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| Purpose Principle  | This procedure provides instructions forperforming the Sweat Chloride assay.\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_The ChloroChek™ Chloridometer® operates according to the principle of coulometric titration. Two silver electrodes—the generator electrodes (anode and cathode) –are dipped into a measuring vessel filled with working solution. The working solution consists of an acid buffer and a colloid stabilizer that keeps the silver chloride, which arises later on, in suspension.Since the buffer does not contain any silver ions, the silver ion concentration, and thus the indicator current (see below) is brought to a specific end point. By means of a constant current (generator current) between the two silver electrodes, a constant amount of silver ions is released at the anode. The silver ion concentration is maintained by the measurement electrodes (indicator electrodes), which are dipped into the solution. By adding a chloride sample, the free silver ions form a non-soluble silver chloride precipitate together with the free chloride ions of the sample.The indicator current drops, and by controlling the generator current, silver ions are released until all chloride ions are precipitated as silver chloride. This restores the original silver ion concentration (end point).The period of flow of the generator current is measured during the titration process and is proportional to the chloride ion concentration. |

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| Scope | This procedure is intended for Clinical Laboratory Scientist (CLS) who are trained and competent in performing the Sweat Chloride assay. |

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| Policy | The Sweat Chloride assay will be performed every Tuesday and Thursday of the week.Titrate all specimens (Controls, Calibrators, Blanks, Patients) in duplicate and average the values (when there is adequate volume). |

Ensure duplicate titrations from the same patient site agree within 10% or ±5 mmol/L, whichever is greater. The duplicate titrations should be averaged for results reporting.

Do not allow any portion of the materials used in the analysis to contact the hands. Only powder-free gloves should be worn.

 Analyze the calibrators, controls, and patient specimens at room temperature.

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| Specimen sources | Human sweat collected with Elitech Group’s Macroduct®Collector |

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| Specimen collection | Specimens are collected by the Pulmonary Laboratory Department. |

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| Specimen transport | Sweat samples will be transported to the 4867 Laboratory by the Pulmonary Laboratory Department. |

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| Specimen storage | Sweat is stable for at least 72 hours across a reasonable temperature range, (2-25˚C) without significant evaporation when stored in 0.2-mL microcentrifuge tubes with snugly fitting caps. |

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| Specimen rejection | Reject specimen under any of the following conditions:* Specimens less than 15ul
* Multiple insufficient samples must be rejected and not pooled for analysis.

Sweat Rejection Incidence Rate:For patients older than three months of age, the annual insufficient rate should not exceed 5%. For patients three months of age or younger, the rate of insufficient samples should not exceed 10%. If these rates are exceeded, the collection procedure should be reevaluated for consistency with the CLSI document C34. The most common cause of insufficient samples is the use of inappropriate collection devices. |

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| Reagents and/or Media | **Description** | **Preparation** | **Stability** |
| 100 mmol/L NaCl/H2O Standard Solution | 1 ml clear glass One Point Cut (OPC) ampleReady to use. | Up to expiration date indicated on the packaging.Stability is ½ hour after ampule has been opened. The standard solution must be stored at 5ᵒ-45ᵒC. |
| Acid Buffer Solution | 10 ml of diluted acetic acid and nitric acid, pH 1.12Ready to use. | Up to expiration date indicated on the packaging.The acid buffer solution must be stored at 10ᵒ-30ᵒC. |
| Gelatin Solution | 30 ml of Gelatin Solution with pH 5.7Ready to use. | Up to expiration date indicated on the packaging.The gelatin solution must be stored at 10ᵒ-30ᵒC. |

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| Materials and supplies | * Titration beaker
* Magnetic Stir Bar
* Magnetic Stir Bar Retriever
* 10 ul fixed volume pipette
* Pipette tips
* Ampule organizer
* Distilled water
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| Equipment | * ChloroChek™Chloridometer®
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| Safety | All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, and the safety of others and adhering to all departmental and medical center safety policies and procedures.* For standard precautions and safety practices in the laboratory; see LAMC-PPP-0123, specifically, but not limited to, equipment safety, proper body

 mechanics, sharps exposure and proper use of personal protective  equipment (PPE).* For Universal Body Substance precautions, see LAMC-PPP-0128, specifically, but not limited to, exposure to body fluids.
* For proper handwashing, see LAMC-PPP-0132, specifically, not limited to, proper handwashing.
* For proper infection control, see LAMC-PPP-0127, specifically, but not limited to, proper use of gloves.
* For proper handling of regular and infectious waste, see LAMC-PPP-0129, specifically, but not limited to, proper disposal of regular and biohazardous waste.
* For proper cleaning of work area, see LAMC-PPP-0130 – Cleaning Work Areas.
* For proper handling of chemicals and reagents, see the Chemical Hygiene Plan.
* For proper storage and disposal of chemical hazardous waste, see LAMC-PPP-0134.
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A fresh Working Solution must be prepared each day before using the

 ChloroChek™Chloridometer®

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| Preparation of Working Solution | **Step** | **Action** |
| **1** | Add 20 drops of Gelatin Solution to one bottle of Acid Buffer Solution. |
| **2** | Put the cap back on the bottle and carefully swirl or invert it to mix it thoroughly. This forms the “Working Solution.” |
| **3** | Place the Magnetic Stir Bar in the Titration Beaker. Pour the freshly prepared Working Solution into the Titration Beaker.  |
|  | Users can run up to 50 samples in each series before needing a fresh Working Solution. A freshly prepared Working Solution should be a transparent red color.The stability of a used working solution is 24 hours. As a Working Solution is used it will appear cloudy. If a freshly prepared Working Solution appears cloudy it should be discarded. The titration beaker should then be carefully cleaned and a new Working Solution prepared. |
| Conditioning | Conditioning must take place whenever a new Working Solution is prepared and used. A new Working Solution should be prepared and used each day.

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| **Step** | **Action** |
| **1** | With the instrument on, press MEASURE on the display. |
| **2** | Place the filled titration beaker in the beaker receptacle. |
| **3** | Lower the handle to bring the electrode set down into the Working Solution. |
| **4** | Press the READY button on the display.**Note:** Pressing CANCEL ends the conditioning cycle and returns you to the Main screen. If this is done, the Working Solution must be replaced.  |
| **5** | In the following display you will be asked to wait until the system has reached its Working Point. A runtime display under the message visualizes this process. Proceed immediately to the next step.After the working point is reached, you will be instructed to pipette 10 µL of the 100 mmol/L NaCl/H2O Standard into the Working Solution.To do so, hold the One Point Cut (OPC) ampule of 100 mmol/L NaCl/H2O Standard steady in one hand. With the other hand, carefully break off the head of the ampule with only slight pressure across the printed point (blue dot) on the neck of the ampule. Use a protective plastic sleeve when opening the ampule. |
| **6** | Place a correctly-sized pipette tip onto a 10 µL fixed-volume piston pipette. Elitech Group has an appropriate pipette (AC-178) and pipette tips (SS-253) available. Make sure the tip is securely fitted to the pipette.  |
| **7** | Place the pipette tip well into the 100 NaCl/H2O mmol/L Standard Solution, holding the tip in the solution during the entire filling phase. Avoid drawing air into the pipette during the filling phase. Allow the piston to smoothly and slowly retract until it has returned to its initial position. Wait for about one second and then remove the pipette tip from the solution. If you see drops on the outside of the pipette tip, carefully remove them with a lint free tissue—do not draw out any solution from the pipette tip (through capillary forces of the paper tissue)—or if necessary, repeat the process with a new pipette tip.  |

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| CalibrationQuality Control |

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| **Step** | **Action** |
| **8** | Using one of the pipette guides found on the sensor carriage, place the loaded pipette tip as near as possible in the center of the stirring Working Solution. Dispense the sample above the working solution, allowing the sample to drop into the working solution. Avoid dispensing the sample near the electrodes or the sides of the beaker. Press the pipette piston down to the second pressure point to completely eject the solution. Remove the pipette from pipette guide and discard the tip. |
| **9** | When the ChloroChek recognizes the added standard solution, the display will read: PROCEEDING WITH CONDITIONING. When the conditioning is complete, the ChloroChek is ready to use. **Note:** In cases where conditioning is not successful, you must clean the electrodes and prepare a new Working Solution. |

No calibration of the system is necessary for performing a Sweat Chloride assay.3 Levels of the Sweat Chloride Controls and the 100 mmol/L NaCl/H2O Standard must be ran on each day of patient testing. When Quality Control tolerance limits are exceeded based on the QC criteria defined in LGM 2022 Qulaity Control (QC) Policy, corrective action must be taken and documented before analyzing patient samples.

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| **Controls** | **Preparation** | **Stability** |
| Sweat Control Solutions (Levels 1,2 & 3) | Ready to use. | Up to expiration date indicated on the packaging.The sweat control solutions must be stored at 15ᵒ-25ᵒC. |
| 100 mmol/L NaCl/H2O Standard | Ready to use. | Up to expiration date indicated on the packaging.Stability is ½ hour after ampule has been opened.The standard solution must be stored at 5ᵒ-45ᵒC. |

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| Procedure | Follow the steps below to run Sweat Chloride Quality Controls and the 100 mmol/L NaCl/H2O Standard |
| **Step** | **Action** |
| 1 | Place a correctly-sized pipette tip onto a 10 µL fixed-volume piston pipette.  |
| **2** | Place the pipette tip well into the Level 1 control ampule, holding the tip in the sample during the entire filling phase. Avoid drawing air into the pipette during the filling phase. Allow the piston to smoothly and slowly retract until it has returned to its initial position. Wait for about one second and then remove the pipette from the solution. If you see drops on the outside of the pipette tip, carefully remove them with a lint free tissue—do not draw out any solution from the pipette tip (through capillary forces of the paper tissue)—or repeat the process with a new pipette tip. |
| **3** | Using one of the pipette guides found on the sensor carriage, place the loaded pipette tip as near as possible in the center of the stirring Working Solution. Avoid expressing solution near the electrodes or the sides of the beaker. Press the piston down to the second pressure point to completely eject the solution, and then remove the pipette tip with the piston still pressed. |
| **4** | The ChloroChek recognizes the added sample; the display will start counting up from 0 until it reaches the final value. The final value remains on the screen until another sample is pipetted into the Working Solution, or until you press STANDBY on the screen.  |
| **5** | Repeats Steps 1 through 4 all other control levels of Sweat Controls and the 100 mmol/L NaCl/H2O Standard. |
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|  |  | **If…** | **Then…** |  |
|  |  | Values fall outside of the defined ranges | Clean the measurement electrodes or check the accuracy of the pipette, then repeat quality control testing. |
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|  |  Note: Rerun standards if a significant delay occurs between testing within one day. |
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 Follow the steps below to run Sweat Chloride on patient samples.

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| **Step** | **Action** |
| 1 | Place a correctly-sized pipette tip onto a 10 µL fixed-volume piston pipette.  |
| **2** | Place the pipette tip well into the sample, holding the tip in the sample during the entire filling phase. Avoid drawing air into the pipette during the filling phase. Allow the piston to smoothly and slowly retract until it has returned to its initial position. Wait for about one second and then remove the pipette from the solution. If you see drops on the outside of the pipette tip, carefully remove them with a lint free tissue—do not draw out any solution from the pipette tip (through capillary forces of the paper tissue)—or repeat the process with a new pipette tip. |
| **3** | Using one of the pipette guides found on the sensor carriage, place the loaded pipette tip as near as possible in the center of the stirring Working Solution. Avoid expressing solution near the electrodes or the sides of the beaker. Press the piston down to the second pressure point to completely eject the solution, and then remove the pipette tip with the piston still pressed. |
| **4** | The ChloroChek recognizes the added sample; the display will start counting up from 0 until it reaches the final value. The final value remains on the screen until another sample is pipetted into the Working Solution, or until you press STANDBY on the screen.  |
| **5** | After a final value is reached, you can pipette another sample into The Working Solution following Steps 1-3 above. The ChloroChek automatically recognizes each sample when injected and the value for the last sample injection is displayed on the screen. **Note:** If the ChloroChek fails to automatically recognize the sample in Step 4, the chloride concentration in the sample is less than 10 mmol/L. Verify this by adding 10 µL of the 100 mmol/L NaCl/H2O Standard Solution. The reading will likely be above 100 mmol/L. Then subtract 100 mmol/L from this final reading for the approximate final result of the sample. Keep in mind that this result is below the specified linearity range of the ChloroChek and should be recorded only as <10 mmol/L. |
| **6** | The ChloroChek will automatically go into STANDBY mode if no sample has been measured for at least 5 minutes. Press CONTINUE to restart stirring and continue with more measurements.**Note:** In the STANDBY Menu, if CANCEL is pressed this will cancel the measurement sequence and the Working Solution should be discarded.  |
| **7** | Refer to the ChloroChek Chloridometer Maintenance Procedure for performing Daily Maintenance after testing is concluded for the day. |

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| Clinical Significance | The quantitative measurement of the chloride in sweat (commonly called the “sweat test”) is used to confirm the laboratory diagnosis of cystic fibrosis (CF). With an approximate incidence of 1:3200 in Western Europe and the USA, CF is the most common life-threatening genetic disease within the Caucasian population. It is an autosomal recessive disorder characterized by viscous secretions that affect the exocrine glands, primarily in the lungs and pancreas. Patients with CF have an increased concentration of sodium, chloride, and potassium in their sweat. The criteria for the diagnosis of CF include the presence of one or more characteristic phenotypic features, a history of CF in a sibling, or a positive newborn screening result; and an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions, or identification of two CF-causing mutations or demonstration of abnormal nasal epithelial ion transport.  |

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| Reference Range |

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| **Age** | **Normal Range** | **Intermediate Range** | **Indicative of CF Range** |
| Infants (0-6 months) | Cl- ≤ 29 mmol/L | Cl- 30-59 mmol/L | Cl- ≥ 60 mmol/L |
| 6 months-18 years | Cl- ≤ 29 mmol/L | Cl- 30-59 mmol/L | Cl- ≥ 60 mmol/L |
| Adults (>18 years)\* | Cl- ≤ 29 mmol/L | Cl- 30-59 mmol/L | Cl- ≥ 60 mmol/L |

Sweat Chloride values less than 30 mmol/L have been documented in  genetically proven CF patients. Clinical correlation is necessary.  |  |
|  |  **Clinical Reportable Range:** 10-160mmol/L **Note:** Even though the analytical instrument may have a higher upper limit of its  AMR, sweat chloride concentrations > 160 mmol/L are not physiologically  possible. Results of sweat chloride testing greater than 160 mmol/L must not be  reported, and the patient must be retested. |  |

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| Limitations | Any salts containing chloride or other halides (halogens) such as fluoride, bromide, or iodide will interfere and cause an elevated reading. CLSI acknowledges this in the C34-A3 guideline. “In addition to chloride, other halides such as bromide and iodide are also detected using a chloridometer. Therefore, if a sweat sample contains other halides in addition to chloride, they will be detected and can falsely elevate the sweat chloride result.” 2. Halides including chloride may be present in lotions or creams, so it is important that the patient’s skin is properly cleaned prior to collecting the sweat. Improperly cleaned skin prior to sweat collection can lead to higher than normal results, thus leading to false intermediate or false positive results. |

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| Controlled Documents | The following controlled documents support this procedure. |
| Document No. | Name of Documents |
| LAMC-PPP-0748 | ChloroChek™Chloridometer® Maintenance |
| LAMC-PPP-0259 | Specimen Collection, Handling, Packaging and Transportation |
| LAMC-PPP-0026 | Quality Control (QC) Policy |
| LAMC-PPP-0123 | Safety Practices |
| LAMC-PPP-0127 | Infection Control |
| LAMC-PPP-0128 | Universal Body Substance Precautions |
| LAMC-PPP-0129 | Handling of Regular and Infectious Waste |
| LAMC-PPP-0130 | Cleaning Work Areas |
| LAMC-PPP-0132 | Hand washing Policy |
| LAMC-PPP-0134 | Storage and disposal of Chemical Hazardous Waste |

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| Non-Controlled Documents | The following non-controlled documents support this procedure. |

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| Document No. | Name of Documents |
|  | ChloroChek™Chloridometer® Sweat Chloride Analyzer Model 3400 User’s Manual 57-2006-01C |
|  | CLSI. Sweat Testing: Specimen Collection and Quantitative Chloride Analysis. 4th ed. CLSI guideline C34. Wayne, PA: Clinical and Laboratory Standards Institute; 2019 |
|  | Pulmonary Lab Procedure for using Macroduct® Collector |

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| Author(s) | Rosalie I. Fajardo, MS, CLS (ASCP) |