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SYMPOSIUM PAPER

Analysis of mixed stones is prone to error: a study with US laboratories using micro CT for verification of sample content

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Abstract This project sought to test the ability of commercial stone analysis laboratories to correctly analyze urinary stones. Human stone specimens were cleaved into pieces, and the pieces of each specimen were verified as being similar using micro-computed tomography (micro CT), a non-destructive method. Thus, similar specimens from 25 stones were sent to five laboratories, and a sixth piece was kept for analysis in our laboratory using Fourier-transform infrared spectroscopy (FT-IR). The results showed that laboratories were very good at analyzing pure specimens, but with mixed specimens the accuracy and consistency varied. In six stones containing apatite, a mineral easily identified using micro CT, apatite was missed 20% of the time. Struvite content in the specimens was inconsistently reported, with laboratories differing in their reports of the presence of struvite in six of the 25 stones (24%). A mixed stone containing atazanavir was not reported by any of the laboratories as containing that drug. Nomenclature differed among the laboratories, especially with regard to apatite, which was variously reported as hydroxyapatite, carbonate apatite, or as apatite with calcium carbonate. One laboratory reported protein in every stone, while for all others protein was reported in only one stone. We conclude that physicians need to be aware that reports on mixed stones, which represent >90% of all calculi, can be erroneous. It is likely that supplying a greater amount of stone material will assist a laboratory in making a correct analysis of mixed stones. Also, standardization of nomenclature could assist in analysis reproducibility, but this remains to be tested.

Keywords Calculi · Stone composition · Infrared spectrophotometry · Urolithiasis

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Introduction

Analysis of stone composition is a routine assessment as part of the medical management of persons suffering from urolithiasis [1, 2]. While this practice has been challenged as being unnecessary for most stone formers [3], the broader view is that the stone analysis can provide important information about the underlying cause for stone formation in most patients [4–6].

A more serious challenge to stone analysis is the question of the accuracy of laboratory reports. Kasidas et al. [6] reported error rates of 30% in their series, in which they sent samples of known composition to stone laboratories for testing. A more extensive study by Hesse et al. [7] tested over 100 laboratories by sending identical specimens of powdered mineral. Ignoring the results for labs using wet-chemical methods (which are now less frequently used) Hesse et al. [7] reported errors for pure substances ranging from 0 to 30%. For mixed samples, error rates tended to be higher; for example, the presence of apatite in a mixed specimen was missed about 10% of the time when

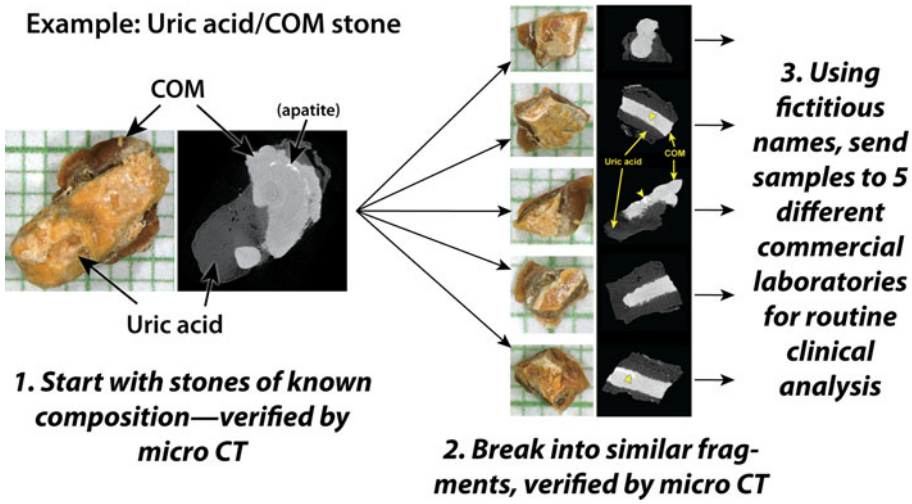

Fig. 1 Outline of procedure for sending comparable pieces of a stone to multiple laboratories for analysis. See text for details. *COM* calcium oxalate monohydrate

infrared methods were used, and 25–30% of the time when X-ray diffraction methods were used [7].

However, neither of these studies utilized real, mixed stones for testing analysis accuracy. Instead, they used powdered mineral standards and mixed them together to simulate a complex stone. These prior methods did not, then, test the ability of the laboratory to adequately analyze a real stone specimen, a process that requires proper examination of the stone or stone fragments to identify portions for analysis in order to detect minor components [8, 9].

The objective of the present study was to test stone analysis laboratories using samples of human stones that were judged by micro CT to be of similar composition [10]. Micro CT enables the non-destructive visualization of minerals within stones [11, 12], and it is especially useful for detecting small amounts of mineral with contrasting X-ray attenuation values, such as apatite within calcium oxalate stones [13]. The results suggest that the analysis of mixed stones is especially problematic for commercial laboratories. Recommendations for improving the usefulness of results for stone analysis include the submission of as much stone material for analysis as possible, communication of patient data to the stone laboratory such as prescription of anti-viral drugs that may yield stones, and standardization of methods and nomenclature for stone analysis.

Methods

Briefly [10], human stone samples were chosen from a library of stone analysis laboratory discards. Samples were de-identified but retained their initial analysis results, and thus a representative set of 46 larger stones was obtained. These stones were scanned by micro CT (Skyscan 1172 system, using voxel size of 7 μm), and then broken by hand into six pieces. The resulting pieces were again scanned by micro CT, and the scans examined to verify that each piece contained similar materials; i.e., the micro CT scans were used to determine whether all pieces were representative of the original stone. An example of this process is shown in Fig. 1. Of the 46 stones taken through this process, only 25 were judged to have pieces sufficiently alike to be used for this study.

For each of these 25 stones, one piece was sent out to each of five commercial stone laboratories, using fictitious patient names to blind the laboratories to the study. For additional verification of the stone's composition, an additional piece of each stone was retained for thorough assessment utilizing micro CT (of just the retained fragment alone), stereomicroscopy, dissection, and transmission Fourier-transform infrared spectroscopy (FT-IR).

Variability of results is described using coefficient of variation. The ability of laboratories to distinguish stone contents was tested using two-way analysis of variance, and the Tukey method was used for post hoc tests. Significance was assumed when $P < 0.05$.

Results

All of the laboratories accurately reported the compositions of relatively pure stones, as shown in Fig. 2 for pure uric acid and cystine stones (top two and bottom two stones in the figure). The two brushite stones in this study, though mixed with other minerals, were also identified correctly by all the laboratories. However, one of the mixed calcium oxalate monohydrate (COM) and uric acid stones (fourth row down in Fig. 2) demonstrated disagreement among the laboratories with one of the laboratories indicating that it was pure uric acid and the others reporting it to be a mixed stone (Fig. 1). The micro CT images in Fig. 1 clearly demonstrate the presence of both COM and uric acid in all the fragments sent out. Thus, each of the laboratories received a mixed stone with a substantial proportion of uric acid and

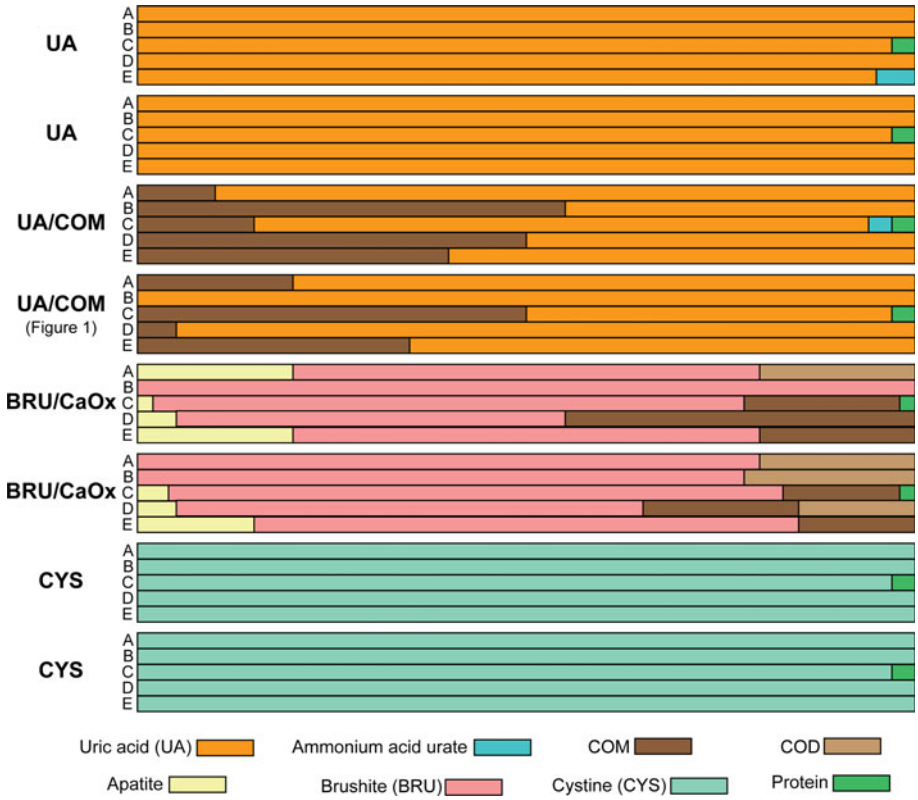

Fig. 2 Analysis results for stones composed of uric acid, uric acid/COM, brushite, and cystine. Letters A–E specify commercial laboratories, and *bar* to right shows reported composition of stone with lengths of color regions proportional to percentages reported by that laboratory. *CaOx* calcium oxalate

COM, and one laboratory missed the COM. In such a patient, a physician might choose not to treat hypercalciuria in the absence of a calcium salt in the stone [14], and so the missed stone analysis could affect clinical treatment.

Just as uric acid and calcium oxalate are easily distinguished by micro CT, apatite can be seen easily with micro CT in the presence of calcium oxalate [13, 15], and even a small portion, <1% by volume, can be correctly identified in most stone types ([11, 16] and unpublished observations). Figure 3 shows four examples of micro CT imaging of stone fragments composed primarily of calcium oxalate: one sample with no detectable apatite, and three others with apatite distributed through every test fragment. The analysis results of these stones are shown along with five others in Fig. 4; the percentage of apatite for each stone is shown on the left, as determined by micro CT [16] for all of the test fragments of the stone scanned as a group. With regard to apatite, the top three stones in Fig. 4 showed no visible apatite by micro CT, and the volume percent of voxels in the absorbance range of apatite was 0.16, 0.44, and 0.30%, respectively. Despite this evidence for lack of apatite in these stones, one laboratory reported 5–10% apatite for each. Overall, the apatite results for these nine calcium oxalate stones tracked with the content measured for the fragments as a group by micro CT ($P < 0.0001$), but the coefficient of variation for apatite determination averaged $140 \pm 69\%$, indicating dramatic variability of results for apatite.

Critique of the details of calcium oxalate results is not as easy. In a positive vein, we can say that all of the laboratories correctly identified the stones in Fig. 4 as containing calcium oxalate. The differences in reported content of COM and COD (calcium oxalate dihydrate) could well represent differences among the fragments sent out, as distinguishing COD from COM is sometimes possible by micro CT [11], but in many cases these forms are probably mixed together (as shown in Fig. 3).

Similarly, differences among the fragments sent out to the laboratories could have accounted for some of the CaOx stones in Fig. 4 being reported as >50% apatite. The fragments were scanned by micro CT as a group, and it was determined that they all had similar compositions, but the relative proportions of components were not determined for each fragment. (Thus, the values for volume % of apatite indicated in the figures were an average for all the fragments together). In any case, the data in Fig. 4 demonstrate that it is possible to send a representative sample of a calcium oxalate stone to a laboratory and have the result reported as predominately apatite stone composition. Such a report could influence the decision to use potassium citrate for such a patient [17].

Finally, Fig. 5 demonstrates the results for stones reported to contain struvite, which is universally recognized as a product of infection [18]. The stars on the right side of the figure mark reports of struvite; note that the marked reports disagreed on struvite content of these stones in six

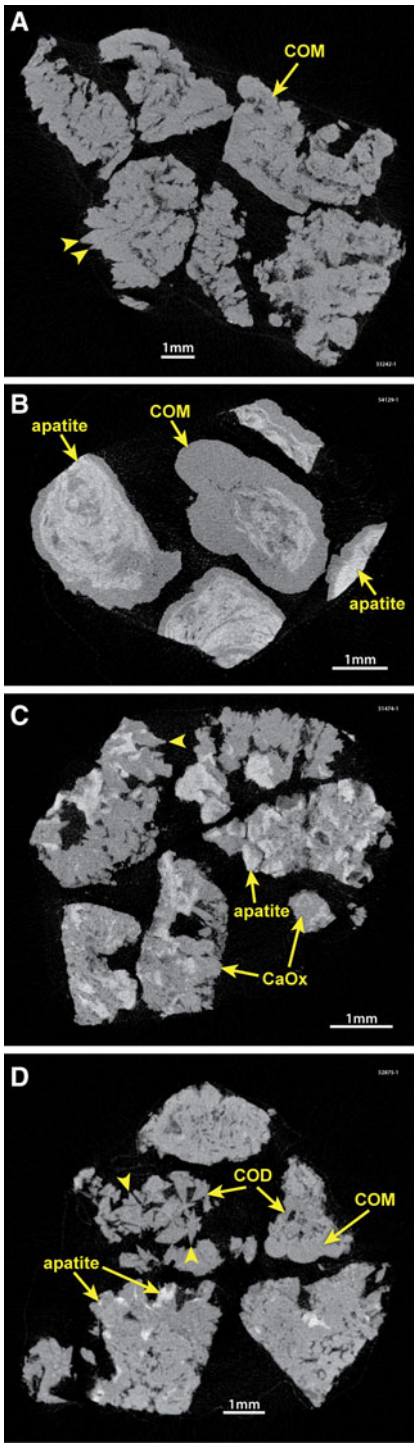

Fig. 3 Micro CT image slices for fragments of four of the samples of CaOx stone sent out to commercial laboratories for analysis. Total apatite content measured in these fragments was 0.16% (**a**), 40.9% (**b**), 17.4% (**c**), and 2.7% (**d**). Similar morphologies and apatite contents were seen for all fragments from a given stone sample. Some fragments show polyhedral crystals (*arrowheads*) indicating CaOx dihydrate (COD), and these were not distributed evenly among fragments. In one specimen, **d**, COM and COD are distinguishable by X-ray attenuation [11] (*gray* value)

of the eight cases. It is difficult to identify struvite by micro CT unless the stone is rather pure [11], so we cannot be certain that all of the stone fragments in Fig. 5 either contained or did not contain struvite. However, these stones were divided into fragments that were judged to be similar in morphology and composition, so the results of the laboratory analyses are still concerning, with disagreement among the laboratories regarding struvite content in 75% of the stones in this compositional class.

Results for apatite content in the stones shown in Fig. 5 are similar to those mentioned earlier. That is, in Fig. 5, for the stones reporting an average apatite content of less than 50%, coefficient of variation was 101%, indicating large variability. Similarly, for five of the eight stones in Fig. 5, laboratories disagreed as to whether the stone was majority apatite or not.

The mixed stone shown at the bottom of Fig. 5 was unusual in that it contained the antiviral drug, atazanavir (AZ). None of the laboratories reported this content, but the fragment retained in our laboratory for confirmatory analysis did contain atazanavir (as verified on FT-IR by a tapered peak at 3428 cm^{-1}, and peaks at 1,724, 1,652, 1,514, 1,240, 778, and 764 cm^{-1}). However, the commercial laboratories were not informed of the possible inclusion of this drug. Such information could be provided by the physician with submission of a specimen, if the patient is taking drugs that are known to form urinary deposits.

Mention should be made of some other anomalies in the data shown earlier. One laboratory reported protein in all 25 stones, while protein (or some other organic material) was reported only once by the other four laboratories. Protein is a component of all stones [19], and our experience is that spectroscopic evidence of its presence is frequently seen, but laboratories apparently differ in whether this is worth reporting.

Laboratories also differed in how apatite was reported. All apatite in stones contains carbonate [20, 21], but some investigators have distinguished apatite with low carbonate from that with high carbonate content [22]. This distinction was used by two of the laboratories in this study, which reported apatite as either hydroxyapatite or carbonate apatite. Another lab reported apatite only as hydroxyapatite, and yet another only as carbonate apatite. The fifth lab reported apatite but usually included an additional quantity of calcium carbonate in the analysis, and this carbonate was considered as part of the apatite content for the present study.

Discussion

The overall result of this study in which real stone specimens were sent to commercial laboratories for analysis is

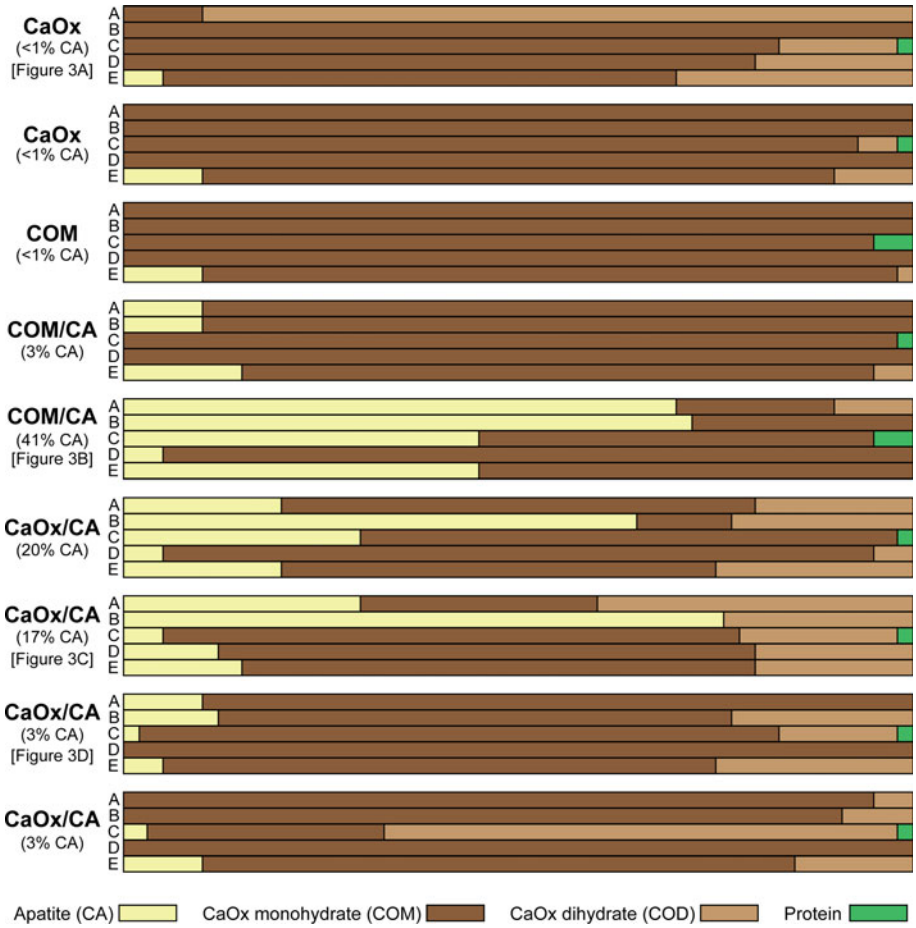

Fig. 4 Analysis results for laboratories A–E for a series of CaOx stones. Total apatite content (volume % by micro CT) measured for fragments as a group is shown in parentheses

that all of the laboratories did well in analyzing pure stones, but the results were not as accurate for mixed stones, and some of the disparate results had the potential to alter treatment rendered to the patient.

A unique aspect of this study is the preparation of stone specimens using micro CT to non-destructively analyze the specimens before sending them out to laboratories for analysis. This allowed the use of real stones, even those with mixed composition, for these tests. This method is especially powerful for characterizing some mixed stones (such as COM/uric acid) and for recognizing the presence of small amounts of some materials (specifically apatite in most stones), but it is less effective for other components (such as identifying struvite in a mixed stone). Thus, some of the results point to actual errors in analysis by commercial laboratories, but others point only to errors that could be in the sampling procedure or in the analysis by the laboratories. This second set of errors is still instructive, such that sampling errors by the surgeon submitting the stone for analysis could contribute to the inaccuracy of stone composition reporting for mixed stones. For this reason, we recommend that the surgeon submit as much material as possible for a stone specimen, and that laboratories examine all portions of the specimen, prerequisites that are likely to give the analysis laboratory the best chance of identifying all the components of a mixed stone.

A question commonly asked when we have presented this work is, "What methods were used by the commercial laboratories?" The answer is that none of the laboratories specified the methods used on its reports. All of the labs state in their literature that they utilize at least one physical method (X-ray diffraction or FT-IR, or both) but no specific analytical sequence is described by any of them. Reviews of methods used for analyzing stones agree that X-ray diffraction and FT-IR are both powerful methods for identifying minerals in stones [9, 23], but it is also clear that proper dissection of the stone material is essential [22, 24]. However, Gault et al. [25] recommend additional wet-chemical analysis for ammonia to enable the detection of struvite when it is in low concentration. It is not known if any of the commercial laboratories routinely utilize such a test, and we are not aware of any study that has verified the work of Gault et al. [25] on struvite detection.

A further concern is that the laboratories in our study did not use the same nomenclature for listing the minerals found in stones. Some of these differences such as the use

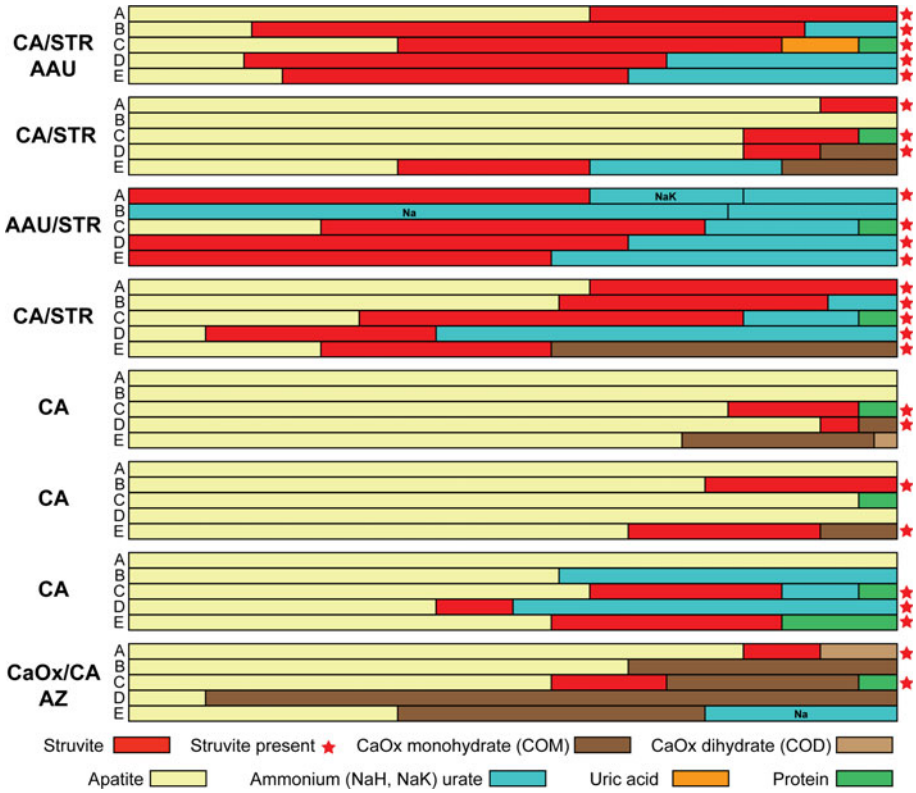

Fig. 5 Analysis results for laboratories A–E for a series of mixed stones, all of which received at least two reports of the presence of struvite. *CA* apatite (carbapatite), *STR* struvite, *AAU* ammonium acid urate, *AZ* atazanavir

of 'whewhellite' or 'COM' are easily reconciled by the referring physician. Other differences are more difficult to interpret, and the variation in naming of different forms of apatite is probably the most serious nomenclature problem we noted in this study. The usefulness of carbonate content in apatite as a marker of infection has been challenged [26], but more recent work suggests that high carbonate in apatite does indeed correlate with infection [21]. In the present study, only one of the laboratories attempted to report the quantitative carbonate content of apatite (although somewhat obscurely as 'calcium carbonate'); the laboratories that reported apatite either as hydroxyapatite or carbonate apatite did not specify how they distinguished these two minerals. While it is clear that more work should be done to determine whether this distinction has clinical utility, it would make such studies easier to conduct if laboratories used the same names for these mineral forms.

In summary, submission of genuine stone samples to commercial laboratories in the United States demonstrates that laboratories accurately identify pure stones, but that analysis of mixed stones showed great variability. Standardization of methodology and nomenclature would be helpful. Exploration of the reliability of the detection of struvite is needed, as the tests in the present study showed that laboratories disagreed on the presence of this infection marker in over a fourth of all stones submitted.

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References

1. Miller NL, Lingeman JE (2007) Management of kidney stones. BMJ 334:468
2. Hall PM (2009) Nephrolithiasis: treatment, causes, and prevention. Clevel Clin J Med 76:583
3. Henderson MJ (1995) Stone analysis is not useful in the routine investigation of renal stone disease. Ann Clin Biochem 32(Pt 2):109
4. Smith CL (1998) Renal stone analysis: is there any clinical value? Curr Opin Nephrol Hypertens 7:703
5. Coe FL, Evan AP, Worcester EM (2005) Kidney stone disease. J Clin Invest 115:2598
6. Kasidas GP, Samuell CT, Weir TB (2004) Renal stone analysis: why and how? Ann Clin Biochem 41:91
7. Hesse A, Kruse R, Geilenkeuser WJ et al (2005) Quality control in urinary stone analysis: results of 44 ring trials (1980–2001). Clin Chem Lab Med 43:298
8. Daudon M, Bader CA, Jungers P (1993) Urinary calculi: review of classification methods and correlations with etiology. Scanning Microsc 7:1081
9. Mandel G, Mandel N (1996) Analysis of stones. In: Coe FL, Favus MJ, Pak CYC et al (eds) Kidney Stones: medical and surgical management. Lippencott-Raven, Philadelphia, pp 323–335
10. Krambeck AE, Khan NF, Jackson ME et al (2010) Inaccurate reporting of mineral composition by commercial stone analysis laboratories: implications for infection and metabolic stones. J Urol 183:1459–1463
11. Zarse CA, McAteer JA, Sommer AJ et al (2004) Nondestructive analysis of urinary calculi using micro computed tomography. BMC Urol 4:15
12. Pramanik R, Asplin JR, Jackson ME et al (2008) Protein content of human apatite and brushite kidney stones: significant correlation with morphologic measures. Urol Res 36:251

13. Miller NL Jr, Williams JC, Evan AP et al (2010) In idiopathic calcium oxalate stone-formers unattached stones show evidence of having originated as attached stones on Randall's plaque. BJU Int 105:242
14. Coe FL (1983) Uric acid and calcium oxalate nephrolithiasis. Kidney Int 24:392
15. Williams JC Jr, Matlaga BR, Kim SC et al (2006) Calcium oxalate calculi found attached to the renal papilla: preliminary evidence for early mechanisms in stone formation. J Endourol 20:885
16. Williams JC Jr, Zarse CA, Jackson ME et al (2006) Variability of protein content in calcium oxalate monohydrate stones. J Endourol 20:560
17. Straub M, Strohmaier WL, Berg W et al (2005) Diagnosis and metaphylaxis of stone disease—consensus concept of the National Working Committee on stone disease for the upcoming german urolithiasis guideline. World J Urol 23:309
18. Miano R, Germani S, Vespasiani G (2007) Stones and urinary tract infections. Urol Int 79:32
19. Boyce WH (1968) Organic matrix of human urinary concretions. Am J Med 45:673
20. Hesse A, Heimbach D (1999) Causes of phosphate stone formation and the importance of metaphylaxis by urinary acidification: a review. World J Urol 17:308
21. Carpentier X, Daudon M, Traxer O et al (2009) Relationships between carbonation rate of carbapatite and morphologic characteristics of calcium phosphate stones and etiology. Urology 73:968
22. Prien EL, Frondel C (1947) Studies in urolithiasis: I, The composition of urinary calculi. J Urol 57:949
23. Schubert G (2006) Stone analysis. Urol Res 34:146
24. Daudon M, Protat MF, Reveillaud RJ (1978) Analyse des calculs par spectrophotométrie infrarouge. Advantages et limites de la méthode. Ann Biol Clin (Paris) 36:475
25. Gault MH, Ahmed M, Kalra J et al (1980) Comparison of infrared and wet chemical analysis of urinary tract calculi. Clinica Chimica Acta 104:349
26. Brien G, Berg W, Schubert G et al (1988) Need for the differentiation of apatite and carbonate apatite. Zeitschrift fur Urologie und Nephrologie 81:605