An AI-enhanced universal lateral flow assay (AuLFA) system for the detection of neurotoxin exposures in diverse military-relevant samples

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ABSTRACT

Organophosphate (OP) neurotoxins, such as Chlorpyrifos, Diazinon, and Fenthion, pose severe long-term risks to military personnel, causing cognitive and motor impairment consistent with Gulf War Illness (GWI) through chronic, low-level exposure in field environments. Rapid and sensitive point-of-care detection is critical to prevent irreversible neurological damage; however, gold-standard analytical methods like GC-MS and HPLC are too costly, slow, and labor-intensive for field deployment. While lateral flow assays (LFAs) offer a rapid, portable alternative, conventional test strips suffer from severe matrix dependency. Variations in sample composition, hydration, and environmental conditions disrupt capillary flow and antibody-antigen binding kinetics across military-relevant media, such as urine, soil, food surfaces, and wastewater. This necessitates manual, sample-specific calibrations that increase operational complexity and risk user error.
To eliminate matrix-induced errors without compromising field usability, we developed an AI-enhanced universal LFA system (AuLFA). The platform integrates a fully automated microfluidic module for standardized sample sampling and pretreatment with a deep learning framework designed for whole-process analysis (WPA), automatically identifying matrix types and applying real-time data correction. Preliminary evaluations demonstrate robust detection of Chlorpyrifos down to 10 ng/mL across multiple challenging matrices, establishing AuLFA as a scalable, field-deployable diagnostic tool for continuous environmental exposure monitoring and force health protection.

BACKGROUND

Organophosphate (OP) neurotoxins like chlorpyrifos are toxic pesticides linked to severe neurological damage and Gulf War Illness in deployed military personnel. A 2020 study estimated 740,000 unintentional pesticide poisonings, resulting in 7446 deaths across 141 countries. While rapid field monitoring is critical to prevent health risks, current methods face a major gap. Gold-standard laboratory tests are far too slow for frontline deployment, whereas portable alternatives like traditional lateral flow assays (LFAs) fail due to severe matrix dependency. Our AI-enhanced universal LFA (AuLFA) system integrates an automated microfluidic pretreatment unit with a deep learning network for whole-process analysis. Preliminary data shows robust detection of chlorpyrifos down to 10 ng/mL in buffer solution, confirming its reliability for rapid, direct analysis in variable field samples.

METHOD

The lateral flow assay was prepared in two main steps: membrane functionalization and gold nanoparticle (GNP) conjugation. First, goat anti-mouse IgG (2 mg/mL, control line) and chlorpyrifos-BSA (0.05 mg/mL, test line) were dispensed onto the nitrocellulose membrane using a dispenser strip, then incubated overnight at 37 °C to dry. For conjugation, 40 nm GNPs were adjusted to pH 9.0 with carbonate buffer, incubated with chlorpyrifos monoclonal antibody for one hour at room temperature, and blocked with 10% BSA. The resulting conjugate was washed three times by centrifugation at 4,000 × g (15 minutes at 4 °C) and resuspended in 0.5% BSA in PBS. For sample detection, the antibody-GNP conjugate was combined with the sample and optimized running buffer, allowed to react at room temperature, and developed using a dipstick format for visual evaluation.

CONCLUSIONS

This study demonstrates a deep-learning-enhanced AuLFA platform for rapid, high-precision quantification of Chlorpyrifos. The automated framework eliminates reading errors and serves as a scalable foundation for future multiplexed toxin detection.

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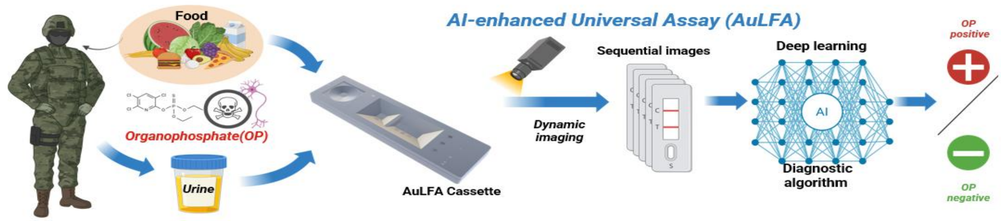
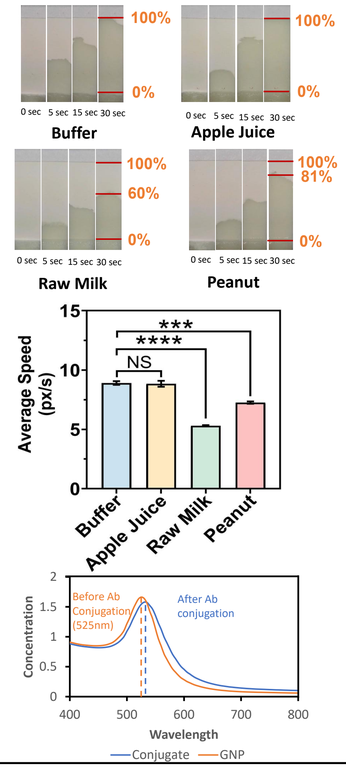
SCHEMATIC

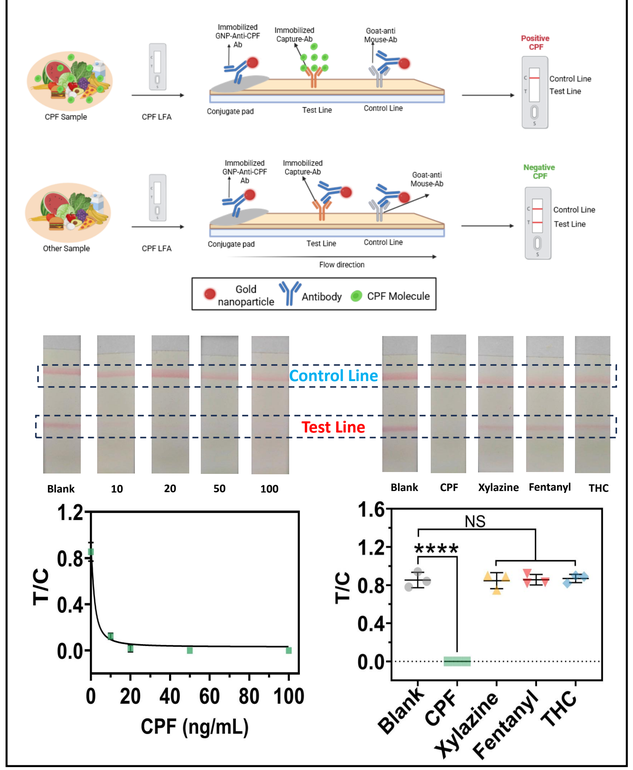


Schematic workflow of the AI-enhanced Universal Assay (AuLFA) system

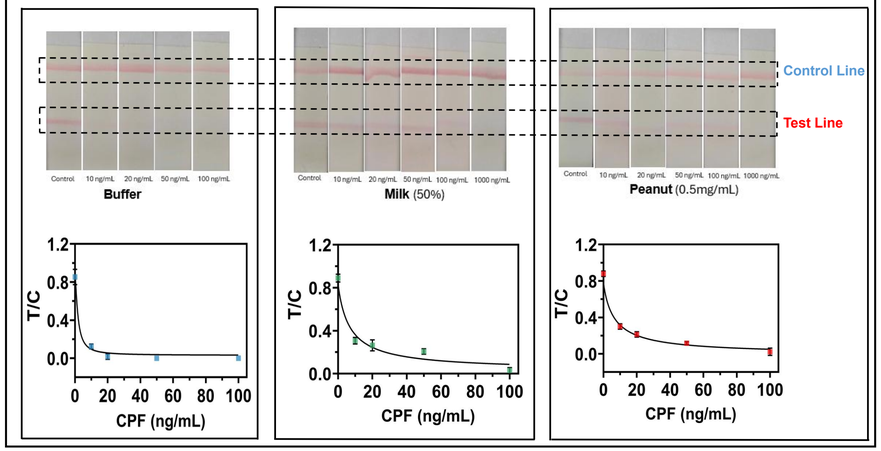
ASSAY REAGENT CHARACTERIZATION



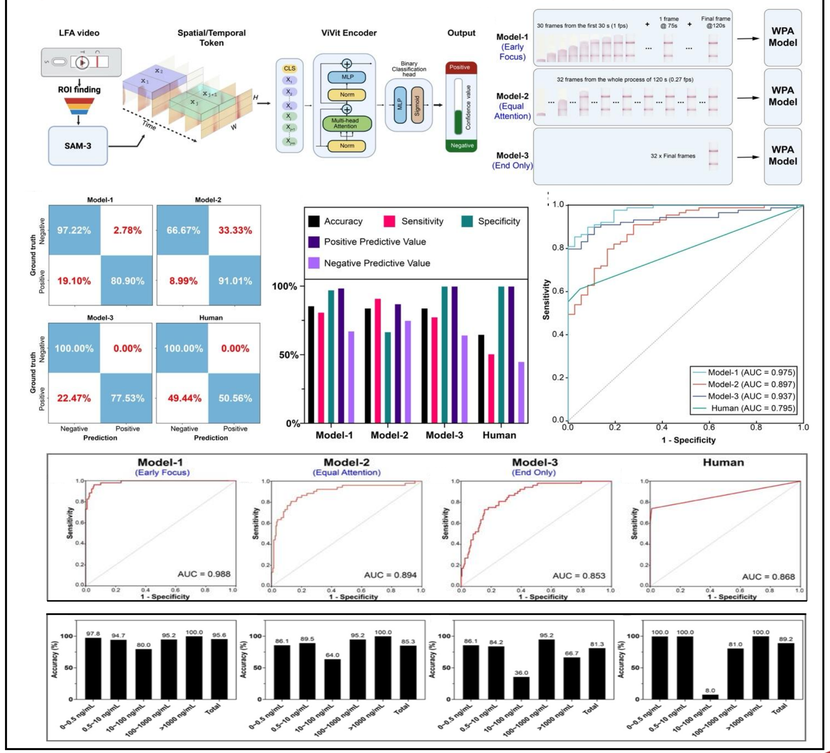
DEVELOPMENT & ANALYTICAL PERFORMANCE OF THE ASSAY



EVALUATION OF ASSAY PERFORMANCE UNDER DIFFERENT MATRIX



RAPID SIGNAL INTERPRETATION WITH DEEP LEARNING MODEL (ADOPTED FROM PREVIOUS XYLAZINE PROJECT)



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