Evaluation of agreement between a laboratory-based and a field-based blood analyser for analysis of selected biochemical analytes in farmed Atlantic salmon (*Salmo salar* L.)

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Abstract
Agreement between a point of care device and a laboratory blood analysis machine for parameters lactate, sodium, potassium and chloride was evaluated using samples from two different Atlantic salmon populations. The results were summarised using scatter plots and calculations of Lin´s concordance correlation coefficient, and Pearson´s correlation coefficient. Good concordance and correlation were found for lactate, while the concordance and correlations for the blood ions were very low.

Introduction

Clinical biochemistry is a well-established diagnostic tool in both human medicine and veterinary medicine of terrestrial animals. Serum or plasma from a blood sample can be analysed for panels of indicators of organ function. Typically, the technique involves measurement of an enzyme, a metabolic product or an ion. The combined information from the panel of analytes can give diagnostic and prognostic information and can be used to assess the progression of a disease and assist in making decisions about treatment and management (Thrall, 2012). In salmonids, gill diseases such as amoebic gill disease (AGD) and tenacibaculosis have been shown to affect plasma levels of chloride, sodium, potassium, cortisol and lactate (Hvas et al., 2017) and lactate (Powell et al., 2005), respectively. Increased lactate levels were found in salmon with infectious salmon anemia (Olsen et al., 1992) and in fish with myopathies such as in pancreas disease (PD) (Rodger et al., 1991). Lactate is also used as an indicator of stress during handling (Iversen et al., 2005). The application of clinical chemistry in clinical fish medicine is still relatively limited (Braceland et al., 2017), however, availability of blood analysis devices that are easy to use and deliver results rapidly may contribute to changing this.

Handheld and portable devices for "point-of-care" (POC) analysis have been developed for clinical chemistry in human and veterinary

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medicine. Such devices have the potential to be used at the fish farm, yielding rapid information about fish health without the delay caused by sending samples for laboratory analysis. However, there is limited knowledge about the practical use and reliability of such POC-devices in Atlantic salmon. The iSTAT (Abaxis Europe GmbH) is a POC-device used in human and small animal veterinary medicine which has the advantage of being able to analyse a wide range of blood analytes, including ions, enzymes and gases. It is easy to use, and the small size means it can easily be transported to fish farms or hatcheries for on-site analysis. The aim of this study was to test the agreement between the point of care device iSTAT in the field and a laboratory blood analysis machine for the parameter's lactate, sodium, potassium and chloride.

Materials and Methods

Blood samples were collected at two different occasions from two different farmed fish populations in Norway. First, 21 Atlantic salmon post-smolts weighing approximately 200 g kept in seawater with a temperature of 10°C at NIVA Research Facility at Solbergstrand were sampled. Four fish had skin ulcers and a proportion of the fish were below normal body condition or had erosions of the fin tips. Fish were intended for use in another research project and fish selected for sampling came from 3 different tanks. Euthanasia was achieved with an overdose of benzocaine (Benzoak vet, ACD Pharmaceuticals AS, Leknes, Norway). Blood was subsequently collected from the caudal vein (21 fish) and additional blood was drawn from the heart (2 fish), directly into lithium-heparinised tubes (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria).

The second sampling was performed at a sea site in Møre og Romsdal (north western Norway) where grossly visible gill lesions and a history of gill disease had been recently reported. At the time of the sampling the 41 sampled fish weighed from 2.4 to 4.9 kg and no clinical signs of disease or elevated mortality rates were evident. The sea temperature was measured at 5.9 and 6°C on the two sampling days. Fish were sedated with benzocaine (Benzoak vet, ACD Pharmaceuticals AS) as per producer recommendations for lice counting (sea lice, *Lepeophtheirus salmonis*). After lice counting fish were placed in an approximately 1000 L salt-water tank and sedated with isoeugenol (Aqui -S vet. Scan Aqua, Årnes, Norway) at 5 mL per cubic meter water and subsequently euthanised with an overdose of metacaine (Finquel vet. Scan Aqua) in an anesthetic bath with an approximate concentration of 200 mg/L. Fish were euthanised one at a time and were considered dead when no gill movement was observed in the anesthetic bath. Euthanasia was immediately followed by drawing of blood from the caudal vein directly into lithium-heparinised tubes (S-Monovette LH, Sarsted, Germany).

Approximately 120 or 250 µL of whole blood (120 µL per cartridge) was used for iSTAT analysis. The remaining heparinised blood was cooled and centrifuged in batches at 5000 rpm for 10 min. Plasma was transferred into 2 mL Eppendorf tubes and either chilled until arrival at the lab and subsequently frozen (sampling 1) or stored at -20°C until shipping to the Fish Vet Group lab on ice (sampling 2).

At sampling 1 iSTAT analysis was performed as soon as possible after sampling, however, time from sampling to analysis varied from

a few mins to approximately 1.5 h. The delay between sampling and analysis is likely to have led to an increased temperature of the affected samples. The analyser was acclimatised to the ambient temperature for at least 30 min prior to the first analysis and all analysis was performed at room temperature (approximately 20-22°C). Each blood sample was analysed with two separate cartridges; CHEM8 and CG4+ (Abaxis Europe GmbH; Griesheim, Germany) on a VetScan iSTAT1 handheld blood analyser (Abaxis Europe GmbH; Griesheim, Germany). Parameters measured included sodium (Na), potassium (K), chloride (Cl), hematocrit (Hct) for CHEM8 and lactate for CG4+. For sampling 2, iSTAT analysis was performed within 5 min after sampling. Samples analysis was performed in the boat cabin, and ambient temperature varied throughout the two days of analysis estimated at approximately 13-15°C and increasing to 20-25°C as the cabin was heated by the engine and the sun. Each sample was analysed using a single cartridge (CHEM8). The iSTAT measures sodium, chloride and potassium by direct ion-selective-electrode (ISE) potentiometry. Hematocrit and lactate are measured indirectly by whole-blood conductometry and by amperometry of hydrogen peroxide after conversion of lactate to hydrogen peroxide and pyruvate by lactate oxidase, respectively.

Laboratory blood analysis was performed on plasma (4°C) after thawing of previously frozen samples. Analyses were performed for sodium, potassium, chloride, urea and lactate using ABX Pentra kits (Horiba Medical, Irvine, CA) on the ABX Pentra C400 (Horiba Medical, Irvine, CA) according to instrument protocols. In this device, ions are measured by direct ion-selective-electrode (ISE) potentiometry, while lactate was measured using spectrophotometry (colorimetry) with lactate oxidase/peroxidase as reagent.

Statistical analysis of results was undertaken in STATA (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.). Scatterplots were produced for each parameter to provide a visual presentation of iSTAT versus Pentra values. Agreement was analysed per sampling event and across sampling events, including all samples. Direct agreement (concordance) between the laboratory-based machine and the POC-device was assessed by the concordance correlation coefficient (Lin, 1989). Correlation between the two readings were also assessed by Pearson's correlation coefficient. Both correlation coefficients can range between 1 and -1, with 0 indicating no agreement. The concordance correlation coefficient more directly compares two sets of test results compared to the Pearson's correlation coefficient which ignores the scales of the test sets. A concordance correlation coefficient of 1 means perfect agreement between two test sets, while a Pearson's correlation coefficient of 1 and -1 means total positive or negative linear correlation, respectively (Dohoo et al., 2014).

Results

Results of blood and plasma analysis are summarised in Table 1. The iSTAT gave no valid readings from a relatively high proportion of the samples, 19/62 (31%) and10/62 (16%) for potassium and chloride, respectively. Additionally, a large fraction of readings were below the highest or lowest detectable levels for both ions, leaving only 10 duplicate measurements for chloride and 33 duplicates for potassium. In contrast all samples analysed with the

Table 1. Mean values for analytes as measured by the POC-device and laboratory analyser. Standard deviation is given in parentheses. Mean values are calculated for numerical values only. Calculation of mean values was not possible for the analyte chloride for one or both samplings due to no and few samples with numerical values. ND = not done. †Converted values generated by multiplying original values with a conversion factor C (C = 1– hematocrit/PCV).

	Na mmol/L (Lab)	Na mmol/L (POC)	K mmol/L (Lab)	K mmol/L (POC)	Cl mmol/L (Lab)	Cl mmol/L (POC)	Lactate mmol/L (Lab)	Lactate mmol/L (Lab con†)	Lactate mmol/L (POC)
Sampling 1	155.7 (17.8) n = 20	156.5 (8.78) n = 21	1.78 (1.29) n = 20	2.0 (0.4) n = 3	138.8 (16.6) n = 20	130.7 (6.1) n = 11	2.36 (0.76) n = 20	1.66 (0.50) n = 20	1.57 (0.51) n =21
Sampling 2	167.7 (11.5) n = 41	166.4 (6.0) n = 31	1.70 (1.25) n = 41	3.2 (0.8) n = 31	141.6 (9.6) n = 41	- n = 0	ND	ND	ND

Table 2. Agreement between the POC-device and laboratory analyser. For each analyte concordance correlation coefficient and Pearson's correlation coefficient (in parentheses) and number of observations are listed.

	Na mmol/L	K mmol/L	Cl mmol/L	Lactate mmol/L	Lactate converted mmol/L
Sampling 1	0.16 (0.23) n =20	-	- 0.43 (- 0.52) n= 10	0.49 (0.95) n = 20	0.89 (0.91) n = 20
Sampling 2	0.43 (0.47) n = 31	0.09 (0.30) n = 30	-	-	-
Over all samples	0.38 (0.44) n = 51	-0.03 (-0.07) n = 33	- 0.52 (- 0.43) n = 10	0.49 (0.95) n = 20	0.89 (0.91) n = 20

Pentra resulted in presumed valid readings for all analytes. Both the correlation and concordance correlation coefficient (Table 2) and scatter plot indicated poor agreement between the two devices for chloride (data not shown), sodium (Figure 1) and potassium (Figure 2).

The iSTAT lactate measurements ranged from 0.69 to 2.42 mmol/L. Plasma lactate measured by the Pentra ranged from 1.03 to 3.47 mmol/L. Using the conversion method reported by Gallagher et al. (2010) whole blood equivalents (converted lactate values) were generated by multiplying plasma lactate values by 1 – hematocrit. The scatterplots (Figure 3 and 4) and Pearson correlation coefficient shows a high correlation between devices for both converted and unconverted lactate measurements, while concordance is high only between iSTAT and converted Pentra lactate values (Table 2).

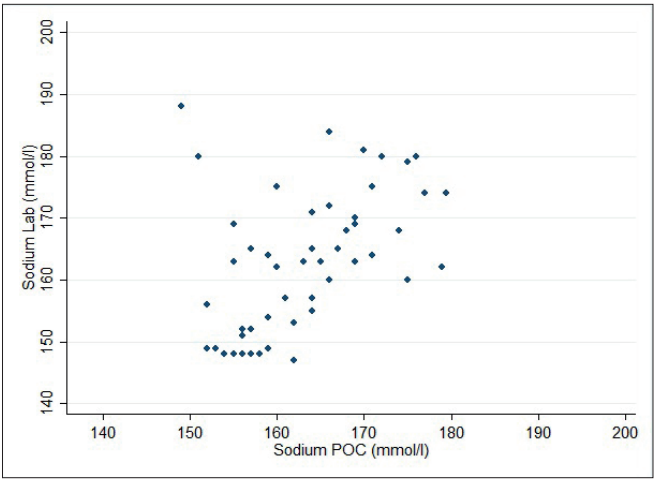

Figure 1. Scatterplot of sodium levels as measured by iSTAT and Pentra. One outlier sample excluded for better visualisation

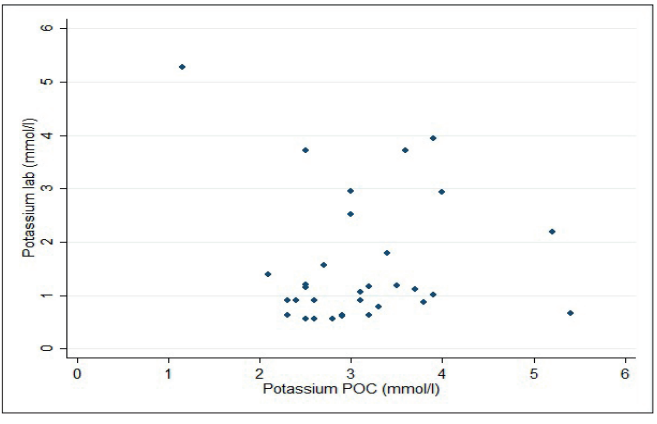

Figure 2. Scatterplot of potassium levels as measured by iSTAT and Pentra

Discussion

Our results indicate that the Vetscan iSTAT POC-device does not perform well for analysis of the electrolytes chloride or potassium in the blood of Atlantic salmon in saltwater. A large number of samples were missing or below the highest or lowest detectable levels for these parameters, and the settings for upper and lower limits of detection for several analytes seems to be unsuited for the blood concentrations found in Atlantic salmon. There was a good agreement between the iSTAT and the converted Pentra C400 values for lactate, though values from the POC-device cannot be directly compared to plasma values from the laboratory analyser because they use different source materials (whole blood versus plasma).

The iSTAT measures lactate levels in whole blood as opposed to plasma and this likely ex-

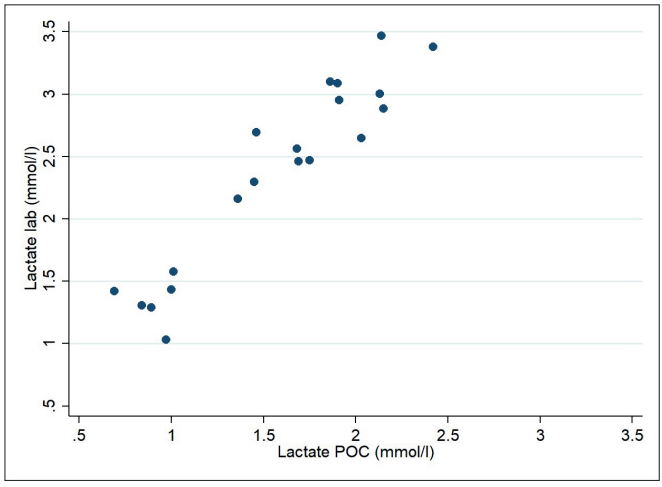

Figure 3. Scatterplot of lactate levels as measured by iSTAT and Pentra. (Data from pilot 1 only).

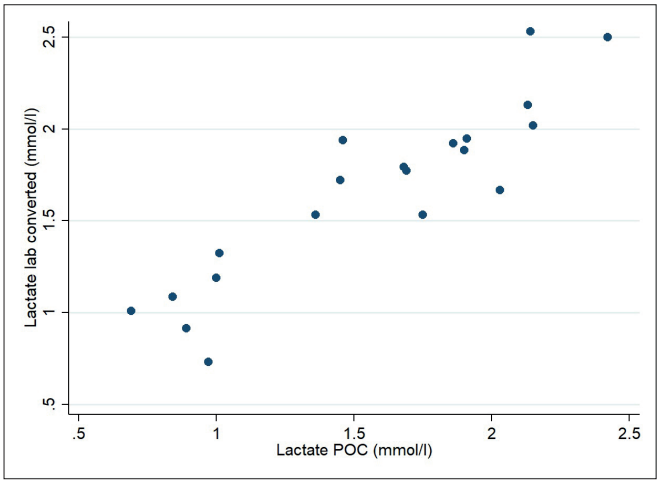

Figure 4. Scatterplot of lactate levels as measured by iSTAT and converted lactate levels measured by Pentra. (Data from pilot 1 only).

plains the higher concentrations of lactate measured by the laboratory analyser (Goodwin et al., 2007). A lower lactate concentration is found in the extracellular fraction of whole blood if analysers use a volume dependent measurement method due to the dilution effect of erythrocytes in the sample. Using a conversion method for plasma lactate substituting hematocrit for packed cell volume (Gallagher et al., 2010) the agreement between lactate values measured between the two devices was good (concordance correlation coefficient 0.893), but not excellent as reported by the said authors. However, this conversion requires that packed cell volume or

hematocrit measurements are also available. In our case hematocrit was measured by iSTAT which does not directly measure packed cell volume, and previous research (Borissov et al., 2019; Harter et al., 2014) has shown that hematocrit in fish is not accurately measured by the iSTAT when compared to conventional laboratory methods. The use of centrifugation for assessing packed cell volume and using these values for generation of the converted plasma lactate values might have improved the concordance between the two devices. In addition, both the plasma and whole blood concentrations of lactate in some samples in this study are likely falsely elevated due to variable delay in sample analysis during sampling 1. In humans lactate levels will increase rapidly if samples are not immediately analysed or frozen (Andersen et al., 2003), but in our case the time prior to whole blood analysis and plasma separation and freezing was almost identical, thus this problem has likely not influenced the agreement analysis to a large extent.

The iSTAT measured analyte levels in whole blood immediately after sampling while frozen and thawed plasma was used for Pentra C400. These differences in pre-analytic treatment of the samples may lead to real differences in analyte levels (Braceland et al., 2017; Braun et al., 2015). In fact, Braceland et al. (2017) showed small but statistically significant differences in sodium, potassium and chloride concentrations in duplicate samples of serum and plasma from Atlantic salmon. A minimal effect of several freeze thaw cycles was also found. While significant differences are found when measuring lactate levels in whole blood versus plasma, this effect in not so clear for the ions sodium, chloride and potassium. A study comparing whole blood and plasma as sources for measurement of sodium in humans showed good agreement between measurements, but a positive bias of iSTAT measurements when analysing whole blood from hyponatremic patients (Geoghegan et al., 2015). Two studies with human patients using paired samples and comparing POC-devices measuring analyte levels in whole blood to a central laboratory device measuring analyte levels in plasma found a small mean difference for sodium, potassium and chloride levels (King and Campbell, 2000). However, a significant difference was found in individual sodium, chloride, and potassium levels measured by the two devices, and a significant difference was still evident when plasma instead of whole blood was used as a sample source for the POC-device (Morimatsu et al., 2003).

The Pearson correlation coefficient ignores the scales of the two sets of results being compared, while the concordance correlation coefficient does not (Dohoo et al., 2014). The concordance correlation coefficient better reflects the level of agreement between two sets of test results and is preferred for comparing such results, however it is sensitive to differences between the sample materials or the handling of samples as in this study. However, any difference caused by the analysis of plasma versus whole blood, storage and freezing of samples would likely cause a deviation in the same direction, i.e. analytes should be systematically increased or decreased in one sample set compared to the other. A high correlation between the values generated by the two analysers would then be expected if both analysers were performing well. Thus, even if the use of plasma and whole blood and differences in pre-analytical treatment of the samples could partially account for the low

agreement between the two analysers in our study, differences in sodium, potassium and chloride are also likely due to real differences in measurements performed by these devices.

The iSTAT is portable and easy to use, however the device is sensitive to ambient temperature which can affect the analysis (pers. comm. Elizabeth Norstrøm, Kruuse AS) and requires time to acclimatise onsite. This makes the use of the device less practical in clinical practice as time for farm visits may be limited and a space suitable for blood analysis with stable temperature could be difficult to find. During the first day of the second sampling at the sea farm the temperature in the room where iSTAT analysis was performed was rising rapidly due to sunlight. This is a possible explanation for the device not producing any values for potassium and chloride for seven fish (sampling 2, sample 13-19), while all results for sodium for these fish were above the upper limit of 180 mmol/L that day. These potential problems with the device do not change the results of the statistical analysis as the missing values were not included in final analysis. The use of a room with stable temperature and improved standardisation of sampling and analysis could potentially have strengthened the test results obtained from the iSTAT, however the aim of this study was to test the applicability of the iSTAT in clinical practice which often offers less than ideal conditions.

Our results indicate that the iSTAT is not a reliable tool for assessment of the selected ions in Atlantic salmon and are in line with earlier results in other fish species. Stoot et al. (2014) reviewed studies using or validating POC-devices for use in fish, birds and mammals. Previous work performed on rainbow trout, *Oncorhynchus mykiss,* by Harter et al. (2014) attempted to validate the iSTAT system for use in this species. In this study CG8+ cartridges were used and iSTAT was found to give meaningful results for pH, while the values were not meaningful for hematocrit or parameters sodium, partial pressure of carbon dioxide, partial pressure of oxygen or bicarbonate. Similarly, a study in cod (*Gadus morhua*) comparing the iSTAT with conventional laboratory techniques found that the POC-device was inaccurate for measurement of pH, pO_2, hematocrit, sodium, potassium, calcium and hemoglobin (Borissov et al., 2019). A validation study in Seminole killifish (*Fundulus seminolis*) (DiMaggio et al., 2010) found that iSTAT could not be used to assess hematocrit or plasma ions in this species. Harrenstien et al. (2005) attempted to validate the iSTAT for two rockfish species (*Sebastes melanops* and *Sebastes mystinus*), but found significant differences between measurements performed with the iSTAT and conventional laboratory methods. These differences have been related to the fact that the iSTAT and its algorithms and constants were developed for use in humans (Harter et al., 2014). Differences in red blood cell morphology and blood temperature between humans and fish, as well as other physiological differences, are likely to explain erroneous measurements in fish. An effect of different blood temperatures, hematocrit and/or PCO_2 on measurement of ions was also found and based on this the authors did not recommend the use of conversion factors to correct these iSTAT-measurements (Borissov et al., 2019; Harter et al., 2014).

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The authors LØ; MA, MK work at Fish Vet Group Norge AS, a commercial diagnostic laboratory that offers clinical chemistry as a service to the aquaculture industry.

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