Rapid and High Sensitivity InstaStrip Technology for Detection of Emerging Drug Threats

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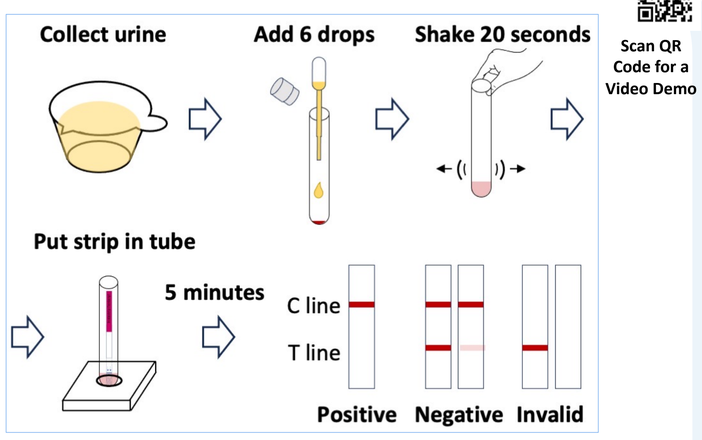
Background: Emerging drug threats such as fentanyl, fentanyl analogs and xylazine affect 24 million people annually. Fentanyl laced with xylazine was designated as an emerging threat to the US in 2023. The majority of fentanyl-related overdoses are caused by illicit fentanyl and/or fentanyl analogs, either alone or in combination with other narcotics. Xylazine is a non-opioid veterinary tranquilizer that has not been approved for human use but been increasingly reported to link to human overdose deaths in the US. Rapid identification of fentanyl, fentanyl analogs and xylazine in both human and environment samples is of paramount importance to ensure the safety, rapid triage, and treatment of potentially exposed or overdosed civilians and warfighters[3].

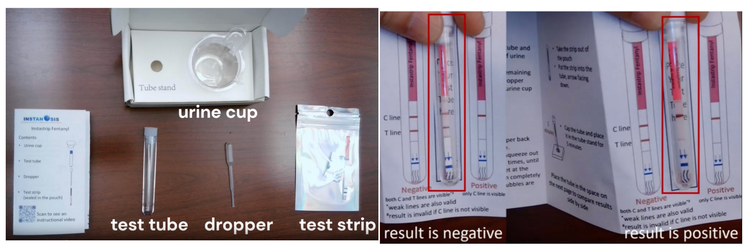
We have developed proprietary and high affinity antibodies for these emerging drugs threats, and patent-pending technology platforms[1] for both in-lab and in-the-field detection of fentanyl, fentanyl analogs and xylazine in minutes (Figs 1&2). Detection limit is at sub-picograms to low nanograms per milliliter. Extensive human and environmental sample testing demonstrated high sensitivity and specificity. Our peer-reviewed publication demonstrated clinical sensitivity in human urine samples is 100% (95% confidence interval (CI) 75.8-100%), and clinical specificity is 99.5% (95% CI 97.3-99.9%)[2]. Usability assessment demonstrated friendliness for lay users.

InstaStrip-Fentanyl (FDA Breakthrough Device Designation)

Human Urine Testing: rapid and portable in-the-field use



Scan QR Code for a Video Demo

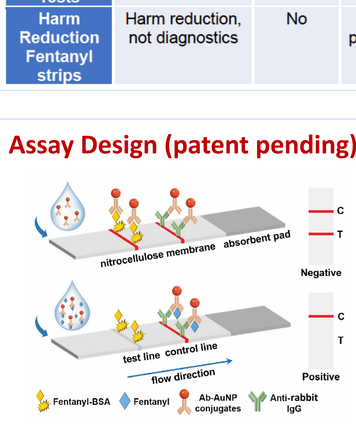


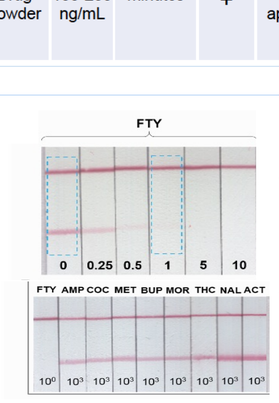
Human Urine Testing using InstaStrip Technology. Results are read with naked eye in 5 min

Table 1. Competitive Advantages for InstaStrip-Fentanyl Rapid Test

Products	Market/Setting	Instrument Required	Sample	Cutoff	Speed	Cost	Regulatory Status
InstaStrip-Fentanyl Rapid Test	Diagnostics (professional & OTC)	No	Human urine	1 ng/mL	5 minutes	+	Presubmission 510(k), OTC use
Core Lab Fentanyl Screening Tests	Diagnostics (Professional use only)	Yes	Human urine	1 ng/mL	Hours	++	FDA cleared, prescription use only
Harm Reduction Fentanyl strips	Harm reduction, not diagnostics	No	Drug powder	100-200 ng/mL	minutes	+	Not FDA approved or cleared

Assay Design (patent pending)





- Cutoff is 1 ng/mL, matching clinical/FDA requirement
- No cross reactivity with other drugs

Cross-reactivity with Fentanyl Analogs

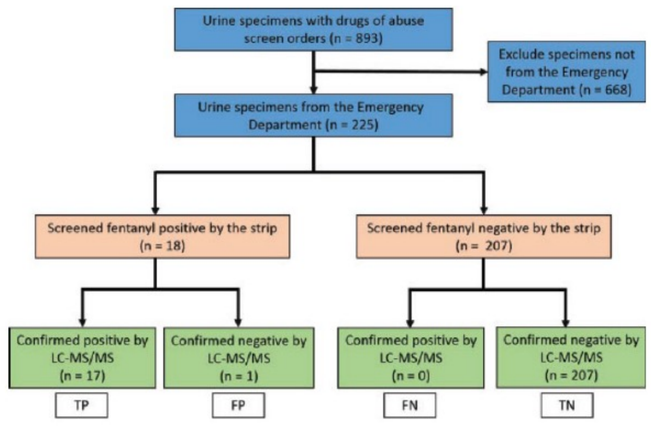
Compound name	Concentration approximately equivalent to the cutoff (ng/ml)	Percent cross reactivity (%)
Fentanyl	1	100
Acryl fentanyl	1	100
Butyryl fentanyl	1	100
Furanyl fentanyl	1	100
Tetrahydrofyranyl fentanyl	1	100
o-Fluorofentanyl	1	100
Benzodioxole fentanyl	1	100
Ocfentanil	1	100
Valeryl fentanyl	10	10
Crotonyl fentanyl	10	10
Para-Fluorofentanyl	10	10
Para-chloroisobutyryl fentanyl	100	1
Despopionyl 2' fluoro-ortho-fluorofentanyl	500	0.2
Norfentanyl	>10000	<0.01
Remifentanyl acid	>10000	<0.01
Sufentanyl citrate	>10000	<0.01
N-benzyl furanyl norfentanyl	>10000	<0.01
N-benzyl parafluoro cyclopropyl norfentanyl	>10000	<0.01

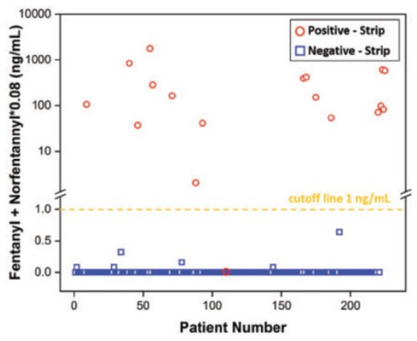
Precision

Fentanyl Concentration (ng/ml)	Relative % Cutoff	N	Lot 1			Lot 2			Lot 3		
			P	N	Positive%	P	N	Positive %	P	N	Positive %
0	-100	60	0	60	0	0	60	0	0	60	0
0.25	-75	60	0	60	0	0	60	0	0	60	0
0.5	-50	60	0	60	0	0	60	0	0	60	0
0.75	-25	60	24	36	40	20	40	33	18	42	30
1	cutoff	60	54	6	90	55	5	92	55	5	92
1.25	+25	60	60	0	100	60	0	100	60	0	100
1.5	+50	60	60	0	100	60	0	100	60	0	100
1.75	+75	60	60	0	100	60	0	100	60	0	100
2	+100	60	60	0	100	60	0	100	60	0	100

Clinical Urine Testing

Clinical Study 1:

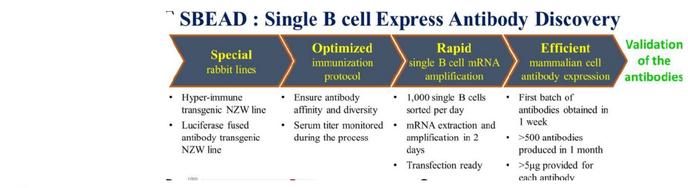


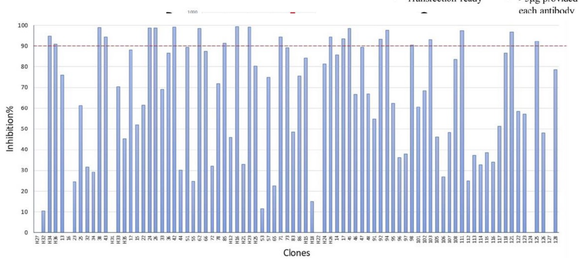


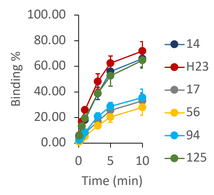
Clinical Sensitivity: 100% (95% confidence interval (CI) 75.8-100%)
Clinical Specificity: 99.5% (95% CI 97.3-99.9%)

Clinical Study 2: 187 consecutive clinical urine samples freshly received at the Hospital of the University of Pennsylvania were tested using InstaStrip-Fentanyl. One hundred and eighty (180) fresh urine samples tested negative and 7 samples tested positive, all concordant with clinical history and/or LC-MS/MS results. One urine sample tested negative using InstaStrip despite a positive result from the predicate immunoassay (ARK Fentanyl II). LC-MS/MS confirmed the InstaStrip result as a true negative result.

High Affinity Antibody Development

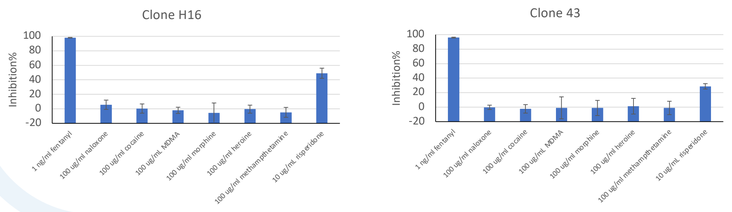






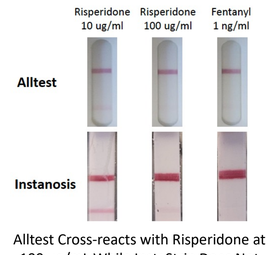
Antibody Affinity Screening: Higher Percentage of Inhibition Corresponds to Higher Affinity

Selected Antibody Reaction Kinetics



InstaStrip-Fentanyl vs Alltest Fentanyl Strip Comparison

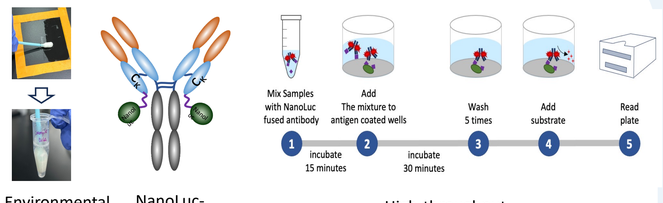
InstaStrip Achieves Higher Sensitivity, Higher Line Intensity and Sharper Transition Around Cutoff



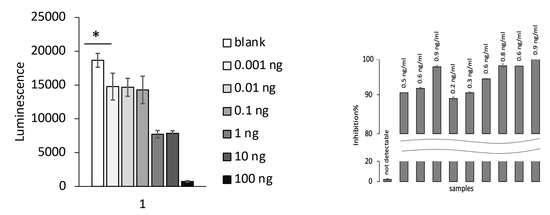
Alltest Cross-reacts with Risperidone at 100 µg/mL While InstaStrip Does Not

Alltest Generates False Positives in Clinical Urine Samples While InstaStrip Does Not

Environmental Testing: rapid and high-throughput



Detection of Fentanyl and its Analogs in Environmental Samples



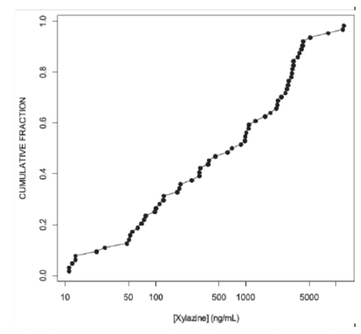
Detection of Fentanyl at Below 1 pg/mL in Environmental Samples

Detection of Fentanyl in Human Urine Samples

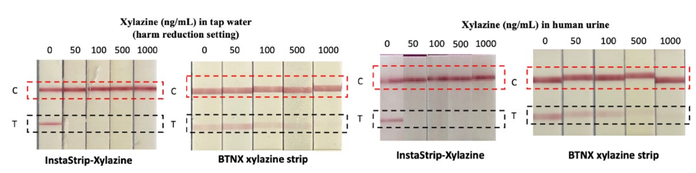
InstaStrip-Xylazine

Competitive Advantage of InstaStrip-Xylazine

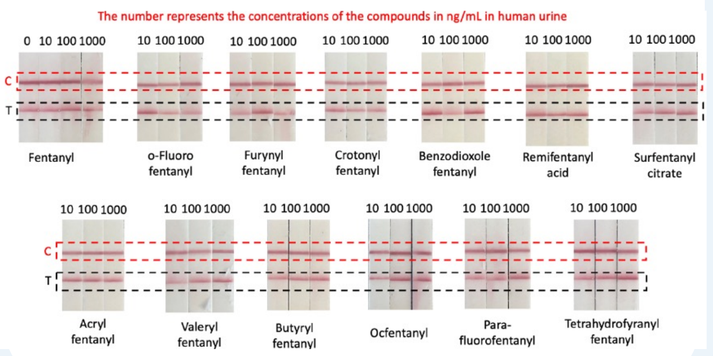
Products	Market/Setting	Sample	LOD	Speed	Cost
InstaStrip-Xylazine Test	Diagnostics (professional & OTC), forensics, harm reduction	Human urine	≤10 ng/mL	5 minutes	+
Mass Spec Testing for Xylazine	Central lab only	Human urine	≤10-200 ng/mL	Days (not useful for immediate clinical management)	+++
BTNX Xylazine strip	Harm reduction, not diagnostics	Drug powder	1000-2000 ng/mL	minutes	+



Distribution of Xylazine in Hospitalized Patients Positive for Fentanyl (Korn et al. 2021)



Comparison Between InstaStrip-Xylazine and BTNX Xylazine Strip Performance in Harm-Reduction and Clinical Setting



InstaStrip-Xylazine Does not Cross-react with Fentanyl or its Analogs, with which Xylazine is Often Mixed in Drug Supplies

Conclusions:

- Complementary technologies: the InstaStrip technology is rapid and portable for in-the-field use; the chemiluminescence technology is high through-put for in-lab use.
- The platforms are easy-to-use, affordable, and can be adapted to other emerging small compounds and drugs.

References:

1. Wang P, Li Z, Chen H and Liu K: Devices and Methods for Rapid Screening of Drugs of Abuse and Other Analytes. Patent Number US 62/874,643, 2019.
2. Li, Z., et al., *Development and Clinical Validation of a Sensitive Lateral Flow Assay for Rapid Urine Fentanyl Screening in the Emergency Department.* Clin Chem, 2020. **66**(2): p. 324-332.
3. Volkow, N.D., et al., *Testing for Fentanyl - Urgent Need for Practice-Relevant and Public Health Research.* N Engl J Med, 2023. 388: 2214-2217.
4. Gupta, R., D.R. Holtgrave, and M.A. Ashburn, *Xylazine - Medical and Public Health Imperatives.* N Engl J Med, 2023. 388:2209-2212

Funding

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