

Zyel et al.
Supplementary Methods

LFA assay

A lateral flow assay (LFA) was used to detect CD30 using LFA strips and detection particles manufactured in-house. LFA strips were made by striping mouse anti-CD30 antibody (R&D systems; cat# MAB291) on nitrocellulose membrane using a BioDot dispenser (BioDot; cat# XYZ30600124). Detection particles were made by conjugating red latex particles (ThermoFisher; cat# DR104) with affinity-purified Polyclonal goat anti-CD30 (R&D Systems, cat# AF229). Seroma fluid was diluted to specified concentrations in LFA running buffer, mixed with detection particles, and pipetted onto the sample pad of CD30 LFA strips. After 5 minutes, each strip was washed with additional LFA running buffer. A positive result was a red test line whose intensity corresponded to the concentration of CD30. A control line ensured the flow of detection particles and confirmed a negative result. The detailed LFA procedure is provided below.

CD30 strip preparation

To manufacture LFA strips, CN95 membrane (Sartorius; cat# 1UN95ER100025NTB) was assembled on a 30-cm backing adhesive card (DCN Diagnostics; cat# MIBA-020) along with an absorbent pad (Ahlstrom; cat# 440) and a sample pad (Ahlstrom; cat# 8980). The assembled membrane was striped using a BioDot dispenser (BioDot; cat# XYZ30600124), at a flow rate of 1 μ L/cm and a dispensed volume of 30 μ L per 30 cm card. The test line contained mouse anti-CD30 antibody (R&D systems; cat# MAB291) diluted to a concentration of 1 mg/mL in 1 \times PBS and 0.2% sucrose. The control line contained donkey polyclonal anti-goat antibodies (Jackson ImmunoResearch Laboratories Inc.; cat# 705-005-147) diluted to a concentration of 0.75 mg/mL in 1 \times PBS and 0.2% sucrose. Striped membranes were dried in a Robbins Scientific Micro Hybridization Incubator 2000 at 37°C for 30 min, then stored overnight at 20°C in a desiccator chamber (Totech; SuperDry Desiccant Cabinet; cat cat# SD-151-21) at 5% humidity. The striped card was cut into 3 mm strips using a KinBio ZQ2000 Guillotine Cutter and stored at 20°C in sealed 50 mL conical tubes (USA Scientific; cat# 5622-7261) with desiccant packs (Intertek Packaging; cat# IN1G51).

Conjugation of latex particles with CD30 antibody

Carboxylate-modified red latex particles (ThermoFisher; cat# DR104) were functionalized with affinity-purified Polyclonal goat anti-CD30 (R&D Systems, cat# AF229) using standard EDC-NHS chemical activation. Briefly, 100 μ L particles at 0.5% solids were centrifuged (10 min at 16,500 \times g) and washed twice with 50 mM MES buffer, pH 5.8. The particles were resuspended in 95 μ L MES buffer and sonicated until no visible aggregates remained. Particles were activated by EDC (ThermoFisher; cat# A35391) and NHS (Millipore Sigma; cat# 130672) at a molar ratio of NHS:carboxyl groups of 20 (1.11 μ L of 50 mg/mL NHS) and EDC:carboxyl groups of 2.5 (1.2 μ L of 10 mg/mL EDC). NHS and EDC were added to the resuspended particle mixture sequentially (NHS followed by EDC) and placed on a benchtop rotator for 30 min at 20°C. After activation, the particles were washed twice by centrifugation in 1 \times PBS and resuspended by sonication. 25 μ g of antibody (25 μ L of 1 mg/mL suspended in 1 \times PBS) was added to the particles and incubated at 20°C on a benchtop rotator for 2 h. The mixture of particles and antibody was centrifuged (10 min at 16,500 \times g), the supernatant was removed, and particles were resuspended in 4% BSA in 1 \times PBS and incubated for 1 hr at 20°C on a benchtop rotator. The particles were washed three times by centrifugation with 1 \times PBS, 1% BSA solution, resuspended at 0.5% solids in 100 μ L 1 \times PBS, 1% BSA, and stored at 4°C.

LFA running protocol for IL-10

30 μ L of sample was prepared by mixing 6 μ L of a concentrated LFA running buffer (5 \times PBS (pH 7.4), 2.5% BSA (Millipore Sigma; cat# A9418), 1.25% Tween-20 (Millipore Sigma; cat# P1379), 1.5% PEG 3000 (Millipore Sigma; cat# 81227) with 24 μ L of diluted seroma fluid (diluted as specified with diH₂O). The sample was then pipetted onto the sample pad of the LFA strip. After 5 minutes, 2.5 \times 10⁹ reporter particles diluted in LFA running buffer (1 \times PBS (pH 7.4), 0.5% BSA, 0.25% Tween-20, and 0.3% PEG 3000)

(20 μ L total volume) were pipetted onto the sample pad of the LFA strip. After 5 minutes, the strips were washed with an additional 20 μ L of LFA running buffer. The strips were run for a total of 15 minutes before imaging with a smartphone camera (iPhone 12).