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Screening, Confirmation, Prevalence, and Fatality of Digoxin in Finland in 2012-2025

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Abstract

Digoxin, a cardiotonic glycoside derived from Digitalis lanata, is widely prescribed for the treatment of congestive heart failure and atrial fibrillation. However, it has a narrow therapeutic index and a significant risk of toxicity. Digoxin toxicity manifests as gastrointestinal, neurological, and cardiac symptoms, with risk factors including renal impairment, drug-drug interactions, and electrolyte disturbances. Post mortem redistribution complicates the interpretation of digoxin concentrations, necessitating careful correlation with clinical history and case circumstances.

This study evaluated analytical methods used for digoxin detection in post mortem (PM) blood samples in Finland during 2012 to 2025. Screening was initially performed using immunoassays, which were later supplemented and eventually replaced by liquid chromatography–tandem mass spectrometry (LC-MS/MS) to improve analytical specificity. In total, more than 34,000 analyses were conducted. Positive rates ranged from 1.8% with immunoassays to 25.5% with LC-MS/MS. Comparative analysis of 234 samples analyzed using both methods demonstrated high immunoassay specificity (99.1%), although concentrations were systematically underestimated compared with LC-MS/MS.

The median digoxin concentration in positive cases was 1.5 µg/L. The 90th and 95th percentiles (3.8 µg/L and 5.1 µg/L, respectively) corresponded well with concentrations typically considered toxic and fatal. A total of 36 fatal digoxin intoxications were identified (0.11% of all cases), with a mean concentration of 14.1 µg/L, predominantly occurring in elderly individuals.

These findings demonstrate the importance of LC-MS/MS for reliable post mortem quantification of digoxin, illustrate the prevalence of digoxin in forensic autopsy cases, and emphasize the relevance of concentration thresholds when distinguishing therapeutic use from toxic or fatal exposure.

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Introduction

Digoxin is a cardiotonic plant glycoside found in Digitalis lanate, the molecular structure of which is shown in Figure 1. It is used to treat congestive heart failure usually in combination with a diuretic and an Angiotensin Converting Enzyme (ACE) inhibitor and for ventricular rate control in atrial fibrillation [1,2]. Digoxin inhibits Na^+/K^+ ATPase, increasing intracellular sodium and calcium via the Na^+/Ca^{2+} exchanger, resulting in increased myocardial contractility (positive inotropy). It also increases vagal tone, thereby slowing atrioventricular nodal conduction (negative chronotropy).

Clinical symptoms of digoxin toxicity include nausea, vomiting, anorexia, confusion, weakness, blurred vision, yellow green visual halos, bradycardia, and ventricular arrhythmias. Risk factors for toxicity include renal impairment, drug interactions (e.g., amiodarone, verapamil), and electrolyte disturbances, particularly hypokalaemia and hypomagnesemia [3].

The therapeutic reference range of digoxin in serum is relatively narrow (0.5-2.0 µg/L), although higher concentrations up to 13.7 µg/L have occasionally been observed in patients exhibiting toxic effects [4,5]. In acute overdose cases with survival, serum concentrations ranging from 11 to 42 µg/L have been reported [6-12]. Digoxin has an elimination half-life of approximately 30-48 hours and a volume of distribution of 5.1-7.4 L/kg [6].

In post mortem cases, average serum digoxin concentrations of approximately 1.4 µg/L (range 0.7-2.9 µg/L) have been reported from femoral vein samples [13]. In post-mortem blood Karjalainen et al. [14], found an average of 4.6 µg/L (range 1.3-8.2 µg/L) in 13 samples of blood obtained from an unidentified source using an extraction-radioimmunoassay procedure.

In fatal overdose cases the serum concentrations of digoxin can be very high, from 3.5 up to 282 µg/L [13,15-24]. Observational studies often show increased all-cause and cardiovascular mortality in digoxin users, especially at higher serum levels. The large, randomized DIG trials found a neutral effect on mortality but a reduction in heart failure hospitalizations, suggesting that excess deaths in observational data may reflect sicker baseline populations rather than direct drug harm [25-29]. Digoxin benefit varies in heart failure hospitalization due heterogeneity [30]. U.S. National Poison Data (2012-2020) shows hundreds of reported exposures annually, with a significant proportion in elderly patients [31]. Annual deaths of 18-36 is attributed to digoxin toxicity, higher than other narrow therapeutic index drugs like lithium or warfarin. Many cases are due to chronic accumulation rather than acute overdose. Digoxin levels in PM heart blood can be up to three times higher than ante mortem peripheral blood in the same patient [32]. This is due to diffusion from myocardium and other tissues after death. Consequently, elevated post mortem concentrations do not necessarily reflect antemortem toxicity, and interpretation must always consider clinical history, case circumstances, and the possibility of post mortem redistribution.

Digoxin is commonly analyzed using immunoassays, although cross reactivity with structurally similar cardiac glycosides can occur [33-38]. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) has become increasingly favoured due to its superior analytical specificity and sensitivity [39-44].

The aim of this study was to evaluate the performance of immunological screening methods and LC-MS/MS confirmation for the analysis of digoxin in post mortem blood samples. A further objective was to assess the prevalence of digoxin detection in forensic autopsy cases and the occurrence of fatal digoxin intoxications in Finland.

Materials and Methods

Data were collected from all medico legal forensic autopsies performed in Finland between 2012 and 2025. Digoxin screening and quantification were initially performed using immunoassays (Siemens Immulite 1000, Immulite 2000, and Advia Centaur). Positive findings and selected samples were later confirmed using LC-MS/MS (Sciex 4000 QTrap and Agilent 6475 instruments).

The immunoassays employed Siemens DIGOXIN assay kits specific to each analyzer, with a Limit of Quantification (LOQ) of 0.5 µg/L. LC-MS/MS analysis was performed using a Kinetex 1.7 µm EVO C18 column (50 mm × 2.1 mm). The mobile phases consisted of 10 mmol/L ammonium acetate (pH 3.2) with 0.1% formic acid (Eluent A) and 0.1% formic acid in methanol (Eluent B). The gradient was run 40% Eluent B at 0 min, 90% Eluent B 2-3 min and dropped to 40% again at 3.2 min and kept until 4min, achieving a total run time of 4 min using flow of 0.4 mL/min and column temperature of 40°C. Mass spectrometric detection was performed using electrospray ionization in negative Multiple Reaction Monitoring (MRM) mode. The monitored transitions were m/z 781.6 → 651.6 and 113.2 for digoxin, and m/z 784.6 → 654.4 for digoxin D_3 (internal standard). The method was validated according to accepted bioanalytical validation guidelines. Samples were considered positive at concentrations >0.3 µg/L, and the LC-MS/MS LOQ was 0.01 µg/L.

Results and Discussion

Digoxin analysis was performed exclusively using an immunoassay between 2012 and 2018, applying the selection criterion that the deceased person was 65 years of age or older. This resulted in nearly 19,000 analyses (2,410 per year), of which 906 were positive (4.8%). Consequently, there was a clear need to reduce the number of analyses with negative findings and to refine the selection criteria.

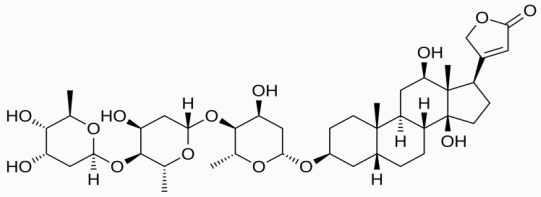

Figure 1: Molecular structure of digoxin.

Table 1: The numbers of analysis of digoxin with different methods in 2012-2025.

Method	2012-2019	2019-2024	2024 - 2025	Sum
Immunological, screening and quantitation	18978	0	0	18978
Immunological, screening only	0	12998	0	12998
LC-MS/MS	0	1481	787	2268
Sum	18978	14479	787	34244

Starting in July 2019, an LC-MS/MS method was introduced for confirmation of positive immunoassay results and for samples with limited volume or advanced putrefaction, owing to the higher specificity of LC-MS/MS. During this period, the total number of analyses was approximately 14,500 (2,630 per year), representing a slight increase compared with the previous years. The positive rate obtained with the immunoassay was only 1.8%, whereas it reached 25.5% when using LC-MS/MS. Thus, there remained a need to further reduce unnecessary analyses, especially those yielding negative results.

From June 2024 onwards, LC-MS/MS was used as the sole method for both screening and confirmation, and new selection criteria were introduced: prescribed digoxin, suspected poisoning by any substance, or death occurring in a hospital or nursing home, combined with an age over 50 years. By October 2025, this approach resulted in a total of 1,199 analyses, corresponding to approximately 900 analyses per year. This represents a significant decrease from the 2,400 analyses per year conducted during the immunoassay period. The overall positive rate was 4.3%.

Correlation between the immunoassay and LC-MS/MS methods was assessed using data from 2019 to 2024, when positive screening results by immunoassay were confirmed by LC-MS/MS. In total, 234 samples were analyzed using both techniques. Only two of these samples were negative by LC-MS/MS, yielding an immunoassay specificity of 99.1%. Sensitivity could not be determined, as negative immunoassay results were not confirmed by LC-MS/MS (Table 1).

In general, immunoassay concentrations were approximately 25% lower than those obtained using LC-MS/MS, with differences ranging from 82% to 53%. In 26 cases, the immunoassay result was higher than that obtained by LC-MS/MS, whereas in the remaining 208 cases it was equal to or lower. Consequently, it is possible that some cases with negative immunoassay results would have been positive if analyzed by LC-MS/MS. However, these cases would most likely not represent clinically relevant intoxications, as the LC-MS/

Table 2: Positive cases of digoxin 2012-2025 and its statistical concentrations.

Method	N		Concentration, µg/L				
	Positive cases	All cases	Mean	Median	90th percentile	95th percentile	97.5th percentile
Immunological	1139	31976	2.15	1.5	3.9	5.2	6.8
LC-MS/MS	431	2680	2.25	1.6	3.4	5.1	6.3
Both methods	1336	34656	2.17	1.5	3.8	5.1	6.6

Table 3: Intoxications with digoxin observed with immunological and LC-MS/MS methods in 2012-2025.

Method	N	Concentration, µg/L		Fatal rate, %		Age, years	
		Mean	Range	All cases	Positive cases	Mean	Range
Immunological	23	14.6	4.1- 73	0.07	2	69.5	25-89
LC-MS/MS	13	13.2	3.7-63	0.49	3	74	63-91
Both methods	36	14.1	3.7-73	0.11	2.7	71.1	25-91

MS limit of detection is very low relative to the upper limit of the therapeutic range.

The linear correlation coefficient (R^2) was 0.725, and the difference between methods was statistically significant (two-tailed t test, $p<0.001$). Thus, although moderate correlation was observed, a systematic difference between the methods remained. Based on these findings, refined selection criteria were established for digoxin analysis: prescribed digoxin, suspected poisoning by any substance, or death occurring in a hospital or nursing home, combined with an age over 60 years. Under these criteria, the risk of missing digoxin poisoning is considered minimal.

Table 2 presents the total number of cases and positive cases, as well as the mean and median concentrations and the 90th, 95th, and 97.5th percentiles for both analytical methods. Concentrations were very similar between the two methods, showing no significant differences. The median concentration was 1.5 µg/L and the mean concentration was 2.17 µg/L, slightly above the therapeutic plasma range. The 95th percentile concentration was 5.1 µg/L, which may be regarded as potentially fatal, while the 90th percentile concentration was 3.8 µg/L, which can be considered toxic [45].

Table 3 summarizes fatal intoxication cases from 2012 to 2025. A total of 36 fatal cases were identified, involving either digoxin alone or digoxin in combination with other drugs or substances. These cases represent 0.11% of all analyses and 2.7% of positive findings. In fatal poisonings, the mean digoxin concentration was 14.1 µg/L (median 7.7 µg/L), with a concentration range of 3.7-73 µg/L. In some cases, relatively low concentrations were observed due to multidrug poisonings in which digoxin was only one contributing substance. Nevertheless, these concentrations correlate well with the 90th percentile concentration (3.8 µg/L), which is already considered toxic. The mean age in fatal cases was 71.1 years (range 25-91 years), as expected given that digoxin is predominantly prescribed to elderly patients.

Conclusion

Immunological screening methods resulted in a high number of analyses but a relatively low positive rate. Comparative testing performed between 2019 and 2024 demonstrated that immunoassays exhibited high specificity but systematically underestimated digoxin concentrations by approximately 25% compared with LC-MS/MS.

The transition to LC-MS/MS based screening, combined with refined case selection criteria, substantially reduced unnecessary analyses while improving analytical accuracy and reliability. This approach enhances both resource efficiency and the forensic interpretation of digoxin findings, particularly in elderly populations where digoxin use and the risk of intoxication are most prevalent. Fatal digoxin intoxications occurred primarily in older individuals and were associated with concentrations well above therapeutic levels.

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