SWB-MP1-rev1
Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water

Revised version May 27, 2022

Questions concerning this document should be addressed to:

Charles S. Wong, Ph.D.
Southern California Coastal Water Research Project Authority
3535 Harbor Blvd. Suite 110
Costa Mesa CA 92626
714-355-3239
charlesw@sccwrp.org

and

Scott Coffin, Ph.D.
State Water Resources Control Board
1001 I St.
Sacramento, CA 95814
scott.coffin@waterboards.ca.gov

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1.0 Scope and Application

1.1 This method is for the determination of microplastics (State Water Resources Control Board, 2020) greater than 50 µm in size in treated drinking water using visual microscopy for particle counts, and Infrared (IR) spectroscopy for chemical identification of counted particles. IR spectroscopy can include, but is not limited to, Fourier Transform IR (FTIR), Laser Direct Infrared (LDIR) Imaging, Optical-Photothermal IR (O-PTIR), and other techniques capable of measuring microplastic particles as small as 50 µm. This method is for use in treated drinking water, as per the California EPA's data gathering and monitoring programs and Section 116376 of the California Health and Safety Code. The method is based on peer-reviewed literature and the results and recommendations from an international microplastic method evaluation study carried out by the Southern California Coastal Water Research Project Authority (SCCWRP).

1.2 Sample collection protocols are not within the scope of this method. Example procedures for sampling drinking water are available (ASTM, 2020), e.g., volumes that can range up to 1,500 L collected by inline sieving in which water is passed through sieves directly from the site sampled. This protocol presumes that samples are in a form amenable to sieving and filtration in the laboratory (i.e., relatively low volumes of up to 20 L, including those in samples collected from inline sieving into collection containers that can then be processed by this method).

1.3 The lowest particle size reliably detected by this method is 50 µm, with a maximum size of 5,000 µm (i.e., the State definition of microplastics, State Water Resources Control Board, 2020) based on the sieves specified in the method. While the extraction procedures in this method have been applied to particles <50 µm (De Frond et al., 2022), this method has not been validated for this size fraction.

1.4 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The MRL for this method is the minimum detectable amount (MDA), which is the minimum number of microplastic particles that must present in a sample to give a specified power, 1-β, of 0.95, for Type II errors (false negatives, MARLAP, 2004). MDA values for microplastics using this method, based on analysis of 0.450 L interlaboratory comparison single-concentration blank samples (De Frond et al., 2022) ranges from 47 to 88 particles for the sizes of particles covered by this method, and is dependent on the size fraction. More MDA data by various size fractions and blank levels is listed in Table 1. These values are independent of the extracted water volume. Each laboratory must establish its own MDA values, which may differ from the values noted here from the SCCWRP interlaboratory evaluation study (De Frond et al., 2022). A batch detection limit for individual batches of samples can also be computed with a single laboratory blank sample for use in quality assurance/quality control.

1.5 Microplastics are present in indoor air, and it is impossible to eliminate background contamination from airborne particles, plastic or otherwise, within the

laboratory. This method includes suggestions for improvements in facilities and analytical techniques to maximize the ability of the laboratory to report reliable microplastic particle counts and minimize particle contamination throughout sample processing and analysis (Section 4.0).

1.6 This method shall be used only by analysts who are experienced in the use of microscopic and spectroscopic techniques and who are thoroughly trained in the sample handling and instrumental techniques described in this method. Each analyst who uses this method must demonstrate the ability, using this procedure as detailed in Section 11.0, to generate acceptable results as noted in the quality assurance in Section 9.0.

2.0 Summary of Method

This method extracts microplastic particles from drinking water samples, and other water samples with low levels of suspended particulate matter and organic material, using sieving and vacuum filtration. Each sample is split into size fractions with separation at 500 µm, 212 µm and 20 µm (to maintain consistency between Raman and IR methods), and particles are collected onto filters or into glass containers prior to microscopic and spectroscopic analysis. Processed samples are viewed using stereomicroscopy and particles are counted. For the identification of material type, a representative set of subsamples of particles is selected and prepared for IR spectroscopy by presentation either on a filter surface or on a glass slide. Each subsampled particle is measured and photographed to make a permanent record of the sample, then chemically identified individually using IR spectroscopy. The instrument is calibrated and run through performance checks prior to use, and spectra are matched using relevant spectral reference libraries. The proportion of particles confirmed to be microplastics via IR spectroscopy is applied to total counts from microscopy to provide an estimate of microplastic particles per liter. This method can reliably detect microplastic particles down to 50 µm in size.

3.0 Definitions

Air blank – A sample of airborne particles collected within the laboratory to determine airborne laboratory background levels, with units of flux i.e., counts/(collection area × collection time). The air blank is taken by passively collecting airborne particles with an appropriate apparatus such as a petri dish, a filter or a slide. Collected particles are counted using microscopy. One or more air blanks must be present during sample processing and analysis to assess background levels during these procedures. The duration for collecting an air blank can vary depending on laboratory conditions, e.g., daily or weekly collection.

Analysis batch – A set of samples, excluding QC samples, extracted together by the same person during a workday (e.g., 8 hours) using the same lot of solvents, reagents, and consumables. An analysis batch can consist of 20 field samples at maximum. Samples collected from the same sampling event, with the same field blank, must be put in a same analysis batch.

Batch spectroscopy – Spectroscopy done on an area defined by the analyst, in which spectra of all particles in the area are taken, and particles in the area are counted by instrumental software.

Field blank (FB) – An aliquot of MAG water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FB is to determine if method analytes or other interferences are introduced into the samples during shipment and collection. At least one FB must be sent out for each sampling event and analyzed with the samples from the analysis batch. The volume of the FB must be similar to that of actual samples collected and processed by this method. FBs differ from trip blanks in that the FB evaluates contamination during both shipment and collection, while the trip blank only accounts for contamination during shipment.

HEPA filter – High-efficiency particle absorbing filter, capable of removing 99.97% of atmospheric particles of 0.3 μm diameter or greater.

Laboratory Reagent Fortified Blank (LRFB) – Sample of MAG water of a similar volume as test samples, to which known quantities and types of microplastic particles have been added. The LRFB is analyzed in the same manner as a sample, including the preservation procedures in Section 8. The LRFB is used during the Initial Demonstration of Capability to verify method performance for precision and accuracy.

Laboratory Reagent Blank (LRB) – Sample of MAG water of a similar volume as test samples that is run through the same laboratory procedures as the test samples. The LRB is used to monitor particles that may be introduced via procedural contamination and to calculate MDA.

Match score – The quality of a particle's spectrum compared to library reference spectra for materials it is suspected to be, as determined by the library search functions and software of a spectroscopic instrument. The higher a match score, the closer the measured spectrum is to the material's spectrum, with 100% being an absolute match.

Minimum detectable amount (MDA) – The MDA is the minimum number of microplastic particles that must present in a sample to give a specified power, 1-β, where β is the probability of a Type II error (false negative). The MDA is calculated with $\beta = \alpha = 0.05$, where α is the significance level (i.e., the probability of a Type I error or false positive) (MARLAP, 2004). The use of these standard values for α and β allows for meaningful comparison of analytical procedures. The MDA may be calculated with replicated LRB measurements (Section 9.2.1.2), and a batch detection limit can be computer with a single LRB measurement (Section 9.3.1.1)

Microplastics – Solid[1] polymeric materials[2] to which chemical additives or other substances may have been added, which are particles which have at least three dimensions that are greater than 1 nm and less than 5,000 µm. Polymers that are derived in nature that have not been chemically modified (other than by hydrolysis) are excluded (State Water Resources Control Board, 2020).

Microplastics-analysis-grade (MAG) water – Water that has been filtered through a filter with pore-size of 1µm or smaller, of any appropriate material that does not shed particles, and used as reagent water and to rinse apparatus in this procedure. Appropriate sources of water to generate MAG water are deionized water, reverse osmosis water, or 18 MΩ cm water (e.g., nanopure/MilliQ water).

Minimum Reporting Level (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value in a sample following analysis. The MRL for this method is the MDA, except as noted (Section 9.2.4).

Single particle spectroscopy – Spectroscopy done manually by the analyst on a single particle at a time.

Trip Blank – A sample of MAG water of a similar volume as test samples, taken from the laboratory to the sampling site and returned without having been exposed to sampling procedures and the environment outside of the lab. The trip blank is to assess contamination introduced during shipping and storage only and must be present for each set of field samples from a sample collection period.

[1] 'Solid' means a substance or mixture which does not meet the definitions of liquid or gas. 'Liquid' means a substance or mixture which (i) at 50 degrees Celsius (˚C) has a vapor pressure less than or equal to 300 kPa; (ii) is not completely gaseous at 20 ˚C and at a standard pressure of 101.3 kPa; and (iii) which has a melting point or initial melting point of 20 ˚C or less at a standard pressure of 101.3 kPa.
'Gas' means a substance which (i) at 50 ˚C has a vapor pressure greater than 300 kPa (absolute); or (ii) is completely gaseous at 20 ˚C at a standard pressure of 101.3 kPa.

[2] 'Polymeric material' means either (i) a particle of any composition with a continuous polymer surface coating of any thickness, or (ii) a particle of any composition with a polymer content of greater than or equal to 1% by mass. 'Particle' means a minute piece of matter with defined physical boundaries; a defined physical boundary is an interface. 'Polymer' means a substance consisting of molecules characterized by the sequence of one or more types of monomer units. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. A polymer comprises the following: (a) a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or other reactant; (b) less than a simple weight majority of molecules of the same molecular weight. 'Monomer unit' means the reacted form of a monomer substance in a polymer. 'Monomer' means a substance which is capable of forming covalent bonds with a sequence of additional like or unlike molecules under the conditions of the relevant polymer-forming reaction used for the particular process.

4.0 Interferences

4.1 Physical interferences

4.1.1 Preventing water samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in quantifying microplastics within drinking water samples. It is not possible to confidently eliminate all contamination from samples during laboratory processing. It is imperative that extreme care be taken to minimize contamination when collecting and analyzing water samples for microplastics. Controlling particle contamination during sample processing requires strict adherence to protocols for contamination control as outlined below in Section 4.2.

4.1.2 Major sources of particle contamination within the laboratory include, but are not limited to: fibers from clothing and textiles (including lab coats, apparel worn by lab personnel, carpets, and furniture), particles deposited from the air within the laboratory environment, particles settled on equipment prior to or during use, non-MAG water, water used to clean equipment prior to use, sponges or brushes used to clean equipment prior to use, synthetic polymer gloves, and plastic sample container lids .

4.2 Contamination Control

4.2.1 Laboratories must use as much plastic-free equipment as possible, except where allowed as noted in Sections 4.2.1.3 to 4.2.1.7.

4.2.1.1 Laboratory personnel must use equipment throughout the process composed of glass (e.g., beakers, petri dishes) or metal (e.g., foil, forceps), except as noted in Sections 4.2.1.3 to 4.2.1.7.

4.2.1.2 All materials used for cleaning of equipment prior to use must be made of natural/non-plastic materials (e.g., natural-based material sponge).

4.2.1.3 If plastic materials are used, inspect their integrity. LRBs exist to help account for any procedural contamination from plastics used during processing. Examples of plastics commonly used in microplastics analysis that are acceptable as they do not shed polymer particles are listed in Sections 4.2.1.4 and 4.2.1.5.

4.2.1.4 Use of hard plastic tubing (e.g., Tygon® or clear PVC tubing) to dispense water used to make MAG water is acceptable.

4.2.1.5 Typical laboratory-grade solvent squeeze bottle (e.g., Teflon or polyethylene) are also suitable to dispense MAG water for the rinsing of sieves, filters, and equipment as long as they are used similarly for QA/QC samples. Minimal contamination has been attributed to these sources.

4.2.1.6 Purple nitrile gloves (e.g., Kimtech®) have minimal contamination potential and their purple color makes it easy to distinguish if any contamination can be attributed to them.

4.2.1.7 All plastic apparatus shall be evaluated on a monthly basis for potential to shed microplastics by the procedures noted in Section 9.

4.2.2 Keep a clean environment.

4.2.2.1 Wipe surfaces down before and after use with MAG water and a towel made from low-shedding natural fibers that do not meet the definition of 'microplastics' (e.g., cotton or paper towels).

4.2.2.2 Clean laboratory floors daily when microplastics analysis is being done, and maintain a record of cleaning frequency. Cleaning can be done by mopping with clean water (does not need to be MAG water) and mops made of natural-fiber materials. Ideally, a HEPA filter vacuum cleaner can also be used to clean floors at the end of each working day to minimize interference from the possible resuspension of particles into the air.

4.2.2.3 Clean all labware thoroughly with low-foam soap and water, and triple-rinse with MAG water before use. Dry-ashing glassware (except volumetric flasks) and metal items at ≥ 450 °C for at least 1 hour is acceptable.

4.2.2.4 Installing a HEPA filtration system in your laboratory is recommended to minimize airborne particulates. Be sure to change the HEPA filter regularly based on manufacturer recommendations.

4.2.3 Minimize use of synthetic textiles in the laboratory

4.2.3.1 Do not wear synthetic clothing when processing samples. Wear cotton lab coats, ideally of a noticeable color not commonly found in environmental samples (e.g., pink) to allow clear identification within samples as contamination.

4.2.3.2 Remove furniture (e.g., chairs, stools, carpets) with padding or fabric. If removal is not possible, then synthetic surfaces may be covered with natural materials, or a material that does not shed plastic particles.

4.2.4 Clean all equipment thoroughly before use.

4.2.4.1 Before using any glassware or tools, and between processing individual samples, wash with low-foam soap and hot water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water and then three times with MAG water.

4.2.4.2 Heavy-duty aluminum foil can be used to cover cleaned apparatuses and tools such as forceps to protect from airborne particulate contamination. Foil may be pre-ashed at ≥ 450 °C for at least 1 hour before use to destroy all organic material, then stored in a covered non-plastic container. Ash heavy-duty foil only, as the lightweight foil will disintegrate at high temperatures. Discard foil after use.

4.2.4.3 Cover all equipment when not in use with glass or clean aluminum foil or store it upside down.

4.2.4.4 Pressurized air can be used to remove possible contamination on the surface of equipment prior to use. If compressed gas is used to blow-dry equipment or samples for microplastics, ensure that the air is clean (e.g., put a 1 µm metal filter between the source and the outlet).

4.2.5 Recommend working in a covered environment.

4.2.5.1 Process samples in a biosafety cabinet, laminar flow hood, a clean cabinet, or other fully enclosed space. A covered environment, even without active air convection, helps to reduce airborne particulate contamination. While chemical fume hoods can reduce airborne

particulate contamination by up to 50%, a laminar flow hood, clean cabinet, or enclosed space can reduce contamination by 95% (Brander et al., 2020). Caution and characterization of blank levels (Section 9) is needed with the use of only a chemical fume hood unless its air source is filtered, as it will continuously move air, and any suspended particulates present, up and across all surfaces in the hood.

5.0 Safety

5.1 No analytes or reagents of concern are used within this method. However, instrument-specific solvents or reagents may be necessary depending on the specific IR technique.

5.2 The following Personal Protective Equipment (PPE) are mandatory for method Sections 11.1 and 11.2 to minimize contamination from analysts:

a. Cotton lab coat
b. Nitrile gloves
c. Cryogenic gloves and safety face shield for filling IR instruments with liquid nitrogen, if applicable.

5.3 IR instrumentation suitable for this method may need small quantities of liquid nitrogen to cool the instrument's detectors. Appropriate PPE (safety goggles, cryogenic gloves, cotton lab coat), cryogenic flasks, transfer equipment, and ventilation are required when the instrument's liquid nitrogen reservoir is refilled. Cryogenic safety equipment is not required during instrument operations, so long as manufacturer instructions regarding the use of cryogenic fluids are followed.

6.0 Equipment and Supplies

References to specific brands or catalogue numbers are included as examples only and do not imply endorsement of the product. Such reference does not preclude the use of other vendors or suppliers.

6.1 Cleaning

Item	Suggested Materials
Low-foam dish soap	Alcojet, Fisher Catalog no. 16-000-110
Sponge made of natural materials	Loofah, cellulose, natural sponge. Amazon "Natural sea sponge, 6-7in"
Cotton cloths and paper towels	-
Mop with natural-fiber head or HEPA-filter vacuum cleaner (consumer-grade is ok)	-

Item	Suggested Materials
Surface mat for doors	ULINE no. H-15678BLU

6.2 Sieving

Item	Suggested Materials or equivalent
Heavy-duty aluminum foil	-
Laboratory labelling tape	Fisher Catalog no. 15901A
Squirt bottle (Teflon, polypropylene or LDPE)	VWR no. 16651-904
Metal sieve (8” diameter)	Hogentogler Catalog no. 5202 (500 µm mesh size) Hogentogler Catalog no. 5207 (212 µm mesh size) Hogentogler Catalog no. 5257 (20 µm mesh size)
Metal sieve pan	Same diameter as sieve
Glass beakers or jars	> 500 mL size, ULINE, glass canning jars (32 Ounce). Non-plastic lids (e.g., metal) are preferred (use aluminum foil to cover containers that do not have lids, such as beakers)

6.3 Vacuum filtration

Item	Suggested Materials or equivalent
1 µm pore-size filters	47 mm diameter, Sterlitech Polycarbonate Track Etch (PCTE) membrane filters, Catalog no. PCT1047100
20 µm pore-size filters	47 mm diameter, Sterlitech PCTE membranes filters, Catalog no. 1270175
Vacuum filtration system (without rubber or plastic parts exposed to sample water; Teflon O-rings are acceptable). The following describes typical systems for sample sizes of 1-2 L. Different sizes of funnels and flasks may be used for other sample sizes as appropriate. Systems typically consist of: 1 Vacuum pump 2 Lengths of plastic tubing 2 1000 mL Glass filtering flasks 1 filtering funnel 1 filter holder with stainless steel support screen 1 metal clamp 1 venting valve or T-adapter with shut-off valve that connects tubing between the filtering flask	Vacuum pump: GAST model DOA-P704-AA or an equivalent vacuum system Plastic tubing: Tygon® S3™ Laboratory Tubing, VWR Catalog no. 89403-878 Filtration Apparatus includes 300mL graduated funnel, aluminum clamp, support base, removable 47mm stainless steel support screen, and 1000 mL filtering flask: VWR Catalog no. 89426-770

Item	Suggested Materials or equivalent
and vacuum pump (or laboratory bench-vacuum valve).	
Glass Petri dish(es) (55 mm bottom diameter)	VWR Catalog no. 25354-025 *(For use with a 47 mm diameter filter)*

6.4 Visual Microscopy

Item	Suggested Materials or equivalent
Glass Petri dishes (95 mm bottom diameter)	VWR Catalog no. 25354-069
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon – "Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)" *Suggested item for systematic counting*
Glass microscope slides	VWR Catalog no. 48300-026
Metal teaspoon	Amazon – "4.5" Stainless Steel Teaspoon, Set of 6"
Stereoscope	With interchangeable black and white base, and/or variable color/intensity selection of light source. Eyepiece magnification typically 10×, and objective magnification typically 4.5×.

6.5 Images and Measurements

Description	Example
Microscope digital camera attachment	e.g., ToupTek® touptek.com/product/product.php?lang=en&class2=56
Computer software for digital imaging and measurements	e.g., - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

6.6 QC materials

Item	Suggested Materials or equivalent
Thermo Scientific™ ChromoSphere™-T Certified Size Standards (colored polymer microspheres)	https://www.fishersci.com/shop/products/chromosphere-t-certified-size-standards/p-4530441

Item	Suggested Materials or equivalent
Thermo Scientific™ 4000 Series Monosized Particles	https://www.fishersci.com/shop/products/4000-series-monosized-particles/p-4530417
Polymer Kit 1.0 (Center for Marine Debris Research, Hawaii Pacific University)	Polymer Kit 1.0 contains 22 plastic materials typically found in the environment, and reference data e.g., spectra. https://commerce.cashnet.com/cashnetg/selfserve/EditItem.aspx?PC=CM-POLYKIT++&ItemCount=0
Cospheric blue polyethylene microspheres, 600-710 μm.	https://www.cospheric.com, catalog# BLPMS-1.00 600-700um.
Cospheric green polyethylene microspheres, 300-355 μm.	https://www.cospheric.com, catalog# GPMS-0.98 300-355um.
Cospheric red polyethylene spheres, 75-90 μm	https://www.cospheric.com, catalog# REDPMS-0.98 75-90um.
Generated fibers	See Section 17.4.
Color key	See Table 17.1.
Morphology key	See Sections 17.2 and 17.3.

6.7 Infrared spectroscopy instrument system

6.7.1 The spectroscopy instrument must be able to collect spectra for particles ≥ 50 μm.

6.7.2 The instrument must be able to image particles to the size limit of the method, and image an area on the slide or filter for determination of particle count, size and morphology.

6.7.3 The instrument must be able to compare collected spectra with reference library spectra to provide a match score to ascertain chemical identity.

6.7.4 The instrument must be able to hold a sample slide and/or filter safely on a horizontal platform to avoid moving, losing and damaging particles.

IR system	Example (vendor and model)
Fourier transform infrared (FTIR)	Thermo Fisher Nicolet iN10 Infrared Microscope
Laser Direct Infrared (LDIR)	Agilent 8700 LDIR
Optical-photothermal infrared (O-PTIR)	Photothermal mIRage IR Microscope

7.0 Reagents and Standards

7.1 MAG water is required throughout the sieving and filtration process to rinse sieves and filter apparatus, and to ensure that all particles from the sample have been collected. The MAG water is be collected and stored in a clean vessel (see Section 4.2.4.1) and covered (Section 4.2.4.2) until use.

7.2 The LRFB may be created by the laboratory or commercially purchased. The LRFB must have a minimum of 10 particles per size fraction, at least two size fractions, and at least two different types of synthetic polymers. If created by the laboratory, it is recommended to include both fibers and particles, however, fibers do not have recovery requirements. In brief, particles are manually added, using fine-tipped tweezers, to an appropriate volume of MAG water under a stereoscope, to generate the LRFB. Deploy an air blank during the creation of the LRFB, and quantify particulates in the air blank under the microscope as per Section 11.2 to assess contamination during LRFB creation.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection should be done following procedures noted in ASTM Method 8332-20 (ASTM, 2020), following protocols for low-suspended-matter waters. Samples collected from inline sieving following ASTM Method 8332-20 consist of particulates suspended in water and go into glass sample containers as noted below, prior to processing steps in the laboratory (Section 11). This method can also be used for grab samples (< 20 L), which are processed in an identical manner. For samples that already exist on filters or slides, sieving and filtering is not necessary, and counting and identification of particle materials may proceed from Sections 11.2-11.4.

8.2 During storage, water samples must be kept liquid (i.e., not frozen) at low temperature (e.g., 4 ± 2 °C), to prevent bacterial growth. Samples must also be kept away from direct sunlight or bright light. Samples that must be stored near direct sunlight or bright light should be stored in opaque containers or containers covered by aluminum foil.

8.3. Glass containers with non-plastic lid liners (PTFE is acceptable), pre-cleaned as with other apparatuses (Section 4.2.4) in this method, and of a size appropriate to the volume needed (Section 6.2), must be used to collect and store samples to minimize microplastic contamination from the container when feasible. Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts if possible; if not, then ensure that containers are sealed prior to shipment. Shipping samples on ice (< 6 °C) is preferred, but samples may be shipped at room temperature.

8.4 Water samples should be processed as soon as possible after collection and extraction to minimize the opportunity for algal growth. A maximum 28-day holding time from sample collection to analysis for treated drinking water is allowed. Freezing of samples is not permitted.

8.5 Trip Blanks shall accompany sample bottles to the sampling site and back to the laboratory after sample collection. Do not open Trip Blanks in the field; Trip Blanks must remain sealed until analysis. Trip Blanks may be used to identify

potential sources of contamination occurring from shipping the sample container to the site and back, and do not need to be analyzed unless evidence of contamination during shipment arises from analysis of LRFBs.

8.6 Field Blanks must accompany each set of sample containers taken from the laboratory to the sampling site and back. At the beginning of the sampling event and for its duration, keep the FB container open at the site while collecting the sample. At least one FB must be transported and analyzed for each sampling event.

9.0 Quality Control

QC requirements include the Initial Demonstration of Capability (IDC) and ongoing QC requirements. This section describes each QC parameter, its required frequency and the performance criteria that must be met in order to satisfy the method's quality objectives. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs. Compliance with the requirements of the IDC must be demonstrated for each size fraction that the laboratory intends to report. Particles < 50 µm and fibers do not have QC requirements.

9.1 QC measures for this method include collection and analysis of LRBs, use of LRFBs, the use of color and morphology keys to standardize particle characterization (see Section 17), and the documentation in variability of analyst count, color, and morphology characterization.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – The IDC must be successfully demonstrated by the analyst or analytical group prior to allowing to analyzing the field samples. Prior to conducting the IDC, the analyst shall be familiar with the calibration requirements outlined in Section 10. The IDC must be completed for each size fraction. Prior to conducting the analysis, the laboratory must analyze at least 4 LRFBs, spiked with particles appropriate for each size fraction. Average recovery efficiency by visual microscopy of particles must be at least 50%, with a precision of 40% relative standard deviation (RSD).

9.2.1 DEMONSTRATION OF LOW SYSTEM BACKGROUND – Analyze at least 4 LRBs and calculate the MDA for each size fraction. Set the laboratory's MRL at the MDA. Recalculate the MDAs annually at a minimum, using routine LRBs analyzed within the last year.

9.2.1.1 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target value for the MRL (i.e., the MDA) for each size fraction based on the intended use of the data. An MDA must be established from LRB measurements. A minimum number of replicate LRB measurements (e.g., 4 replicates) are needed for determining the mean and standard deviation of LRB background particle counts. Using this data, the MDA is determined from low-background Poisson statistics, using the Poisson-normal approximation approach (MARLAP, 2004; Currie, 1968). This guidance is intended to minimize the

occurrence of reporting results that are false positives (Type I error, α = 0.05) and false negatives (Type II error, β = 0.05).

9.2.1.2 The laboratory MDA by size fraction can be calculated as follows:

$$MDA = Mean + 3 + 3.29 \times SD \times \sqrt{1 + \frac{1}{n}}$$

where *mean* and *SD* denote the average and standard deviation, respectively, of background particle counts for the LRBs, and *n* denotes the number of replicate LRB measurements.

9.2.2 DEMONSTRATION OF PRECISION – Prepare and analyze at least 4 replicate LRFBs. LRFBs must have at least 10 particles for each size fraction. The %RSD of the concentrations of the replicate analyses must be ≤ 40% for all size fractions greater than 50 µm.

$$\%RSD = \frac{Standard\ Deviation\ of\ Measured\ Concentrations}{Average\ Concentration}\ x\ 100$$

9.2.3 DEMONSTRATION OF ACCURACY – Calculate the average percent recovery for each size fraction using the same set of replicate data generated for Section 9.2.2. The average recovery of the replicate analyses must be at least 50% of the true value and must not exceed 150%.

$$\%Recovery = \frac{Average\ Measured\ Concentration}{Fortified\ Concentration}\ x\ 100$$

9.3 ONGOING QC REQUIREMENTS – This section describes the ongoing QC procedures that must be followed when processing and analyzing field samples.

9.3.1 LABORATORY REAGENT BLANK (LRB) – LRBs must be quantified for particle count, size, color, and morphology (see Section 17 for guide). The content of the blank samples must be recorded, and this data shall be associated with the samples processed in the same analysis batch.

9.3.1.1 One LRB is analyzed for every analysis batch. A batch detection limit can be computed with this LRB data.

$$batch\ detection\ limit = N_b + 3 + 4.65\sqrt{N_b}$$

where N_b is the background particle count of the LRB.

9.3.1.2 If the batch detection limit (Section 9.3.1.1) is greater than the MRL, the laboratory must take corrective action with regards to reducing contamination (Section 4) and analyze a new LRB until this criterium is met.

9.3.1.3 Blank correction is not permitted, given the many factors that can go into blanks (e.g., it is possible to have many blue fibers, for example, in the blank, but few such fibers in actual samples).

9.3.2. LABORATORY REAGENT FORTIFIED BLANK (LRFB) – LRFBs consist of MAG water spiked with microplastic particles of known and representative count, size range, color, morphology, and material composition, with known spectra. Appropriate particles for creating LRFBs are available (see Section 6.6). An LRFB using particles colored by size-fraction can also be created from other vendors, such as listed in Section 6.6. A minimum of 10 particles per size fraction must be included if created by the laboratory.

Analyze at least one LRFB per analyst performing sieving or filtration (Section 11.1) at a minimum of once per week or once every 20 field samples, whichever is more frequent. Recovery efficiency by visual microscopy of particles in the LRFB must be at least 50% and must not exceed 150%. Document recovered microplastic count, size range, color, and morphology. If the LRFB recovery exceeds these criteria, the source of the problem must be identified and corrected prior to continuing with analysis.

9.3.3 FIELD BLANK (FB) – A FB must be included with each set of samples collected at the same site and time, and analyzed to assess contamination during shipping and storage. Microplastics levels must be below the MRL; if not, the batch of samples associated with the FB must be flagged accordingly.

9.3.4 TRIP BLANK – Trip blanks do not need to be analyzed unless the FB shows evidence of contamination. In that event, the requisite trip blank must be analyzed to determine if the contamination was due to shipping.

9.3.5 COUNTING VARIABILITY – Analysts in a laboratory shall quantify the total number of microplastics of each color and morphology (as per the keys in Section 17, which include appropriate collapsing of some colors and morphologies) on a sample of known content via visual microscopy once per month or every 100 samples, whichever is more frequent. Client samples containing at least 20 particles for each size fraction are recommended, however, if no samples contain more than 20 particles, a LRFB may be utilized as an alternative. If particles are counted by a single analyst, replicate counts must be done and be within 5% of the known sample composition. If more than one analyst counts particles, a comparison among each analyst's count of particle enumeration must fall within 10% of each other. If not, identify the source of the error and perform additional counts utilizing at least three consecutive LRFBs, until quantification falls within 10% between analysts.

9.3.6 AIR BLANKS – During analysis of LRBs and client samples, leave one filter uncovered near the area where the analyst is working, covering the filter when work is not being conducted. Analyze the air blank sample can determine levels and sources of airborne contamination in the laboratory. Analyze the air blank sample by visual microscopy only, at the same frequency as the LRFB.

If particulate counts calculated by associating with the exposure area and time of the air blank are greater than the laboratory's MRL, the laboratory must take corrective action with regards to reducing contamination (Section 4), and flagging any impacted sample results. Maintain a log of air blanks and take corrective action with regard to reducing contamination (Section 4) if trends are identified.

9.3.7 CONTAMINATION CONTROL VERIFICATION – It must be verified that plastic equipment that comes into contact with samples does not shed particles. Rinse each piece of equipment three times with MAG water and test the rinse as a blank sample. If the rinse blank results in a particle count greater than the MRL, the equipment may not be used. Rinse blanks may also be utilized to identify potential sources of contamination in the laboratory. Conduct this verification monthly (Section 4.2.1.7).

10.0 Calibration and Standardization

Calibration procedures for spectroscopy instruments, which are vendor-specific, measure spectra of a built-in reference material for comparison to reference spectra; these shall be recorded in the laboratory's SOP. Calibration shall be performed daily according to the manufacturer's instructions to ensure an accurate collection of spectra. Any vendor changes to instrument calibration shall be documented to allow for traceability.

For particle photographs and measurements, the use of imaging software is recommended. For this purpose, the microscope should be connected to a camera and computer. Here, the scale bar must be calibrated before use. This can be done by placing a stage micrometer under the microscope, comparing ruler measurements to the scale bar on the screen and adjusting accordingly.

Analysts shall be familiar with the color and morphology keys in Section 17. Particles in samples, blanks, and LRFBs shall be characterized using these keys for reference (See Section 9.3.7).

11.0 Procedure

Large amounts of particles in a sample may block sieves and overload filters, and may thus overwhelm counting. A preliminary test may determine if it is necessary to analyze not the whole sample, but only part of a sample, which may be taken from a well-mixed original sample.

Polycarbonate filters should not be used if pyrolysis gas chromatography-mass spectrometry will be done on the same sample at some later point.

11.1 Filtering (See Figures 1-2 for flow diagram)

11.1.1 Rinse off the outside of the sample container with MAG water, to remove all particulates that may interfere, including those from packing materials. Discard this rinse. If the sample has not been size-fractionated, then proceed with step 11.1.2. If the sample has already been size-fractionated, skip step 11.1.2, and go to 11.1.3.

11.1.2 Place the cleaned and rinsed 500 µm sieve on top of the 212 µm sieve, which in turn is on top of the 20 µm sieve, which in turn is placed on top of the sieve pan (Figure 1).

11.1.2.1 Pour the sample into the sieve stack. After the sample is completely drained into the sieve pan, place the sieves on another clean sieve pan. Pour the contents of the first sieve pan through the sieve stack. Place the sieves back onto the first sieve pan and repeat this procedure at least three times. After the last pour, rinse the sieve pan with MAG water into the sieve stack.

11.1.2.2. Triple rinse the inside, rim, and lid of the sample container into the sieve using MAG water. Rinse the sieve stack gently with MAG water and tap the sieve gently to move everything through to its appropriate size fraction.

11.1.2.3 Rinse the contents of each sieve into a (cleaned and labelled) glass container using MAG water. This will collect a size fraction >500 µm, a size fraction 212-500 µm, a size fraction 20-212 µm, and a size fraction of <20 µm, if the lab will be analyzing this size fraction. It is recommended to rinse minimal amounts of MAG water into each size fraction/jar. To do this, tilt the sieve towards the analyst and rinse the contents of the sieve to pool all particles into one area. Then rinse the pooled particles into the jar.

11.1.2.4 If collection of a size fraction <20 µm is not desired, then the contents of the sieve pan may be discarded. It may be valuable to keep this size fraction for re-analysis if particulate loss is observed.

11.1.3 Particles are filtered onto appropriately sized filter.

11.1.3.1 Assemble the filter flask with the glass filter holder. Place a 20 µm filter (e.g., the PCTE filter) onto the glass filter holder and secure the filtering funnel on top using the metal clamp. If measurement of the <20 µm size fraction is desired, then the 20 µm filter for this step is replaced for this size fraction with a 1 µm filter.

11.1.3.2 Attach the vacuum tubing to the filtering apparatus and turn on the vacuum pump. Rinse the filtering funnel with MAG water to pre-clean the system. This step can be helpful for determining if there are any leaks in the system.

11.1.4 Pour the appropriate sample (e.g., sieve pan contents that have been transferred to a beaker) through the filtration system. Triple-rinse the beaker with MAG water into the filtration system once the final sample volume has

been emptied. Ensure that the filtering flask does not overfill, as this may lead to sample loss.

11.1.4.1 Keeping the vacuum pump on, triple rinse the sides of the filtering funnel with MAG water in a circular motion. Release the vacuum pressure by slowly opening the venting valve or the shut-off valve of the T-adapter. Do not open the venting valve too quickly, because this can lead to blow-back and particulate loss. Triple-rinse the filtering funnel again with MAG water in a circular motion. Close the venting valve and triple-rinse the filtering funnel a third time with MAG water in a circular motion.

11.1.4.2 Slowly open the venting valve to break the vacuum. Remove the metal clamp. Slowly and gradually detach the filtering funnel from the glass filter holder to prevent the filter from sticking to the funnel. Forceps may also be used to ensure the filter is not removed with the filtering funnel as you do this.

11.1.4.3 Slowly close the venting valve and carefully rinse the base of the filtering funnel onto the filter using MAG water. Aim to rinse small sections of the funnel base onto the center of the filter so that particle loss is minimized. NOTE: spherical particulates such as those that may be used for the LRFB may roll off if the filter gets too dry.

11.1.4.4 Slowly open the venting valve, and turn off the vacuum pump. Remove the filter from the filtration system with tweezers and place it into a clean, labeled petri dish and cover it. It is recommended that the petri dish be held level with the glass filter during transfer and slowly, horizontally slide the filter into the petri dish to minimize particulate loss.

11.1.4.5 Pour and triple rinse the contents of the filtering flask into a clean beaker and cover. Refilter these contents if there is any sign of a leak in the filtration system, or if particle recovery is low.

11.1.4.6 Repeat all steps in 11.1.3, 11.1.4, and 11.1.5 as appropriate for the remaining size fractions.

11.2 Visual microscopy

11.2.1 Prepare materials for visual identification of particles; attach the grid sticker to the outer base of the petri dish, if not present already.

11.2.2 Bring all size fractions of the sample and materials (Section 6.4) for visual identification over to the microscope.

11.2.3. Deploy an air blank for the duration of visual microscopy ensuring the air blank is covered when switching between samples, if analyst steps away from microscope, or if analyst has completed analyses for the day. Quantify particulates under the microscope immediately after completing analyses for the day, as per Section 11.2, to assess contamination during filter analyses.

11.2.4. Set up microscope with appropriate magnification, background and illumination settings. Adjust illumination until the grid lines of the grid sticker are clearly visible through the filter.

11.2.5. Particles may be directly counted and visually characterized from the filter surface. Start with the largest size fraction and identify, count, number and visually characterize (by color and morphology) all particles that are observed within the sample. Use the grid lines of the grid sticker as a reference to count and characterize particles systematically. Use the color and morphology keys in Section 17 for guidance on visual characterization and categorization of particles. Record all counted particles on the data sheet. A minimum of 2 different slide backgrounds (e.g., black/white), light colors or light filters is required to ensure that all particles are recorded.

11.2.5.1 Be sure to check visually for particles around the inner edges of the petri dish that might have moved from the filter surface during transition to the microscope.

11.2.5.2 To help avoid double counting particles, image the entire sample in sections at the appropriate magnification for each size fraction to use as reference if moving sample (e.g., to switch the stage plate color). Ensure images are read-only to avoid tampering.

11.2.6 If the analyst is performing single particle spectroscopy, then whilst visually identifying, counting and characterizing particles using microscopy, randomly select at least 3 sets of subsamples per size fraction, each consisting of ≥ 30 particles irrespective of color and morphology (De Frond et al. 2022) for single particle spectroscopy (Section 11.4.3.3). The subsampled particles can be marked and left on the filter surface for later imaging, measurement, and chemical identification (Sections 11.4, and 11.5). Alternatively, subsampled particles ≥ 212 µm may be placed on a proper substrate to facilitate further instrumental measurement and numbered to identify them. If there are fewer than 30 particles present, then this step is not necessary, as all particles shall be analyzed by spectroscopy (Section 11.4) This step is not necessary if the analyst is performing batch spectroscopy (Section 11.4.3.5).

11.2.7 Repeat 11.2.4 through 11.2.6 with remaining size fractions.

11.3 Images and Measurements

11.3.1 **(IF NEEDED)** If taking reference images (Section 11.2.5.2), use an appropriate magnification for each size fraction and methodically image the sample in subsections using integrated camera software or other imaging methods. Use the grid system to ensure all areas of the sample are captured. Particles >500 µm generally require 10-100× magnification. Those 212-500 µm generally require 200× magnification, and those <212 µm generally require 350-450× magnification.

11.3.2 For each particle, record a clear image and measure the length and width at the longest dimension, using computer software that is compatible with the

camera attached to the microscope. If an image contains >1 particle, appropriately document this the data sheet.

11.3.2.1 For fibers, do not measure frayed projections and use segmented/curved lines to measure length where necessary. If a particle has broken apart, use the analyst's best judgment e.g., measure three lengths and one width for a fragment that has fractured along its length. Make note of the method used for measurement in this case.

11.4 IR Spectroscopy

Visual microscopy alone cannot provide information on particle material types. Therefore, analysis of particles using IR spectroscopy is required to differentiate microplastic and non-microplastic particles, and to determine their chemical identities. Ideally, all particles are characterized for material composition; however, this is often not practical. It is recommended that analysts prioritize chemical identity of particles over as many particles as possible, including by means such as batch spectroscopy.

11.4.1 Applicability of the IR spectroscopy instrument is based on meeting site requirements for installation.

11.4.1.1 There shall be no floor vibrations from air conditioners, pumps, motors, etc. Install the instrument on a bench-top vibration-damping pad (e.g., from McMaster-Carr) or a vibration isolation workstation.

11.4.1.2 No exposure to excessive dust or airborne particulate matter is allowed. No intense magnetic fields or static-producing materials (e.g., carpet) nearby.

11.4.1.3 Laboratory temperature must be 15-25 °C with a stability: ±1 °C.

11.4.1.4 Ensure that the instrument is in an operational state before use, e.g., purge gas on or liquid nitrogen filled into the tank of the detector as appropriate.

11.4.2 Calibrate the instrument prior to use as per procedures in Section 10, relevant to its make and model.

11.4.3 Particles may be chemically identified individually (i.e., single particle spectroscopy), or via batch spectroscopy, in which multiple particles are identified through scanning a sample area to locate and collect IR spectra for all particles within that area.

11.4.3.1 Particles may be loaded on a glass slide, or onto a filter held in a petri dish.

11.4.3.2 For spectra collection, select appropriate collection parameters, background and detector if applicable, and select a target particle for single particle spectroscopy, or an area of the slide or filter for batch spectroscopy.

11.4.3.3 For spectroscopy of individual particles, chemical identities of all subsampled particles from 11.2.6 must be determined using IR spectroscopy. If less than 30 particles were present, chemical identities of all particles must be determined.

11.4.3.4 Use attenuated total reflectance (ATR) or reflectance mode for particles >212 μm, and reflectance mode for particles <212 μm, as applicable. NOTE: Use of ATR mode may destroy the particle.

11.4.3.5 For batch spectroscopy, choose multiple zones (e.g., ≥ 4) located evenly on the slide or filter, then select an area (e.g., ≥ 1 × 1 mm) within each zone. Scan each area using reflectance mode to acquire an IR image for that area, then use the instrument's software tools to find and chemically identify the particles. The software typically provides total particle number in the selected area, as well as particle dimensions and other statistical data (e.g., proportion of each identified polymer types) (Section 12.2.2).

11.4.4 Library searches are necessary, as the chemical identity of a particle must be determined by matching spectra between reference libraries and the collected spectra.

11.4.4.1 Include reference libraries relevant to both microplastics (virgin and environmentally aged) and natural materials that may be misidentified as microplastics via microscopy. The laboratory should also utilize its own collected spectral library using any known plastic materials (e.g., particles listed in Section 6.6).

11.4.4.2 The laboratory may utilize any collection technique (e.g., Reflection, ATR). Upon collecting a spectrum, the laboratory must save a base spectrum before applying any corrective techniques (e.g., Kamers-Kronig).

11.4.4.3 A match score of 60% or above should be used as the threshold for an accurate spectral match. The laboratory must attempt a spectral match with an uncorrected spectrum prior to applying any corrective techniques. If the match score is between 30% and 60% even after making appropriate corrections, it is recommended to collect another spectrum from a different part of the particle.

11.4.4.4 If two spectra collected at different spots of the same particle still result in a match score between 30% and 60%, the particle should be flagged as "Possible" and notes must be provided on why this match was reported (i.e., signal interference from additives or dyes, or potentially from biofilm material [rare but possible in drinking water] impacted accuracy of spectral matching). If no spectral matches result in a hit quality greater than 30%, report the particle as "undetermined".

11.5 Storage of samples

Samples shall be stored in containers that will minimize disturbance of picked particles or filters and avoid contamination (e.g., picked particles on glass slides shall be stored in new clean glass petri dishes with the cover on, and filters shall be stored

in original clean, glass petri dish with the cover on). It is impossible to guarantee that particles on filters will never be jostled, moved, or dislodged, so all images and measurements must be taken prior to long-term storage of samples.

12.0 Data Analysis and Calculations

12.1 Data acquisition from visual microscopy analysis:

12.1.1 For every counted particle: Assign each particle a unique code. Record color, morphology, and size (length and width). Note: reporting color is recommended but optional if polymer identification is provided for >90% of particles.

12.1.2 Images and Measurements
For all subsampled particles: length and width and one clear image of the particle with a scale bar.

12.1.3 Calculations:
12.1.3.1 Calculate the following data for each size fraction, and for each sample by summation of data of each size fraction:
a. Total particle count.
b. Total particle count for each morphology (i.e., fragment, sphere, fiber).
c. Total particle count for each color (see Section 17.1)
d. Average size (± standard deviation) of the particles by total particles, morphology, and color.

12.1.3.2 If the results in Section 12.1.3.1 are calculated from a representative subsample (Section 11.0), estimate the counts in the entire sample via extrapolation from the measured count data.

12.1.3.3 Divide the count data by the sample volume to convert into sample volume-based particle concentrations.

12.2 Data acquisition from spectroscopic analysis (via either single particle spectroscopy, or batch spectroscopy):

12.2.1 Single particle spectroscopy, if performed, provides library search results, consisting of chemical identity and match score, for each particle analyzed. Save the raw spectrum along with the spectral match result for each particle.

12.2.2 Batch spectroscopy for selected areas, if performed, provides the total area particle count, as well as each particle's dimensions and image, chemical identity, and match score. The software may also provide further statistical data (e.g., proportion for each identified polymer types). For each area, save raw spectra for particles identified, as well as spectral match results.

12.2.3 Calculations:
Both single particle spectroscopy and batch spectroscopy may provide the chemical identities for the measured particles. The fraction of each polymer type in the size fractions and the entire sample may be estimated via

extrapolation from the measured data. The variability in subsampling, if done, can be determined by the standard deviation of the fraction of each polymer type in each sub-sample, whether taken by subsampling and single-particle spectroscopy or by batch spectroscopy, and extrapolation from the measured data.

12.2.3.1 For single particle spectroscopy, calculate the fraction of total particles in the size fraction for each identified polymer type, based on the results of Sections 12.1.3 and 12.2.1.

12.2.3.2 By using the dimension data and associated image from spectroscopy, the morphologies of the particles may be identified. The total numbers and proportions of each type of morphology may be obtained.

12.2.3.3 For batch spectroscopy, the total particle number and the total particle number for each polymer type for the entire slide or filter may be estimated from the batch spectroscopy data (Section 12.2.2) with the following formula:

$$Particle\ count\ on\ the\ slide\ or\ filter = \frac{(\Sigma\ particle\ count\ in\ the\ selected\ areas)}{Area\ fraction\ of\ all\ the\ selected\ areas}$$

12.2.3.4 The total numbers and proportions of each type of morphology from 12.2.3.2 and total particle number from 12.2.3.3 may be used as alternatives to the results from the visual microscopy analysis (Section 12.1.3).

12.2.3.4 For batch spectroscopy, the proportion and variability for each identified polymer type from 12.2.2 may be estimated by taking the average and standard deviation, respectively of those proportions from the selected areas.

12.2.3.5 The total particle number and variability of each polymer type for the entire slide or filter may be calculated via the total particle number from Section 12.1.3 or 12.2.3.3 multiplied by the proportion and standard deviation, respectively, of the polymer type from Section 12.2.3.1 or 12.2.3.2.

12.2.3.6 The total particle number and variability of each morphology type for the entire slide or filter may be calculated via the total particle number from Section 12.2.3.2 multiplied by the proportion and standard deviation, respectively, of the morphology type from Section 12.2.3.4.

12.2.3.7 Divide the count data by the sample volume to convert into sample volume-based particle concentrations.

13.0 Method Performance

This method was validated via a blind sample intercalibration study organized by SCCWRP in 2019-2020, with 22 participating laboratories (De Frond et al., 2022). The clean water matrix used was deionized water, to which microplastic particles of various sizes (3-2000 µm), colors, polymers, and morphologies (e.g., fragments,

spheres, fibers) were added along with natural particles (e.g., sand, shell fragments, cotton fibers, animal fur) serving as false-positive materials. Most microplastic particles came in individual gelatin capsules containing sodium bicarbonate and malic acid to facilitate dissolution; others were added manually. Laboratories analyzed these samples up to 11 months after creation. Method performance data from these samples is applicable for reagent water, finished drinking water, and raw source water. This method has not been evaluated for water high in ionic strength or total dissolved solids (> 0.2 M), or water containing substantial levels of natural matrices (e.g., surface water, wastewater).

Method performance can be divided into two aspects: (1) optical microscopy alone, and (2) spectroscopic confirmation of particle composition. These parameters are applicable for particles 50-5000 µm in size.

For optical microscopy, the accuracy of reported suspected plastic particle counts (i.e., recovery) was 92 ± 57% for 22 laboratories for all particle sizes > 50 µm (De Frond et al., 2022). This protocol, however, requires the use of spectroscopy to confirm the composition of particles analyzed. For particles > 50 µm, FTIR spectroscopy can accurately identify the surface composition of a particle 93% of the time; if it is a plastic particle, correct identification is 95%. Precision data for IR (using FTIR in the SCCWRP study, De Frond et al., 2022) is not available due to the large variation in the number of particles identified by the various participants in the validation study. Fibers are a morphology more difficult to identify correctly by FTIR (76% accuracy overall for both plastic and natural fibers). Further details on accuracy and precision of this method, based on De Frond et al. (2022), are listed in Table 2 for the size fractions used in this method.

14.0 Pollution Prevention

14.1 All extracted microplastics may be disposed of as stated in Section 15.3 so as not to contribute to microplastic pollution of waterways.

15.0 Waste Management

This section describes the minimization and proper disposal of waste and samples.

The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrix of concern is finished drinking water. However, laboratory waste management practices must be conducted consistent with all applicable rules and regulations and that laboratories protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

15.1 Clean drinking water remaining after particle extraction is unlikely to contain microplastics > 20 microns, and removal of those particles < 20 microns remaining is not economically feasible. This water can be disposed down the drain.

15.2 All waste including used filter papers, projector paper, and tape can be disposed of in solid waste intended for landfill.

15.3 When appropriate (i.e., when all particles have been identified, results reported and the samples are no longer required), dispose of the extracted and identified particles in solid waste intended for landfill.

16.0 References

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Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water

from: https://www.waterboards.ca.gov/board_decisions/adopted_orders/resolutions/2020/rs2020_0021.pdf

17.0 Tables, Diagrams, Forms, Flowcharts, and Validation Data

This section contains all the method, tables, figures, diagrams, example forms for data recording, and flowcharts. This section will also contain validation data referenced in the body of the method.

Table 1: Summary of MDAs calculated from background particle counts of blank blind samples from the clean water matrix of the SCCWRP measurement intercalibration exercise (De Frond et al., 2022). The range of background particle counts is 3 to 107 across all size fractions, with an MDA as calculated in Section 9.2.1.2 of 152 particles.

Count range	**Background particle count ≤10**			
Size fraction	**> 500 µm**	**212-500 µm**	**20-212 µm**	**1-20 µm**
MDA (particles)	88	47	80	11

Table 2: Summary of recovery by visual microscopy with RSDs, overall accuracy of identifying particle as plastic vs. non-plastic, and accuracy of correctly identifying the type of polymer for a microplastic particle. Data is based on 0.45 L spiked blind-samples of clean water matrix from the SCCWRP measurement intercalibration exercise (De Frond et al., 2022). Spectroscopy data was normalized by pooling results from all laboratories (De Frond et al., 2022) to determine the proportion of correct chemical IDs for certain particle types (i.e., overall, plastic, natural), classified into size fractions by the measured largest dimension. Relative standard deviations of accuracy results are not available as all analyzed particles were pooled across laboratories and no standard deviation may be calculated. Method Performance Criteria were not developed in De Frond et al. (2022) for the <50 µm fraction given the limited amount of data reported by participating laboratories. N/A = not applicable. n = total number of particles chemical identified among all laboratories.

Size fraction	**Recovery (%)**	**Recovery (%RSD)**	**Accuracy (overall, n)**	**Accuracy (plastic, n)**
>500 µm	125.82	95.74	97.99%, 547	99.42%, 522
212-500 µm	122.30	63.63	90.54%, 391	92.72%, 371
50-212 µm	79.91	74.95	86.57%, 201	89.14%, 175
< 50 µm	31.90	153.26	N/A	N/A

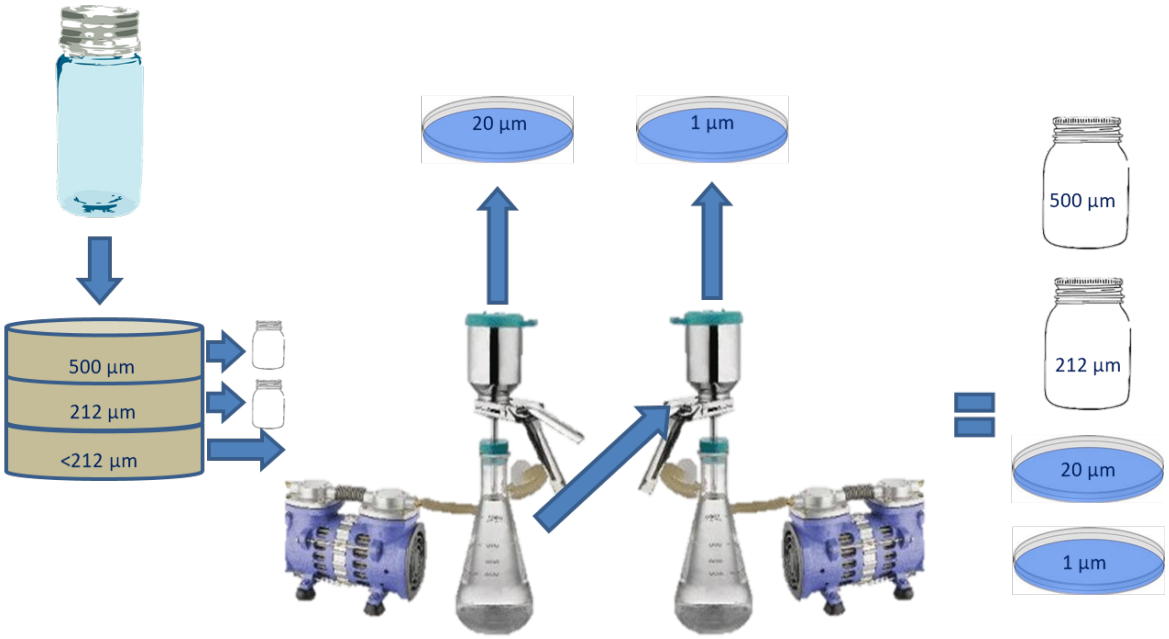


Figure 1: Flow diagram schematic of filtration procedure without a 20 µm sieve (Section 11.1).

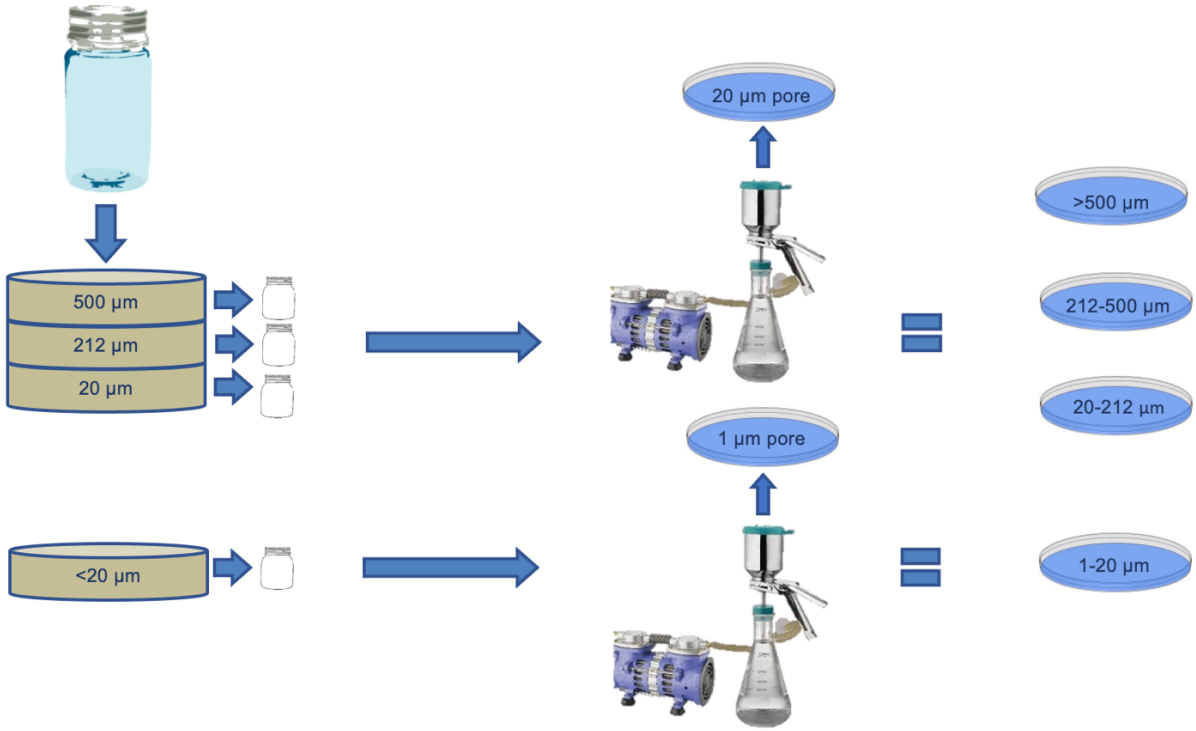


Figure 2: Flow diagram schematic of filtration procedure with a 20 µm sieve (Section 11.1).

17.1 Color key. This color key is to be used to characterize colors of microplastic particles in samples. All particles described as clear, grey, silver, or white are categorized as white, and all gold, orange, or yellow particles are described as orange. For a more detailed breakdown of further developed color characterizations see Figure 1 of Martí et al., 2020.

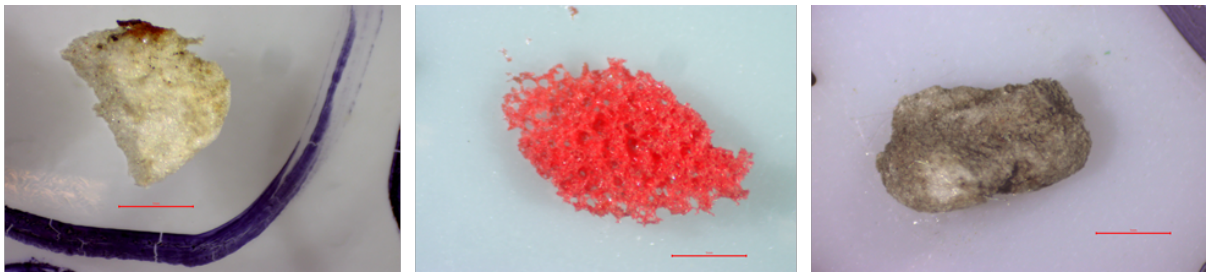
Color Key	HEX Values	RGB
Black	#000000	rgba(0,0,0,255)
Blue	#add4ee #0ab2f0 #0b31d1	rgba(173,212,238,255) rgba(10,178,240,255) rgba(11,49,209,255)
Brown	#ad6800 #7f4800 #522e06	rgba(173,104,0,255) rgba(127,72,0,255) rgba(82,46,6,255)
Green	#00f727 #00a509 #005b01	rgba(0,247,39,255) rgba(0,165,9,255) rgba(0,91,1,255)
Multicolor (2+ colors)		
Pink	#fc9cf7 #e651d3 #c608b1	rgba(252,156,247,255) rgba(230,81,211,255) rgba(198,8,177,255)
PURPLE	#c887fe #b656e4 #7d0bc4	rgba(200,135,254,255) rgba(182,86,228,255) rgba(125,11,196,255)
Red	#fd3334 #e51c0f #bd0501	rgba(253,51,52,255) rgba(229,28,15,255) rgba(189,5,1,255)
Clear, Grey, Silver, White WHITE	#fcfcfc #c4c4c4 #787474	rgba(252,252,252,255) rgba(196,196,196,255) rgba(120,116,116,255)
Gold, Orange, Yellow ORANGE	#ffe501 #ffd600 #ffc001	rgba(255,229,1,255) rgba(255,214,0,255) rgba(255,192,1,255)

17.2 Morphology Key. This morphology key is to be used to characterize microplastics particles in samples. All foams, films, fragments, or pellets are categorized as fragments, and fibers and fiber bundles are categorized as fibers. See Section 17.3 for examples of these morphologies.

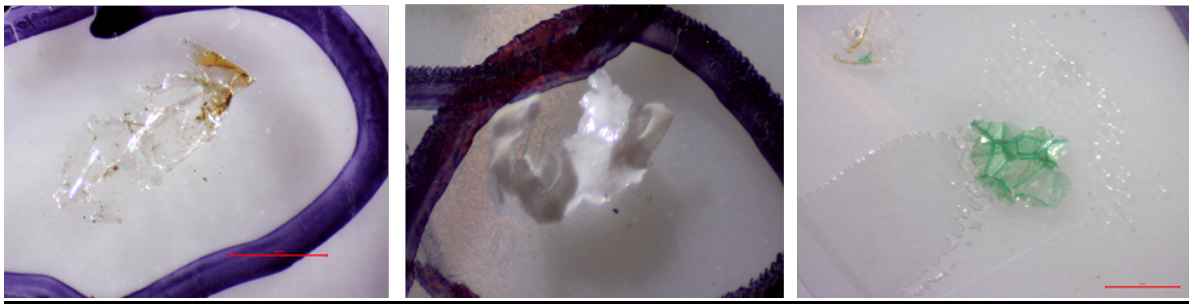
Specific Morphology	Morphology Name to use for Reporting
Foam Film Fragment Pellet	Fragment
Fiber Bundle Fiber	Fiber
Sphere	Sphere
Fragment with rubbery constituency, often black but not always	Rubbery fragment

17.3 Images and Descriptions of Morphology Key. This morphology key is to be used to characterize microplastics particles in samples in conjunction with Section 17.2 (Images courtesy of Keenan Munno and Hannah De Frond).

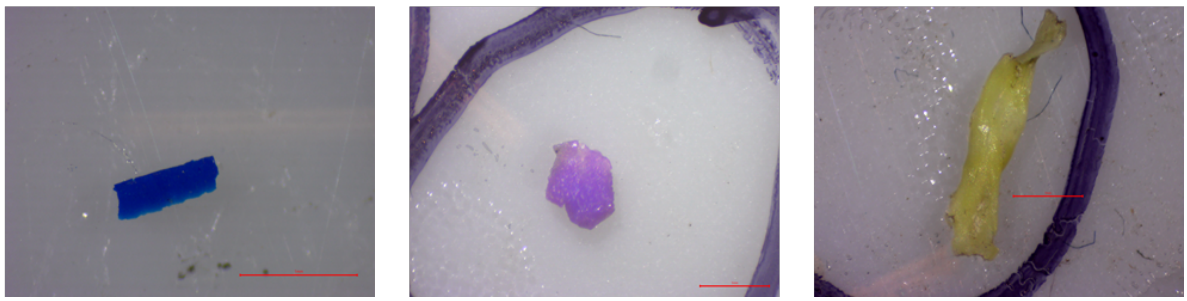
Foam: holes within particle structure; bouncy; 'cloud like' appearance



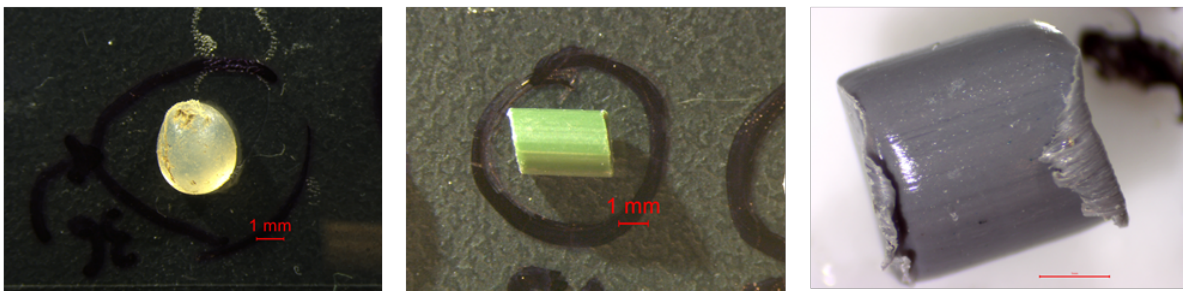
Film: thin, flat, flexible sheets; can fold or crease



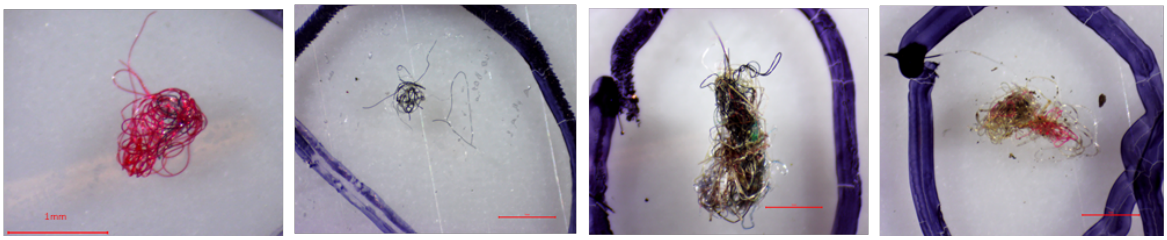
Fragment: rigid edges; irregular shapes; hard



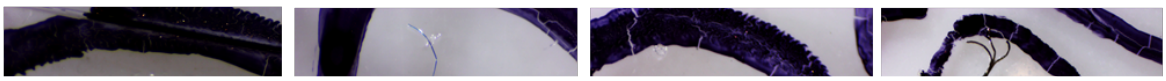
Pellet: can be different shapes; clear 'machine cut' edge; typically, larger than spheres



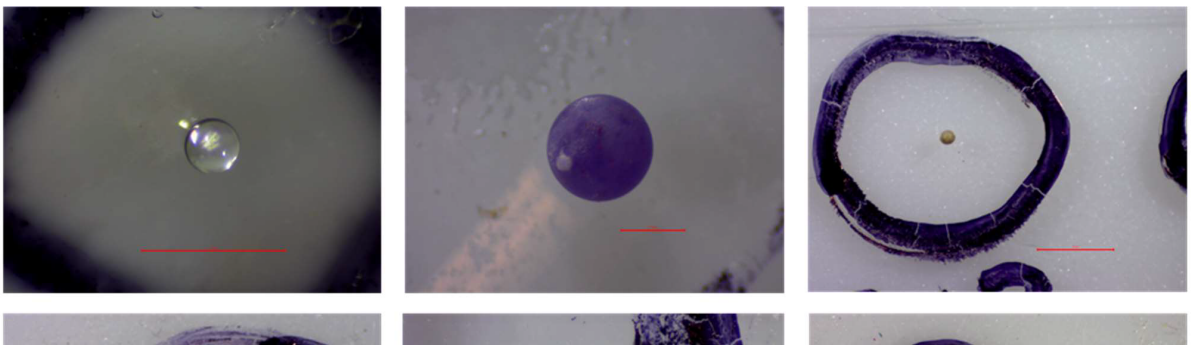
Fiber bundle: >20 tightly wound individual fibers; cannot be teased apart



Fiber: strand or string-like; often equally thick; easily bent and twisted; ends can be flat, pointed, or fraying



SPHERE
Perfectly spherical
Can be hemispheres (broken spheres)
No irregularities
Smooth, often shiny surface



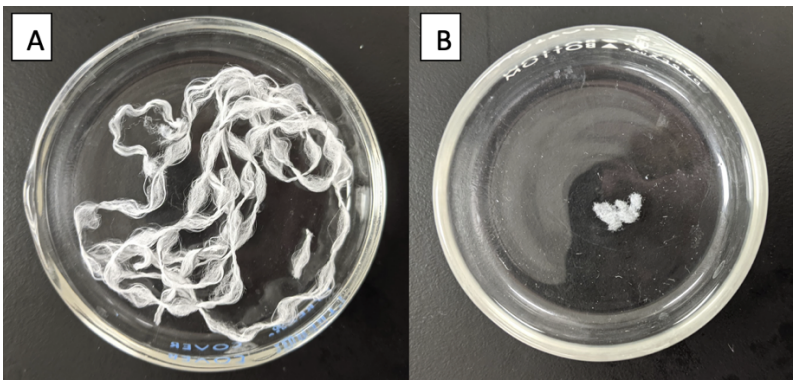
Rubbery fragment: fragment (see fragment images above for general morphology) with rubbery consistency; often black, but not always

Section 17.4: Generating fibers for LRFBs.

Item	Suggested Materials or equivalent
Glass petri dish	VWR Catalog no. 25354-025
Starting material for fibers	Any preferred material (e.g., PET). Bright colors recommended for easy identification.
Curved forceps	Forceps
Vannas scissors	Amazon: “OdontoMed2011 McPherson VANNAS Micro Scissors 7MM Blade 4" Straight ODM”
Regular scissors	-
Squeeze bottle filled with MAG water	VWR no. 16651-904
200-250mL beaker MAG water	-
70% ethanol: MAG water (by volume)	-
Kim wipes	-

This method of generating fibers takes thirty minutes to cut 1mg of starting material, which generates roughly 10,000 fibers. The fibers on average measure about 200µm in length and 20µm in width.

1. Clean and rinse all materials and working surfaces first with MAG water, then 70% ethanol:MAG water.
2. Remove several strands of fabric material from the larger cloth by cutting out a small portion of fabric (~4 × 4 in) and unraveling thick strands as carefully as possible.
3. Once a strand has been isolated (Figure A), hold the strand with forceps and dampen it by dipping it into the beaker filled with MAG water. Dab the fiber on a Kim wipe to remove excess water.
4. While continuing to hold the strand with forceps, begin cutting the end of the strand haphazardly. Continue to cut as finely as possible. Well-cut fibers should resemble the consistency of lint (Figure B).
5. Once the desired number of fibers have been cut, allow fibers to dry overnight. The next day, weigh dry fibers using a microscale to determine the total mass.



17.5 Data table. Sample data reporting with suggested columns, as per De Frond et al. (2022). PHOTOID refers to the name of the file with an image of the particle.

sizefraction	particleid	morphology	color	photoid	instrument	chemid	length (µm)	width (µm)	comments
>500 µm	CW_1_500_1	Sphere	Green	CW_1_500_1-1	FTIR	PE	0.123	0.60	comment
212-500 µm	CW_1_212-500_1	Fragment	White	CW_1_212-500-1-1	FTIR	PS	0.312	0.123	comment
212-500 µm	CW_1_212-500_2	Fiber	Brown	CW_1_212-500-2-2	Raman	PET	0.250	0.018	comment
20-212 µm	CW_1_20-500_1	Fiber	Red	CW_1_20-500-1-1	Raman	PP	0.120	0.010	comment