

# Navigating ISO/FDIS 16094-2 for Microplastic Analysis in Water Using Vibrational Spectroscopy



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## Introduction

Regulatory focus on environmental pollution now includes emerging contaminants like microplastics. Analyzing microplastics is challenging due to their varied sizes, forms, and polymer compositions. Accurate assessment requires advanced techniques.

Agencies are developing guidelines and testing protocols to monitor microplastics in drinking water, emphasizing the importance of reliable analytical methods. Key efforts to standardize analysis include vibrational spectroscopy, as highlighted in ISO/DIS 16094-2 for water samples containing low-content suspended solids.<sup>1</sup>

This white paper presents how the Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system (Figure 1), utilizing quantum cascade laser infrared microscopy (QCL-IR), meets these analytical requirements.



**Figure 1.** The Agilent 8700 LDIR chemical imaging system allows high-speed routine analysis of microplastics in drinking water, including the number of particles present in the sample, and their size and chemical composition.

## ISO methods: Key language and applicability

To implement an ISO method, one must first understand its applicability and terminology. The ISO document<sup>1</sup> contains a link to a web page containing a foreword with helpful guidance.<sup>2</sup>

The document states that these standards are voluntary and do not override national laws, which remain the priority. For instance, laboratory testing performed under the EU directive on microplastics in drinking water for human consumption<sup>3</sup> would take precedence over these standards.

Secondly, it clarifies some language used, stating that:

- The word "shall" indicates a requirement.
- The word "should" indicates a recommendation.
- The word "may" is used to indicate that something is permitted.
- The word "can" is used to indicate that something is possible; for example, that an organization or individual is able to do something.

It goes on to note that:

- A "requirement" is defined as an "expression, in the content of a document, that conveys objectively verifiable criteria to be fulfilled and from which no deviation is permitted if conformance with the document is to be claimed."
- A "recommendation" is defined as an "expression, in the content of a document, that conveys a suggested possible choice or course of action deemed to be particularly suitable without necessarily mentioning or excluding others."

These distinctions help a laboratory know which parts of the standard are required and which are merely suggestions. The standard also includes several annexes labeled "informative"—meaning they serve solely as guidance and information.

## Layout of the standard

The standard offers comprehensive guidance regarding the implementation, validation, and reporting aspects of the method, and follows a defined format that is common among similar ISO documents. Furthermore, it applies to a variety of instruments appropriate for vibrational spectroscopy analysis. This white paper specifically focuses on the application of the 8700 LDIR within laboratories utilizing this method, and will address only the pertinent sections. Unless explicitly stated otherwise, it is assumed there are no barriers to employing the LDIR for this purpose.

Likewise, as dictated by the standardized format, common topics like wavelength range may be addressed in several sections. In this white paper, we will address these by topic with the relevant sections of the standard noted for the purpose of cross referencing. The sections of the standard that are addressed in this white paper are listed in Table 1.

**Table 1.** Sections of the standard covered in this white paper.

Section Number	Section Title
3	Terms, definitions, and abbreviations
5	Interferences
7	Precautions for the laboratory environment, equipment, and materials
8	Sampling
9	Operating protocol
10	Method characterization and verification
11	Quality check of analytical control blanks in test series
12	Expression of results
13	Test report
Annex F	QCL-IR microscopy

## General information

The first parts of the standard set out some general information about the method, the types of samples it applies to, types of results, and the scope. For example, it specifies that it is applicable to the determination of microplastics in the range of 1 to 5,000  $\mu\text{m}$ , and lists the key polymers of interest. Table 2 shows some of these key parameters.

**Table 2.** Summary of microplastic analysis requirements across four methods using vibrational spectroscopy.

Method	ISO/DIS 16094-2
Type of Sample	<ul style="list-style-type: none"> <li>– Ultrapure water</li> <li>– Water intended for human consumption</li> <li>– Raw groundwaters</li> </ul>
Polymers of Interest	<ul style="list-style-type: none"> <li>– Polyethylene (PE)</li> <li>– Polypropylene (PP)</li> <li>– Polyethylene terephthalate (PET)</li> <li>– Polycarbonate (PC)</li> <li>– Polystyrene (PS)</li> <li>– Polytetrafluoroethylene (PTFE)</li> <li>– Polyvinyl chloride (PVC)</li> <li>– Polyamide (PA)</li> <li>– Polymethyl methacrylate (PMMA)</li> <li>– Polyurethane (PU)</li> </ul>
Results	<ul style="list-style-type: none"> <li>– Number of particles</li> <li>– Polymer ID for each particle</li> <li>– Particle size</li> </ul>
Minimum Measurable Particle Size	<ul style="list-style-type: none"> <li>– 5 <math>\mu\text{m}</math> (Raman)</li> <li>– 20 <math>\mu\text{m}</math> (infrared)</li> </ul>
Maximum Measurable Particle Size	5 mm
Analytical Techniques	Infrared apparatus coupled to microscope, such as Fourier transform infrared, quantum cascade laser infrared microscopy, or Raman coupled to microscope

## Suitability of the LDIR

### Use of a QCL

The standard addresses both Raman and infrared (IR) spectroscopy, particularly when these methods are used in combination with a microscope. Since the LDIR system operates via IR, the relevant sections pertain to IR techniques and can be found throughout the document—including Section 3 "Terms, definitions, and abbreviations", Section 4 "Principle", and Section 7.2 "Equipment". Additionally, Annex F specifically focuses on QCL-based systems.

Sections 3 and 4 offer broad information without restricting the use of QCL-based systems like the LDIR. More specific details appear in section 7.2.2, "IR apparatus coupled to microscope," which includes summarized requirements in Table 3. Section 7.2.1 also states that procedures may be carried out manually or automatically.

Section 7 of the standard pertains to equipment specifications, while Section 9 outlines operating protocols, providing additional relevant details. Section 9.6.9, titled "IR spectra acquisition and particle identification," discusses acquisition modes and wavelength ranges, noting transmittance as a suitable method. The section acknowledges that some instruments feature spectral ranges narrower than the specified 4,000 to 750  $\text{cm}^{-1}$ , with reference to Annex F, which focuses on QCL-based systems. It is important to note, however, that some non-QCL systems also operate within more limited spectral parameters. Furthermore, commonly used filters such as those made from aluminum oxide (Anodisc) may eliminate signals within the 1,200 to 750  $\text{cm}^{-1}$  range.

**Table 3.** Section 7.2.2 "IR apparatus coupled to microscope" and how the LDIR meets the requirements.

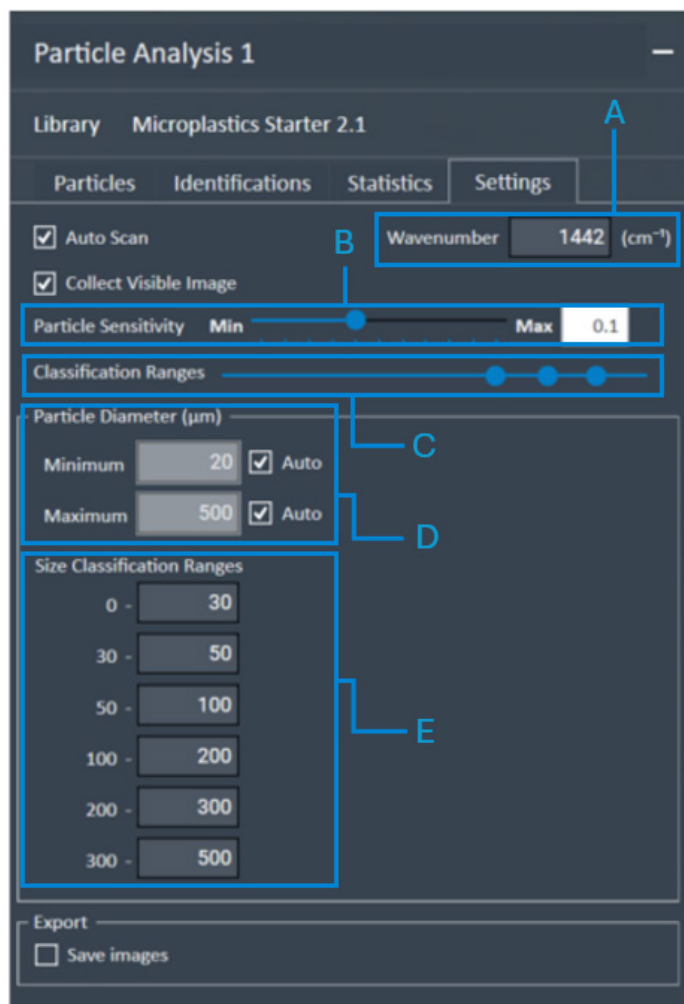
Requirement	LDIR
<ul style="list-style-type: none"> <li>– Optical microscope</li> <li>– Objectives enabling at least 4<math>\times</math> magnification</li> <li>– Able to work in visible light</li> </ul>	Fully meets requirements
High-resolution camera able to display particles on a computer screen	Fully meets requirements
Suitable positioning system, to move the filter under the IR light beam	Fully meets requirements
<ul style="list-style-type: none"> <li>– Calibrated infrared spectrometer</li> <li>– Able to record a spectrum in transmission, reflection or attenuated total reflectance (ATR) mode</li> <li>– Infrared range, between 4,000 and 750 <math>\text{cm}^{-1}</math>, with resolution better than or equal to 8 <math>\text{cm}^{-1}</math></li> <li>– The correct operation of the equipment should be regularly checked by analyzing a spectral reference</li> </ul>	<ul style="list-style-type: none"> <li>– Fully meets requirements</li> <li>– Meets requirements: reflection, transfection, and ATR</li> <li>– Meets requirements: Operates in the fingerprint region of 1,800 to 975 <math>\text{cm}^{-1}</math></li> <li>– Wavelength range is further discussed in Section 9 and Annex F</li> <li>– LDIR collects spectra at 0.5 <math>\text{cm}^{-1}</math> resolution</li> <li>– Fully meets requirements: contains internal spectral references and automated regular validation systems</li> </ul>
Software enabling the acquisition of spectra, the correction of filter background noise, and their comparison with a spectral database	Fully meets requirements
Spectral database that contains at least several types of spectra of PE, PET, PP, PS, PC, PVC, PMMA, PTFE, PA, PU	Fully meets requirements

Annex F, introduced in a 2022 draft standard but based on a 2021 regional version, is general in nature in that it applies to any QCL-based system. The details, however, are not relevant for the 8700 LDIR. Of course, it must also be remembered that such annexes are informative only.

Annex F draws attention to three areas of concern for QCL-based systems:

- Paragraph 2 states that there is insufficient laboratory testing to show a similarity between QCL and  $\mu$ FTIR/ $\mu$ Raman results. While this may be true for other QCL-based instruments, laboratories using the LDIR have participated in several recent interlaboratory studies. The most recent study, results for which were published early in 20254, includes several laboratories using the LDIR, and results clearly confirm the LDIR's satisfactory performance within the group.
- Point 1 "Sample preparation" raises a concern with potential sample loss when using a glass slide (a low-e or "Kevley" slide) as the analysis substrate. It is unclear why this point is raised in Annex F. The use of such a slide is not limited to QCL-based systems; indeed, it is common in IR spectroscopy, including traditional FTIR systems. Any laboratory using this method would be required to demonstrate suitable recovery as set out in Section 10.6. Furthermore, the LDIR is supplied with a sample holder suitable for either gold or aluminum filters, on which samples can be analyzed directly at the completion of the final filtration step.
- Point 2 "Method parameters for automated spectral acquisition" raises a concern that some QCL-IR systems have limited setting options that may somehow impact the results, but fails to describe what options are missing. In addition to lacking detail, the point is made redundant by the requirement that the method (regardless of the instrument used) is validated according to Section 10 "Method characterization and verification".

The LDIR offers a range of suitable settings that the user may adjust to ensure the analysis meets requirements. These include the ability to adjust spectral acquisition speed, the IR wavelength used for particle detection, the sensitivity of particle detection, the minimum and maximum particle size, and so on (Figure 2). It also allows for incredibly fast and easy reprocessing of spectra using alternative libraries.



**Figure 2.** (A) Wavenumber selection for particle detection, (B) Particle detection sensitivity, (C) Classification (HQI) ranges, (D), minimum and maximum particle size, (E) Size classification ranges for reporting.

Given these issues, Annex F does not add value and should be disregarded. Laboratories should follow Section 10 of the main standard for validating methodologies, which applies to all relevant techniques including QCL systems like LDIR.

## Suitability of the LDIR: Other requirements

The standard defines a range of other instrument requirements as follows, all of which are met by the LDIR (Table 4).

**Table 4.** Summary requirements for an IR instrument, as set out in Section 9.

Requirement	LDIR
Section 5 discusses interferences such as sources of external contamination, while section 9.6.1 discusses precautions to be taken during the physical transfer of the filter from the filtration device to the microscope.	The LDIR uses a closed and purged analysis chamber, so that once in the LDIR, the chamber and sample are protected from contamination. Additionally, this protects the sample from potential loss of particles.
9.4 Adjustment and calibration of instruments. 9.4.1 Infrared microscope.	The LDIR runs a daily automated test protocol with results recorded and retained. Additional checks are run at production, at installation, and annually, covering all relevant functions.
9.6.2 Choice of the optical objectives. An objective between 4x and 100x should be used to provide sufficient signal and contrast. If multiple objectives are used, they should be suitably aligned.	The objectives used in the LDIR meet this criterion and are more than sufficient to achieve the required resolution. The LDIR uses both visible and IR channels that are aligned to a defined tolerance and frequently checked.
9.6.9 IR spectra acquisition and particle identification: – Use one of transmission (transflectance), reflection, and ATR. Transmission or reflection acquisition modes are preferred. – Background signal subtraction is applied. – Spectra are compared with a spectral database (library) in the same acquisition mode.	<ul style="list-style-type: none"> <li>– The LDIR uses transflection mode for automated analysis, while both reflection and ATR modes are available.</li> <li>– The background is taken on the filter surface at multiple points continuously throughout the measurement.</li> <li>– A library of transflection spectra is supplied with the LDIR, while other libraries are available. In addition, users can create their own libraries or add to existing libraries.</li> </ul>
9.8 Spectra treatment: – Does not define treatments to be used, but notes that several options are available. – Does advise to use only those needed to avoid potential false identification.	<p>LDIR uses minimal spectral treatment:</p> <ul style="list-style-type: none"> <li>– Baseline subtraction</li> <li>– Spectra collected at <math>0.5\text{ cm}^{-1}</math>, smoothed to <math>8\text{ cm}^{-1}</math></li> <li>– Spectra normalized</li> <li>– First derivative used for matching</li> </ul>
9.9 Criteria of chemical composition identification: – Broad criteria for matching. – Notes a range of classification options but recommends Pearson's correlation. – Discusses HQI and, in Section 10.4, details a method for determining a suitable HQI. – Gives an example of a HQI of about 80% (0.8) for automated analysis.	<ul style="list-style-type: none"> <li>– The LDIR fulfills the requirements of this section as demonstrated in a recent white paper<sup>5</sup> and confirmed in interlaboratory studies.<sup>4</sup></li> <li>– While the LDIR matching system is based on cosine similarity, the white paper demonstrates it is well aligned.</li> <li>– While a laboratory should establish its own HQI threshold, it is demonstrated that 0.8 is a suitable guideline for automated analysis by LDIR.</li> </ul>
9.10 Spectral interferences: Specifically addresses common interferences such as polyamide versus natural proteins (9.10.3) and polyethylene versus other molecules with long CH chains (9.10.4). Recommends inclusion of suitable materials in libraries and verification by a qualified operator.	<ul style="list-style-type: none"> <li>– The LDIR can adequately deal with these issues as demonstrated in a previous white paper<sup>5</sup> and application note.<sup>6</sup></li> <li>– The spectral library contains relevant materials of all types.</li> <li>– Manual verification is simple in the LDIR spectral viewer, and includes options for quick and easy assessment with additional libraries.</li> </ul>

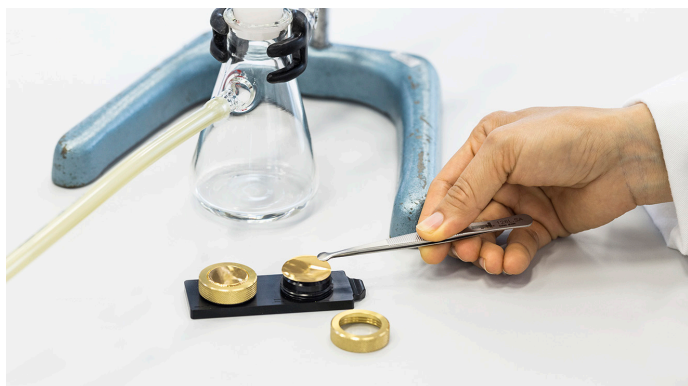
## Sampling (pre-analysis)

Sampling is a critical part of microplastic analysis and can have an impact on recovery of materials, levels of nontarget materials retained from the sample, and external contamination. While a large proportion of sampling and sample preparation processes are common between instruments and techniques, some distinctions remain.

Section 7.2.5 describes the characteristics of filters, such as type and size. For example, it notes that filters shall have the following characteristics:

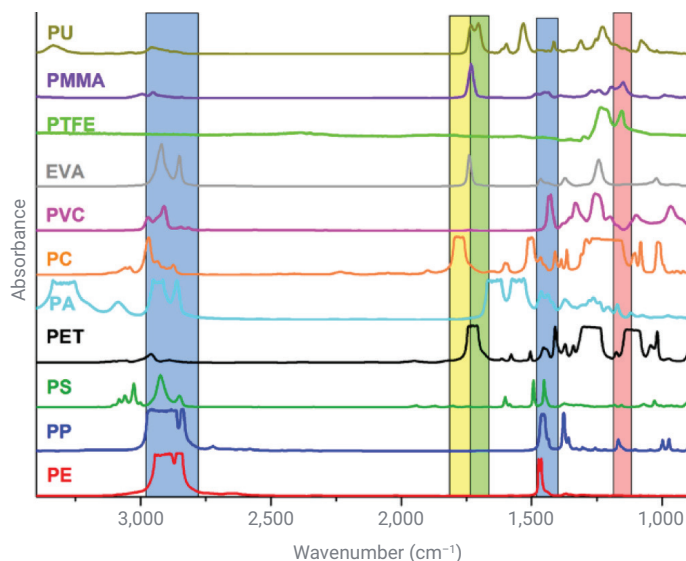
- Compatible with the measurement type (e.g., IR) and mode (e.g., transfection)
- Do not present spectral bands that interfere with the target microplastics
- Appropriately flat, noting that special holders can assist
- Do not release any microplastics that may impact background noise
- Heat and mechanical resistant (as required) and resistant to oxidation
- Suitable porosity (pores diameter and distance between them)

Both gold- and aluminum-coated filters<sup>7,8</sup> (25 mm diameter) can be used with the LDIR (Figure 3). The coatings are resistant to most common solvents as well as oxidizers if used in dilute concentrations. They cover the full surface of the filter, ensuring there is no interference either from spectral artefacts or fragments from the substrate. Equipped with a high density of 0.8  $\mu\text{m}$  pores, they ensure practical speeds of filtration and limit clumping and clogging. The two coating types provide high-quality signal reflection with minimal interference or distortion.



**Figure 3.** Gold-coated (as shown) and aluminum-coated filters are offered with the LDIR.

This is not the case for at least one of the filter types commonly used with FTIR systems, the aluminum oxide Anodisc filter. This filter absorbs strongly from 1,200  $\text{cm}^{-1}$ , rendering this part of the spectrum unusable and thereby eliminating the ability to detect PTFE – which is only detectable by bands in the 1,174 to 1,087  $\text{cm}^{-1}$  region (Figure 4).<sup>9</sup>



**Figure 4.** IR spectra of most common synthetic polymers (transmission mode). While most have peaks in the fingerprint region (1,800 to 975  $\text{cm}^{-1}$ ), PTFE can only be identified using the spectral range of 1,174 to 1,087  $\text{cm}^{-1}$  (red).<sup>9</sup>

The LDIR is provided with a special filter holder that ensures the filter is held flat. In addition, the LDIR automatically detects if the sample is not flat enough in the system and warns the user.

The filters are compatible with commercially available filtration apparatus that are compliant with the requirements of Section 9.2 "Sample filtration protocol."

## Software and reporting

The standard goes into some detail about the processes of analysis and how results are reported. In advanced and fully automated instruments such as the LDIR, these functions are largely software controlled.

### Selection of particles or areas to be analyzed

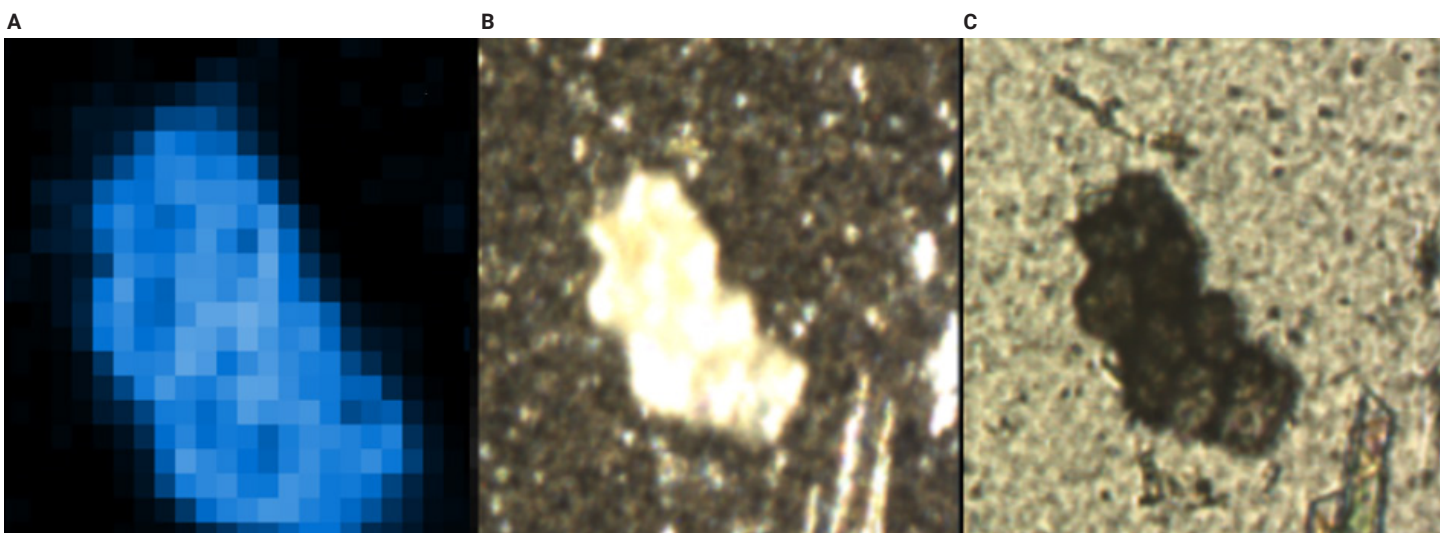
Particle-focused analysis of microplastics can be time consuming. Samples can contain high numbers of particles; therefore, particles should, at least somewhat, be separated to allow systems to detect and isolate them for characterization and analysis.

Section 9.6.3 "Selection of particles to be analyzed or choice of the analyzed surface area" sets out the guidelines for this, as discussed in Table 5.

Section 9.6 presents sampling models, highlighting the "TOTAL" model—where the entire sample is analyzed—as the preferred approach. The LDIR adopts this model due to its rapid automated analysis capabilities. Alternative subsampling models are available but considered less effective, mainly compensating for slower, more laborious systems. Operators should assess their suitability, especially given the typical lack of homogeneity in microplastic samples.<sup>10</sup>

**Table 5.** Summary of microplastic analysis requirements across four methods utilizing vibrational spectroscopy.

Requirement	LDIR
<p>Detection of particles: The first paragraph deals with detection of particles but the language used is relevant for systems that use the visible image for this task, as is the case with most systems.</p> <p>It also notes the importance of eliminating the impact of "pores, filter rugosity, or other light artefacts" in identification of the particles.</p>	<p>The LDIR is the only instrument that uses IR light for this process—a far superior process. When coupled with the highly reflective backgrounds of the filters and slides, unparalleled contrast is achieved.</p> <p>The use of the IR image as the primary identification method eliminates interference from the sample substrate, and so on (Figure 5)</p>
<p>The second paragraph deals with particle size, and notes that size shall be correctly characterizable, especially with regards to the impact of color and transparency.</p>	<p>Using the IR image for this process, the LDIR eliminates issues related to color and transparency.</p>
<p>The third paragraph deals with sample flatness and the impact on focus.</p>	<p>The LDIR eliminates these concerns in two ways:</p> <ul style="list-style-type: none"> <li>– Regardless of the use of filters or slides, the sample holders are designed to ensure that the sample surface is as flat as possible. Notably, the filter holders use an innovative locking mechanism to ensure they are both flat and secure.</li> <li>– The automated workflow within Agilent Clarity software (the LDIR operating software) performs automated sample flatness checks at the commencement of analysis and warns the analyst if this is not within tolerance.</li> </ul>



**Figure 5.** Infrared (A), on-axis (B) and off-axis (C) images of a particle.

## Expression of results

Section 12 deals with the expression of results, while Section 13 deals with the test report itself. As is common with these types of standards, other sections also touch on these topics, especially with regards to definitions. Much of the detail in this section is generic in that it is applicable to all methodologies, but some points are notable:

- It is important to ensure that the reporting limits are displayed. How these are calculated is detailed in Section 10.
- The number of particles in the sample can only be qualified (that is, reported) if it is higher than the reporting limit.
- When calculating the number of particles, subtraction of the blank value is not permitted.
- Likewise, correction for recovery rate is not permitted.

The LDIR complies with these requirements in that it does not perform corrections for blanks or recovery rates in any of the calculations or reporting.

This section also notes that results must be expressed according to their nature; that is, polymer type and size. The LDIR software allows the user to define (and save for future use) the required minimum and maximum size ranges, as well as the individual classification ranges (Figure 6). These are reflected in the results.

In addition, all results can be downloaded in a Microsoft Excel file for additional reporting. This report includes additional parameters that may be used to determine particle shape, among other things (Figure 7).

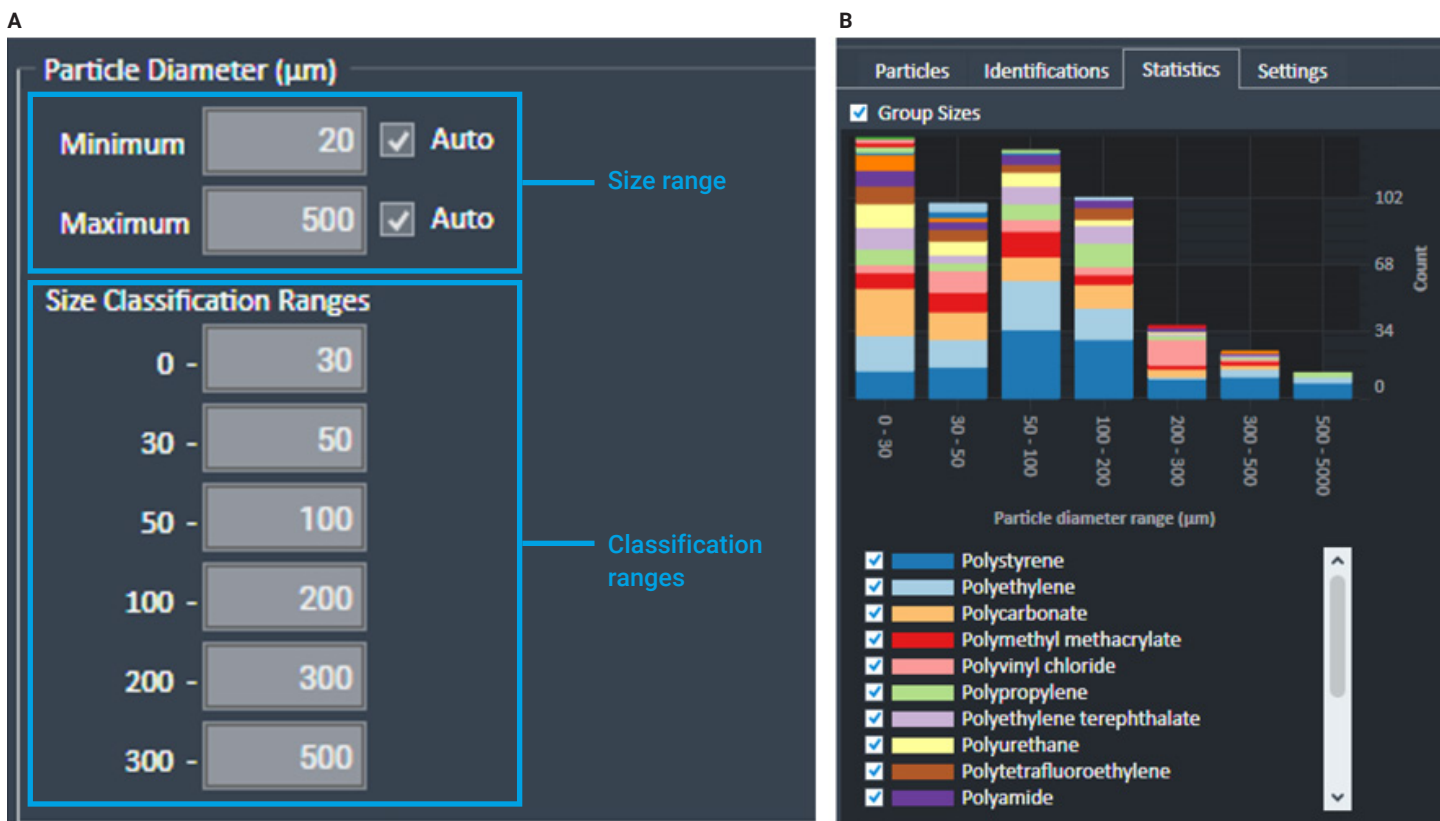


Figure 6. An example of results expression in the LDIR.

#	Id	Width (µm)	Height (µm)	Diameter (µm)	Aspect Ratio	Area (µm <sup>2</sup> )	Perimeter (µm)	Eccentricity	Circularity	Solidity	Identification	Notes	Match Type	Quality	Is Valid
1	A1	319	150	180.72187	2.134599262	25651.4137	1251.164346	0.557037974	0.205917117	0.685786253	Polycarbonate (PC)		Auto	0.819660559	true
2	A2	346	134	155.1396658	2.581549625	18903.21112	1256.680788	0.805443702	0.150416506	0.496481542	Polycarbonate (PC)		Auto	0.853483665	true
3	A3	121	209	143.7762008	0.57735382	16235.43347	807.148436	0.806069906	0.313160468	0.820757455	Rubber		Auto	0.664502823	true
4	A4	127	142	116.4099098	0.892498502	10643.14028	649.2839931	0.696181463	0.317256302	0.76378926	Polycarbonate (PC)		Auto	0.791846815	true
5	A5	87	133	103.9666991	0.650617355	8489.427285	430.4798969	0.677555942	0.57568181	0.908286214	Polycarbonate (PC)		Auto	0.736232699	true
6	A6	124	160	119.2085059	0.772881356	11161.03244	598.0980646	0.817559274	0.392075261	0.862054851	Polycarbonate (PC)		Auto	0.840211686	true
7	A7	153	104	109.7763171	1.468931335	9464.707436	613.2498047	0.584875404	0.31625851	0.803592186	Polycarbonate (PC)		Auto	0.778167911	true
8	A8	64	160	78.52923376	0.39824582	4843.425246	511.9070217	0.941390813	0.232263139	0.648998915	Polycarbonate (PC)		Auto	0.839841591	true
9	A9	130	69	77.55654183	1.871283228	4724.183447	437.9128136	0.512071115	0.309571742	0.709412857	Polycarbonate (PC)		Auto	0.891068745	true
10	A10	53	101	65.10229508	0.528117021	3328.759967	315.0612339	0.862438708	0.42140764	0.847812231	Natural Polyamide		Auto	0.9297209	true
11	A11	116	64	74.03258624	1.8030557	4304.628966	317.8767316	0.519266675	0.535338046	0.884696841	Polycarbonate (PC)		Auto	0.680473079	true
12	A12	47	87	58.28621023	0.54398489	2668.219282	255.5698286	0.87440005	0.513348407	0.860636277	Polycarbonate (PC)		Auto	0.870308025	true
13	A13	68	60	57.23481112	1.1344931	2572.825842	244.5634069	0.476089241	0.54055178	0.866957686	Polycarbonate (PC)		Auto	0.844240091	true
14	A14	86	42	48.9010416	2.025316463	1878.13195	320.2240031	0.57378099	0.230159126	0.703385158	Polycarbonate (PC)		Auto	0.868342381	true
15	A15	32	55	35.81115644	0.587082253	1007.225177	165.9876002	0.749411596	0.459393816	0.780604678	Polycarbonate (PC)		Auto	0.822053494	true

Figure 7. Results can also be exported into a detailed Excel worksheet for further analysis.

## Method characterization and verification

Section 10 deals with method characterization and verification. Many of the details are common to all techniques, but are nonetheless important. Indeed, it has been noted that many early works on microplastics lack detail regarding methods and how they have been validated. As a result, studies are difficult to compare, limiting their usefulness in developing a comprehensive understanding of the issue.

Section 10.1 is a general introduction noting that a "laboratory shall demonstrate that it is proficient." The details of how to do this are contained in Sections 10.2 to 10.5:

- 10.2 Verification of the particle size measurement accuracy
- 10.3 Verification of microplastics identification and classification at claimed size
- 10.4 Determination of minimal HQI for automatic identification
- 10.5 Determination of the reporting limits of the method

In addition, Section 11 discusses the use of analytical control blanks.

### Verification of the particle size measurement accuracy (Section 10.2)

It is required that the laboratory verifies the trueness of the size measurement using a certified standard. The fully automated workflow of the LDIR determines the size of the particle during the analysis. Two parameters reported are width and height—being two axes, long and short, of the particle. The instrument also offers an onboard ruler function that can be used to verify those measurements (Figure 8).

The onboard ruler can be verified using the supplied USAF Target with bars of a known width (Figure 9). These targets are the standard tool used for verifying resolutions in instruments such as these.<sup>11</sup>

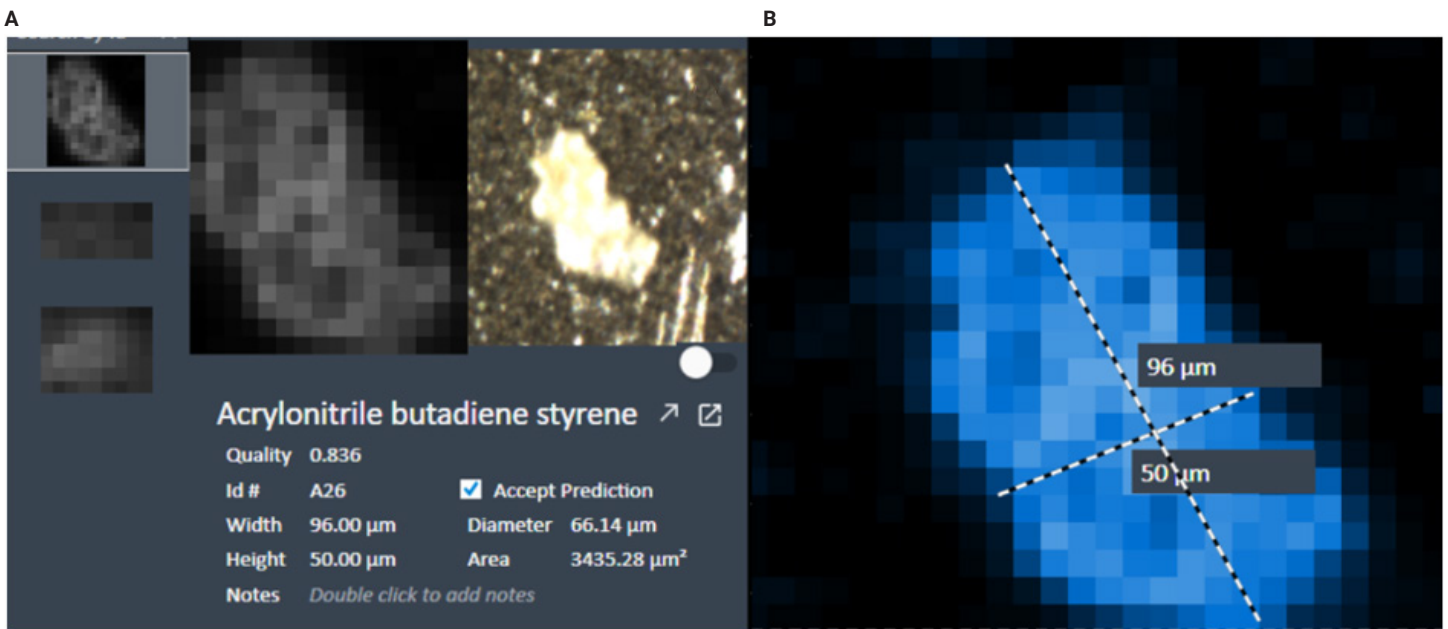


Figure 8. The onboard ruler function.

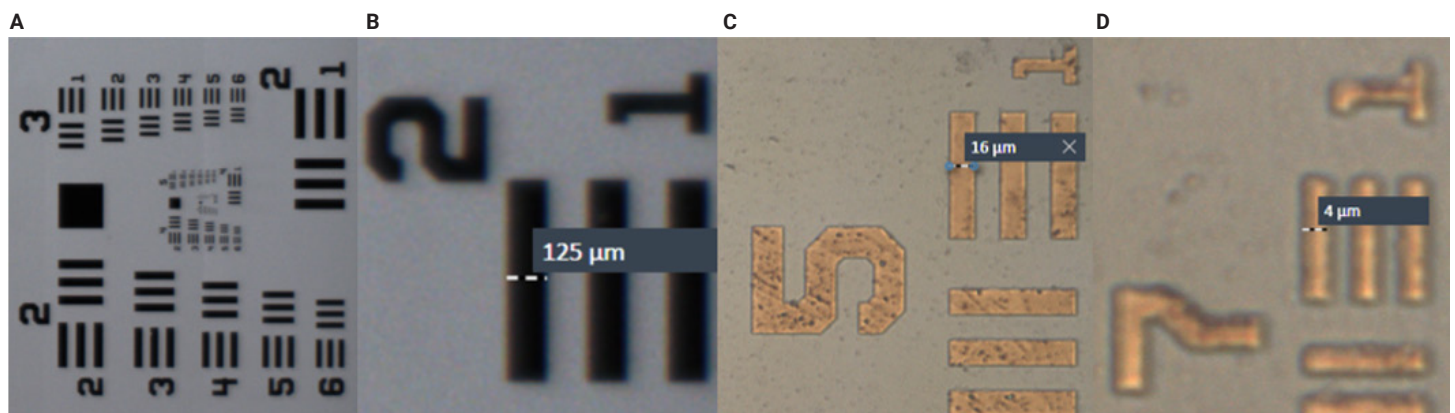


Figure 9. (A) The USAF Target has a set of bars of known width. Bars in (B) Group 2 Element 1 are 125  $\mu\text{m}$  wide, in (C) Group 5 Element 1 are 15.63  $\mu\text{m}$ , and in (D) Group 7 Element 1 are 3.91  $\mu\text{m}$ . Note that the images in A and B utilize the standard camera in the LDIR, while C and D are taken with the high-magnification lens.

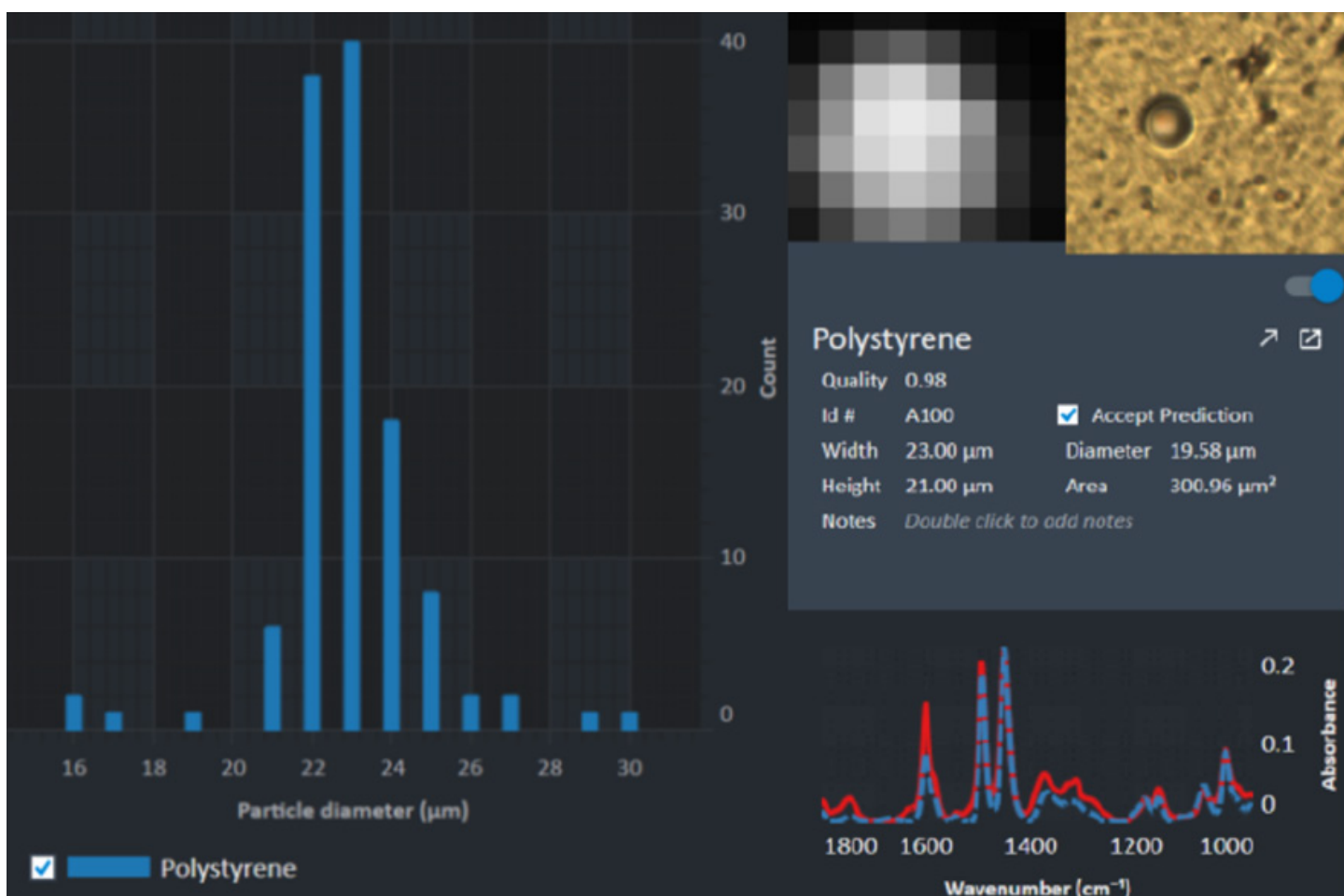
### Verification of microplastics identification and classification at claimed size (Section 10.3)

The key requirement of this section is verifying that the instrument correctly determines the size of particles within the claimed size range. Specifically, it requires the identification of at least four different types of microplastics at the claimed lower size range. It notes that this can be achieved using either natural samples or spiked samples.

If using natural samples in the LDIR for this task, the size can be easily verified using the onboard ruler, as shown in Section 10.2. In Figure 10, a sample spiked with NIST-certified 20  $\mu\text{m}$  diameter beads is used for the task.<sup>12</sup>

### Determination of minimal HQI for automatic identification (Section 10.4)

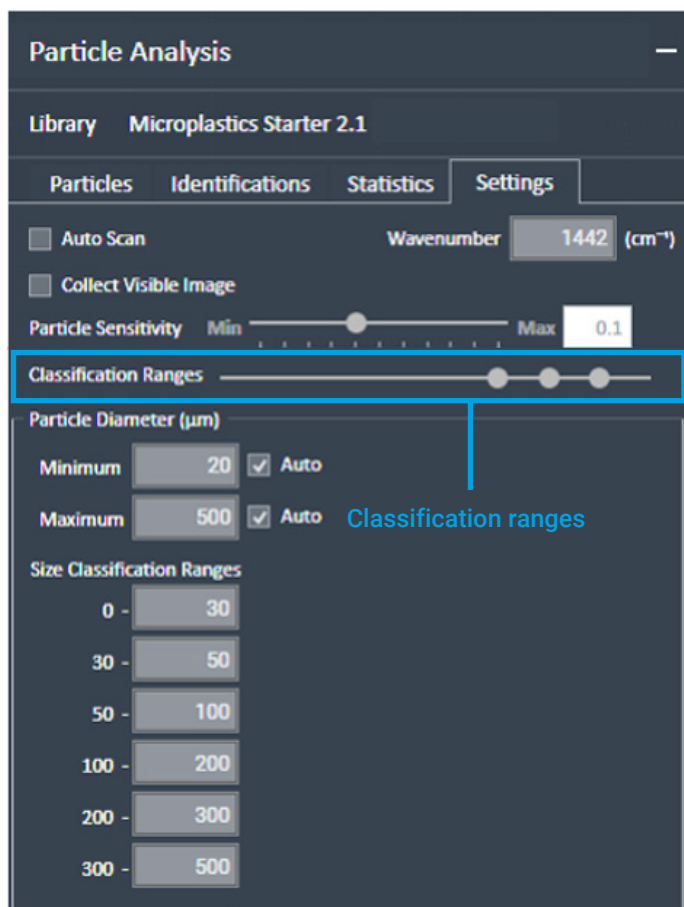
Section 10.4 sets out a procedure for the determination of a suitable HQI, which is easily followed in the LDIR. Specifically, it requires analysis of at least three samples using both the automated methodology and manual verification by a qualified operator. It notes that the samples may be natural or spiked. These results are compared with regards to the HQI to determine a suitable HQI threshold at which there are not more than 30% false negative or false positive results when the automated results are compared to the manually verified ones.



**Figure 10.** Examples of 20  $\mu\text{m}$  polystyrene beads (NIST traceable) measured using the automated Particle Analysis workflow of the Agilent 8700 LDIR chemical imaging system.

This task is easily performed with the LDIR using the results exported in the Excel file. As it contains both the polymer identification and the HQI score, when compared to the manually validated results the suitable HQI can easily be calculated.

The standard then suggests that the laboratory further determines a range between which a result may be accepted following verification by a skilled operator and a HQI score below which a result would be rejected. As an example, it suggests that a result may be accepted without further verification if the HQI is about 80% (or 0.8 as expressed by the LDIR), rejected if it is below 60%, and verified if it is between these. The LDIR allows the user to set such classification ranges, and these settings can be saved for later use. While the ISO standard suggests three ranges, the LDIR allows up to four (Figure 11). Notably, for those in the lowest range, the LDIR automatically records the result as "Undefined" and does not report the best match to avoid the chance of false reporting.



**Figure 11.** Classification ranges can be easily set and custom ranges saved for later use.

### Determination of the reporting limits of the method (Section 10.5)

The standard defines how to establish the reporting limit. This process is common among instruments and has no specific implications for LDIR users. However, the standard notes that that the reporting limit applies to both the minimum number (count) of particles detected and the minimum particle size. Once this minimum particle size is determined and verified, the LDIR has the capability to automatically exclude particles smaller than the established reporting limit to avoid misreporting data.

Note that while this section asks the user to determine the minimum size, it does suggest a minimum of 5 µm for Raman and 20 µm for IR instruments. The automated workflow in the LDIR has been demonstrated to be reliable for particles down to 10 µm in diameter<sup>12</sup>; however, this standard does require each laboratory to verify this themselves.

### Quality check of analytical control blanks in test series (Section 11)

Section 11 addresses the very important topic of analytical control blanks. These are also discussed in Section 9.3. As with other sections, this is common among instrument types, and much of the detail relates to general laboratory practice.

One important aspect is that analytical control blanks are identically treated. The LDIR's two-place filter holder allows the laboratory to run an analytical control blank run beside a sample, without removing the sample or blank from the instrument. This analysis can be run in the fully automated workflow without operator intervention. This feature is not only convenient but it can also greatly enhance the accuracy of the blank reporting.

## Conclusion

In conclusion, the implementation of LDIR technology, guided by the ISO standard, offers laboratories a robust and reliable approach for the identification and quantification of microplastics in water samples. The ability to customize and save classification ranges for HQI scores enhances both flexibility and standardization in reporting, ensuring that results are consistent and defensible. The LDIR's automated exclusion of particles below the verified reporting limit minimizes the risk of misreporting and supports compliance with internationally recognized best practices. Additionally, the integration of analytical control blanks into the automated workflow, facilitated by the instrument's two-place filter holder, streamlines quality control and increases the accuracy of blank measurements without additional manual intervention.

Adhering to the ISO recommendations for minimum particle size and the rigorous verification of reporting limits ensures that laboratories can confidently report results that meet regulatory and scientific standards. The features and workflow optimizations provided by the LDIR not only simplify compliance with these standards but also allow for efficient, high-throughput analysis. As the field of microplastics analysis continues to evolve, such advancements in instrumentation and methodology are essential for generating reliable data to inform environmental monitoring and regulatory decision-making.

Ultimately, the adoption of standardized procedures and advanced technologies like the LDIR will continue to play a pivotal role in advancing the science of microplastic detection and quantification. These tools empower laboratories to produce high-quality data, support harmonization of methods across the scientific community, and contribute to the global effort to understand and mitigate microplastic pollution in water resources.

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