Actim™PROM, AmniSure®, and ROM +plus®: Rupture of membrane kits tested on amniotic fluid from women at C-section: a comparative study

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Abstract

Objectives: To confirm three point of care commercial tests for rupture of membranes consistently and accurately detect amniotic fluids from pregnant women, and they are practical and easy to use.

Methods: Samples of fresh amniotic fluid were collected in a syringe taken at time of C-section and run on kits (Actim™PROM, AmniSure®, and ROM +plus®) from three commercially available point of care rupture of membrane tests. Samples were sent to an independent laboratory for ELISA analysis of these amniotic fluid proteins (insulin-like growth factor binding protein (IGFBP-1) and alpha-Fetal protein (AFP). Test results of known amniotic fluid on Actim™PROM, AmniSure®, and ROM +plus®, along with ease of use of comments. False negatives were used to determine accuracy of tests.

Results: Correct results were obtained in 96.8%, 95.8%, and 98.9% respectively for Actim™PROM, AmniSure®, and ROM +plus®. Users reported ROM +plus® was easiest to use.

Conclusions: ROM +plus®. provided the least amount of false negative testing on known samples of amniotic fluid. ROM +plus® was considered most simple and easiest to use and detects two different amniotic fluid proteins while the other tests detect one protein.

Key words: amniotic fluid, rupture of membranes, C-section, Actim™PROM, AmniSure®, ROM +plus®, IGFBP-1, PP12, AFP.

Introduction

Premature rupture of membranes (PROM), defined as spontaneous rupture of membranes (ROM) before the onset of uterine contractions, is one of the most common diagnostic dilemmas in contemporary obstetric practice. Premature rupture of membranes can occur at any gestational age, and preterm PROM (PPROM, defined as PROM before 37 weeks) is responsible for 20-40% of preterm births[1]. Early and accurate diagnosis of PROM would allow for gestational age-specific obstetric interventions designed to optimize perinatal outcome and minimize serious complications such as cord prolapse, preterm delivery, fetal distress and infectious morbidity (chorioamnionitis, neonatal sepsis)[2,3]. A false-negative result could lead to misdiagnosis and potential fetal demise. Conversely, a false-positive diagnosis of PROM may lead to unnecessary obstetric interventions, including hospitalization, administration of antibiotics and corticosteroids, and even induction of labour. Therefore, the correct and timely diagnosis of this disorder is of critical importance to the clinician because PROM and PPROM may be associated with serious maternal and neonatal consequences.

The diagnosis of fetal membrane rupture is conventionally made by clinical vaginal examination to observe whether amniotic fluid is leaking from the cervical os. If leaking is absent, subsequent measures to reach a diagnosis include visual inspection of the posterior vaginal fornix for pooling of amniotic fluid, nitrazine/pH testing of the vaginal fluid and microscopic examination of the fluid for the presence of 'ferning'[4,5]. Immunoassay tests have become more widely accepted in clinical practice but questions still remain about the specificity and sensitivity of these tests since there is no reliable gold standard to which they may be compared. These immunoassay tests depend on the detection of specific amniotic fluid proteins in vaginal secretions at specified levels that may indicate the membranes have ruptured.

The present study was designed to assess the accuracy of three commercially available tests to detect specific proteins found uniquely in amniotic fluid. Actim™ Prom (Oy Medix Biochemica Ab Kauniainen, Finland), AmniSure® (AmniSure Intl. LLC/Qiagen, 24 School Street, 6th floor, Boston, MA 02108), and ROM+plus® (Clinical Innovations LLC 747 West 4170 South, Murray, Utah 84123 USA) were used for the comparative studies. These tests are all rapid, point-of-care, qualitative, immunochromatographic tests developed for the detection of amniotic fluid in cervico-vaginal secretions of women with suspected rupture of the membranes. Actim™ Prom detects the protein IGFBP-1, AmniSure® detects the protein PAMG-1 and ROM+plus® detects a combination of proteins IGFBP-1(also known as Placental Protein 12=PP-12) and AFP (alpha-

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fetoprotein). There is widespread agreement that IGFBP-1 and PAMG-1 are essentially the same protein molecule.[5-7] Significant concentrations of these proteins are present in amniotic fluid of pregnant women in all trimesters of pregnancy and are used as specific markers for the diagnosis of rupture of the membranes[8-10].

The main objective of the study was to assess and compare the capability of the three test kits to consistently and accurately diagnose rupture of the membranes (ROM) in samples of known amniotic fluid. In addition, information was sought regarding which test was preferred by the clinicians in terms of ease of use and convenience.

Methods

Inclusion criteria for the study were healthy pregnant women 16 years and older in which a sample of known amniotic fluid not contaminated with blood was collected during caesarean delivery. Samples that were contaminated with blood were repeated and excluded from the study. Careful collection technique was used to obtain a sample of clear amniotic fluid using a sterile 50cc syringe inserted into the opened amniotic sac at cesarean section. The clinicians who performed the tests were practicing labour and delivery staff members who normally assessed patients for possible ROM. They were all familiar with the methods of performing the tests according to the manufacturers' instructions.

The specimens were individually assessed for positive or negative results using all three test kits and the results recorded on a data form along with a comment survey concerning test kit simplicity, ease of use, and kit preference. Photographs of the three kit results were taken for all testings. Photographs A and B show typical results for specimens taken and tested at the time of sampling. In addition to the actual amniotic fluid (AF) sample testing, particular attention was paid to kit storage and positive and negative control testing.

Following point-of-care testing, each sample was placed in a stabilized preservative tube, refrigerated and subsequently sent to a laboratory for enzyme-linked immunosorbent assay (ELISA) (R and D Systems, 614 McKinley Place NE Minneapolis, MN 55413 USA) which is commercially available with a sensitivity of 31.2 pg/ml for IGFBP-1 and 46 pg/ml for AFP with interassay coefficient of variation of < 10%. The study was approved by the Hospital's Institutional Review Board, and approved for collection and use of the amniotic fluid samples and information for research purposes. Informed consent (and a detailed history) was obtained from all women prior to the caesarean section.

Statistical method and findings

The aim of the statistical analysis was two fold. First, to demonstrate the wide variability of concentrations of proteins used by diagnostic tests to determine rupture of membrane (ROM), using uncontaminated samples of amniotic fluid obtained directly from the placenta during c-section deliveries. Protein concentrations were determined by the ELISA method. Second, compute the sensitivity of three commerical diagnostic tests to detect amniotic fluid in these samples. These tests were Actim™Prom, AmniSureR and ROM+plus®. Since all samples were actual amniotic fluid, there should be no negative test results, so all negative results represent false negative conclusions. The assessment of specificity was not possible with this study design, since no samples of fluid other than amniotic fluid was used in the assessment. The standard definition of sensitivity was used, and two-sided 95% confidence intervals for sensitivity were calculated using the Wilson score method. The sensitivity was statistically compared between the three diagnostic tests, two at a time, using a paired sample McNemar test, with no adjustment for multiple comparisons.

Results

During the period from October 2011 to May 2012, 95 women from whom clear amniotic fluid samples were obtained were enrolled into the study. The gestational ages of the pregnancies ranged from 16 to 42 weeks.

Table 1 displays the results of the testing of the amniotic fluids using all three test kits together with the corresponding ELISA test result for each sample. Negative readings were recorded in three cases using the ActimTMProm test kit, in four cases using the AmniSure® kit and in one using the ROM+plus® kit. There were no equivocal or invalid results recorded for any of the kits. The sample and data for patient 47 was lost and therefore was removed from the study.

The results demonstrate a sensitivity for ActimTMProm of 96.8% (95% confidence interval, 91.0% to 98.9%), for AmniSure® of 95.7% (95%CI, 89.6% to 98.3%), and for ROM+plus® of 98.9% (95%CI, 94.2% to 99.8%) (Table 2).

Concentrations of the samples from the ELISA testing showed IGFPB-1/PAMG-1 levels in the range of 9 to 23688 ng/ml and an AFP range of >10 to 39276 ng/ml. The threshold for the lower limit of detection for the test kits are, respectively, Actim™ Prom = IGFBP-1 25 ng/ml, AmniSure® = PAMG-1 5 ng/ml and ROM+plus® = IGFBP-1 5 ng/ml, AFP 150 ng/ml. Thus, all amniotic fluid samples tested were above the lower threshold limit except for one (No 75 in Table 1) tested with the Actim™Prom kit. Furthermore, the ELISA results showed that the protein concentration levels in the fluid samples were all below the upper limits given for the three test kits.

The threshold for lower limit of detection for the kits are as follows: Actim™PROM =25ng/ml IGFBP-1, AmniSure® =5 ng/ml PAMG-1, ROM +plus® = 5ng/ml IGFBP-1,and 150 ng/ml AFP. As shown in Table 1 and 2, all amniotic fluid samples as analyzed by ELISA were above the threshold except for one sample for

Table 1. Results of actim™PROM, AmniSure®, ROM +plus®, and ELISA on the amniotic fluid samples

Amniotic Fluid Sample ID	actim™PROM	AmniSure®	ROM +plus®	PP12	AFP
1	+	+	+	1710	1636
				9	
2	+	+	+	287	1002
3	+	+	+	682	486
4	+	+	+	1618	2429
				4	
5	+	+	+	2039	1380
				5	
6	+	+	+	1931	1583
				6	
7	+	+	+	1526	75
				0	
8	+	+	+	433	469
9	+	+	+	1393	382
				1	
10	+	+	+	1499	847
				5	
11	**+**	**-**	**+**	**1018**	**258**
12	+	+	+	1313	956
				2	
13	+	+	+	203	1623
14	+	+	+	1470	1583
				5	
15	+	+	+	4574	1324
16	+	+	+	2131	1927
				4	
17	+	+	+	2205	719
				8	
18	+	+	+	2153	364
				9	
19	+	+	+	1632	1281
				2	
20	+	+	+	837	1380
21	+	+	+	1569	815
				1	
22	+	+	+	1829	1662
				7	
23	+	+	+	2365	2096
				4	
24	+	+	+	212	940
25	+	+	+	308	130
26	+	+	+	646	469
27	+	+	+	1847	1287
				5	6
28	+	+	+	540	311
29	+	+	+	256	2155
30	+	+	+	671	1032
31	+	+	+	9	1583
32	+	+	+	4974	112
33	+	+	+	4277	670
34	+	+	+	444	894
35	+	+	+	269	486
36	+	+	+	1558	1122
				3	
37	+	+	+	226	1002
38	+	+	+	1607	434
				4	
39	+	+	+	4545	1062
40	+	+	+	1465	1077
				3	
41	+	+	+	544	3585
42	+	+	+	2105	971
				8	
43	**-**	**+**	**+**	**450**	**1610**
44	+	+	+	220	185
45	+	+	+	1013	1017
46	+	+	+	406	799
48	+	+	+	699	1393
49	+	+	+	143	1352
50	**+**	**-**	**+**	**12**	**719**
51	+	+	+	1483	1570
				6	
52	+	+	+	1817	2166
				9	
53	+	+	+	199	1136
54	+	+	+	1922	1421
				4	
55	+	+	+	461	1778
56	+	+	+	2278	503
				2	
57	+	+	+	560	2259
58	+	+	+	1607	1309
				4	
59	**+**	**-**	**+**	**90**	**<10**
60	+	+	+	395	894
61	+	+	+	1398	554
				2	
62	+	+	+	523	2000
63	**+**	**+**	**-**	**2368**	**<10**
				8	
64	+	+	+	272	986
65	+	+	+	1436	554
				7	
66	+	+	+	4648	637
67	+	+	+	510	1380
68	+	+	+	90	240
69	+	+	+	1705	1421
				2	
70	+	+	+	798	<10
71	+	+	+	547	1281
72	+	+	+	4806	986
73	**-**	**-**	**+**	**807**	**799**
74	+	+	+	4829	486
75	**-**	**+**	**+**	**9**	**294**
76	+	+	+	843	347
				9	
77	+	+	+	1337	130
				9	
78	+	+	+	1367	1092
				9	
79	+	+	+	1989	1267
				8	
80	+	+	+	1859	18
				4	
81	+	+	+	4263	185
82	+	+	+	1999	1309
				0	
83	+	+	+	4619	<10
84	+	+	+	1385	537
				5	
85	+	+	+	2288	<10
				2	
86	+	+	+	2355	<10
				3	
87	+	+	+	2173	1421
				2	
88	+	+	+	336	<10
89	+	+	+	120	1092
90	+	+	+	931	3927
					6
91	+	+	+	691	2188
					0
92	+	+	+	2248	8093
				4	
93	+	+	+	1018	4696
94	+	+	+	4998	3345
95	+	+	+	325	1952

Summary: False negatives:

actim™PROM = 3 AmniSure® = 4
ROM +plus® = 1

Table 2. Summary of false negative diagnostic test results (negative for ROM) for all amniotic fluid samples where at least one test had a false negative result (out of N=94 samples tested)

Test (Lower Threshold of Test) X denotes false negative result			*ELISA Determined Concentration*		
Actim™PROM (IGFBP-1 25 ng/ml)	AmniSure® (PAMG-1 5 ng/ml)	ROM+plus® (IGFBP-1 5 ng/ml or AFP 150 ng/ml)	PP-12* ng/ml	AFP** ng/ml	Amniotic Fluid Sample Identication Number
X			9	294	75
	X		12	719	50
	X		90	<10	59
X			450	1610	43
X	X		807	799	73
	X		1018	258	11
		X	23688	<10	63

*PP-12 = protein IGFBP01, also known as Placental Protein 12. PAMG-1 and IGFBP01 widely agreed to be essentially the same protein.

**AFP = alpha-fetoprotein

Table 3. Sensitivity of diagnostic tests to detect aminotic fluid removed directly from the uterus during c-section delivery (N=94 samples)

Test	*Sensitivity (%)*	*95% confidence interval**
Actim™PROM	96.8 (91/94)	91.0 - 98.9
AmniSure®	95.7 (90/94)	89.6 - 98.3
ROM plus®	98.9 (93/94)	94.2 - 99.8

Sensitivity comparisons using paired sample McNemar test

Actim™PROM versus AmniSure®, p > 0.99

Actim™PROM versus ROM+plus®, p = 0.62

AmniSure® versus ROM+plus®, p = 0.38

*Two-sided 95% confidence interval for sensitivity calculated using the Wilson score method

the Actim™PROM test. AmniSure® test kits resulted in the most false negatives, with Actim™ PROM resulting in the next most false negatives and ROM +plus® resulted in the least with only one false negative.

The wide variability of proteins in amniotic fluid is presented in frequency table format in Figures 1 and 2. When the proteins are this variable in amniotic fluid in the placenta, after which the variability is increased in the vagina after ROM due to dilution, contamination, and absorption, this initial variability adds to the difficulty of designing a diagnostic test to detect ROM.

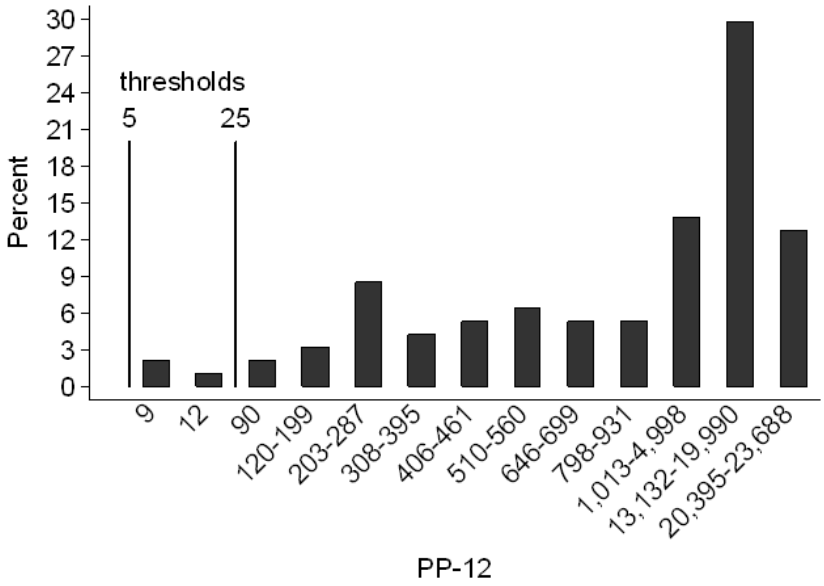
According to the comments submitted on the data collection form by the participants the ROM+plus® test kit was considered the most convenient and easiest to use and was the preferred kit by all participants due to the shorter collection and preparation time, easier dispensing mechanism of the cassette platform, non-spill buffer vial design and because of the integrated timer.

Discussion

Clinical diagnosis of PROM and PPROM can be difficult or inconclusive in pregnant women who have contradictory signs or symptoms of rupture of the membranes, especially when the gestation is less than 34 weeks[11]. Except when rupture of the membranes is clinically overt (i.e. amniotic fluid is observed escaping directly from the cervical os), biochemical markers are required to help confirm or exclude PROM or PPROM.

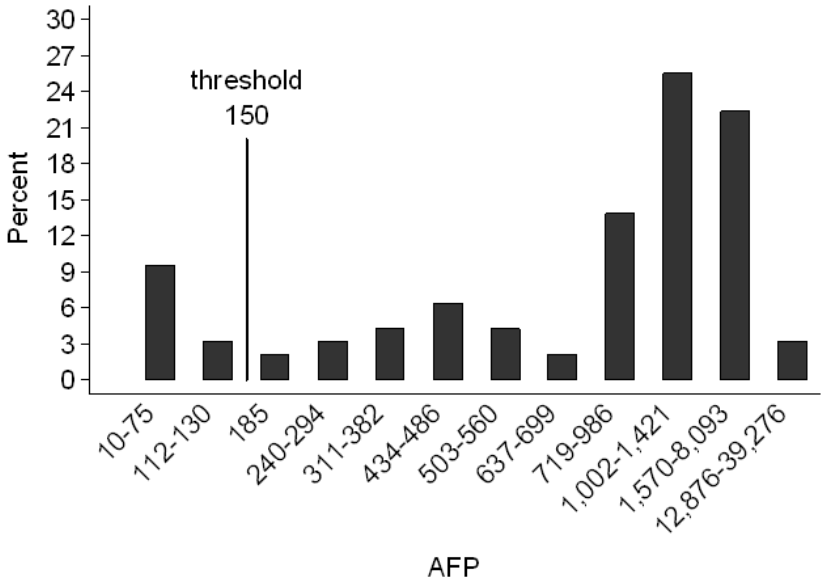
The purpose of this study was to assess the efficacy of three commercially available point-of-care immunoassay tests to show a positive result when known amniotic fluid was used. Given that the test samples were all fresh amniotic fluid collected at cesarean section, the expectation was that all three immunoassay tests would have shown a positive reading for the protein in every case. However, in the present study false negative results were recorded with each of the commercial kits tested. Similarly, it

Figure 1. **Histogram of PP-12 in amniotic fluid removed directly from placenta during c-section delivery. Vertical lines are threshold values where the diagnostic tests return a positive rupture of membranes determination.**



Photographs of test results for amniotic fluid samples 62 and 11.

Figure 2. **Histogram of AFP in amniotic fluid removed directly from placenta during c-section delivery. Vertical line is threshold value where the diagnostic test return a positive rupture of membranes determination, while simultaneously considering value of PP-12.**



has been reported elsewhere that false positive test results may occur due to the passage of small quantities of amniotic fluid into the vagina through microperforations in the membrane wall[12] or from derivatives of the amniotic proteins produced by decidual cells[13]. With this in mind, the clinician must be aware of the limitations of immunoassay tests when interpreting the results and arrive at a diagnosis as to whether the membranes are ruptured or intact based on an assessment of all relevant clinical findings and not solely on the result of the immunoassay test.

There have been reports of negative AmniSure® in cases of clinical ROM (false negative results)[14]. It would be important to determine the incidence of failure of these tests to show positive results when known amniotic fluid is being detected. ROM +plus® demonstrated the least false negative rate. An explanation for these results could be the fact that ROM +plus® test uses monoclonal and polyclonal antibodies while both actim™PROM, and AmniSure® use only monoclonal antibodies. Polyclonal antibody assays are more sensitive because they can cover the surface of a complex antigen protein more uniformly, thus improving the detection capability. The more binding sites available when the protein flows through the device the better chance that the labeled protein will have something to bind to, showing a more visible line. In addition, monoclonal antibodies can bind to only one type of epitope on the surface of the protein, increasing the possibly of reducing the level of coating. The potential then exists to give up a certain level of sensitivity, and when too much protein is available, the possibility of a hook (prozone) effect increases. Furthermore, the ROM +plus® detects a second protein, AFP, which may also increase the sensitivity of the test to show a positive line on the test kit.

Conclusion

Amniotic fluid from 94 c-section patients were tested on three different immunoassay tests for the detection of rupture of membranes in order to evaluate the rate of false negatives from the testing. Actim™PROM, AmniSure®, and ROM +plus® are available commercially for detection of membrane rupture. ROM +plus®. Which uses both monoclonal and polyclonal antibodies and two protein detection instead of one proved to be the most accurate and provided the least amount of false negative testing on known samples of amniotic fluid. ROM +plus® was also rated most simple and easiest to use and detects two different amniotic fluid proteins.

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