Analytical and operational evaluation of the ROM-Plus test for rupture of fetal membranes

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Abstract

Background: ROM-Plus (Clinical Innovations) is a recently approved test for rupture of fetal membranes, intended for use at point-of-care. The test is a lateral flow sandwich immunoassay for detection of either placental protein 12 (PP12, or insulin-like growth factor binding protein-1, IGFBP-1) or alpha-fetoprotein (AFP) in vaginal pool fluid, as markers for presence of amniotic fluid. We investigated analytical and operational characteristics of the assay: sensitivity for detection of controls, stability of controls, dilution factor of swab samples, and titre of near-term amniotic fluid and of biological fluids other than amniotic fluid.

Methods: Operation of the assay was according to manufacturer's instructions, using either direct application (DA) of fluids to the application point (as for controls) or by application after swab transfer (ST) to diluent (as for samples), as noted. Controls are provided in sealed glass ampules within pliable plastic holders. For positive control, release of solvent by breakage of the ampule subsequently dissolves lyophilized protein within the holder. The holder is also a dropper device for direct application of fluid to the application point. For vaginal pool samples, a plastic holder with diluent is provided wherein a swab sample is placed for elution of sample from the swab. After score-point breakage of the tip which then remains in the diluent, the holder has an attached dropper cap wherewith sample is applied. A dye-diffusion timer on the device is activated by finger. Samples are to be read as positive or negative by appearance of a line at the test position not more than 20 min after sample application.

Results: Mass-carrying capability of swab for 7 g/dL albumin solution was on average 79±13 µL (n = 6); given diluent volume of 380 µL, this indicated an average minimum dilution for ST samples of 18%. Positive control (stated concentrations [AFP] = 600 ng/mL, PP12 = 20 ng/mL) was positive (DA) to 1:30 dilution, consistent with stated device analytical sensitivity ([AFP] = 150 ng/mL, PP12 = 5 ng/mL) for ST when accounting for ST dilution. Control 1:8 titre remained positive (DA) after 10 days storage either refrigerated or frozen. By ST, near-term pregnancy pooled, previously frozen amniotic fluid (submitted for fetal lung maturity testing) was positive to titre less than 1:3000. EDTA-whole blood samples from males, non-pregnant females (<36 years of age), and near-term pregnant females were all positives by ST. Pooled plasma specimens for near-term pregnant females were positive by ST to titre between 1:3 and 1:10. Urine from near-term pregnancy was negative (ST).

Conclusions: Positive ROM-Plus ST results for samples other than amniotic fluid are likely due to high test sensitivity for IGFBP. Compared to amniotic fluid, near-term pregnancy plasma samples were positive by ST application only at low titre (>1:10). Thus, barring bloody samples, a test positive by ST is likely the result of the presence of amniotic fluid, in accordance with premise and intent of the assay. Analytical performance results verified manufacturer's FDA-approval studies. The operational design of the ROM-Plus assay was judged to be highly suitable for use as point-of-care testing.

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Introduction

ROM-Plus (Clinical Innovations) is a recently FDA-approved test for rupture of fetal membranes, intended for operation at point-of-care [1].

The test is a lateral flow sandwich immunoassay for detection of either placental protein 12 (PP12, or insulin-like growth factor binding protein 1, IGFBP-1) or alpha-fetoprotein (AFP) in vaginal pool fluid, as markers for presence of amniotic fluid. Both AFP and IGFBP-1 are expected to be decreasing in near-term AF [2, 3]. Nonetheless, both analytes are expected to be 10- to 1000-fold more concentrated in AF than in plasma at any advanced stage of pregnancy [1].

We investigated analytical and operational characteristics of the ROM-Plus assay: sensitivity for detection of controls, stability of controls, dilution factor of swab samples, and titre of near-term amniotic fluid and in biological fluids other than amniotic fluid.

Methods

Operation of the assay was according to manufacturer's instructions, using either direct application (DA) of fluids to the application point (as for controls) or by application after swab transfer (ST) to diluent (as for samples), as noted.

Controls are provided in sealed glass ampules within pliable plastic holders. For positive control, release of solvent by breakage of the ampule subsequently dissolves lyophilized protein within the holder. The holder is also a dropper device for direct application of fluid to the application point.

For vaginal pool samples, a plastic holder with diluent is provided wherein a swab sample is placed for elution of sample from the swab. After score-point breakage of the tip which then remains in the diluent, the holder has an attached dropper cap wherewith sample is applied.

A dye-diffusion timer on the device is activated by finger.
Samples are to be read as positive or negative by appearance of a line at the test position not more than 20 min after sample application.

Results

Extent of sample dilution by swab transfer

Mass-carrying capability of swab for 7 g/dL albumin solution was on average 79±13 mL (n=6); given diluent volume of 380 mL, this indicated an average minimum dilution for ST samples of 18%.

Titre of positive control

Positive control (stated concentrations [AFP]=600 ng/mL, PP12=20 ng/mL) was positive to 1:30 dilution by direct application (DA), consistent with stated device analytical sensitivity ([AFP]=150 ng/mL, PP12=5 ng/mL) for swab transfer (ST) when accounting for ST dilution. (**Figure 1**)

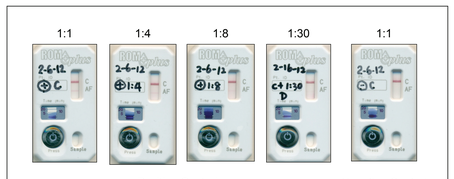


Figure 1. Controls

Results (cont'd)

Stability of control after dissolution

After dissolution, control 1:8 titre remained positive (DA) after 10 days storage either refrigerated or frozen. (**Figure 2**)

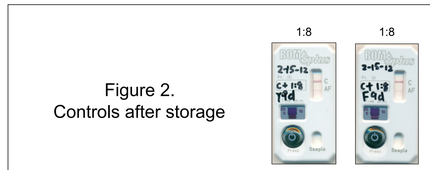


Figure 2.
Controls after storage

Titre of near-term pooled amniotic fluid

By ST, near-term pregnancy pooled, previously frozen amniotic fluid (submitted for fetal lung maturity testing) was positive to titre less than 1:3000. (**Figure 3**)

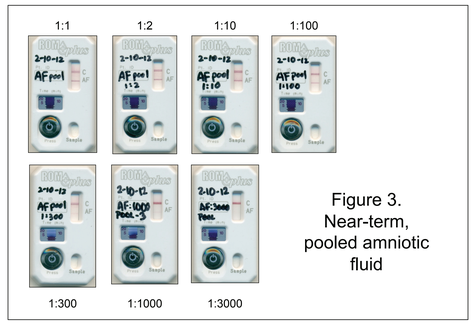
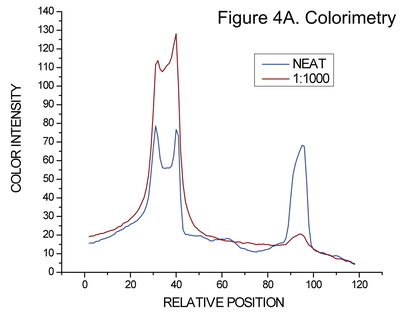


Figure 3.
Near-term, pooled amniotic fluid

Colorimetry

Although the test is intended solely as a qualitative (binary) test, a colorimetric dose-response curve for the amniotic fluid test results may be observed by image analysis (**Figure 4, A**). For the pooled amniotic fluid samples, the dose-reponse curve across dilution was more steep than a pure hyperbolic function (response = dilution/(dilution + *K*)) that would characterize a single saturable ligand-receptor (antigen-antibody) interaction (**Figure 4, B**).



Results (cont'd)

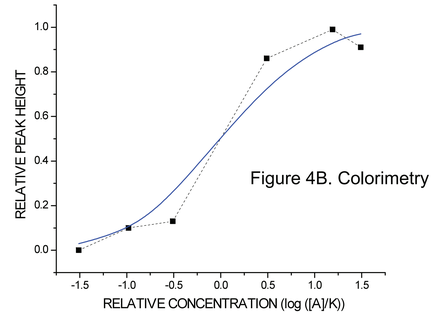


Figure 4B. Colorimetry

Positivity of samples other than amniotic fluid

EDTA-whole blood samples from males, non-pregnant females (<36 years of age), and first-trimester pregnant females were all positives by ST. (**Figure 5**)

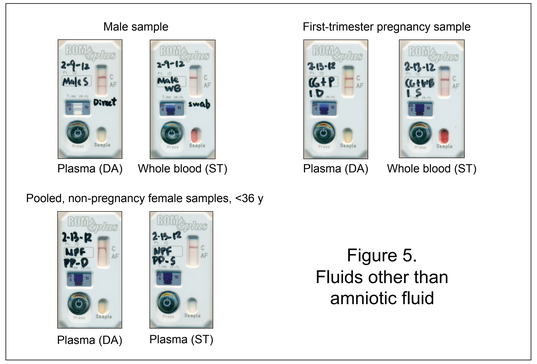


Figure 5.
Fluids other than amniotic fluid

Near-labor whole blood, pooled plasma

Whole blood for near-labor pregnant subjects was positive by ST. Pooled plasma specimens for near-labor pregnant subjects were positive by ST to titre between 1:3 and 1:10. (**Figure 6**)

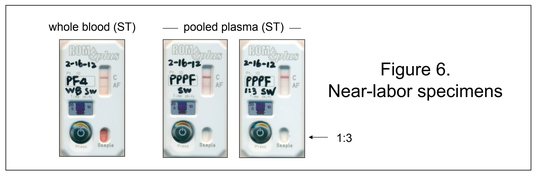


Figure 6.
Near-labor specimens

Urine from near-term females

Urine from near-term pregnancy was negative by ST. (**Figure 7**)

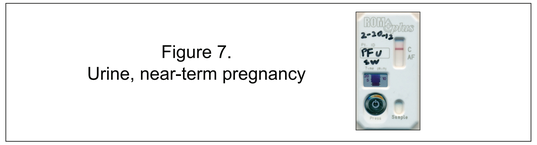


Figure 7.
Urine, near-term pregnancy

Results (cont'd)

Commercial bovine serum albumin solution (22%, Immunocor) was positive at titre 1:3. (**Figure 8**)

Figure 8.
Commercial BSA (7%), (DA)

Visibility of control lane on test units prior to use

It was noted that the control lane is clearly visible on unused test units. Kit instructions criterion for a successful test use refers only to appearance of a line. Users must recognize that the appropriate criterion would be appearance of a red line. (**Figure 9**)

Figure 9.

Control line, unused, compared to test positive

Conclusions

Positive ROM-Plus ST results for samples other than amniotic fluid are likely due to high test sensitivity for IGFBP. Specifically, the variety of test-positive samples were unlikely to be positive due to AFP.

Compared to amniotic fluid, near-term pregnancy plasma samples were positive by ST application only at low titre (>1:10). Barring bloody samples, or recent copulation, a test positive by ST is likely the result of the presence of amniotic fluid, in accordance with premise and intent of the assay.

Overall, analytical performance results verified data in manufacturer's kit insert.

The operational design of the ROM-Plus assay was judged to be highly suitable for its use as POCT. Specifically, the kit is self contained; there is no need for refrigerated storage, pipettors or timers; the device is stable on a table surface without holder.

References

[1] Kit insert, ROM-Plus, Clinical Innovations, Salt Lake City, UT.

[2] Lau HL, Linkins SE. Alpha-fetoprotein. Am J Obstet Gynecol 1976;124(5):533-54.

[3] Rutanen EM, Bohn H, Seppala M. Radioimmunoassay of placental protein 12: levels in amniotic fluid, cord blood, and serum of healthy adults, pregnant women, and patients with trophoblastic disease. Am J Obstet Gynecol 1982;144(4):460-3.