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Forensic Laboratories Feel the Impact of the "CSI Effect"

By James L. Caruso

A recently popular topic in the general media and to some extent in the scientific literature is the "CSI effect." The phrase refers to the significant impact the immensely popular television show *CSI: Crime Scene Investigation* and its imitators, centered around the crime lab and the morgue, have had on the public's perception of the forensic sciences. A literature search for information on the CSI effect resulted in few citations for medical and scientific journals and abundant coverage in *USA Today*, *Newsweek*, *National Geographic News*, *MSNBC*, *BBC News*, and other popular media outlets.

There is no debating that shows based on crime scene investigation and the collection and processing of evidence have dominated the television ratings for the past few years. The public's long-standing fascination with crime and punishment has morphed from the somewhat simplistic *Perry Mason* era, through a lighter-hearted, if not soap opera-ish, *L.A. Law* phase, to the modern genre of shows like the three currently available flavors of *CSI*, *Crossing Jordan*, and *Bones*. *Perry Mason* usually relied on the guilt-ridden accused to confess his or her crime when caught in a lie or presented with circumstantial evidence. *CSI*-style shows focus much more on physical evidence; usually a single individual or a team collects, analyzes, interprets, and testifies about it.

Public expectations

The CSI effect involves the expectation by the public that every crime scene investigation, every trial, and every prosecution will contain all the elements that have become familiar through the television facsimiles of the forensic sciences. The public expects that there will always be hard evidence, such as fingerprints, ballistics, toxicology, and DNA, and

that this evidence and the methods used to analyze it will withstand all scrutiny because the state-of-the-art forensic laboratory leaves no room for error.

Furthermore, the test results will always be at hand promptly, even immediately in some cases. During one recent episode of *CSI*, DNA testing was required to prove paternity. When one of the putative mothers was told that this test would be necessary, she countered, "Will this take long; he doesn't usually stay up late." The mother was reassured that they would put a rush on it. And, true to form, complete allelic profiles for all interested parties were available in just a few television hours (a few minutes for the viewer).

At the Office of the Armed Forces Medical Examiner (AFME), what we call a "stat DNA" usually takes 36–48 hours in the best of hands because some aspects of the testing methodology, such as amplification steps, take time. An analogous situation would be a clinician calling the hospital laboratory to request that an erythrocyte sedimentation rate be rushed! Some analytic processes are time-consuming and tedious, especially if done properly. Most of my fellow medical examiners would be envious of the turnaround time I can get on a DNA specimen if I really need it. Compared with the television portrayal of DNA technology, however, two days is an eternity.

A persuasive medium

Television can be an extremely persuasive medium. My early influences in medicine included such popular television legends as *Marcus Welby, M.D.*, who always had the correct diagnosis and whose patients adored him (perhaps in part because they

Continued on page 3

Inside...

Multidimensional GC-MS	2
Digoxin Interference	5

Multidimensional GC-MS Offers Improved Sensitivity

By Jeri D. Ropero-Miller

Multidimensional gas chromatography-mass spectrometry is an emerging technology that can offer improved separation, reproducibility, and sensitivity. For clinical and forensic toxicologists who must analyze complex biological matrices for drugs and other compounds, it is an analytical option that warrants investigation.

Analytes of interest are often present in trace amounts in matrices predominated by other compounds. This creates a problem of co-elution and low analyte-to-background signal, which is difficult to detect and quantify using traditional gas chromatography-mass spectrometry (GC-MS). Tandem mass spectrometry (MS-MS, MS-MS-MS) was introduced to improve sensitivity, but many laboratories cannot afford these instruments for routine analyses. GC-GC-MS offers comparable chromatographic improvements at less expense.

While multidimensional gas chromatography (MDGC) has been used for decades in the petroleum industry, a refined version was only recently investigated for forensic and clinical applications. MDGC uses successive chromatographic columns of different phases (e.g., polar and nonpolar) to improve the resolution quality of the system by increasing the selectivity factor (α) as described by the equation:

$$R = \sqrt{N} * ((\alpha - 1) / \alpha) * (k / (1 + k))$$

where R is resolution, N is the number of theoretical plates, and k is the capacity of the column to retard the passage of a component.

Earlier "switching valves" in these instruments were fraught with catalytic activity of metal parts before Deans introduced a stream switching system using pneumatic pressure balancing in 1968 (1). MDGC systems employing the Deans switch are now commercially available, and kits are available to retrofit GC-MS systems for approximately \$10,000.

Transferring peaks

Multidimensional separation techniques improve separation and analytical quality by transferring or "cutting" chromatographic peaks of interest from the primary (one-dimensional) column to the secondary (analytical or two-dimensional) column. These mechanisms focus on a short region of solute for further separation.

"Heartcutting" employs a device (such as a Deans switch or valve) to pass the solute of a pre-

selected retention time region from the primary column to the secondary column by directing the carrier flow from a monitoring detector (usually a flame ionization detector) or purge outlet to an analytical detector (e.g., mass spectrometer). Usually, the primary column is more polar than the secondary column, promoting retention of background matrix proteins on the primary column. When the switch occurs, the analyte of interest passes through to the less polar secondary column.

The method's complexity can be increased by including a second oven for the secondary column, cryogenic focusing to trap and re-inject solute into the second dimension, and effluent splitting to multiple detectors (2,3). Heartcut selectivity can be combined with column switching by using a packed primary column for greater introduction of specimen volume, followed by heartcutting of the analyte region to the secondary capillary column to maximize efficiency and sensitivity.

Another method, known as a flow reversal or backflushing mode, uses a separate auxiliary carrier flow to the monitoring (first) detector and the secondary column to force retention of heavier volatile compounds on the primary column and passage of lighter volatile analytes of interest. With a valve switch, the larger auxiliary flow counters the carrier's forward flow of the primary column, pushing the heavier compounds back. Finally, comprehensive two-dimensional gas chromatography analysis includes the entire sample, after a single introduction to the primary column, for two different separations through the use of valve modulation (e.g., longitudinal modulated cryogenic system or cryo-jet modulation).

Applications

A review of the literature shows several recent clinical and forensic applications of GC-GC-MS. In 2003, Kueh et al. reported that comprehensive GC-GC-MS could effectively separate and quantitate 27 drugs in doping control samples (4). Similarly, Moore et al. used two-dimensional GC coupled to MS with electron capture chemical ionization to detect the marijuana metabolite, THC-COOH, in hair at concentrations of 0.05 pg/mg (3). This year, Sanchez and Sack used two-dimensional gas chromatography coupled to time-of-flight mass spectrometry to analyze breath samples for biomarkers for active tobacco use. Approximately 250 different compounds were observed, of which 142 were correctly identified. Three particular biomarkers (2,5-dimethylfuran, 2-methylfuran, and furan) were found in easily measurable concentrations in samples taken up to two hours after smoking (5).

Multidimensional GC-MS may play an important role in the future of clinical and forensic toxicology. Its use of dissimilar chromatographic columns and switching mechanisms can result in selected separation of solute regions containing analytes of interest without background matrix compounds.

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CSI Effect

Continued from page 1

never seemed to have to pay for his services), and the icon of television forensics, *Quincy M.E.* Scientific methods were a part of the Quincy series and perhaps there was a bit of CSI effect in the way Dr. Quincy solved the mysterious deaths that baffled all others. His trusty laboratory scientist, Sam, always provided some key pieces to the puzzle, but the role of the forensic laboratory usually took a back seat to circumstances and confessions (and perhaps even Dr. Quincy's houseboat).

There are definitely some positive aspects to the numerous television shows that center around forensic medicine and the crime lab. The number of applications for degree programs and vocational training positions in the forensic sciences is increasing exponentially. A new group of young, energetic scientists

has entered the field, armed with excellent training and able to gain experience from the "graybeards."

In addition, one can hope for a more educated jury pool. After all, not everything about the forensic laboratory as portrayed on television is fictional. The technology demonstrated is basically sound and every show has technical advisors to keep the writers from straying too far off course. Perhaps there would have been a different outcome in one very high profile murder trial several years ago if the jury understood that a failure to preserve critical DNA evidence appropriately would have been far more likely to provide uninterpretable results rather than a DNA profile that matched the defendant's.

Unfortunately, the television persona of the crime scene investigator is far from realistic. On *CSI* the investigator personally goes to crime scenes, collects the evidence, analyzes evidence, confronts the suspects, and testifies in the courtroom. In the typical crime lab no single person usually performs all of these roles.

The eager, newly minted forensic scientist will receive a reality check when faced with the abundant documentation and regulatory requirements of the job. No one on *CSI* ever seems to run controls or perform quality assurance checks. The glamour and glitz of a Las Vegas casino, nine times out of ten, will most likely be replaced by a filthy apartment, an alley, or a compromised crime scene in a public thoroughfare.

Immediate results

How does the CSI effect impact the forensic laboratory, particularly the forensic toxicology laboratory? Again, I am fortunate enough to be associated with one of the premier forensic toxicology laboratories in the world, but as capable and efficient as the AFME laboratory is, their efforts pale in comparison to what transpires during prime-time television. Our toxicology laboratory cannot provide me with thorough toxicology results prior to my completion of the autopsy, mainly because unlike what appears to take place during television postmortem examinations, I collect the specimens as I perform the autopsy. (While some specimens may be collected via a percutaneous route, that practice is definitely frowned on if an autopsy is to be performed.)

I am unaware of a medical examiner office that submits toxicology specimens prior to completion of the autopsy, except for those rare cases where a test result may dictate the performance of an additional procedure. This reality doesn't seem to faze the television version of the forensic laboratory, where toxicology reports are handed to the patholo-

gist even before the Y-incision is completed.

The fan of television forensics expects the medical examiner to be armed with toxicology results almost immediately. More than one family member and law enforcement agency has called me within a day or two of the autopsy expecting as much. Granted, in appropriate settings, some analyses such as a screening test for carboxyhemoglobin saturation may be run as a stat. In general, I feel fortunate to have complete toxicology results seven to ten days after completing the autopsy, as would nearly all medical examiners.

Testing for everything

Another myth is that all specimens will be tested for all possible substances. To those of us who work in the field of laboratory medicine, it is ingrained to think about such things as screening vs. confirmation testing, batching of specimens, and performing only tests that yield relevant results. Unfortunately, the most competent and up-to-date television-drama laboratory performs such exhaustive testing that all substances present are identified, qualitatively and quantitatively, within minutes.

Do the misconceptions about the forensic laboratory impact the way laboratory professionals do their job? According to one of the senior toxicologists in our laboratory, Dr. Mick Smith, any deviation in the handling of specimens may influence how far the prosecution will take a case, even if it can be shown that there was zero influence on the testing itself. The jury expects all aspects of specimen collection and testing to be performed flawlessly, with 100 percent accuracy and precision. We also may be asked to perform additional tests in response to the unrealistic expectations of jurors rather than follow protocols dictated by science.

I have already alluded to problems with the next of kin. Unfortunately, many families' expectations may be molded by what they have "learned" by watching television. To say that there may be some unrealistic expectations would be an understatement. Some of the best examples are again found in the area of toxicology. If the laboratory didn't test for the presence of all substances that might possibly be present, how can a case of poisoning with a rare toxin be completely excluded? Surely what is being called a suicide might be a homicide staged to look like a suicide and no investigator short of Gil Grissom, CSI, would be able to tell the difference.

Courtroom effects

More importantly, the CSI effect may come into play in the courtroom. A jury, guided by the popular media representation of the forensic sciences, may

have unrealistic expectations of how evidence is recovered and processed. There have been examples in the popular media of juries discounting eyewitness accounts because they expected to have fingerprints or trace evidence available to corroborate the story. The jury was reluctant to convict without such evidence because television has "taught" them to demand the presence of physical evidence.

According to lawyers interviewed for the popular media coverage of the CSI effect, it is not unusual to ask potential jurors about their television viewing habits. The *USA Today* article cited one study that found 70% of 500 potential jurors were viewers of *CSI* or similar television shows.

In an effort to educate juries, some attorneys have resorted to bringing in experts for the sole purpose of testifying that it is not unusual to have minimal physical evidence at a crime scene. Military lawyers have told me that the unrealistic expectations the public has developed regarding forensic science has had a definite impact on how they prepare and present their cases, increasing the cost in both time and money.

Not surprisingly, the CSI effect has also influenced the way defense attorneys prepare their cases. Jurors may be unwilling to accept that technical or human errors can compromise the validity of scientific evidence. After all, the television crime lab personnel never seem to run into any difficulties with even the most complex extraction and identification procedures. Because of the public's fascination with television forensics, the presence of any physical evidence, even if its relevance to the guilt of the defendant is minimal, may have an exaggerated influence on a jury.

Budget effects

An unfortunate fact is that crime labs and forensic science programs in general are underfunded and overworked. Law enforcement agencies are submitting an ever-increasing number of specimens for testing to a system that is already backlogged in many jurisdictions. Budget constraints do not allow the average crime lab to purchase every new technological innovation that comes on the market. Increasing the number of laboratory tests performed, especially if they are of questionable relevance, only adds to this backlog.

Of course taking some liberties with the actual science is necessary and expected in the television portrayal of a crime lab and the forensic sciences. After all, with the time out for commercials, there is less than 45 minutes available to develop the plot and solve the crime. It would be extremely difficult to work in the complex analytic procedures, ex-

pected delays, and routine frustrations experienced in even the best of crime labs. The technical advisors to shows such as *CSI* are expected to make sure the truth isn't stretched too far, though they are not always successful.

Conclusion

The *CSI* effect has had both positive and negative influences on the forensic sciences. Everyone in the field who testifies in the courtroom, interacts with families, or simply answers inquiries from individuals who may be biased from the television portrayal of the crime lab should be prepared to provide a more realistic explanation of how laboratory testing is carried out. The public's increased familiarity with the methodology and capabilities of the laboratory can be beneficial to the forensic science field, as long as the real (vs. reel) laboratory professionals are able to help separate fact from fantasy.

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Digoxin-Like Substances Interfere with Immunoassays

By Amitava Dasgupta

Digitalis glycosides have been used in medicine for more than 200 years. The main pharmacological effects include a dose-dependent increase in myocardial contractility and a negative chronotropic action. Digitalis also increases the refractory period and decreases impulse velocity in certain myocardial tissue (such as the AV node). The electrophysiological properties of digitalis are reflected in the ECG by a shortening of the QT interval.

Both digoxin and digitoxin have narrow therapeutic indices, and therapeutic drug monitoring is essential to achieve optimal efficacy and avoid toxicity. The therapeutic range of digoxin is usually considered to be 0.8–2.0 ng/mL, but there is substantial overlap between therapeutic and toxic concentrations. Moreover, mild to moderate renal failure can also significantly increase risk during digoxin therapy (1).

Digoxin toxicity can occur with lower digoxin levels in the presence of hypokalemia, hypomagnesemia, or hypothyroidism. Likewise, the concomitant

use of drugs such as quinidine, verapamil, spiro-lactone, flecainide, and amiodarone can increase serum digoxin levels and increase the risk of digoxin toxicity. A recent clinical trial in women found that serum concentrations of 0.5–0.9 ng/mL had a beneficial effect on morbidity and no excess mortality, whereas serum concentrations at or over 1.2 ng/mL appeared harmful (2).

Although digoxin concentrations in serum or plasma can be detected accurately by sophisticated analytical techniques such as high performance liquid chromatography combined with tandem mass spectrometry, in clinical laboratories digoxin immunoassays are the preferred method because of their ease of automation and rapid turnaround time. Both exogenous and endogenous digoxin-like immunoreactive substances (DLIS) can interfere with the immunoassays due to their structural similarity to digoxin.

The major endogenous DLIS are found in elevated concentrations in volume-expanded patients (uremia, liver disease, pregnancy, etc.). Major exogenous substances that interfere with serum digoxin immunoassays are spironolactones, potassium canrenoate, and various Chinese medicines. The magnitude of the interference varies with the kind of antibody used (monoclonal or polyclonal) and the assay design. The fluorescence polarization immunoassay (FPIA) by Abbott Laboratories is affected the most.

Discovery of endogenous DLIS

After the discovery of endorphins, the endogenous equivalent of opiates, it was proposed that there might be an endogenous equivalent of cardiac glycosides. It was further hypothesized that an anti-digoxin antibody might be able to detect the presence of DLIS in body fluids. Gruber et al. first demonstrated the presence of DLIS in volume-expanded dogs in 1980 (3). Craver and Valdes later reported an unexpected increase in the serum digoxin concentration of a patient with renal failure who was already on digoxin, with digoxin still measurable for a week after its discontinuation (4). DLIS were found in various human body fluids and tissues, including cord blood, placenta, amniotic fluid, bile, meconium, cerebrospinal fluid, and saliva.

DLIS cross-react with anti-digoxin antibodies and inhibit Na^+/K^+ -ATPase. Early reports also indicated cross-reactivity of DLIS with the Abbott Laboratories FPIA. Most investigators used commercially available digoxin assays for detecting DLIS in body fluid. However, Panesar used bufalin as an antigen and developed polyclonal antisera for detecting DLIS (5). Lin et al. developed a polyclonal-antibody-based ouabain enzymeimmunoassay for detecting DLIS (6). They also developed a Fab fragment of the anti-

digoxin-antibody-based enzymeimmunoassay for this purpose. They concluded that a polyclonal-antibody-based ouabain assay was more efficient in detecting DLIS in human blood.

DLIS concentrations in health and disease

Usually concentrations of DLIS in healthy subjects are below detection limits even using the FPIA assay for digoxin, which has high cross-reactivity with DLIS. Volume expansion is a major cause of elevated DLIS in blood. Elevated concentrations of DLIS have been reported in uremia, essential hypertension, hypertension of water volume expansion, liver disease, pre-eclampsia, liver and kidney transplant, congestive heart failure, prematurity, and other conditions. Howarth et al. reported elevated plasma concentrations of DLIS in intensive-care patients, measured using an FPIA assay (7). Although some patients showed either hepatic or renal dysfunction, 42 patients who showed elevated DLIS had neither. The DLIS concentrations in 16 patients with coexisting hepatic and renal dysfunction were 0.0–1.32 ng/mL (detection limit 0.20 ng/mL), while 38 patients with hepatic dysfunction but normal renal function showed a range of 0.0–0.60 ng/mL.

Impact of DLIS interference on TDM

The presence of DLIS in various patient groups is significant in the light of the narrow therapeutic window of digoxin. Positive interference of DLIS with the Abbott FPIA is well-documented in the literature. Avendano et al. reported an 89% false-positive rate for digoxin in blood drawn from peripheral veins of newborn babies and a striking 100% false-positive rate in the corresponding cord blood when FPIA (Digoxin II) was used (8). The authors also observed a 60% false-positive rate in patients with severe hepatic disease and concluded that digoxin levels must be interpreted very carefully in these patients.

Way et al. evaluated the Vitros digoxin assay (Johnson & Johnson) for interference from DLIS by comparing it with Roche OnLine and MEIA (microparticle enzymeimmunoassay, Abbott Laboratories) assays using 26 adult patients receiving digoxin (9). They observed mean digoxin concentrations of 1.30 ± 0.69 ng/mL (SD) by the Roche assay, 1.34 ± 0.58 ng/mL by the Abbott assay, and 1.46 ± 0.68 ng/mL by the Vitros digoxin assay. The mean digoxin concentrations found by using the Vitros were significantly different by the student t-test. The authors concluded that the positive bias in the Vitros assay compared with the Roche OnLine assay was probably due to DLIS.

Although most investigators reported positive

interference of DLIS with serum digoxin measurement, negative interference (falsely lower digoxin values) has been reported in the MEIA (10). This interference could be a serious problem if a clinician increased a digoxin dose based on a falsely low digoxin concentration.

Elimination of interference by ultrafiltration

Digoxin exhibits poor protein binding (25%), but Valdes and Graves reported strong serum protein binding of DLIS (11). DLIS is usually absent from protein-free ultrafiltrate, so both positive and negative interference can be completely eliminated by measuring protein-free ultrafiltrate (12).

Interference of spironolactone and its metabolites

Spironolactone, a competitive aldosterone antagonist, has been used clinically in the therapy of hypertension and congestive heart failure for a long time. Spironolactone is rapidly and extensively metabolized and the metabolite canrenone is pharmacologically active. Because spironolactone and digoxin may be used concurrently in the management of a patient, interference of spironolactone and canrenone in therapeutic monitoring of digoxin is troublesome.

Morris et al. first reported positive interference of spironolactone in digoxin measurement using FPIA in 1988 (13). Later, other authors verified the interference of spironolactone and canrenone in FPIA and other commonly used immunoassays for digoxin. Steimer et al. described negative interference of canrenone in digoxin measurement (14). Canrenone and spironolactone caused falsely low digoxin values due to negative interference in serum digoxin measurement when an MEIA was used. On several occasions, misleading subtherapeutic measurements of digoxin led to improper dosing, leading to serious digoxin toxicity in the patients. Taking advantage of strong protein binding of spironolactone and its metabolites and poor protein binding of digoxin, it might be possible to eliminate interference of these compounds in serum digoxin measurement by monitoring free digoxin.

Interference by Chinese medicines

Traditional Chinese medicines are readily available without prescription from herbal stores and local Chinese stores. One such Chinese medicine is Chan Su, which is prepared from the dried white secretion of the auricular glands and skin glands of Chinese toads (*Bufo melanostictus Schneider* and *Bufo bufo gargarinas Gantor*). Chan Su is a major component of traditional Chinese medicines Liu-Shen-Wan and Kyushin, which are used for the treatment of tonsillitis, sore throat, palpitation, and

more. Chan Su is also used to stimulate myocardial contraction, as an anti-inflammatory, and for pain relief. Chan Su's cardiotoxic effect is due to its major bufadienolides, such as bufalin, cinobufagin, and resbufogenin. Bufalin is known to block vasodilation and increase vasoconstriction, vascular resistance, and blood pressure by inhibiting Na^+/K^+ -ATPase. Panesar reported an apparent digoxin concentration of 1124 pmol/L (0.88 ng/mL) in a healthy volunteer who ingested Liu-Shen-Wan pills (15). An apparent digoxin concentration of 4.9 ng/mL was reported in one woman who died from ingestion of Chinese herbal tea containing Chan Su (16).

Chan Su extracts also falsely increased serum digoxin measurements in vitro when FPIA (Digoxin II, TDx analyzer, Abbott Laboratories) was used (17). In contrast, serum digoxin levels were falsely lowered when the MEIA assay (AxSYM analyzer, Abbott Laboratories) was used. However, the components of Chan Su responsible for digoxin-like immunoreactivity are significantly bound to serum proteins (>80%), so their interference can be eliminated by monitoring free digoxin.

Another Chinese herb, danshen, is prepared from the root of *Salvia miltiorrhiza* and used for various cardiovascular diseases, including angina pectoris. More than 20 diterpene quinones known as tanshinones have been isolated from danshen, and these compounds are structurally similar to digoxin. Feeding danshen to mice caused digoxin-like immunoreactivity in sera; however, the extent of the activity was less remarkable than that caused by feeding them Chan Su (18). In vitro studies indicated that danshen can falsely increase serum digoxin levels by FPIA. In contrast, digoxin values were falsely lower with the MEIA assay. Because the components of danshen that cause DLIS activity are strongly protein bound, monitoring free digoxin can eliminate their interference.

There is also a case report of interference by Siberian ginseng (see "Case Report" in next column).

These Chinese medicines and other herbal remedies interfere with serum digoxin using immunoassay measurement due to structural similarity of certain components. Table 1 summarizes the immunoassay effects of each.

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Case Report: Ginseng and Digoxin

There is one case report of interference of Siberian ginseng in serum digoxin measurement (1). A 74-year-old man had a steady serum digoxin level of 0.9–2.2 ng/mL (1.15–2.81 nmol/L) for 10 years. His serum digoxin increased to 5.2 ng/mL (6.66 nmol/L) on one occasion after taking Siberian ginseng. Although the level was toxic, the patient did not experience any signs or symptoms of digoxin toxicity. When he stopped taking Siberian ginseng, his digoxin level returned to normal.

However, other reports indicate that Siberian, Asian, American, and Indian ginseng interfere only modestly with FPIA (falsely elevated digoxin values) and MEIA (falsely lowered digoxin values) while showing no cross-reactivity with Roche, Bayer, and Beckman digoxin assays (2).

— Amitava Dasgupta

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Table 1. Interference of herbal products and Chinese medicines in therapeutic drug monitoring of digoxin

Product	Effect*	Comments
Siberian, Asian, and Indian ginseng	Moderate	Falsely elevated digoxin concentration. Components of Siberian ginseng may cross-react with antibody used in digoxin assay
Kyushin	Significant	Possible interference with digoxin assay
Chan Su	Moderate	Active components like bufalin cross-react with assay
Uzara root (diuretic)	Significant	Additive effect with digoxin. Also interferes with assay
Oleander	Significant	Oleandrin and related components interfere
Danshen	Moderate	Interferes with FPIA and MEIA assay

*Moderate=interferes with only a few digoxin assays (polyclonal-based such as FPIA)
Significant=interferes with most digoxin immunoassays

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