to any collection of culture specimens that may be indicated.

Rupture of membranes should be ruled out prior to performance of the fFN Immunoassay, since fFN is found in both amniotic fluid and the fetal membranes.

The fFN Immunoassay result should not be used as the sole basis for making decisions regarding patient care. All other available clinical and laboratory evidence should be considered when making decisions regarding patient management.

White blood cells, red blood cells, bacteria, and bilirubin and the use of douches may interfere with the assay results.

Viral infections may affect fFN Immunoassay results.

Results obtained using the fFN Immunoassay are valid only when specimens are collected with the Adeza Biomedical Specimen Collection Kit. The Specimen Collection Kit contains a sterile Dacron swab and a tube containing extraction buffer which has been optimized for use in the fFN Immunoassay. No other swab or extraction buffer can be used in place of the Dacron swab and extraction buffer provided in the Specimen Collection Kit. Use of materials other than those provided in the Specimen Collection Kit has not been evaluated.

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Lubricants, soaps, and disinfectants may interfere with absorption of the specimen by the swab or with the antibody-antigen reaction of the fFN Immunoassay. Therefore, care must be taken not to contaminate the swab or cervicovaginal secretions with such products.

VII. Summary of Studies

A. Nonclinical Studies

Nonclinical laboratory studies were conducted to determine the purity and specificity of the reagents as well as to define assay performance characteristics.

1. Characterization of the Antigen

The primary antigen used for monoclonal antibody production was prepared from human hepatoma HuH-6 cells as previously described (Matsuura, 1985). The antigen was characterized by amino acid and carbohydrate composition determination, inhibition immunoassay techniques, SDS-polyacrylamide gel electrophoresis, and immunoblotting of the

whole molecule and its proteolytic fragments (Matsuura, 1985, 1988).

2. Specificity of the Antibodies

a. Monoclonal Antibody

Specificity of the murine monoclonal antibody FDC-6 to fFN was demonstrated by using electrophoretic and immunoblotting techniques. The results are as follows:

- Anti-FDC-6 reacts with the III-CS region epitopes containing fibronectin of human hepatoma HuH-6 and HuH-7 cells and WI-38 fibroblasts. It does not react with hepatic fibronectin which lacks the III-CS region eptitopes (Matsuura, 1985, 1988);

- Anti-FDC-6 reacts minimally (i.e., it did not have a signal different from background) with well-defined proteolytic fragments of plasma and cellular fibronectins as well as

with fibronectin isolated from the culture medium of human hepatoma HuH-6 and HuH-7 cells and WI-38 fibroblasts (Matsuura, 1985, 1988);

- Anti-FDC-6 reacts with fibronectin in amniotic fluid, but not with plasma fibronectin.

b. Polyclonal Antibody

Affinity purified polyclonal antibody was tested for reactivity against plasma fibronectin, fFN, and other proteins using conventional immunoblotting techniques. Affinity purified polyclonal antibody was found to react with plasma fibronectin and fFN, but not with other plasma or amniotic fluid proteins.

3. Laboratory Performance Characteristics

Laboratory performance studies were conducted at Adeza Biomedical and three clinical laboratory sites: University of Utah Medical Center, University of Colorado Health Science

Center, and Prentice Women's Hospital, Northwestern University. The laboratory performance sites are identified by codes in tables and text. The codes for each of the laboratory sites participating in this portion of the evaluation are shown in Table 1.

Table 1 Codes for Laboratory Performance Sites

Laboratory Site	Code
Adeza Biomedical	ADEZA
University of Utah	UTAH
Northwestern University	UOCP
University of Colorado	UOCD

a. Minimum Detectable Absorbance

The minimum detectable absorbance that can be statistically distinguished from the Negative Reference (containing 0 μg/mL fFN) was calculated at 3 clinical sites and ADEZA using three lots of the fFN Immunoassay containing different lot combinations of microtiter plates and conjugate. The minimum detectable absorbance is defined as the absorbance that is 2 standard deviations above the average absorbance calculated for 21 to 24 replicates of the Negative Control. The minimum detectable absorbance calculated for the fFN Immunoassay was determined to be 0.070 units of optical density.

b. Reproducibility

(1) Intra-Assay Reproducibility (Within Assay)

Intra-assay reproducibility was assessed at 3 clinical sites and at Adeza Biomedical using 3 lots of fFN Immunoassay Kits containing different lot combinations of microtiter plates and antibody conjugate. Five reference materials were assayed at least 20 times in each of the 3 lots.

The concentration of the Positive Reference was 0.050 μg/mL fFN. The approximate fFN concentrations of the remaining reference materials were:

Neg Ctl	0.015 μg/mL
Pos Ctl	0.080 μg/mL
Ref 1	0.150 μg/mL
Ref 2	0.500 μg/mL

The results from the studies, summarized in Table 2, showed intra-assay reproducibilities [all coefficients of variance (CVs) of individual sites] of < 7 percent.

Table 2 Summary of fFN Immunoassay Intra-Assay Reproducibility

		Absorbance Units (550 nm)			
Clinical Site	Pos Ref	Neg Ctl	Pos Ctl	Ref 1	Ref 2
ADEZA					
Avg	0.139	0.075	0.166	0.279	0.704
Std	0.004	0.003	0.007	0.011	0.026
%CV	2.9%	4.0%	4.2%	3.9%	3.7%
N	66	66	66	66	66
UOCD					
Avg	0.131	0.080	0.154	0.229	0.550
Std	0.005	0.004	0.007	0.009	0.019
%CV	3.8%	5.0%	4.5%	3.9%	3.5%
N	66	66	66	66	66
UOCP					
Avg	0.139	0.078	0.167	0.225	0.553
Std	0.006	0.003	0.009	0.007	0.023
%CV	4.3%	3.8%	5.4%	3.1%	4.1%
N	71	71	72	72	72
UTAH					
Avg	0.111	0.073	0.128	0.214	0.550
Std	0.005	0.005	0.005	0.011	0.023
%CV	4.5%	6.8%	3.9%	5.1%	4.2%
N	66	64	64	66	66
Pooled					
Avg	0.130	0.077	0.154	0.237	0.598
Std	0.005	0.004	0.007	0.010	0.023
%CV	3.8%	5.2%	4.5%	4.2%	3.8%
N	269	267	267	270	270

(2) Inter-Assay Reproducibility (between run)

Inter-assay reproducibility was assessed at 3 clinical sites and at Adeza Biomedical. Six reference materials were assayed either 19, 20, or 24 times using three different kit lots of the fFN Immunoassay. The 6 reference materials used in the inter-assay reproducibility study were assayed in duplicate (tests/run). No more than 2 runs were performed per day (runs/day). The number of days required for each site to complete the inter-assay reproducibility varied between 10 and 20 days.

The concentrations of the Negative and Positive Reference materials were 0 and 0.050 μg/mL fFN, respectively. The approximate fFN concentrations of the remaining reference materials were:

Neg Ctl	0.015 μg/mL
Pos Ctl	0.080 μg/mL
Ref 1	0.150 μg/mL
Ref 2	0.500 μg/mL

The results from the studies, summarized in Table 3, showed inter-assay

reproducibilities (all CVs of individual sites) of < 13 percent.

Table 3 Summary of fFN Immunoassay Inter-Assay Reproducibility

Absorbance Units (550 nm)

Lab Site	Pos Ref	Neg Ref	Neg Ctl	Pos Ctl	Ref 1	Ref 2
ADEZA						
Avg	0.139	0.062	0.079	0.170	0.264	0.672
SD	0.013	0.005	0.006	0.020	0.025	0.059
%CV	9.4%	8.1%	7.6%	11.8%	9.5%	8.8%
N	72	72	72	72	72	72
UOCD						
Avg	0.116	0.061	0.074	0.136	0.200	0.491
SD	0.007	0.004	0.003	0.008	0.014	0.041
%CV	6.0%	6.0%	4.5%	5.9%	6.8%	8.4%
N	60	60	60	60	60	60
UOCP						
Avg	0.129	0.063	0.077	0.158	0.233	0.577
SD	0.015	0.004	0.006	0.018	0.027	0.069
%CV	11.6%	6.3%	7.8%	11.4%	11.6%	12.0%
N	60	60	60	60	60	60
UTAH						
Avg	0.121	0.059	0.076	0.143	0.212	0.541
SD	0.012	0.005	0.007	0.016	0.024	0.069
%CV	9.9%	8.5%	9.2%	11.2%	11.3%	12.8%
N	58	60	60	60	60	60
Pooled						
Avg	0.126	0.061	0.077	0.152	0.227	0.570
SD	0.012	0.005	0.006	0.016	0.023	0.060
%CV	9.5%	8.2%	7.8%	10.5%	10.1%	10.5%
N	250	252	252	252	252	252

c. Specimen Dilution

Two specimens of amniotic fluid containing known concentrations of fFN were serially diluted using extraction buffer (containing 0 μg/fFN) from the Specimen Collection Kit. Each dilution was assayed in triplicate using each of three lots of the fFN Immunoassay. These dilution experiments indicate that the relationship between concentration and absorbance followed the expected pattern for immunoassays and that the dose-response curve allowed sufficient discrimination between positive (≥ 0.050 μg/mL fFN) and negative (< 0.050 μg/mL fFN) samples.

d. Specificity and Interfering Substances

Studies were conducted to assess the specificity of the fFN Immunoassay. Hepatic fibronectin, surgical lubricant, the contrast agent indigo carmine, and therapeutic agents (i.e., prostaglandin E_2 up to 100 μg/mL, ampicillin up to 100 μg/mL, cephalexin up to 18 μg/mL, erythromycin up

to 10 μg/mL, gentamycin up to 4 μg/mL, dexamethasone up to 200 μg/mL, magnesium sulfate up to 50 μg/mL, oxytocin up to 100 U/mL, terbutaline up to 1 mg/mL, and ritodrine up to 100 μg/mL) did not interfere with the ability of the fFN Immunoassay to qualitatively detect fFN in reference samples. The fFN Immunoassay gave a positive result for preparations containing fFN in the presence of potential interfering substances and remained negative for preparations not containing fFN.

Semen was also evaluated for assay interference. In one experiment, forty-one sperm samples were obtained from healthy males and tested for fetal fibronectin. Twenty-one of the samples (51 percent) tested positive for fetal fibronectin. In a second experiment, twenty-two samples were collected from the posterior fornix of non-pregnant women within one hour of intercourse. The amount of fetal fibronectin in the samples was quantitated. Five of the 22 samples (23 percent) tested positive for fetal fibronectin. As a result

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of these studies and because it has been suggested that seminal proteins are negligible or undetectable 24 or more hours after coitus (Haimovici, 1995), patient specimens should not be tested if the patient has had sexual intercourse within 24 hours prior to the sampling time.

e. Stability

The stability of the fFN Immunoassay was determined by testing three different lots of the fFN Immunoassay Kits containing different lot combinations of plates and conjugate after storage for various periods of time. The three lots of the fFN Immunoassay were subjected to elevated storage temperatures of approximately 25°C, 37°C and 45°C. Additional real time stability studies were performed over 13 months for reagents stored at 2-8°C, the recommended storage temperature for the fFN Immunoassay. Accelerated and real time stability studies support a shelf-life dating of 12 months for the fFN Immunoassay Kit at 2-8°C.

B. Clinical Studies

A multicenter clinical trial was conducted to prospectively evaluate the association of the fFN Immunoassay result to delivery in ≤ 7 days and ≤ 14 days of specimen collection in self-selected, symptomatic patients between 24 weeks, 0 days and 34 weeks, 6 days gestation with intact amniotic membranes and minimal cervical dilatation (<3 cm). Each of the facilities and the institution code describing each institution in this section are provided in Table 4. The institutions participating in this evaluation of the fFN Immunoassay are located in distinct regions of the United States.

Table 4 Clinical Study Sites and Codes

Clinical Study Site	Code	Total No. of Subjects Evaluated
Good Samaritan Hospital, Phoenix	GSAM	52
Hartford Hospital, Hartford	HART	52
Hennepin County Medical Center, Minneapolis	HCMC	140
University of Texas, Houston - LBJ Hospital	LBJH	45
Ohio State University, Columbus	OHIO	65
Stanford University, Stanford	STAN	58
University of North Carolina, Chapel Hill	UNCC	81
University of Alabama, Birmingham	UOAB	90
Northwestern University - Prentice Hospital	UOCP	138
Louisville University, Louisville	UOLL	42
University of Texas, Dallas - Parkland Hospital	PARK	200
	TOTAL	963

1. Subjects Evaluated for Safety and Effectiveness

A total of 1745 symptomatic subjects who presented to Labor and Delivery or the Emergency Room at each of the investigational sites were evaluated as potential participants in Study 031. Of these 1745 subjects, 371

evaluated did not meet the inclusion criteria and were not offered the opportunity to participate. A total of 1374 subjects met the inclusion criteria and were enrolled in Study 031 pending final verification of their eligibility during the clinical examination. The fFN sample was collected during the clinical examination prior to the evaluation of the cervix and amniotic membranes, thus many subjects were enrolled and then deemed ineligible. During the clinical examination at enrollment, subjects who were found to have one of the following were deemed ineligible and were not included in the analysis:

- received tocolytic medications for treatment of threatened preterm delivery prior to collection of the cervicovaginal specimen for fFN determination,
- cervical dilatation ≥ 3 centimeters
- suspected placenta previa
- gestational age less than 24 weeks, 0 days or greater than 34 weeks, 6 days
- rupture of amniotic membranes
- cervical cerclage in place
- a symptom not associated with idiopathic threatened preterm delivery, e.g., trauma

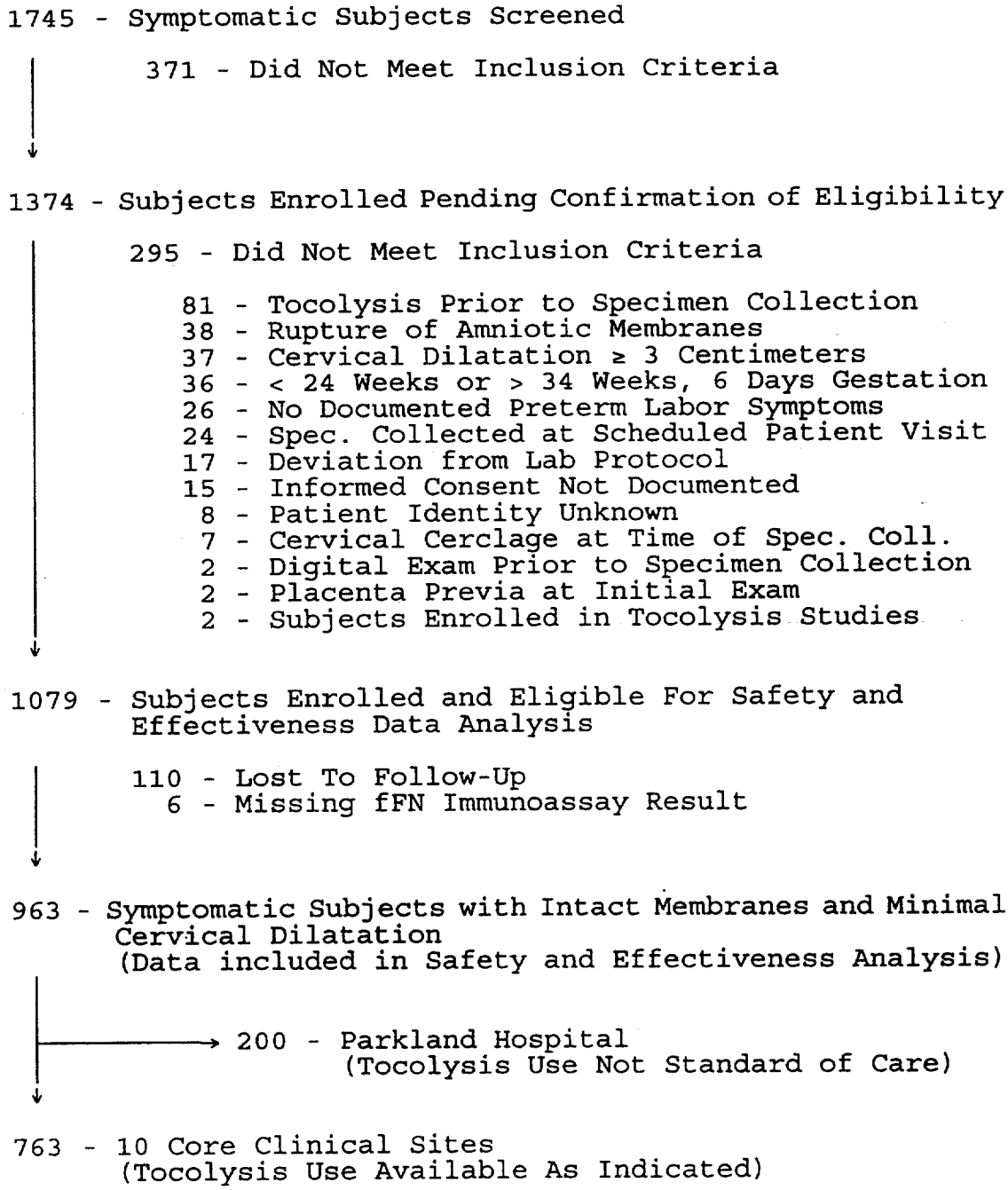
- digital exam prior to specimen collection
- enrollment in tocolysis studies

There were a total of 295 subjects who were eligible at the initial screen but later found to be ineligible. Reasons for subject ineligibility are included in a summary of Study 031 shown in Figure 1. Of the remaining 1079 subjects, 110 were lost to follow-up and thus did not have delivery information available. Finally, cervicovaginal specimens were lost and no fFN Immunoassay result was available for 6 subjects.

A total of 963 subjects met all eligibility criteria, had delivery information available, and had complete results. These patients were included in the data analyzed to determine the safety and effectiveness of the fFN Immunoassay. This included 763 subjects from the 10 core clinical sites (CORE SITES) (where tocolytic medications are commonly used as the "standard of care" for treatment of threatened preterm

delivery) and 200 subjects from Parkland Hospital in Dallas, Texas (where tocolytic medications are not routinely used as the "standard of care" for patients with threatened preterm delivery).

Figure 1 Flow Chart of Subject Recruitment and Outcomes



2. Safety and Effectiveness Data

The safety and effectiveness of the fFN Immunoassay were assessed in 963 symptomatic subjects* meeting the following criteria:

Table 5

INCLUSION	EXCLUSION
· presented for emergency obstetrical care at an emergency room or labor & delivery unit · between 24 weeks, 0 days and 34 weeks, 6 days gestation · informed consent in accordance with the IRB at each institution · intact amniotic membranes · < 3 cm cervical dilatation · at least 18 yrs old or emancipated consenting minor	· presented for regularly scheduled obstetrical care with symptoms · < 24 weeks, 0 days or > 34 weeks, 6 days gestation · rupture of amniotic membranes · ≥ 3 cm cervical dilatation · cervical cerclage · suspected placenta previa · < 18 yrs old and not emancipated consenting minor

*Patient complaints included:

- Uterine contractions, with or without pain
- Intermittent lower abdominal pain; dull backache; pelvic pressure
- Bleeding during the second or third trimester
- Menstrual-like or intestinal cramping, with or without diarrhea
- Change in vaginal discharge--amount, color, or consistency
- Patient is not "feeling right"

The number of subjects from each clinical site is shown in Table 4. The study was conducted using the same clinical trial protocol at all sites. The data were split into two distinct groups and analyzed separately for comparative purposes. Data collected from PARK were analyzed separately from the 10 remaining clinical sites, as physicians at PARK do not routinely use tocolytic medications as the "standard of care" for the management of patients with threatened preterm delivery.

Physicians at the other 10 sites typically use tocolytic medications as "standard of care" for treatment of threatened preterm delivery as indicated by patient need and physician preference. The purpose of this analysis was to determine the potential effect posed by use of tocolytic medications on the association of the fFN Immunoassay result to delivery ≤ 7 days of specimen collection. The results reported from the 10 clinical sites are referred to as the CORE SITES while results from Parkland are referred to as PARK.

Results from CORE SITES:

The average estimated gestational age at the time of study entry and specimen collection for the 763 patients from the CORE SITES was 30.3 ± 3.0 weeks. Of the 763 subjects, 22 (2.9 percent) delivered ≤ 7 days of specimen collection for the fFN Immunoassay. A total of 30 (3.9 percent) subjects delivered ≤ 14 days of specimen collection. There were 150 (19.7 percent) positive fFN Immunoassay results for the 763 subjects.

The univariate association of the fFN Immunoassay test result to delivery status is shown in Table 6. For delivery ≤ 7 days, the sensitivity, specificity, positive and negative predictive values of the fFN Immunoassay were 86.4 percent, 82.3 percent, 12.7 percent, and 99.5 percent, respectively ($p<0.00001$ by Fisher's Exact Test). For delivery ≤ 14 days, the sensitivity, specificity, positive and negative predictive values of the fFN Immunoassay were 83.3 percent, 82.9 percent, 16.7 percent, and 99.2 percent, respectively ($p<0.00001$ by Fisher's Exact Test).

Table 6 Univariate Association of the fFN Immunoassay Result to Delivery in ≤ 7 Days and ≤14 Days from Specimen Collection for the CORE SITES

	fFN +	fFN -	
Del ≤ 7 Days	19	3	22
Del ≤ 14 Days	25	5	30
Del > 14 Days	125	608	733
	150	613	763

Measure	Estimate (95% C.I.) ≤ 7 Days	Estimate (95% C.I.) ≤ 14 Days
Sensitivity	86.4% (66.4%, 95.3%)	83.3% (66.3%, 93.7%)
Specificity	82.3% (79.4%, 84.9%)	82.9% (80.0%, 85.4%)
Pos PV	12.7% (4.2%, 33.7%)	16.7% (7.3%, 33.7%)
Neg PV	99.5% (98.7%, 99.8%)	99.2% (98.3%, 99.6%)
Fisher's Exact Test, p<0.0000001		

A total of 162 of the 763 subjects (21.2 percent) delivered prematurely, i.e., before 37 weeks gestation. The sensitivity, specificity, positive and negative predictive values of the fFN Immunoassay were 41.3 percent, 86.2 percent, 44.7 percent, and 84.5 percent, respectively, for delivery ≤ 36 weeks.

Subjects delivering ≤ 7 days of specimen collection gave birth to infants having significantly lower

average birth weight (1904 v. 3188 grams, p=0.0001). Infants born ≤ 7 days of specimen collection had significantly more prematurity related complications than infants born > 7 days after specimen collection. These complications included respiratory distress, neonatal sepsis, and patent ductus arteriosus (p<0.05 for all by Fisher's Exact Test). The greater prevalence of complications experienced by infants born ≤ 7 days of specimen collection is reflected in their higher admission rate to neonatal intensive care units (70.8 percent v. 12.4 percent, p<0.00001 by Fisher's Exact Test) and higher mortality rate (8.3 percent v. 1.0 percent, p=0.040 by Fisher's Exact Test).

Subjects with positive fFN Immunoassay results had infants weighing significantly less than infants born to subjects with negative fFN Immunoassay results (2804.0 v. 3242.8 grams, p=0.0001). Moreover, proportionately more infants weighing < 1500 grams (7.1 percent v. 0.9 percent, p=0.00005 by Fisher's Exact Test), < 2000 grams (16.2 percent v. 3.3 percent, χ^2_1=35.49, p<0.0000001), and < 2500 grams (37.0 percent v. 10.8 percent, χ^2_1=61.37, p<0.0000001) were delivered of subjects with

positive fFN Immunoassay results. In addition, subjects with positive fFN Immunoassay results delivered infants with significantly more prematurity related complications than infants delivered of subjects with negative fFN Immunoassay results. These included respiratory distress (χ^2_1=15.80, p=0.00008), neonatal sepsis (p=0.0011 by Fisher's Exact Test), necrotizing enterocolitis (p=0.025 by Fisher's Exact Test), and patent ductus arteriosus (p=0.030 by Fisher's Exact Test). The greater prevalence of complications experienced by infants delivered of subjects with positive fFN Immunoassay results is reflected by their higher admission rate to neonatal intensive care unit (28.6 percent v. 10.7 percent, χ^2_1=31.12, p<0.0000001) and greater mortality (3.9 percent v. 0.6 percent) p=0.006 by Fisher's Exact Test).

The number of subjects, distribution of positive fetal fibronectin test results, sensitivity, and predictive value of a positive test for delivery in ≤ 7 days from specimen collection is shown in Table 7 for symptomatic pregnant women < 32 weeks and ≥ 32 weeks.

Table 7 Distribution of Subjects and Fetal Fibronectin Enzyme Immunoassay Results Before and After 32 Weeks Gestation[a]

EGAS (Weeks)	Subjects (n)	fFN +[b] n (%)	Sensitivity (fFN+/Del≤7 Days)	Pred Val + (%) (Del≤7 Days/fFN +)
< 32 Weeks	483	91 (18.8%)	8/9 (88.9%)	8/91 (8.8%)
≤ 32 Weeks	280	59 (21.0%)	11/13 (84.6%)	11/59 (18.6%)
TOTAL	763	150(19.7%)	19/22 (86.4%)	19/150 (12.7%)

[a]Estimated Gestational Age at Specimen Collection
[b]fFN + = positive Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay result

Table 8 summarizes the clinical efficacy for assessing the risk of delivery ≤ 7 days of the initial evaluation using the fFN Immunoassay results, uterine activity, vaginal bleeding, cervical dilatation, and presence of vaginal infection (bacterial vaginosis). As univariate markers, all were statistically associated with delivery ≤ 7 days of specimen collection in this population of symptomatic subjects with intact amniotic membranes and minimal cervical dilatation. Uterine activity ≥ 4 contractions per hour, cervical dilatation > 1 cm, vaginal bleeding, and bacterial vaginosis were specific but not sensitive indicators of delivery ≤ 7 days of specimen collection. Compared to cervical dilatation, uterine activity, vaginal bleeding, and bacterial vaginosis, the fFN Immunoassay was more sensitive and more specific for assessing the risk of delivery ≤ 7 days of specimen collection.

The results of the univariate analysis presented in Table 8, 9, and 10 indicate that the fFN Immunoassay can aid in assessing the risk of delivery ≤ 7 days of specimen collection. The analysis also indicates that uterine activity, cervical dilatation, vaginal bleeding, and

bacterial vaginosis were specific but not sensitive assessors of the risk of delivery ≤ 7 days of specimen collection. This analysis, however, does not address the potential ability of the combined clinical factors to assess the risk of delivery. Since these clinical factors could have been dependently expressed, a stepwise multiple logistic regression model was devised to evaluate the independent association of these clinical factors to delivery ≤ 7 days of specimen collection. The fFN Immunoassay result provided the best assessment of risk of delivery ≤ 7 days of specimen collection. Vaginal bleeding and uterine activity ≥ 4 contractions per hour were also significant in assessing the risk of delivery ≤ 7 days of specimen collection. Cervical dilatation and bacterial vaginosis failed to significantly improve the assessment of risk of delivery ≤ 7 days of specimen collection above the risk attributed to the fFN Immunoassay result, uterine activity, and vaginal bleeding.

The results of the univariate and multivariable analysis demonstrated that the fFN Immunoassay can be used as an aid in assessing the risk of preterm delivery in ≤ 7 days or ≤ 14 days from the time of

sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and cervical dilatation (< 3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5 percent and 99.2 percent, for delivery in ≤ 7 and ≤ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7 percent and 16.7 percent for delivery in ≤ 7 and ≤ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

Results from PARK:

Parkland Hospital is the main teaching hospital of the Southwestern Medical School of the University of Texas. Parkland Hospital participated in this study specifically because of the fact that tocolytic medications are not used as "standard of care" for treatment of threatened preterm delivery. The inclusion of Parkland Hospital as an investigational site was done specifically to

determine the influence of withholding tocolytic medications (for threatened preterm delivery) on the endpoint, i.e., delivery ≤ 7 days of specimen collection. Parkland Hospital conducted its clinical evaluation of the fFN Immunoassay using the same protocol as all other investigational sites participating in this study, however.

The average estimated gestational age at the time of study entry and specimen collection for the 200 patients from PARK was 30.7 ± 2.6 weeks. Of the 200 subjects at PARK, 8 (4.0 percent) delivered ≤ 7 days of specimen collection for the fFN Immunoassay. There were 30 (15.0 percent) positive fFN Immunoassay results for the 200 subjects.

The univariate association of the fFN Immunoassay to delivery status is shown in Table 9. The sensitivity, specificity, and positive and negative predictive values of the fFN Immunoassay for assessing the risk of preterm delivery ≤ 7 days of specimen collection at PARK were 62.5 percent, 87.0 percent, 16.7 percent, and 98.2 percent, respectively (p=0.0022 by Fisher's Exact Test).

Although the clinical sensitivity of the fFN Immunoassay is somewhat lower in the PARK population compared to the CORE SITES, this is probably due to the small number of deliveries ≤ 7 days of specimen collection observed at PARK. Other parameters which describe the association, i.e., specificity, and positive and predictive values, of the fFN Immunoassay result to delivery ≤ 7 days of specimen collection are similar as can be seen in Table 10.

Table 8 Sensitivity, Specificity, and Positive and Negative Predictive Values for Risk Factors and Assessing the Risk of Delivery ≤ 7 Days of Specimen Collection

Risk Factor	Positive Test Defined[a]	Sensitivity (95% CI)[b]	Specificity (95% CI)	Pos PV (95% CI)	Neg PV (95% CI)
fFN Immunoassay n=763	≥ 0.05 µg/mL	86.4% (66.4%, 95.3%)	82.3% (79.4%, 84.9%)	12.7% (4.2%, 33.7%)	99.5% (98.7%, 99.8%)
Uterine Activity n=750	≥ 4 cntx/hr	54.5% (34.5%, 73.1%)	75.3% (72.0%, 78.3%)	6.3% (1.4%, 24.3%)	98.2% (96.9%, 98.9%)
Cervical Dilatation n=757	> 1 cm and < 3 cm	38.1% (20.6%, 59.4%)	88.3% (85.8%, 90.4%)	8.5% (2.1%, 27.9%)	98.0% (96.7%, 98.8%)
Vaginal Bleeding n=759	Any Bleeding	40.9% (23.0%, 61.6%)	85.2% (82.4%, 87.6%)	7.6% (1.9%, 26.3%)	98.0% (96.7%, 98.7%)
Ascending Genital Tract Infection n=763	Bacterial Vaginosis	9.1% (2.5%, 27.8%)	84.1% (81.2%, 86.5%)	1.7% (0.1%, 17.6%)	97.3% (95.9%, 98.2%)

[a]Cutoff used to define a positive test result for determining sensitivity, etc.
[b]95% Confidence Interval (Lower Limit, Upper Limit)

Table 9 Clinical Efficacy of fFN Immunoassay Results for Delivery ≤ 7 Days at Parkland Hospital

	fFN +	fFN -	
Delivery ≤ 7 Days	5	3	8
Delivery > 7 Days	25	167	192
	30	170	200

Measure	Estimate
Sensitivity	62.5%
Specificity	87.0%
Pos PV	16.7%
Neg PV	98.2%
Fisher's Exact Test, p=0.0022	

Table 10 Comparison of the Sensitivity, Specificity, and Positive and Negative Predictive Values Observed at the CORE SITES and PARK for Delivery ≤ 7 Days

Measure	CORE SITES	PARK
Sensitivity	86.4%	62.5%
Specificity	82.3%	87.0%
Pos PV	12.7%	16.7%
Neg PV	99.5%	98.2%

Summary of Clinical Trial:

A prospective, multicenter clinical study was successfully conducted to determine the safety and effectiveness of the fFN Immunoassay as an aid in assessing the risk of preterm delivery in ≤ 7 days or ≤ 14 days of specimen collection for subjects with intact membranes and minimal cervical dilatation who sought unscheduled obstetrical care between 24 weeks, 0 days and 34 weeks, 6 days with obstetrical complications associated with imminent preterm delivery. The presence or absence of fFN was determined for a single cervicovaginal specimen obtained from the posterior vaginal fornix at study entry. The endpoints of Study 031 were the association of fFN Immunoassay result to delivery ≤ 7 days and ≤ 14 days of specimen collection. Additional clinical data were obtained on the relationship of the fFN Immunoassay test result to delivery ≤ 36 weeks, neonatal status, and before and after 32 weeks gestation, and the ability of other clinical factors to assess the risk of preterm delivery.

The primary hypothesis of the clinical trial was confirmed: the fFN Immunoassay results are

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statistically associated with aiding in assessing the risk of preterm delivery in ≤ 7 days or ≤ 14 days of specimen collection.

VIII. Conclusions Drawn from the Studies

The results of the clinical studies support the use of the fFN Immunoassay as an aid in assessing the risk of preterm delivery in ≤ 7 or ≤ 14 days from the time of sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (< 3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5 percent and 99.2 percent, for delivery in ≤ 7 and ≤ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7 percent and 16.7 percent for delivery in ≤ 7 and ≤ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

Antigen characterization information and antibody specificity information were found to be adequate. Non-clinical laboratory studies show that intra- and inter-assay reproducibility of the assay are < 7 percent and < 13 percent, respectively. The minimum detectable absorbance of the assay is 0.070 units of optical density. Specimen dilution experiments show that the dose-response curve allows sufficient discrimination between positive and negative samples. Hepatic fibronectin, the contrast agent indigo carmine, and therapeutic agents commonly associated with pregnancy were not found to interfere with the assay. The stability studies support a shelf-life dating of 12 months for the fFN Immunoassay Kit at 2-8°C. In conclusion, the results of the nonclinical studies were found to be adequate in supporting the safety and effectiveness of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay.

IX. Panel Recommendation

The Clinical Laboratory Devices Panel recommended at the panel meeting on April 6, 1995 that the PMA for the fFN Immunoassay be approved with the following conditions:

1. Recalculate confidence intervals using a more exact method and submit an amendment. The recalculations are required for the data included in the SSED and the labeling.

2. Reanalyze the data to include the 56 subjects who had incomplete follow-up, and determine if there is a signficant change in the data.

3. Provide data/literature references to substantiate the claim that specimens collected 24 hours after sexual intercourse are acceptable for use. Additionally, provide the protocol(s) for the semen studies.

4. Address concerns of analyte stability at recommended shipping and storage conditions; ensure the labeling emphasizes this. Provide further clarification on the specimen stability studies,

specifically why the data for day 2 were less than ideal and apparently excluded.

5. Provide a summary of stability data for the buffer and characterize its components.

6. Provide a separate clinician's brochure and submit for our review.

7. Address the concerns (listed below) raised by the panel members:

   a. Revise wording in the insert and clinician's brochure to reflect benefits from positive and negative results.

   b. Ensure the insert mentions that testing was conducted on a single specimen from the patient.

   c. Emphasize that results should not be used as the only criterion for medical decisions.

   d. Include the affects of bloody specimens in the insert and clinician's brochure.

e. Include a breakdown of data to indicate predictability by gestational age and birth weight in the insert or clinician's brochure.

f. Emphasize in the labeling why a test should not be performed if the patient is bleeding, has rupture of membranes, has advanced dilatation, etc.

8. Submit the revised Summary of Safety and Effectiveness Data and package insert.

X. CDRH Action on the Application

CDRH concurred with all the recommendations of the Panel and issued an approval letter on _____.

FDA concluded that the device had been shown to be safe and effective for stated indication based on the data and scientific literature submitted by the applicant and issued an approval letter to Adeza Biomedical on _____ _____.

The applicant's manufacturing and control facilities were inspected on March 30, May 5, and May 15, 1995, for compliance with the Device Good Manufacturing Practice

Regulations (GMPs) and the facilities were found to be in compliance.

The shelf life of the fFN Immunoassay has been established at 1 year when stored at 2°-8°C.

XI. Approval Specifications

Directions for use: See Attached Labeling

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order.

XII. References

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Matsuura H, et al J Biol Chem 263:3314-3322, 1988.

Morrison JC, et al Am J Obstet Gynecol 168:538-542, 1993.

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For *In Vitro* Diagnostic Use Only
Store at 2° to 8°C

Fetal Fibronectin Enzyme Immunoassay

For Professional Use Only
Kit size: 96 wells

Cat. No.: 00480
Patented

INTENDED USE

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is a device to be used as an aid in assessing the risk of preterm delivery in ≤ 7 or ≤ 14 days from the time of sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (<3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5% and 99.2%, for delivery in ≤ 7 and ≤ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7% and 16.7% for delivery in ≤ 7 and ≤ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

CONTRAINDICATIONS

The Fetal Fibronectin Enzyme Immunoassay should not be used for symptomatic women who have:

- advanced cervical dilatation (≥3 centimeters)
- rupture of amniotic membranes
- cervical cerclage
- moderate or gross vaginal bleeding

Delivery typically occurs imminently when the cervix is dilated more than 3 centimeters or if the amniotic membranes are ruptured. Additional diagnostic testing is usually not necessary to confirm risk for women with advanced cervical dilatation or rupture of amniotic membranes. Moderate or gross vaginal bleeding is an independent risk factor for preterm delivery and may be associated with other severe obstetrical or medical problems. Clinical attention should be focused on identification of the origin of bleeding rather than immediate assessment of delivery risk. At this time, there is insufficient information characterizing the association of vaginal fetal fibronectin expression to delivery for women with cervical cerclage.

SUMMARY AND EXPLANATION OF THE TEST

Of the approximately 4,000,000 deliveries that occur annually in the United States, about 400,000 are premature. Preterm delivery, defined by the American College of Obstetricians and Gynecologists as delivery prior to the 37th week of gestation, is responsible for the majority of non-chromosomal perinatal morbidity and mortality (1-4). Symptoms of threatened preterm delivery include uterine contractions, change of vaginal discharge, vaginal bleeding, backache, abdominal discomfort, pelvic pressure, and cramping. Diagnostic modalities for identification of threatened preterm delivery include uterine activity monitoring and performance of a digital cervical examination, which allows estimation of cervical dimensions. These methods have been shown to be limited, as minimal cervical dilatation (<3 centimeters) and uterine activity occur normally and are not necessarily diagnostic of imminent preterm delivery (5-7). While several serum biochemical markers have been evaluated, none have been widely accepted for practical clinical use (8,9).

Fetal fibronectin (fFN), an isoform of fibronectin, is a complex adhesive glycoprotein with a molecular weight of approximately 500,000 daltons (10-12). Matsuura and co-workers have described a monoclonal antibody called FDC-6 which specifically recognizes III-CS, the region defining the fetal isoform of fibronectin (10,11). Immunohistochemical studies of placentae have shown that fFN is confined to the extracellular matrix of the region defining the junction of the maternal and fetal units within the uterus (5,13).

Fetal fibronectin can be detected in cervicovaginal secretions of women throughout pregnancy by use of a monoclonal antibody-based immunoassay. Fetal fibronectin is elevated in cervicovaginal secretions during the first 24 weeks of pregnancy but diminishes between 24 and 34 weeks in normal pregnancies. The significance of its presence in the vagina during the first 24 weeks of pregnancy is not understood. However, it may simply reflect the normal growth of the extravillous trophoblast population and the placenta. Detection of fFN in cervicovaginal secretions between 24 and 34 completed weeks gestation is reported to be associated with preterm delivery in symptomatic pregnant women (5-7).

PRINCIPLE OF THE TEST

The Fetal Fibronectin Enzyme Immunoassay is a solid-phase enzyme-linked immunosorbent assay (ELISA). During the assay, cervicovaginal samples are incubated in microtiter wells coated with FDC-6, a monoclonal antibody specific for fFN (5-7). The resulting antibody-antigen complex is washed to remove non-specifically bound material and then reacted with an enzyme-labeled antibody directed against human fibronectin. Following formation of the antigen-antibody "sandwich", the microtiter well is washed to remove unbound labeled antibody. It is then incubated with an enzyme substrate. The presence or absence of fFN in the specimen is determined spectrophotomstrically.

PRECAUTIONS AND WARNINGS

Note:

Specimens must be stored/transported refrigerated at 2° to 8°C and assayed within three (3) days of collection to avoid degradation of the analyte. Store appropriately and avoid extreme temperatures. Do not freeze samples or expose to elevated temperatures.

1. For *in vitro* diagnostic use only.
2. Carefully follow the instructions and procedures described in this insert.
3. Pre-warming References and specimens to 37°C is necessary to ensure homogeneity. (See *Procedure*: Assay Protocol.) However, prolonged warming may result in degradation of fFN and should be avoided.
4. Do not use glass tubes or pipets, as fetal fibronectin binds to glass. Tubes and pipets of polypropylene or polyethylene are acceptable.
5. Reagents in this kit contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. Thus, when disposing of these reagents, always flush the drain with large volumes of water to prevent azide build-up.
6. Do not mix materials from different kit lots.
7. Do not use reagents beyond the kit expiration date printed on the kit box.
8. Do not use kit if reagents are cloudy or discolored.
9. Avoid cross-contamination of reagents. When using a micropipet, change tips between each Reference and sample. Recap reagents tightly with the correct color-coded caps.
10. Source material used to prepare the Positive Reference is of human origin. The donors were tested and found to be negative for human immunodeficiency virus (HIV) antibody and hepatitis B surface antigen (HBsAg) using established methods. No known test method can offer total assurance that HIV, hepatitis B virus or other infectious agents are absent. Handle these reagents and all patient specimens as if potentially infectious.

STORAGE

All Fetal Fibronectin Enzyme Immunoassay reagents should be refrigerated at 2° to 8°C. All unused strips should be resealed immediately with the desiccant pack in their foil pouch to ensure stability.

STABILITY

The shelf life of the Fetal Fibronectin Enzyme Immunoassay Kit is one year from date of manufacture. Unopened assay reagents may be used until the expiration date printed on the kit box. Once opened, they should be used within 12 weeks. However, they should not be used if they are cloudy, and the Substrate should not be used if it has turned pink in color.

SPECIMEN COLLECTION AND PREPARATION

The specimen should be obtained from the posterior fornix of the vagina during a sterile speculum examination. ***The Adeza Biomedical Specimen Collection Kit is the only acceptable specimen collection kit which can be used to collect specimens for this assay.*** The Dacron swab provided in the Specimen Collection Kit should be inserted into the vagina and lightly rotated across the posterior fornix for approximately 10 seconds to absorb the cervicovaginal secretions. Once the specimen is obtained, carefully remove the swab from the vagina and place it into the tube of buffer provided with the Specimen Collection Kit. The method of collection is also described in the directional insert provided with the Adeza Biomedical Specimen Collection Kit. Use only one Specimen Collection Kit per patient. Label the Specimen Transport Tube with the patient's name and any other identifying information required.

- **SPECIMENS SHOULD BE OBTAINED PRIOR TO DIGITAL CERVICAL EXAMINATION OR VAGINAL PROBE ULTRASOUND EXAMINATION AS MANIPULATION OF THE CERVIX MAY CAUSE THE RELEASE OF fFN.**
- Care must be taken not to contaminate the swab or cervicovaginal secretions with lubricants, soaps, or disinfectants (e.g., K-Y® Jelly, Betadine®, hexachlorophene). These substances may interfere with absorption of the specimen by the swab or with the antibody-antigen reaction of the Fetal Fibronectin Enzyme Immunoassay.
- Collection of vaginal specimens for microbiologic culture frequently requires aggressive collection techniques which may abrade the cervical or vaginal mucosa. Since cellular debris may potentially interfere with sample preparation, specimens for the Fetal Fibronectin Enzyme Immunoassay should be collected prior to collection of culture specimens.
- Patient specimens should not be tested if the patient has had sexual intercourse within 24 hours prior to the sampling time.
- Specimens for testing with the Fetal Fibronectin Enzyme Immunoassay should not be obtained from patients with suspected or known placental abruption or placenta previa.
- The Fetal Fibronectin Enzyme Immunoassay is not intended for use in the management of patients with medium or gross vaginal bleeding. The presence of vaginal bleeding judged by the caregiver to be medium or gross in amount may contribute to difficulty in interpreting the Fetal Fibronectin Enzyme Immunoassay result.
- Rupture of membranes should be ruled out prior to performance of the fFN Enzyme Immunoassay since fFN is found in both amniotic fluid and the fetal membranes.
- The Fetal Fibronectin Enzyme Immunoassay is not intended for use in patients with cancers of the reproductive tract.

ASSAY PROCEDURE

Reagents/Materials Provided

1. Microtiter Strips: twelve 8-well strips coated with affinity purified mouse monoclonal antibody to human fetal fibronectin in a sealed pouch containing desiccant. Store at 2° to 8°C.
2. Strip Holder: one (packaged in the microtiter strip pouch).
3. Plate Cover: one.
4. Antibody Conjugate: one blue-capped bottle containing 10 ml of affinity purified goat polyclonal antibody to human fibronectin conjugated to calf intestine alkaline phosphatase in a buffer matrix with sodium azide as a preservative. Store at 2° to 8°C.
5. Substrate: one red-capped bottle containing 10 ml of phenolphthalein monophosphate substrate in buffer with sodium azide as a preservative. Store at 2° to 8°C.
6. Positive Reference: one green-capped bottle containing 2.5 ml of 0.05 µg/ml human fetal fibronectin in a stable protein matrix with sodium azide as a preservative. Store at 2° to 8°C.
7. Negative Reference: one yellow-capped bottle containing 2.5 ml of stable protein matrix without fetal fibronectin, with sodium azide as a preservative. Store at 2° to 8°C.
8. Wash Buffer Concentrate: one white-capped bottle containing 20 ml of concentrated buffer salts with detergent and with sodium azide as a preservative. Store at 2° to 8°C.
9. Stopping Solution: one white-capped bottle containing 10 ml of buffer with a chelating agent. Store at 2° to 8°C.
10. Sample Filters: forty-eight.
11. Sample Storage Tubes: forty-eight.
12. Directional Insert: one.

Materials Required But Not Provided

1. Microtiter plate reader (spectrophotometer) with a 550 nm filter (linear to 2.0 A).
2. Aspiration or decantation device.
3. 37°C incubator or water bath.
4. 2 L graduated cylinder or volumetric flask.
5. 2 L wash bottle or other container for storage of diluted Wash Buffer Concentrate.
6. Distilled or deionized water.
7. Test tube or other container for decanted Substrate.
8. Timer.
9. 50 and 100 µl micropipettors with disposable plastic tips.
10. Fetal Fibronectin Enzyme Immunoassay Control Kit (Cat. No. 00481)

REAGENT PREPARATION

To reconstitute the Wash Buffer Concentrate, add the entire contents (20 ml) of the Wash Buffer Concentrate bottle to a 2 L graduated cylinder or volumetric flask. Dilute the Wash Buffer Concentrate with distilled or deionized water to a final volume of 2 L. Mix well. Transfer to a 2 L bottle or other container for storage. Label container with the date and lot number. The diluted wash buffer should be stored capped at room temperature (20° to 25°C) and may be used until the expiration date on the kit box.

Recommended Materials Available from Adeza Biomedical

1. Fetal Fibronectin Enzyme Immunoassay Control Kit (Cat. No. 00481)

Assay Protocol

NOTE: Patient samples and References should be tested at the same time. Do not use the Reference results from one assay to determine patient sample results from a second plate or assay. Incubation times must be accurate in order to avoid erroneous results. The microtiter plate reader should be set to read at 550 nm.

1. Remove kit components from the box, and allow the components to equilibrate to room temperature (20°-25°C) prior to use.
2. Prewarm all References and Specimen Transport Tubes. Allow approximately 20 minutes in a 37°C incubator or 10 minutes in a 37°C water bath.
3. Gently vortex or mix Specimen Transport Tube prior to removing the swab.
4. Open the Specimen Transport Tube cap and swab assembly. The swab shaft should be seated in the cap. Express as much fluid from the swab as possible. Dispose of the swab and cap in a manner consistent with the handling of potentially infectious material.
5. Snap a Sample Filter into the top of the Specimen Transport Tube.
6. Filter the sample by squeezing the entire contents of the Specimen Transport Tube through the filter tip into a clean, labeled Sample Storage Tube.
   Note: Although most samples pass through the Sample Filter easily, there are occasional samples which do not pass through. Such samples should be centrifuged at 550xg (for example 2500 rpm in a microcentrifuge) at room temperature for 5 minutes instead of filtering and the supernatant tested in the same manner as filtered samples.
7. Calculate the number of 8-well strips needed for testing, based on testing References and samples in duplicate.
8. For unopened kits, follow these steps:
   A. Remove the 96-well plate from the foil pouch.
   B. Leave the required number of 8-well strips for the assay in the plate holder starting with Row A, Column 1.
   C. Carefully remove the remaining 8-well strips, if any, that will not be needed for the assay and place the strips back in the foil pouch with the desiccant.
   D. Reseal the pouch tightly and return to the refrigerator immediately.
9. For previously opened kits, follow these steps:
   A. Remove only the required number of 8-well strips from the foil pouch.
   B. Place strips in the plate holder starting with Row A, Column 1.
   C. If unused strips remain, leave them in the pouch, reseal the pouch tightly, and immediately return it to the refrigerator.
10. Gently invert the prewarmed Positive and Negative References several times to mix.
11. Pipet 100 µl of the yellow-capped Negative Reference into each of 2 wells.
12. Pipet 100 µl of the green-capped Positive Reference into each of 2 wells.
13. Pipet 100 µl of each sample into each of 2 wells.
14. Cover the plate and incubate for 1 hour at room temperature (20°-25°C).
15. Wash and thoroughly aspirate the wells three times with the diluted Wash Buffer Concentrate, being sure to fill the wells completely each time.
16. Select the blue-capped Antibody Conjugate bottle. Pipet 100 µl into each well.
17. Cover the plate and incubate 30 minutes at room temperature (20°- 25°C).
18. Wash and aspirate the plate as in Step 15.
19. Select the red-capped Substrate vial. Decant the appropriate volume of Substrate into a separate tube or other container. The Substrate should appear pale yellow. Pipet 100 µl of Substrate into each well. Do not return unused Substrate to the original bottle.
20. Cover the plate and incubate for 30 minutes at room temperature (20°- 25°C).
21. Do not aspirate or wash. Pipet 50 µl of Stopping Solution into each well.
22. Gently rotate the plate to mix, being careful not to spill fluid out of the wells.
23. Gently wipe the bottom of the microtiter wells with a cloth or tissue to remove any moisture and/or fingerprints.
24. Read absorbance at 550 nm within one hour, using a microtiter plate reader.

'TERPRETATION OF RESULTS

Compute the average absorbance of duplicate wells for each Reference provided in the assay kit and each patient specimen. Values for duplicate determinations below 0.2 OD units should be within 0.05 OD units of the mean OD. Duplicate values greater than 0.2 OD units generally should be expected to be within 20% of the mean OD. The average absorbance of the Positive Reference should be at least 0.02 absorbance units greater than the average absorbance of the Negative Reference. If it is not, review the assay protocol, and be sure that the correct reagents were used at each step. Repeat the assay. If the problem is not corrected, please call your Adeza Biomedical representative for technical help. The sample result is positive if the average absorbance is greater than or equal to the average absorbance of the Positive Reference. The sample result is negative if the average absorbance is less than the average absorbance of the Positive Reference (See Table 1 for examples of positive and negative results).

Table 1
Examples of Patient Results

Sample	Mean O.D.	Result
Positive Kit Reference	0.120	---
Negative Kit Reference	0.056	---
Patient Sample #1	0.097	Negative
Patient Sample #2	0.120	Positive
Patient Sample #3	0.252	Positive

Use caution when interpreting results from specimens containing trace amounts of blood. Although specimens should not be collected from patients with moderate or gross vaginal bleeding, the distinction between trace and moderate bleeding is subjective, so some specimens with moderate bleeding may inadvertently be collected. The Fetal Fibronectin Enzyme Immunoassay is not intended for use in the management of patients with moderate or gross vaginal bleeding.

A positive fetal fibronectin test result suggests elevated risk of early delivery with its attendant neonatal consequences. In contrast, a negative fetal fibronectin test result is strongly associated with prolonged gestation and term delivery. Symptomatic women with a negative fetal fibronectin test result have less than a 1% chance of delivering in ≤ 7 or ≤ 14 days from the time of specimen collection. Thus, in the absence of other clinical evidence, a negative fetal fibronectin test result indicates a reduced risk of preterm delivery. This should be considered in light of other information in making patient management decisions.

QUALITY CONTROL PROCEDURES AND MATERIALS

Current good laboratory practice includes the periodic use of controls to monitor assay performance. The Fetal Fibronectin Enzyme Immunoassay Control Kit (Catalog No. 00481), available from Adeza Biomedical, contains two Controls: one Positive Control and one Negative Control. The Control Kit is recommended for use in monitoring the performance of the Fetal Fibronectin Enzyme Immunoassay. In order to ensure accurate and reliable test results, performance testing should be done only with the Controls from the Fetal Fibronectin Enzyme Immunoassay Control Kit. The recommended frequency of use of the Controls is once per microtiter plate per assay. Any deviation from the recommended frequency of Quality Control testing must be validated by the laboratory.

LIMITATIONS

The Fetal Fibronectin Enzyme Immunoassay result should not be interpreted as absolute evidence for the presence or absence of a process that will result in delivery in ≤ 7 or ≤ 14 days from specimen collection. A positive Fetal Fibronectin Enzyme Immunoassay result may be observed for patients who have experienced cervical disruption caused by, but not limited to, events such as sexual intercourse, digital cervical examination, or vaginal probe ultrasound. The Fetal Fibronectin Enzyme Immunoassay result should always be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as cervical examination, cervical microbiological culture, assessment of uterine activity, and evaluation of other risk factors.

- Modification of the assay protocol described herein may yield erroneous results.
- The assay has been optimized with specimens taken from the posterior fornix. Samples obtained from other locations should not be used.
- The safety and effectiveness of using a cutoff other than that provided by the Positive Reference (0.050 µg/ml) has not been established.
- There are no prospective clinical trials to support use of the Fetal Fibronectin Enzyme Immunoassay as a method for screening asymptomatic pregnant patients for risk of preterm delivery.
- Assay interference from the following components has not been ruled out: douches, white blood cells, red blood cells, bacteria and bilirubin.
- The presence of infections has not been ruled out as a confounding factor to risk of preterm delivery.
- Test results are invalid if the specimen contains semen or if the specimen was collected less than 24 hours after coitus. Two studies were conducted establishing that intercourse and presence of semen may lead to positive test results at the cutoff of 0.05 µg fFN/mL. In the first study, fetal fibronectin was detected in 23% of post-coital vaginal specimens obtained from 22 non-pregnant women. In the second study, fetal fibronectin was detected in 21 of 41 sperm samples obtained from healthy male volunteers. These results suggest that sperm (or semen) may contain sufficient concentration of fetal fibronectin to result in a positive fetal fibronectin test result.

EXPECTED VALUES

Elevated levels (≥0.050 µg/ml) of fFN between 24 weeks, 0 days and 34 weeks, 6 days, indicates increased risk of delivery in ≤ 7 or ≤ 14 days from sample collection. The cut-off of 0.050 µg fFN/ml was established in a multicenter study conducted to evaluate the association between fetal fibronectin expression during pregnancy and preterm delivery (5). Only subjects with symptoms of preterm labor or preterm rupture of membranes were eligible for this study. Of the total study population, the association of vaginal fetal fibronectin expression to preterm delivery was evaluated for 117 symptomatic women with intact amniotic membranes. The strength of this association was

termined at a variety of cutoffs using receiver operator characteristic (ROC) curves. These results show that the optimal sensitivity and specificity is provided at a cutoff of 0.050 μg fFN/ml. Subsequent studies evaluating the fFN Enzyme Immunoassay test as a predictor of preterm delivery have confirmed that the optimal cutoff is 0.050 μg fFN/ml (6,7).

CLINICAL PERFORMANCE CHARACTERISTICS

A prospective study of 763 pregnancies was conducted at 10 clinical sites in the United States to assess the association of cervicovaginal expression of fetal fibronectin to preterm delivery.

Assessment of Risk for Symptomatic Patients

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay was used for identification of preterm delivery risk for symptomatic pregnant women meeting the following clinical criteria:

- Present for unscheduled obstetrical care
- Have signs and symptoms of threatened preterm delivery limited to:
  - Uterine contractions (with or without pain)
  - Intermittent lower abdominal pain, dull backache, pelvic pressure
  - Vaginal bleeding during the second or third trimester
  - Menstrual-like intestinal cramping (with or without diarrhea)
  - Change in vaginal discharge (amount, color, or consistency)
  - Vague sense of discomfort characterized as "not feeling right"
- Have a gestational age between 24 weeks, 0 days and 34 weeks, 6 days
- Have intact amniotic membranes
- Have minimal cervical dilatation (< 3 centimeters)

Relationship of Fetal Fibronectin Expression to Delivery Endpoints

The safety and effectiveness of the Adeza Biomedical Fetal Fibronectin Immunoassay was evaluated in a population of 763 pregnant patients with signs and symptoms commonly associated with threatened preterm delivery. The relationship of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay test result to delivery in ≤ 7 and ≤14 days is shown in Table 2. For delivery ≤ 7 days, the sensitivity, specificity, positive and negative predictive values of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay were 86.4%, 82.3%, 12.7%, and 99.5%, respectively. For delivery ≤ 14 days, the sensitivity, specificity, positive and negative predictive values of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay were 83.3%, 82.9%, 16.7%, and 99.2%, respectively.

Table 2

Sensitivity, Specificity, and Positive and Negative Predictive Values of Fetal Fibronectin for Delivery ≤ 7 and ≤ 14 Days (n = 763)

Delivery	n (%)	Sensitivity (95% CI)*	Specificity (95% CI)	Pred Val + (95% CI)	Pred Val - (95% CI)
≤ 7 Days	22 (2.9%)	86.4% (66.4,95.3)	82.3% (79.4,84.9)	12.7% (4.2,33.7)	99.5% (98.7,99.8)
≤14 Days	30 (3.9%)	83.3% (66.3,93.7)	82.9% (80.0,85.4)	16.7% (7.3,33.7)	99.2% (98.3,99.6)

*Proportion of deliveries at each endpoint are calculated using 763 as the denominator

Additional Clinical Data

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay test result was also related to other clinical features, including delivery in ≤ 36 completed* weeks of gestation (preterm delivery) and neonatal status. The sensitivity, specificity, positive and negative predictive values of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay were 41.3%, 86.2%, 44.7%, and 84.5%, respectively, for delivery ≤ 36 weeks (162 or 21.2% of the 763 subjects delivered ≤ 36 weeks).

The relationship of the fetal fibronectin test result to neonatal status is shown in Table 3.

Table 3

Neonatal Status Stratified by Fetal Fibronectin Test Result*

		fFN +	fFN -	p-value
Total Subjects, n (%)		150 (19.7%)	613 (80.3%)	--
Infant Weight (grams)				
	Avg	2804.0	3242.8	0.00010
	SD	776.2	582.7	
	n	154	636	
	Range	625-4280	835-5800	
Infant Weight (grams)				
n (%)	<1500	11 (7.1%)	6 (0.9%)	0.00005
	<2500	57 (37.0%)	69 (10.8%)	<0.00001
Perinatal Morbidity, n (%)				
Respiratory Distress		18 (11.7%)	22 (3.5%)	0.0001
NICU Admission, n(%)		44 (28.6%)	68 (10.7%)	<0.000001
Neonatal Hospital Days		5.9 ± 11.1	2.9 ± 7.1	0.01

*Neonate information available for only 753 of the 763 subjects

The number of subjects, distribution of positive fetal fibronectin test results, sensitivity, and predictive value of a positive test for delivery in ≤ 7 days from specimen collection is shown in Table 4 for symptomatic pregnant women < 32 weeks and ≥ 32 weeks. The

proportion of positive tests and the sensitivity of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is the same for women before and after 32 weeks. The number of symptomatic women seeking care increases gradually with advancing gestational age as does the number of deliveries.

The ability of other clinical factors to assess risk of preterm delivery were also evaluated in this prospective trial and compared to the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay. The sensitivity, specificity, positive predictive values, and negative predictive values and their 95% confidence intervals for delivery in ≤ 7 days for the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay, cervical dilatation, uterine activity, vaginal bleeding, and ascending genital tract infection (bacterial vaginosis) are provided in Table 5.

Table 4

Distribution of Subjects and Fetal Fibronectin Enzyme Immunoassay Results Before and After 32 Weeks Gestation[a]

EGAS (Weeks)	Subjects (n)	fFN +[b] n (%)	Sensitivity (%) (fFN +/Del ≤ 7 Days)	Pred Val + (%) (Del ≤ 7 Days/fFN +)
<32 Weeks	483	91 (18.8%)	8/9 (88.9%)	8/91 (8.8%)
≥32 Weeks	280	59 (21.0%)	11/13 (84.6%)	11/59 (18.6%)
TOTAL	763	150 (19.7%)	19/22 (86.4%)	19/150 (12.7%)

[a]Estimated Gestational Age at Specimen Collection
[b]fFN + = positive Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay result

Table 5

Sensitivity, Specificity, Positive and Negative Predictive Values for All Risk Factors

Risk Factor[a]	n	Sensitivity (95% CI)[b]	Specificity (95% CI)	Pred Val + (95% CI)	Pred Val - (95% CI)
fFN Enzyme Immunoassay	763	86.4% (66.4,95.3)	82.3% (79.4,84.9)	12.7% (4.2,33.7)	99.5% (98.7,99.8)
Uterine Activity	750	54.5% (34.5,73.1)	75.3% (72.0,78.3)	6.3% (1.4,24.3)	98.2% (96.9,98.9)
Cervical Dilatation	757	38.1% (20.6,59.4)	88.3% (85.8,90.4)	8.5% (2.1,27.9)	98.0% (96.7,98.8)
Vaginal Bleeding	759	40.9% (23.0,61.6)	85.2% (82.4,87.6)	7.6% (1.9,26.3)	98.0% (96.7,98.7)
Ascend Gen Tract Inf	763	9.1% (2.5,27.8)	84.1% (81.2,86.5)	1.7% (0.1,17.6)	97.3% (95.9,98.2)

[a]Cutoff used to define a positive test result for determining sensitivity, etc (fFN Enzyme Immunoassay, ≥ 0.05 μg/mL; uterine activity, ≥ 4 contractions per hour; cervical dilatation, > 1 centimeter; vaginal bleeding, evidence of any bleeding; ascending genital tract infection, bacterial vaginosis
[b]95% Confidence Interval (% Lower Limit, % Upper Limit)

LABORATORY PERFORMANCE CHARACTERISTICS

Within-Run Precision

Within-run precision was determined using three different lots of Fetal Fibronectin Enzyme Immunoassay Kits. Five samples of reference materials containing varying concentrations of fFN were assayed at least 20 times each in at least two different runs. The within-run coefficients of variation for the pooled data ranged from 2.9% to 4.2%. The data for a representative assay are presented below.

Within-Run Precision

Sample	1	2	3	4	5
Number of Replicates	66	66	66	66	66
Mean Absorbance (550 nm)	0.139	0.075	0.166	0.279	0.704
Standard Deviation	0.004	0.003	0.007	0.011	0.026
% Coefficient of Variation	2.9	4.0	4.2	3.9	3.7

Between-Run Precision

Between-run precision was determined using three different lots of Fetal Fibronectin Enzyme Immunoassay Kits. Six samples of reference materials containing varying concentrations of fFN were assayed at least 20 times each in at least two different runs. The between-run coefficients of variation for the pooled data ranged from 7.6% to 11.8%. Data from a representative site that ran three assays are presented below.

Between-Run Precision

Sample	1	2	3	4	5	6
Replicates/assay	72	72	72	72	72	72
Mean Absorbance (550nm)	0.139	0.062	0.079	0.170	0.264	0.672
Standard Deviation	0.013	0.005	0.006	0.020	0.025	0.059
% Coefficient of Variation	9.4	8.1	7.6	11.8	9.5	8.8

Interfering Substances

Care must be taken not to contaminate the swab or cervicovaginal secretions with lubricants, soaps, or disinfectants (e.g., K-Y® Jelly, Betadine®, hexachlorophene). These substances may interfere with absorption of the specimen by the swab or with the antibody-antigen reaction of the Fetal Fibronectin Enzyme Immunoassay.

Diagnostic and Therapeutic Agents

Various concentrations of pharmacologic agents were added to specimens containing 0 to 1.0 μg fFN/mL and assayed in duplicate. The drugs added were: prostaglandin E_2 (up to 100 μg/mL), ampicillin (up to 100 μg/mL), cephalexin (up to 18 μg/mL), erythromycin (up to 10 μg/mL), gentamycin (up to 4 μg/mL), dexamethasone (up to 200 μg/mL), magnesium sulfate (up to 50 μg/mL), oxytocin (up to 100 U/mL), terbutaline (up to 1 mg/mL), and ritodrine (up to 100 μg/mL). These drugs did not interfere with the assay at the concentration limits cited.

Minimum Detectable Absorbance

The Minimum Detectable Absorbance (MDA) of fetal fibronectin is approximately 0.070 O.D. units. The MDA is defined as the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the Negative Reference.

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TECHNICAL SERVICE AND ORDERING INFORMATION

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Clinician's Brochure for the Fetal Fibronectin Enzyme Immunoassay

ADEZA BIOMEDICAL
FETAL FIBRONECTIN ENZYME IMMUNOASSAY

INFORMATION FOR HEALTH CARE PROVIDERS

ASSISTS IN THE ASSESSMENT OF PRETERM DELIVERY RISK

Prepared by:

Adeza Biomedical Corporation
1240 Elko Drive
Sunnyvale, CA 94089

TABLE OF CONTENTS

This brochure was prepared by Adeza Biomedical to familiarize you with clinical interpretation of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay. In conjunction with other clinical information, testing for the presence of fetal fibronectin in cervicovaginal secretions of women with suspected preterm labor will help you and your patients gain valuable information about their pregnancies, including assessment of risk of preterm delivery. Additional copies of this brochure are available free of charge by calling Adeza Biomedical at 1-408-745-0975.

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INTENDED USE

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is a device to be used as an aid in assessing the risk of preterm delivery in ≤ 7 or ≤ 14 days from the time of sample collection in pregnant women with signs and symptoms of early preterm labor, intact amniotic membranes and minimal cervical dilatation (<3 cm), sampled between 24 weeks, 0 days and 34 weeks, 6 days gestation.

The negative predictive values of 99.5% and 99.2%, for delivery in ≤ 7 and ≤ 14 days respectively, make it highly likely that delivery will not occur in these time frames. In addition, although the positive predictive values were found to be 12.7% and 16.7% for delivery in ≤ 7 and ≤ 14 days, respectively, this represents an approximate 4-fold increase over the reliability of predicting delivery given no test information.

SUMMARY: FETAL FIBRONECTIN AS A CLINICAL TOOL

In a prospective clinical study of 763 symptomatic pregnant women with signs and symptoms of preterm labor, it was determined that the expression of fetal fibronectin in cervicovaginal secretions can be used to assess the risk of preterm delivery in ≤ 7 or ≤ 14 days of specimen collection (as described in Intended Use). In this same trial, it was also determined that expression of fetal fibronectin is also related to other clinical features, including delivery in ≤ 36 completed weeks (preterm delivery) and neonatal status. A positive fetal fibronectin test result suggests elevated risk of early delivery with its attendant neonatal consequences. In contrast, a negative fetal fibronectin test result is strongly associated with prolonged gestation and term delivery. Symptomatic women with a negative fetal fibronectin test result have less than a 1% chance of delivering in ≤ 7 or ≤ 14 days from the time of specimen collection. Thus, in the absence of other clinical evidence, a negative fetal fibronectin test result indicates a reduced risk of preterm delivery. This should be considered in light of other information in making patient management decisions.

PRETERM DELIVERY: THE CLINICAL DILEMMA

Of the approximately 4,000,000 deliveries that occur annually in the United States, about 400,000 are premature. Preterm delivery, defined by the American College of Obstetricians and Gynecologists as delivery prior to the 37th week of gestation, is responsible for the majority of non-chromosomal perinatal morbidity and mortality (1-4). Symptoms of threatened preterm delivery include uterine contractions, change of vaginal discharge, vaginal bleeding, backache, abdominal discomfort, pelvic pressure, and cramping. Diagnostic modalities for identification of threatened preterm delivery include uterine activity monitoring and performance of a digital cervical examination, which allows estimation of cervical dimensions. These methods have been shown to be limited, as minimal cervical dilatation (<3 centimeters) and uterine activity occur normally and are not necessarily diagnostic of imminent preterm delivery (5-7). While several serum biochemical markers have been evaluated, none have been widely accepted for practical clinical use (8,9).

FETAL FIBRONECTIN: PRESENCE AT THE MATERNAL-FETAL INTERFACE

Fetal fibronectin, an isoform of fibronectin, is a major component of the extracellular matrix of the membranes of the amniotic sac. Fetal fibronectin can be distinguished from other members of the fibronectin family by the presence of a unique region, known as the III-CS domain. Scientists have developed a monoclonal antibody, called FDC-6, which specifically recognizes the III-CS domain of fetal fibronectin (10,11). Immunohistochemical studies of placentae show that fetal fibronectin is confined to the extracellular matrix of the interface between the maternal and fetal units within the uterus; this interface is also known as the choriodecidual junction (5,12). These studies suggest that fetal fibronectin is a product of extravillous chorionic trophoblasts which cover the periphery of the amniotic sac and attach to the maternal decidua of the uterus. Because of its unique localization adjacent to the placenta and amniotic sac, a number of clinical trials have been conducted using the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay to determine the association of cervicovaginal fetal fibronectin expression to risk of preterm delivery.

Fetal fibronectin can be detected in cervicovaginal secretions of women throughout pregnancy by use of a monoclonal antibody-based immunoassay. Fetal fibronectin is elevated in cervicovaginal secretions during the first 24 weeks of pregnancy but diminishes between 24 and 34 weeks in normal pregnancies. The significance of its presence in the vagina during the first 24 weeks of pregnancy is not understood. However, it may simply reflect the normal growth of the extravillous trophoblast population and the placenta. Detection of fFN in cervicovaginal secretions between 24 and 34 completed weeks gestation is reported to be associated with preterm delivery in symptomatic pregnant women (5-7).

FETAL FIBRONECTIN: CLINICAL TRIAL RESULTS

A prospective study of 763 pregnancies was conducted at 10 clinical sites in the United States to assess the association of cervicovaginal expression of fetal fibronectin to preterm delivery.

Assessment of Risk for Symptomatic Patients

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay was used to assess risk of preterm delivery for symptomatic pregnant women meeting the following clinical criteria:

- Present for unscheduled obstetrical care
- Have signs and symptoms of threatened preterm delivery limited to:
  - Uterine contractions (with or without pain)
  - Intermittent lower abdominal pain, dull backache, pelvic pressure
  - Vaginal bleeding during the second or third trimester
  - Menstrual-like intestinal cramping (with or without diarrhea)
  - Change in vaginal discharge (amount, color, or consistency)
  - Vague sense of discomfort characterized as "not feeling right"
- Have a gestational age between 24 weeks, 0 days and 34 weeks, 6 days
- Have intact amniotic membranes
- Have minimal cervical dilatation (< 3 centimeters)

Relationship of Fetal Fibronectin to Delivery Endpoints

The safety and effectiveness of the Adeza Biomedical Fetal Fibronectin Immunoassay was evaluated in a population of 763 pregnant patients with signs and symptoms commonly associated with threatened preterm delivery. The relationship of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay test result to the primary endpoint of delivery in $\leq$ 7 and $\leq$14 days is shown in **Table 1**. For delivery $\leq$ 7 days, the sensitivity, specificity, positive and negative predictive values of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay were 86.4%, 82.3%, 12.7%, and 99.5%, respectively. For delivery $\leq$ 14 days, the sensitivity, specificity, positive and negative predictive values of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay were 83.3%, 82.9%, 16.7%, and 99.2%, respectively.

Table 1

Sensitivity, Specificity, and Positive and Negative Predictive Values of Fetal Fibronectin for Delivery $\leq$ 7 and $\leq$ 14 Days (n=763)

Delivery	n (%)	Sensitivity (95% CI)	Specificity (95% CI)	Pred Val + (95% CI)	Pred Val - (95% CI)
$\leq$ 7 Days	22 (2.9%)	86.4% (66.4, 95.3)	82.3% (79.4, 84.9)	12.7% (4.2, 33.7)	99.5% (98.7, 99.8)
$\leq$ 14 Days	30 (3.9%)	83.3% (66.3, 93.7)	82.9% (80.0, 85.4)	16.7% (7.3, 33.7)	99.2% (98.3, 99.6)

*Proportion of deliveries at each endpoint are calculated using 763 as the denominator

Additional Clinical Data

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay test result was also related to other clinical features, including delivery in ≤ 36 completed weeks of gestation (preterm delivery) and neonatal status. The sensitivity, specificity, positive and negative predictive values of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay were 41.3%, 86.2%, 44.7%, and 84.5%, respectively, for delivery ≤ 36 weeks (162 or 21.2% of the 763 subjects delivered ≤ 36 weeks).

The relationship of the fetal fibronectin test result to neonatal status is shown in **Table 2.**

Table 2

Neonatal Well-Being Stratified by Fetal Fibronectin Test Result

		fFN +	fFN -	p-value
Total Subjects	n (%)	150 (19.7%)	613 (80.3%)	–
Infant Weight (grams)				
	Avg	2804.0	3242.8	0.00010
	SD	776.2	582.7	
	n	154	636	
	Range	625-4280	835-5800	
Infant Weight (grams)				
n (%)	<1500	11 (7.1%)	6 (0.9%)	0.00005
	<2500	57 (37.0%)	69 (10.8%)	<0.00001
Perinatal Morbidity	n (%)			
Respiratory Distress		18 (11.7%)	22 (3.5%)	0.0001
NICU Admission	n (%)	44 (28.6%)	68 (10.7%)	<0.000001
Neonatal Hospital Days		5.9±11.1	2.9±7.1	0.01

*Neonate information available for only 753 of the 763 subjects
The p-value is not computed on the basis of "Unknown" being a category

The number of subjects, distribution of positive fetal fibronectin test results, sensitivity, and predictive value of a positive test for delivery in ≤ 7 days from specimen collection is shown in **Table 3** for symptomatic pregnant women < 32 weeks and ≥ 32 weeks. The proportion of positive tests and the sensitivity of the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is the same for women before and after 32 weeks. The number of symptomatic women seeking care increases gradually with advancing gestational age as does the number of deliveries.

Table 3

Distribution of Subjects and Fetal Fibronectin Enzyme Immunoassay Results Before and After 31 Completed Weeks Gestation[a]

EGAS[a] (weeks)	Subjects n	fFN +[b] n (%)	Sensitivity (fFN+/Del≤7 Days) (%)	Pred Val + (Del≤7 Days/fFN+) (%)
< 32 Weeks	483	91 (18.8%)	8/9 (88.9%)	8/91 (8.8%)
≥ 32 Weeks	280	59 (21.0%)	11/13 (84.6%)	11/59 (18.6%)
TOTAL	763	150 (19.7%)	19/22 (86.4%)	19/150 (12.7%)

[a]Estimated Gestational Age at Specimen Collection
[b]fFN + = positive Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay result

The ability of other clinical factors to assess risk of preterm delivery were also evaluated in this prospective trial and compared to the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay. The sensitivity, specificity, positive predictive values, and negative predictive values and their 95% confidence intervals for delivery in ≤ 7 days for the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay, cervical dilatation, uterine activity, vaginal bleeding, and ascending genital tract infection (bacterial vaginosis) are provided in Table 4.

Table 4

Sensitivity, Specificity, Positive and Negative Predictive Values for Risk Factors

Risk Factor	n	Positive Test Defined[a]	Sensitivity (95% CI)[b]	Specificity (95% CI)	Pred Val + (95% CI)	Pred Val - (95% CI)
fFN Enzyme Immunoassay	763	≥ 0.05 μg/mL	86.4% (66.4%, 95.3%)	82.3% (79.4%, 84.9%)	12.7% (4.2%, 33.7%)	99.5% (98.7%, 99.8%)
Uterine Activity	750	≥ 4 cntx/hr	54.5% (34.5%, 73.1%)	75.3% (72.0%, 78.3%)	6.3% (1.4%, 24.3%)	98.2% (96.9%, 98.9%)
Cervical Dilatation	757	> 1 cm	38.1% (20.6%, 59.4%)	88.3% (85.8%, 90.4%)	8.5% (2.1%, 27.9%)	98.0% (96.7%, 98.8%)
Vaginal Bleeding	759	Any Bleeding	40.9% (23.0%, 61.6%)	85.2% (82.4%, 87.6%)	7.6% (1.9%, 26.3%)	98.0% (96.7%, 98.7%)
Ascend Gen Tract Inf	763	Bacterial Vaginosis	9.1% (2.5%, 27.8%)	84.1% (81.2%, 86.5%)	1.7% (0.1%, 17.6%)	97.3% (95.9%, 98.2%)

[a]Cutoff used to define a positive test result for determining sensitivity, etc.
[b]95% Confidence Interval (Lower Limit, Upper Limit)

CLINICAL SIGNIFICANCE OF FETAL FIBRONECTIN

Clinical experience with the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay has been limited to observational studies establishing the association between the test result and likelihood of delivery. As a result, no studies have been conducted to determine the therapeutic efficacy of using the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay in conjunction with other clinical information for threatened preterm delivery. In the absence of such studies, it is not possible to make recommendations regarding specific treatment options.

Management of Women with a Positive Fetal Fibronectin Test Result

Women with a positive fetal fibronectin test are at increased risk for delivery in ≤ 7 days, ≤ 14 days of specimen collection, and for preterm delivery in ≤ 36 completed weeks. Thus, a positive fetal fibronectin test enhances the ability of the clinician to predict preterm delivery in a population with equivocal presenting symptoms. Identification of women with elevated risk would allow for increased surveillance and triage of patients who are otherwise clinically

unremarkable. Increased surveillance and subsequent early identification of additional clinical symptoms would likely result in the earlier, efficacious management of treatable symptoms. Finally, and perhaps most important, a positive fetal fibronectin test is associated with adverse neonatal outcome, particularly respiratory distress syndrome. Thus, early identification of women at risk would be likely to improve delivery of corticosteroid therapy to progressively symptomatic patients who ultimately deliver a premature baby.

Management of Women with a Negative Fetal Fibronectin Test Result

The absence of fetal fibronectin in cervicovaginal secretions between 24 and 34 weeks gestation is associated with delivery at or near term. Symptomatic women with a negative fetal fibronectin test result have $< 1\%$ probability of delivering in ≤ 7 or ≤ 14 days from the time of specimen collection, and approximately a 15% chance of delivery in ≤ 36 completed weeks. Clearly, the majority of women with a negative fetal fibronectin test result who deliver prematurely, deliver after 34 completed weeks, when serious perinatal morbidity is unlikely though possible. More judicious use of tocolytic drugs lowers the probability of maternal and fetal toxicity associated with these medications and preserves their effectiveness for a time when they may be more critically needed. In addition, lowering delivery risk through lifestyle changes, e.g., bedrest or work restrictions, which can have significant social, economic, and emotional effects, may be less severe for symptomatic women with a negative test result. It is important to note, however, that symptomatic patients with a negative fetal fibronectin test are still at increased risk for prematurity, simply because they present for unscheduled care, thus increased patient education and surveillance are critical components of patient management.

MECHANISMS OF RELEASE OF FETAL FIBRONECTIN

The exact mechanisms underlying the onset of labor and delivery in humans are unknown, therefore, it is impossible to conclusively describe a mechanism by which fetal fibronectin appears in the vagina. Immunohistochemical studies have shown that fetal fibronectin is localized in the extracellular matrix of the maternal-fetal interface also known as the choriodecidual junction. The immunolocalization of fetal fibronectin in the placenta and amniotic sac, particularly in the lower uterine segment, suggests that fetal fibronectin may be extravasated or "leaked" into the vagina. Two possible pathways may lead to the appearance of fetal fibronectin in the vagina. In the first pathway, the mechanical stress caused by uterine contractions and cervical change leads to choriodecidual separation which, in turn, promotes loss of fetal fibronectin from the interface. Accumulating clinical evidence suggests the existence of a second pathway in which localized inflammation of the choriodecidual interface, resulting perhaps from occult ascending bacterial infiltration, promotes maternal host defense. If the infectious stimulus and maternal response are sufficiently powerful, the resulting inflammation could promote degradation of the choriodecidual extracellular matrix and weakening of the amniotic membranes. Such a process has been discussed in the literature and can be summarized as follows: 1) an ascending bacterial infiltration from the lower genital tract results in the recruitment of leukocytes to the decidua and membranes; 2) bacterial and leukocyte-derived proteases degrade decidual and chorionic extracellular matrix; 3) degradation of extracellular

matrix proteins results in the extravasation of fetal fibronectin into the vagina and, if the degradation is severe, premature rupture of the amniotic membranes occurs; 4) the same ongoing inflammatory process promotes localized release of prostaglandins and cytokines, resulting in cervical ripening and contractions (13-18). Thus, fetal fibronectin appearance in the vagina is likely attributable to various processes associated with choriodecidual separation and the onset of labor, regardless of the whether the stimulus is infectious or mechanical.

WHY THE SYMPTOMATIC PATIENT POPULATION IS RESTRICTED

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is intended to be used as an aid to assess risk of delivery for women with symptoms of preterm labor who have intact amniotic membranes and minimal cervical dilatation (<3 centimeters). The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay should be used in conjunction with all other clinical information to assess risk of delivery, e.g., uterine contractions, cervical dilatation, ascending genital tract infection, vaginal bleeding, etc.

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is not intended to be used for symptomatic women who have advanced cervical dilatation (≥3 centimeters), rupture of amniotic membranes, cervical cerclage, or visual evidence of moderate or gross vaginal bleeding. Delivery typically occurs imminently when cervical dilatation exceeds 3 centimeters or if the amniotic membranes are ruptured. Therefore, additional diagnostic testing is usually not necessary to confirm risk for women with advanced cervical dilatation or rupture of amniotic membranes. Moderate or gross vaginal bleeding is an independent risk factor for preterm delivery and may be associated with other severe obstetrical or medical problems. Clinical attention should be focused on identification of the origin of bleeding rather than on immediate assessment of delivery risk. Currently, there is insufficient information characterizing the association of cervicovaginal fetal fibronectin expression to delivery for women with cervical cerclage.

CAN FETAL FIBRONECTIN ALONE BE USED TO IDENTIFY RISK OF DELIVERY?

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is an objective test that can be used as an aid in the prediction of delivery in ≤ 7 or ≤ 14 days of specimen collection. Detection of fetal fibronectin in cervicovaginal secretions should not be interpreted alone to assess risk of imminent delivery. The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay should be used in conjunction with other clinical tests and information to assess overall risk of preterm delivery and assure appropriate patient management.

SPECIMEN COLLECTION

The specimen should be obtained from the posterior fornix of the vagina during a sterile speculum examination. *The Adeza Biomedical Specimen Collection Kit is the only acceptable specimen collection system which can be used to collect specimens for this assay.* The Dacron® swab provided in the Specimen Collection Kit should be inserted into the vagina and lightly rotated across the posterior fornix for approximately 10 seconds to absorb the cervicovaginal secretions. Once the specimen is obtained, carefully remove the swab from the vagina and place it into the tube of buffer provided with the Specimen Collection Kit. Use only one Specimen Collection Kit per patient. Label the Specimen Transport Tube with the patient's name and any other identifying information required.

To safely interpret the Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay test result, **the sample must be collected before performance of any other activities or procedures which might disrupt the cervix**, e.g., coitus, digital cervical examination, vaginal ultrasound, microbiologic culture of cervical secretions, or pap smear. Finally, the test result is invalid if the swab is contaminated by lubricants, soaps, or disinfectants, e.g., K-Y® Jelly, Betadine®, hexachlorophene. The method of collection is also described in the directional insert for the Adeza Biomedical Specimen Collection Kit.

PRINCIPLE OF THE FETAL FIBRONECTIN ENZYME IMMUNOASSAY

The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay is a solid-phase enzyme-linked immunosorbent assay (ELISA). During the assay, cervicovaginal samples are incubated in microtiter wells coated with FDC-6, a monoclonal antibody specific for fetal fibronectin (5-7). The resulting antibody-antigen complex is washed to remove non-specifically bound material and then reacted with an enzyme-labeled antibody directed against human fibronectin. Following formation of the antigen-antibody "sandwich", the microtiter well is washed to remove unbound labeled antibody and then incubated with an enzyme substrate. The presence or absence of fetal fibronectin in the specimen is determined spectrophotometrically at a wavelength of 550 nanometers.

DEFINITION OF A POSITIVE TEST

Patient specimens having an absorbance greater than or equal to the absorbance of the Positive Reference (containing ≥ 0.050 μg/ml fFN) are defined as positive for the presence of fetal fibronectin. Patient specimens having an absorbance less than the absorbance of the Positive Reference are defined as negative for the presence of fetal fibronectin.

LIMITATIONS

The Fetal Fibronectin Enzyme Immunoassay result should not be interpreted as absolute evidence for the presence or absence of a process that will result in delivery in $\leq$ 7 days from specimen collection. A positive Fetal Fibronectin Enzyme Immunoassay result may be observed for

patients who have experienced cervical disruption caused by, but not limited to, events such as sexual intercourse, digital cervical examination, or vaginal probe ultrasound. The Fetal Fibronectin Enzyme Immunoassay result should always be used in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures such as cervical examination, cervical microbiological culture, assessment of uterine activity, and evaluation of other risk factors.

- Modification of the assay protocol described herein may yield erroneous results.
- The assay has been optimized with specimens taken from the posterior fornix. Samples obtained from other locations should not be used.
- The safety and effectiveness of using a cutoff other than that provided by the Positive Reference (0.050 μg/ml) has not been established.
- There are no prospective clinical trials to support use of the Fetal Fibronectin Enzyme Immunoassay as a method for screening asymptomatic pregnant patients for risk of preterm delivery.
- Assay interference from the following components has not been ruled out: douches, white blood cells, red blood cells, bacteria and bilirubin.
- The presence of infections has not been ruled out as a confounding factor to risk of preterm delivery.
- Test results are invalid if the specimen contains semen or if the specimen was collected less than 24 hours after coitus. Two studies were conducted establishing that intercourse and presence of semen may lead to positive test results at the cutoff of 0.05 μg fFN/mL. In the first study, fetal fibronectin was detected in 23% of post-coital vaginal specimens obtained from 22 non-pregnant women. In the second study, fetal fibronectin was detected in 21 of 41 sperm samples obtained from healthy male volunteers. These results suggest that sperm (or semen) may contain sufficient concentration of fetal fibronectin to result in a positive fetal fibronectin test result.

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The Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay, the fetal fibronectin epitope and all related applications to pregnancy are patented and/or patent pending.

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