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Discovering Cross-Reactivity in Urine Drug Screening Immunoassays through Large-Scale Analysis of Electronic Health Records

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BACKGROUND: Exposure to drugs of abuse is frequently assessed using urine drug screening (UDS) immunoassays. Although fast and relatively inexpensive, UDS assays often cross-react with unrelated compounds, which can lead to false-positive results and impair patient care. The current process of identifying cross-reactivity relies largely on case reports, making it sporadic and inefficient, and rendering knowledge of cross-reactivity incomplete. Here, we present a systematic approach to discover cross-reactive substances using data from electronic health records (EHRs).

METHODS: Using our institution's EHR data, we assembled a data set of 698 651 UDS results across 10 assays and linked each UDS result to the corresponding individual's previous medication exposures. We hypothesized that exposure to a cross-reactive ingredient would increase the odds of a false-positive screen. For 2201 assay–ingredient pairs, we quantified potential cross-reactivity as an odds ratio from logistic regression. We then evaluated cross-reactivity experimentally by spiking the ingredient or its metabolite into drug-free urine and testing the spiked samples on each assay.

RESULTS: Our approach recovered multiple known cross-reactivities. After accounting for concurrent exposures to multiple ingredients, we selected 18 compounds (13 parent drugs and 5 metabolites) to evaluate experimentally. We validated 12 of 13 tested assay–ingredient pairs expected to show cross-reactivity by our analysis, discovering previously unknown cross-reactivities affecting assays for amphetamines, buprenorphine, cannabinoids, and methadone.

CONCLUSIONS: Our findings can help laboratorians and providers interpret presumptive positive UDS results. Our data-driven approach can serve as a model for high-throughput discovery of substances that interfere with laboratory tests.

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Urine drug screening (UDS)[3] immunoassays are one of the primary methods to assess exposure to drugs of abuse. Although UDS assays are fast, simple, and relatively inexpensive, they often cross-react with compounds they were not designed to detect. This cross-reactivity can cause the screen to be presumptive positive in the absence of the target drug, and is one reason presumptive positive results should be confirmed by a more specific technique, such as LC-MS/MS. However, many clinical laboratories do not perform their own confirmatory testing, and even if they do, results are generally not available until several days later. Consequently, patient care decisions (e.g., in emergent situations) may be made on the basis of the screen alone. False-positive screens can lead providers to make incorrect assumptions about drug exposure and damage the relationship between provider and patient. A comprehensive list of which compounds cross-react on which UDS immunoassays could markedly improve the reliability of UDS results and thereby improve patient care.

Currently, the identification of new cross-reactivities relies largely on false-positive screens catching the attention of a laboratorian, who may then check for drugs in common on the patients' medication lists and decide which drug(s) to test for cross-reactivity experimentally *(1)*. This case-based approach is inefficient and unlikely to identify cross-reactivity of infrequently used medications. Efforts involving more comprehensive chart review have focused on estimating the frequency of false-positive screens caused by known cross-reactants, not on discovering and validating new ones *(2–4)*. An approach based on analysis by high-resolution mass spec-

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[3] Nonstandard abbreviations: UDS, urine drug screening; EHR, electronic health record; IRB, Institutional Review Board; SD, Synthetic Derivative; OMOP, Observational Medical Outcomes Partnership; OR_{FP}, odds ratio false positive; OR_{TP}, odds ratio true positive.

trometry has shown promise on a small scale *(5)* but is labor- and cost-intensive and limited by the completeness of compound databases. Computational approaches based on molecular similarity *(6, 7)* suffer from the limitation that some cross-reactants are not structurally similar to the assay's target compound *(1, 8)*. Thus, many cross-reactivities likely remain unknown.

An increasingly valuable resource for biomedical discovery is the electronic health record (EHR), which documents the course of each patient's clinical care. Large-scale analyses of EHR data have revealed associations between treatments and outcomes or adverse events *(9–11)*, as well as drug–drug interactions *(12)*. Although EHR data are observational, making causal inference a challenge *(13, 14)*, evidence from large-scale analyses can help prioritize hypotheses for further investigation *(12, 15)*.

In this study, we sought to identify cross-reactive substances based on statistical analysis of EHR data. We combined >5 years' worth of UDS results and documented drug exposures to quantify the potential cross-reactivity of hundreds of drugs on 10 screening assays at our institution. We then validated the cross-reactivity of selected compounds experimentally.

Materials and Methods

Code and summary-level data for this study are available at https://doi.org/10.6084/m9.figshare.8079944. Access to individual-level EHR data was restricted by the Institutional Review Board (IRB). The Vanderbilt IRB reviewed and approved this study as nonhuman subjects research (IRB nos. 081418 and 190165).

EXTRACTION OF UDS RESULTS AND DRUG EXPOSURES FROM EHR DATA

We extracted EHR data from the Synthetic Derivative (SD), a database of deidentified clinical data from Vanderbilt University Medical Center *(16)* that is formatted according to the Observational Medical Outcomes Partnership (OMOP) Common Data Model *(17)*. As part of the deidentification process to create the SD, dates are shifted backward by a number of days between 0 and 364. The date shift is constant for all events related to a given person, but varies from person to person. We extracted results for UDSs and corresponding confirmations in the SD dated September 21, 2013 or later, to ensure that all UDS results in our data set were collected after Vanderbilt University Medical Center began using the screening assays currently in use. Although the screen result becomes available in the medical record before the corresponding confirmation result, the two results ultimately receive the same timestamp in the SD. Therefore, we used the timestamp to link screen and confirmation results for a given urine sample from a given person.

At our institution, samples with a presumptively positive UDS result are reflexively sent for confirmatory testing (assuming adequate sample volume), but physicians can also directly order confirmatory testing with or without a UDS panel. We included only UDS results in which the sample (*a*) screened negative and was not sent for confirmatory testing, (*b*) screened presumptive positive and confirmed negative (which we called a false positive), or (*c*) screened presumptive positive and confirmed positive (which we called a true positive). We excluded results in which the sample confirmed positive and either was not screened or screened negative.

The confirmation assays were based on GC-MS (opiates, oxycodone, amphetamines, barbiturates, methadone, cannabinoids, cocaine, and tricyclic antidepressants) or LC-MS/MS (benzodiazepines and buprenorphine). All drug screen results and most confirmation results were qualitative. All quantitative results were from confirmation assays related to the buprenorphine screen: buprenorphine, buprenorphine-glucuronide, norbuprenorphine, and norbuprenorphine-glucuronide. For those assays, we considered the confirmation for a given sample positive if the result from at least 1 of the 4 assays was numeric (indicating a measured concentration above the cutoff) or included the string "positive," and negative if the results from all 4 assays started with "<" (indicating a measured concentration less than the cutoff) or included the string "negative."

In general, not every screening assay target is included in the confirmation panels. In addition, the confirmation is in some cases only marginally more analytically sensitive than the screen. In the case of tricyclic antidepressants, the screen detects parent drugs as well as active metabolites. Thus, the total amount of detected compounds in a sample could meet the cutoff for a positive screen even if no compound individually meets the cutoff for a positive confirmation, which would lead to an apparent false-positive result.

For each person in the data set, we identified drug exposures documented between 1 and 30 days before each UDS result. We excluded UDS results that occurred <30 days after the person's first ever visit at Vanderbilt University Medical Center because we would lack a previous 30 days of documented drug exposures. Documented drug exposures are available as structured data in the SD and come primarily from medication lists, but also from physician-administered drugs, prescriptions written, Current Procedural Terminology (CPT) codes corresponding to drugs, inpatient administrations, prescriptions dispensed in pharmacy, and patient self-reports. Exposures from patient self-reports (which are contained in problem lists) and medication lists were previously extracted into the OMOP-structured data using a validated algorithm called MedEx *(18)*. We mapped each drug to its active ingredient(s) using RxNorm *(19)*,

creating a list of the distinct ingredients to which a person was exposed in the 30-day period before providing the urine sample.

We did not limit the drug exposures to any particular type, e.g., inpatient administrations. We also did not verify that drugs documented in medication lists were actually taken or that prescriptions written were actually filled (e.g., by an outpatient pharmacy) and then taken because these data do not exist. Furthermore, the date on which the exposure is documented does not necessarily correspond to the date(s) on which a person has actually been exposed (e.g., for medication lists that are updated during outpatient encounters or outpatient prescription orders). In addition, the vast majority of documented drug exposures lack information for dose, quantity, and refills. Thus, having a documented exposure within 30 days is only a proxy for being exposed at the time of the UDS.

STATISTICAL ANALYSIS OF UDS RESULTS AND DRUG EXPOSURES

To quantify associations between ingredients and false-positive screens, we used logistic regression. For an assay–ingredient pair, we fit a logistic regression model in which the dependent variable corresponded to the UDS result (negative or false positive) and the independent variable corresponded to presence or absence of previous exposure to the ingredient. We fit each model using Firth's method, which reduces bias in maximum likelihood estimates and is especially apt for rare events *(20, 21)*. We used the resulting coefficient to calculate an odds ratio (equal to the exponentiated coefficient), and used the 95% CI based on the penalized profile likelihood. An odds ratio of 2 meant that the odds of a false-positive screen (as opposed to a negative screen) on that assay doubled if the person had a documented exposure to that ingredient between 1 and 30 days previously. We fit a model for an assay–ingredient pair only if at least 5 individuals exposed to the ingredient had a false-positive screen on the assay. We quantified associations between ingredients and true-positive screens in the same way.

The large size of our data set caused small effects to become highly statistically significant. Therefore, although Firth logistic regression provides a P value for each model coefficient, we focused on the ingredients with the strongest effects by sorting on the lower bound of the 95% CI of the odds ratio.

For the ingredients most strongly associated with false-positive results on a screening assay, we calculated the percentage of exposures to one ingredient for which the person was also exposed to a second ingredient. We considered only exposures preceding negative and false-positive screens. To distinguish the effects of concurrent exposure to multiple ingredients, we added terms to the logistic regression model that corresponded to each individual ingredient and all pairwise interactions.

We defined known cross-reactants as substances whose cross-reactivity was (*a*) described in the package insert for the screening assay or (*b*) validated in a scientific publication for any screening assay designed to recognize the same drug(s).

EXPERIMENTAL VALIDATION OF CROSS-REACTIVITY

We evaluated each compound's cross-reactivity by spiking a reference standard into drug-free urine at various concentrations and testing the spiked urine samples in singlicate on the panel of 10 screening assays on an Abbott Architect c16000 chemistry analyzer. The buprenorphine assay was a cloned enzyme donor immunoassay with detection at 660 nm. All other assays were homogeneous enzyme immunoassays with detection at 340 nm (Table 1). We considered a compound's cross-reactivity on an assay validated if the concentration of target drug registered by the assay ever reached the cutoff used to call a UDS result presumptive positive. We used linear interpolation to estimate the concentration of the test compound at which the assay registered a concentration equal to the cutoff.

We purchased reference standards from Sigma-Aldrich, MedChemExpress, Toronto Research Chemicals, and LGC Standards. We prepared stock solutions of each standard in saline when possible, or in methanol when solubility in saline was negligible. We spiked the urine samples using a fixed volume of 20% spiking solution made of a combination of diluent and stock solution, including one sample per compound with only diluent to serve as a negative control. In most cases, we tested the maximum technically feasible concentration for a compound, given the limits of solubility, the concentration of the reference material, and the fixed 20% spiking volume.

Results

BUILDING A DATA SET OF UDS RESULTS AND DRUG EXPOSURES

We first assembled a data set of UDSs and confirmations performed since our institution implemented the screening assays currently in use. Our data set contained 698 651 UDS results for 10 assays and 40 741 individuals (Table 1). The false-positive rates of the assays (percent of presumptive positive screens that confirmed negative) varied from 0% to 43%. The highest false-positive rates came from the assays for amphetamines, buprenorphine, and tricyclic antidepressants.

We next added to our data set, for each person, all documented drug exposures occurring between 1 and 30 days before a UDS result. Our data set included exposures to 2027 ingredients. Each UDS result was

Table 1. Characteristics of UDS immunoassays in this study.

Target drug(s)	Format	Manufacturer/Brand	Cutoff, ng/mL	Number of UDS results		
				Negative screen	Presumptive positive screen, positive confirmation	Presumptive positive screen, negative confirmation
Amphetamines	Homogeneous enzyme immunoassay	Abbott MULTIGENT	500	66 747	3641	1393
Barbiturates	Homogeneous enzyme immunoassay	Abbott MULTIGENT	200	70 532	1191	47
Benzodiazepines	Homogeneous enzyme immunoassay	Abbott MULTIGENT	200	58 922	12 412	196
Buprenorphine	CEDIA®[a] immunoassay	Thermo Scientific	5	62 046	6608	2231
Cannabinoids	Homogeneous enzyme immunoassay	Abbott MULTIGENT	50	61 875	9573	228
Cocaine metabolite	Homogeneous enzyme immunoassay	Abbott MULTIGENT	300	68 284	3571	0
Methadone	Homogeneous enzyme immunoassay	Abbott MULTIGENT	300	70 292	1100	148
Opiates	Homogeneous enzyme immunoassay	Abbott MULTIGENT	300	47 474	19 050	306
Oxycodone	Homogeneous enzyme immunoassay (DRI®)	Thermo Scientific	300	47 636	11 377	19
Tricyclic antidepressants	Homogeneous enzyme immunoassay	Abbott MULTIGENT	300	65 548	3571	2633

[a] CEDIA, cloned enzyme donor immunoassay.

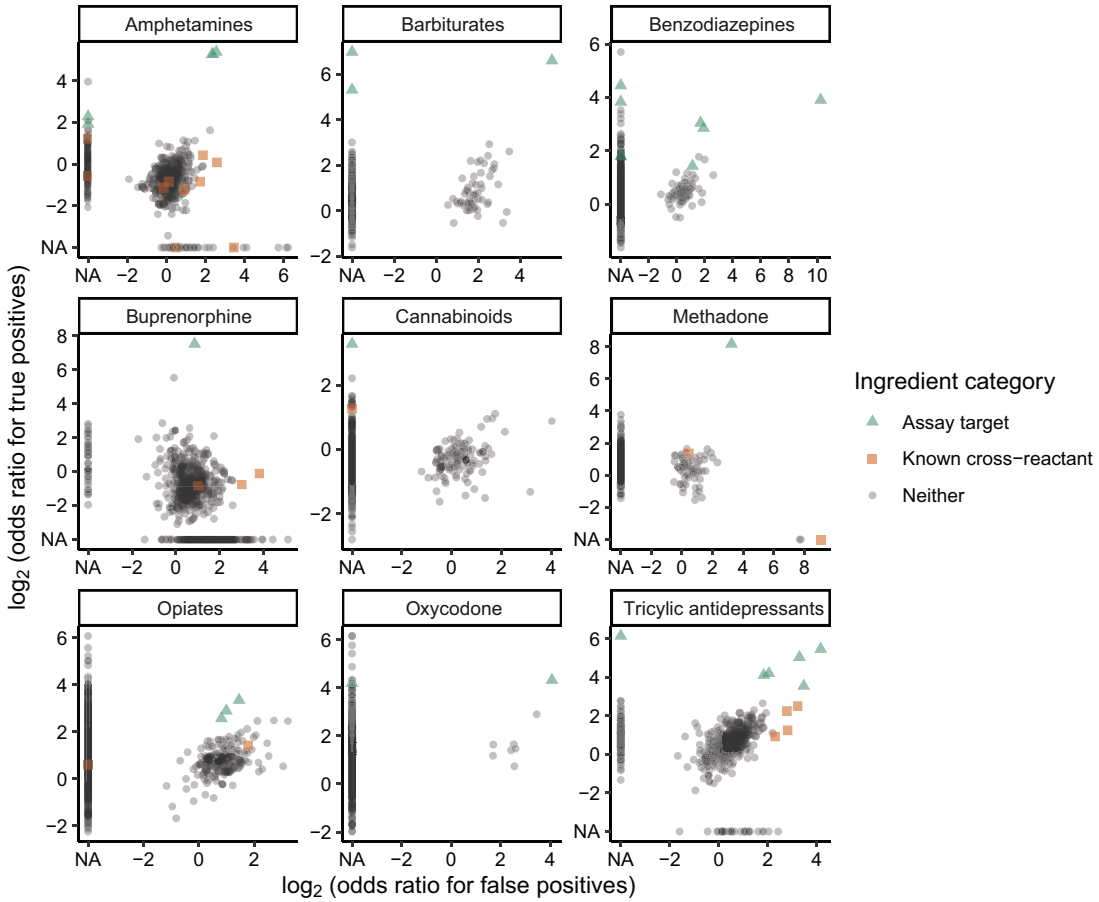

Fig. 1. Establishing validity of the data and approach.

Each plot corresponds to a screening assay, and each point corresponds to an ingredient. A $\log_2$ (odds ratio) of NA indicates that the association was not tested, as <5 individuals had a false-positive (NA on *x* axis) or true-positive (NA on *y* axis) UDS result preceded by exposure to the given ingredient. The green triangle in the upper right of the benzodiazepines plot corresponds to clobazam, which is detected by the screen but not by the confirmation. See Materials and Methods for an explanation of why some other assay targets (e.g., several tricyclic antidepressants) had increased odds ratios for false-positive screens.

preceded by exposure to a median of 3 ingredients (see Fig. 1 in the online Data Supplement), and the median number of UDS results preceded by exposure to a specific ingredient was 190 (see Table 1 in the online Data Supplement).

QUANTIFYING ASSOCIATIONS BETWEEN UDS RESULTS AND DRUG EXPOSURES

We hypothesized that exposure to a cross-reactive ingredient would increase the odds of a false-positive screen. To quantify the association between exposures to an ingredient and false-positive results on a screening assay, we used logistic regression to calculate an odds ratio (which we call OR_{FP}) and 95% CI. We used the same approach to quantify associations between ingredient exposures and true-positive screens (for which we call the odds ratio OR_{TP}), which we hypothesized would identify assay targets. Altogether, we calculated OR_{FP} for 2201 assay–ingredient pairs (see Table 2 in the online Data Supplement) and OR_{TP} for 6464 assay–ingredient pairs (see Table 3 in the online Data Supplement).

To validate our data and approach, we examined the odds ratios (for false-positive screens, OR_{FP}; for true-positive screens, OR_{TP}) of known cross-reactants and targets for each screening assay (Fig. 1). Known cross-reactants generally had among the highest OR_{FP} for a given assay and were either not tested for association with true-positive results or had $OR_{FP} > OR_{TP}$. In addition, assay targets generally had the highest OR_{TP} and were either not tested for association with false-positive find-

Table 2. Top-ranked ingredients associated with false positives on the amphetamines and buprenorphine screens.[a]

Screening assay	Ingredient	Odds ratio	95% CI Lower	95% CI Upper	Exposure frequency	Previous status
Amphetamines	Ceftaroline	73.8	37.4	150.4	$4.8 \cdot 10^{-4}$	Unknown
	Ceftaroline fosamil	52.5	23.4	119.0	$3.4 \cdot 10^{-4}$	Unknown
	Procainamide	69.5	21.0	251.2	$1.5 \cdot 10^{-4}$	Unknown
	Imatinib	17.0	10.0	27.7	$1.1 \cdot 10^{-3}$	Unknown
	Methyldopa	15.2	9.2	24.4	$1.3 \cdot 10^{-3}$	Unknown
	Esmolol	10.7	6.1	17.8	$1.3 \cdot 10^{-3}$	Unknown
	Mexiletine	10.9	5.6	19.5	$9.8 \cdot 10^{-4}$	Cross-reactant
	Trazodone	6.0	5.2	6.8	$4.6 \cdot 10^{-2}$	Cross-reactant
	Dextroamphetamine	5.2	3.6	7.3	$5.6 \cdot 10^{-3}$	Assay target
	Amphetamine	5.0	3.5	7.0	$5.6 \cdot 10^{-3}$	Assay target
Buprenorphine	Methoxsalen	34.5	13.9	87.9	$2.8 \cdot 10^{-4}$	Unknown
	Hydroxychloroquine	14.1	11.2	17.5	$5.7 \cdot 10^{-3}$	Cross-reactant
	Propafenone	15.1	8.1	27.2	$7.2 \cdot 10^{-4}$	Unknown
	Rotigotine	21.3	7.3	59.1	$2.2 \cdot 10^{-4}$	Unknown
	Levofloxacin	8.0	7.1	9.1	$2.9 \cdot 10^{-2}$	Cross-reactant
	Cytarabine	9.2	6.9	12.3	$3.9 \cdot 10^{-3}$	Unknown
	Decitabine	11.8	6.6	20.3	$9.0 \cdot 10^{-4}$	Unknown
	Belimumab	15.5	6.0	37.0	$3.1 \cdot 10^{-4}$	Unknown
	Posaconazole	9.5	5.2	16.6	$9.3 \cdot 10^{-4}$	Unknown
	Sulfamethoxazole	6.0	5.2	6.9	$2.5 \cdot 10^{-2}$	Unknown

[a] Ingredients are sorted by lower bound of the 95% CI of the odds ratio (OR_{FP}). All tested associations for all screening assays are in Table 2 of the online Data Supplement.

ings or had $OR_{FP} < OR_{TP}$. One exception was clobazam, which had $OR_{FP} > OR_{TP}$ for the benzodiazepines screen, consistent with the fact that clobazam is detected by the screening assay, but not by our institution's benzodiazepine confirmation assay (thus leading to apparent false-positive results). Taken together, these findings indicate that our approach captures the effects of drug exposure on UDS results, and that OR_{FP} is a metric for potential cross-reactivity.

To focus on the ingredients with the strongest evidence for cross-reactivity, we ranked the associations for each screening assay by the lower bound of the 95% CI of OR_{FP}. The top-ranked ingredients included known cross-reactants and assay targets, but also substances whose cross-reactivity had not previously been described (Table 2). Defining ingredient exposures based on the previous 15 days or 60 days, instead of 30 days, gave very similar results (see Tables 4 and 5 in the online Data Supplement).

We reasoned that an ingredient that did not cross-react on a particular assay could still be associated with false-positive screens if individuals exposed to that ingredient were frequently exposed to another ingredient that did cross-react. To investigate this possibility, we calculated the coexposure frequency: the percentage of UDS results preceded by exposure to one ingredient that were also preceded by exposure to a second ingredient (see Fig. 2 in the online Data Supplement). Many ingredients previously not known to be cross-reactive had a low coexposure frequency with known cross-reactants and assay targets, providing additional evidence that these substances are true cross-reactants.

Several top-ranked ingredients on the buprenorphine screen (including cytarabine) had a high coexposure frequency with the known cross-reactant levofloxacin *(8)*. To revise our estimate of potential cross-reactivity of each of these ingredients, we extended the logistic regression model to account for levofloxacin exposure. This adjustment tended to result in a smaller OR_{FP} and a wider 95% CI that spanned $OR_{FP} = 1$ (see Fig. 3 in the online Data Supplement), suggesting that these substances were unlikely to be cross-reactive on their own. In addition, we could not distinguish the potential cross-reactivity of sulfamethoxazole and trimethoprim because the

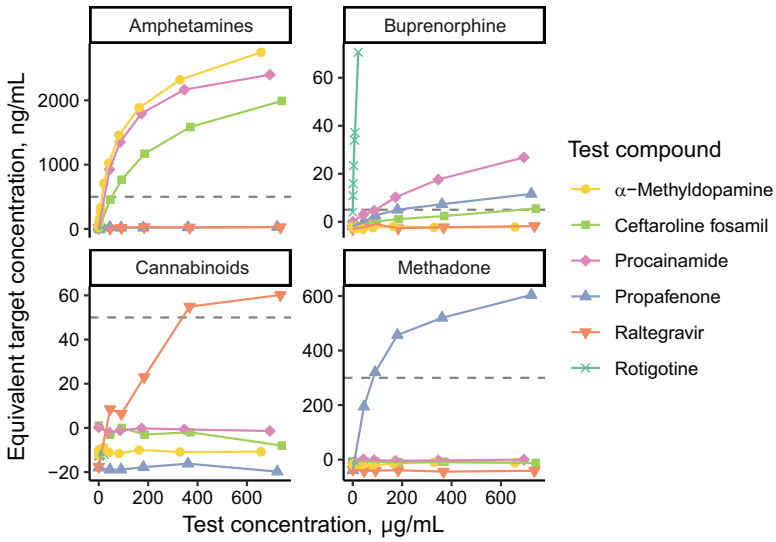

Fig. 2. Validating cross-reactivity by spiking compounds into drug-free urine.

Dashed lines show the cutoff for each screening assay. Plots show only a subset of the tested compounds and a subset of the assays. Data from all spiking experiments are in Table 6 of the online Data Supplement.

two ingredients had coexposure frequencies with each other near 100%.

VALIDATING PREDICTED CROSS-REACTIVITY EXPERIMENTALLY

To test the hypotheses raised by this analysis, we experimentally evaluated the cross-reactivity of 18 compounds (13 parent drugs and 5 metabolites). Overall, we validated the cross-reactivity of 15 assay–ingredient pairs (in which the ingredient's cross-reactivity could be due to the parent drug or a metabolite), including 12 of 13 tested pairs for which cross-reactivity was expected based on our analysis (Fig. 2 and Table 3 here and also Table 6 in the online Data Supplement). Only donepezil (and its metabolite 6-o-desmethyldonepezil) on the amphetamines screen failed to show sufficient cross-reactivity to cause a presumptive positive at the concentrations tested. Trimethoprim, but not sulfamethoxazole, cross-reacted on the buprenorphine screen. As expected, cytarabine did not cross-react on the buprenorphine screen. Furthermore, most metabolites showed similar cross-reactivity profiles to their respective parent drugs, with one exception: α-Methyldopamine (a metabolite of methyldopa) cross-reacted on the amphetamines screen, although methyldopa itself and another metabolite, 3-o-methyldopa, did not. Altogether, the newly discovered cross-reactivities affect the screening assays for amphetamines, buprenorphine, cannabinoids, and methadone.

Four ingredients were cross-reactive on multiple assays: ceftaroline fosamil and procainamide on the amphetamines and buprenorphine screens, rotigotine on the buprenorphine and cannabinoid screens, and propafenone on the buprenorphine and methadone screens. The cross-reactivity of procainamide on the buprenorphine screen and rotigotine on the cannabinoid screen was unexpected because, owing to low numbers of UDS results, we had not quantified the associations. For the same reason, we had not calculated OR_{FP} of ceftaroline fosamil (a prodrug) on the buprenorphine screen, although OR_{FP} of ceftaroline (the active metabolite) was in the top 20.

To estimate how many false-positive screens on a particular assay could be explained by various ingredients, we calculated the percentage of false-positive screens preceded by exposure to an assay target, known cross-reactant, or newly identified cross-reactant (Fig. 3A). Altogether, these ingredients could explain between 5.3% and 52.6% of false-positive screens in our data set, depending on the assay (Fig. 3B).

Discussion

Although the issue of cross-reactivity in UDS assays is well known, the identification of new cross-reactivities has relied on serendipity, making it sporadic and inefficient. We developed and validated an approach to systematically discover cross-reactivity using large-scale analysis of EHR data. Our approach also enabled comprehensive estimates of the fraction of false-positive screens explained by exposure to various ingredients.

Table 3. Experimental validation of cross-reactivity, including parent drugs and metabolites.[a]

Screening assay	Compound tested	Parent drug	Concentration causing a presumptive positive, μg/mL
Amphetamines	α-Methyldopamine	Methyldopa	13.6
	Procainamide	–	23.2
	Ceftaroline fosamil	–	53.1
	N-acetyl-3-hydroxyprocainamide	Procainamide	92.2
	Imatinib	–	216.6
	Esmolol	–	237.3
	Esmolol acid	Esmolol	446.4
	Methyldopa	–	NA
	3-o-Methyldopa	Methyldopa	NA
	Donepezil	–	NA
	6-o-Desmethyldonepezil	Donepezil	NA
Buprenorphine	Rotigotine	–	0.13
	Trimethoprim	–	47.2
	Procainamide	–	92.8
	N-acetyl-3-hydroxyprocainamide	Procainamide	126.2
	Propafenone	–	180.7
	Ceftaroline fosamil	–	681.5
	Donepezil	–	709.6
	Sulfamethoxazole	–	NA
	Cytarabine	–	NA
Cannabinoids	Raltegravir	–	339.5
	Rotigotine	–	415.1
Methadone	Propafenone	–	83.2
	Pazopanib	–	198.4

[a] NA indicates the compound was not sufficiently cross-reactive to cause a presumptive positive on the given screening assay at the concentrations tested. Cytarabine was not expected to be cross-reactive. In addition, based on the EHR data analysis, the potential cross-reactivity of trimethoprim and sulfamethoxazole could not be distinguished.

Our data-driven approach produces hypotheses about cross-reactivity based on statistical associations. A strong association means the probability of a false-positive screen is increased by previous exposure to a given ingredient, but does not necessarily mean the false-positive screen is caused by the ingredient itself. One alternative possibility is that the association is due to a metabolite, as we found on the amphetamines screen with methyldopa. A second possibility is that the association is spurious and caused by coexposures with another ingredient that is cross-reactive, as we found on the buprenorphine screen with cytarabine. To be considered conclusive, the hypotheses raised by our approach should be validated experimentally.

Our findings suggest that the sources of many false-positive UDS results remain to be discovered. In the future, it may be possible to refine statistical associations from EHR data by leveraging structural or pharmacological similarity *(6, 7)* or knowledge of shared metabolites. Future work could also explore the likely scenario that some false-positive results are caused by multiple cross-reactive drugs.

The large size of our data set allowed us to discover ingredients that, although infrequently used, are strongly cross-reactive. However, because our approach relies on exposures documented in the EHR (e.g., in the medication list), it is likely less sensitive to cross-reactivity of drugs that are typically taken over-the-counter, especially if they are taken for only a short time and not reported to a provider. In addition, because our approach considers all documented exposures to a given drug, cross-reactivity caused by rare cases of overdose may be masked by a lack of cross-reactivity under typical dosing.

To assess cross-reactivity efficiently, we chose a wide concentration range for spiking each compound in urine (in most cases, up to the maximum technically feasible

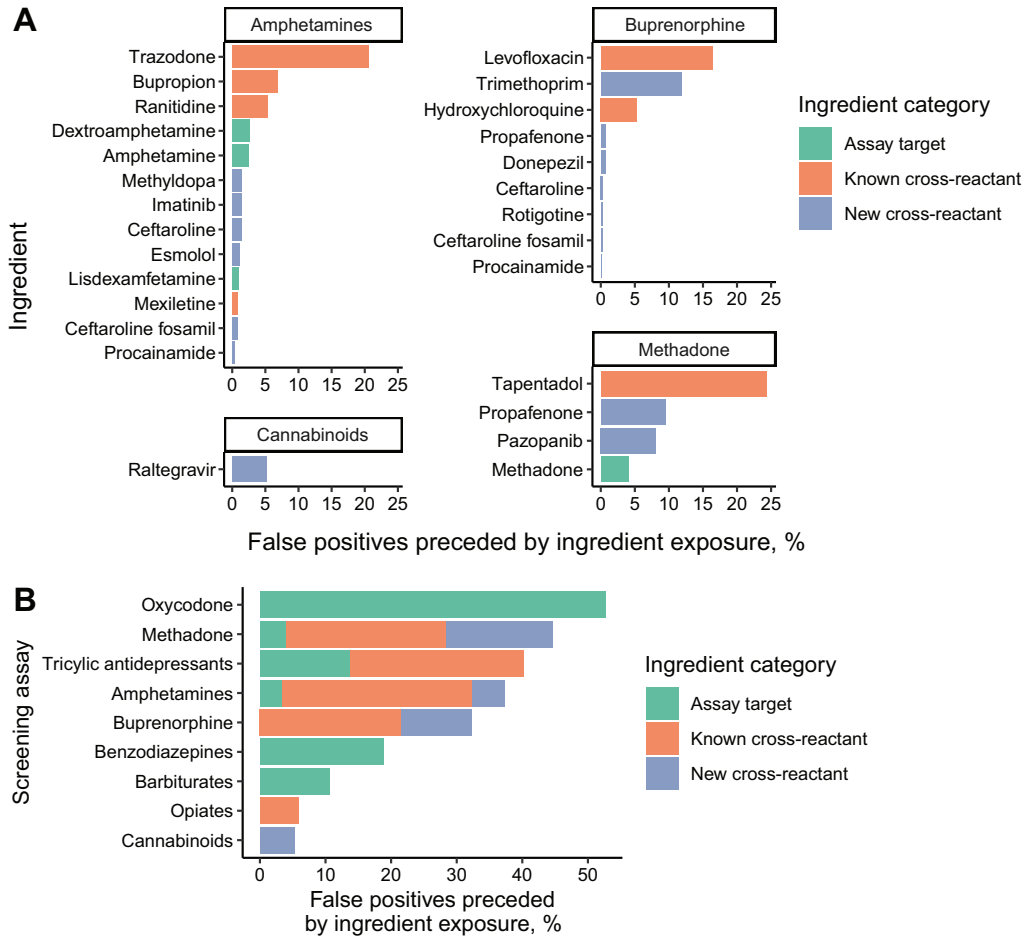

Fig. 3. Estimating percentages of false-positive results explained by (A) ingredient and (B) ingredient category.

Plots include only known cross-reactants whose 95% CI lower bound of odds ratio (OR_{FP}) was ≥2. Exposures are not mutually exclusive, so percentages in (A) could sum to >100. Exposures in (B) followed the hierarchy assay target > known cross-reactant > new cross-reactant, so each exposure was counted once.

concentration). For some compounds, the tested concentrations are well within the range expected with standard dosing *(22–24)*. For others, either the concentration required to produce a presumptive positive result is higher than would be expected with standard dosing or the expected concentration range in urine is not well established *(25–30)*. Regardless, the combination of (*a*) empirical association between false-positive screens and previous ingredient exposure (not explained by coexposures) and (*b*) experimental validation of cross-reactivity provides strong evidence that exposure to the ingredient is causal for some fraction of false-positive screens.

We envision multiple future applications of our work. First, because our institution's EHR data are made available to IRB-approved researchers in a standard format called OMOP *(17)*, it should be possible to apply our approach to OMOP-formatted EHR data from other institutions that may use different screening assays. Second, rather than being a one-time analysis, our approach can be applied on an ongoing basis as evidence for existing drug accumulates and as new drugs and assays become available. Such postmarketing surveillance could guide analytical specificity testing in assay development. Finally, to achieve the promise of a learning health system *(31)*, growing knowledge of cross-reactivity must be incorporated into the EHR so that a patient's recent drug exposures can be used to automatically inform providers when a false-positive screen is likely, even before a confirmation result is available. This could improve patient care in emergent or other situations in which UDS results directly influence clinical decisions.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

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