STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Reagent Grade Water

DOCUMENT NOTES:

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STCL-SOP-053
REAGENT GRADE WATER

1 PURPOSE

1.1 Various procedures done within each clinical laboratory require reagent water of differing degrees of purity. The NCCLS defines three specific levels of water purity based on resistivity, bacterial content, pH, silica content, particulate matter and organic content. Type I reagent grade water is the purest, and should be used for the preparation of reagents and standards. Its use in test methods is generally limited to tests requiring minimal interference from contaminants. Type II reagent grade water is used for laboratory tests not requiring Type I water. Type II reagent grade water is the type used in the Stem Cell Laboratory (STCL). Type III reagent grade water is the least pure. It can be used as source water to produce Type I or II water. The specifications for each type of reagent grade water are given in the table, ***"Specifications/Monitoring."***

1.2 To meet NCCLS specifications for the required grade of water, STCL contracts with Pure Flow Water Systems Inc. to produce the appropriate grade water. The system uses the process of deionization. Deionization involves passing water over insoluble resins which exchange ions from the resin surface for charged impurities within the water. In STCL, the system uses both anion and cation resins together as "mixed bed resins" capable of producing water with a resistivity of >2.0 megohms/cm. After the water has been de-ionized, it is filtered through a submicron filter to remove only impurities or microorganisms that are larger than the filter pore size, which is usually 0.2 um.

2 INTRODUCTION

2.1 To meet NCCLS and CAP specifications, water must be tested at frequent and specific time intervals. The two most frequent tests, resistivity and microbiological content, are performed within the laboratory. Procedures for these are given below.

(**NOTE**: Starting in December 2014, Pure Flow, Inc. will begin testing the microbial content of the water bi-annually (instead of quarterly) as part of preventative maintenance (PM) process. As a result of this change, the steps outlined in *Section 8.3* of this procedure will no longer be performed by STCL staff on a quarterly basis.)

2.2 Testing for silicate content, particulate matter and organic matter is done by outside consultants. NCCLS and CAP do not specify the frequency of such testing. In our laboratory, these tests will be performed when a new system is installed or after modification of the local water source.

3 SCOPE AND RESPONSIBILITIES

3.1 This procedure is used to insure that the reagent grade water used within STCL meets the purity requirements set by NCCLS.

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3.2 The Medical Director, Lab Manager, and designated lab staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	NCCLS	National Committee for Clinical Laboratory Standards
4.2	STCL	Stem Cell Laboratory
4.3	CAP	College of American Pathologists
4.4	CFU	colony forming units

5 MATERIALS

5.1 REAGENTS, SPECIAL SUPPLIES

5.1.1 A resistivity light is built into the existing water supply system.

5.1.2 Millipore HPC Water Tester (Cat. # MHPC 100 25, supplied by the Clinical Microbiology Laboratory.

5.1.3 A Megohm/cm meter is affixed to the system above the sink.

5.1.4 Alcohol prep pads or equivalent

6 EQUIPMENT

6.1 Deionized Water System

7 SAFETY

7.1 N/A

8 PROCEDURE

8.1 DAILY Maintenance

8.1.1 Check the resistivity lights on the system daily. Record whether or not it is illuminated on the water quality maintenance log.

8.1.2 If the red light is illuminated instead of the green, contact Pureflow for service. A mixed bed deionizer tank replacement may be scheduled. Record information on the troubleshooting log located on the reverse side of the *DI Water Checks* form.

Pure Flow, Inc. (1-800-242-9430)

NOTE: According to Pure Flow, the resistivity light, installed in each system, when lit, will guarantee resistivity of >2.0 megohm/cm (which is the resistivity standard for Type II water according to the College of American Pathologists (CAP). Pure Flow will measure the resistivity of the water at each regular service visit (performed quarterly); they will take readings before (pre-) and after (post-) the tanks are changed. These resistivity checks will be documented on the service reports. Service Reports are filed with equipment QC and maintenance records in the Stem Cell Laboratory.

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8.2 WEEKLY Maintenance

8.2.1 Check the reading on the megohm/cm meter and record the result on the water quality maintenance log. It should read 10 or above. If the meter reads less than 10, contact Pureflow. A mixed bed deionizer tank replacement may be scheduled. Record information on the troubleshooting log located on the reverse side of the *DI Water Checks* form.

8.2.2 Prime spigot # 1 (deionized & filtered water) and spigot # 2 (deionized water only) for a minimum of 30 seconds so the water can flow through the system.

8.2.3 Clean spigot # 1 (both deionized & filtered water) and spigot # 2 (deionized water only) using an alcohol prep pad or equivalent.

8.3 QUARTERLY Maintenance (*Effective 12/2014, Pure Flow, Inc (not STCL) will start performing this maintenance procedure twice per year instead of quarterly*)

8.3.1 Quarterly, or whenever a component is changed, culture the water to determine the microbial content.

8.3.1.1 Label the water tester with a permanent marker to include the date, the testing technologist's initials, and the location of the water supply being tested.

8.3.1.2 Clean each of the spigots using an alcohol prep pad or equivalent.

8.3.1.3 Collect the water sample. Turn the water on to approximately 1/4 - 1/2 maximum flow and allow the water to run for 30 seconds.

8.3.1.4 Carefully fill the sampler case to the 18 ml mark with the water being tested.

8.3.1.5 Insert the tester paddle into the water filled case, pushing firmly into place to form a leak proof seal.

8.3.1.6 Carefully place the tester on a level, flat surface with the membrane side facing down. Allow the tester to remain undisturbed for 30 seconds. Make certain that the entire membrane becomes saturated with water.

8.3.1.7 Hold the tester upright and carefully remove the paddle from the case. With a firm snap of the wrist, shake off any excess water.

8.3.1.8 Empty the case and carefully reinsert the paddle. Make certain that the paddle is properly seated in the case, with an airtight seal.

8.3.1.9 Place the inoculated sampler grid side down at 37°C (no CO2) in a location where it is unlikely to be disturbed. Place a *Do Not Disturb* sign over the tester(s).

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(**NOTE**: Since there is not a 37°C incubator (with no CO2) located in the STCL, the paddles will have to be incubated in the Microbiology Lab in the Carl Building).

8.3.1.10 Leave tester undisturbed for 72 hours.

8.3.1.11 Inspect the testers. If the paddle is light grey, the tester has dried out and the test is invalid. Discard the tester and repeat the test.

8.3.1.12 If the paddle does not show any evidence of drying, estimate the bacterial colonies present using the comparison charts below.

8.3.2 Record the number of colonies as colony-forming units per milliter (CFU/mL) on the *DI Water Checks* form. An acceptable count is less than 10 CFU/ml.

NOTE: Although the specification for Type II water is less than 1,000 CFU/ml, the STCL monitors microbial content at less than 10 CFU/ml.

8.3.3 If the colony count is greater than 10, clean and prime the spigot and repeat the cultures. Document all actions on *DI Water Checks* form.

COLONY COUNT COMPARISON CHART
SMALL COLONIES

To obtain approximate count, align Sampler with photo showing same density of colonies.

10

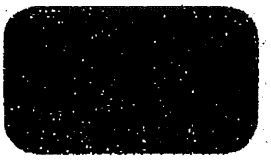
30

50

SMALL COLONIES

100

300



TNTC*
*Too numerous to count

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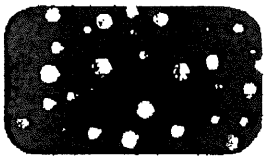
COLONY COUNT COMPARISON CHART

LARGE COLONIES

To obtain approximate count, align Sampler with photo showing same density of colonies.

10



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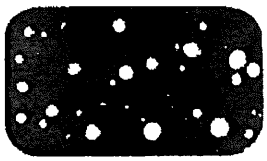

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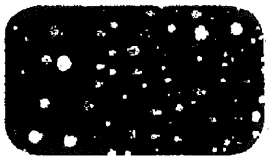
LARGE COLONIES



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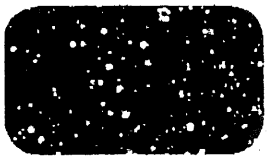

300

TNTC*
*too numerous to count

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Specifications and Monitoring

Parameter	Water Type	Spec	Production Device	Monitoring Device	Monitoring Schedule
Ion Content (Resistivity)	I	>10.0	1. Carbon Tank 2. Mixed Bed Deionizers	500K ohm quality light resistometer	Daily
"	II	>2.0	"	500K ohm quality light	Daily
"	III	>0.1	"	NA	NA
Particulate Matter (um)	I	<0.2	1.0 um Pre-filter	NA	NA
"	II	NA	1.0 um Pre-filter	NA	NA
"	III	NA	NA	NA	NA
Microbial Content (CFU/ml)	I	<10	NA	Millipore HPC Water Tester	Quarterly
"	II	<1000	NA	Millipore HPC Water Tester	Quarterly
"	III	NA	NA	NA	NA
Maximum silicate (pg/L SiO_2)	I	0.05	Mixed Bed Deionizers	Analyzed by PAR Laboratories, Inc. via Pureflow	Initially verified as acceptable
"	II	0.1	Mixed Bed Deionizers		
"	III	1.0	Mixed Bed Deionizers		

9 RELATED DOCUMENTS / FORMS

9.1 *DI Water Checks* form

10 REFERENCES

10.1 National Committee for Clinical Laboratory Standards: *Preparation and testing of reagent water in the clinical laboratory.* 2nd ed. Proposed Guideline C3-P2. Villanova, PA: NCCLS, 1985.

10.2 Hamlin WB. Reagent Water Specifications. Commission on Laboratory Inspection and Accreditation. Skokie, IL: College of American Pathologists, 1978.

10.3 Linke EG, Henry JB. Clinical pathology/laboratory medicine purposes and practice. In: Henry JB, editor. Clinical diagnosis and management by laboratory methods. Philadelphia: Saunders, 1984.

10.4 Bernes EW Jr, Young DS. General laboratory techniques and procedures. In: Tietz NW, editor. *Fundamentals of clinical chemistry.* Philadelphia: Saunders, 1987.

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11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
03	B. Waters-Pick	Section 2.1 – Introduction added "**NOTE**: Starting in December 2014, Pure Flow, Inc will begin testing the microbial content of the water in the STCL bi-annually (instead of quarterly) as part of the preventative maintenance (PM) process so this testing will no longer be performed by STCL staff". Section 3.1 - Added "This procedure is used to insure that the reagent grade water used within STCL meets the purity requirements set by NCCLS." Section 8.3 – Added "(*Effective 12/2014, Pure Flow, Inc (not STCL) will start performing this maintenance procedure twice per year instead of quarterly*)" Section 11 - Revision History added

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Signature Manifest

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STCL-SOP-053 Reagent Grade Water

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Manager

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Barbara Waters-Pick (WATE02)		23 Oct 2014, 12:08:56 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
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Quality

Name/Signature	Title	Date	Meaning/Reason
John Carpenter (JPC27)		30 Oct 2014, 01:22:40 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		17 Nov 2014, 05:54:28 PM	Approved