Lot-to-Lot Variations in a Qualitative Lateral-Flow Immunoassay for Chronic Pain Drug Monitoring

To the Editor:

Recently, Algeciras-Schimnich et al. highlighted the lot-to-lot variability of a quantitative immunoassay and the failure of frequently used protocols to detect these variations (1). We have discovered that lot-to-lot variations also present an important issue in qualitative immunoassays used for chronic pain monitoring. These variations impact the reliability of these assays in clinical practice.

Over 7 months, we analyzed a total of 1681 urine samples across 5 lots of the same CLIA-waived, lateral-flow immunoassay (ABMC RapidTOX®), according to manufacturer instructions. We chose this device based on its low cost, its high negative predictive value, and that it includes an assay for the use of buprenorphine. We also analyzed these samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (2). Each lot of immunoassay was used to analyze a similar number of different urine samples (mean across 5 lots = 336, range 329–354) with a similar number of samples measured by each lot containing opiates (mean = 150, range 140–156), oxycodone (mean = 155, range 133–164), or methadone (mean = 73, range 70–78). As the comparative method, LC-MS/MS was deemed the gold standard and used to categorize samples that should test positive for opiates (morphine, hydrocodone, codeine, hydrocodeone, 6-monooctylmorphine, or glucuronide metabolites), oxycodone (oxycodone, oxymorphone, or oxymorphoneglucuronide), or methadone. The immunoassay was considered falsely positive when the immunoassay was positive and there were no detectable relevant analytes by LC-MS/MS, and falsely negative when the immunoassay was negative and the LC-MS/MS detected analytes present at concentrations above the cutoffs listed in the immunoassay package insert.

Fig. 1 illustrates the false-positive and false-negative rates for each of the 5 lots across 3 immunoassays on the lateral-flow device. Each immunoassay, there was statistically significant lot-to-lot variability in the false-positive and false-negative rates, which were calculated as the number of false-positive results divided by the total number of positive results and the number of false-negative results divided by the total number of negative results, respectively. For example, the methadone false-positive rate jumped from a mean of 4% for the first 4 lots to 19% in lot 5 (P < 0.001, G-test). As a result, the positive predictive value fell from 84% (mean across lots 1–4) to 51% in lot 5. The prevalence of drug positivity would affect false-positive rates, false-negative rates, and the positive predictive value, but there were no between-lot differences in drug positivity rate for the analytes and samples analyzed in this study (P > 0.57 for each immunoassay, G-test). Not all immunoassays on the device were affected similarly by lot-to-lot variations. For example, the oxycodone false-positive rate was 26% in lot 2 and 16%.

Fig. 1. Lot-to-lot variability of a lateral-flow immunoassay device. Rates of false-positive and false-negative results are shown for 5 lots of a CLIA-waived multiplex immunoassay device.

For each immunoassay, the results were compared against a LC-MS/MS assay. Also shown are the results of the statistical analyses by G-test, which tests whether there is lot-to-lot variability of the proportion of false-positive or false-negative results (among all positive or negative results, respectively) across the 5 lots for each immunoassay tested.
in lot 5 (P = 0.001, Fisher exact test), whereas the methadone false-positive rate was 4% in lot 2 and 19% in lot 5 (P < 0.001, Fisher exact test). The between-lot discrepancies in false-positive rates between assays on the same device reduced the likelihood that changes in operator behavior over time (either intra- or interoperator) explain the lot-to-lot variability observed. In addition, each device was reviewed the next day by a single technologist, with <1% of results being changed on review, which also limited the likelihood that operator error was an important contributor to interlot variability. However, the fact that different urine samples were analyzed on each lot of the lateral-flow device must be recognized as a limitation of our study and prevents us from ruling out operator variability as an important contributor to our results.

Lot-to-lot variability was also observed in false-negative rates across the 5 lots. In particular, lot 4 showed an oxycodone false-negative rate 5-fold higher than the mean false-negative rate for the other 4 lots (15% vs 3%, P < 0.001). This led to a drop in negative predictive value from a mean of 96% to 77%.

We also found that the cross-reactivity of specific analytes in specific assays on the lateral-flow device showed substantial lot-to-lot variability. This is best illustrated by hydrocodone, which cross-reacts with the oxycodone immunoassay per the package insert and leads to 56% of the oxycodone false-positive results (from hydrocodone measured by LC-MS/MS). However, detectable hydrocodone (>10 ng/mL) caused a false-negative oxycodone result 90% of the time in all lots but lot 4 (only 60%).

To mitigate the cost of testing to patients, we have designed a risk-based reflexive strategy. Non-compliant lateral-flow screening results are confirmed with LC-MS/MS (2). As a result of this strategy, we find the lot-to-lot variations in the false-positive and false-negative rates acceptable. However, laboratories need to be aware of these issues and may need to consistently rely on confirmatory testing to properly assess compliance in high-risk patients.

Note: The Clinical Chemistry editorial office invited American Bio Medica Corporation to submit a Reply to this letter but received no response.

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References


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Engineered Human Lipocalin as an Antibody Mimetic: Application to Analysis of the Small Peptide Hormone Hepcidin

To the Editor:

Anticalin® proteins are human lipocalins that are engineered to bind relevant targets with high affinity and specificity (1). By applying a mutation and selection process, it is possible to select Anticalin proteins that can specifically bind to very diverse protein targets in a monovalent fashion. Anticalin proteins are also suited for engaging small and compact ligands owing to their cuplike binding pocket. Compared with monoclonal antibodies, Anticalin proteins are about 8 times smaller and can be recombinantly produced in bacterial cells in large amounts.

The hepatic peptide hormone hepcidin is a highly conserved molecule of only 25 amino acids with 4 disulfide bridges that plays a central role in body iron metabolism (2). Therefore, it could become a useful biomarker. Numerous hepcidin assays, by use of either mass spectrometry (MS)-based1 techniques or traditional immunochemical assays,