LZI SPICE I (JWH-018/JWH-073/AM2201) Enzyme Immunoassay – EU Only

REF 0500 (100/37.5 mL R_1/R_2 Kit)
0501 (1000/375 mL R_1/R_2 Kit) 2ºC 8ºC

IVD For In Vitro Diagnostic Use Only

Lin-Zhi International, Inc.

Intended Use

The Lin-Zhi International, Inc. (LZI) SPICE I (JWH-018/JWH-073/AM2201) Enzyme Immunoassay is intended for the qualitative and semi-quantitative determination of JWH-type synthetic cannabinoids in human urine using JWH-018 N-(5-hydroxypentyl) metabolite as calibrator at the cutoff values of 20 ng/mL. The assay is designed for use with a number of automated clinical chemistry analyzers.

The assay provides a rapid screening procedure for determining the presence of synthetic cannabinoids in urine. The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

Summary and Explanation of Test

In the past few decades, research has confirmed the presence of an endogeneous endocannabinoid system or ECS in humans (3). Endocannabinoids are produced within the human body and activate two known cannabinoid receptors, CB_1 and CB_2 (4). The CB_1 receptor is localized primarily to the brain and is thought to be responsible for the euphoric and anticonvulsive effects of cannabis whereas the CB_2 receptor is found primarily in the immune system and thought to be responsible for the anti-inflammatory effects of Δ^9-THC (5-7).

Due to the role the ECS may play in a number of physiological processes, much interest has been developed in the use of synthetic cannabinoids (synthetic ECS) ligands for therapeutic purposes such as CB_1 receptor mediated anti-emetic, appetite stimulating, and pain-relieving properties (7-10). Unfortunately, these compounds have become new drugs of choice as many were not initially regulated and are not detected in common drug screening assays. Beginning around 2004, a wide variety of products marketed as "legal" smoking blends or mixtures have been gaining popularity worldwide. These products, with popular brand names such as "SPICE" and 'K2', are typically labeled as "incense" and "not for human consumption," or "for aromatherapy only."

Early reports document undesirable symptoms not commonly associated with marijuana use, including extreme agitation, syncope, tachycardia, and visual and auditory hallucinations (11-14).

The JWH-018 compound is the archetypical naphthoylalkylindole type of synthetic cannabinoid. These synthetic cannabinoids are rapidly processed by the body with the majority of metabolites monohydroxylated on one of many carbons throughout the molecule, and a smaller metabolite group consists of dihydrodiols resulting from arene oxidation of the naphthalene ring system (15). The major metabolite, a monohydroxylated product is then essentially entirely glucuronidated. Additionlly, other metabolites in the human urine include forms monohydroxylated at the ω-1alkyl site, monohydroxylated on the indole group, or carboxylated on the ω alkyl site. These metabolites have longer serum half-lives, similar to that of 11-COOH-THC (16-19).

Assay Principle

The LZI SPICE I (JWH-018/JWH-073/AM2201) Enzyme Immunoassay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (20). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, JWH-018-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody would bind to free drug; the unbound JWH-018-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at 340 nm.

Reagents Provided

Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-JWH-018 metabolite antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09 %) as a preservative.
Enzyme-drug Conjugate Reagent (R_2): Contains JWH-018-metabolite-labeled glucose-6-phosphate dehydrogenase (G6PDH), stabilizers, and sodium azide (0.09 %) as a preservative.

Calibrators and Controls*

**Calibrators and Controls are sold separately and contain negative human urine with sodium azide as a preservative.*

SPICE I (JWH-018 N-[-5-hydroxypentyl] metabolite) Calibrators	REF
THC Negative Calibrator	0002c
Low Calibrator: Contains 10 ng/mL JWH-018 N-(5-hydroxypentyl) metabolite	0502
Cutoff Calibrator: Contains 20 ng/mL JWH-018 N-(5-hydroxypentyl) metabolite	0503
Intermediate Calibrator: Contains 35 ng/mL JWH-018 N-(5-hydroxypentyl) metabolite	0504
High Calibrator: Contains 50 ng/mL JWH-018 N-(5-hydroxypentyl) metabolite	0505

SPICE I (JWH-018 N-[-5-hydroxypentyl] metabolite) Controls	REF
Level 1 Control: Contains 15 ng/mL JWH-018 N-(5-hydroxypentyl) metabolite	0507
Level 2 Control: Contains 25 ng/mL JWH-018 N-(5-hydroxypentyl) metabolite	0508

Precautions and Warning

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes, always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (21).*
- *Do not use the reagents beyond their expiration dates.*

Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be at 2-8ºC when not in use.

Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (22-24). Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days. For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown JWH-type synthetic cannabinoid samples in urine are stable at -20ºC for up to 216 days (25). Samples should be equilibrated to room temperature (18-25ºC) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and both samples should be forwarded to the laboratory for testing.

Handle all urine specimens as if they are potentially infectious.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at 340 nm and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Beckman Coulter® AU400e. If other instruments are used, performance will need to be validated by the laboratory (26, 27).

Assay Procedure

Typical assay parameters used for the Beckman Coulter AU400e analyzer include a 12 µL sample, 120 µL of antibody reagent (R_1), 45 µL of enzyme conjugate reagent (R_2), 10 µL dilution following addition of R_2 in 37ºC incubation temperature, 16-20 reading points, and 340 nm primary wavelength.

For qualitative analysis use the 20 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 15 ng/mL and 25 ng/mL.

Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A preliminary positive test result does not necessarily mean a person took illegal drugs, and a negative test result does not necessarily mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 20 ng/mL of JWH-018 N-(5-hydroxypentyl) metabolite, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (ΔmAU) equal to or greater than that obtained with the cutoff calibrator is considered preliminary positive. A sample with a change in absorbance (ΔmAU) lower than that obtained with the cutoff calibrator is considered negative.

Semi-Quantitative: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for verification by a confirmatory method such as GC/MS, LC/MS or (2) permitting laboratories to establish quality control procedures. When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of JWH-018 N-(5-hydroxypentyl) metabolite in the sample may then be estimated from the calibration curve.

Limitations

1. *A preliminary positive result from the assay indicates only the presence of JWH-018 N-(5-hydroxypentyl) metabolite.*
2. *The test is not intended for quantifying this single analyte in samples.*
3. *A preliminary positive result does not necessarily indicate drug abuse.*
4. *A negative result does not necessarily mean a person did not take illegal drugs.*
5. *Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test results.*
6. *Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
7. *The test is designed for use with human urine only.*
8. *The test is not for therapeutic drug monitoring.*

Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU400e automated chemistry analyzer.

Precision:

Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

Concentration	Within Run (N=20)			Run-to-Run (N=80)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	0.1	0.6	76.0 %	0.1	0.9	N/A
5 ng/mL	4.4	0.7	16.9 %	4.4	0.9	20.5 %
10 ng/mL	10.0	0.7	6.5 %	10.0	0.9	8.5 %
15 ng/mL	14.7	0.7	4.6 %	14.7	0.8	5.4 %
20 ng/mL	19.9	0.6	2.8 %	19.9	0.6	3.2 %
25 ng/mL	24.7	0.7	2.9 %	24.7	0.8	3.2 %
30 ng/mL	29.2	0.6	2.1 %	29.2	0.7	2.5 %
35 ng/mL	34.8	0.6	1.8 %	34.8	0.8	2.4 %
40 ng/mL	41.4	1.0	2.3 %	41.3	1.1	2.6 %

20 ng/mL Cutoff Result:		Within Run (N=20)		Total Precision (N=80)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	20	20 Neg	80	80 Neg
5 ng/mL	25 %	20	20 Neg	80	80 Neg
10 ng/mL	50 %	20	20 Neg	80	80 Neg
15 ng/mL	75 %	20	20 Neg	80	80 Neg
20 ng/mL	100 %	20	11 Neg/ 9 Pos	80	37 Neg/ 43 Pos
25 ng/mL	125 %	20	20 Pos	80	80 Pos
30 ng/mL	150 %	20	20 Pos	80	80 Pos
35 ng/mL	175 %	20	20 Pos	80	80 Pos
40 ng/mL	200 %	20	20 Pos	80	80 Pos

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

Concentration	Within Run (N=20)			Run-to-Run (N=80)		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	-7.3	2.2	-36.5 %	-7.3	3.1	-42.2 %
5 ng/mL	10.1	2.5	26.8 %	10.1	3.2	32.2 %
10 ng/mL	28.9	2.5	8.7 %	28.9	2.8	9.7 %
15 ng/mL	50.1	2.6	5.2 %	50.1	3.4	6.8 %
20 ng/mL	72.7	3.3	4.6 %	72.7	3.9	5.4 %
25 ng/mL	100.3	3.2	3.2 %	100.3	3.9	3.9 %
30 ng/mL	126.2	4.4	3.5 %	126.1	5.1	4.0 %
35 ng/mL	157.6	3.0	1.9 %	157.6	4.2	2.7 %
40 ng/mL	185.0	3.9	2.1 %	185.0	5.5	3.0 %

20 ng/mL Cutoff Result:		Within Run (N=20)		Total Precision (N=80)	
Concentration	% of Cutoff	# Samples	EIA Result	# Samples	EIA Result
0 ng/mL	0 %	20	20 Neg	80	80 Neg
5 ng/mL	25 %	20	20 Neg	80	80 Neg
10 ng/mL	50 %	20	20 Neg	80	80 Neg
15 ng/mL	75 %	20	20 Neg	80	80 Neg
20 ng/mL	100 %	20	16 Neg/ 4 Pos	80	54 Neg/ 26 Pos
25 ng/mL	125 %	20	20 Pos	80	80 Pos
30 ng/mL	150 %	20	20 Pos	80	80 Pos
35 ng/mL	175 %	20	20 Pos	80	80 Pos
40 ng/mL	200 %	20	20 Pos	80	80 Pos

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine with 95 % confidence, was determined to be 3 ng/mL in both qualitative and semi-quantitative analyses.

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control (see semi-quantitative results section), a drug-free pool of processed urine was spiked with JWH-018 N-(5-hydroxypentyl) metabolite to 50 ng/mL and was subsequently diluted. Each sample was run in 10 replicates and the average was used determine percent recovery compared to the expected target value. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follow:

$y = 0.9956x + 0.0622$, $r^2 = 0.999$

% Dilution	Expected Value (ng/mL)	Observed Value (ng/mL)	% Recovery
100 %	0	0.8	N/A
94 %	3	3.5	116.7 %
90 %	5	4.9	98.8 %
80 %	10	9.7	97.0 %
70 %	15	14.6	97.4 %
60 %	20	19.5	97.6 %
50 %	25	24.8	99.3 %
40 %	30	29.4	97.8 %
30 %	35	34.6	98.9 %
20 %	40	40.1	100.3 %
10 %	45	46.1	102.3 %
0 %	50	49.6	99.1 %

Specificity: Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually to various concentrations and evaluated against the cutoff calibrator.
The table listed the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator or the maximal concentration of the compound tested that gave a response below the response of the cutoff calibrator.

Structurally Related Synthetic Cannabinoid Compounds:

Compound	Spiked [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross Reactivity
AB-PINACA	10,000	1.8	0.0 %
AM-2201 6-hydroxyindole metabolite	50	21.9	43.7 %
AM-2201 N-(4-hydroxypentyl) metabolite	25	25.8	103.0 %
AM-694 N-(5-hydroxypentyl) metabolite	25	19.1	76.4 %
JWH-007	35	19.6	56.0 %
JWH-015	35	25.4	72.4 %
JWH-022	35	26.4	75.4 %
(±) JWH-018 N-(4-hydroxypentyl) metabolite	25	22.3	89.2 %
JWH-018 5-hydroxyindole metabolite	25	22.0	88.0 %
JWH-018 N-(5-hydroxypentyl)-b-D-glucuronide	35	22.5	64.1 %
JWH-018 N-(pentanoic acid) metabolite	25	24.9	99.6 %
JWH-019 N-(5-hydroxyhexyl) metabolite	25	23.3	93.0 %

Structurally Related Synthetic Cannabinoid Compounds: continued

Compound	Spiked [ ] (ng/mL)	EIA [ ] (ng/mL)	% Cross Reactivity
(±) JWH-073 N-(3-hydroxybutyl) metabolite	25	25.6	102.2 %
JWH-073 6-hydroxyindole metabolite	25	19.0	75.8 %
JWH-073 N-(4-butanoic acid) metabolite	25	26.1	104.2 %
JWH-073 N-(4-hydroxybutyl) metabolite	25	24.3	97.2 %
JWH-073 N-(4-hydroxybutyl)-b-D-glucuronide	45	24.0	53.2 %
JWH-081 N-(5-hydroxypentyl) metabolite	25	21.6	86.4 %
JWH-122 N-(5-hydroxypentyl) metabolite	25	24.4	97.4 %
JWH-203 N-(5-hydroxypentyl) metabolite	45	22.2	49.2 %
JWH-210 N-(5-hydroxypentyl) metabolite	25	21.6	86.4 %
JWH-250 N-(5-hydroxypentyl) metabolite	100	18.5	18.5 %
JWH-398 N-(5-hydroxypentyl) metabolite	25	21.8	87.0 %
MAM-2201 N-(4-hydroxypentyl) metabolite	35	20.7	59.4 %
Methaqualone	2000	22.2	1.1 %
1'-Naphthoyl Indole	20	24.7	123.3 %
RCS-4 N-(5-hydroxypentyl) metabolite	50	19.1	38.1 %
THJ	10,000	3.1	0.0 %
THJ-018	100	26.6	26.6 %
THJ-2201	50	23.9	47.7 %
UR-144 N-(5-hydroxypentyl) metabolite	600	19.4	3.2 %
UR-144 N-(pentanoic acid) metabolite	550	21.6	3.9 %
XLR-11-144 N-(4-hydroxypentyl) metabolite	550	22.3	4.1 %

Structurally Unrelated Pharmacological Compounds: Various structurally unrelated compounds that are potential interferents were tested for cross-reactivity with the assay. Test compounds were spiked into a pool of processed drug free urine to the desired concentrations and then JWH-018 N-(5-hydroxypentyl) metabolite was spiked to a final concentration of 0 ng/mL or the negative control concentration of 15 ng/mL, or the positive control concentration of 25 ng/mL. The table below lists the concentration measured of each test compound.

Interfering Substances	Spiked [ ] (ng/mL)	JWH-018 N-(5-hydroxypentyl) metabolite		
		0 ng/mL (ng/mL)	15 ng/mL Control (ng/mL)	25 ng/mL Control (ng/mL)
Acetaminophen	100,000	-0.9	15.0	24.5
6-Acetylmorphine	10,000	-1.2	13.5	25.8
Acetylsalicylic Acid	100,000	0.6	15.4	25.5
Alpha-hydroxy-alprazolam	10,000	0.5	15.2	25.0
Amitryptyline	100,000	0.3	14.0	25.6
Amobarbital	100,000	0.5	14.9	25.8
Amphetamine	100,000	-1.9	13.3	23.5
Benzoylecgonine	100,000	2.0	13.9	25.7
Buprenorphine	20,000	3.4	17.4	26.2
Burpropion	50,000	3.6	16.5	27.3
Caffeine	100,000	-0.3	15.2	24.9
Chlorpheniramine	100,000	-1.0	15.5	26.2
Chlorpromazine	100,000	-0.1	15.5	27.9
Cocaine	100,000	-0.3	15.7	26.1
Codeine	100,000	0.5	15.2	26.7
Dextromethorphan	100,000	0.2	15.3	26.0
Diazepam	10,000	-1.2	15.0	26.0
Ecgonine Methyl Ester	100,000	0.6	15.0	23.5
d,l-Ephedrine	100,000	1.0	16.0	24.5
Hydrocodone	10,000	-1.7	14.5	24.1
Hydromorphone	10,000	-1.8	13.4	23.7
Imipramine	100,000	0.8	15.8	25.1
Lidocaine	100,000	2.4	17.9	27.4
Lorazepam	10,000	-2.2	14.1	24.3
MDMA	100,000	0.6	14.9	23.8
Meperidine	100,000	-1.6	14.3	24.3
Methadone	100,000	-0.1	15.4	25.5
Methamphetamine	100,000	-2.7	14.4	24.2
Morphine	100,000	-3.5	14.2	23.1
Nobuprenophine	10,000	0.1	13.7	25.1
Nordiazepam	10,000	0.2	13.7	23.9
l-11-Nor-Δ9-THC-9-Carboxylic Acid	10,000	-1.7	13.5	24.5
Nortriptyline	100,000	-0.7	16.0	25.2
Oxazepam	100,000	-1.1	15.5	24.7
Oxycodone	10,000	-1.0	14.2	24.7
Oxymorphone	10,000	-1.1	14.0	24.8

Structurally Unrelated Pharmacological Compounds: continued

Interfering Substances	Spiked [ ] (ng/mL)	JWH-018 N-(5-hydroxypentyl) metabolite		
		0 ng/mL (ng/mL)	15 ng/mL Control (ng/mL)	25 ng/mL Control (ng/mL)
Phencyclidine	100,000	-0.7	14.7	24.9
Phenobarbital	100,000	-2.6	14.3	25.3
Promethazine	100,000	0.8	15.7	25.1
Propoxyphene	100,000	-1.1	15.0	26.1
Ranitidine	100,000	0.3	15.4	25.4
Secobarbital	100,000	0.8	15.8	25.3
Valproic Acid	100,000	-0.1	15.3	25.4
Zolpidem	4000	3.1	17.3	26.6
Zopiclone	5000	-1.0	14.3	24.4

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.

Interference: Endogenous Substances

The following potentially interfering compounds were spiked into a pool of processed drug free urine to the desired concentrations and then spiked with JWH-018 N-(5-hydroxypentyl) metabolite to a final concentration of 0 ng/mL or the negative control concentration of 15 ng/mL, or the positive control concentration of 25 ng/mL. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff value of 20 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked [ ] (ng/mL)	JWH-018 N-(5-hydroxypentyl) metabolite		
		0 ng/mL (ng/mL)	15 ng/mL Control (ng/mL)	25 ng/mL Control (ng/mL)
Acetone	1000	3.5	17.9	27.4
Ascorbic Acid	400	-1.8	15.4	25.7
Creatinine	500	-1.1	16.0	26.5
Ethanol	1000	1.0	16.1	26.1
Galactose	10	-1.1	15.0	24.5
γ-Globulin	500	-0.4	14.9	25.3
Glucose	3000	-0.6	14.2	24.8
Hemoglobin	300	-0.4	16.4	26.8
Human Serum Albumin	500	-0.3	16.5	26.5
Oxalic Acid	100	-1.3	15.2	24.9
Riboflavin	0.3	-1.1	15.4	25.8
Urea	2000	-0.6	14.9	25.3
Sodium Chloride	2000	-2.5	12.3	23.2
pH 3	N/A	-1.6	13.9	24.4
pH 4	N/A	-0.4	13.9	25.6
pH 5	N/A	-0.5	15.0	25.6
pH 6	N/A	-0.1	15.2	25.3
pH 7	N/A	0.3	16.4	25.3
pH 8	N/A	0.2	15.1	25.4
pH 9	N/A	0.0	15.6	25.0
pH 10	N/A	0.6	16.4	26.1
pH 11	N/A	1.3	16.6	25.2

Specific gravity: Samples ranging in specific gravity from 1.000 to 1.030 were spiked with JWH-018 N-(5-hydroxypentyl) metabolite to a final concentration of 0 ng/mL, the negative control concentration of 15 ng/mL, or the positive control concentration of 25 ng/mL. No interference was observed.

Specific Gravity Value	0 ng/mL	15 ng/mL Control	25 ng/mL Control
1.000	2.0	12.6	22.9
1.005	1.9	12.4	22.1
1.007	1.5	11.1	21.3
1.010	1.1	14.9	25.0
1.015	0.1	13.3	22.9
1.018	-0.5	13.9	24.0
1.020	-1.5	14.3	23.8
1.025	-0.7	12.3	21.8
1.027	-1.3	11.6	21.2
1.030	-1.8	13.0	22.4

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