Accuwik® Ultra Membrane for the Direct Collection, Storage, and Efficient Rapid Release of Biological Samples for Clinical Diagnostics

PALL Life Sciences

Galina Fomovska, Stephane Perreault, Richmond Lo, Martin A. Smith. Pall Corporation, Port Washington, NY

Abstract

Objectives: Assess Accuwik Ultra membrane as a tool for direct sample collection, storage, and diagnostic analysis of protein composition and clinical biomarkers from biological samples such as saliva and plasma. **Materials and Methods:** Analytes: The quality and diagnostic utility of samples collected and recovered by Accuwik Ultra membrane has been evaluated by measuring clinically relevant analytes in saliva samples: alpha-amylase activity using the enzyme kinetic reaction kit (Salimetrics LLC, State College, PA), and protein profiles by 4-12% linear gradient SDS PAGE using IgG and human albumin as controls; in plasma samples: Troponin I by sandwich ELISA kit (Life Diagnostics, Inc, West Chester, PA) and 2DE profile after depletion of abundant proteins. Saliva sample collection: Samples were collected in 50 mL conical tubes by passive drool. Each sample was applied to a 25 mm disc of Accuwik Ultra by three different techniques. The first method simulated a sample collection from a patient's mouth followed by analysis in the lab on the same day of collection. Accuwik Ultra discs were soaked in the saliva samples, placed into Pall Forensic Nanosep® centrifugal devices, and stored on the bench for 0.5-1 hour. The second technique simulated sample collection from a patient's mouth in a physician's office where overnight shipment to an analytical lab is necessary. Accuwik Ultra discs were soaked in the saliva samples and stored on the bench at room temperature for 24 hours. The third collection protocol simulated conditions where samples are dried and stored long term. Saliva was applied to Accuwik Ultra discs and dried at 37 °C for 1 hour. After storage at room temperature for 3 days, samples were reconstituted by applying 200 µL of water to the disc for 10 minutes. After collection and storage, all saliva samples were recovered from the Accuwik Ultra discs by centrifugation in an Eppendorf* centrifuge at 13,000 rpm for 7 minutes and frozen at -20 °C prior to analysis. Saliva samples were collected by passive drool and stored under the same conditions for both the controls and Accuwik Ultra discs. **Results:** Protein profiles generated by 2DE of plasma samples collected by Accuwik Ultra were statistically identical to that of centrifuged plasma. Alpha-amylase activity measured in the saliva samples after 1 hour, 24 hours, and three days of room temperature storage on Accuwik Ultra was 0.29+/-0.031, 0.29+/-0.042, and 0.21+/-0.034 nKat/L respectively (n = 9); the values are statistically the same as the enzyme activity found in control samples 0.29+/-0.025 (1 hour control, n = 3) and 0.28+/-0.015 (24 hours control, n = 3). Analysis of IgA and albumin bands on 1D SDS-PAGE electrophoresis demonstrates that protein profiles of the samples collected by Accuwik Ultra are very similar to those of samples collected by passive drool (data not shown). **Conclusions:** Assessment of Accuwik Ultra illustrates that the membrane is a reliable tool for direct collection of clinical samples including saliva and plasma, enables stability during sample storage, and allows for rapid and efficient release of clinically relevant protein biomarkers suitable for diagnostic analyses.

Introduction

Accuwik Ultra Membrane—a robust material for lateral flow and direct dipstick applications

Common design features of a lateral flow device:

- Includes four overlapping pads – 1) wicking, 2) conjugation, 3) reaction membrane, 4) absorption.
- When dipped into a solution, or swabbed with a sample, the wicking pad carries the fluid to and rehydrates the specific detection reagent (colloidal gold, colored latex) dried on the conjugation pad.
- The rehydrated detection reagent then migrates to the membrane where it is captured by the capture antibodies and forms a colored line.
- The intensity of the line is an indicator of how much of the targeted molecule was in the sample solution.

Advantages of using Accuwik Ultra membrane as wicking or conjugate release pads for lateral flow diagnostics:

- Accuwik Ultra is a fibrous, hydroxylated polyester material and is inherently water wettable resulting in consistent flow rates.
- Uniform wicking front with wicking rate typically below 8 seconds/2 cm provide assay consistency.
- Low protein binding yields increased signal intensity.
- High conjugate capacity and quick release result in less media required per mL of sample.
- Free of binders and surfactant that can interfere with assay sensitivity and results.

Additional requirements to wicking materials for direct dipstick applications:

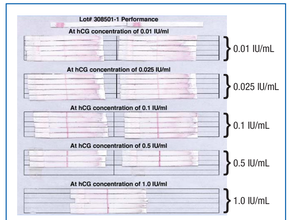
Oral fluids including saliva, gingival crevicular fluid, and mucosal transudates have been widely used for monitoring drugs, hormones, and a variety of other molecules and chemical substances for over 50 years. During the past decade, the use of oral fluids also has been advocated as a noninvasive alternative to the collection of blood for the detection of antibodies to a number of specific bacterial, viral, and fungal agents.

Development of direct dipstick immunoassays for oral fluids that include specimen collection from the patient's mouth requires special consideration when selecting the materials employed, specifically for sample collection and storage. These considerations include ensuring that the material is/has:

- of binders or surfactants to ensure effective conjugate release and consistent flow rates.
- Extremely low protein binding to minimize non-specific binding of the target analyte(s).
- High efficiency recoveries of analytes of interest.
- Archival ability without degrading the protein profile.
- Not a glass medium.

The study presented has been designed to demonstrate the consistency and robustness of Accuwik Ultra membrane for direct dipstick applications and sample collection.

Figure 1.
Accuwik Ultra Membrane Provides Consistent Conjugate Release Without Compromising Assay Sensitivity



Test strips were composed of Accuwik Ultra conjugate release pad over nitrocellulose membrane. Antibodies specific to hCG were applied at the test line. Test strips were tested with samples of hCG at the following concentrations (IU/mL) of hCG: 0.01, 0.025, 0.1, 0.5, and 1.0. Following the completion of the assay, there was complete release of the colloidal gold conjugate from the Accuwik Ultra pad. The sensitivity of the assay was 0.01 IU of hCG per mL.

Accuwik Ultra Membrane for Sample Collection and Storage for Alpha-Amylase Activity Measuring in Saliva

Saliva sample collection and storage:

- Samples were collected by passive drool from healthy volunteers.
- Each sample was applied to a 25 mm disc of Accuwik Ultra membrane.
- The discs were stored under three different conditions that simulated different clinical lab settings.

Condition 1: Simulates sample collection/analysis in the clinical lab.

Accuwik Ultra membrane discs were soaked in the collected saliva samples, placed into Pall Forensic Nanosep centrifugal devices, and stored on the bench for 0.5-1 hour.

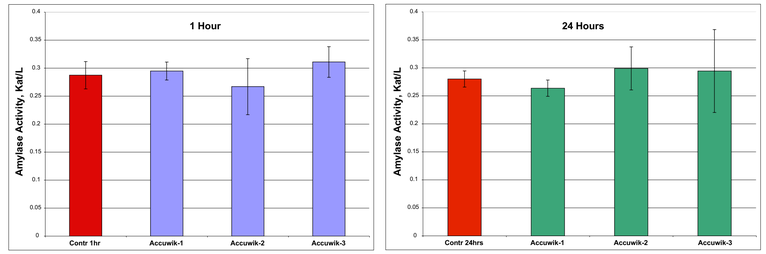
Condition 2: Simulates sample collection in a physician's office where overnight shipment to an analytical lab is necessary.

Accuwik Ultra discs were soaked in the saliva samples and stored on the bench at room temperature for 24 hours.

Condition 3: Simulates conditions where samples are to be stored long term before analysis (e.g., field study, over weekend storage).

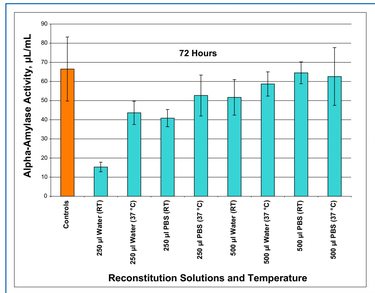
Saliva samples were applied to Accuwik Ultra membrane discs and dried at 37 °C for 1 hour. Following drying, samples were stored at room temperature for 3 days. Dry samples were reconstituted by applying reconstitution solutions to the membrane discs for 15 minutes at room temperature or 37 °C.

Figure 2.
Protein Activity Levels Are Not Altered When Using Accuwik Ultra Membrane for Sample Collection and Storage



After collection, storage, and reconstitution, all saliva samples were recovered from the Accuwik Ultra membrane discs by centrifugation in an Eppendorf centrifuge at 13,000 rpm for 7 minutes and frozen at -20 °C prior to analysis. Saliva samples collected by passive drool and stored under the same conditions as the samples on the Accuwik Ultra membrane discs were used as controls. Alpha-Amylase activity was measured using the enzyme kinetic reaction kit and protocol from Salimetrics LLC (State College, PA).

Figure 3.
Accuwik Ultra Membrane Provides Stable Long Term Storage Material for Diagnostic Assays



24 aliquots of a saliva were applied onto 24 Accuwik Ultra membrane discs. Eight different reconstitution conditions were compared in order to achieve complete alpha-amylase enzyme recoveries in triplicate. Aliquots frozen immediately after sample collection and thawed on the day of measurement were used as a control. Both water and PBS solutions were successfully used for complete recovery of alpha-amylase from samples stored long term on Accuwik Ultra media. Full recovery is also achieved at room temperature as well as 37 °C. The restoration conditions presented on the chart are described in Table 1.

Table 1.
The Restoration Conditions Used to Recover the Saliva Samples from Accuwik Ultra Discs After 3-day Dry Storage

Sample ID on the Chart	Solution Restored	Volume (µL) Restored	Restoration Conditions
Controls	None	250	Thawed after freezing and storage at -20 °C
250 µL Water (RT)	Water	250	Incubated at RT on shaker for 30 mins
250 µL Water (37 °C)	Water	250	Incubated at 37 °C in oven
250 µL PBS (RT)	PBS	250	Incubated at RT on shaker for 30 mins
250 µL PBS (37 °C)	PBS	250	Incubated at 37 °C in oven
500 µL Water (RT)	Water	500	Incubated at RT on shaker for 30 mins
500 µL Water (37 °C)	Water	500	Incubated at 37 °C in oven
500 µL PBS (RT)	PBS	500	Incubated at RT on shaker for 30 mins
500 µL PBS (37 °C)	PBS	500	Incubated at 37 °C in oven

Protein Profiles of Stored Plasma Samples Recovered from Accuwik Ultra Membrane vs. Centrifuged Plasma

Plasma samples were separated from whole fresh EDTA blood by centrifugation.

200 µL of centrifuged plasma was applied to a 25 mm disc of Accuwik Ultra membrane and left at room temperature for 0.5-1 hour.

Plasma was recovered from the Accuwik Ultra membrane discs by centrifugation in an Eppendorf centrifuge at 13,000 rpm for 7 minutes and frozen at -20 °C prior to analysis using Pall Forensic Nanosep centrifugal devices.

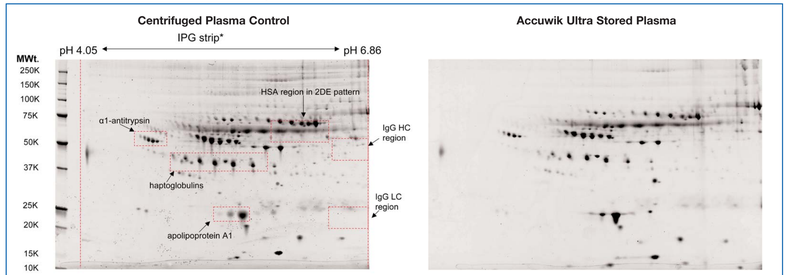
Plasma samples recovered from Accuwik Ultra membrane, as well as centrifugation, were analyzed by 2D Electrophoresis following abundant protein (HSA and IgG) depletion by Pall Enchant™ Multi-Protein Affinity Separation Kit. The total protein concentration in plasma samples before and after HSA/IgG depletion is shown in Table 2.

Table 2.
Low Non-Specific Binding of Target Analytes in Accuwik Ultra Stored Plasma

Plasma ID	µg Total Protein in 25 µL Plasma Sample	µg Total Protein in Depleted Sample	% Depletion
Accuwik Ultra Stored Plasma	1217	389	68
Centrifuged Plasma (Control)	1064	359	66

Plasma protein concentrations before and after HSA/IgG depletion.

Figure 4.
Equivalent Protein Profiles Obtained from Plasma Samples Collected, Stored, and Recovered in Accuwik Ultra Membrane



Analysis: A 2DE protocol of very high resolution of the acidic pI, medium molecular weight region where most of the known cardiac biomarkers are located was employed as a first dimension with pH 4-7 NL IPG strips followed by a second dimension separation on 10.5-14% SDS PAGE. There are no statistical differences seen between the 2D protein profiles of the control centrifuged plasma and Accuwik Ultra membrane stored plasma.

Conclusions

These studies have illustrated that Accuwik Ultra membrane is a reliable tool for direct collection of clinical samples including saliva and plasma. Furthermore, the use of this material enables stability during sample storage and allows for rapid and efficient release of clinically relevant protein biomarkers suitable for diagnostic analyses.

Contact Information

Phone: 734.665.0651
Toll free in USA: 888-676-7255
Web address: www.pall.com/oem
E-mail: oem@pall.com

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