Data on hydroxychloroquine interference with urine laboratory testing

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ABSTRACT

Hydroxychloroquine is a medication used to treat rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune disorders. Previous studies have shown that hydroxychloroquine and the structurally related drug chloroquine have the potential to interfere with some common urine chemistry tests, especially at high concentrations. In the related research article, we observed suspected interference with urine drug of abuse testing in a patient who ingested approximately 12 g of hydroxychloroquine in an acute overdose, with urine hydroxychloroquine concentrations exceeding 500 mg/L. This case prompted a more detailed investigation of the effects of hydroxychloroquine spiked into pooled de-identified urine specimens from a hospital clinical laboratory. The data in this article provides the raw data for 24 urine assays that were investigated. The analyzed data is provided in the tables included in this article. The dataset reported is related to the research article entitled "Diagnostic Pitfalls and Laboratory Test
Interference After Hydroxychloroquine Intoxication: A Case Report” [1].
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### 1. Data

We investigated the effect on urine assays of hydroxychloroquine at concentration up to 1000 mg/L spiked into pools of de-identified urine specimens from the university medical center central clinical laboratory. This followed from observation that a patient with a large overdose of hydroxychloroquine showed suspected interference for some urine laboratory tests obtained for clinical care [1]. There is limited published data that hydroxychloroquine can interfere with urine protein dipstick methods [2,3] and with some urine drug screening tests [4]. A detailed review of package inserts for drug of abuse and therapeutic drug monitoring assays did not find hydroxychloroquine reported as an interferent in any of the assays [5,6].
The 24 specific urine assays analyzed are as follows: amphetamines drug screen, amylase, benzodiazepines drug screen, buprenorphine drug screen, calcium, chloride, cocaine metabolite screen, creatinine screen, creatinine, glucose, human chorionic gonadotropin (hCG), magnesium, microalbumin, myoglobin, NGAL, opiates drug screen, oxytocin drug screen, pH, phosphorus, potassium, protein, sodium, tetrahydrocannabinol (THC) drugs screen, and urea nitrogen. Technical details on these 24 urine assays are available elsewhere [1]. The assays were initially screened in triplicate for a single de-identified pooled urine sample. Thirteen of the assays showed no absorbance or other alarms/errors and also did not have any results that differed by more than 15% from the control without hydroxychloroquine. The raw data for these 13 assays is in Table 1. Raw data for the remaining 11 assays is in Table 2. These were all tested in triplicate for a total of 4 separate pooled urine samples.

Fig. 1 shows data for 4 of the assays (amphetamine screen, benzodiazepine screen, buprenorphine screen, and cocaine screen). An absorbance alarm was evident for all 4 samples containing 1000 mg/L hydroxychloroquine for the buprenorphine screen (Fig. 1B). Fig. 2 shows data for another 4 of the assays (cotinine screen, microalbumin, myoglobin, and opiates screen). Positive bias and absorbance errors were evident for the cotinine assay for all 4 specimens (Fig. 2A). Biphasic effects of hydroxychloroquine were evident for the microalbumin assay, especially for samples 1, 2, and 4 (Fig. 2B). A negative bias was evident for the myoglobin assay for sample 2 (Fig. 2C). The remaining urine samples had <21 ng/mL myoglobin and did not show any evident effect of hydroxychloroquine, although a negative bias would not be detectable in these 3 samples with the myoglobin concentrations below the lower limit of quantitation. Fig. 3 shows data for the remaining 3 assays analyzed in detail. Absorbance errors were evident for the oxycodone for all 4 samples spiked with 1000 mg/L (Fig. 3A). A positive bias from hydroxychloroquine was evident for all 4 samples analyzed for tetrahydrocannabinol (THC; Fig. 3B), although all 4 of these samples would still be negative in this qualitative assay.

2. Experimental design, materials, and methods

All analyses were performed on Roche Diagnostics cobas 8000 analyzers (c501, c502, c602, and c701). The complete list of assays with vendor name, methodology, and assay version are summarized elsewhere [1]. All assays were run in accordance with package insert instructions. A total of 4 pooled urine specimens were prepared using de-identified specimens from the clinical laboratory. All 24 assays were tested in triplicate for 1 of the urine pools. As described above, 11 of the assays were then tested and triplicate for the remaining 3 urine pools. The raw data consists of concentration or absorbance units for the specimens. It should be noted that the urine total protein method used for the data in this report uses a biuret complex method using divalent copper in alkaline solution [7]. This is in contrast to previous studies using tetrabromophenol blue [3] and pyrogallol red-molybdate methods [2] that showed interference by hydroxychloroquine.

Table 1
Raw data for 13 urine assays on a single pooled urine samples.

| Assay     | Units | Hydroxychloroquine concentration |
|-----------|-------|----------------------------------|
|           |       | 0 mg/L | 1 mg/L | 10 mg/L | 100 mg/L | 500 mg/L | 1000 mg/L |
| Amylase   | U/L   | 121    | 121    | 123     | 122      | 121      | 122       |
| Calcium   | mg/dL | 10.4   | 10.4   | 10.6    | 10.8     | 9.9      | 10.0      |
| Chloride  | mmol/L| 69.0   | 70.7   | 68.0    | 70.3     | 72.7     | 76.7      |
| Creatinine| mg/dL | 76.9   | 79.3   | 79.3    | 77.8     | 82.3     | 80.7      |
| Glucose   | mg/dL | 6.0    | 5.5    | 5.4     | 5.3      | 6.0      | 6.0       |
| hCG       | mU/mL | 1.0    | 1.1    | 1.1     | 1.1      | 1.0      | 1.1       |
| Magnesium | mg/dL | 4.8    | 4.7    | 4.7     | 4.7      | 4.3      | 4.2       |
| NGAL      | ng/mL | 12.0   | 11.0   | 11.5    | 12.0     | 11.0     | 11.5      |
| pH        | pH units | 7   | 6.8    | 6.9     | 6.9      | 6.9      | 6.9       |
| Phosphorus| mg/dL | 20.4   | 21.3   | 22.0    | 21.6     | 19.6     | 20.1      |
| Potassium | mmol/L| 35     | 35     | 34      | 35       | 39       | 39        |
| Protein   | mg/dL | 55     | 57     | 57      | 58       | 54       | 49        |
| Sodium    | mmol/L| 60     | 62     | 61      | 62       | 65       | 66        |
Table 2
Raw data for 13 urine assays on four separate pooled urine samples.

| Assay                  | Units     | Sample # | Hydroxychloroquine concentration |
|------------------------|-----------|----------|----------------------------------|
|                        |           |          | 0 mg/L  | 1 mg/L  | 10 mg/L | 100 mg/L | 500 mg/L | 1000 mg/L |
| Amphetamines Drug Screen | Relative absorbance units | 1 | –268 | –286 | –276 | –281 | –271 | –250 |
|                        | (positive: >0) | 2 | –302 | –323 | –309 | –314 | –298 | –291 |
|                        |           | 3 | –273 | –283 | –284 | –279 | –281 | –264 |
|                        |           | 4 | –299 | –299 | –295 | –298 | –283 | –265 |
| Benzodiazepines Drug Screen | Relative absorbance units | 1 | –187 | –178 | –176 | –174 | –171 | –164 |
|                        | (positive: >0) | 2 | –263 | –204 | –209 | –201 | –185 | –58  |
|                        |           | 3 | –210 | –181 | –183 | –176 | –176 | –172 |
|                        |           | 4 | 102  | 108  | 106  | 105  | 105  | 107  |
| Buprenorphine Drug Screen | Relative absorbance units | 1 | –339 | –441 | –441 | –472 | –534 | Absorbance error |
|                        | (positive: >0) | 2 | –283 | –421 | –410 | –429 | –472 | Absorbance error |
|                        |           | 3 | –283 | –393 | –398 | –420 | –418 | Absorbance error |
|                        |           | 4 | –258 | –389 | –395 | –405 | –451 | Absorbance error |
| Cocaine Drug Screen | Relative absorbance units | 1 | –548 | –538 | –554 | –557 | –565 | –540 |
|                        | (positive: >0) | 2 | –575 | –587 | –570 | –581 | –578 | –558 |
|                        |           | 3 | –553 | –543 | –546 | –546 | –537 | –536 |
|                        |           | 4 | –558 | –560 | –568 | –570 | –551 | –552 |
| Cotinine Screen | Relative absorbance units | 1 | >2000 | >2000 | >2000 | >2000 | >2000 | Absorbance error |
|                        | (positive: >0) | 2 | 1294 | 786  | 810  | 784  | 705  | Absorbance error |
|                        |           | 3 | –250 | –194 | –182 | –202 | –268 | Absorbance error |
|                        |           | 4 | 229  | 398  | 397  | 348  | 119  | Absorbance error |
| Microalbumin | mcg/mg creatinine | 1 | 293  | 273  | 273  | 281  | 315  | 329  |
|                        |           | 2 | 155  | 105  | 105  | 112  | 144  | 189  |
|                        |           | 3 | 24   | 20   | 23   | 24   | 26   | 30   |
|                        |           | 4 | 215  | 225  | 224  | 231  | 249  | 274  |
| Myoglobin | ng/mL | 1 | <21  | <21  | <21  | <21  | <21  | <21  |
|                        |           | 2 | 1008 | 899  | 900  | 876  | 752  | 599  |
|                        |           | 3 | <21  | <21  | <21  | <21  | <21  | <21  |
|                        |           | 4 | <21  | <21  | <21  | <21  | <21  | <21  |
| Opiates Drug Screen | Relative absorbance units | 1 | 88   | 103  | 101  | 101  | 96   | 89   |
|                        | (positive: >0) | 2 | –520 | –497 | –496 | –496 | –469 | –436 |
|                        |           | 3 | –483 | –477 | –455 | –475 | –458 | –459 |
|                        |           | 4 | –479 | –446 | –453 | –447 | –426 | –392 |
| Oxycodone Drug Screen | Relative absorbance units | 1 | –158 | –159 | –160 | –158 | –158 | Absorbance error |
|                        | (positive: >0) | 2 | 28   | 31   | 32   | 31   | 27   | Absorbance error |
|                        |           | 3 | –154 | –159 | –159 | –157 | –156 | Absorbance error |
|                        |           | 4 | –155 | –155 | –156 | –153 | –153 | Absorbance error |
| Urea nitrogen | mg/dL | 1 | 859  | 862  | 884  | 877  | 866  | 863  |
|                        |           | 2 | 684  | 696  | 692  | 694  | 673  | 647  |
|                        |           | 3 | 951  | 927  | 930  | 926  | 930  | 923  |
|                        |           | 4 | 541  | 529  | 531  | 519  | 519  | 518  |
| THC Drug Screen | Relative absorbance units | 1 | –178 | –165 | –162 | –163 | –155 | –126 |
|                        | (positive: >0) | 2 | –221 | –191 | –186 | –180 | –167 | –152 |
|                        |           | 3 | –225 | –206 | –206 | –207 | –195 | –201 |
|                        |           | 4 | –225 | –210 | –211 | –212 | –194 | –180 |
Fig. 1. Analysis of hydroxychloroquine effects on urine assays for (A) amphetamine screen, (B) benzodiazepine screen, (C) buprenorphine screen, and (D) cocaine screen. Four separate de-identified pooled urine samples were tested in triplicate at the indicated hydroxychloroquine concentrations.
Fig. 2. Analysis of hydroxychloroquine effects on urine assays for (A) cotinine screen, (B) microalbumin screen, (C) myoglobin assay, and (D) opiates screen. Four separate de-identified pooled urine samples were tested in triplicate at the indicated hydroxychloroquine concentrations.
Fig. 3. Analysis of hydroxychloroquine effects on urine assays for (A) oxycodone screen, (B) tetrahydrocannabinol (THC) screen, and (C) urea nitrogen. Four separate de-identified pooled urine samples were tested in triplicate at the indicated hydroxychloroquine concentrations.
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None.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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