# Development of a lateral flow assay for the detection of β-actin in whole cell extracts

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## BACKGROUND

The Lateral Flow Assay (LFA) is a rapid, point-of-care immunodiagnostic test commonly seen as the home pregnancy test. This method of diagnosis is convenient due its user-friendly format and ease of use without specialized personnel. In typical LFAs, gold nanoparticles are conjugated to antibodies that capture the target analyte from the sample.

As the sample flows through the LFA membrane, the gold nanoparticle-antibody-target complex is captured onto the antibodies immobilized on the test line on the membrane of the strip. The development of the color on the test line indicates a positive test; a downstream control line confirms that the test has been conducted correctly and sample was added to the strip.

## OBJECTIVE

The primary objective of my summer project (SURF) was to gain hands-on experience in LFA development for cancer diagnostics and more specifically the development of an LFA for the detection of β-actin in whole cell extracts. I initially worked on a model LFA for the detection of human Chorionic Gonadotropin (hCG). Then, I developed an LFA for the detection of β-actin in CA46 human B-cell lymphoma cells. Cultured cancer cell lines have significant relevance in cancer research by serving as models for preclinical tests.

Drs. Kourentzi and Willson are working towards the development of a rapid Acute Promyelocytic Leukemia (APL) LFA test for blood. APL is a distinct subtype of leukemia that involves severe coagulopathy that rapidly results in life-threatening lung or brain bleeding in 40% of patients. Highly effective therapies exist, however, and patients can reliably be saved and eventually cured if diagnosed early.

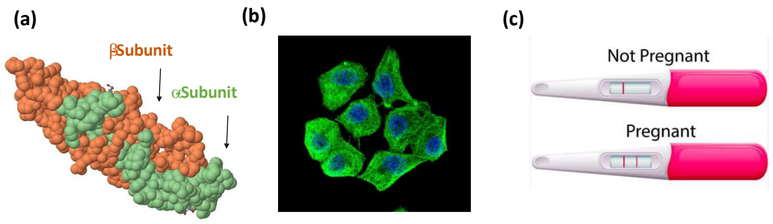



**Figure 1. (a) Chemical structure of human chorionic gonadotropin (hCG) consisting of two subunits. (b) Cellular β-actin in cultured cells was immunostained with an anti β-actin antibody labeled with Alexa 488 fluorophore; www.novus.com (c) A typical over-the-counter pregnancy test.**

### Human Chorionic Gonadotropin (hCG)

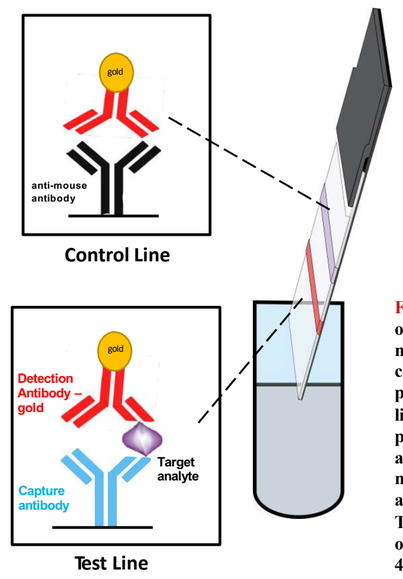
hCG (MW: 40 kDa) is an important hormone synthesized by cells in the placenta when a woman is pregnant. Elevated concentrations of hCG indicate pregnancy and hCG is detected in the common over-the-counter pregnancy tests.

### Actin

β-actin is one of most abundant cellular proteins and a key protein present in all cells that participates in a variety of cellular processes such as cell division, cell signaling and cell maintenance. Detection of β-actin protein in whole cell extracts is an essential control due to the fact that expression levels do not vary drastically between different types of cells. Thus, actin is commonly used for confirming reproducibility of sample preparation and normalization of the number of cells in specimens.

## PROCEDURE & APPROACH

### Lateral Flow Assay (LFA)




Target analyte at varying concentrations is mixed with LFA Running Buffer (0.1% Tween-20, 1% BSA, and 1% PEG in 1x PBS). The anti-target specific detection antibody conjugated to gold nanoparticles is mixed with the target; an LFA strip is inserted into the tube. As the sample travels up the membrane, two lines could be observed: Test Line if the target is present and Control line if the strip was run correctly. After drying, the strips are imaged and analyzed.

**Figure 2. Lateral Flow Assay for the detection of hCG and Actin. hCG was captured by mouse monoclonal anti-β hCG antibodies conjugated to gold nanoparticles and goat polyclonal anti-α hCG antibodies on the test line (TL). For the detection of Actin, rabbit polyclonal anti-β actin antibodies (abcam, ab8227) were deposited on the TL and mouse monoclonal anti-β actin antibodies (abcam, ab6276) were conjugated to gold nanoparticles. The control line (CL) for both LFAs consisted of anti-mouse antibodies.**
**4-mm wide lateral flow strips were prepared on a Biodot XYZ3060 Dispensing Platform. The strip consists of a 43-mm long FF80HP nitrocellulose membrane and a 22-mm CF5 absorbent pad.**

### Whole Cell Extracts

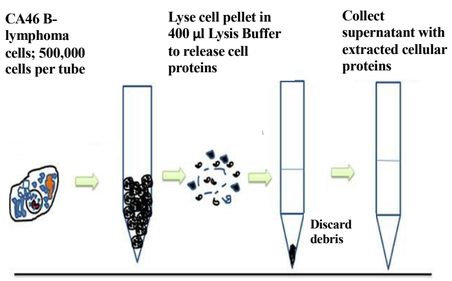
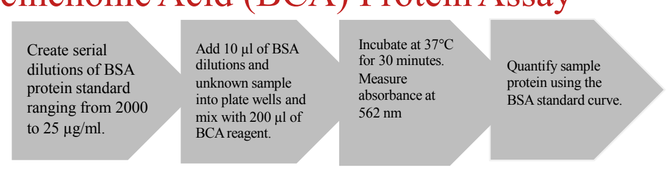



**Figure 3. Whole cell extracts of CA46 human B-cell lymphoma cultured cells were prepared using the Cell Lysis Buffer under non-denaturing conditions following the manufacturer instructions.**

Cell Lysis Buffer (Cell Signaling Technology; #9803): 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM $Na_2EDTA$, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM $Na_3VO_4$, 1 µg/ml leupeptin

### Bicinchonic Acid (BCA) Protein Assay




The BCA assay is a colorimetric method to determine protein concentration in a sample using the reduction of $Cu^{2+}$ to $Cu^{1+}$ by proteins and the detection of $Cu^{+1}$ cation by the BCA reagent. The unknown sample is assayed alongside a standard protein (typically BSA). The total protein in the the CA46 whole cell extracts was estimated at 546 µg/mL. The amount of protein per cell was 87.4 pg/cell.

## RESULTS & DISCUSSION

A model lateral flow assay for hCG was used to study the effect of cell lysis buffer in the LFA. A dilution series of hCG target protein along with no protein, negative samples were run in parallel.

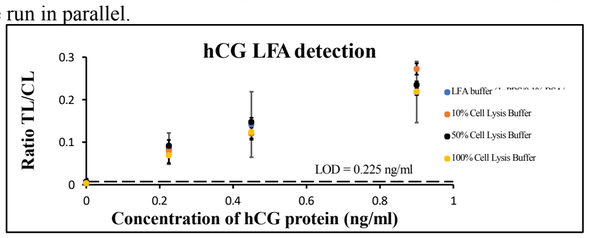



**Figure 4. hCG was mixed with Cell Lysis Buffer diluted in LFA running buffer and gold nanoparticles. We observed that the Cell Lysis Buffer did not affect the hCG Limit of Detection (LOD), the lowest analyte concentration that gave a signal (TL/CL) clearly distinguishable from the no-target sample signal ($TL/CL_{no\text{-}target} + 3*SD_{no\text{-}target}$).**

We designed a lateral flow assay for the detection of actin in whole cell extracts.

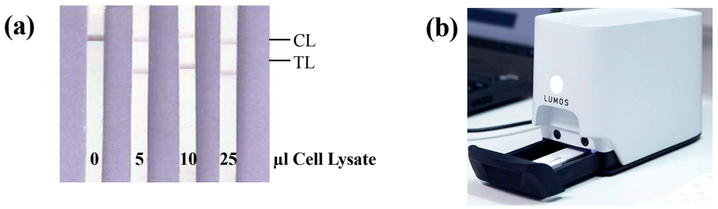



**Figure 5. (a) LFA detection of β-actin protein in CA46 human B-cell lymphoma cultured cells on anti β-actin (abcam, ab8227) test lines with anti-mouse control lines; detector: anti β-actin (abcam, ab6276)/gold. Scanned images of strips show darker TL signal as the amount of cell lysate was increased. (b) LFA strips were imaged and the brightness of the lines was quantified using the Lumos Leelu reader (results are shown in Figure 6).**

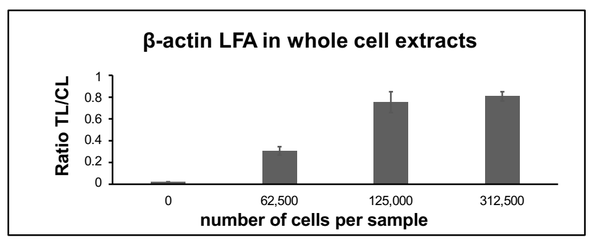



**Figure 6. Whole cell extracts were mixed with LFA Running buffer and gold nanoparticles in a total volume of 50 µl, and then introduced to the LFA strip. Desalting the sample through a Zeba spin column and heating at 70°C for 10 minutes prior running the LFA provided the best signal for the β-actin LFA.**

## CONCLUSIONS

- LFA strips were constructed and a model hCG LFA was run with gold nanoparticle reporters.
- For the hCG LFA the LOD was estimated at 0.225 ng/ml in buffer; we confirmed that the LOD was not affected by the presence of Cell Lysis Buffer.
- We identified a β-actin antibody pair that works well in the LFA. Little gold nanoparticle aggregation was observed on the strips.
- β-actin signal on the TL was improved by desalting/conditioning the cell lysate using Zeba columns and heating at 70°C for 10 minutes.
- Higher amounts of cell lysate gave a stronger TL signal.

## REFERENCES

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