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Shortening of incubation times in Streptavidin plate production

Implementation of one-piece flow production

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Implementation of one-piece flow production

Thesis explores the feasibility of shortening the incubation times used in Streptavidin (SA) plate manufacturing process. During the incubation process the Streptavidin protein is coated onto polystyrene surface of microwell plate cup using passive adsorption. Manufactured SA plates are used as base for analyte specific coating process of Point-of-Care (POC) immunoassay tests. Aim of this study is also to examine how SA plates would be produced with shortened incubation times and how it would affect to the production output.

Topic of the thesis is formed based on implementation of one-piece flow manufacturing strategy of POC tests. Implementation of one-piece flow strategy in the production of POC immunoassay tests requires shortening of the incubation times used in the SA plate manufacturing. Production of the POC immunoassay tests is complex process containing multiple process phases with long incubation times and storage steps which increases lead time of the manufacturing process.

Effects of shortened incubation times to SA plates performance and quality was evaluated with quality control tests. Process flow simulations were prepared to estimate how SA plates would be manufactured with shortened incubation times and how it would affect to the production output. The study includes both theoretical and technical aspects, including alternative incubation methods and quality control tests.

Results of study indicate that the incubation times used in SA plate manufacturing could be shortened with 81 – 59 % allowing to reduce lead time of SA plate manufacturing from 3 days to 1 day. The adsorption of Streptavidin to polystyrene surface could be improved by increasing the incubation temperature. Shortening the incubation times does not significantly impact the quality and performance of the SA coated plates. However, the reduction in incubation times is only beneficial for smaller batch sizes. For the smaller batch sizes the solution manufacturing processes of SA plate coating need to improved so that the shortening of incubation times would be profitable.

Keywords:

Streptavidin, coating, immune assay, process optimization

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List of abbreviations

Abbreviation	Explanation of abbreviation
A	Total process time in seconds
B	Amount of processed plates in pieces (PCS)
c	Concentration
D	Takt time, seconds/plate
X	Biotin Binding capacity in pmol/cup
n	Sample Size
$t_{(0)}$	Time point of testing (Release test)
$t_{(1)}$	Time point of testing (Stability test)
Y	Value for Biotin binding capacity (pmol/cup)
z	Value for confidence level
AS	Assay Solution / buffer
Bio-Eu	Biotinylated Europium
CAL	Calibration plate or cup
CPS	Counts Per Second
CS	Coating solution
CV	Coefficient of Variation
IL	Insulation Layer
POC(T)	Point Of Care (Testing), diagnostic testing
REF	Reference

SA	Streptavidin
SD	Standard Deviation
UV	Ultraviolet

1 Introduction

Purpose of the thesis is to investigate possibility of shortening incubation process step in SA plate manufacturing. The manufactured SA plates are used as component in point-of-care test manufacturing. Topic of the thesis is formed from new one-piece flow manufacturing strategy of point-of-care tests. Implementation of one-piece flow strategy in the production of POC immunoassay tests requires shortening of the incubation times used in the SA plate manufacturing. Production of the immunoassay tests is complex process containing multiple process phases with long incubation times and storage steps which increases lead times and uses limited resources for example incubation cabinets and production space. Due the production cycle, manufactured SA plates are stored usually at least some days always up to shelf life of the SA plates before entering next process step. By one-piece flow production strategy and Lean principles storage is considered as waste in the process. By shortening incubation times storage steps could be removed. These are some of the reasons why the current process is not suitable for one-piece flow strategy.

The objective of the study is to research feasibility to shorten the incubation times used in SA plate manufacturing. Target is to shorten incubation times from up to 81 % from current incubation times with minimal process changes and investigate alternative incubation methods. The goal was also to investigate how manufacturing process could be performed with the shorter incubation times and how it affects to the production output and lead time of the SA plate manufacturing.

Possibility of incubation time shortening is investigated by manufacturing SA plates from the same components and reducing only the incubation time and incubation method used in SA plate manufacturing. The effects of incubation method and time reduction on SA plate performance is evaluated with quality control methods. Also effects of shortened SA plate incubation times on end product are evaluated. Production flow charts for SA plate manufacturing with

shortened incubation times are prepared to see how the SA plates could be manufactured with shorter incubation times and how it would affect to the production output of the SA plate manufacturing.

The thesis includes theoretical and technical part. Theoretical part includes chemistry of the streptavidin coating, theory of quality control tests and future development ideas. In the technical part alternative SA plates are manufactured with shorter incubation times and methods and performance of SA plates are tested. Also the effect of shortened incubation times and methods to the performance end product is evaluated with analyte X. Performance of the SA plates is verified with stability, homogeneity, background and biotin binding capacity tests. Also for the end product stability testing, homogeneity, background and release testing is performed for the analyte X test and calibration cups.

2 Point-of-care testing

Point-of-care testing (POCT) is laboratory testing performed close to the patient care, typically where treatment is provided. The primary benefit of POCT is the rapid turnaround time of test results, which allows for the prompt implementation of appropriate treatment and can lead to improved clinical or economic outcomes when compared to traditional laboratory testing. (Price 2001) The traditional laboratory testing process involves several steps, including collecting patient samples at the bedside or clinic, transporting them to a centralized laboratory, and subjecting them to various processing steps causing delays to decision making and treatment. (Kost 1999)

The POCT process can be divided into three primary stages: pre-analytical, analytical, and post-analytical. During the pre-analytical phase, healthcare workers collect, transport, prepare, and load the sample for testing. The analytical phase is where the actual testing sequence of a POCT takes place, using the handheld device to perform the rapid test. Finally, the post-analytical phase begins when the test result is available, and the result is communicated to the treatment team to guide appropriate actions and interventions (Larkins 2023.)

The POCT analyzer can cover an extensive range of critical biomarkers like for heart failure, myocardial infraction, venomous thromboembolism, infection and pregnancy.

In company’s immunoassay tests patients’ blood sample tube is inserted into analyzer. The analyzer dispenses the blood sample into test cup. Target antigens binds on biotinylated antibodies that are binded onto Streptavidin surface in cup and labeled antibodies containing Europium binds on target antigens also, see Figure 1. After processing the cup with patients’ blood sample, the excitation with UV light is done for the test cup and the analyzer reads the emission signal of Europium particles from cup.

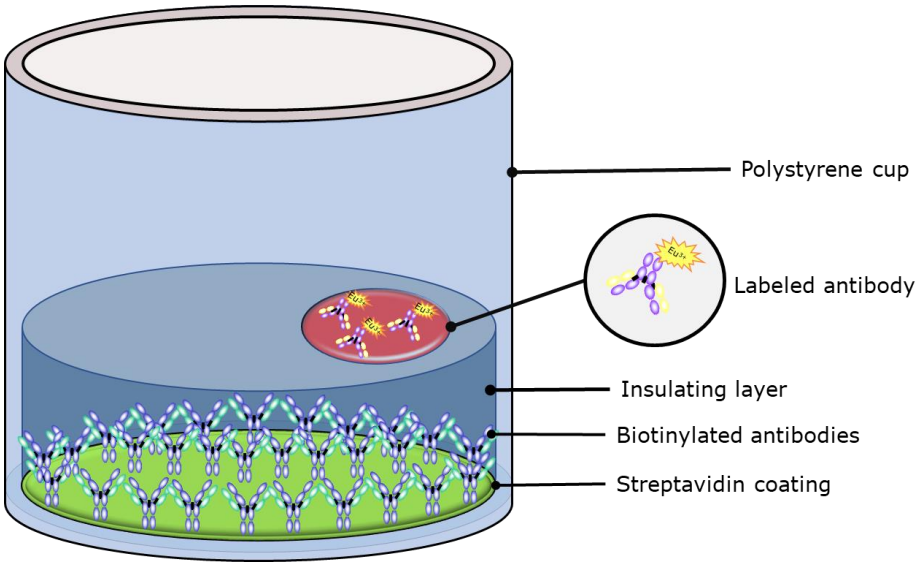

Figure 1 Content of TEST cup

2.1 Streptavidin

Streptavidin is a tetrameric protein that is produced as a secreted protein from the bacterium called *Streptomyces avidii.* By size the Streptavidin is large, approximately 60 000 Daltons. Streptavidin was first discovered in the 1960s when examining the byproducts of *S. avidii* fermentation. The protein was named streptavidin, because, like avidin from egg white, it has the inherent property of binding four biotin molecules to itself (Välimaa 2008.)

The complete form of streptavidin is a sequence of approximately 180 amino acids, of which the first 24 amino acids code for excretion. However, a significant step in terms of biotin binding is the post-translational changes to the structure of streptavidin, which the S. avidin proteases are responsible for. These are able to modify the full form of the protein into so-called nuclear streptavidin, whose biotin binding capacity is significantly higher than long-chain streptavidin, and the majority of commercial streptavidin's are post-translationally modified. Among other things, pepsin and chymotrypsin can

cause a similar change in the structure of streptavidin as with S. avidin's own protease (Välimaa 2008.)

The use of streptavidin in diagnostic methods is based on the ability of streptavidin to immobilize on a solid surface. The hydrophobic areas on the surface of streptavidin enable adhesion to, for example, a polystyrene surface, thereby providing a solid adhesion surface for biotin derivatives (Ylikotila 2009, 271–277.)

2.2 Biotin

Biotin, also known as vitamin H, is a small water-soluble molecule with a double-ring structure and a carboxyl acid side chain. The side chain can be extended with different linker molecules and active groups enabling chemical coupling of biotin to other molecules. It occurs widely throughout nature, and it has been found to be an essential nutrient for all living things. Biotin participates in the body as a coenzyme in the transport of carboxyl groups (Ensminger & Esminger, 1993.)

2.3 SA coating on polystyrene surface

Passive adsorption of proteins like streptavidin to polystyrene surfaces has been studied to be driven by hydrophobic forces. Passive adsorption is a widely used method because it is simple and flexible to perform. (Le 2018)

96-well microtitration plates or single cup plates are widely used as solid phase material for binding of specific antigen or antibody for immunoassays. These plates or cups are typically manufactured from polystyrene. For the immunoassay purposes, the cups surfaces are precoated with capturing protein like antibody to allow analyte immobilization.

It is studied that the direct adsorption of antibodies to the polystyrene surface may destroy the functional sites of antibodies to even less than one-tenth of the original activity of antibody. (Butler 1992, 77–90)

Studies have demonstrated that preserving protein activity is achievable by immobilizing the capturing antibody as a secondary layer over a primary coated layer, using streptavidin-biotin linkage. This is why streptavidin coated polystyrene plates or cups are favored over antibody or antigen coated cups. Furthermore, streptavidin-coated cups offer a versatile immobilization surface for any biotinylated molecule, which eliminates the need for time-consuming optimization of adsorption conditions for multiple antibodies or antigens (Välimaa 2003, 103–111.)

In noncompetitive, two-site immunoassays, the solid phase has a significant impact. The surface's binding capacity must be sufficient to ensure a surplus of reagents, a broad dynamic range without a high-dose hook effect, and rapid kinetics (Välimaa 2003, 103–111.)

2.4 Biotin binding on streptavidin surface

The bond formed by streptavidin and biotin is one of the strongest known non-covalent bonds. Breaking the bond requires harsh conditions. However, some chemical solutions combined with heating are able to separate SA and biotin from each other (Välimaa 2008.)

2.4.1 Application in diagnostics

SA-biotin binding is a widely used method in diagnostics, especially in the industrial production of immuno assays. Attaching biotin to an antibody or antigen by means of a chemical reaction is called biotinylation. By means of biotinylation, the antibody or antigen can be attached to the SA surface, which can then be dispensed into, for example, a polystyrene cups surface (Välimaa 2008.)

The use of fluorescent markers together with specific antibodies is a very typical way of using the SA-biotin structure in a diagnostic assay. The benefit of the complex is also mentioned in other diagnostic studies, such as increasing the sensitivity of immunohistochemical methods (Dabbs 2013.)

3 Manufacturing process

Manufacturing process of immunoassay test begins with SA plate coating process. SA cups are used as base for coating analyte specific biotinylated antibodies in dry cup manufacturing. Analyte specific test and calibration cups are then packed into cartridges to be in suitable form for analyzer. Test cups are used for the biomarker testing of patient blood samples and calibration cups are used for calibration of analyzer. Manufactured batches are tested to confirm the performance and quality. After that, the cartridges are packed as test kits for the customers.

The high-level production flow of POC test cups and more detailed production flow of SA cup production flow is shown in Figure 2.

The SA cups are manufactured with the automation line and the main production flow of SA cups is divided into four days. In the first day the coating buffer for SA coating solution is prepared.

At the second day SA stock solution is prepared, mixed with the coating buffer to denature the SA and the solution is filtered before dispensing into polystyrene cups. The SA coating solution is dispensed into polystyrene cups and incubated in the airtight incubation chambers to prevent evaporation of SA coating solution at room temperature. Also wash solution and saturation solution are prepared at second day.

At the third day after the minimum SA coating solution incubation time is reached the incubated plates are fed back into automation line and the excess SA coating solution is aspirated from cups and the cups are washed to remove excess unbonded SA coating solution. After that the saturation solution is dispensed into washed cups and plates are once again placed in the incubation chambers.

At the fourth day after the minimum saturation solution incubation time is reached the incubated plates are fed back into automation line and the excess saturation solution aspirated from cups. After the aspiration the SA plates are

either dried or moved to the storage to wait the analyte coating (Private communication 2024.)

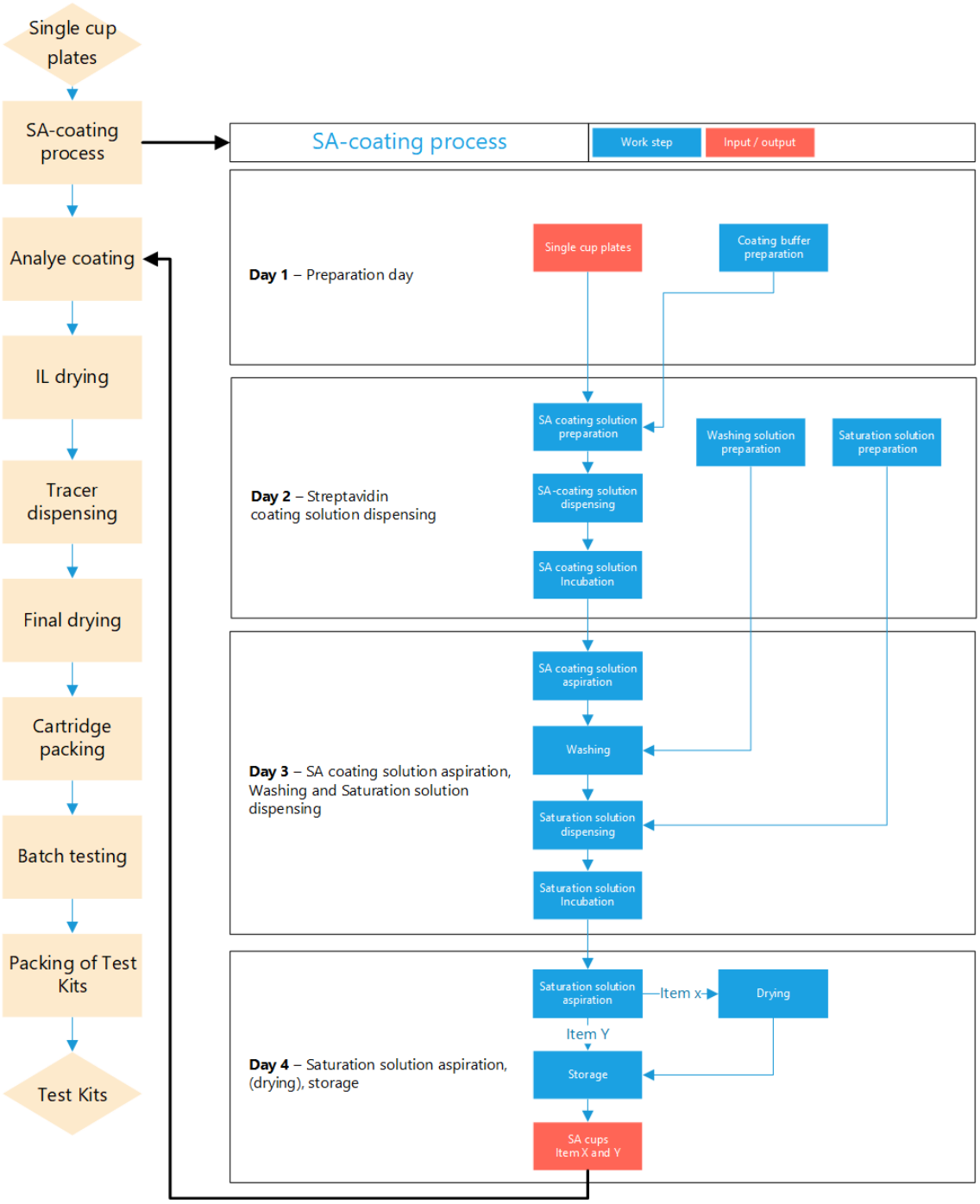

Figure 2 SA cup manufacturing process (Private communication 2024)

3.1 Critical factors in SA coating

The mixing of SA into the buffer solution is one of the first critical steps in the manufacturing process. A minimum duration has been defined for the mixing to ensure homogenous SA coating solution, but there is no maximum mixing time defined and the effect of an extended mixing time has not been carefully studied. Since the maximum mixing time for SA coating solution is not defined it can be considered also as variation source in the process (Private communication 2024.)

All of the dispensing steps during the SA plate production are considered as critical factors to ensure high quality of plates. It is critical that the concentration of streptavidin in SA coating solution is correct. Also the dispensed volume of SA coating solution needs to be sufficient to ensure coating of the required area of the cups surface. To remove the unattached SA the volume of wash solution needs to be correct. If the dispensing volumes of the saturation solutions are not within limits, it will affect to the performance of the SA plate. Purpose of the saturation solution is to cover uncoated surfaces of the cup and prevent non-specific binding to the cup.

Also the incubation time is considered to be critical factor. There is a difference of 13 hours between the lower and upper limits of the incubation time, and the variation in duration may have an effect on the creation of a higher binding capacity.

4 Test methods

The effects of shortened SA coating solution and saturation solution incubation times to the SA cups performance were evaluated with quality control tests. Homogeneity, background signal and biotin binding capacity of SA cups were determined.

SA cups with shorten incubation times were also manufactured to the end product with analyte X. To evaluate does shorter SA and saturation solution incubation times have effect to the quality of end product, homogeneity, background, barcoding, release and stability tests were performed to the analyte cups manufactured from characterization batch 2.

4.1 SA cup homogeneity test

The homogeneity of the signal given by the SA cups is determined by the homogeneity test.

The test is performed on SA plates, which have been picked from the batch at regular intervals. For the test, a dilution of the biotinylated antibody is prepared in the buffer solution, as well as a dilution of the insulation layer solution, the homogeneity solution, and the Eu-labeled antibody. A dilution of the biotinylated antibody is first pipetted into the SA cups. The cups are incubated with mixing to allow the biotinylated antibody to attach to the streptavidin. After incubation, the cups are washed with a plate washer and the Eu-antibody dilution is pipetted into cups. The cups are incubated with mixing to maximize antibody binding. After this, the cups are washed, dried with hot air and allowed to cool before measuring. The time-resolved fluorescence signal of the cups is determined with a Victor plate reader. The relative variation within plates is calculated using Excel calculation sheet. Relative variation within plates is used as acceptance criteria (Private communication 2019.)

4.2 SA cup background test

The background signal of SA cups is determined with a test which applies analyte X in the determination. For the test, a dilution of the biotinylated antibody is prepared in a buffer solution and a dilution of the Eu-labeled antibody in an insulation layer solution suitable for the analyte. Cups are incubated in a SA-biotin mixture to optimize binding. The Eu-labeled antibody is only diluted shortly before the end of the incubation of the first plate due to stability of the solution. After incubation, the Eu-antibody dilution is pipetted into the cups, which are incubated with mixing to maximize binding of the labeled antibody. The incubated cups are washed, dried and cooled before measuring. The time-resolved fluorescence of the cups is measured with Victor, and the mean background signal (counts) are calculated using Excel calculation sheet. Mean background signal is used as acceptance criteria for the test (Private communication 2019.)

4.3 Biotin binding capacity test

The determination of the biotin binding capacity of SA cups is performed using the Bio-Tekes-Eu. Bio-Tekes-Eu is a reagent used to determine the biotin binding capacity of SA cups by time-resolved fluorescence. It is a biotinylated europium chelate manufactured by company itself. A nine-step dilution series is prepared from the reagent for the assay, which is pipetted into prewashed SA cups. Each dilution is pipetted into six parallel cups, and the cups are incubated with mixing to maximize Bio-Tekes-Eu binding to streptavidin surface. At the end of the incubation, the cups are washed to remove unbound reagent. DELFIA Enhancement solution is dispensed into the washed cups with a DELFIA plate dispenser. The Enhancement solution is incubated with mixing so that the bound europium detaches from the bound Bio-Tekes-Eu. After incubation, the plates are measured with a Victor to determine the time resolved fluorescence of the Enhancement solution contained in the cups. The biotin

binding capacity is used as acceptance criteria for the test (Private communication 2019.)

4.4 Analyte cup Homogeneity testing

The purpose of the test is to determine the signal homogeneity of the 96 cups on the plate for TEST and CAL plates, which is measured by relative standard deviation (CV%). In the assay a homogeneity solution is used as a sample for TEST plates. The homogeneity solution contains the antigen to be measured which is the same compound that the customers measures form the blood samples. For the CAL plates only assay buffer is used since the CAL cups already contains antigen to be measured (Private communication 2023.)

In addition, the dispersion of the signal of plates columns and rows is examined. These criteria can be used to determine the signal differences between the cups caused by the production processes and what kind of dispersion there would be in the packed cartridge. In addition, the relative signals of the individual cups are checked to see if there are rising or falling trends within the plate or between plates, which could cause false patient results Private communication2023.)

The relative standard deviation (CV%) is used as acceptance criteria for the TEST and CAL plate homogeneity and the limits varies within plate types. (Private communication 2023)

4.5 Analyte cup background testing

The purpose is to determine the signal obtained from the TEST cups when there is no sample in it by measuring the cups with the analyzer. In this way, it is possible to find out if the tracer droplet has been absorbed through the insulation layer during tracer dispensing or has the insulation layer drying been

successful. The median background signal level is used as an acceptance criteria for the test. If too high values are obtained from the background signal level, dispersion or individual results, it may appear as false negative results in customers (Private communication 2023.)

4.6 Analyte cup barcoding

The purpose of barcoding is to determine the concentration for the manufactured CAL cups. In practice, by reading the barcode and measuring the CAL cartridge, the customer calibrates the analyzer to be at the right level (signal to concentration ratio is the same) so that the patient results are reliable. The assay is performed with analyzers by using TEST and CAL cartridges, AS buffer and standards and controls with a known concentration are used as a sample (Private communication 2023.)

At the end of the runs, the signals are applied to the calculation program which calculates analyzer specific concentrations of controls and CAL cups against the standard curve. To ensure the functionality of the assay and the correct level of results, background signal level, sensitivity, concentration and variance limits for controls, standards and CAL cartridges are used as acceptance criteria for the test (Private communication 2023.)

4.7 Analyte cup release test

In release test control samples are run with the analyzer, just as the customer runs patient samples. In the release test the correctness of the calibration barcode is verified. The analyzer is calibrated with the barcode generated in the barcoding and control samples with a known concentration are run against the calibration (Private communication 2023.)

At the end of the runs, the results are applied to the calculation base and the correct result level is verified. To ensure the functionality of the assay and the correct level of results, background signal level, control concentrations and control concentration variations (CV%) are used as acceptance criteria (Private communication 2023.)

4.8 Analyte cup onboard stability test

The manufactured TEST and CAL cartridges has a claimed onboard shelf life which means how many days they can be stored inside of analyzer. The onboard stability test is used to evaluate does process changes effect to the onboard time and to predict effects on shelf life of the product. Onboard stability test used in this study was based on the release test protocol. In practical the release test is repeated by using same analyzers, control samples and the analyzer calibration performed in release test. In the onboard stability test the sample TEST and CAL cartridges were stressed at +32 °C, 80 % RH for claimed onboard shelf life + 1 day before the assay.

Same acceptance criteria as in release test are applied for onboard stability study. In addition, to evaluate the stability of a TEST cartridges a measurand drift is calculated as percentage bias to 0-point. The mean is calculated for each control per test (n = 30) and measurand drift is calculated by using following equation:

$$\text{Measurand drift} = \frac{c_{\mathrm{t}\,(1)} - c_{\mathrm{t}(0)}}{c_{\mathrm{t}(0)}} \times 100\ \% \qquad (1)$$

where

c is mean concentration of control sample

$t_{(0)}$ is release test time point

$t_{(1)}$ stability test time point

Also for CAL cartridges a measurand drift drift is calculated as percentage bias to 0-point by using the mean signal of CAL cups and used as acceptance criteria for the onboard stability test. The measurand drift is calculated separately for each analyzer (*n* = 8). The measurand drift for CAL cartridges is calculated by using following equation:

$$\text{measurand drift} = \frac{CPS_{t(1)} - CPS_{t(0)}}{CPS_{t(0)}} \times 100\ \% \qquad (2)$$

where

CPS is mean Counts Per Second of CAL cup signal

$t_{(0)}$ is release test

$t_{(1)}$ stability time point

Also similarity between characterization and REF batches is evaluated with JMP equivalence test which uses Two One-Sided Tests approach. Two one-sided *t* tests are constructed for the null hypotheses that the difference between the true mean and the hypothesized value exceeds the threshold. If both null hypotheses are rejected, this implies that the true difference does not exceed the threshold. The similarity is evaluated by calculating 95% confidence intervals of measurand drift for each characterization batch and REF batch results. The confidence intervals shall overlap to conclude that the result is acceptable. The confidence interval is calculated according to following equation:

$$\frac{\left(c_{t\,(n1)} - c_{t\,(0)} \pm z_{0.025}^{N_{t\,(1)}+N_{t\,(0)}-2}\sqrt{\frac{SD_{t(1)}^2}{N_{t(1)}} + \frac{SD_{t(0)}^2}{N_{t(0)}}}\right)}{c_{t(0)}} \times 100\ \% \qquad (3)$$

where

c is mean concentration of control sample

$t_{(0)}$ is release test

$t_{(1)}$ stability time point

z is value for the confidence level

SD is standard deviation at release test and stability time point

5 Characterization tests

In the characterization testing of SA coating solution and saturation solution incubation two different incubation times were studied, Time 1 and Time 2. Characterization batches were manufactured alongside with the production batches and the quality and performance of the characterization batches were compared to the production batches by using quality control tests specified for SA and analyte cup quality control.

The characterization batches included two batches for SA cup coating and one batch for analyte coating. The study entity can be seen in Figure 3. The manufacturing of characterization batches and purpose of each batch is presented in more detail following paragraphs.

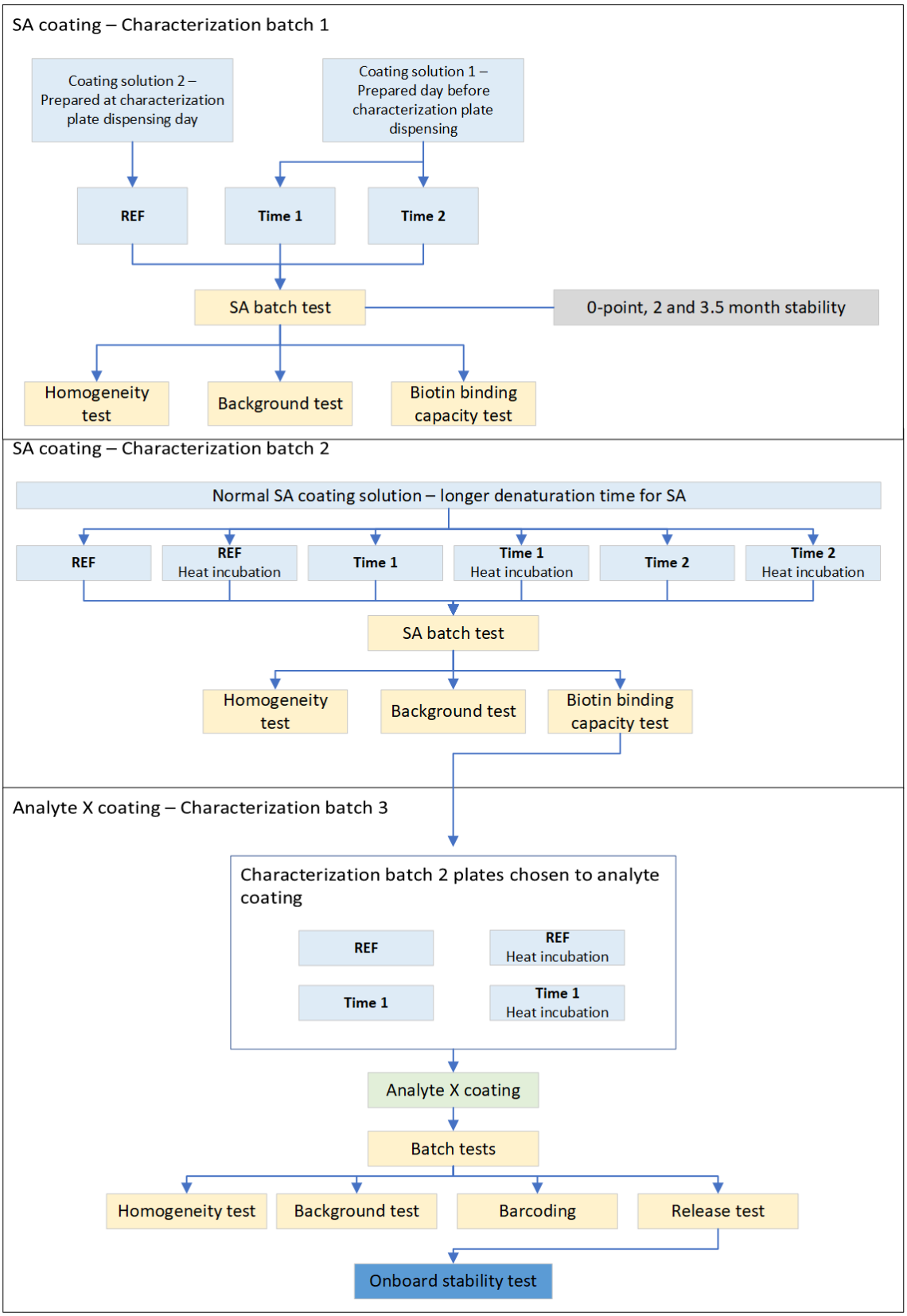

Figure 3 Characterization study entity

5.1 Characterization batch 1 – SA cups

In the first characterization batch SA plates with shorter incubation times were manufactured (Time 1 and Time 2). From the manufactured SA plates the effect of shorter incubation times to the quality and shelf life of SA plates was studied. The self-life was studied by performing the quality control test right after manufacturing and after 2 months and 3.5 months storage. In the same characterization batch also the effect of denaturation time of Streptavidin in coating buffer was studied by preparing two different SA coating solutions:

- **Coating solution 1**: Same solution that was prepared for production batch, but the solution was dispensed into cups 24 hours later than for production batch (longer denaturation time).
- **Coating solution 2**: Solution that was prepared for only characterization batch plates. The solution was prepared as the same way as the solution for production batch and with same components, but the manufacturing volume was not validated. Streptavidin was added to coating buffer at the dispensing day and solution was filtered. It was noted that the Streptavidin might coat onto surface of glass beaker, but it is considered that the time used for preparation of solution (mixing, filtering and dispensing) is short and it does not have effect on solutions properties since the solution contains an excess of Streptavidin.

5.1.1 Manufacturing of plates

Test plates with shorter incubation times were manufactured alongside with the production batch. Since the test plates were manufactured in conjugation with production batch, the SA coating solution dispensing for test plates was done with multichannel pipette. This was done to synchronize the test and production batch plates coating solution aspiration, washing and saturation solution dispensing steps with production batch. Also, this way it was possible to use same wash and saturation solution for the test and production batch plates. Manufacturing flow of characterization batch 1 can be seen in Appendix 1.

The realized SA coating and saturation solution incubation times for test plates were exceeded for some plates due the manufacturing problems. In Table 1 can be seen if the incubation target incubation times were achieved and how much the incubation times were exceeded.

Table 1 Realized incubation times in characterization batch 1

Planned incubation time	Stacker number	SA coating solution incubation time	Saturation solution incubation time
Time 2	1	Target	Target
	2	Target	Target
	3	Target	Target
Time 1	4	Exceeded + 9 %	Exceeded + 17 %
	5	Target	Target
	6	Targe	Exceeded + 15 %

5.1.2 Homogeneity test results

Homogeneity testing for each characterization and REF plates was performed after SA plate coating. Homogeneity tests were also performed 2 and 3.5 months after the coating as stability tests to see does the incubation time shortening affect to shelf life of the SA plates.

Homogeneity tests results of each time point can be seen in Figure 4. As seen from the homogeneity test results test plates coated with coating solution 2 exceeded the acceptance limit with both incubation times while test plates coated with coating solution 1 showed lower variance than reference batch. The difference between coating solutions was the denaturation time of Streptavidin and manufactured batch size. Coating solution was not manufactured in validated batch size, so it was considered that either or both of these factors have affected homogeneity of the plates. Figure 4 also shows a decreasing

trend in CV% during the storage of the plates. With REF batch trend can be seen more clearly than with test batches. The trend may be caused by the test method, or it may be cause of plate storage.

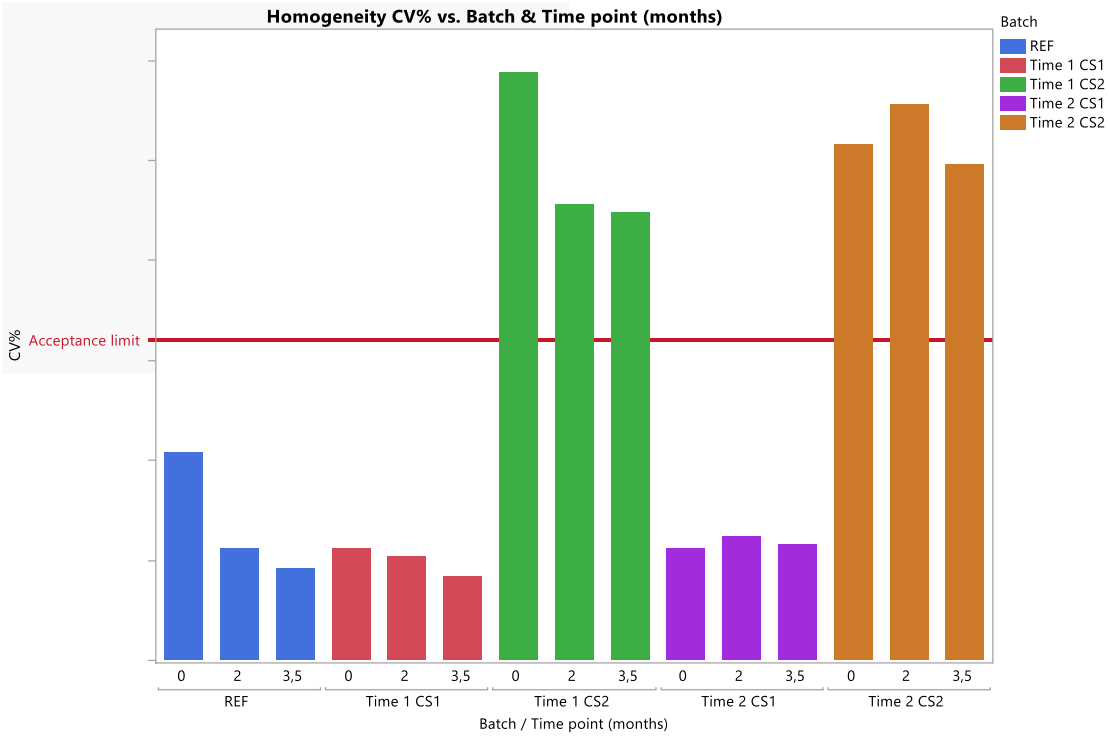

Figure 4 Characterization batch 1 – Homogeneity test results

5.1.3 Background test results

Background testing for each test plate combination and REF plates was performed after SA plate coating. Background tests were also performed 2 and 3.5 months after the coating as stability tests to see does the incubation time shortening affect to shelf life of the SA plates by increasing the background level of the plates.

Background tests results of each time point can be seen in Figure 5. All of the tested batches were within control and acceptance criteria in all tested time points. There was no significant difference in background signal levels between batches. Slight increase in background levels was observed between 0 point and 3.5 months storage with all tested batches except with Time 2 incubation with coating solution 1. Anyhow the increase in background levels is not significant.

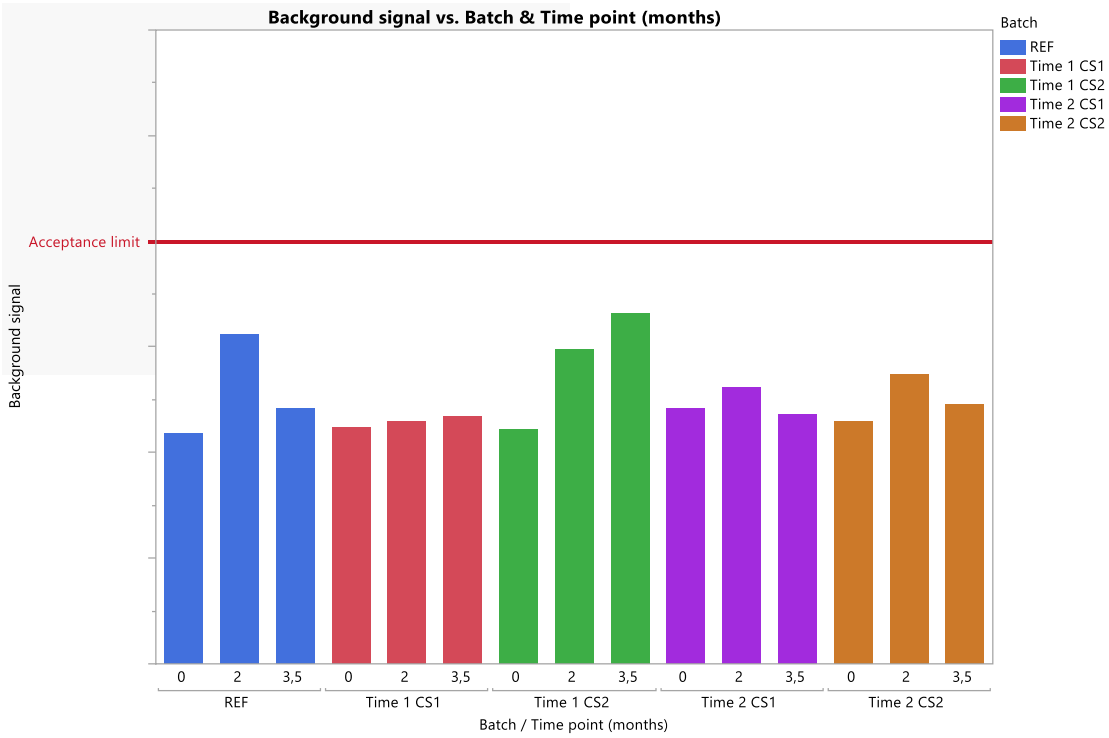

Figure 5 Characterization batch 1 – Background signal results

From the background results it can be assumed that also the shortened saturation solution incubation time has been sufficient to maintain low background level.

5.1.4 Capacity test results

Bio-TEKES-Eu binding capacity testing for each characterization batch and REF plates was performed after SA plate coating. Binding capacity tests were also performed 2 and 3.5 months after the coating as stability tests to see does the incubation time shortening affect to shelf life of the SA plates by decreasing the biotin binding capacity.

Biotin binding capacity test results of all tested time points can be seen in Figure 6. From the test results can be seen that the shortening of incubation time seems to have effect on adsorption of Streptavidin to cups polystyrene surface since the longest incubation time (REF) resulted highest concentration of Streptavidin in cup. With coating solution 1 biotin binding capacity in cups was just within acceptance criteria with both tested incubation times. With coating solution 2 sufficient adsorption of Streptavidin was not achieved with either of the tested incubation times.

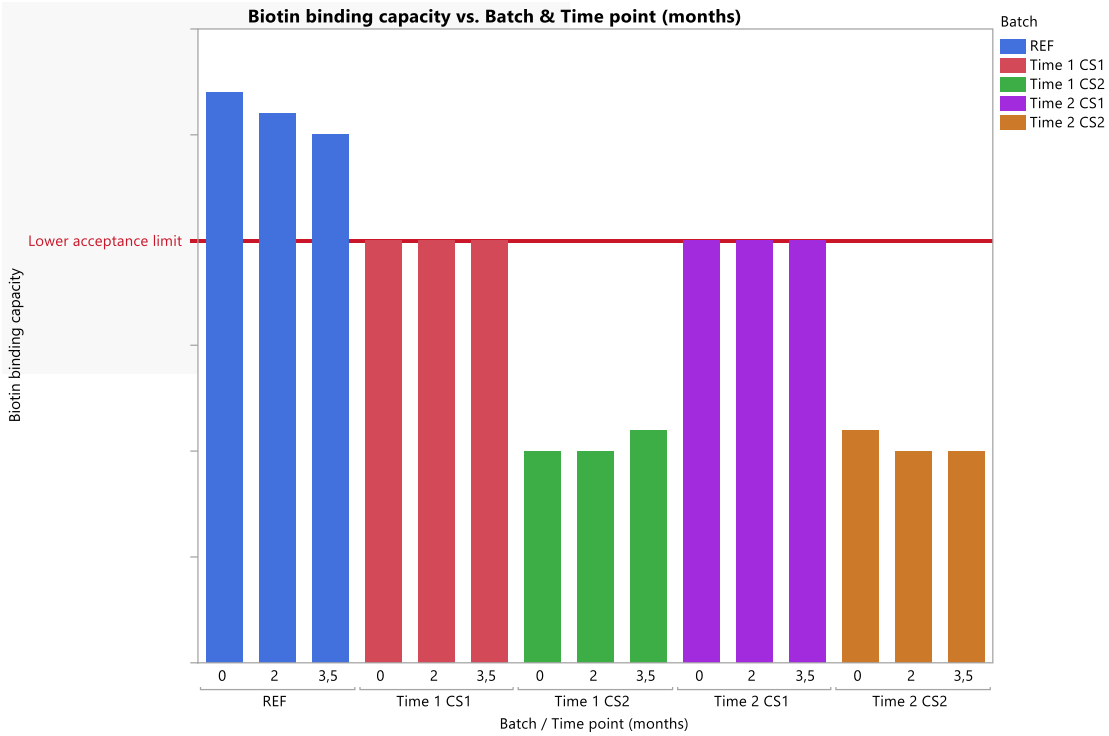

Figure 6 Characterization batch 1 – Biotin binding capacity results

Biotin binding capacity test results indicates that the denaturation time of Streptavidin has not been long enough with coating solution 2 because the realized incubation times were same than with coating solution 1 and plates coated with coating solution 1 resulted higher Streptavidin adsorption.

5.1.5 Characterization batch 1 conclusions

From characterization batch 1 results can be concluded that the shortening of SA coating solution and saturation solution incubation times does not affect to the shelf life of SA coated plates. Shortening of incubation times do have effect on the Streptavidin adsorption on cups polystyrene surface, but with coating solution 1 the adsorption has been sufficient enough with shortened incubation times to maintain high enough concentration of Streptavidin in cups.

5.2 Characterization batch 2 – SA cups

In this characterization batch the same SA coating solution and saturation solution incubation times were used as were used in batch 1. In previously performed characterization batch 1 the streptavidin binding capacity and homogeneity results did not meet with the acceptance criteria. It was considered that the SA coating solution was faulty, because it was not manufactured in validated volume or the denaturation time of Streptavidin in coating buffer with the shorter incubation times was not sufficient to achieve homogenous SA coating with necessary biotin binding capacity.

In characterization batch 2 shorter incubation times were tested so, that the SA coating solution is manufactured earlier to increase the denaturation time of Streptavidin in solution container. The characterization batch plates with shorter incubation times were manufactures so that the denaturation time is equal to the REF plates, but the contact time of SA coating solution and cups polystyrene surface is shortened.

It was also considered that the incubation of SA coating solution and saturation at higher temperatures might increase the adsorption of SA to polystyrene. In the characterization batch 2 the heat treatment during SA coating solution and saturation solution incubations was also studied.

5.2.1 Manufacturing of plates

Plates in characterization batch 2 were not manufactured in conjugation with the production batch like in characterization batch 1. For characterization batch 2 only one SA coating solution was prepared, and all characterization and REF plates were SA coated with same solution. This time the SA coating solution was prepared in minimum validated batch size to ensure correct solution manufacturing. Also same saturation and washing solutions were used for REF and characterization batches.

In Table 2 are shown the realized incubation times of SA coating solution and saturation solution as difference percentage when characterization batch incubation times are compared to the REF plates incubation times. Also the calculated contact times of Streptavidin and coating buffer (time when Streptavidin was added to coating buffer – time when SA coating solution incubation ended) are shown in Table 2. REF plates minimum SA coating solution incubation time was exceeded but with characterization plates the planned incubation times were achieved. Saturation solution incubation times were exceeded only with the incubation Time 1.

Table 2 Characterization batch 2 - Realized incubation times

Planned incubation time	Incubation condition	Streptavidin denaturation time[A)]	SA incubation time[B)]	Saturation solution incubation time[C)]
REF	Normal	N/A	N/A	N/A
	Heat incubation	+ 1.4 %	+ 1.3 %	– 1.7 %
Time 2	Normal	– 10.3 %	– 71,6 %	– 68.5 %
	Heat incubation	– 9.1 %	– 71.6 %	– 68.4 %
Time 1	Normal	– 8.2 %	– 81.5 %	– 75.1 %
	Heat incubation	– 8.6 %	– 82.9 %	– 75.0 %

A) Contact time of Streptavidin and coating buffer
B) Contact time of SA coating solution and cup
C) Contact time of saturation solution and cup

Normal SA coating solution and saturation solution incubations for characterization and REF plates were performed in stacker incubation chambers like as in normal process. The heat treatment incubations were performed in condition cabinet at higher temperature. Plates for the heat treatment incubations were stacked inside plastic coating boxes (45 plates/box). Plastic film was placed on the top plates to minimize the evaporation during incubation and the box was sealed with lid, see Figure 7 for illustration.

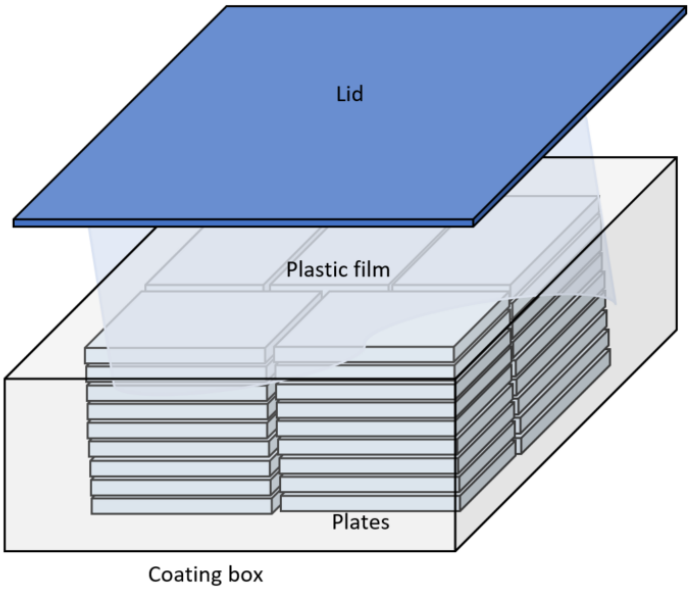

Figure 7 SA plate incubation box

5.2.2 Homogeneity test results

Homogeneity testing for each characterization batches and REF batch was performed after SA plate coating. Homogeneity test results (mean CV% of all tested plates / batch, *n* = 6) for all tested batches can be seen in Figure 8.

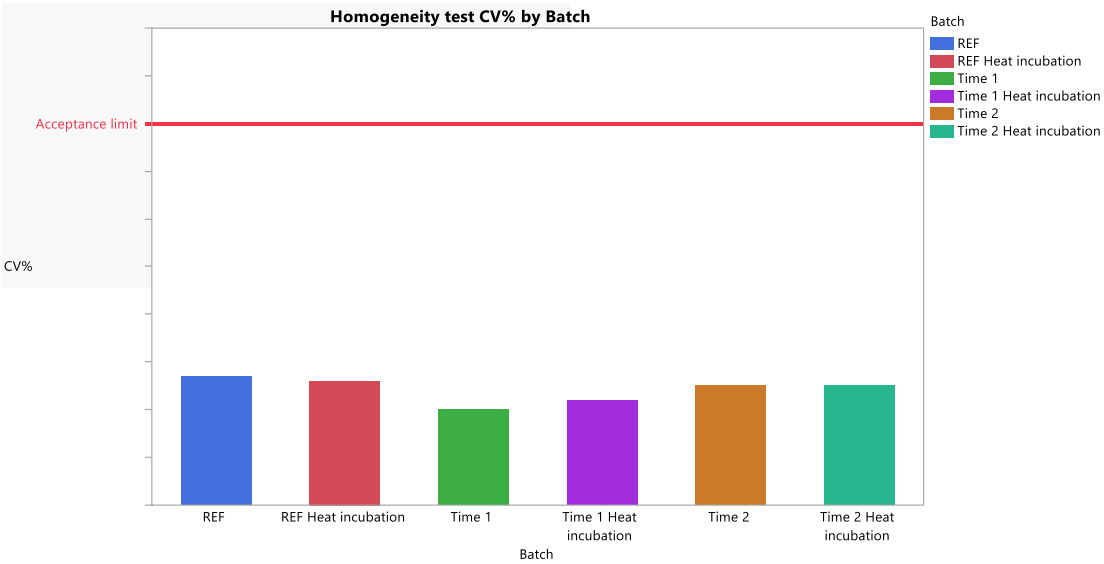

Figure 8 Characterization batch 2 – Homogeneity test results

All of the tested batches were within acceptance criteria and all characterization batch plates resulted lover CV% than REF batch. Anyhow, there were no significant differences in CV% between REF and characterization batches. Based on the homogeneity test results it can be concluded that with each tested incubation time or temperature, homogenous plates can be produced.

5.2.3 Background test results

Background testing for each for each characterization batches and REF batch was performed after SA plate coating. Mean (*n* = 6) background signal for all tested plates per batch can be seen in Figure 9.

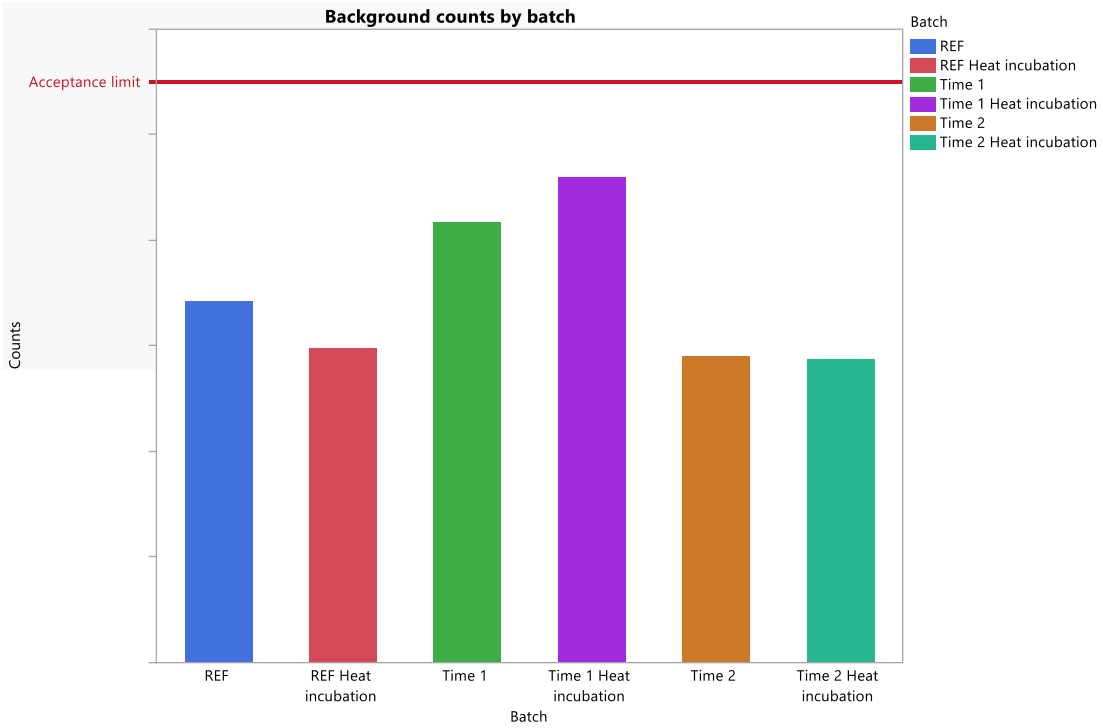

Figure 9 Characterization batch 2 – Mean SA plate background signals

SA plates incubated with Time 1resulted slightly higher background signal than REF plates or plates incubated with Time 2. Similar phenomenon was not observed in characterization batch 1 background testing. Reason for higher

background signal for SA plates incubated with Time 1 was not found in the production process of SA plates. Higher background signal can also be results of the background test testing method. Anyhow, all tested characterization and REF batch plates were within control and acceptance criteria and that's why further testing was not performed for characterization batch plates with Time 1 incubation time. From the background results it can be assumed that the SA coating solution and saturation solution incubation at higher temperature does not increase the background signal levels. Also the saturation solution incubation time has been sufficient to maintain low background levels.

5.2.4 Capacity test results

Bio-TEKES-Eu binding capacity testing for each characterization batch and REF plates was performed after SA plate coating. Mean (n = 4) biotin binding capacity results of each tested characterization batch and REF batch plates can be seen in Figure 10.

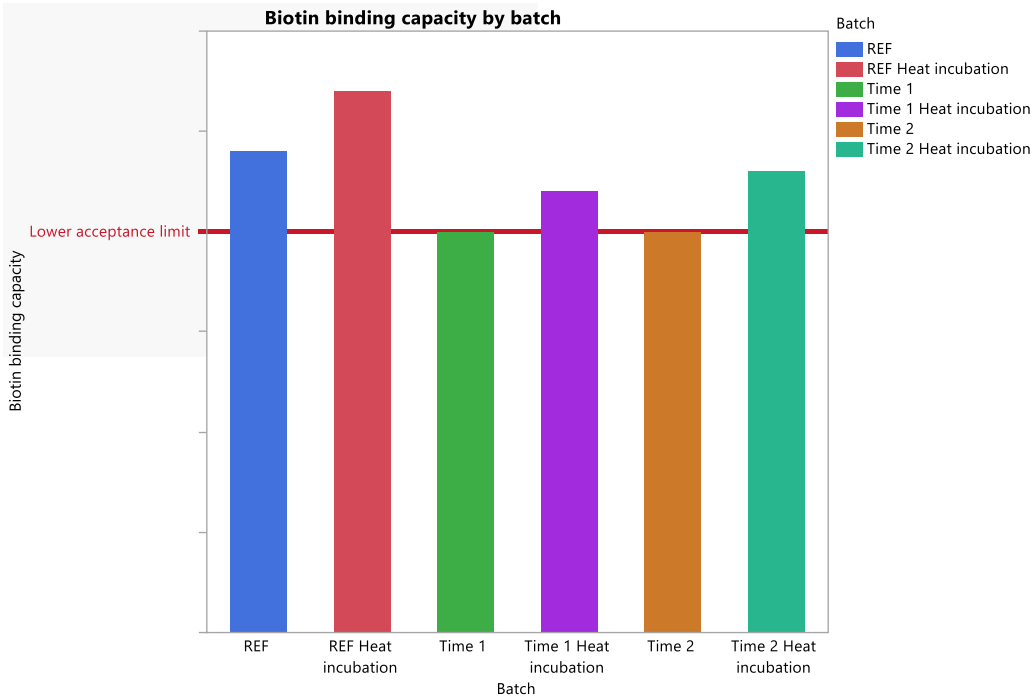

Figure 10 Characterization batch 2 – Mean SA plate biotin binding capacity

All of the tested characterization and REF plates were within acceptance criteria, resulting minimum biotin binding capacity. Like in characterization batch 1, REF plates resulted higher biotin binding capacity due the longer incubation time. Also the heat incubation resulted higher biotin binding capacity with all tested incubation times when compared to normal incubation.

To compare the differences between biotin binding capacity between characterization batches and REF batch, relative bias % was calculated according to Tartu 2024. Results of calculations can be seen in Table 3.

$$bias\ (\%) = \frac{Y_{\text{characterzation batch}} - Y_{\text{REF batch}}}{Y_{\text{REF batch}}} \times 100\% \qquad (4)$$

Where

Y is biotin binding capacity (pmol/cup)

Table 3 Relative bias% results

Batch	Relative bias %
REF Heat incubation	+ 12.5
Time 2	– 16.7
Time 2 Heat incubation	– 4.2
Time 1	– 16.7
Time 1 Heat incubation	– 8.3

5.2.5 Characterization batch 2 conclusions

All of the batch test results were within acceptance criteria for each tested characterization and REF batches. Significant differences between REF and characterization batches could not be seen in homogeneity of SA plates.

From the background results it can be assumed that the incubation at higher temperatures does not increase the background signal levels. Also the saturation solution incubation time has been sufficient to maintain low background levels.

Based on the biotin binding capacity test results the heat incubation seemed to enhance the adsorption of Streptavidin to cups polystyrene surface since heat incubated SA plates resulted higher biotin binding capacity with all tested incubation times when compared to normal incubation.

Characterization batch 2 results indicated possibility to shorten incubation times from REF incubation times to tested incubation times.

5.3 Characterization batch 3 – Analyte cups

SA plates manufactured in characterization batch 2 were used for analyte X coating of characterization batch 3. For the analyte X coating following characterization batch 2 plates were chosen:

- REF
- REF Heat incubation
- Time 1
- Time 1 Heat incubation

SA plates incubated at higher temperatures were chosen for analyte coating to see does the heat incubation affect to the analyte coating properties of analyte X. Characterization batch plates with Time 1 incubation were chosen for analyte coating since it was the target incubation time, and it acts also as worst-case scenario. Also, there were no major differences between characterization batch plates incubated with Time 1 or Time 2.

Characterization batch 2 SA plates were stored up to claimed shelf life of SA plates in SA plate storage before beginning of the analyte X coating. Storage time represents the worst-case scenario.

Characterization batch 3 was analyte coated in conjugation with sales batch. Same materials and equipment were used for characterization and sales batch plates in analyte coating, IL drying, tracer dispensing and cartridge packing. Only difference between the characterization batches and sales batch was the SA plates.

5.3.1 Homogeneity results

Within (Figure 11) and between plate (Figure 12) CV%:s were calculated for characterization plates based on only two whole plates which were measured during characterization batch homogeneity testing. The sample size for Sales batch was higher and for that reason it results significantly lower between plate CV%. There were no column or row CV% acceptance criteria exceedings with REF or characterization batch TEST plates. Also within and between plate CV% were within acceptance criteria with all tested characterization batch and REF TEST plates.

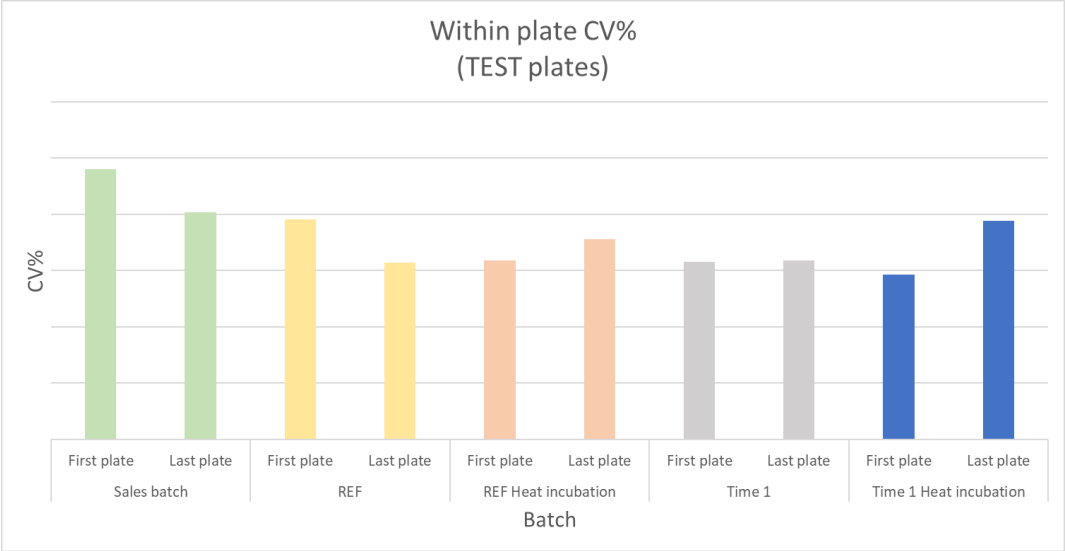

Figure 11 Characterization batch 3 TEST plate homogeneity – Within plate CV%

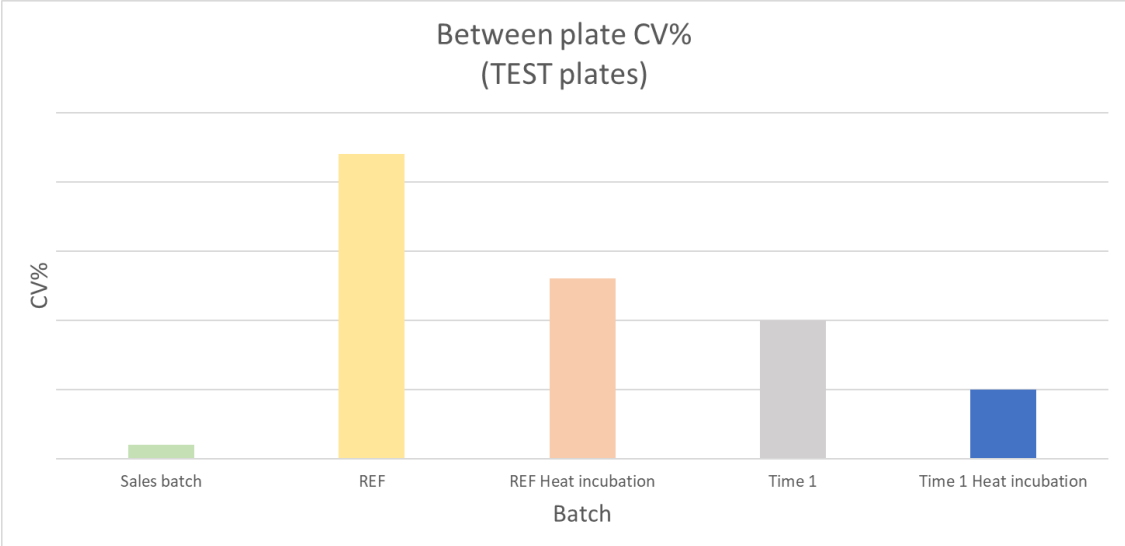

Figure 12 Characterization batch 3 TEST plate homogeneity – Between plate CV%

There were no acceptance limit exceedings in within plate CV%:S with the characterization or REF CAL plates, see Figure 13. Also there were no significant differences between Sales batch, REF or characterization batches.

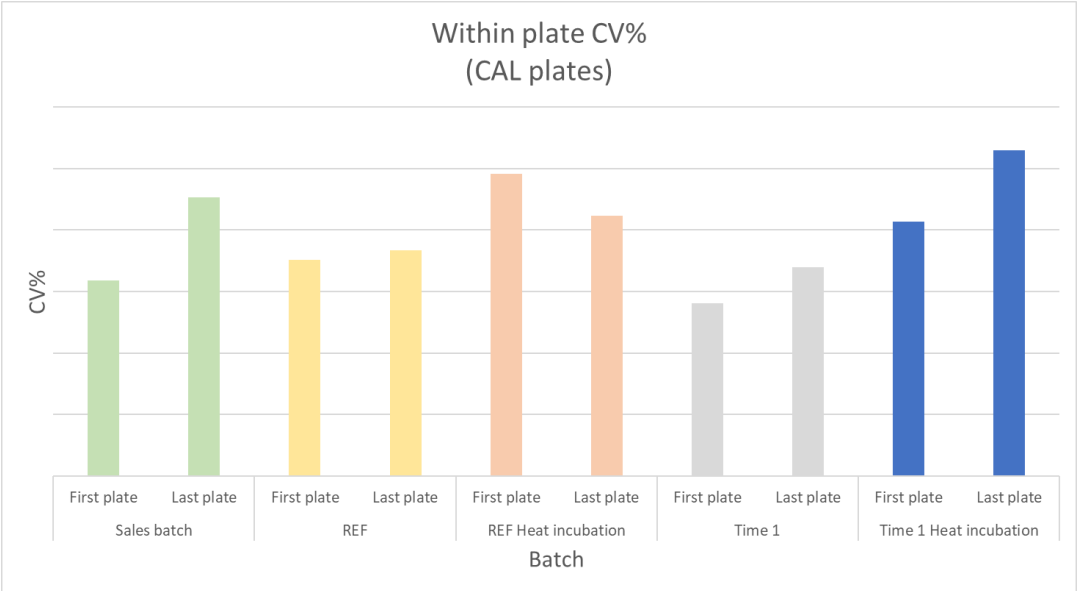

Figure 13 Characterization batch 3 CAL plate homogeneity – Within plate CV%

Within plate CV% were within acceptance criteria for all tested batches. All characterization batch plates resulted slightly lower CV% than REF batch. Anyhow the difference between characterization batches and REF batch were not remarkable.

Between plate CV% was calculated only based on two plates, since only two plates were tested during the characterization. All results were within acceptance criteria. Sales batch resulted significantly lower CV% for between plate CV% than characterization batch plates. Also the characterization batch plates with Time 1 incubation time resulted lower CV% than the REF plates. Within plate CV% for CAL plates were within acceptance criteria for all tested batches. There were no major differences between tested batches.

Based on the homogeneity test results, the incubation time has no effect on the homogeneity of SA plates. Anyhow, the sampling for the homogeneity test was

reduced and it might be that the homogeneity would result differently with larger batch size and sampling.

5.3.2 Background test results

In background test only the background signal level for each batch was observed. In Figure 14 is shown the mean (*n* = 32) background signal for each batch by used analyzer. From the results can be seen that the background signal varies between the used analyzers which is normal phenomena.

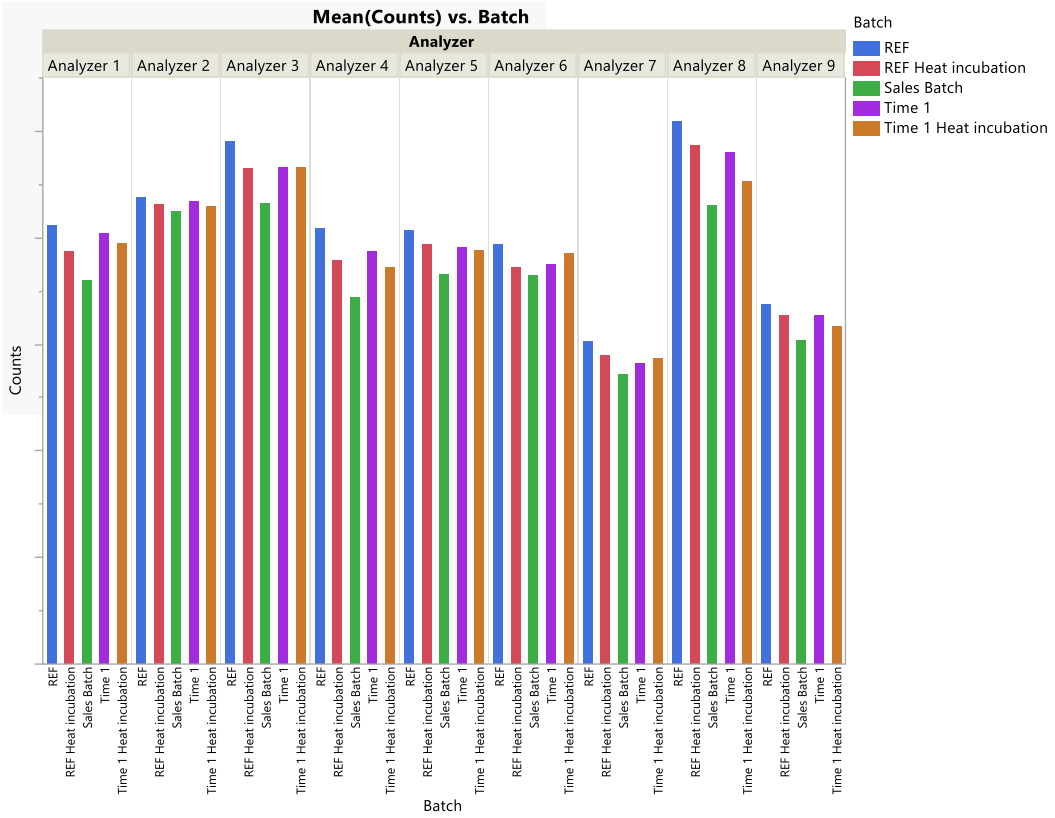

Figure 14 Characterization batch 3 TEST cartridge background signal by analyzer.

In Figure 15 is shown the mean (all analyzers, *n* = 9) background signal by batch. All batches were within acceptance criteria and there were no significant differences between characterization batches, REF or sales batches.

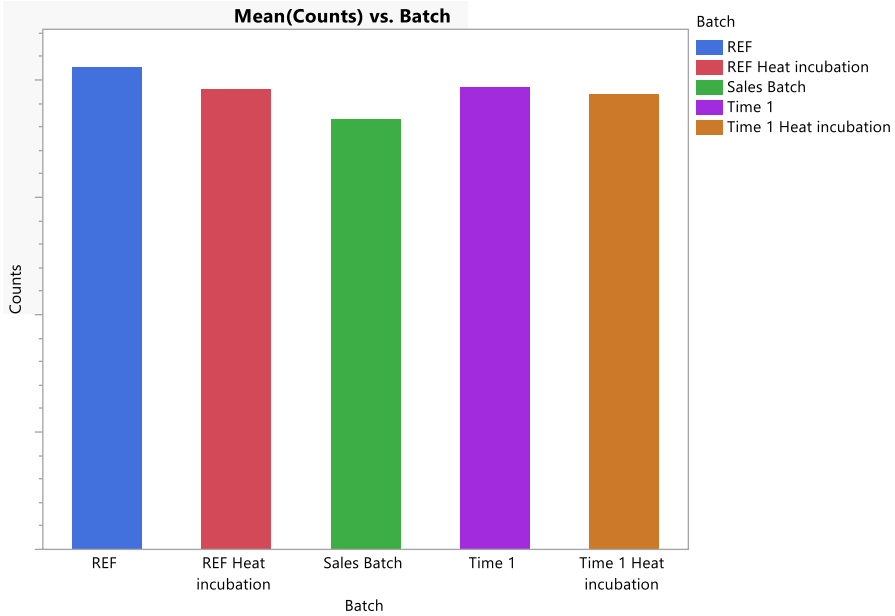

Figure 15 Characterization batch 3 TEST cartridge mean background signal by batch

5.3.3 Barcoding test results

In Figure 16 are shown the results of mean background signal for each tested batches. There were no control or acceptance limit exceedings in background signal level for any of the batches. Also significant differences in background signal level were not observed between batches. Sensitivity for all tested batches were within acceptance criteria and there were no any difference between batches.

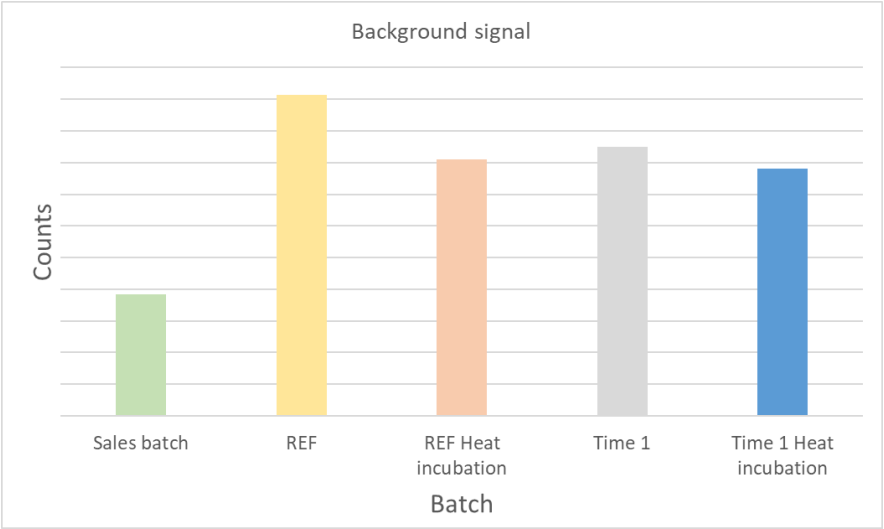

Figure 16 Characterization batch 3 - Barcoding background signal

In Figure 17 are shown the CAL cartridge concentrations and in Figure 18 deviation (CV%) of CAL cartridges concentration for each tested batch. As seen in the results the batches coated on heat incubated SA plates resulted slightly lower CAL cartridge concentration. Anyhow, the difference in CAL cartridge concentration is not critical factor for product performance since it is determined in barcoding test and there are no acceptance criteria for the CAL cartridge concentration. Deviation in CAL cartridge concentrations were within acceptance criteria for each tested batch.

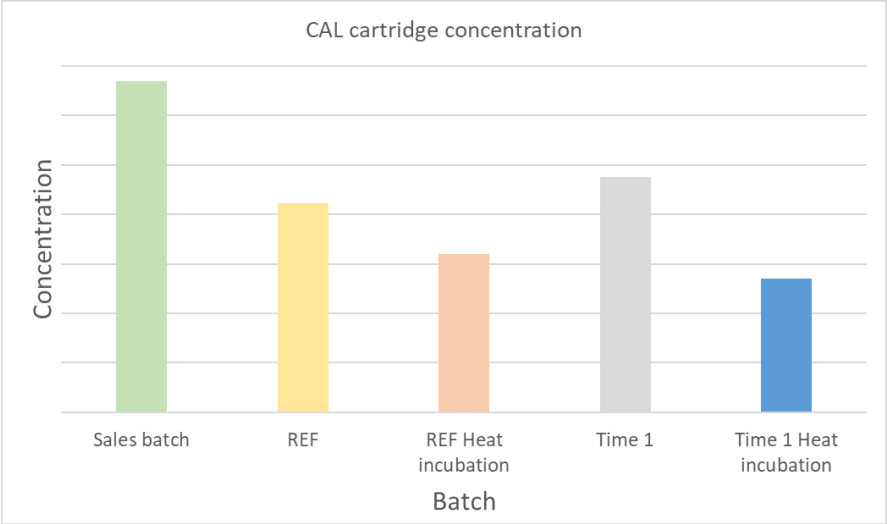

Figure 17 Characterization batch 3 – CAL cartridge concentration

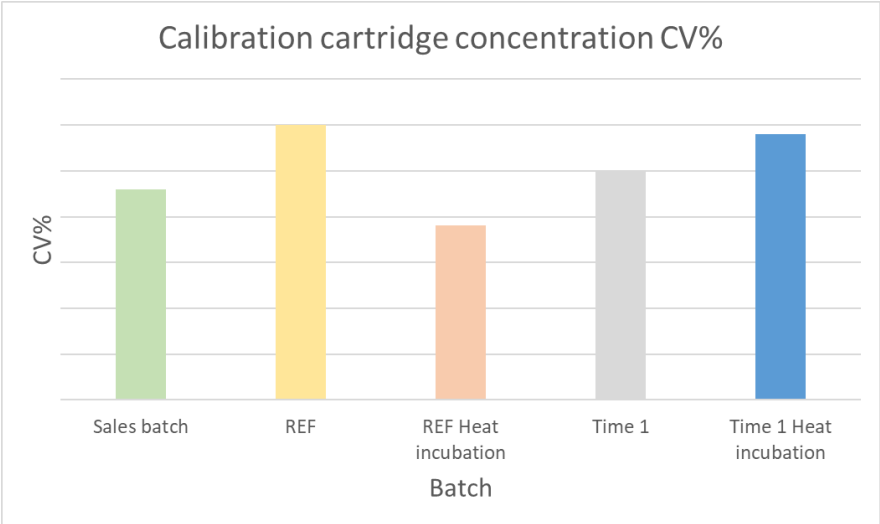

Figure 18 Characterization batch 3 – Variation of CAL cartridge concentration

5.3.4 Release test results

Release test for each characterization, REF and sales batch were performed using same materials and analyzers. In the release testing all result were within acceptance criteria. There were no major differences in background signal levels between characterization batches, REF and sales batch. The background signal levels can be seen in Figure 19.

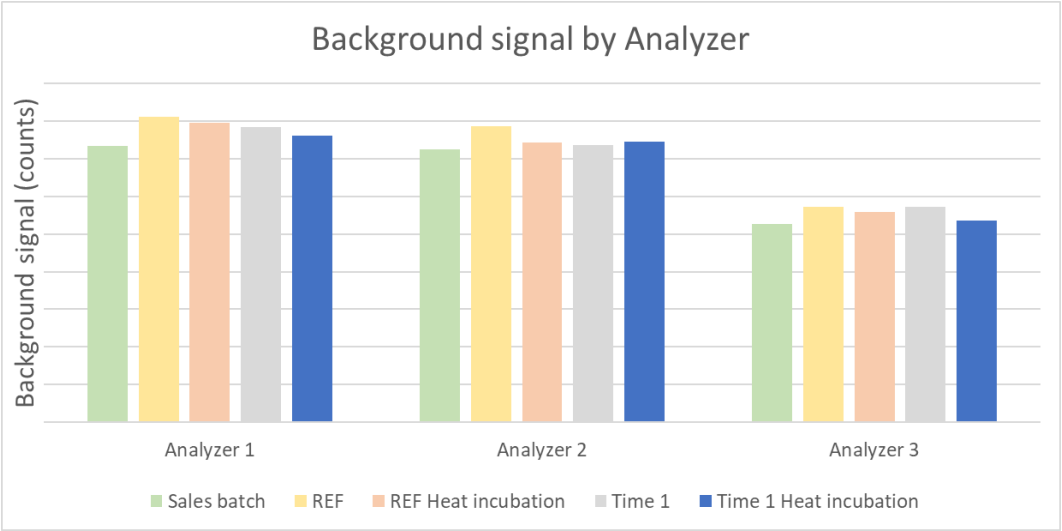

Figure 19 Characterization batch 3 – Release test background signal results

The variation of control concentrations was within acceptance criteria with all tested batches and there was no significant difference between characterization batches and REF batch. The variation of control concentrations can be seen in Figure 20.

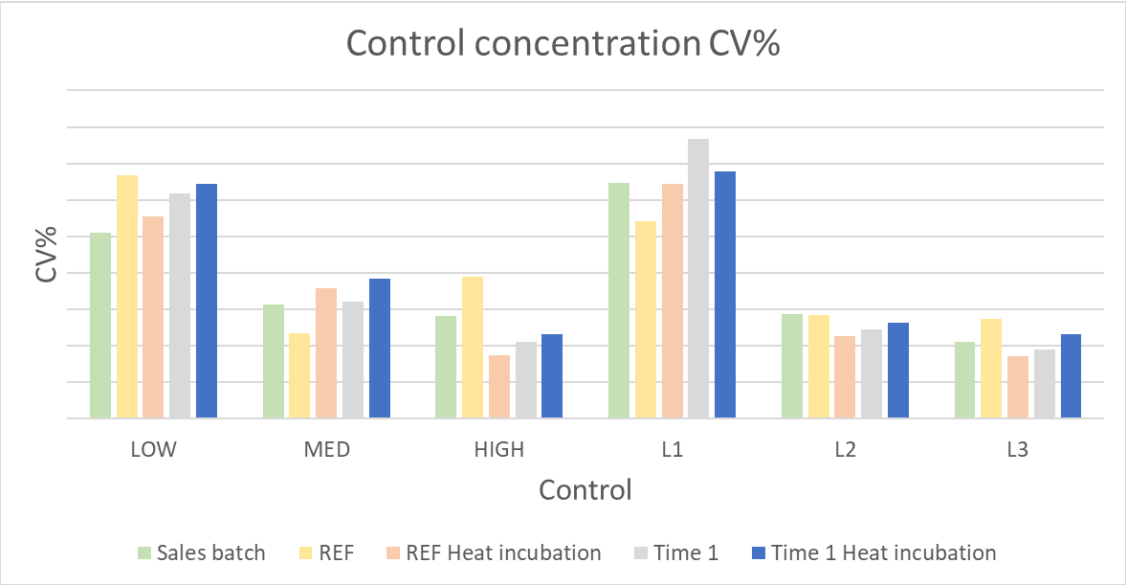

Figure 20 Characterization batch 3 – Release test control concentration variation results

For CAL cartridges the mean background signal (Figure 21) and mean CAL cup signal (Figure 22) was calculated for each used analyzer (*n* = 8).

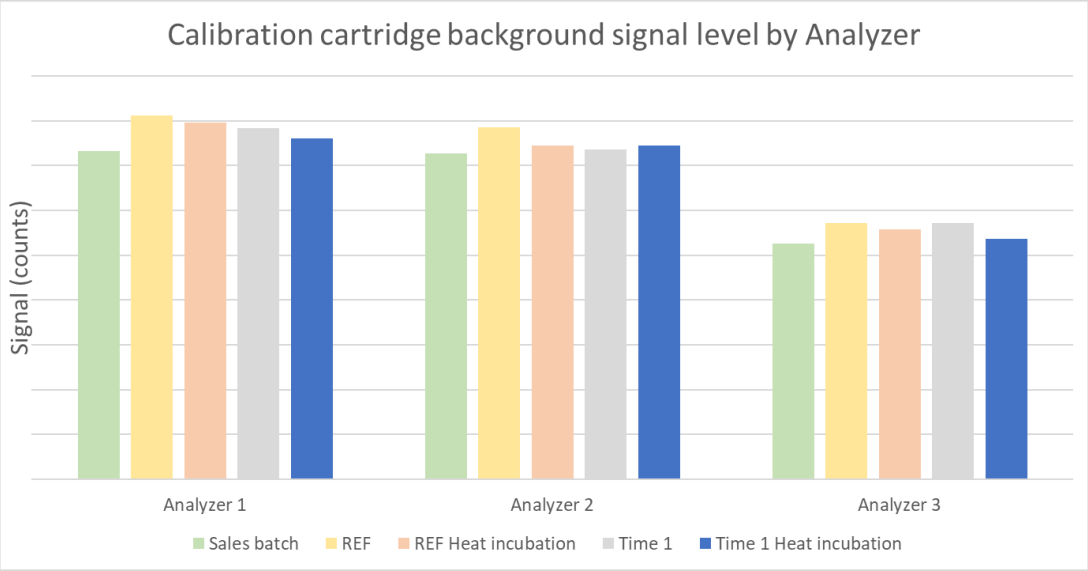

Figure 21 Calibration cartridge background signal level by Analyzer

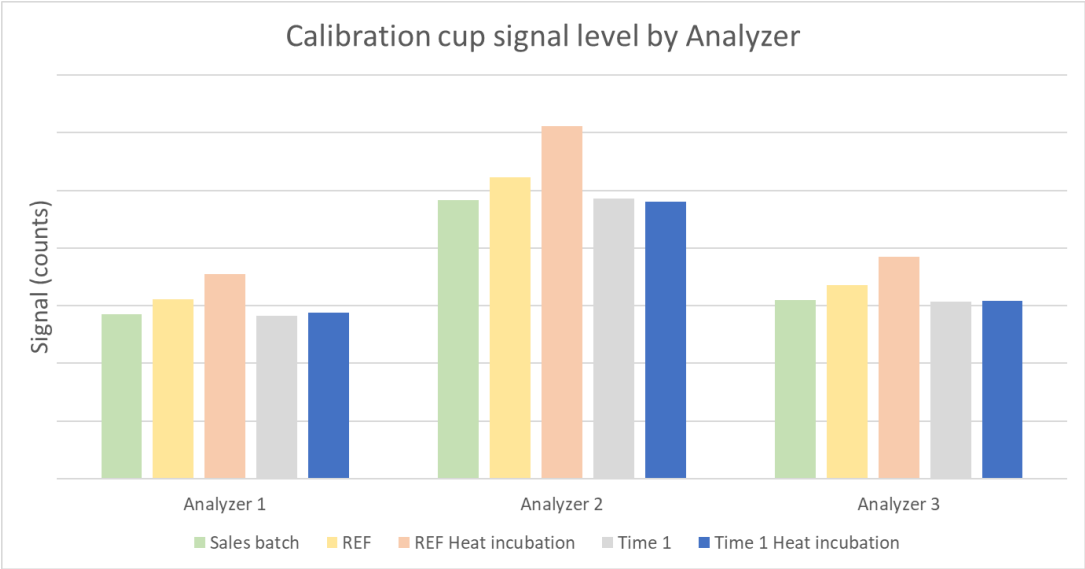

Figure 22 Calibration cup signal level by Analyzer

For the CAL cartridges there are no acceptance criteria for the background signal level or CAL cup signal level, but these values were used for onboard stability evaluation.

5.3.5 Onboard stability test results

Onboard stability testing for all batches was performed as described in paragraph 4.8. except characterization batches REF cartridges and REF heat incubation cartridges were measured only with 2 analyzers due the malfunction of analyzer 1. Same control samples were used for onboard stability testing as were used in release tests.

In Figure 23 can be seen the mean background signal levels by batch measured during release test and onboard stability test. As seen from the results the background signal does not significantly change between the release test and onboard stability test.

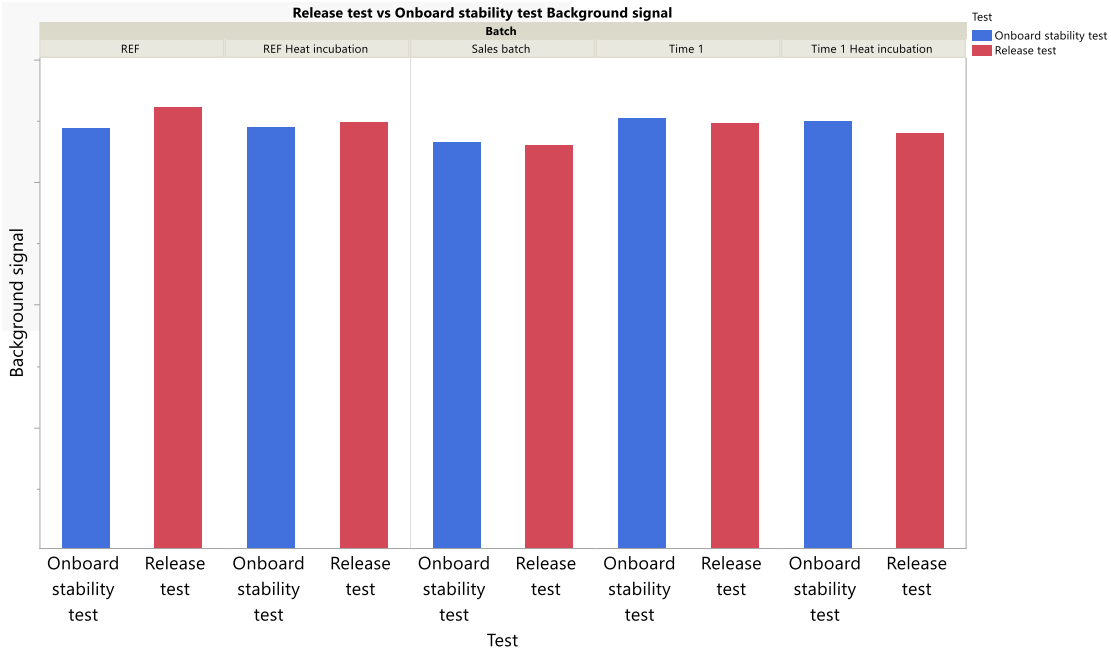

Figure 23 Release and Onboard stability test background signal

Concentration difference (measurand drift) between the release test results and stressed cartridges was calculated for every control level. In Table 4 can be seen the overall measurand drift for each tested batch. Due the malfunction of analyzer 1, the overall measurand drift was calculated based on the results of 3 and 2 analyzers.

Table 4 Onboard stability results, measurand drift

Batch	Test cartridge drift-%		CAL cartridge drift-%	
	2 Analyzers	3 Analyzers	2 Analyzers	3 Analyzers
Sales batch	– 7.1	– 7.4	+ 0.4	+ 1.1
REF	– 6.4	-	– 1.3	-
REF Heat incubation	– 5.9	-	– 0.7	-
Time 1	– 6.5	– 7.7	+ 2.2	+ 3.6
Time 1 Heat incubation	– 2.9	– 4.9	+ 2.5	+ 2.1

For the test cartridges all characterization batches resulted smaller measurand drift when results are compared based on 2 analyzers, which indicates better onboard stability for the characterization batch test plates. The difference in measurand drift between the Sales batch, REF and characterization batches with shorter SA coating solution and saturation solution incubation times can be considered as negligible. From the results can also be noted that the characterization batches that were incubated at higher temperature resulted better onboard stability than batches incubated at normal conditions. CAL cartridges resulted mostly fine signal increase with sales batch and characterization batches with shorter incubation times.

To evaluate the equivalency between REF and characterization batches the TOST test with JMP was performed for Test and CAL cartridge measurand drift results. The TOST test results for TEST cartridges can be seen in Figure 24 and for CAL cartridges in Figure 25. The TOST test results can be visually assessed, closer the legend (red line) is to the center line of graph, the more similar batches are with each other.

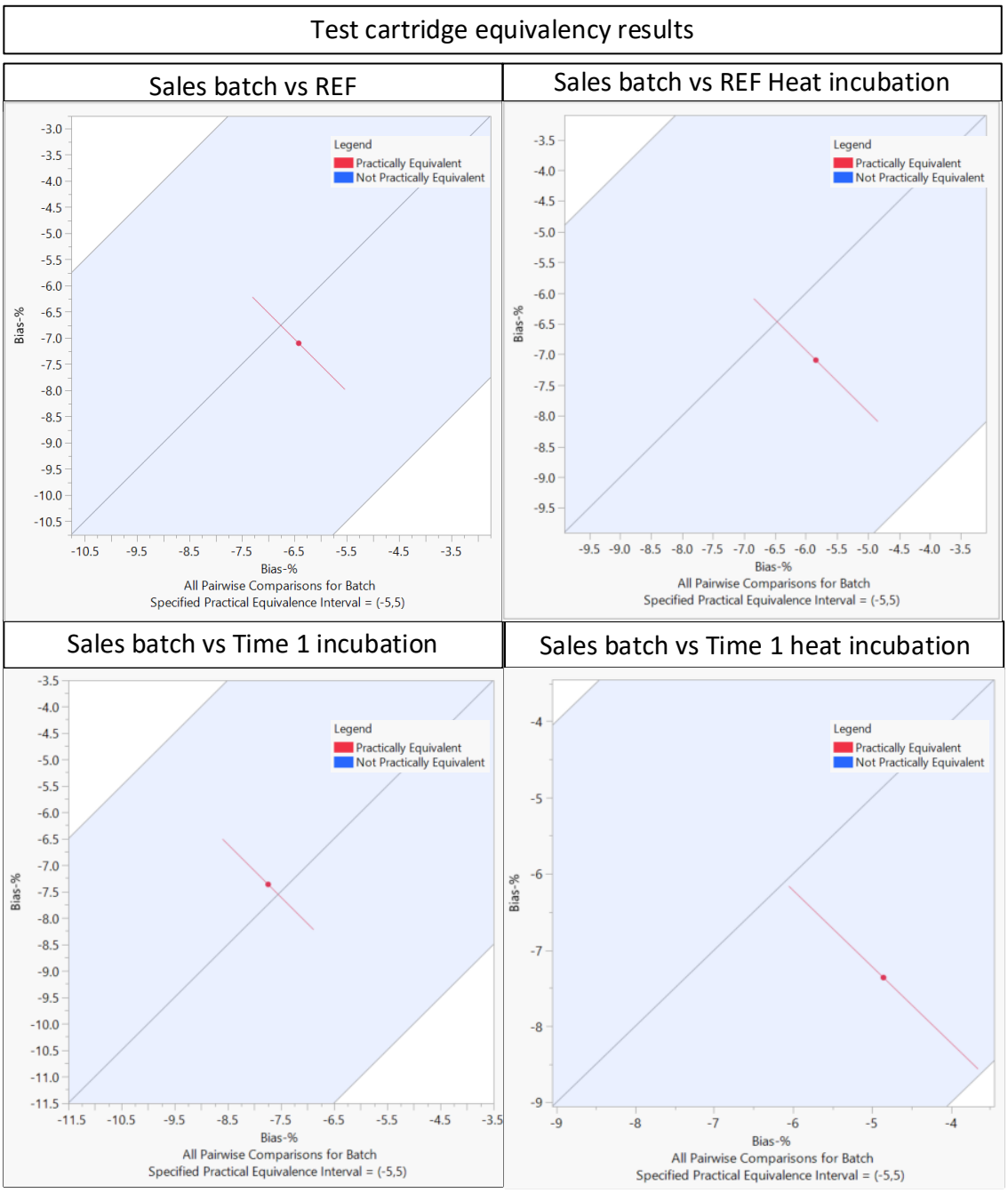

Figure 24 Test cartridge equivalency test results

Based on the TOST test results all of characterization batch TEST cartridges are practically equivalent with the REF batch.

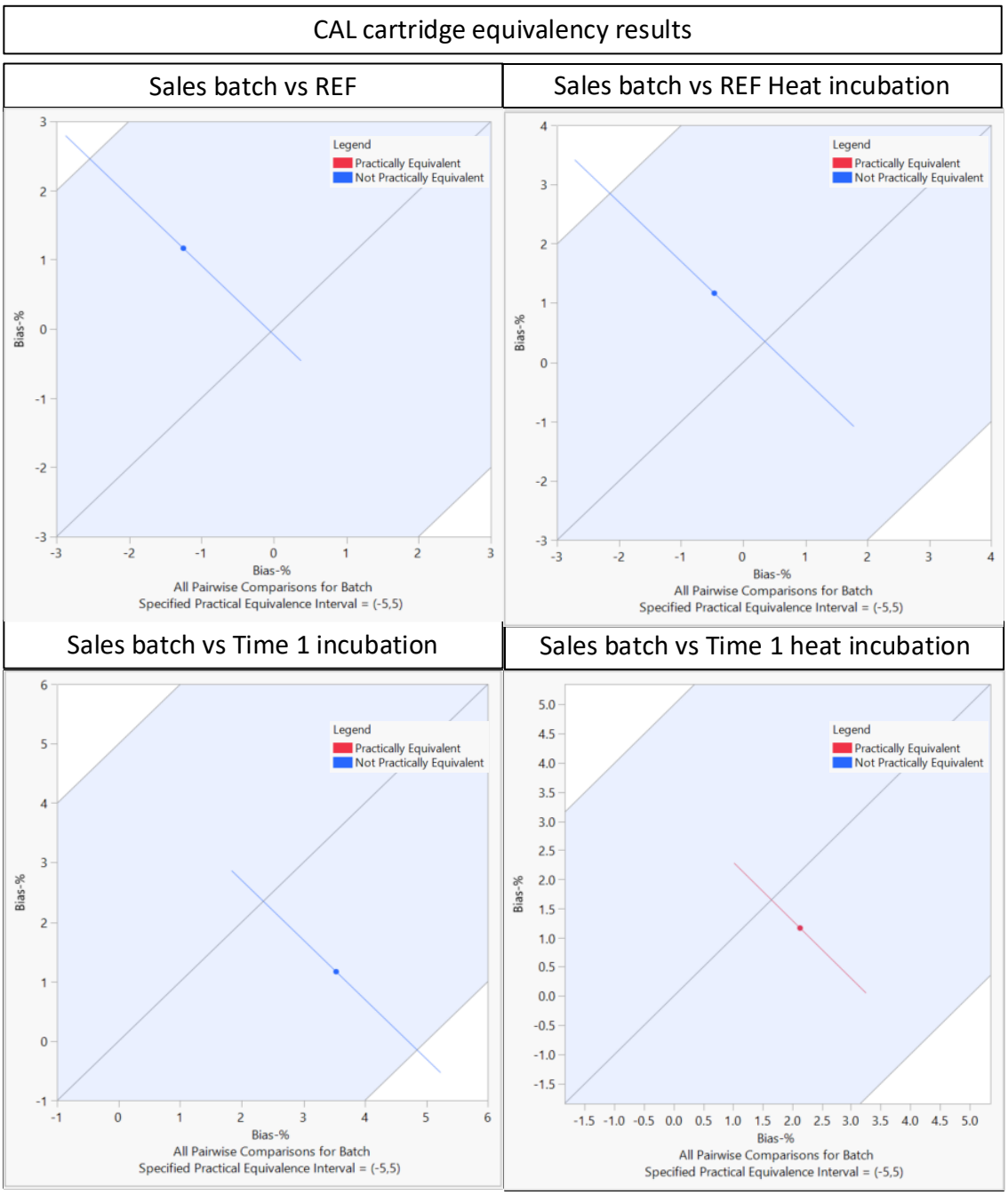

Figure 25 CAL cartridge equivalency test results

5.3.6 Characterization batch 3 conclusions

Based on the quality control test results and onboard stability test results, all of the tested characterization batch combinations passed the acceptance criteria.

Anyhow, for the homogeneity testing the amount of plates tested was reduced from the normal sample amount. For that reason the homogeneity test results are indicative.

Also for the background testing the sample amount was reduced from the normal sample amount but the background levels were observed also during the barcoding, release and onboard stability testing. Based on the test results the background signal were within the acceptance criteria and the analyte X cups manufactured from SA plates with shortened incubation times resulted slightly lower background signal level which indicates that the saturation solution incubation time does not have effect on the product performance.

From the barcoding results can be observed that the shortened SA coating solution and saturation solution incubation times does not have effect on the CAL cups performance. There were no major differences in the sensitivity, concentration or CV% between characterization batch and REF batch CAL cups.

In the release test there were no major differences in background signal levels between characterization batches and REF batch. The variation of control concentrations was within acceptance criteria with all tested batches and there was no significant difference between characterization and REF batch.

By onboard stability test results shorter SA and saturation solution incubation times does not have negative effect on onboard stability of analyte X. All characterization batches resulted smaller measurand drift when, which indicates better onboard stability for the characterization batch TEST and CAL plates. The difference in measurand drift between the REF and characterization batches with shorter SA coating solution and saturation solution incubation times can be considered as negligible. By the TOST test results the REF and characterization batches can be considered as equivalent.

6 Process optimization

The cycle time of the SA cup production process was calculated based on the retrospective data. The processing time of one SA plate was calculated with following equation. The mean processing time was calculated based on four production batches.

$$D = \frac{A}{B} \qquad (5)$$

where

A is total process time (First – last plate) in seconds
B is amount of processed plates in pieces (PCS)
D is a takt time, seconds/plate

Calculated processing times can be seen in Table 5.

Table 5 Processing times of SA plates

Processing day	Day 1	Day 2	Day 3
Processing time (s/plate)	4.0	3.8	4.0

For the shortened incubation time process simulations the batch sizes of 1900 plates (SA plates for one analyte batch) and 3800 plates (SA plates for 2 analyte batches) were selected. The restrictions for the simulations were the available time per day (16 hours), solution manufacturing and takt time of SA coating line.

In the Table 6 are shown the calculated processing times of each process phase for the selected simulation batch sizes. Takt times shown in Table 5 were used for the calculations.

Table 6 Processing times of each step in SA process

Batch size (PCS)	Processing time (hh.mm)		
	SA coating solution dispensing	Washing and saturation solution dispensing	Saturation solution aspiration
1900	02.06	02.00	02.06
3800	04.12	04.00	04.12
10000	12.48	12.06	12.48

Process flow simulations with shortened SA coating and saturation solution incubation times can were prepared. In the process flow simulations the solution manufacturing, line preparations and processing times of each process phase were taken into account. According to process simulations, it is possible to manufacture 1900 or 3800 SA plates during 16 hours work shift.

For the 1900 plates batch size the SA coating solution and saturation solution incubations must be shorten to Time 1 if all of the solutions are prepared during the coating day. Based on the batch simulation of 1900 plates with incubation Time 1 there would be approximately 1 hour and 30 minutes buffer for the batch production during the 16 hours shift. If the batch size to be manufactured is increased from 1900 plates, the shortening of incubation times to Time 1 is not beneficial.

If the manufactured batch size is increased from 1900 plates it would require that the SA coating solution and saturation solution incubation were shorten to Time 2 and the solution manufacturing must be performed the day before coating day since there is no enough time to prepare the solutions during the coating day and zero buffer for the batch production.

6.1 Production capacity

Maximum production capacity of SA plates for the shorter Time 1 and Time 2 incubation times and current incubation times are calculated using takt time of SA coating line and current shift model. In calculations theoretical available production time of 50 weeks is used and remaining two weeks are reserved for maintenance of the production line. Based on the batch simulations for the shorter incubation times, 7 batches per week could be produced with the batch size of 1900 or 3800 plates. With the current production method 2 batches with the batch size of 11 500 plates could be produced. Based on these assumptions, the calculated maximum SA plate amount can be seen in Table 7.

Table 7 Maximum produced plate amount

Batch size (PCS)	Output Plates / year	Output change to current method (%)
1900	665 000	– 42.6
3800	1 150 000	+ 15.7
11500	1 330 000	-

Based on the calculations output of SA plates with Time 1 incubation time would decrease by 42.6 % and with the Time 2 incubation time it would increase by 15.7 % when compared to the current maximum output.

Other aspect of the SA plate production is the solution manufacturing which is performed separately for each produced batch. The active time that operators use for solution manufacturing for each batch was estimated to be 7 hours. Even though the batch size would be decreased from 11 500 plates to 1900 plates, the steps that operators perform during the solution manufacturing would be same and thus the time spent on each batch solution manufacturing would also remain the same.

By using the same estimates of two 11 500 plate batches per week and seven 1900 or 3800 plate batches per week, the time active operator time spent on solution manufacturing was calculated.

Two batches produced with the current production method would mean 700 hours of active operator time spent on solution manufacturing annually. While with the shorter incubation times seven batches produced weekly, it would mean 2450 hours spent on solution manufacturing annually. Based on these calculations the time spent on solution manufacturing would increase by 250 % if shorter incubation times were implemented and seven batches were manufactured weekly without changes to the solution manufacturing process.

7 Discussion

Characterization batch 1 homogeneity test results indicate that the denaturation time of Streptavidin and manufactured coating solution batch size may have affected the homogeneity of the plates, as test plates coated with coating solution 2 exceeded the acceptance limit. Exceeding the acceptance limit could not be caused by plate manufacturing process, shortened incubation times or test method, since the dispensed solution was the only variable. Anyhow, it remained unclear was the unvalidated solution batch size or denaturation time of SA reason for poor homogeneity, since both variables were fixed for characterization batch 2 solution manufacturing process.

As seen from the biotin binding capacity test results of characterization batch 2 the heat incubation increased the SA adsorption to the polystyrene surface of the cup. With the shorter Time 1 and Time 2 incubation times, heat incubation would be a sufficient incubation method to stay safely above the lower acceptance limit. Anyhow, to verify effect of heat incubation further testing and repetition would still be needed as the sample amount in characterization batch 2 were small. As seen from all characterization batch results, the background levels remained within acceptance criteria which indicated that the saturation solution incubation time could be shortened and performed at normal conditions or at higher temperatures.

Shortened incubation times did not have effect on performance or stability of end product manufactured from analyte X based on the quality control test results of one manufactured batch. Characterization should also be performed with other analytes, since there are differences between biotinylated antibodies and their coating properties to the SA surface. The results obtained in this study indicates that the SA coating solution and saturation solution incubation times could be shortened.

With the current shift model and production equipment the batch sizes of 1900 or 3800 SA plates could be manufactured per day with shortened incubation times. SA plate production output could not be increased remarkably by

shortening the incubation times, but it would increase flexibility in production by adding ability to react quicker to demand as well as reduce the lead time of the final product.

To implement the one-piece-flow production method to POC test manufacturing the whole process should be optimized precisely. By optimizing also the batch size used in the analyte coating process, it would be possible to perform the process steps from SA coating to IL drying in one day. To implement the one-piece-flow production methodology to the current production lines, the high-level process flow simulation from SA coating to IL drying was prepared. Based on the batch simulation batch of 1600 plates could be manufactured during the 16 hours shift from SA coating solution dispensing to IL drying. This would require a preparation day for the solutions used in the SA coating. Also it was noted that there would be some variation within batch in incubation times.

Proceeding to analyte coating directly after the saturation solution aspiration would require further investigation since the effects of removing the SA plate storage step to functionality of end product are unknow. Also to simplify the SA plate manufacturing process the need of drying of SA plate item X should be re-evaluated and removed if possible.

As stated in the previous paragraph, the time used for SA plate production solution manufacturing is not reasonable if the shorter incubation times are implemented. The solutions used for SA coating should be possible to manufacture in larger volumes and to be used for multiple smaller batches. For that reason the shelf life of the solutions should be investigated and revalidated since the current shelf life does not support the of solution for several days.

8 Conclusions

In conclusion, results of the characterization batches quality control tests indicate that shortening of the SA coating and saturation solution incubation times does not have a significant impact on the shelf life, homogeneity, background levels, or performance of the SA-coated plates and end product of Analyte X. Anyhow, the characterization batches were manufactured in small batch sizes and the sample sizes in some quality control tests were reduced, for that reason further testing would be needed. The heat incubation at higher temperatures appears to enhance the adsorption of Streptavidin to cups polystyrene surface. With the shorter Time 1 and Time 2 incubation times, heat incubation would be a sufficient incubation method to stay safely above the acceptance limit. The study results indicate possibility to shorten SA coating and saturation solution incubation times up to 81 % from current incubation times.

As the batch simulations show, the reduction of incubation times to Time 1 is only beneficial for batch size of 1900 plates and 3800 plates for Time 2 incubation times.SA plate production output could not be increased remarkably by shortening the incubation times but it would increase flexibility in production by adding ability to react quicker to demand as well as reduce the lead time of the final product. The time spent on solution manufacturing would increase by 250% if shorter incubation times were implemented and seven batches were manufactured weekly without changes to the solution manufacturing process. Overall, the results suggest that the optimization of incubation times can improve manufacturing efficiency while maintaining the quality and performance of the SA-coated plates.

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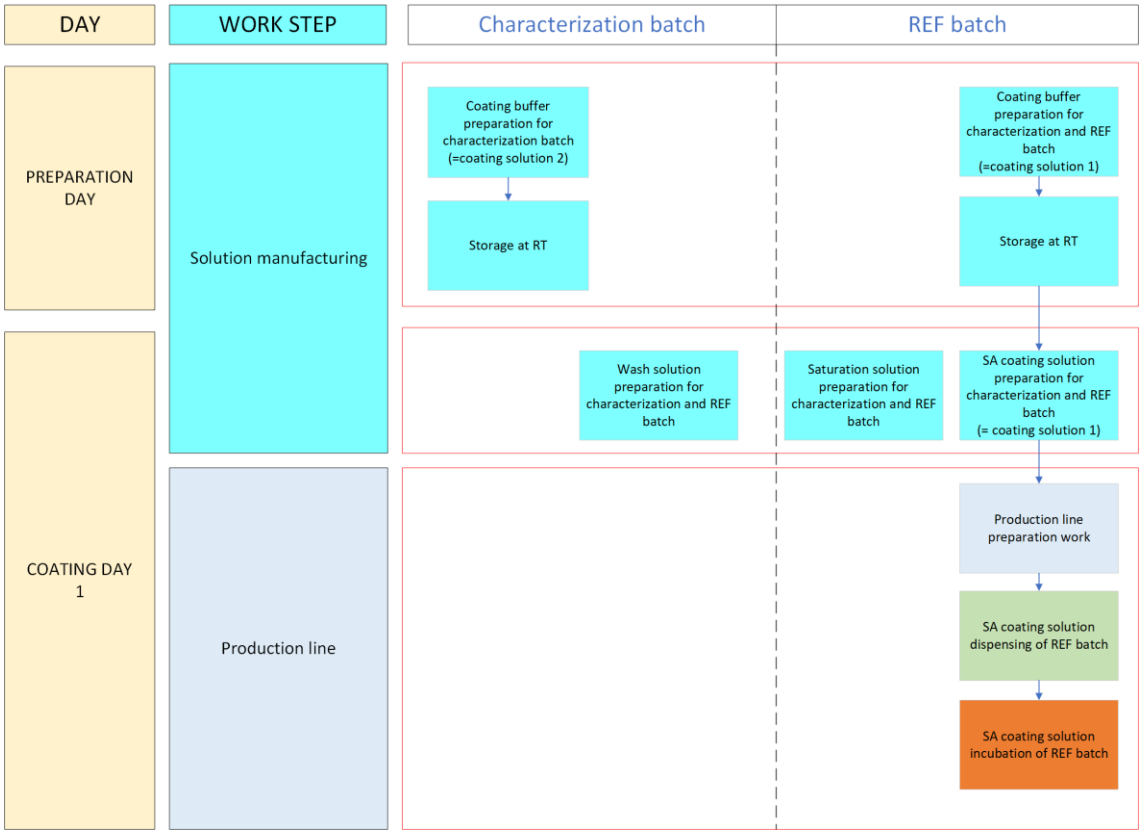
Production flow of characterization batch 1



COATING DAY 2
Solution manufacturing
SA stock solution preparation + addition in coating buffer (=coating solution 2)
Mixing of coating solution 1
Mixing of coating solution 2
Filtration of coating solution 2
Production line + SA coating solution dispensing of characterization plates
Dispensing of coating solution 1 and 2 in characterization batch plates
SA coating solution incubation
Saturation solution incubation
Production line post cleaning and preparation for day 3
Saturation solution aspiration of characterization batch
Production line post cleaning
Storage of characterization batch plates
Production line preparation work
SA coating solution aspiration of characterization and REF batch
Wash solution dispensing and aspiration of characterization and REF batch
Saturation solution dispensing of characterization and REF batch
Saturation solution incubation of REF batch
COATING DAY 3
Production line
Production line preparation work
Saturation solution aspiration of REF batch
Production line post cleaning
Storage of plates