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The CLAM-2000 Challenge: Checking the Accuracy and Reproducibility of Automatic Sample Preparation of Steroids and Glucocorticoids in Plasma

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Introduction

Manual sample preparation of clinical samples is a time consuming work. The CLAM-2000 robot is fully automated sample preparation module for LCMS. It can be used with commercial kits and homebrew methods. So far it intended use is research only.
We selected two steroid hormones (Cortisol, Testosterone) and two glucocorticoid drugs (Dexamethasone, Prednisolone) for closer investigation of the efficiency of automatic sample preparation.
Cortisol and testosterone are hormones routinely tested in new born screening. Dexamethasone and Prednisolone treat many inflammatory and autoimmune conditions , both substances are banned under WADA anti-doping rules.

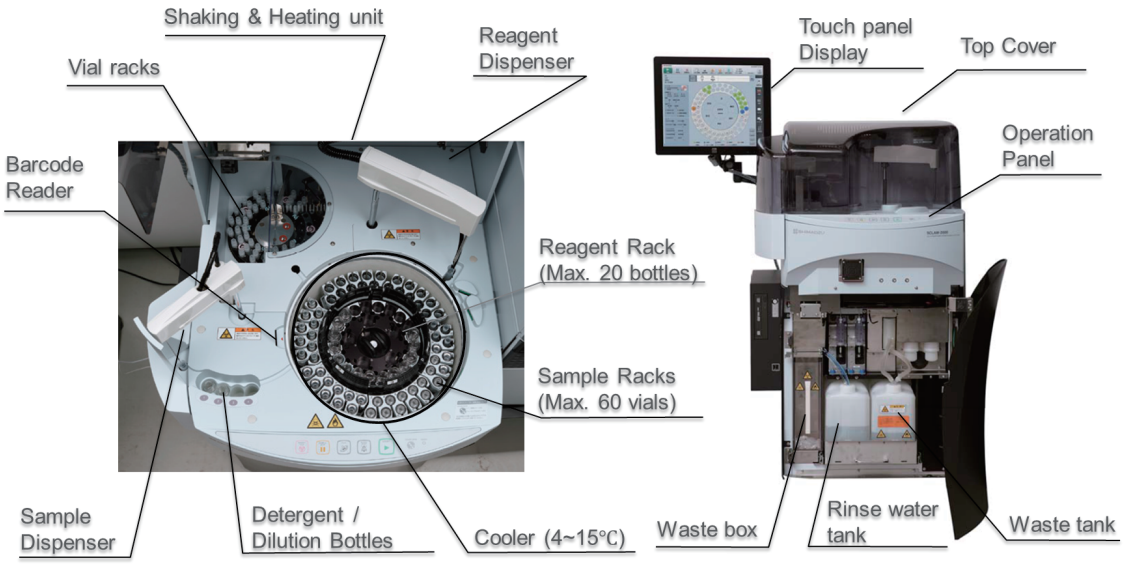

Fig 1 CLAM-2000 configuration

Materials and Methods

Manual Sample Prep:

(1) To 100 uL plasma sample (with spiked compounds) add 200 uL precipitation mix (containing internal standard), **(2)** Mix for 60 sec on vortex mix, **(3)** Centrifugation 5 min at 12000 g, **(4)** Transfer supernatant to autosampler vial and inject 20 uL into LCMS-8045 triple quadrupole coupled to an Nexera-X2 UHPLC. Analysis of triplicates (same sample preparation) in MRM spectrum mode using internal standards for

Automatic Sample Prep using the CLAM-2000:

(1) Conditioning the filtration vial with 20 uL of methanol, **(2)** 50 uL plasma sample (with spiked compounds) added to filtration vial, **(3)** 100 uL precipitation mix (containing internal standard) added to filtration vial, **(4)** Shaking for 60 sec, **(5)** Filtration for 90 sec, **(6)** Transfer flowthrough from collection vial to HPLC autosampler and inject 20 uL into the LCMS-8045 triple quadrupole to an Nexera-X2 UHPLC. Analysis of triplicates (separate sample preparation) in MRM spectrum mode using internal standards for confirmation.

Results and discussion

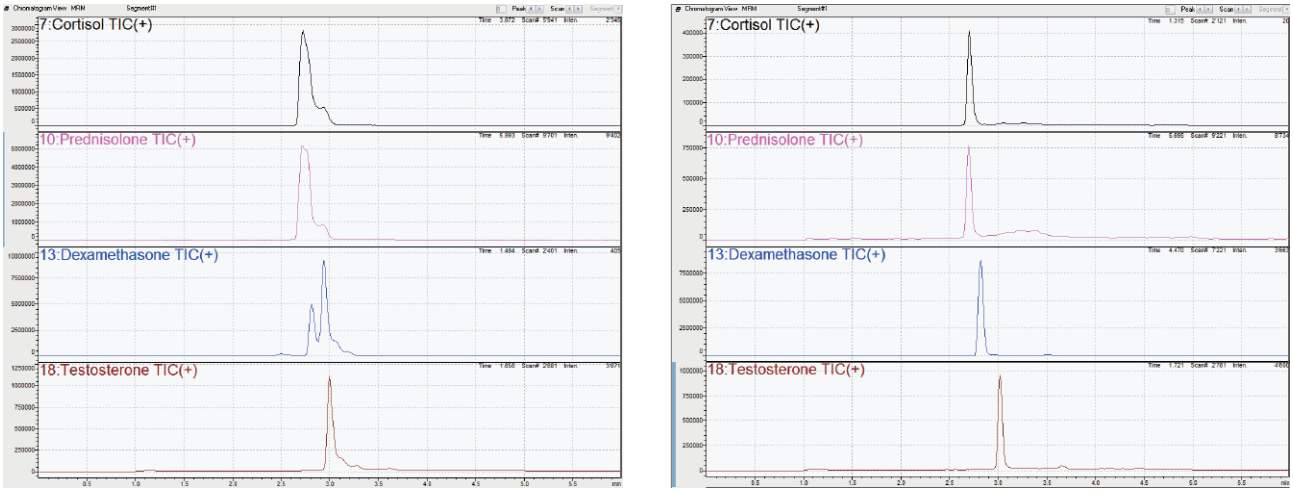

Fig. 2 Chromatograms: left manual prep, right: automatic sample prep. The filtration step during the automatic CLAM sample preparation seems to give sharper peaks for plasma samples.

Suprisingly we found sharper peak shapes for the chromatograms of all compounds using the automatic sample preparation (Figure 2). The check of the reproducibility using control samples showed more variation for automatic sample preparation, this is due to the fact that the CLAM-2000 robot prepares real independent technical replicates.

Table 1: Reproducibility check using five injections of a control sample

	Cortisol manual Conc. [ng/mL]	**Cortisol automatic** Conc. [ng/mL]
Average	602.28	614.75
%RSD	4.35	14.83
Maximum	637.98	688.82
Minimum	559.60	459.17
Std. Dev.	26.19	91.15

	Dexamethasone manual Conc. [ng/mL]	**Dexamethasone automatic** Conc. [ng/mL]
Average	416.02	471.76
%RSD	13.07	25.71
Maximum	518.25	659.82
Minimum	370.08	342.86
Std. Dev.	54.36	121.29

	Testosterone manual Conc. [ng/mL]	**Testosterone automatic** Conc. [ng/mL]
Average	5.18	5.25
%RSD	15.44	11.20
Maximum	6.39	5.98
Minimum	4.30	4.66
Std. Dev.	0.80	0.59

	Predinisolone manual Conc. [ng/mL]	**Prednisolone automatic** Conc. [ng/mL]
Average	392.00	471.77
%RSD	12.61	18.27
Maximum	485.20	580.61
Minimum	349.55	384.32
Std. Dev.	49.44	86.19

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The statistical Passing-Bablok test did not show a significant difference in the results between manual and automatic sample preparation as shown in Figure 3. We found the same LOQ and the same linearity for all four compounds. Additionally automated sample preparation showed less variation of area of the internal standards.

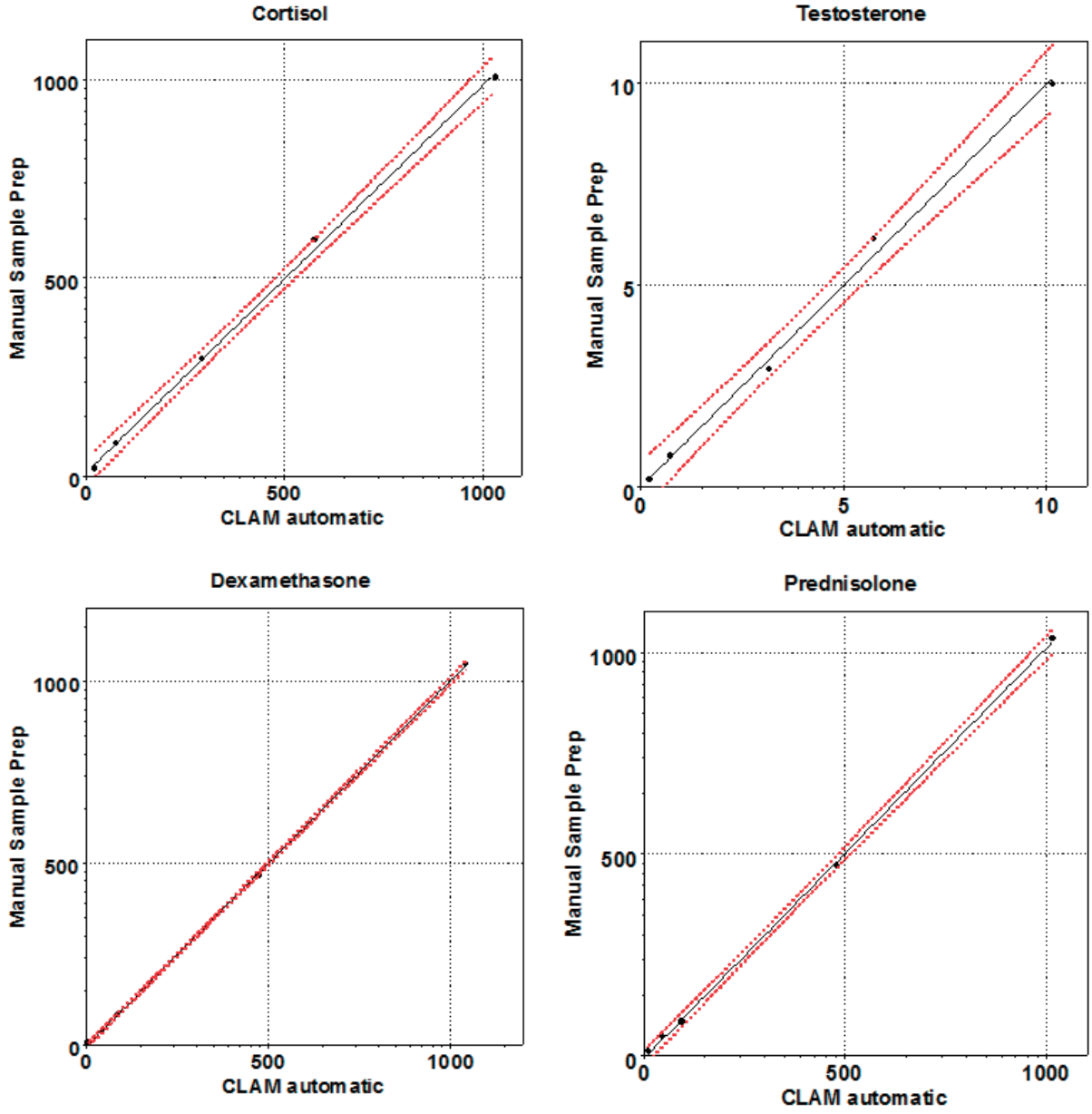

Fig 3 Passing-Bablock test
Red dotted line: 95% Confidence Interval
Black line: Linear regression of results CLAM (y-axis) versus manual sample prep (x-axis)

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Summary

Automatic sample preparation using the CLAM-2000 robot for LCMS gives similar results compared to manual preparation, but reduces the risk of human error and also the consumption of reagents. The filtration step in the automatic sample preparation seem to improve chromatographic peak shape.

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